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Receptor endocytosis: Frizzled joins the ubiquitin club

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Wnt/β-catenin signalling is initiated by binding of secreted Wnt ligands to Frizzled and LRP5/6/Arrow co-receptors. A new study in this issue of *The EMBO Journal* provides compelling evidence that the level of cell surface Frizzled is controlled by a cycle of mono-ubiquitylation–deubiquitylation, the latter being mediated by the deubiquitylating enzyme UBPY/USP8. The amount of Frizzled on the plasma membrane appears to be a major rate-limiting factor in determining a cell's Wnt responsiveness.

During animal development, members of the Wnt family of secreted ligands are sometimes thought to act as morphogens, moving from sites of synthesis to form a gradient, specifying different cell identities in a concentration-dependent manner (Cadigan, 2002). This has generated intense interest in the factors that are required for establishing and maintaining Wnt gradients (Yan and Lin, 2009). These studies often focus on the concentration of the Wnt ligand as the final arbiter of signalling strength, underplaying the possibility that differences in cell responsiveness to the signal may also be important.

In *Drosophila*, it is clear that endocytosis has an important role in removing Wingless (Wg, a fly Wnt) from the extracellular environment (Gagliardi *et al*, 2008). Determining the effect of this removal on signalling activity is complicated by the fact that endocytosis has also been found to promote Wnt/β-catenin signalling in several systems (Gagliardi *et al*, 2008; Kikuchi *et al*, 2009). These studies are often controversial, probably due to the non-specific effects of compromising endocytosis. Sorting things out requires the identification of specific factors that control the intracellular trafficking of Wnt and Wnt receptors. This issue of *The EMBO Journal* contains a beautiful example of how genetics, biochemistry and imaging can be combined to study this complicated problem.

Mukai *et al* used targeted RNAi screening in *Drosophila* to identify dUBPY, an enzyme of the Ubiquitin (Ub)-specific protease family, as a positive regulator of Wnt/β-catenin signalling. In flies, reduction of *dUBPY* severely disrupts Wg signalling, while overexpression of dUBPY activates the pathway. Similar results were also observed in mammalian cells using dominant-negative and constitutively active forms of UBPY.

UBPY had previously been shown to deubiquitylate EGFR (Mizuno *et al*, 2005; Alwan and van Leeuwen, 2007; Niendorf *et al*, 2007; Row *et al*, 2007), which inspired Mukai *et al* to examine whether the Wnt receptor Frizzled (Fz) is ubiquitylated in Hela cells. Transfected Fz4 was mono-ubiquitylated at several sites, the levels of which were greatly enhanced by inhibition of UBPY. Importantly, UBPY deubiquitylates Fz4 *in vitro*. Ubiquitylated Fz4 is rapidly removed from the cell surface and trafficked to the lysosome for degradation. The authors confirm the importance of this Fz modification on Wnt/β-catenin signalling by testing the activity of a Fz4 mutant that cannot be mono-ubiquitylated, owing to mutation of its cytosolic lysine residues. This mutant was several times more active than wild-type Fz4 in mediating Wnt/β-catenin signalling.

The authors then moved back to the fly, examining the regulation of subcellular localization of the Drosophila Fz2 (Dfz2) protein by dUBPY. In the developing fly wing, Dfz2 is rapidly removed from the cell surface and degraded, a process that depended on dUBPY. In dUBPY-depleted cells, Dfz2 was found in a Rab7 intracellular compartment, consistent with accumulation in multivesicular bodies (MVBs).

Mukai *et al* propose a model that is summarized in Figure 1A. At the cell surface, Fz is mono-ubiquitylated by an unknown enzyme. The modified receptor is rapidly endocytosed, whereby it has two potential fates. It can be deubiquitylated by UBPY and recycled back to the cell surface via Rab11-positive endosomes, or it can be trafficked to a Rab7-positive MVB compartment before delivery to the lysosome for degradation.

Mukai *et al* found no evidence that Fz ubiquitylation–deubiquitylation is influenced by Wnt ligand. This is different from the situation with EGFR, wherein mono-ubiquitylation is ligand dependent (Mizuno *et al*, 2005; Alwan and van Leeuwen, 2007; Niendorf *et al*, 2007; Row *et al*, 2007). Rather than acting in response to Wnt, Fz recycling is important for setting the level of responsiveness of the receiving cells to the Wnt ligand.

Another important aspect of the report by Mukai *et al* is the degree of specificity that UBPY has for Fz. The levels of other cell surface receptors in the developing fly wing were not affected by loss or gain of dUBPY function, including the Wg co-receptor Arrow (Arr). Indeed, a severe wing defect caused by reduction of dUBPY was completely rescued by overexpression of Dfz2, indicating that it is the major target of dUBPY action in this tissue. This suggests a model wherein Dfz2 is the limiting partner in Wg signal reception, with Arr being present in excess (Figure 1B and C). Whether this paradigm is true in other contexts, including that of chronic

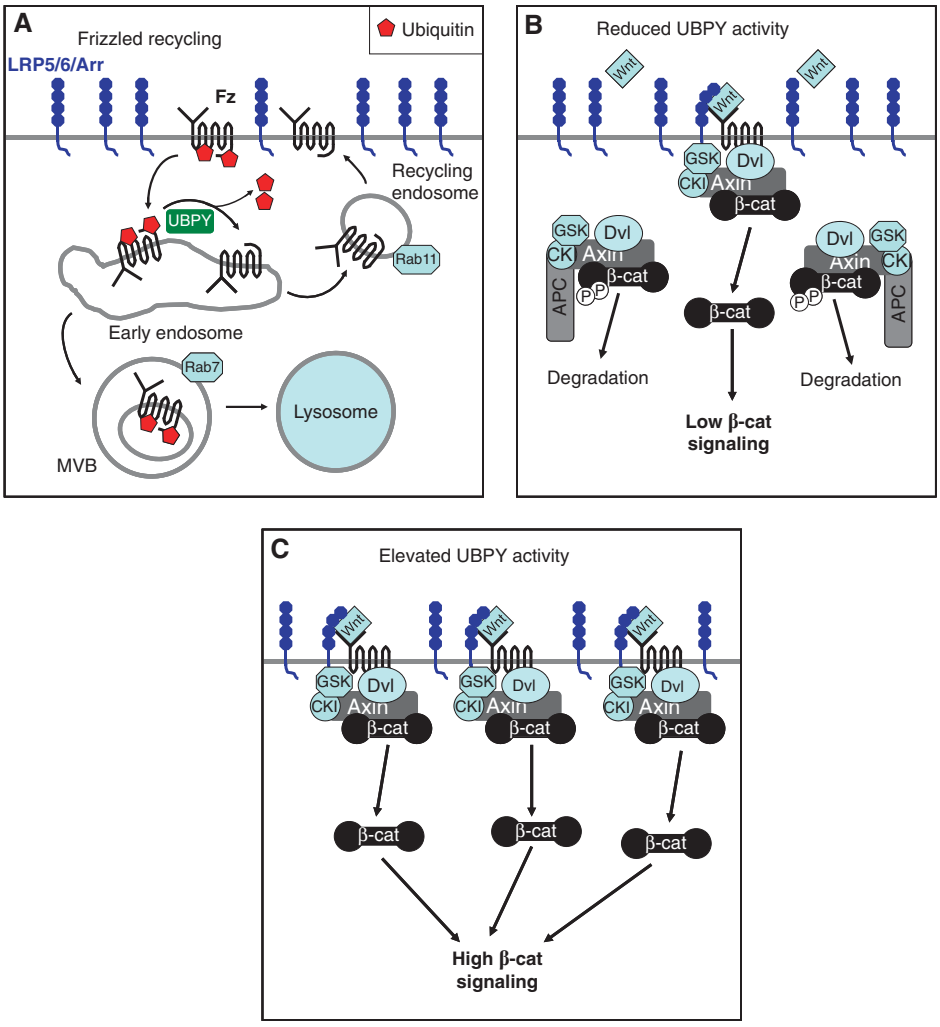

Figure 1 Mono-ubiquitylation of the Wnt receptor Fz determines the responsiveness of cells to Wnt stimulation. (**A**) The Fz ubiquitylation cycle. Mono-ubiquitin is symbolized by the red pentagons. After Fz ubiquitylation, UBPY promotes recycling of Fz by catalyzing its deubiquitylation. See text for further explanation. (**B**) Wnt/β-catenin signalling in cells depleted of UBPY. When UBPY activity is low, there is less cell surface Fz, resulting in limited inhibition of the β-catenin destruction complex by the Wnt activated Fz-LRP5/6/Arr receptor complex. The destruction complex continues to phosphorylate most of the cytosolic β-catenin, targeting it for degradation. (**C**) When UBPY activity is high, more Fz is present on the surface, resulting in stronger destruction complex inhibition, less β-catenin phosphorylation/degradation and thus higher signalling levels. This model assumes that Fz is the rate-limiting factor in the Wnt receptor complex, with Arr present in excess. See additional references for more information on the mechanism of Wnt/β-catenin signalling (Cadigan and Peifer, 2009; MacDonald *et al*, 2009).

lymphocytic leukaemia, for which the authors found elevated levels of UBPY and related proteins, awaits additional studies.

Conflict of interest

The author declares that he has no conflict of interest.

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