Strabismus comes into focus

Jeffrey D. Axelrod

At least one member of the Frizzled (Fz) family of receptors, together with the downstream signalling protein Dishevelled, participates in several distinct, yet closely related signal transduction pathways. Activation of the correct signal transduction pathway is critical. Here, a new study suggests that the transmembrane protein Strabismus participates in this decision.

Like a talented blues musician, evolution has taken a limited set of developmental 'riffs' and played them in new and interesting ways. One well-known riff is cell signalling through the Fz family of seven-span transmembrane receptors. Recent work has highlighted how two variant Fz riffs are interrelated. One is the canonical Wnt signal that modulates gene expression to control cellular differentiation and proliferation, whereas the other transduces a signal that controls cell polarity. On page 20 of this issue[1], Park and Moon demonstrate that Strabismus, a novel transmembrane protein, serves, in effect, as a regulatory switch to suppress the canonical signal and potentiate the polarity signal.

The Fz proteins are a conserved family of receptors that have many functions in development. Fz was identified in *Drosophila melanogaster* as a protein that determines the polarity of cells, as well as the multicellular units visible in the adult fly. For example, nearly every one of the 30,000 epithelial cells that make up the *Drosophila* wing produce a cellular extension, called a trichome. Remarkably, all of these trichomes are neatly organized in an oriented, parallel array. Similarly, the clusters of photoreceptor and other cell types that constitute the repeating units of the compound eye also form oriented, parallel arrays. These phenotypes have been collectively referred to as planar cell polarity (PCP), as cell polarity is manifest within the plane of the epithelium, perpendicular to the cell's apical–basal axes (reviewed in ref. 2).

Subsequently, Fz was also shown to transduce signals mediated by members of the Wnt family of secreted glycoproteins, including *Drosophila* wingless (Wg). Wnt-1 was discovered because it initiates mammary carcinomas in mice, and Wg, because it regulates the embryonic segmentation of *Drosophila*. The Wnt family has been shown to regulate many developmental patterning events in multicellular organisms, where these proteins determine cell fate and regulate proliferation (reviewed in ref. 3).

Fz is required for both the Wg and the PCP signalling pathways. Fz is the lone receptor for an unknown ligand during PCP signalling. However, Fz, together with Fz2 and a coreceptor, LRP (ref. 4), serve as a receptor for Wg, activating what is often called the canonical Wnt pathway (Fig. 1). Both pathways also require the activity of a cytoplasmic protein called Dishevelled (Dsh). Specific *dsh* alleles produce PCP defects, but null alleles reveal that Dsh is also involved in Wg signalling, mimicking the full range of defects seen in *wg* mutants. However, the similarity between these two pathways may end there. The canonical Wnt signal results in stabilization of β-catenin, which then combines with the Tcf family of transcription factors to regulate gene expression. By contrast, the PCP signal requires a distinct set of factors, and results in an asymmetric subcellular localization of signalling molecules, and ultimately an asymmetric reorganization of the cytoskeleton. In some circumstances, the Jun amino-terminal kinase (JNK) signalling pathway is activated in response to the PCP signal[5].

A current challenge is to understand how these two pathways can share a common receptor and signal transducer, and yet be capable of generating such different outputs. Part of this signalling specificity may involve the differential requirement for the LRP coreceptor. Another aspect of this specificity may derive from unique functions for the shared signal transducer,

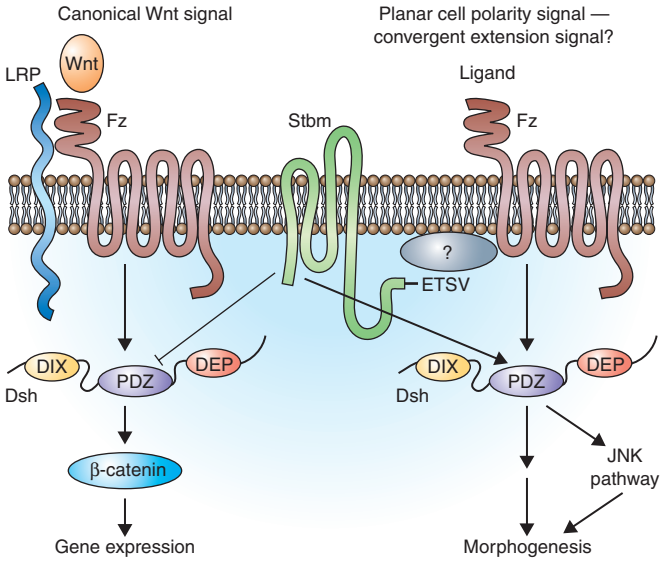

Figure 1 **Two alternative Wnt signalling pathways. Both canonical Wnt signalling and PCP signalling require the receptor Fz and the cytoplasmic signal transducer Dsh. The coreceptor LRP is required for canonical Wnt, but not PCP, signalling[4]. Dsh contains three conserved domains — a DIX (dishevelled and axin) domain, which is required for canonical Wnt signalling; a PDZ (PSD-95, discs-large and ZO-1) domain, the target of both Stbm and CKI binding[1,15]; and a DEP domain (dishevelled, Egl-10 and pleckstrin), required for Dsh membrane localization during PCP signalling[6,12]. Canonical Wnt signalling stabilizes β-catenin, which dimerizes with the Tcf and Lef transcription factors to regulate transcription. By contrast, the PCP pathway requires different factors, and results in morphogenetic changes. JNK signalling is required during PCP signalling in the eye, but not for the polarization of trichomes. Stbm binds the Dsh PDZ domain, but surprisingly, this requires N-terminal sequences and not the potential PDZ-binding peptide (ETSV) at the carboxyl terminus. As the ETSV peptide is required only for activity on PCP-like signalling, it may bind to a PCP-specific factor.**

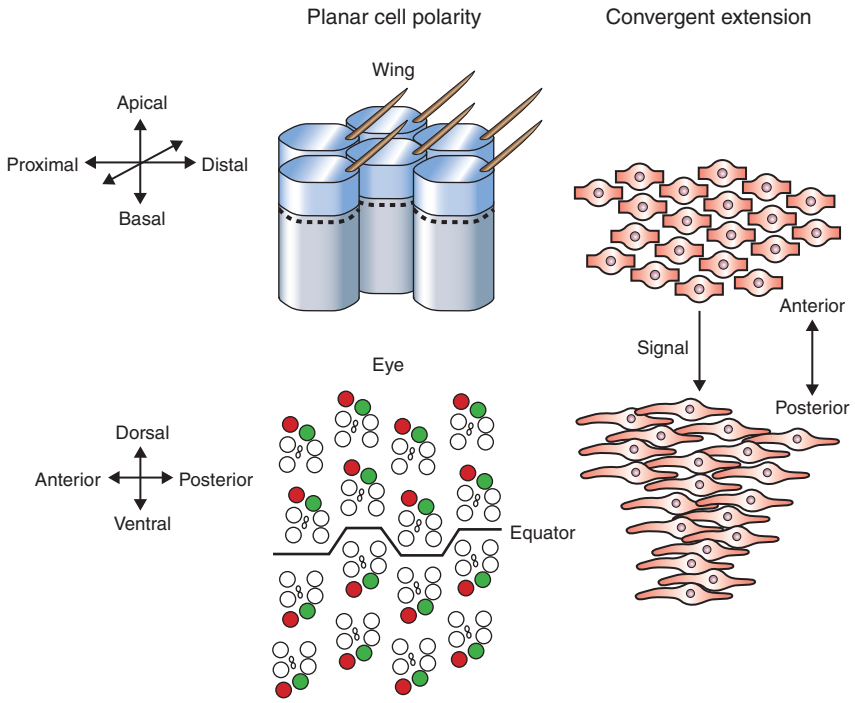

Figure 2 **Planar cell polarity and convergent extension. PCP in the *Drosophila* wing is manifest as the oriented and parallel array of trichomes (brown) — cellular extensions emanating from the distal vertex of each cell. PCP in the eye is manifest as the oriented and parallel array of chiral photoreceptor cell clusters in each hemisphere of the eye. During convergent extension, nearly symmetric cells within the mesoderm and neural ectoderm elongate along an axis orthogonal to the anterior–posterior axis, and by extending filopodia and crawling, intercalate between each other to drive axial elongation. Figure is based on an original from Wallingford *et al*[19].**

Dsh. The two pathways appear to have differential requirements for specific domains within the Dsh protein, as Dsh constructs lacking one or more domains, as well as mutant alleles of Dsh, can function in only one of the two pathways[5–11]. This may reflect a specific requirement for the membrane localization of Dsh during PCP signalling, but not during canonical Wnt signalling[6,12].

Studies of the vertebrate implementations of these two pathways have been revealing. The canonical Wnt pathway has remained highly conserved throughout evolution. In vertebrates, as in *Drosophila*, Wnt signalling is involved at many steps in the determination of cell fate and proliferation. However, during embryogenesis, a noncanonical Fz- and Wnt-mediated signal regulates a morphogenetic movement, called convergent extension. This is a process in which cells elongate and intercalate in a polarized fashion, bringing distant lateral structures closer together (convergence) and elongating the anterior–posterior axis (extension)[7–9]. This signal is noncanonical because it is independent of β-catenin. However, it is less clear whether this signal is homologous to the *Drosophila* PCP signal. The profile of Dsh deletion constructs that modulate convergent extension matches the profile of deletions that function in PCP signalling, but not of canonical Wnt signalling in flies, suggesting that the convergent extension signal may be similar to the PCP signal[7,8,10,11]. The idea that this noncanonical Wnt signal is homologous to PCP signalling is appealing, as the cells driving morphogenetic movement become polarized, much as *Drosophila* epithelial cells do during PCP signalling (Fig. 2). However, the evidence that the molecular mechanisms are similar was, until recently, rather sketchy.

Enter Strabismus. Park and Moon[1] set out to determine what a PCP-like pathway might do in vertebrate development by studying a homologue of a protein that in flies is thought to function only in PCP. Strabismus/Van Gogh is a newly discovered transmembrane protein that was identified almost simultaneously, on the basis of its requirement for planar polarization of the photoreceptor clusters in the fly eye (Stbm; ref. 13) and the hair cells of the fly wing (Van Gogh; ref. 14). Park and Moon studied the function of Stbm in frog and zebrafish, and their findings were both satisfying and surprising. Satisfying, because they found that Stbm modulates convergent extension, as monitored by axial elongation, and by its ability to activate the surrogate marker, JNK signalling. Surprising, because they found that vertebrate Stbm is also an antagonist of canonical Wnt signalling, inhibiting the ability of the canonical Wnt signal to regulate patterning in early neural development, through the activation of the β-catenin and Tef transcriptional targets. The authors provide at least a potential explanation for Stbm's ability to affect both pathways, by demonstrating that Stbm binds to the Dsh PDZ domain.

Because Stbm modulates convergent extension, the case for a similarity between the PCP pathway and the Wnt–Fz pathway controlling convergent extension becomes much stronger. However, the finding that Stbm is also a regulator of canonical Wnt signalling raises a number of questions about the relationship between canonical Wnt and PCP-like signalling. In this respect, Stbm shares functional characteristics with two other proteins, casein kinase I (CKI) and Naked cuticle (Nkd). These proteins may act as molecular switches, toggling the activity of Dsh between its function in these two pathways. CKI can phosphorylate Dsh, and its activity promotes Dsh function in canonical Wnt signalling, but inhibits its activity in convergent extension[15,16]. Conversely, Nkd is a feedback inhibitor that blocks Dsh function in canonical Wnt signalling, but can also activate the PCP-like convergent extension signal in vertebrates[17,18]. Therefore, all three proteins promote the activity of one pathway and inhibit the activity of the other, probably by affecting Dsh.

The ability of Stbm, CKI and Nkd to affect the relative flux through the PCP-like and canonical Wnt pathways would make a neat story. However, several important issues remain unresolved. The evidence that CKI and Stbm affect both pathways involves loss-of-function studies, and is compelling. Aside from their abilities to bind Dsh, however, nothing is known about how Stbm or dominant-negative CKI activate the PCP function of Dsh. By contrast, careful loss-of-function analyses in flies have failed to identify a function for Nkd in PCP signalling. Artificial overexpression of Nkd in both flies and frogs can impact on PCP or PCP-like signalling, but as yet, there is no evidence that this is a physiological effect. How is it, then, that nonphysiological overexpression of Nkd can have this activity? Is this telling us something about how Stbm modulates the PCP-like convergent extension pathway? Clearly, we have much to learn about how Dsh function is activated before we can understand the ability of these three proteins to modulate Dsh function in canoncial Wnt and PCP-like signalling. □

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A crossbridge too far

James A. Spudich and Ronald S. Rock

Myosin V is a marvellous molecular motor that delivers various cargo to specific addresses in eukaryotic cells. Recent developments are leading to a detailed molecular understanding of how this enzyme transduces the chemical energy of ATP hydrolysis into mechanical movement along actin filaments.

Mice and humans missing myosin V are defective in directing melanin-containing granules in melanocytes to their proper locations, resulting in a pigmentation disorder. Neurological disorders are also apparent, presumably owing to the failure to direct important vesicular traffic in neurons. Apart from being highly processive (one molecule of myosin V takes many steps along an actin filament for each diffusional encounter with the actin track[1,2]), myosin V takes larger steps than any other known molecular motor, moving 36 nm for each round of ATP hydrolysis[3]. These unusual properties have allowed the elucidation of the nucleotide-dependent movement of this motor in more molecular detail than is available for any other motor protein. On pp. 59–65 of this issue, Veigel *et al.*[4] take this to an exciting level and show two distinct mechanistic substeps of the overall 36-nm step of myosin V. The first is a working stroke of ~25 nm, which might even consist of two still-smaller substeps, and the second is a diffusional step of ~11 nm. Their work brings to light important aspects of molecular motor movement and protein behaviour in general.

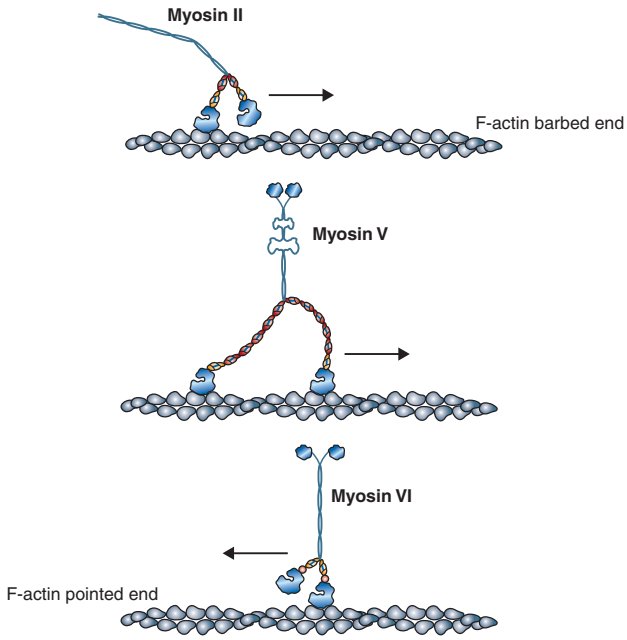

Figure 1 **Three examples of the diverse structures of members of the myosin superfamily.**

Microtubules and actin filaments serve as roads along which >30 varieties of molecular motors travel, each carrying out distinct tasks. As in a city plan, the traffic patterns are highly regulated, with stops that control the moving traffic and addresses that are delivery points for cargo. We know very little about how the many different molecular motors operating in a cell pick up their cargo and deliver them to the right address, nor do we understand much about how the movement of the traffic is regulated. These are wonderful areas for future research. We do know, however, that the entire city plan is highly dynamic, and that motor proteins play pivotal roles in such changes. This happens, for example, every time a cell enters mitosis or when it encounters external signals of various types, which can induce dramatic changes in intracellular organization.

The aspect of the city plan that we know most about at the molecular level is the structure and function of molecular motors themselves and the tracks along which the motors move. The extension of *in vitro* motility assays to the single molecule level has been a major step forward in this regard[5–8]. Single molecule assays allow for the measurement of individual nanometre steps and picoNewton forces as one molecule of a molecular motor interacts with its filamentous track.

Within the actin-based myosin motor superfamily, there are at least 17 different family types. Different motors serve different functions and are built accordingly (Fig. 1). Myosin VI, also probably a cargo-transporting motor, has the unusual feature of moving in the opposite direction along the polar actin filament[9]. Myosin II, found in muscle and in the contractile ring of nonmuscle cells, forms bipolar thick filaments. The myosin II head domains serve as crossbridges between the myosin and actin filaments, to pull actin filaments