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Review Article

Molecular mechanisms mediating asymmetric subcellular localisation of the core planar polarity pathway proteins

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Planar polarity refers to cellular polarity in an orthogonal plane to apicobasal polarity, and is seen across scales from molecular distributions of proteins to tissue patterning. In many contexts it is regulated by the evolutionarily conserved 'core' planar polarity pathway that is essential for normal organismal development. Core planar polarity pathway components form asymmetric intercellular complexes that communicate polarity between neighbouring cells and direct polarised cell behaviours and the formation of polarised structures. The core planar polarity pathway consists of six structurally different proteins. In the fruitfly *Drosophila melanogaster*, where the pathway is best characterised, an intercellular homo-dimer of the seven-pass transmembrane protein Flamingo interacts on one side of the cell junction with the seven-pass transmembrane protein Frizzled, and on the other side with the four-pass transmembrane protein Strabismus. The cytoplasmic proteins Diego and Dishevelled are co-localised with Frizzled, and Prickle co-localises with Strabismus. Between these six components there are myriad possible molecular interactions, which could stabilise or destabilise the intercellular complexes and lead to their sorting into polarised distributions within cells. Post-translational modifications are key regulators of molecular interactions between proteins. Several post-translational modifications of core proteins have been reported to be of functional significance, in particular phosphorylation and ubiquitination. In this review, we discuss the molecular control of planar polarity and the molecular ecology of the core planar polarity intercellular complexes. Furthermore, we highlight the importance of understanding the spatial control of post-translational modifications in the establishment of planar polarity.

Introduction

Many cells require asymmetrical architecture — termed polarity — that allows structures to be compartmentalised within a cell in order to fulfil their function. Apicobasal polarity is common in many cell types such as neurons, where it enables unidirectional signal transduction, and epithelial cells, enabling unidirectional secretion. Cells can also be polarised in an orthogonal plane, which is termed planar polarity, also known as planar cell polarity (PCP). Planar polarity allows the placement of structures to be co-ordinated with the tissue axis and with neighbouring cells. Planar polarity is evolutionarily conserved [1] and has been reported in a range of tissues in both vertebrates and invertebrates, and is vital to proper tissue development and function. Examples of planar polarity enabled processes include cilia mediated convection currents in the oviduct, the arrangement of hair cells in the inner ear, and the overlapping nature of mammalian fur (reviewed in [2–4]). Planar polarity has been reported from as early as epiblast formation in zebrafish [5] and primitive endoderm formation in mouse [6]. Its loss is implicated in neural tube closure defects [7], spermatogenesis defects [8], deafness [9], and heart defects [10]. As such the underlying mechanisms are of broad interest.

At a molecular level, planar polarity principally involves the formation of intercellular complexes with heterologous components. Planar polarity can be genetically regulated by two well-characterised pathways: the Fat-Dachsous (Ft-Ds) pathway and the core planar polarity pathway ('core pathway', see Table 1). In the Ft-Ds pathway, the atypical cadherins Ft and Ds localise to opposite cell ends [11–13], where they interact intercellularly to form a heterophilic complex [14,15]. The *Drosophila* core pathway protein distribution consists of an intercellular homodimer of the atypical cadherin Flamingo (Fmi; also known as Starry Night [Stan]) [16] and asymmetric localisation of associated proteins (summarised in Figure 1A,C). The seven-pass transmembrane protein Frizzled (Fz) and the cytoplasmic proteins Dishevelled (Dsh) and Diego (Dgo) are found on one side of the Fmi homodimer [17–21], and on the other side are the four-pass transmembrane protein Strabismus (Stbm, also known as Van Gogh [Vang]) and the cytoplasmic protein Prickle (Pk) [22,23].

Due to simple husbandry, a small genome and fast reproductive cycle, the fruitfly *Drosophila melanogaster* has been at the forefront of genetic research for over a century. Readily accessible readouts of planar polarity in *Drosophila* include photoreceptor orientation within the eye (ommatidia), the organisation of surface bristles on the body, and wing hair (trichome) placement originating from distal cell edges (Figure 1A,D–F). *Drosophila* genetic tools allow for powerful and versatile experimental design. These include the rapid generation of transgenic animals, the ability to create mosaic mutants (Figure 1G,H) and the ability to express genes ectopically. These experimental paradigms have allowed for the robust assessment of the roles of particular gene products during development, including the elucidation of the genetic control of planar polarity. Here we primarily discuss mechanisms of planar polarity establishment in the developing *Drosophila* wing, which is a simple epithelial sheet, allowing easy imaging of fixed samples (Figure 1F–H), while also being accessible for live imaging.

Tissue level control of patterning in planar polarity

A pervading question within the planar polarity field regards the nature of a global cue to co-ordinate polarity with the tissue axis. Does it exist? And if so, what is it? Structures within epithelial sheets that are hundreds of cells wide are all co-ordinated to be planar polarised on parallel vectors. Global polarity of the Ft-Ds system appears to be based in part upon a vectorial gradient of Four-jointed (Fj) distribution, which is expressed in a graded fashion along the proximodistal axis of the developing wing [24,25]. Both Ft and Ds are phosphorylated by the kinase activity of Fj, with opposing effects on their heterophilic binding affinities. This results in phosphorylated Ft having an increased binding affinity for Ds, but phosphorylated Ds having a decreased affinity for binding Ft [26–28]. This leads to a local imbalance of Ft and Ds localisation within each cell, which in turn propagates to neighbouring cells. A 3% difference in Fj activity between neighbouring cells was found to be sufficient to generate Ft-Ds planar polarity *in silico* [28].

A global cue for the core pathway has been more elusive. Secreted Wnt ligands are known to bind to Fz receptors [29], and Wnts are expressed in gradients in the developing *Drosophila* wing [30–32]. Ectopic overexpression of Wnts can reorient trichomes in the wing [33]. However, an instructive role for Wnts in directing

Table 1 Planar polarity proteins in flies and vertebrates

Drosophila protein	Symbol	Vertebrate homologues
Ft-Ds pathway		
Fat	Ft	Fat
Dachsous	Ds	Dchs
Four-jointed	Fj	Fjx1
Core pathway		
Flamingo/Starry Night	Fmi/Stan	Celsr
Frizzled	Fz	Fz/Fzd
Strabismus/Van Gogh	Stbm/Vang	Vangl
Prickle	Pk	Pk
Dishevelled	Dsh	Dvl
Diego	Dgo	Diversin

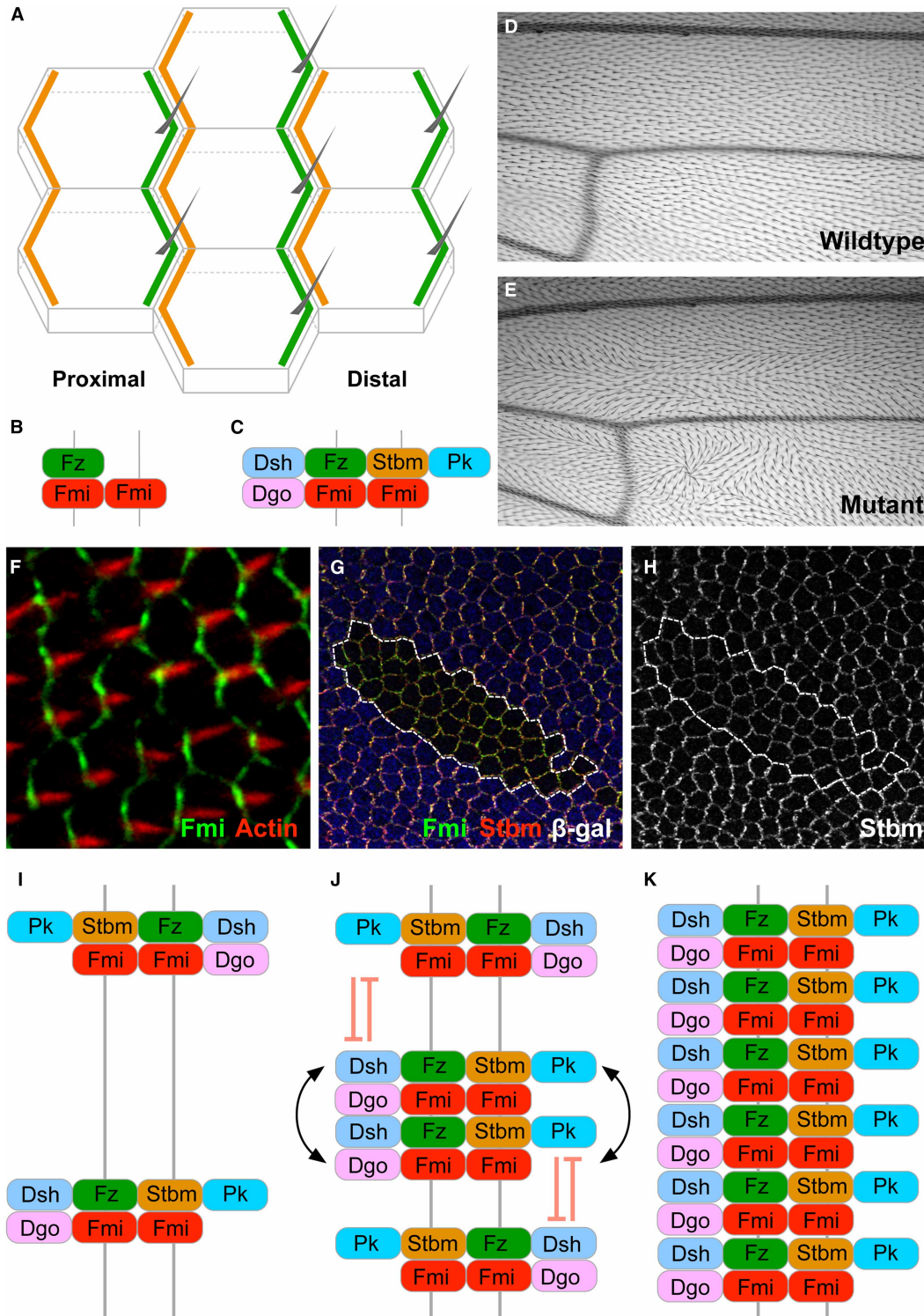**Figure 1. Principles of planar cell polarity.**   Part 1 of 2

(**A**) Schematic of localisation of core planar polarity pathway protein complexes in cells of the *Drosophila* pupal wing. Proximal localisation of Stbm (orange) and distal localisation of Fz (green) at apicolateral cell junctions leads to trichome (grey) emergence from distal cell edges. (**B** and **C**) Evolution of symmetry breaking cluster formation. (**B**) Fmi homodimers are stabilised by Fz localisation on one side of the complex at junctions between neighbouring cells. (**C**) Stbm localises to the apposing cell edge and the cytoplasmic proteins Pk, Dsh and Dgo localise proximally and distally in the complex as shown.

Figure 1. Principles of planar cell polarity. Part 2 of 2

(**D**) Image of dorsal surface of a wild-type *Drosophila* wing, showing uniform distal orientation of trichomes. Proximal is left, and anterior is up. (**E**) Image of dorsal surface of wing from a *pk^pk-sple13^* mutant fly, showing a swirled trichome pattern. (**F**) Confocal microscope image of *Drosophila* pupal wing epithelium, immunolabelled for Fmi (green, localised preferentially to proximodistal cell boundaries) and showing actin-rich trichomes (red, emerging from distal cell edges). (**G** and **H**) Confocal microscope image of a *Drosophila* pupal wing epithelium genetically mosaic for *dsh^V26^* mutant tissue, marked by loss of blue β-gal immunolabelling and outlined in white (**G**). Fmi (green) and Stbm (red, or white in **H**) are asymmetrically localised at proximodistal cell boundaries at the apicolateral cell junctions (left and right cell edges) in wild-type tissue, but lose this asymmetric localisation in mutant tissue. This shows that Dsh activity is required for planar polarisation of core pathway components such as Fmi and Stbm. (**I–K**) Molecularly asymmetric complexes (**I**) are sorted by feedback interactions (**J**) so that they all align in the same orientation (**K**). Complexes of opposite orientation are destabilised (red inhibitory symbols) and complexes of the same orientation are stabilised (black arrows).

trichome orientation has been difficult to demonstrate using loss-of-function mutations (reviewed in [34]). In addition, it has been proposed that the Ft-Ds system acts upstream of the core pathway, either by a direct interaction between the two pathways or by biasing microtubule orientation and Fz/Dsh transport distally within the cell (reviewed in [34]). Nevertheless, the Ft-Ds system only influences trichome orientation over part of the wing, suggesting that other factors must also contribute to generating the global cue for the core pathway. These have been suggested to include growth, morphogenesis and mechanical tension [4,34,35].

Molecular interactions of the core planar polarity pathway proteins

During pupal wing development, core pathway proteins are asymmetrically localised at the apico-lateral membrane, at the adherens zone. This polarisation is evident to some degree throughout development, but becomes more pronounced prior to trichome formation in the wing [36,37]. The establishment of core pathway planar polarity requires a first symmetry breaking step: the decision for Fz to accumulate distally and Stbm proximally around Fmi:Fmi homodimers at proximodistal cell boundaries (Figure 1C). In *fmi* mutant flies there is a reduction in Stbm and Pk at cell boundaries [22,23] and a loss of Fz, Dsh, and Dgo [17–20]. In *fz* mutant flies, Fmi is also mostly lost from the proximodistal cell boundaries and is observed at the apical cell membrane, suggesting that Fz is needed to stabilise Fmi homodimers [38]. Furthermore, Fmi appears to preferentially homodimerise when Fz is on only one side of the complex, suggesting that a Fz-Fmi:Fmi bridge is the backbone of the core pathway (Figure 1B) [38,39]. The cytosolic proteins and Stbm are required for the sorting of these asymmetric molecular bridges to a uniform orientation on proximodistal cell edges [17–20,22,23].

Beyond evidence that there is some polarised transport of Fz and Dsh distally on microtubule arrays [40,41], cellular mechanisms that sort the core proteins across the cell are not known. However, it has been reported that the core pathway proteins can self-organise. At cell boundaries in mosaic analysis experiments, *fz* mutant cells preferentially recruit Fz from their neighbours, whilst in the mutant cell Stbm localises to membranes apposing the wild-type neighbours, leading to a reversal of polarity on the distal side of the mosaic tissue [20,23,42,43]. This *de novo* planar polarity axis can extend across several cells suggesting an intracellular signal propagates away from the boundary cue [44].

Individual molecular interactions have been observed between many of the core pathway proteins. At least *in vitro* Stbm, Pk, Dsh, and Dgo can all interact with one another, while there also is evidence that Fmi may interact directly with Fz or Stbm, and Fz may interact with Stbm and Dsh (Table 2) [21–23,43,45–55]. Broadly speaking, these interactions are thought to underlie self-organisation, where complexes of the same orientation are stabilised, and complexes of opposite organisation are destabilised (Figure 1I–K). Feedback mechanisms driven by selective stabilisation/destabilisation of intercellular complexes could serve to amplify any small polarity bias generated by a global cue, to generate robust polarity [56–59].

Some potential feedback mechanisms acting in core pathway self-organisation have been uncovered. Based on binding interactions, it was proposed that Dgo and Pk compete for binding to Dsh, with Dgo promoting Fz activity and Pk inhibiting it (Figure 2A) [22,49]. Consistent with this, more recent work has shown that Pk acts via Dsh to destabilise Fz in the same cell, and also acts to stabilise Fz via Stbm using trans-interactions in the neighbouring cell (Figure 2A,B) [60,61]. Furthermore, overexpression of Pk leads to the co-ordinated

Table 2 Physical interactions between the core planar polarity proteins

Protein 1	Protein 2	Method	References
Fmi	Fz	co-immunoprecipitation	[43]
Celsr1[1]	Vangl2[1]	co-immunoprecipitation	[52]
Fzd3[1]	Vangl2[1]	co-immunoprecipitation	[51]
Fzd5[1]	Dvl1[1]	PEPSCAN-based ELISA	[55]
Stbm Vangl2[1]	Stbm Vangl1[1], Vangl2[1]	co-immunoprecipitation, GST pulldown, yeast two-hybrid	[46,54]
Stbm Vangl1[2], Vangl2[2]	Pk Pk1[2], Pk2[2]	co-immunoprecipitation, GST pulldown, mass spectrometry of immunoprecipitates	[23,46,53]
Stbm Vangl[3,4] Vangl1[1], Vangl2[1]	Dsh Dvl[3,4] Dvl1[1], Dvl2[1], Dvl3[1]	co-immunoprecipitation, GST pulldown, yeast two-hybrid	[23,45,46,48]
Stbm	Dgo	GST pulldown	[21]
Pk	Pk	GST pulldown	[46]
Pk Pk[3]	Dsh Dvl[3]	GST pulldown	[22,47,49]
Pk	Dgo	GST pulldown	[21]
Dsh Dvl2[1]	Dgo Diversin[1]	co-immunoprecipitation, GST pulldown, yeast two-hybrid	[49,50]

See Table 1 for fly and vertebrate homologues. Interactions were between fly molecules unless otherwise stated.
[1]Mouse;
[2]human;
[3]Xenopus;
[4]zebrafish.

internalisation of Pk, Stbm and Fmi, and if Fz is forced to accumulate on particular cell boundaries in genetic mosaics, Pk promotes exclusion of Stbm from these cell boundaries (Figure 2C) [62]. In addition, Dsh binding to the Fz-Fmi:Fmi-Stbm complex results in the stabilisation of Pk in the adjacent cell [63]: if Dsh is acutely depleted in one cell, free Pk in the adjacent cell then destabilises Dsh-Fz in this cell, and thus leads to a propagating wave of core complex destabilisation (Figure 2D) [63].

In the pupal wing, core pathway proteins are observed along the length of the proximodistal junctional membranes in uneven abundance, and these localised concentrations are termed 'puncta'. Fluorescence recovery after photobleaching (FRAP) experiments on live *in vivo* wing junctions have shown that core proteins in these puncta are more stable than in the non-puncta regions [64,65]. The proteins within these puncta are also highly asymmetrically localised compared with neighbouring non-puncta regions [62,64]. These data support the idea of mutual exclusion of unsorted complexes: antiparallel complexes are excluded from uniformly oriented puncta, while sorted complexes are mutually stabilised (Figure 1J,K).

Strutt and Gamage [65] directly measured the stoichiometry of core pathway proteins in these puncta, by tagging each protein individually with EGFP and comparing intensity in live pupal wings. When compared with immunolabelled Fmi, signal intensity of all six core proteins increased linearly with puncta size. This supports the idea that all puncta have the same composition. The stoichiometry in puncta was determined to be two Fmi (trans-homodimer) with approximately two Dsh, one Fz, and one Dgo on the distal membrane, and six Stbm with one Pk on the proximal membrane of the adjoining cell (Figure 2G). Interestingly, this stoichiometry is consistent between stable puncta and unstable non-puncta regions within the same cell.

Notably, the stoichiometry of the cytoplasmic proteins in puncta is dependent on their expression levels [65]. For example, if Dsh was overexpressed, more was found within both stable puncta and unstable non-puncta regions. However, the other complex components maintained their normal ratios. Likewise, when Dsh levels were halved, the ratio within stable and unstable regions halved for only Dsh [65]. In contrast, halving
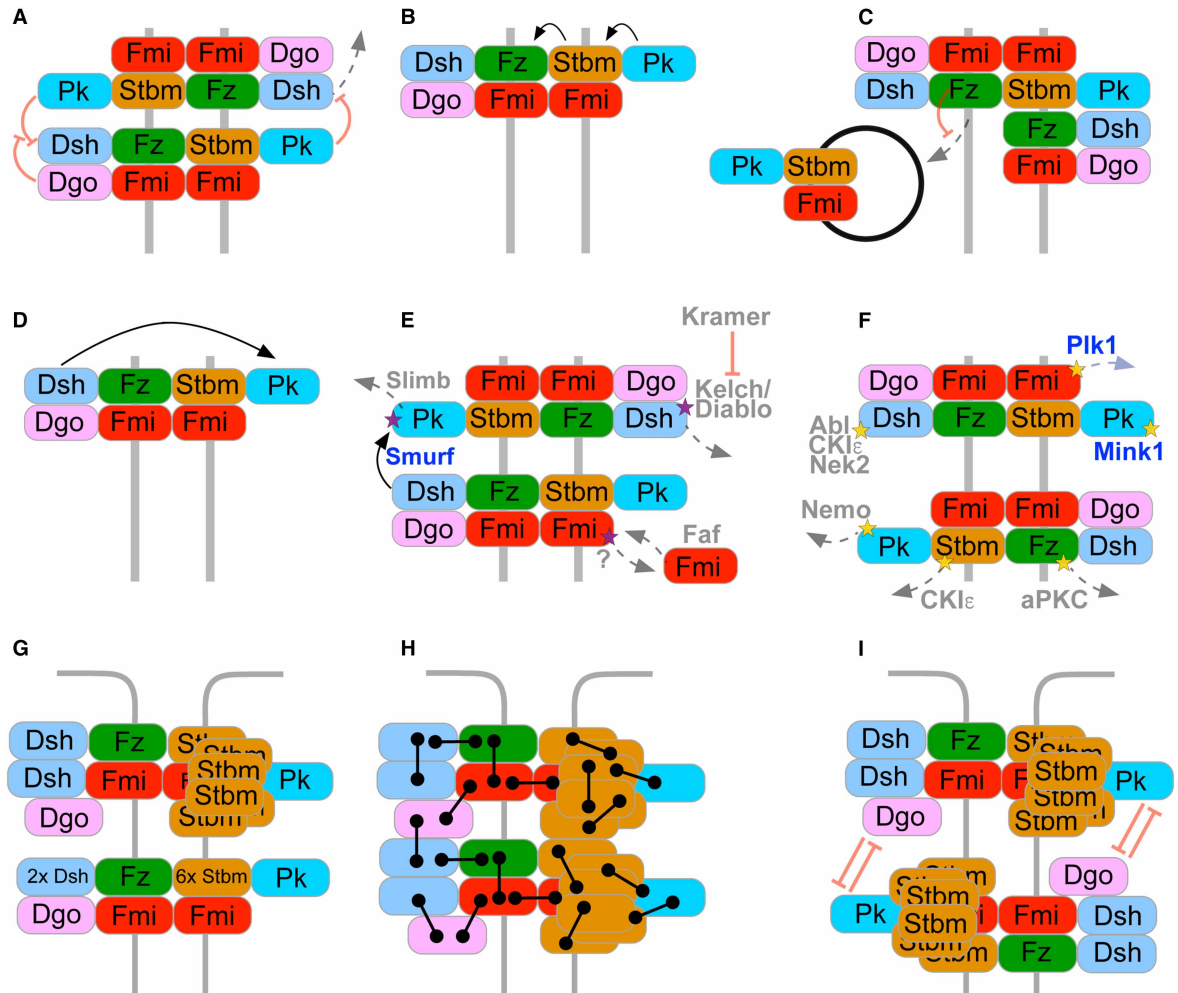
Figure 2. Mechanisms of planar cell polarity establishment.

(**A–D**) Putative stabilising and destabilising interactions between the core proteins on two neighbouring cell membranes (grey solid lines). Black arrows indicate stabilising interactions and red inhibitory symbols indicate destabilising interactions. Dotted grey arrows indicate removal of proteins from the intercellular complex. (**A**) Pk and Dgo compete for Dsh binding. Pk binding to Dsh leads to destabilisation of Fz. (**B**) Pk acts through Stbm to stabilise Fz in the adjacent cell. (**C**) Pk promotes Fmi and Stbm endocytosis in the presence of Fz on the same membrane. (**D**) Dsh stabilises Pk in the adjacent cell. (**E,F**) Post-translational modifications of the core proteins. Purple stars indicate ubiquitination of a core protein (E) and yellow stars indicate phosphorylation (**F**). Modifying enzymes acting in *Drosophila* are in grey and those only known to act in vertebrates are in blue. (**G**) Stoichiometry of core pathway complexes. A homodimer of Fmi associates with one Fz, two Dsh and one Dgo molecule on one side of the complex and with six Stbm and one Pk molecule on the other. (**H**) Clustering of intercellular complexes in parallel arrays results in multiple stabilising interactions, and complexes undergo a phase transition to form stable puncta. (**I**) Outside of puncta, core protein complexes are in both orientations, and destabilising feedback interactions prevent clustering of complexes, leading to complexes that are less densely packed.

the expression of Fmi resulted in a halving of Fz recruitment into puncta, maintaining the 2 : 1 ratio of Fmi:Fz and supporting the asymmetric molecular bridge hypothesis (Figure 1B). This suggests that the cytosolic proteins form a dosage-dependent 'cloud' around a fixed Fz-Fmi:Fmi backbone. The conclusion of this work is that core protein complexes within both stable puncta and unstable non-puncta coalesce via multivalent protein–protein interactions between the different proteins. Overall, it was suggested that when locally ordered complexes reach a critical density they undergo a phase transition from an unstable, fluid state, to a more solid stable state in puncta (Figure 2H,I) [65].

The Ft and Ds proteins are also organised into puncta, with FRAP experiments showing a more stable population in puncta [28]. Analogous to the core pathway proteins, puncta formation is dependent on formation of intercellular heterodimers between Ft and Ds. Furthermore, puncta stability is dependent on trans-binding affinity [28].

Post-translational modifications influence protein–protein interactions and complex sorting

Post-translational modifications (PTMs) are ubiquitous in mediating feedback interactions in cell polarity, from symmetry breaking in yeast budding, to anteroposterior patterning of PAR proteins in *C. elegans* embryos and apicobasal polarity of epithelial cells [66–68]. The core pathway proteins have a complex network of PTMs that include ubiquitination and phosphorylation (Table 3). Ubiquitination of a protein can target it for proteasomal degradation, or can promote internalisation of transmembrane proteins that are then either recycled back to the plasma membrane or degraded in the lysosome [69]. Therefore, targeted ubiquitination of a core protein could promote destabilisation/removal of that protein from a complex and complex sorting. Phosphorylation can alter protein stability by priming proteins for ubiquitination, but it can also alter protein conformation and/or affinity for binding partners [70]; and thus could also regulate interactions between the core proteins and sorting of complexes. Interestingly, phosphorylation has been identified as a key modulator of phase transitions, and thus could regulate clustering of core protein complexes into puncta [71].

Table 3 Post-translational modifications of the core planar polarity proteins

Substrate	Enzyme	Candidate modification sites	References
Fmi[1]	Fat facets (Faf) deubiquitinase	-	[73]
Celsr1[2]	Polo-like kinase 1 (Plk1)[2]	14 S/T residues in the cytoplasmic domain of mouse Celsr1, including S2741, T2750, and S2752	[92]
Fz	atypical Protein Kinase C (aPKC)	S554, S560 of fly Fz	[95]
Stbm Vangl2[2]	Casein Kinase 1ε (CKIε) Casein Kinase 1δ/ε (CKIδ/ε)[2]	Two clusters of highly conserved S/T residues located in the N-terminal cytoplasmic domain (S5–17, ST113–122 of fly Dsh; S5–17, ST76–84 of mouse Vangl2)	[61,88–91]
Pk	Farnesyl-diphosphate farnesyl transferase (FNTA, FNTB)	C-terminal CaaX motif	[75]
Pk Pk2[2]	Cullin1-SkpA-Slimb Skp1-Cullin1-F-Box[2] ubiquitin ligase	-	[62,75,76]
Pk1[2]	Smurf1[2], Smurf2[2] ubiquitin ligases	-	[77,78]
Pk	Nemo kinase	4 potential MAPK phosphorylation sites in the middle region	[98]
Pk1	Ste20 kinase Mink1	T370 of *Xenopus* Pk1	[53]
Dsh Dvl[3]	Cullin3-Diablo/Kelch Cullin3-KLHL12[3] ubiquitin ligase	-	[72,73]
Dsh	Abelson tyrosine kinase (Abl)	Y473 of fly Dsh	[79,82]
Dsh	Nek2 kinase	multiple sites	[80,81]
Dsh Dvl[2,3,4]	Casein Kinase 1ε (CKIε) Casein Kinase 1δ/ε (CKIδ/ε)[2,3,4]	S236 of fly Dsh	[61,83–87]

See Table 1 for fly and vertebrate homologues. Modifications were of fly molecules unless otherwise stated.
[1]Deduced target, but no direct modification observed;
[2]Mouse;
[3]human;
[4]Xenopus.

Ubiquitination of *Drosophila* Dsh via a Cullin3-Diablo/Kelch ubiquitin ligase complex results in reduced Dsh at cell junctions and its targeting for degradation [72,73]. Dsh ubiquitination is antagonised by the phosphoinositide binding protein Kramer/PLEKHA4, which in vertebrates sequesters the Kelch homologue KLHL12 (Figure 2E) [74]. There is also evidence of ubiquitination promoting internalisation of Fmi, as the deubiquitinase Fat facets (Faf) promotes the recycling of internalised Fmi to the junctional membrane (Figure 2E) [73]. Furthermore, farnesylation of Pk promotes its recruitment into the proximal complex by Stbm [75], while Stbm also promotes degradation of excess Pk via a Cullin1–SkpA–Supernumerary limbs (Slimb) ubiquitin ligase complex (Figure 2E) [62,75,76]. In vertebrates, the HECT ubiquitin ligases Smurf1 and Smurf2 promote proteasomal degradation of Pk [77]. Interestingly, Smurfs also interact specifically with phosphorylated Dvl (the vertebrate Dsh homologue) [77], and Dvl promotes Smurf activity [78]. This leads to a model in which phosphorylated Dsh recruits Smurf, which then ubiquitinates Pk and destabilises complexes of the opposite orientation (Figure 2E).

The abundance of evidence for PTM control of planar polarity comes from assays of protein phosphorylation. In particular, Dsh hyperphosphorylation correlates with its recruitment to junctions by Fz [17,19], and analysis of putative phosphorylation sites in Dsh has revealed multiple redundant phosphorylation sites [79]. Phosphorylation of Dsh by the Abelson tyrosine kinase (Abl), Nek2 and Casein kinase Iε (CKIε, or Discs overgrown [Dco] in flies) has been implicated in regulation of planar polarity (Figure 2F). Nek2 phosphorylates Dsh at multiple sites [80] and loss of Nek2 leads to accumulation of Dsh in intracellular puncta, and reduced core protein asymmetry [81]. Abl phosphorylates Dsh *in vitro* at Tyr473, and loss of Abl disrupts photoreceptor specification and polarity [82]. Finally, CKIε promotes Dsh phosphorylation *in vitro* and *in vivo*, and loss of CKIε abolishes core protein asymmetry [61,83–88].

In addition to phosphorylating Dsh, CKIε also phosphorylates the N-terminus of Stbm, and mutation of the Stbm phosphorylation sites disrupts planar polarity in both flies and vertebrates [61,88–91]. Interestingly, in *Drosophila* mutation of the CKIε phosphorylation sites in Stbm leads to an increase in Stbm stability, while mutation of putative CKIε phosphorylation sites in Dsh decreases its stability (Figure 2F) [61]. This dual action of CKIε would therefore enhance removal of Stbm from complexes and stabilise Dsh in complexes, and thus promote sorting of proximal and distal complex components to opposite cell ends.

In vertebrate skin, the Fmi homologue Celsr1 is phosphorylated on a dileucine endocytic motif by Polo-like kinase 1 (Plk1) [92]. Interestingly, Plk1 is a mitotic kinase and thus promotes internalisation of Celsr1 specifically in mitosis (Figure 2F). Failure of this internalisation perturbs skin planar polarity [92,93]. Internalisation was suggested to allow core proteins to be redistributed equally between the two daughter cells, and also to protect mitotic cells against aberrant signalling while they are rounded up.

Three further kinases, atypical PKC (aPKC), Nemo and Misshapen (Msn) have also been implicated in regulating planar polarity in the *Drosophila* eye (Figure 2F) [94–96]. aPKC can phosphorylate the cytoplasmic tail of Fz, which is thought to inhibit Fz function. This activity of aPKC on Fz is antagonised by the apicobasal polarity protein Bazooka/Par-3 [95]. Bazooka also interacts with Fmi, raising interesting questions regarding cross-talk between apicobasal and planar polarity [97]. Nemo is thought to act in two ways in eye planar polarity: firstly, a direct effect via Pk [98] and secondly by regulating downstream effectors that regulate ommatidial cluster rotation [99]. Pk phosphorylation by Nemo is thought to promote the targeted proteasomal degradation of Pk from planar polarity complexes via Cullin1–SkpA–Slimb (see above) [98]. Finally, in vertebrates Pk phosphorylation by the Msn homologue Mink1 promotes Pk trafficking to the plasma membrane and clustering with the vertebrate Stbm homologue Vangl2 [53].

Future directions

It is interesting to note that despite the long list of known PTMs that affect core protein activity, only in the case of Smurf binding to phosphorylated Dsh has a specific regulatory mechanism been uncovered that implicates a PTM in driving core complex sorting within clusters. Nevertheless, it seems likely that spatiotemporal regulation of PTMs will be a critical mechanism in planar polarity establishment.

Integral to models of core protein sorting via feedback interactions is the idea that complexes in the same orientation are stabilised and those of the opposite orientation are destabilised (Figure 1J). To understand stabilising/destabilising interactions it will be necessary to identify the role of specific PTMs: in particular, how each PTM affects the stability of core proteins at junctions. It will also be important to determine whether and how specific PTMs are regulated: for example if one core protein locally promotes the modification of another protein and this alters its stability then this would constitute evidence for a feedback mechanism.

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Moreover, understanding the complex roles of multiple PTMs will require precise spatiotemporal manipulations of protein activity, in order to distinguish fast acting direct effects from more long term consequences. This will require the development of new methods for acutely activating or removing activity. Finally, we anticipate that advances in microscopy — for instance single molecule imaging — that allow more detailed visualisation of core protein clustering at cell junctions, will be key in understanding the effects of specific PTMs on core protein organisation and sorting.

Such studies will be an important and exciting way forward in unravelling molecular mechanisms of core planar polarity pathway function.

Perspectives

- Planar polarity allows cells and groups of cells to exhibit polarised behaviours that are co-ordinated with their neighbours and the tissue axes. Such coordination of cell behaviours is vital for correct tissue morphogenesis and function.

- Co-ordinated planar polarisation involves the formation of heterophilic intercellular complexes that become asymmetrically localised to opposite cell edges. Self-organising processes involving stabilising and destabilising feedback interactions between intercellular complexes leads to clustering of complexes of the same orientation in puncta. PTMs most likely play a key role in controlling the spatiotemporal dynamics of planar polarity protein interactions.

- Further research is required to elucidate the cellular mechanisms that sort the core proteins across the cell, and how the feedback interactions between the core planar polarity proteins are regulated. The control of PTMs is important in core protein asymmetry, however, the mechanisms controlling PTM activity are poorly understood. Better methods for mapping PTMs and PTM enzyme activity in time and space will be essential for understanding pathway function.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

Literature review, C.H., H.S., H.S. and D.S.; Writing — original draft preparation, C.H. and H.S.; Writing — review and editing, C.H., H.S. and D.S.; Figure preparation, C.H. and H.S.; Table preparation, Hongyu Shao.

Abbreviations

FRAP, Fluorescence recovery after photobleaching; PCP, planar cell polarity; PTMs, Post-translational modifications.

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