An Arrow straight to the heart of Wingless signalling

Over the last few years the Wingless (Wg)/Wnt patway has been shown to function in cell-fate determination and morphogenesis in both vertebrates and invertebrates. Many members of the signalling cascade downstream of Wg/Wnt have been identified, especially in *Drosophila*, and include the seven-transmembrane-span receptor Frizzled (Fz). However, the precise mechanism of Wg/Wnt transduction across the membrane of responding cells is still not clearly understood. Three recent papers, by Wherl *et al.* (*Nature* 407, 527–520; 2000), Tamai *et al.* (*Nature* 407, 530–535; 2000) and Pinson *et al.* (*Nature* 407, 535–538; 2000), identify a new member of the pathway in *Drosophila, Xenopus* and mouse.

Drosophila embryos homozygous for the null allele of *arrow*, which encodes a member of the low-density lipoprotein (LDL) receptor-related protein family, have severe embryonic defects that mimic the phenotype of *wg*-null animals (upper-left picture). Arrow is homologous to *Xenopus*/murine/human LRP6, and mice homozygous for LRP6 exhibit developmental defects, including neural-tube closure, that are very similar to those of homozygous Wnt embryos (upper-right picture). In *Xenopus*, overexpression of LRP6 leads to duplication of the embryonic dorsal axis, induction of Wnt-responsive genes, and enhanced development of neural-crest cells (lower picture). These phenotypes mimic those of Wnt overexpression. From these phenotypic studies it seems that Arrow/LRP6 acts within the Wg pathway, but where?

Experiments conducted using both *Drosophila* and *Xenopus* indicate that Arrow/LRP6 acts in the cells that receive and respond to the Wg/Wnt signal, rather than in those that produce it. Further epistatic experiments in *Drosophila* indicate that *arrow* acts downstream of *wg*, but upstream of *dishevelled (dsh*, an intracellular downstream component of the Wg pathway). It was then demonstrated, using *Xenopus*, that the extracellular domain of LRP6 binds to Wnt-1 and forms a complex with the Fz receptor, but only in a Wnt-1-dependent manner. From this it seems that when Arrow/LRP6 is bound to Wg/Wnt it acts in a complex with Fz to regulate the incoming signal. How Arrow/LRP6 interacts with the proteoglycan molecules that are known to mediate Wg signalling remains to be identified, but yet again the world of Wg/Wnt signalling has become more complicated.

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and an increased probability of anticlockwise flagellar rotation. Thus, smooth swims are extended, and cells migrate up an attractant gradient.

In principle, this situation does not require any interactions beyond those of a receptor dimer with a CheA dimer and two CheW monomers in a 1:1:1 complex. It is adequate to explain responses to large shifts in the concentration of single attractants. Indeed, it has been calculated that the best strategy for an *E. coli* cell with a limited number of receptors (1,500–4,500) to detect chemicals in its environment would be to distribute these receptors uniformly (or randomly) over the cell surface[6].

So compelling was this argument that *E. coli* was selected as a negative control by Maddock and Shapiro[7] in their search for receptor clusters in the asymmetrically dividing species *Caulobacter crescentus*, in which only one daughter cell is flagellated. The result was startling. Receptors in *C. crescentus* indeed cluster at the flagellar pole of the predivisional swarmer cell, but in *E. coli*, which does not sport a polar flagellum, the receptors are also present in polar patches. Furthermore, polar localization of the receptors in *E. coli* diminishes when either CheA or CheW is absent.

These observations beg the question of why *E. coli* chemoreceptors are distributed in a patchy fashion, which is seemingly contrary to sound engineering principles. The answer presumably lies in the nature of the signalling mechanism, as clustering of receptors could account for several unexplained features of chemotaxis. First, a change of less than 1% in receptor occupancy causes a measurable increase in anticlockwise rotational bias[8]. How can inhibition of the activity of only a few CheA molecules associated with attractant-bound receptors be amplified to give a detectable signal? Second, it is unclear how the low-abundance receptors Tap and Trg mediate strong responses to their attractant ligands when they stimulate CheA activity only weakly[9]. Finally, the means by which responses to different attractants or repellents in a chemically heterogeneous environment are integrated at the levels of signalling and adaptation is unknown[10].

These issues have been dealt with previously by Bray *et al.* in a conceptual model that invokes interconnected arrays within receptor patches[11]. Although there is no experimental basis for such extended networks, they are consistent with the existence of receptor patches. However, the identification of reconstituted aggregates of the soluble cytoplasmic domain of a chemoreceptor with CheW and CheA in a stoichiometry of ~7:1:1 may provide a glimpse of greater structural complexity[12].

The model-building exercise reported by Shimizu and colleagues would have been useful even if it just predicted how the individual protein partners interact, but its implications are far greater. The geometry of the proposed receptor–CheW–CheA trigonal complex indicates a straightforward way that it can be extended to form a hexagonal array of indefinite expanse, a clear candidate for the receptor patch. Within such an array, conformational perturbations initiated by the binding of ligand to one receptor dimer could spread in order to amplify or integrate signals from different receptors. The mysterious, but crucially important, linker between the second membrane-spanning segment and the extended cytoplasmic domain of the receptors[13] may allow the bending in an otherwise rigid helix that would be required to form the trimer of receptor dimers.

The model also predicts that an 'adaptation compartment' may exist between the cell membrane and the hexagonal lattice. The ability of CheR methyltransferase[14] and CheB methylesterase[15] to bind to the carboxyl-terminal tail of high-abundance receptors, together with their sequestration in this chamber, would restrict their diffusion away from the site at which their activity is needed. Recent studies using proteins fused to green fluorescent protein (GFP) have indicated that CheY and CheZ concentrate at the polar receptor patches as well[16]. Conveniently,