Off-track takes Frizzled off the canonical path

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Like many other signalling pathways, the Wnt pathway is seen as part of a network that integrates extracellular information mediated by a variety of cell surface proteins to produce cell context-specific outputs. The identification of Frizzled as Wnt receptors (Bhanot *et al*, 1996) provided the essential link between extracellular Wnts and the intracellular components of Wnt signal transduction. Recent observations, however, indicate a rather complex interplay of this 'core' receptor element with an increasing number of co-receptors. In this issue of *The EMBO Journal*, Tolwinski and Borchers (Peradziryi *et al*, 2011) describe PTK7/Off-track as a novel co-receptor element that might govern specificity in cellular Wnt responses.

The β -catenin/TCF pathway is often described as canonical because it mediates the core activities of Wnts in cell specification and tumourigenesis. This distinction is necessary because Frizzled receptors had previously been shown to participate in planar cell polarity (PCP), a process whereby cells acquire polarity within the plane of epithelia (Strutt, 2008). PCP is the best characterised of various alternative Wnt signalling pathways that have collectively been termed non-canonical. Over the years, vertebrate Wnts have been classified into canonical and non-canonical Wnts. For example, vertebrate Wnt3a is an archetypal canonical Wnt while vertebrate Wnt5a has been implicated in PCP signalling. This distinction can be useful but is almost certainly an oversimplification, as the effect of various Wnts is strongly dependent on the nature of the Frizzled receptors present (van Amerongen and Nusse, 2009). It is worth mentioning that, in *Drosophila*, no Wnt has so far been shown to be required for PCP signalling.

One feature that has often been noted is that activation of the two pathways is somewhat exclusive. How PCP and canonical signalling affect each other and what the relative contribution of various Frizzled and Wnts to either pathway is are questions currently under active investigation.

Despite their central importance, Frizzled proteins are far from being the only receptors that modulate or control Wnt signalling. Indeed, members of the LRP (LDL-related protein) family are essential co-receptors for canonical signalling. For example, Arrow, the sole *Drosophila* LRP, is absolutely required for Wingless signal transduction (Wehrli *et al*, 2000). A current view is that LRP family members form a tripartite complex with Wnt and Frizzled. As *arrow* mutations do not cause a PCP phenotype in flies, and vertebrate LRPs do not modulate PCP signalling, this class of co-receptors is seen

as strictly canonical. Another class of single-pass transmembrane proteins, including ROR1 and ROR2, also act as Wnt co-receptors although their effect on canonical signalling is inhibitory (Green *et al*, 2008). Initially, ROR receptors were thought to act by merely sequestering Wnt receptors. However, a recent paper suggests that, in addition to suppressing canonical signalling, these receptors could actively promote PCP signalling in a Frizzled-dependent manner (Grumolato *et al*, 2010). One simple model is that the ROR2/Fz/Wnt complex could trigger PCP signalling while the LRP/Fz/Wnt complex would activate canonical signalling. Individual Wnts would favour the formation of one or the other type of complex thus explaining why some Wnts tend to activate PCP while others activate canonical signalling.

Tolwinski, Borchers and colleagues describe another transmembrane receptor that inhibits canonical signalling and could act in the PCP pathway (Peradziryi *et al*, 2011). This protein, which is known as protein tyrosine kinase-7 (PTK7) in vertebrates and Off-track (Otk) in *Drosophila*, has been shown previously to be deregulated in cancers and to be

Figure 1 Many receptors modulate Wnt signalling. Frizzled proteins are considered the main Wnt receptors. The outcome of the Wnt-Frizzled interaction is determined by co-receptors. Thus, Wnt/Frizzled/LRP activates the canonical β -catenin/TCF signalling pathway, which itself can be modulated by other cell surface proteins (e.g., binding of R-spondins to LGR receptors enhances canonical Wnt signalling). The presence of PTK7 or ROR inhibits β -catenin/TCF signalling. It has been suggested that, in addition, these co-receptors promote PCP signalling. However, the underlying mechanism remains unknown. Some receptors such as Ryk are thought to function independently of Frizzled.

implicated in various morphogenetic movements (see references in Peradziryi *et al.*, 2011). The authors' interest in Otk was piqued by the mutant phenotype in *Drosophila*: reduction of zygotic *otk* leads to a mild excess in canonical Wingless signalling. Conversely, *otk* overexpression inhibits Wingless signalling, although only in regions where Wnt4 is expressed. Such inhibition is almost certainly mediated by Wnt4, because Wnt4 overexpression inhibits canonical signalling in an Otk-dependent manner. This, and the results of immunoprecipitation experiments, led to the suggestion that Otk could preferentially bind to Wnt4 in *Drosophila*. More immunoprecipitation experiments with *Xenopus* proteins show that PTK7 binds to Wnt3a and Wnt8 (which activate canonical signalling in *Xenopus* embryos), and that this interaction requires a Frizzled. Thus, PTK7 could inhibit canonical signalling by displacing LRP from the activating tripartite complex. This is similar to, yet distinct from, the proposed action of ROR2. ROR2 disrupts the canonical Wnt/Frizzled/LRP signalling complex but, at the same time, promotes the formation of a PCP-specific Wnt/Frizzled/ROR2 signalling complex. PTK7 is unlikely to act in the same manner, since it does not bind to Wnt5a and Wnt11, two 'PCP-specific' Wnts. Yet the authors suggest that, through an unknown mechanism, PTK does participate in PCP signalling. In support of this assertion, overexpression of Wnt4 or Otk disrupts PCP signalling in *Drosophila* wings. However, this observation is weakened by the fact that *otk* or *wnt4* loss-of-function has no effect on PCP. Thus, further work is needed to determine whether PTK7/Otk is really a component of a PCP signalling pathway. In particular, it will be necessary to determine its mode of action and to assess the significance of its similarity to receptor tyrosine kinases (RTKs), especially in light of observations, suggesting that

activity of its kinase domain is not required for its role in Wnt signalling. Intriguingly, RORs are also homologous to RTKs, and there may be useful information to be gleaned from comparing directly the function of ROR and PTK7. Here, the power of *Drosophila* genetics may help as a single ROR is encoded by its genome (<http://flybase.org/>). Genetic analysis could assess whether ROR and PTK7/Otk overlap in function or perform distinct roles.

The present study adds to the growing sentiment that the nature and abundance of receptors and co-receptors are key to determining the cellular effects of Wnts (Figure 1). In addition to the receptors discussed above, other Wnt receptors have been reported including Crypto, which too acts in concert with Frizzled albeit in an as yet uncharacterised fashion (Tao *et al.*, 2005), and Ryk, which, in contrast to ROR and PTK7, activates a Frizzled-independent pathway (van Amerongen and Nusse, 2009). Other extracellular regulators of Wnts include secreted antagonists and glypicans (Kawano and Kypta, 2003; Lin, 2004). It is also worth mentioning the recent demonstration that R-spondins, secreted molecules previously known to potentiate canonical Wnt signalling, act through LGR4 and LGR5, two 7-pass transmembrane receptors that have remained orphaned up to now (Carmon *et al.*, 2011; de Lau *et al.*, 2011). The cell surface is rich with factors that control and modulate Wnt signalling activity. We can look forward to understanding how the abundance and activity of these factors is controlled and integrated to provide the cell with meaningful information about its environment.

Conflict of interest

The authors declare that they have no conflict of interest.

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