Frizzled signalling and cell polarisation in *Drosophila* and vertebrates

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Summary

A key aspect of animal development is the appropriate polarisation of different cell types in the right place at the right time. Such polarisation is often precisely coordinated relative to the axes of a tissue or organ, but the mechanisms underlying this coordination are still poorly understood. Nevertheless, genetic analysis of animal development has revealed some of the pathways involved. For example, a non-canonical Frizzled signalling pathway has been found to coordinate cell polarity throughout the insect cuticle, and recent work has implicated an analogous pathway in coordinated polarisation of cells during vertebrate development. This review discusses recent findings regarding non-canonical Frizzled signalling and cell polarisation.

Introduction

There are many instances in animal development where the polarity of individual cells or groups of cells must be correctly coordinated with the polarity of the tissue to which the cells belong. For example, hairs or feathers must point the right way on animal skin, cilia on epithelia must beat in the right direction, and sensory hairs in the vertebrate inner ear must be correctly polarised (Eaton, 1997). The genetic control of such coordinated cell polarisation events has been best studied in the cuticle of the fruitfly *Drosophila*, where signalling pathways downstream of the Frizzled (Fz) receptor have been found to be involved (Gubb and García-Bellido, 1982; Vinson and Adler, 1987; Vinson et al., 1989; Zheng et al., 1995).

Receptors of the Frizzled (Fz) family are well known to be activated by Wnt ligands to signal via a β-catenin-dependent pathway (Wodarz and Nusse, 1998) that is commonly referred to as the 'canonical' Wnt/Fz pathway, to distinguish it from several other Wnt/Fz pathways that do not act through β-catenin. The best characterised of these 'non-canonical' pathways is the Wnt/Ca^{2+} pathway, which was first described in vertebrates (Kühl et al., 2000), and the planar polarity pathway, which was first identified in *Drosophila* (McEwen and Peifer, 2000) (Fig. 1).

Here, I discuss our current understanding of non-canonical Fz activity in controlling coordinated cell polarity decisions in the *Drosophila* cuticle, in what is now thought to be a two-step process (Strutt and Strutt, 2002; Ma et al., 2003). First, a long-range signal is set up that requires both Fz activity and the participation of atypical cadherin molecules. This long-range signal is responsible for the overall coordination of cell polarity within the axes of the tissue. Fz is then involved in a second event that is required for the coordinated polarisation of individual cells and involves a few conserved proteins that assemble into multiprotein complexes. Significantly, much evidence is now emerging that the same molecules act together to coordinate cell polarisation during vertebrate embryogenesis. This review concentrates on the functions of these conserved genes in cell polarisation in flies and vertebrates, and describes recent advances in our understanding of the pathways that act upstream and downstream of them.

Frizzled and planar polarity in flies

The *frizzled* (*fz*) gene of *Drosophila* is required to establish polarity in structures throughout the adult cuticle, but its functions have been best characterised in the wing and eye, where it exhibits both autonomous and non-autonomous patterning functions (Gubb and García-Bellido, 1982; Vinson and Adler, 1987; Zheng et al., 1995). In the wing, *fz* is required for the correct orientation of the hairs (or trichomes) that are produced by each cell. Normally, each cell produces a single hair on its apical surface at the distal vertex of the cell, which then grows out distalwards (Fig. 2A). In the absence of *fz*, hairs form in the centre of the apical surface of the cell and no longer invariably grow out distalwards (Fig. 2B) (Wong and Adler, 1993). This constitutes the cell-autonomous activity of *fz* in the wing.

In the eye, *fz* determines polarity of individual ommatidial units (Strutt and Strutt, 1999). Each ommatidium consists of a group of eight photoreceptor neurons and about 12 supporting cells (Wolff and Ready, 1993). During development, each ommatidium undergoes two distinct events that determine its polarity in the adult eye (Fig. 2C). First, it adopts the correct chirality or 'handedness', according to its position above or below the dorsoventral midline of the eye. Second, it rotates precisely 90° in the appropriate direction. Both of these events require *fz* activity. Correct chirality choice is dependent on the correct balance of *fz* activity in the R3/R4 photoreceptor pair; *fz* activity in only one cell of this pair is sufficient to confer R3 fate and the corresponding chirality on the entire ommatidium (Zheng et al., 1995; Tomlinson and Struhl, 1999). Subsequent rotation of the ommatidium by 90° is dependent on having *fz* activity in at least one photoreceptor (Zheng et al., 1995). Hence, for both chirality and rotation to be correct, *fz* is

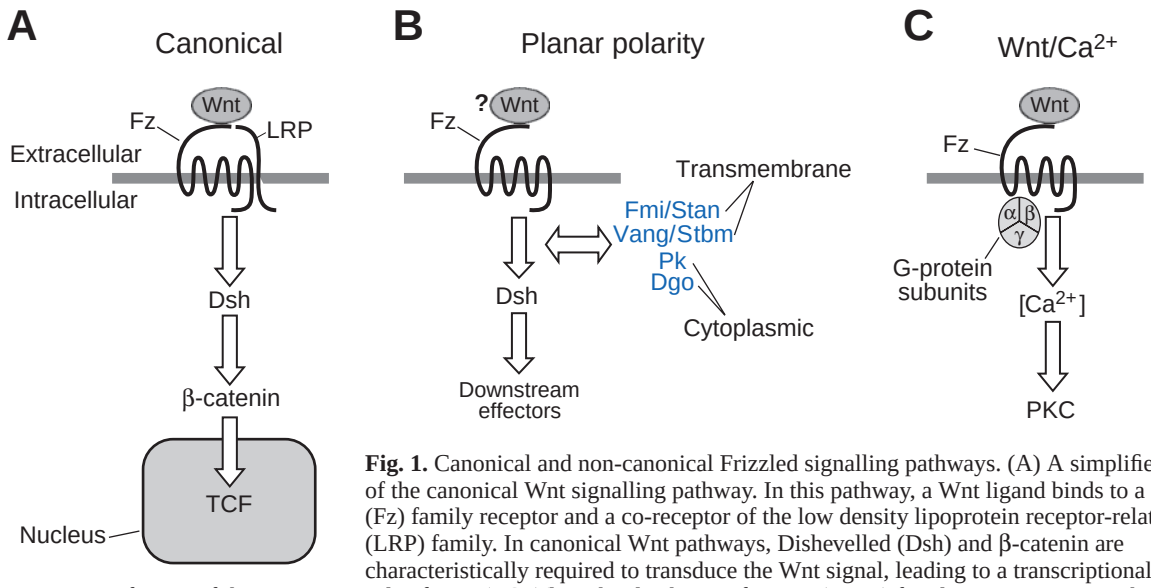

Fig. 1. Canonical and non-canonical Frizzled signalling pathways. (A) A simplified scheme of the canonical Wnt signalling pathway. In this pathway, a Wnt ligand binds to a Frizzled (Fz) family receptor and a co-receptor of the low density lipoprotein receptor-related protein (LRP) family. In canonical Wnt pathways, Dishevelled (Dsh) and β-catenin are characteristically required to transduce the Wnt signal, leading to a transcriptional response mediated by transcription factors of the ternary complex factor (TCF)/lymphoid enhancer factor 1 (LEF1) family. Fz receptors might also couple to heterotrimeric G proteins in this pathway (see text for more details). (B) Planar polarity involves a non-canonical β-catenin-independent Wnt/Fz pathway that requires Dsh. A Wnt ligand for this pathway has yet to be identified in *Drosophila* ('?'), although Wnt ligands have been found to activate an analogous pathway in vertebrates. There is also no evidence, as yet, for heterotrimeric G proteins being involved. This pathway involves the core planar polarity proteins (blue) Flamingo/Starry Night (Fmi/Stan), Van Gogh/Strabismus (Vang/Stbm), Prickle (Pk) and Diego (Dgo). These proteins are thought to modulate the activity of this pathway by forming a multiprotein complex with Fz/Dsh that spans cell-cell junctions, rather than being cascade components. Fmi/Stan and Vang/Stbm are both multipass transmembrane proteins, whereas Pk and Dgo are cytoplasmic proteins. The *prickle* (*pk*) locus produces two protein isoforms, Pk and Spiny Legs (Sple), which vary in activity from tissue to tissue (only Pk is shown here for simplicity). (C) The Wnt/Ca^{2+} pathway probably signals via heterotrimeric G-proteins (α, β, γ subunits), to mobilise intracellular Ca^{2+} and, in some contexts, to stimulate protein kinase C (PKC). Whether this pathway requires Dsh remains controversial (see text for more details). In vertebrates, Wnt/Ca^{2+} signalling is activated by the same ligands as the planar polarity pathway, suggesting that these pathways may overlap to some extent.

required in at least the R3 photoreceptor. This again constitutes a cell-autonomous activity of *fz* in polarity patterning.

In both cases, the manifestation of this polarity that is controlled by *fz* is an example of 'planar polarity', also referred to as 'tissue polarity' or 'planar cell polarity (PCP)'. This is because the axis of polarity adopted is in the plane of the tissue. In the case of the wing, hairs polarise in the proximodistal axis of the wing epithelium. In the case of the eye, ommatidial polarity is coordinated relative to the dorsoventral and anteroposterior axes of the eye epithelium. It is noteworthy that in these cases, planar polarity is established in monolayer epithelia (which are already polarised on the apicobasal axis) and so constitutes polarisation in additional axes of already polarised cells.

An interesting aspect of *fz* function in planar polarity patterning is that it also exhibits long-range non-autonomous effects on cell polarisation. This was first noted in the wing, when groups or 'clones' of cells were generated that lacked *fz* function in otherwise wild-type tissue that retained *fz* activity. As a result, the cells of the clone failed to produce correctly polarised hairs (owing to their lack of *fz* activity), and, in addition, cells around the clone were also mispolarised, producing hairs that pointed towards the clone rather than distally (Gubb and García-Bellido, 1982; Vinson and Adler, 1987) (Fig. 2B). The evidence suggests that this is due to a distinct non-autonomous activity of *fz*. First, the cell-autonomous and non-autonomous activities of *fz* can be separated by mutation (Vinson and Adler, 1987); second, whereas cells lacking *fz* activity within the clone produce a hair in the centre of the apical surface, those surrounding the clone still produce hairs at the cell edges (albeit not the correct edges); third, the two functions can be temporally separated, with the non-autonomous function preceding the autonomous function (Strutt and Strutt, 2002). A similar non-autonomous phenotype of *fz* clones is observed in the eye (Zheng et al., 1995). Taken together, these observations suggest that *fz* first functions in the setting up or maintenance of a long-range patterning system that coordinates cellular polarity with that of the tissue as a whole, and then acts subsequently in a cell-autonomous fashion in the interpretation of these cues to ensure the local coordination of cell polarity (Vinson and Adler, 1987; Strutt and Strutt, 2002).

The cloning of *fz* revealed that it encodes a seven-pass transmembrane receptor that lacks homology to the better-characterised sevenpass G-protein coupled receptors (GPCRs) (Vinson et al., 1989). Fz is now known to be the founder member of a large family of receptors for Wnt ligands that are conserved throughout the animal kingdom (Bhanot et al., 1996; Wodarz and Nusse, 1998). Interestingly, Fz itself is able to function redundantly with its homologue Frizzled 2 as a receptor for canonical β-catenin-dependent Wnt signalling (Kennerdell and Carthew, 1998; Müller et al., 1999), in addition to its non-canonical functions in regulating planar polarity (Fig. 1).

The 'core' planar polarity proteins

Several proteins are thought to act together with Fz in the

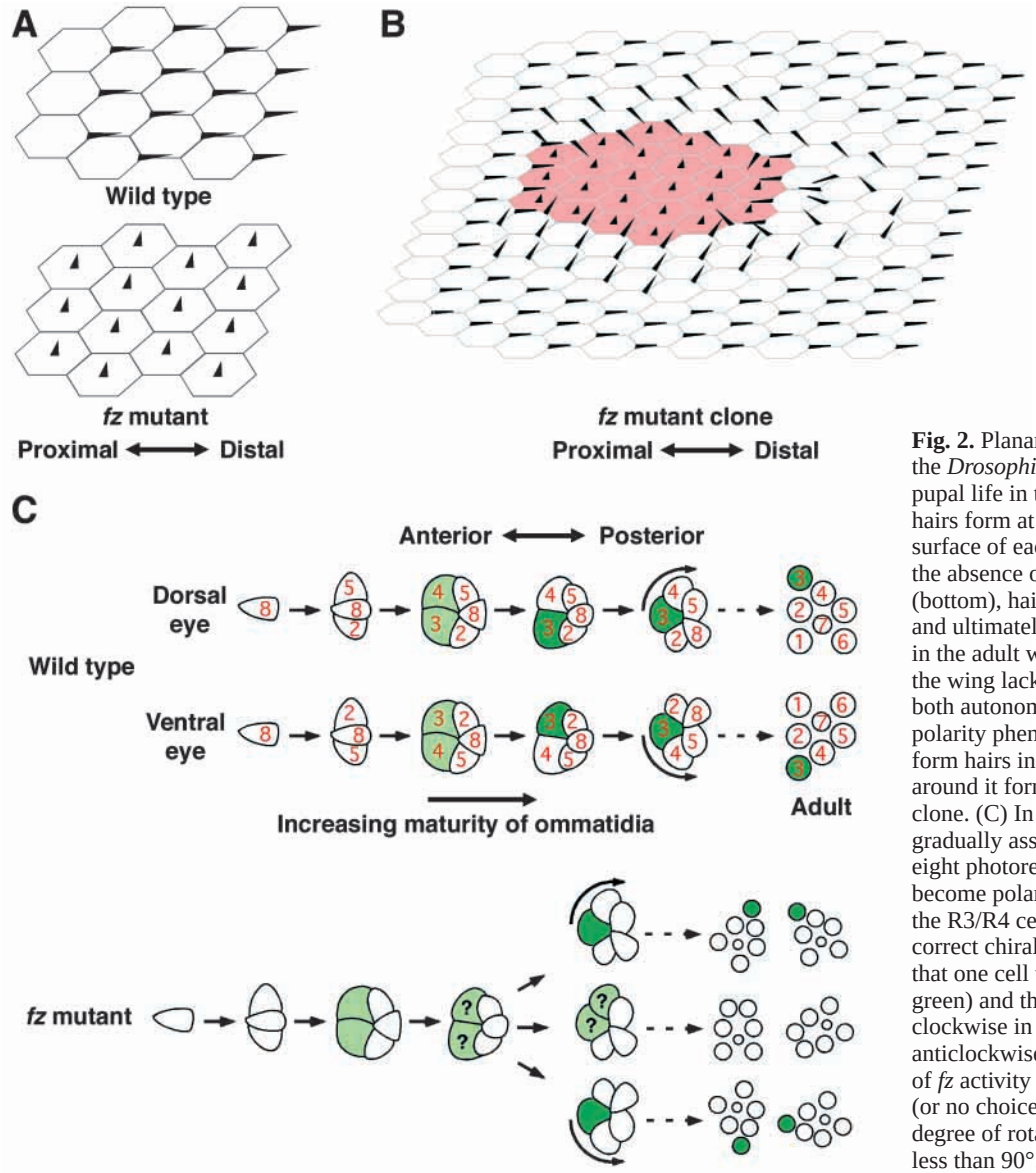

Fig. 2. Planar polarity and *fz* phenotypes in the *Drosophila* wing and eye. (A) During pupal life in the wild-type wing (top), hairs form at the distal vertex on the apical surface of each cell and point distally. In the absence of *frizzled* (*fz*) activity (bottom), hairs form in the centre of cells and ultimately adopting swirling patterns in the adult wing. (B) A clone of cells in the wing lacking *fz* activity (pink) shows both autonomous and non-autonomous polarity phenotypes. Cells in the clone form hairs in the centre of the cell. Cells around it form hairs that point towards the clone. (C) In the eye, ommatidia are gradually assembled by the recruitment of eight photoreceptor cells (R1-R8), and become polarised. *fz* activity is required in the R3/R4 cell pair (light green) for a correct chirality decision to occur, such that one cell takes on the R3 fate (dark green) and the ommatidium rotates 90° clockwise in the dorsal half of the eye or anticlockwise in the ventral half. Absence of *fz* activity leads to a randomised choice (or no choice) of R3 fate and a randomised degree of rotation that is either greater or less than 90°.

second step of polarisation when individual cells make a coordinated polarity decision. These proteins are encoded by the *dishevelled* (*dsh*), *prickle* (*pk*), *Van Gogh*/*strabismus* (*Vang*/*stbm*), *flamingo*/*starry night* (*fmi*/*stan*) and *diego* (*dgo*) genes, and are often referred to as components of a planar polarity 'pathway' or 'cascade', although they probably act as constituents of a multiprotein complex. A lack of any one of these genes results in similar autonomous polarity defects in the wing and eye, and often in other tissues (Gubb and García-Bellido, 1982; Vinson and Adler, 1987; Theisen et al., 1994; Zheng et al., 1995; Taylor et al., 1998; Wolff and Rubin, 1998; Gubb et al., 1999; Chae et al., 1999; Usui et al., 1999; Feiguin et al., 2001). Furthermore, their protein products all adopt similar asymmetric subcellular localisations in polarising cells of the wing and eye (Usui et al., 1999; Axelrod, 2001; Feiguin et al., 2001; Shimada et al., 2001; Strutt, 2001; Das et al., 2002; Strutt et al., 2002; Tree et al., 2002; Rawls and Wolff, 2003; Bastock et al., 2003).

Genetic epistasis experiments indicate that *dsh* acts downstream of *fz* (Krasnow et al., 1995). The *dsh* locus encodes a cytoplasmic protein that contains conserved DIX (Dishevelled-Axin), PDZ (PSD95-Discs Large-ZO1) and DEP (Dishevelled-EGL10-Pleckstrin) domains (Klingensmith et al., 1994; Theisen et al., 1994). Studies of the domains of Dsh and of its vertebrate homologues have established that Dsh couples to at least two pathways, the β-catenin-dependent canonical Wnt pathway and the non-canonical planar polarity pathway; the DEP domain was found to be most critical for planar polarity function and the DIX domain for canonical Wnt signalling (Yanagawa et al., 1995; Axelrod et al., 1998; Boutros et al., 1998; Li et al., 1999; Moriguchi et al., 1999; Rothbächer et al., 2000; Penton et al., 2002).

Unlike *fz* and *dsh*, the *pk*, *Vang/stbm*, *fmi/stan* and *dgo* genes are implicated only in non-canonical signalling in *Drosophila*. Notably, they do not have simple epistatic relationships with *fz* and *dsh* (e.g. Krasnow et al., 1995; Taylor et al., 1998; Chae et al., 1999), arguing against their functioning in a linear cascade with *fz/dsh*. Furthermore, the molecular homologies of

their encoded proteins do not indicate likely functions in cell polarisation (Table 1) (Wolff and Rubin, 1998; Chae et al., 1999; Usui et al., 1999; Gubb et al., 1999; Feiguin et al., 2001).

Interestingly, Pk and Vang/Stbm are also implicated in acting with Fz in the first step of cell polarisation, when a long-range polarity cue is generated. Clones of cells lacking *Vang/stbm* activity in the wing show a similar non-autonomous phenotype to that of *fz* clones, although with opposite polarity (Taylor et al., 1998). Similarly, *pk* clones also show non-autonomous phenotypes, although weaker than those seen around *fz* or *Vang/stbm* clones (Gubb and García-Bellido, 1982; Gubb et al., 1999; Adler et al., 2000). Considering these phenotypes and the genetic interactions between these loci, it has been proposed that they act together to regulate a long-range polarity signal (Adler et al., 2000). It is also possible that *fmi*/*stan* functions in propagating polarity cues, as very weak non-autonomous phenotypes have been observed around *fmi*/*stan* clones (Chae et al., 1999; Usui et al., 1999), but their significance remains unclear.

Asymmetric localisation of polarity proteins

Pioneering experiments in the fly wing have established that the second (autonomous) activity of *fz* is required in this tissue to promote actin accumulation and thus hair initiation at the correct cellular site (Wong and Adler, 1993; Krasnow and Adler, 1994). These studies showed that whereas loss of *fz* function leads to hair formation in the centre of the apical surface of wing cells, an excess of *fz* activity causes excess hairs to form at the cell edges. From these results, a model was proposed in which local Fz signalling via Dsh at the distal cell edge was the cue for hair formation (Wong and Adler, 1993; Krasnow and Adler, 1994; Krasnow et al., 1995).

It was subsequently demonstrated that localised *fz* activity at the distal cell edge is a result of the Fz receptor being preferentially localised here (Strutt, 2001). Dsh colocalises with Fz in this location (Axelrod, 2001; Shimada et al., 2001). Thus, Fz/Dsh signalling activity is necessarily restricted to this part of the cell. Therefore, to understand the role of Fz in cell polarisation, we need to establish the mechanism by which it becomes asymmetrically localised. Almost certainly relevant to this is that Fmi/Stan, Vang/Stbm, Pk and Dgo proteins also become asymmetrically localised on the proximodistal axis of polarising wing cells. Fmi/Stan and Dgo are thought to localise both proximally and distally (Usui et al., 1999; Feiguin et al., 2001), whereas Vang/Stbm and Pk are found at proximal cell edges (Bastock et al., 2003; Tree et al., 2002) (Fig. 3A).

Asymmetric subcellular localisations of polarity proteins are also observed in photoreceptors in the developing eye during the establishment of ommatidial polarity (Das et al., 2002; Strutt et al., 2002; Yang et al., 2002; Rawls and Wolff, 2003),

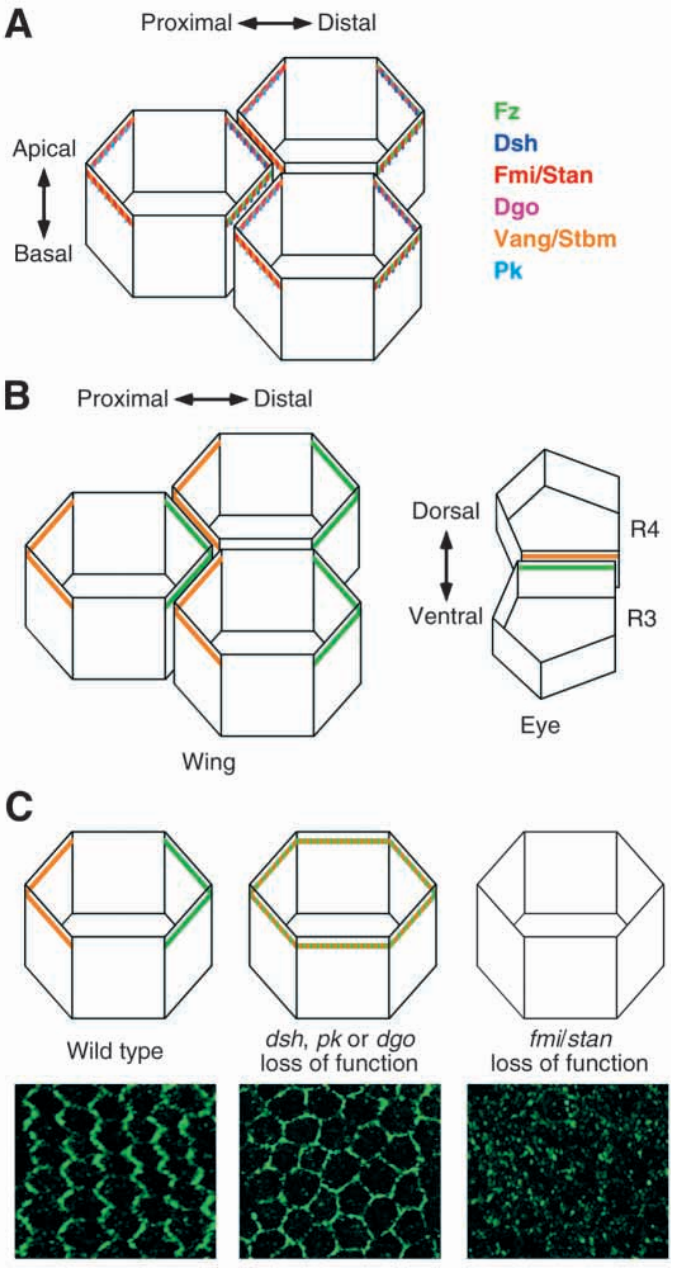

Fig. 3. Asymmetric localisation of core planar polarity proteins in the *Drosophila* wing and eye. (A) Distribution of core planar polarity proteins in cells of the pupal wing between ~24 and 32 hours after prepupa formation (APF). Frizzled (Fz) and Flamingo/Starry Night (Fmi/Stan) are found in distal apicolateral membranes at the level of the adherens junctions, and Van Gogh/Strabismus (Vang/Stbm) and Fmi/Stan are found in proximal membranes. Other proteins are recruited from the cytoplasm to the cell cortex either distally [Dishevelled (Dsh)] or proximally [Prickle (Pk)], or both [Diego (Dgo)]. Actin accumulates and hairs form at the distal cell vertex at ~32 hours APF. (B) Comparison of Fz and Vang/Stbm distribution in the wing and eye. Fz is distal and Vang is proximal in pupal wing cells. In the third instar eye disc, Fz is localised on the R3 side of the R3/R4 cell-cell boundary and Vang/Stbm is localised on the R4 side. (Fz and Vang/Stbm are probably also on other membranes of these cells, but this has not been fully characterised.) Thus, an intercellular complex forms across the R3/R4 cell-cell boundary that is probably functionally equivalent to the asymmetric complex across distal-proximal cell boundaries in the wing. (C) Loss of Fmi/Stan largely blocks Fz and Vang/Stbm recruitment to apicolateral cell regions (right, top and bottom). Loss of Dsh, Pk or Dgo blocks the formation of asymmetric proximodistal complexes between 18 and 32 hours APF (middle, top and bottom), resulting in Fz and Vang/Stbm remaining distributed around the circumference of the cells. Photomicrographs at the bottom are confocal sections through the apical regions of pupal wing cells at ~28 hours of pupal life, showing Fz-GFP distribution in the adherens junction zone in wild type (left), in a $pk^{pk\text{-}sple\text{-}13}$ mutant (middle) and in a fmi^{E59} mutant (right).

Table 1. Genes involved in planar polarity patterning in the *Drosophila* wing

Name	Symbol	Core planar polarity function?	Long-range patterning function?	Molecular homologies	Presumed function in planar polarity determination	Subcellular localisation in wing	Known planar polarity functions of vertebrate homologues	References
frizzled	*fz*	Yes	Yes	Sevenpass transmembrane receptor	Receptor for polarity signal? Recruits Dsh to membranes	Distal	Fish/frog CE	Gubb and García-Bellido, 1982; Vinson and Adler, 1987; Vinson et al., 1989; Strutt, 2001; Deardorff et al., 1998; Djiane et al., 2000; Medina et al., 2000
dishevelled	*dsh*	Yes	No	DIX, PDZ and DEP domains	Transducer of Fz signalling	Distal	Fish/frog CE Mammalian neural tube closure	Klingensmith et al., 1994; Theisen et al., 1994; Krasnow et al., 1995; Axelrod, 2001; Shimada et al., 2001; Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000; Hamblet et al., 2002
prickle	*pk*	Yes	Yes	Two protein products (Pk and Sple) each with a PET domain, 3 LIM domains, C-terminal prenylation motif	Unknown; may mediate protein-protein interactions; Pk and Sple isoforms have different activities in different tissues	Proximal	Fish/frog CE	Gubb and García-Bellido, 1982; Gubb et al., 1999; Tree et al., 2002; Takeuchi et al., 2003; Veeman et al., 2003
Van Gogh/ strabismus	*Vang/stbm*	Yes	Yes	Fourpass transmembrane protein, C-terminal PDZ-binding domain	Unknown; may recruit Pk and Dsh to membranes	Proximal	Fish/frog CE Mammalian neural tube closure Sensory hair cell polarity in vertebrate ear.	Taylor et al., 1998; Wolff and Rubin, 1998; Bastock et al., 2003; Darken et al., 2002; Goto and Keller, 2002; Jessen et al., 2002; Park and Moon, 2002; Kibar et al., 2001; Murdoch et al., 2001; Montcouquiol et al., 2003
flamingo/ starry night	*fmi/stan*	Yes	Uncertain	Sevenpass transmembrane protein homologous to secretin family of GPCR's, extracellular cadherin repeats	Intercellular adhesion and/or signalling? Required for localisation of other proteins to adherens junction	Distal and proximal	Mammalian neural tube closure Sensory hair cell polarity in vertebrate ear	Chae et al., 1999; Usui et al., 1999; Curtin et al., 2003
diego	*dgo*	Yes	No	Ankyrin repeats	Unknown; may mediate protein-protein interactions	Distal and proximal?	Fish CE	Feiguin et al., 2001; Schwarz-Romond et al., 2002
widerborst	*wdb*	No	Uncertain	Protein phosphatase 2A B′ regulatory subunit	Links core polarity gene asymmetric localisation to long-range patterning?	Early proximal, late distal	Fish CE	Hannus et al., 2002
four-jointed	*fj*	No	Yes	Type II transmembrane protein, C-terminus may be cleaved and secreted	Unknown, may act as secreted ligand or intracellular enzyme?	Unknown	Unknown	Brodsky and Steller, 1996; Villano and Katz, 1995; Ashery-Padan et al., 1999; Zeidler et al., 1999; Zeidler et al., 2000; Strutt and Strutt, 2002; Yang et al., 2002; Ma et al., 2003
dachsous	*ds*	No	Yes	Atypical cadherin	Cell adhesion or cell-cell signalling?	Uniform at adherens junctions	Unknown	Clark et al., 1995; Adler et al., 1998; Casal et al., 2002; Rawls et al., 2002; Strutt and Strutt, 2002; Yang et al., 2002; Ma et al., 2003
fat	*ft*	No	Yes	Atypical cadherin	Cell adhesion or cell-cell signalling?	Uniform at adherens junctions	Unknown	Mahoney et al., 1991; Adler et al., 1998; Casal et al., 2002; Rawls et al., 2002; Strutt and Strutt, 2002; Yang et al., 2002; Fanto et al., 2003; Ma et al., 2003
atrophin	*atro*	No	Yes	Transcriptional co-repressor	Mediates transcriptional response downstream of Fat?	Cytoplasmic and nuclear	Unknown	Zhang et al., 2002; Fanto et al., 2003

CE, convergent extension; DIX, Dishevelled-Axin; PDZ, PSD95-Discs Large-ZO1; DEP, Dishevelled-EGL10-Pleckstrin; PET, Prickle-Espinas-Testin; LIM, Lin11-Isl1-Mec3; GPCR, G-protein-coupled-receptor.

particularly in the R3/R4 photoreceptor pair. In these cells, Fz preferentially localises on the R3 side of the R3/R4 boundary, whereas Vang/Stbm preferentially localises on the R4 side (Strutt et al., 2002). Hence, the R3/R4 boundary appears to be functionally equivalent to the distal/proximal cell boundary between cells in the wing (Fig. 3B).

Finally, it should be noted that the asymmetric localisation of polarity proteins in *Drosophila* is not restricted to tissues that give rise to the adult cuticle. During development of the fly embryo, dorsal epidermal cells converge towards the dorsal midline in a process known as dorsal closure. These converging epithelial cells exhibit planar polarisation of their cytoskeleton and also show asymmetric localisation of core planar polarity proteins; furthermore, there is some evidence for non-canonical Wnt signalling regulating this process (Kaltschmidt et al., 2002). Although the precise activities of the core planar polarity genes in dorsal closure have not been established, these observations support a conserved role for a planar polarity pathway in *Drosophila* embryo morphogenesis.

Stages of asymmetric localisation

Results to date show that the activities of all six asymmetrically localised proteins are required for the correct localisation of each of the other proteins (Usui et al., 1999; Axelrod, 2001; Shimada et al., 2001; Strutt, 2001; Feiguin et al., 2001; Tree et al., 2002; Bastock et al., 2003), suggesting that these molecules act together in a multiprotein complex. However, different proteins play different roles in the process of asymmetric localisation (Fig. 3C). Based on existing data, we have proposed that they act in a heirarchy to bring about asymmetric protein localisation (Bastock et al., 2003). Fmi/Stan function is at the top of the hierarchy and is responsible for recruiting the other transmembrane proteins, Fz and Vang/Stbm, to the apicolateral adherens junction zone of wing cells (Strutt, 2001; Bastock et al., 2003). The recruitment is probably via direct protein-protein interactions, but Fmi/Stan, Fz and Vang/Stbm are certainly required for the recruitment of the three putative cytoplasmic proteins, Dsh, Pk and Dgo, to the cell cortex. Once all six proteins have been recruited to apicolateral regions, they become asymmetrically distributed on the proximodistal axis of the cells.

Other support for these molecules forming a multiprotein complex comes from analyses of their physical interactions. For example, Fz is able to recruit Dsh from the cytoplasm to membranes in a heterologous cell type (Axelrod et al., 1998). Furthermore, Dsh and Pk interact in vitro (Tree et al., 2002); and Vang/Stbm is able to recruit both Pk and Dsh to membranes in COS7 cells, and these proteins also co-immunoprecipitate together (Bastock et al., 2003). Physical interactions have also been reported between vertebrate homologues of Stbm and Dsh (Park and Moon, 2002) and of Pk and Dsh (Takeuchi et al., 2003).

Notably, some of these in vitro interactions are not predicted by our knowledge of the composition of the asymmetric complex at proximodistal cell boundaries. In this complex, Dsh localises to distal cell boundaries, whereas Stbm and Pk are proximal. What, then, is the significance of the direct interactions between Dsh and Stbm, and Dsh and Pk? Assuming that they do occur in vivo, there are two possible explanations. The first is that at an earlier phase of development, prior to the redistribution of the proteins on the proximodistal axis, the complexes have a different composition, which might reflect a distinct biochemical function. The second is that the in vitro binding reflects in vivo interactions that are only transient and act in some way to promote asymmetric complex formation. Indeed, it has been suggested that the interactions between Dsh and Pk are part of a mechanism for blocking Dsh localisation in proximal cell regions (Tree et al., 2002).

Overall, the mechanism by which the proteins become asymmetrically distributed on the proximodistal axis remains a mystery. It is clear that they must be redistributed in response to the long-range signal that coordinates polarity with the axes of the tissue, but the molecular nature of this signal remains unclear, which itself is a barrier to understanding its mechanism of action.

Fz/Dsh signalling and asymmetric localisation of the core polarity proteins

The elusive long-range signal has generally been thought to be a secreted factor, possibly a ligand for the Fz receptor (Adler et al., 1997; Axelrod, 2001; Strutt, 2001) that might exist in a gradient across the wing, such that each cell has a gradient of Fz signalling activity across its proximodistal axis. The asymmetric localisation of the core polarity proteins would then occur as part of a feedback amplification system via Fz/Dsh signalling that turns the initially shallow gradient of signalling into a peak of signalling at only the distal cell edge. A recent refinement to this model suggests that Pk is also involved in the feedback loop (Tree et al., 2002). This model is attractive because it provides a mechanism for amplifying a gradient of a long-range signal to produce an unambiguous cellular cue for hair placement. Furthermore, it fits well with the mechanisms thought to be used by chemotactic cells in responding to shallow gradients of extracellular signals (Servant et al., 2000).

However, more evidence is required to verify this model. It is not yet clear that the polarity cue is in the form of a shallow gradient that requires amplification. There is no direct evidence that Fz/Dsh signalling is required for the asymmetric distribution of any of the polarity proteins, largely because there is no assay for Fz/Dsh signalling. It is known that point mutations in these molecules that abrogate their function also block the asymmetric distribution of the core polarity proteins (Axelrod, 2001; Strutt, 2001), but this could be due to a failure of physical interaction rather than of signalling. Furthermore, the biochemical function of Pk is unknown (Gubb et al., 1999), and so there is no direct evidence that it is involved in signalling. Genetic epistasis evidence has been proposed to support the case for feedback loops (Tree et al., 2002). However, these results are equally consistent with a model in which all the core polarity proteins are required to form a fully functional multiprotein complex.

It remains possible that localisation occurs independently of Fz/Dsh signalling, with Fz/Dsh being transported to the distal cell edge by another mechanism. In this case, Fz/Dsh signalling could be activated by a uniformly distributed extracellular ligand (possibly a Wnt) that did not itself impart directional information. Alternatively, Fz/Dsh signalling might actually become activated as a result of their

incorporation into asymmetric complexes at the proximodistal cell boundaries. This could be due to ligand-independent activation of signalling, perhaps by receptor clustering, as is thought to occur when Fz is overexpressed (Krasnow et al., 1995; Adler et al., 1997). Or to other members of the asymmetric complex, such as Fmi/Stan or Vang/Stbm, could interact directly with the Fz receptor and act as ligands. Support for the idea that Fz signalling is Wnt-independent during fly planar polarity comes from a study in which overexpressing all seven *Drosophila* Wnt homologues had no effect on planar polarity patterning in the abdomen (Lawrence et al., 2002).

These issues will be resolved only by more detailed study of the biochemical and enzymatic properties of the proteins involved and from a better understanding of the composition of the protein-protein complexes that form during asymmetric localisation.

Widerborst and asymmetric protein localisation

An important publication reported recently that a *Drosophila* protein phosphatase 2A (PP2A) regulatory subunit, encoded by the *widerborst* (*wdb*) gene, becomes distally localised to apicolateral microtubules in polarising wing cells (Hannus et al., 2002). This distal localisation apparently precedes that of the core planar polarity proteins and is independent of their function. Furthermore, *wdb* activity is required for the asymmetric proximodistal localisation of the other planar polarity proteins. Notably, Wdb does not completely colocalise with the other proteins and its loss-of-function phenotypes are not identical to theirs, hence it is not a component of the planar polarity protein asymmetric complex. Rather, Wdb appears to act upstream of the core planar polarity proteins, possibly as a link to long-range patterning cues.

Thus, core planar polarity protein function is not required for all aspects of the proximodistal patterning of wing cells. Instead, it appears to play a role downstream of other manifestations of cellular proximodistal polarity, as part of a mechanism for specifying the site of hair outgrowth. As Wdb becomes both distally positioned and localises with microtubules, it is tempting to speculate that proteins can be directionally transported to the distal (and possibly also the proximal) ends of cells via microtubule motors.

Atypical cadherins and long-range patterning

The first step in cell polarisation, which sets up a long-range coordinating signal and requires *fz*, *Vang/stbm* and *pk*, is now known to involve additional genes. In particular, Adler and colleagues have reported that the atypical cadherins encoded by the *dachsous* (*ds*) and *fat* (*ft*) loci show non-autonomous defects in planar polarity in the wing (Adler et al., 1998). The type II transmembrane protein encoded by the *four-jointed* (*fj*) locus (Brodsky and Steller, 1996; Villano and Katz, 1995) is also known to non-autonomously regulate planar polarity in both the eye and wing (Zeidler et al., 1999; Zeidler et al., 2000) (Table 1).

Work from several groups has led to a model in which gradients of *ds*, *ft* and *fj* activity in the developing wing, eye and abdomen generate a long-range polarity signal (Zeidler et al., 1999; Zeidler et al., 2000; Casal et al., 2002; Rawls et al., 2002; Strutt and Strutt, 2002; Yang et al., 2002; Fanto et al., 2003; Ma et al., 2003). Mechanistic details are still lacking, but epistasis studies suggest that this pathway acts in parallel to *fz*, *Vang/stbm* and *pk* (Strutt and Strutt, 2002). There is evidence that *ft* acts through the transcriptional co-repressor Atrophin (Fanto et al., 2003) and that *fj* may be controlling cell adhesion by modulating Ds/Ft heterophilic interactions (Strutt and Strutt, 2002; Ma et al., 2003). Whether these events ultimately lead to the secretion of a Fz ligand in a gradient or coordinate long-range polarity by another mechanism remains to be elucidated.

In addition to the early long-range patterning activities of *fz* and *fj*/*ft*/*ds*, it has also been proposed that the cell to cell propagation of asymmetric polarity protein complexes is important for the long-range propagation of polarity information (Axelrod, 2001; Strutt, 2001; Tree et al., 2002; Ma et al., 2003). But as evidence has also been presented against this view (Strutt and Strutt, 2002), further work is still required to clarify this issue.

Downstream effectors of planar polarity

The asymmetric localisation of polarity proteins is just one step in a process that leads to the polarisation of diverse structures in tissues such as the *Drosophila* eye and wing. Individual cells undergo complex morphological changes during polarisation, which are achieved by the core planar polarity genes regulating the activity of downstream effector genes. These effectors are distinguished from the core planar polarity genes by several criteria. First, their protein products are not assembled into asymmetric complexes and are not required for complex formation. Second, they often only act in a subset of tissues where polarity is regulated by the core polarity genes. Third, they often only control a subset of the downstream responses to core planar polarity protein activity. As the functions of these downstream effectors have been well reviewed recently (Adler, 2002; Axelrod and McNeill, 2002), they will only be dealt with briefly here (Table 2).

In the wing, loss of the polarity genes *inturned* (*in*), *fuzzy* (*fy*) and *multiple wing hairs* (*mwh*) leads to two distinct hair defects: the hairs are mispolarised and each cell produces more than one hair (Gubb and García-Bellido, 1982; Wong and Adler, 1993; Park et al., 1996; Collier and Gubb, 1997). Other reported downstream effectors of planar polarity in the wing only seem to affect hair number, such as the p21 GTPase RhoA (Rho1 – FlyBase) (Strutt et al., 1997) and its putative effector, the *Drosophila* Rho-associated kinase, Drok (Winter et al., 2001). In the eye, the core planar polarity genes control both cell fate decisions in the R3/R4 photoreceptor pair and the normal 90° rotation of each ommatidium. The R3/R4 decision is mediated by modulating levels of *Notch* and *Delta* activity in these two cells (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999), although the actual mechanism for this remains controversial (Das et al., 2002; Strutt et al., 2002). Ommatidial rotation is partly controlled by the genes *nemo* and *roulette* (Choi and Benzer, 1994), and by *RhoA* (Strutt et al., 1997; Strutt et al., 2002) and *Drok* (Winter et al., 2001). It has been proposed that a JNK cascade acts downstream of RhoA in the control of ommatidial polarity, leading to activation of the DJun (Jra – FlyBase) transcription factor (Strutt et al., 1997; Boutros et al., 1998; Weber et al., 2000), although the evidence for this remains inconclusive (see Strutt et al., 2002).

Table 2. Downstream effectors of planar polarity

Name	Symbol	Molecular homology	Requirement in wing/eye	Planar polarity effector in vertebrates?	References
inturned	*in*	Predicted transmembrane protein	Wing hair number and polarity	Unknown	Gubb and García-Bellido, 1982; Wong and Adler, 1993; Park et al., 1996
fuzzy	*fy*	Predicted fourpass transmembrane protein	Wing hair number and polarity	Unknown	Gubb and García-Bellido, 1982; Wong and Adler, 1993; Collier and Gubb, 1997
multiple-wing-hairs	*mwh*	Unknown	Wing hair number and polarity	Unknown	Gubb and García-Bellido, 1982; Wong and Adler, 1993
RhoA	*RhoA*	p21 GTPase of Rho family	Wing hair number; ommatidial rotation	*Xenopus* CE	Strutt et al., 1997; Wünnenberg-Stapleton et al., 1999
Rho-associated kinase	*Drok*	Protein kinase that acts as Rho effector	Wing hair number; ommatidial rotation	Zebrafish CE	Winter et al., 2001; Marlow et al., 2002
Notch	*N*	Transmembrane receptor	Ommatidial chirality	Unknown	Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999
Delta	*Dl*	Notch ligand	Ommatidial chirality	Unknown	Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999
nemo	*nmo*	Serine/threonine kinase	Ommatidial rotation; (also wing hair polarity?)	Unknown	Choi and Benzer, 1994; Verheyen et al., 2001
roulette	*rlt*	Unknown	Ommatidial rotation	Unknown	Choi and Benzer, 1994
basket (and other JNK cascade components)	*bsk*	Jun N-terminal kinase	No phenotype	Unknown	Strutt et al., 1997; Boutros et al., 1998; Strutt et al., 2002
Djun	*Djun*	Jun/Ap-1 transcription factor	Weak phenotype in ommatidial rotation and chirality	*Xenopus* CE	Boutros et al., 1998; Weber et al., 2000; Strutt et al., 2002

CE, convergent extension; JNK, Jun-N-terminal-kinase.

Non-canonical Wnt signalling in vertebrates

Wnt ligands in vertebrates can activate at least two downstream pathways. One group of Wnts, represented by Wnt1, Wnt3a, Wnt8 and Wnt8b, can transform mammalian cells (Wong et al., 1994) and induce axis duplication in amphibian embryos (Christian et al., 1991; Du et al., 1995). Another group, typified by Wnt4, Wnt5a and Wnt11, do not have transforming or axis-duplication activity, but instead cause defects in cell movement during gastrulation when injected into *Xenopus* embryos, and ultimately result in a shortened body axis (Moon et al., 1993; Du et al., 1995). The transforming Wnt proteins signal via the canonical β-catenin-dependent Wnt/Fz signalling pathway, whereas the non-transforming group is implicated in activating the non-canonical Wnt/Ca^{2+} pathway (Kühl et al., 2000) (Fig. 1). This pathway is thought to act through heterotrimeric G proteins, leading to the activation of protein kinase C (PKC) (Slusarski et al., 1997; Sheldahl et al., 1999), and has generally been considered to be independent of the activity of Dsh (Kühl et al., 2000; Winklbauer et al., 2001). However, new evidence suggests that this pathway may be Dsh dependent (Sheldahl et al., 2003).

Recent work suggests that the non-transforming Wnt ligands, and in particular Wnt5a and Wnt11, also activate a Dsh-dependent pathway that is homologous to that involved in *Drosophila* planar polarity determination and that regulates cell polarisation during vertebrate gastrulation (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000; Wallingford et al., 2001; Kilian et al., 2003). This vertebrate equivalent of the *Drosophila* planar polarity pathway is now also implicated in the processes of neural tube closure and in the polarised orientation of sensory hair cells in vertebrate ears (Box 1) (Kibar et al., 2001; Murdoch et al., 2001; Wallingford and Harland, 2001; Goto and Keller, 2002; Hamblet et al., 2002; Curtin et al., 2003; Montcouquiol et al., 2003).

Conserved genes control convergent extension

The gastrulation of vertebrate embryos involves complex cell movements and rearrangements that are mediated by a variety of processes. One of these processes is called ‘convergent extension’ (CE), which describes the narrowing and lengthening of a group of cells (Fig. 4 and see also movies at http://dev.biologists.org.cgi/content/full/126/20/4547/DC1 and http://dev.biologists.org/cgi/content/full/130/5/873/DC1). This process is particularly important in the lengthening of the anteroposterior axis of embryos, but also contributes to other events, such as neurulation and organogenesis. Axis elongation has been best studied in the mesoderm of amphibian embryos, where cells are seen to ‘converge’ towards the midline at the same time as the tissue ‘extends’ along the anteroposterior axis. In other organisms, such as fish embryos, ‘convergence’ in the form of directed migration of cells towards the midline occurs prior to ‘extension’, when the cells intercalate to extend the anteroposterior axis (see Keller, 2002; Myers et al., 2002; Wallingford et al., 2002; Glickman et al., 2003).

CE can be considered as a manifestation of planar polarity, as cells become polarised in the plane of the gastrulating tissue. However, there are significant differences between CE and planar polarity in *Drosophila*. So far in flies, only epithelial

Box 1. Conservation of the *Drosophila* planar polarity pathway in vertebrates

In *Drosophila*, it is well established that there is a non-canonical Frizzled (Fz) signalling pathway that controls planar polarity in the adult cuticle. In this context, Fz signals via Dishevelled (Dsh) to activate downstream effectors. An important feature of this pathway is the polarised subcellular localisation of Fz and Dsh into asymmetric protein complexes that also contain the 'core' planar polarity proteins Flamingo/Starry Night (Fmi/Stan), Van Gogh/Strabismus (Vang/Stbm), Prickle (Pk) and Diego (Dgo). (See main text for references.)

All these molecules are conserved in vertebrates, where they have been implicated in controlling developmental patterning events that are broadly analogous to planar polarity in the fly: the process of convergent extension (CE) during gastrulation and the coordinated orientation of sensory hair cells in the inner ear.

In amphibians and fish, homologues of Fz, Dsh, Vang/Stbm, Pk and Dgo are all believed to act during CE (Deardorff et al., 1998; Djiane et al., 2000; Medina et al., 2000; Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000; Darken et al., 2002; Goto and Keller, 2002; Jessen et al., 2002; Park and Moon, 2002; Takeuchi et al., 2003; Veeman et al., 2003; Schwarz-Romond et al., 2002) in a non-canonical Wnt/Fz signalling pathway. As in fly planar polarity, the activity of this pathway is dependent on the DEP (Dishevelled-EGL10-Pleckstrin) domain of Dsh, and Dsh translocates to membranes and becomes hyperphosphorylated in cells where the pathway is active. Furthermore, in both contexts, epistasis studies have placed Vang/Stbm function parallel to Fz and Dsh in this pathway.

Neural tube closure in vertebrate embryos probably requires CE movements of neural tissues. Consistent with this, manipulations of Dsh or Vang/Stbm activity in frogs that disrupt CE result in failure of the neural tube to close (Wallingford and Harland, 2001; Goto and Keller, 2002). Notably, the loss of activity of Dsh, Vang/Stbm and Fmi/Stan homologues in mouse also results in neural tube defects, suggesting that these genes act in a conserved pathway controlling CE in mammals (Kibar et al., 2001; Murdoch et al., 2001; Hamblet et al., 2002; Curtin et al., 2003). Mouse homologues of Vang/Stbm and Fmi/Stan are also required for correct polarity of sensory hair cells in the ear (Curtin et al., 2003; Montcouquiol et al., 2003); whether homologues of the other core planar polarity genes are involved remains unresolved.

The functions and interactions of the vertebrate homologues of the core planar polarity genes are not yet sufficiently understood to say with certainty that a single conserved planar polarity pathway exists that acts in cell polarisation events from flies to humans; however, evidence to date supports such a hypothesis.

cells of the external cuticle are known to exhibit Fz-dependent planar polarity. During CE, both surface ectodermal cells and internal mesenchymal cells become polarised. Furthermore, in the wing, planar polarity controls the production of a stable actin structure (the hair), whereas during CE, cells form dynamic lamelliform protrusions that are involved in attaching to and crawling over adjacent cells (Keller, 2002; Wallingford et al., 2002). In fact, CE may be more analogous to planar polarity in the *Drosophila* eye. Here, ommatidia rotate within an epithelium, requiring cell movement and rearrangement, although the role of cell polarisation has not yet been characterised. The overall common feature of these processes

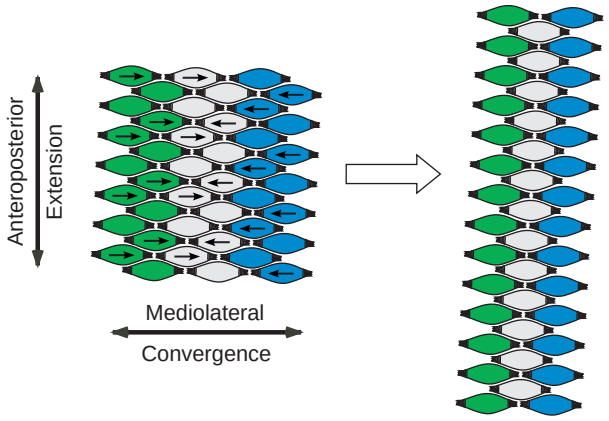

Fig. 4. Polarisation of cells in convergent extension of vertebrate embryos. Simplified scheme of convergent extension in the mesoderm of *Xenopus* during gastrulation. Cells become polarised in a bipolar fashion on the mediolateral axis and intercalate, such that they converge together on the mediolateral axis and the tissue extends on the anteroposterior axis.

is the production of actin-rich structures at particular cell faces in a polarised fashion.

In addition to Wnt5a and Wnt11 being implicated in control of vertebrate gastrulation, the *Xenopus* Dsh homologue Xdsh has long been known to control morphogenetic movements (Sokol, 1996), and more than one Fz homologue has been found to regulate CE via non-canonical pathways (Deardorff et al., 1998; Djiane et al., 2000; Medina et al., 2000). The evidence that this non-canonical pathway might be equivalent to that controlling planar polarity in *Drosophila* has come from several observations. First, it was found that both in fish and frogs, CE is selectively disrupted by mutations in Dsh that were predicted from *Drosophila* studies to affect planar polarity but not canonical Wnt signalling (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000). These Dsh mutations also resulted in a failure of dorsal mesoderm cells to polarise during CE in *Xenopus* embryos, and wild-type Xdsh-GFP was noted to translocate to cell membranes during CE (Wallingford et al., 2000). Such translocation is characteristic of the behaviour of Dsh during planar polarity determination in *Drosophila* (Axelrod, 2001; Shimada et al., 2001), although in *Xenopus* cells, Xdsh-GFP seems to be uniformly associated with the external membrane rather than showing a polarised distribution. In addition, a dominant-negative form of Xwnt11 that disrupts CE leads to reduced Dsh hyperphosphorylation (Tada and Smith, 2000), which is reminiscent of the loss of Dsh phosphorylation that is caused by mutations in *Drosophila* core planar polarity genes (Axelrod, 2001; Shimada et al., 2001).

Further support for a conserved pathway has come from reports that vertebrate homologues of other core planar polarity genes are also required for CE. A combination of overexpression, morpholino knockout and mutant studies have uncovered a role for *Vang/stbm* homologues in regulating gastrulation movements in both fish and frogs (Darken et al., 2002; Goto and Keller, 2002; Jessen et al., 2002; Park and Moon, 2002). Epistasis experiments indicate that the fish homologue of *Vang/stbm*, which is encoded by the *trilobite* gene, is likely to act in parallel to Fz/Dsh rather than in a linear cascade (Jessen

et al., 2002), which fits well with epistasis results in flies (Taylor et al., 1998). Similarly, *pk* homologues have also been shown to be required for CE in fish and frogs (Takeuchi et al., 2003; Veeman et al., 2003). Consistent with the genetic and physical interactions seen between *Drosophila* Pk and Vang/Stbm (Taylor et al., 1998; Bastock et al., 2003), zebrafish Pk and Vang/Stbm homologues interact synergistically in regulating CE (Veeman et al., 2003). In addition, a zebrafish homologue of *diego* has been identified, named Diversin (Schwarz-Romond et al., 2002), which appears to regulate both canonical and non-canonical Wnt signalling, and the loss of function of which leads to defects in gastrulation movements.

Core planar polarity genes in mammals

The vertebrate homologues of core planar polarity genes can also affect the CE of neural tissues in amphibian embryos, the disruption of which leads to subsequent defects in neural tube closure (Wallingford and Harland, 2001; Goto and Keller, 2002). Significantly, mutations in the *Vang/stbm*, *dsh* and *fmi/stan* homologues in mouse also result in defects in neural tube closure, which may occur as a result of abnormal CE of the neural plate in mutant mice (Kibar et al., 2001; Murdoch et al., 2001; Hamblet et al., 2002; Curtin et al., 2003).

A particularly striking manifestation of planar polarity in vertebrates is the arrangement of sensory hair cells in sense organs, as exemplified by the stereocilia in the cochleas of mammalian ears (Lewis and Davies, 2002). Like CE, this process has emerged as being regulated by homologues of the core planar polarity genes *Vang/stbm* and *fmi/stan* (Curtin et al., 2003; Montcouquiol et al., 2003), and a Wnt has also been implicated in it (Dabdoub et al., 2003).

Thus, there is good evidence that a conserved non-canonical Fz pathway acts in coordinating cell polarisation events from flies to mammals. Furthermore, homologues of all the core planar polarity genes have been found to act in vertebrates. However, it is not known whether all the core planar polarity proteins act together in the different contexts in which they function in vertebrates. Indeed, there is good evidence that a *Vang/stbm* homologue directs polarised neuronal migration in zebrafish embryos independently of *dsh* activity (Jessen et al., 2002).

Downstream effectors in vertebrates

There is also evidence that some downstream effectors of planar polarity in *Drosophila* are involved in vertebrate CE. A *Xenopus* RhoA p21 GTPase homologue is required for morphogenetic movements during early embryogenesis (Wünnenberg-Stapleton et al., 1999), which is probably activated via Dsh and the novel adaptor protein Daam1 (Habas et al., 2001). The potential RhoA effector Rho kinase 2 has also been shown to act downstream of Wnt11-dependent non-canonical signalling in regulating CE in zebrafish (Marlow et al., 2002).

It is well documented that mutated forms of Dsh that affect planar polarity in *Drosophila* can also interact with the JNK signalling pathway in vertebrate cells (Boutros et al., 1998; Li et al., 1999; Moriguchi et al., 1999). It has been recently reported that vertebrate homologues of Vang/Stbm, Pk and Diego can activate the JNK pathway (Park and Moon, 2002; Schwarz-Romond et al., 2002; Takeuchi et al., 2003; Veeman et al., 2003). The significance of this is unclear, as components of the JNK pathway play a negligible role in planar polarity determination in flies (Boutros et al., 1998; Weber et al., 2000; Strutt et al., 2002). Nevertheless, in *Xenopus*, there is evidence that JNK might regulate axis elongation through CE (Yamanaka et al., 2002), suggesting that it is an effector of planar polarity in vertebrates. However, JNK has also been reported to be activated in *Xenopus* via a Wnt11-activated non-canonical pathway involving PKC (Pandur et al., 2002), which is not obviously analogous to planar polarity in *Drosophila*.

Does asymmetric subcellular localisation occur in vertebrates?

CE and planar polarity in flies do not involve identical cell behaviours. Thus, some differences in the actions of conserved planar polarity genes in these two contexts would not be surprising. One major difference is that asymmetric subcellular localisation of planar polarity proteins has not been reported to occur during CE. As in *Drosophila* planar polarity, vertebrate Dsh does translocate to the cell cortex during CE (Wallingford et al., 2000), but then apparently does not then become asymmetrically distributed on any axis of the cell. There are two possible explanations for this. The first is that there is a fundamental difference in the way the planar polarity proteins act together in the two contexts: during CE, they might all associate together at the cell cortex, rather than forming asymmetric complexes. This would resemble an earlier phase of action in *Drosophila* prior to asymmetric complex formation. The second explanation is that asymmetric localisation may be less pronounced and/or just more difficult to visualise in vertebrate cells. In the *Drosophila* wing, the asymmetric accumulation of proteins occurs over several hours, in a restricted apicolateral region of static well-tessellated cells, making their visualisation easy. During CE, asymmetric protein localisation would be occurring in dynamically moving cells, probably to broad regions at the cell edge, making its visualisation less likely.

What is the relationship between the Wnt/Ca^{2+} pathway and planar polarity?

The observation that Wnt5A and Wnt11 activate both the Wnt/Ca^{2+} pathway and regulate CE via conserved planar polarity proteins raises the question of whether these are the same, overlapping or independent pathways. One recent study makes a good case that these are distinct pathways during *Xenopus* gastrulation (Winklbauer et al., 2001) by presenting evidence that a Wnt/Ca^{2+} pathway exists downstream of Xfz7 that is Dsh-independent and activates PKC. This pathway is required for the proper separation of the mesoderm and ectoderm during gastrulation, but its inhibition does not directly affect CE. Consistent with this, it has been suggested that the Wnt/Ca^{2+} pathway has an indirect effect on CE by regulating canonical Wnt signalling and determining dorsal cell fates (Kühl et al., 2001). Another study suggests that the Wnt/Ca^{2+} pathway is in fact Dsh dependent, and speculates that the Wnt/Ca^{2+} and planar polarity pathways overlap (Sheldahl et al., 2003). Finally, others have argued for the existence of a Dsh-independent Wnt/Ca^{2+} pathway that activates PKC and that directly affects CE by regulating the activity of the p21 GTPase, Cdc42 (Choi and Han, 2002).

Although this issue has not been resolved, it is noteworthy that whereas PKC has not been implicated in planar polarity in

Drosophila, it has been implicated in an alternative non-canonical Wnt pathway that controls cell migration in the ovary (Cohen et al., 2002). This requires the Wnt ligand Wnt4 and uses Fz2 as a receptor and also Dsh. Hence, it is conceivable that there are multiple undiscovered non-canonical Wnt pathways in vertebrates that use PKC in either a Dsh-dependent or -independent manner, which could impinge on CE in as yet undiscovered ways.

A related issue is that Fz homologues have been implicated as acting as GPCRs that signal via heterotrimeric G proteins in both the Wnt/Ca^{2+}- and, more controversially, the β-catenin-dependent canonical Wnt pathways (Slusarski et al., 1997; Sheldahl et al., 1999; Liu et al., 2001; Malbon et al., 2001). So far no evidence has suggested that Fz receptors act through heterotrimeric G-proteins during planar polarity determination, but this is a possibility.

Concluding remarks

This review discusses a conserved group of genes that have been discovered to regulate cell polarisation during planar polarity establishment in the *Drosophila* cuticle and convergent extension during vertebrate gastrulation. Moreover, there is good evidence that these genes encode components or modulators of a conserved non-canonical Wnt/Fz signalling pathway. The challenge now is to discover whether there really is a conserved mechanism of cell polarisation at work, and if so how widely this is found in nature. As yet, little is understood about the biochemical or enzymatic functions of the core planar polarity proteins or about how they act together to transduce a polarity signal. It is also unclear whether the same 'core' of proteins acts together in all contexts see (Adler, 2002). We therefore need to characterise the protein-protein interactions of these factors and, most importantly, to determine when they occur in vivo in polarising cells in different tissues, as well as to better understand the long-range signals that coordinate cell polarity relative to the axes of the tissue, and the downstream effectors that lead to changes in cell structure and movement.

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