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Planar cell polarity in development and disease

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Abstract

Planar Cell Polarity (PCP) is an essential feature of animal tissues, whereby distinct polarity is established within the plane of a cell sheet. Tissue-wide establishment of PCP is driven by multiple global cues, including gradients of gene expression, gradients of secreted Wnt ligands, and anisotropic tissue strain. These cues guide the dynamic, subcellular enrichment of PCP proteins, which can self-assemble into mutually exclusive complexes at opposite sides of a cell. Endocytosis, endosomal trafficking, and degradation dynamics of PCP components further regulate planar tissue patterning. This polarization propagates throughout the whole tissue, providing a polarity axis that governs collective morphogenetic events such as the orientation of subcellular structures and cell rearrangements. Reflecting the necessity of polarized cellular behaviours for proper development and function of diverse organs, defects in PCP have been implicated in human pathologies, most notably in severe birth defects.

Introduction

Cell polarization refers to the organized establishment of asymmetries within cells. Just as intracellular functions are compartmentalized in organelles, many cellular functions are made more effective by partitioning them along an axis of polarization. Cell polarization typically involves the localization of specific molecular determinants to specific cellular domains, and the coordination of polarity across tissues is essential for the development of specialized form and function in multicellular organisms. Cell polarity is best understood in the context of epithelia, and epithelial cells are generally considered to display two key forms of polarity: apical—basal polarity, which refers to polarized distribution of cellular components and specialized functions between the opposing surfaces of an epithelial sheet, and, the focus of this Review, planar polarity, which refers to organization along the perpendicular axis, in the plane of the epithelial sheet (for comparison between the two polarity axes see Supplementary information S1 (Box)).

Planar tissue patterning is governed by two major signalling pathways: the “core” planar cell polarity (PCP) and “Fat, Dachsous, and Four-Jointed” (Ft-Ds-Fj) modules. These signalling pathways were initially identified in *Drosophila melanogaster* screens for regulators of the coordinated orientation of external bristles and hairs[1–5] (Fig.1a). A key feature of planar polarization through these pathways is the complementary and mutually exclusive distribution of transmembrane signalling complexes, resulting in their asymmetric

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enrichment in distinct cell compartments within each cell of a patterned tissue (Fig.1b). This in turn directs the orientation of subcellular structures and cell behaviours through the regulation of cytoskeletal elements and cellular adhesions. Proper establishment and maintenance of planar polarity is essential during development and involved in tissue homeostasis and repair, and defects in PCP signalling are associated with diverse human pathologies (Box 1).

Box 1

Planar cell polarity genes and human birth defects

Birth defects, associated with developmental abnormalities, are the leading cause of infant mortality in the United States and are among the leading causes of death for children of all ages[120]. As planar cell polarity (PCP) is an important organizer of tissues during morphogenesis, studies of the mechanisms of PCP signalling in embryonic development provide an opportunity to shed light on an important public health issue. For example, failure of convergent extension following PCP disruption in model animals results in neural tube defects (NTDs), and PCP genes are now among the most well-defined genetic risk factors for NTDs in humans – a common birth defect[121]. A flurry of papers has emerged implicating PCP genes in human NTDs. The first identified mutations were in Vang-like protein 1 (VANGL1)[116], but since then, mutations in essentially all the core PCP genes have now been identified in human NTD patients. These individual findings are too numerous to list here but have been comprehensively delineated elsewhere[122,123]. Several studies also provide mechanistic insights, revealing that NTD-associated mutations disrupt known interactions among PCP proteins and/or disrupt their subcellular localization[116,124–126].

In addition to NTDs, PCP gene mutations also contribute to the etiology of Robinow Syndrome, a severe skeletal dysplasia characterized by short limbs and craniofacial anomalies. Studies revealed mutations in ROR2 kinase, which now has an established role in the PCP-mediated elongation of the murine limb in response to Wnt5a[44], as a genetic background for this syndrome [127,128]. More recently, Robinow Syndrome has been associated with genetic variants in the core PCP Dishevelled genes DVL1[129,130] and DVL3[131], as well as in WNT5a itself[132]. Importantly, these gene-association studies are supported by functional assays in model animals, providing mechanistic insights to supplement genotype–phenotype correlations.

Lastly, there is evidence to suggest that PCP signalling functions in the proper formation of the spine and that PCP mutations could lead to the onset of idiopathic scoliosis phenotypes. This link was additionally uncovered in the study of zebrafish ptk7 mutants, which exhibit late-onset severe spinal curvature abnormalities that are likely due to defective cerebrospinal fluid flow, mediated by multiciliated cells[133,134]. A recent survey of a patient cohort with adolescent idiopathic scoliosis (AIS), which screened for mutations in VANGL1, identified two missense mutations of interest were identified as potentially contributing to the AIS phenotypes[135].

This review will focus primarily on discussing the mechanisms underlying the establishment and tissue-wide coordination of PCP patterns. We will also outline specific examples to illustrate how cells can exploit these patterns to execute polarized cell behaviours, thereby shedding light on the molecular etiology of PCP-related human birth defects.

Establishment of core PCP patterns

The establishment of planar polarity involves several fundamental processes, including sensing cues that govern the global orientation of polarity, establishing molecular asymmetries according to these cues, refining polarity patterns throughout neighboring cells, and executing respective polarized cell shape changes and behaviours .

Core PCP signalling components

When considering the roster of molecular players at work in PCP, it is useful to understand that distinct molecules participate in these diverse processes. The transmembrane components of this system allow for exchange of polarity information between cells. In *D, melanogaster* these include Flamingo, ((Fmi, also known as Starry Night (Stan); CELSR in vertebrates), Frizzled (Fz; FZD in vertebrates), and Van Gogh, (Vang also known as Strabismus or Stbm; VANGL in vertebrates). By contrast, the cytoplasmic components encoded by Dishevelled (Dsh; DVL in vertebrates), Prickle (Pk), and Diego (Dgo; ANKRD6 in vertebrates) are involved in amplifying intracellular asymmetries and translating polarity cues into cell behavioral changes[1–5].

The basis of PCP establishment is the localization of mutually exclusive subsets of the core PCP proteins to opposing domains along the cell cortex, forming a pattern that propagates throughout the tissue. PCP protein patterns develop from an initially symmetric distribution that gradually sorts out into two complementary domains, with Fz, Dsh, and Dgo complexes typically accumulating on one side of each cell, and Vang and Pk enriched on the other; Fmi is present on both sides[1–5] (Fig.1c). Asymmetric sorting and symmetry breaking is driven by dynamic trafficking, binding, and signalling interactions that differentially control stable localization of PCP components at discrete regions of the cell, and as detailed in following sections, the resulting stable accumulations ultimately coordinate planar-polarized cell behaviours.

Cell–cell communication and propagation of PCP asymmetry

The asymmetric localization of a molecular cue can be sufficient to polarize a single cell's behaviour, but for groups of cells in an epithelium to *collectively* polarize, polarity information must be transmitted between neighboring cells. Such cell-cell communication of planar polarity is primarily facilitated through the physical interactions of PCP signalling complexes at cellular junctions, of which the seven-pass atypical cadherin Fmi is an essential component[6–9]. Extracellular interactions of cadherin repeat domains promotes the formation of Fmi homodimers across apical cell–cell junctions, which in result become increasingly stable[8–11]. These stable transmembrane accumulations link PCP signalling complexes in neighbouring cells, propagating a uniform, cooperative pattern of polarity information (Fig. 1c).

In addition to serving as the molecular bridges that link *intercellular* polarity, Fmi also promotes the assembly of *intracellular* asymmetric directional signalling complexes. Junctional Fmi helps recruit PCP components Fz (a seven-pass transmembrane Wnt receptor) or Vang (four-pass transmembrane protein) to lateral regions of the apical cell surface[6,12,13]. These interactions help stabilize PCP components at cellular junctions but also mutually influence Fmi localization; Fmi is diffusively apically localized rather than apicolaterally enriched in a *Vang*, *fz* double mutant background, similar to Vang and Fz localizations in *fmi* mutant cells[6,8,12,13]. Once recruited to apicolateral junctions, directional Fmi–Fz and Fmi–Vang signalling complexes can be progressively assembled in a coordinated, collective fashion across the tissue. Fmi molecules seem to preferentially bind Fz over Vang, and a Fmi–Fz complex is more likely to stably associate with a junctional Fmi molecule of an adjacent neighboring cell that is not bound to Fz – that is, it is either bound to Vang or bound to neither Fz nor Vang[7–9,14]. This cell-cell interaction appears to help maintain the junctional localization of these signalling components in both cells, and the association of Vang with such a complex, specifically by binding a Fmi molecule linked to a Fmi-Fz complex in an adjacent cell, appears to further increase the combined apicolateral stability of all signalling components involved[8,11]. This occurs by bolstering resistance to the endocytic flux that these molecules are subject to in the absence of such an assemblage (Fig.2).

Binding of Fmi to Fz or Vang induces differential Fmi signalling behaviours across cellular junctions that promote the propagation of a uniform PCP pattern[7–9,14]. Because signalling through Fmi occurs also when the extracellular domains of Fz and Vang are not intact, this indicates that Fmi signalling can be modulated by the binding to its cytoplasmic partners – with Fz, Vang or neither Fz nor Vang being bound to the C-terminal region of Fmi. Thus, it seems that even though Fmi is localized to both sides of a PCP-patterned epithelial cell, it can exhibit asymmetry in behaviour that will influence the potential interactions between PCP signalling complexes of adjacent cells. Demonstrating this, *fz* mutant cells (over a distance of several cells) can be polarized quite remarkably by a neighbouring clone of cells, as long as both cell populations retain Fmi function and the neighbouring clone expresses *fz* [14]. Vang function does not appear to be required for a cell to be polarized by signals from a nearby clone expressing *fz*, though the effects of this polarizing activity are less strong than when Vang is present in the signal receiving cells (the distance over which the polarization induced by the *fz* expressing clone is maintained is less for *fz* and *Vang* double mutants as compared to *fz* single mutants)[14]. Lastly, *fz* mutant cells can be polarized by Fz positive cells that do not express *Vang*[14]. Cumulatively, this analysis shows that to ensure directional non-autonomous cell polarization, Fmi must be present in both sending and receiving cells, and Fz must be present in at least one of the two cell populations. Vang activity may not be absolutely essential to initiate polarization, but it appears to facilitate the polarization of the receiving cells. Thus, Fmi is essential for the intercellular communication of axial planar polarity and appears to assume one of two signalling states: one – with Fz – required for establishing a stable, vectorial planar polarity signal, and another – either unbound or bound to Vang – that associates with Fmi–Fz in the neighbouring cell across the cell–cell junction[7,8,14].

Feedback amplification of asymmetry

The recruitment of junctional PCP complexes by Fmi is necessary for intercellular communication of polarity, but not sufficient for the establishment of robust PCP patterns within planar-polarized cells. The differential signalling states of Fmi with either Fz or Vang, which promote stability of the oppositely associated complexes across junctions, can set the stage for such patterns[8,9]. However, the concomitant sorting and enrichment of PCP signalling complexes is mediated by positive and negative interactions between the asymmetric PCP components. While some of these interactions may be driven by the membrane components, such as the extracellular domain of Fz potentially acting as a ligand for Vang[8,10], much of the feedback amplification process occurs through activity of the cytoplasmic components Dsh, Dgo, and Pk. These cytoplasmic components act by clustering transmembrane signalling complexes into stable PCP enrichments[11,15–19], which are not necessarily required for intercellular PCP signalling, but rather for locally accumulating these signalling complexes to amplify the asymmetric pattern of PCP signalling complexes[19] (Fig. 1c). Thus, these interactions that promote PCP patterning are often collectively referred to as the feedback amplification of asymmetry[3,20]. Notably, for robust amplification to occur, the activies of all the core PCP components seem to be essential. Significant perturbation of any single core PCP protein is often sufficient to disrupt the asymmetric patterning of them all, adversely affecting tissue structure and collective cell behaviour[19].

In a polarized epithelial cell, one half of the hallmark PCP pattern consists of Fmi, Fz, Dsh and Dgo assembled into asymmetric junctional enrichments (Fig 1c). As Fmi homodimers recruit Fz to apicolateral regions of the cell, Dsh can be recruited as well in a Fz-dependent manner through the binding of the DEP domain of Dsh to the Fz cytoplasmic tail[12,15,16]. In addition, the ankyrin repeat domain of Dgo can bind the PDZ domain of Dsh, serving as an additional interaction that promotes the unilateral clustering of the Fmi–Fz–Dsh–Dgo complex[18]. The asymmetric enrichment of Fz and overall junctional levels of Fmi appear greatly diminished in *dsh* mutant cells[6,8,12,13], likely due to a reduction in junctional stability[11].

Complementary to the Fz, Dsh and Dgo accumulations that decorate one side of a planar-polarized cell, Fmi, Vang and Pk assemble along the opposite half of the apical membrane, generating the characteristic PCP pattern[13,20] (Fig. 1c). Vang and Pk accumulate through interactions of their respective C-terminal regions, mediating the heterotypic binding of Vang with Pk, as well as homotypic Pk and Vang binding[17]. These binding interactions, similarly to with Fz–Dsh[11], facilitate the clustering of Vang and Pk and increase the stability of their junctional localization[21].

The positive interactions that promote the clustering of PCP signalling components appear to be sufficient to drive the enrichment of these complexes in any given junctional region of the cell, yet there must be additional negative interactions in order to develop and maintain the mutually exclusive enrichment of Fmi–Fz–Dsh–Dgo and Fmi–Vang–Pk complexes to the opposing cell membranes. One simple mechanism would be for PCP signalling components to “exclude” the complementary set from localizing in the same region. Indeed, interactions between members of oppositely localizing complexes, namely between Vang, Pk and Dsh and Dgo, were observed[17,18,20,22,23]. Interestingly, it is the C-terminal regions of Pk and

Vang (through which they cluster with each other to accumulate on the one side of the cell) that have been demonstrated to associate with both the PDZ domain of Dsh and ankyrin repeats of Dgo[18,23]. Utilizing the same binding region to interact with members of both asymmetric domains could establish a framework for negative feedback mechanisms, and it has been proposed that Pk inhibits the association of Dsh with Fmi–Vang by competing for the same binding region of Vang, while Dgo competes with Pk for binding to Dsh[17,18,23]. Along with the positive interactions that promote clustering PCP complexes, these negative interactions appear to allow for the self-sorting of PCP asymmetry and short-range propagation of polarity patterns, likely in the absence of any additional molecular inputs or guidance cues[19,24,25].

Endocytosis and endosomal trafficking facilitates asymmetric sorting

It stands to reason that in order to generate and maintain PCP patterns, mislocalized or unstable components need to be removed from the membrane, and endocytosis, endosomal trafficking and degradation can indeed influence core PCP localization and signalling (Fig. 2). Both Rab GTPase Rab5 and Dynamin have been shown to play a role in the internalization of unstable PCP signalling components[8, 26,27]. Inhibition of endocytosis leads to the overaccumulation of Fmi at the plasma membrane, and owing to the apparent role of Fz and Vang in stabilizing junctional Fmi, there appears to be an increased rate at which Fmi is internalized in *fz* and *Vang* mutant wing blade cells[8,11]. Along with Fmi, both Fz and Vang also appear to be subject to membrane turnover, and Fz may facilitate the feedback amplification of asymmetry by promoting removal of Fmi–Vang–Pk complexes where Fz is accumulated[8,11,28]. Ubiquitylation of Pk by an E3 ubiquitin ligase complex consisting of Cullin-1, SkpA and Supernumerary limbs can promote the interalization of Fmi–Vang–Pk complexes, and the Pk-dependent internalization of Fmi in this manner is significantly reduced in *fz* mutants[28] (Fig. 2).

Both Dsh-dependent and -independent mechanisms contribute to control of Fmi internalization[8], and the former is likely to require Fz bound to Fmi due to known Fz-Dsh binding interactions[30]. Interfering with the ability of DVL2 (one of the vertebrate Dsh homologues) to interact with the Clathrin adaptor AP-2 leads to a reduction in internalized FZD4, which in turn results in PCP-defective gastrulation movements in *Xenopus laevis*[29]. As described before, Pk has similarly been shown to mediate the internalization of Fmi and Vang, which may be related to its function in clustering Fmi–Vang–Pk accumulations[28]. Together, the cytoplasmic PCP signalling components appear to have a dual regulatory role on PCP, clustering their associated transmembrane components in some regions and mediating their internalization in others (Fig. 2).

While internalization of PCP proteins is important for patterning of the PCP axis, this is only the first step in the intracellular sorting of these signalling components. Following endocytosis, PCP proteins are subject to one of several downstream processes, including recycling back to the membrane, trafficking to another region of the cell, or degradation. Inhibition of lysosomal maturation leads to the intracellular accumulation of internalized Fmi, suggesting some fraction of Fmi is normally turned over via lysosomal degradation[8,11]. Internalized Fmi colocalizes with endosomal makers such as Rab4, Rab5, Rab7 and Rab11,

and both Rab4 and Rab11 appear to be capable of mediating the recycling of Fmi back to the membrane[8,11,27]. As unstable components are likely continually degraded, it would seem that newly synthesized proteins must be continually added to the plasma membrane to help generate stable PCP patterns. Indeed, factors such as Clathrin adaptor protein AP-1 and trafficking GTPase ARF1, which are thought to facilitate trafficking from the Golgi and endosomes to the plasma membrane, have been identified as important factors in PCP establishment, and their perturbation results in strong PCP phenotypes[31]. These findings further underline the importance of membrane trafficking in the regulation of PCP complex distribution.

Endocytosis is essential not only for establishing PCP patterns, but also for adjusting patterns during cell rearrangements and in the face of frequent mitotic events in proliferative tissues[27]. In the developing mammalian epidermis, PCP components are internalized and redistributed during mitoses, which appears to be essential for the proper patterning of the skin and planar polarization of hair follicles[32]. The events of internalization and redistribution are synced with cell division through the control of CELSR phosphorylation by Polo-like kinase -1 (PLK1), and phosphorylation promotes the internalization of CELSR1 and associated FZD proteins[33]. Thus, the endocytic control of PCP components is an important part of maintaining uniform polarity, particularly during dynamic cellular behaviours.

Polarized microtubule trafficking facilitates PCP protein anisotropy

Feedback amplification and endosomal trafficking mechanisms facilitate the asymmetric sorting of PCP proteins, but these rely upon an initial anisotropic bias in order to become enriched and stable. This initial bias can be subtle, or random even, and with amplification can result in locally aligned asymmetry[19,24,25]. However, in order to uniformly align PCP asymmetry across a given tissue, there must be an additional, reliable input that influences PCP patterning. Microtubules and microtubule-based trafficking are used throughout development to introduce biases that break symmetry and specify axes, and arrays of planar-polarized microtubules have been visualized in various PCP-patterned tissues[24,34,35]. As it turns out, these polarized microtubule arrays can bias the transport of core PCP particles consisting of Dsh, Fz and Fmi towards one side of the cell, aiding the PCP patterning process[36–39]. These Fmi–Fz–Dsh accumulations are preferentially trafficked towards the plus ends of microtubules, showing a bias in directionality similar to plus-end tip tracking proteins such as EB1[38] (Fig. 2). However, while this process has been visualized in cells of the wing blade and likely is working similarly in the abdomen of *D. melanogaster*, the distal bias of microtubule plus ends is diminished in the most distal regions of the wing, demonstrating that this is just one potential mechanism for globally aligning PCP asymmetry.

Interestingly, while polarized microtubules are essential for establishing some PCP asymmetries, they may be dispensable for maintaining these patterns once they have been stably generated[37,40], and this supports the notion that the key role of microtubules in PCP patterning is to introduce an initial directional bias. It has separately been shown that intact PCP patterns are necessary for the polarization of microtubule arrays[24,38,41], demonstrating

that intricate and mutual relationships exist between microtubule polarity and planar polarity patterning.

Post-translational modifications fine-tune PCP protein function

Protein localization and function is often regulated through post-translational modifications. Similar to the PLK1-1 mediated internalization of CELSR1 during murine epidermal divisions mentioned previously, other PCP component phosphorylations are known to occur, and this has been demonstrated to impact PCP signalling in several contexts. VANGL and DVL proteins have multiple phosphorylation sites, modification of which can regulate protein localization and behaviour[5,42]. For example, mouse DVL2 is phosphorylated in response to WNT5a stimulation and can associate with the ubiquitin ligase SMURF2 and apical polarity protein PAR6 as a result of this phosphorylation, and this complex regulates PK1 protein stability by ubiquitylating PK1 and thereby promoting its proteasomal degradation[43]. WNT5a can also promote VANGL2 phosphorylation through tyrosine-protein kinase receptor ROR2, which is essential for governing planar-polarized cellular behaviours in the mouse limb bud[44] (see also below). In addition, serine/threonine kinase misshapen-like kinase 1 (MINK1) phosphorylates a conserved residue of PK1, which promotes membrane localization with VANGL2 and apical enrichment of PK1–VANGL2 complexes[45]. The localization of FZD3 may also be regulated through its phosphorylation, which is disrupted upon loss of DVL1 in murine axon growth cones[46]. On the other hand, VANGL2 promotes the dephosphorylation and internalization of FZD3 in this same context[46].

Transmembrane proteins are often sorted into lysosomes following ubiquitylation, and because both Fmi and Fz accumulate intracellularly upon the prevention of lysosomal maturation, these proteins are likely subject to lysosomal turnover[8] (Fig. 2). Along these lines, a deubiquitylating enzyme, fat facets (faf), was identified in a *D. melanogaster* genetic screen for enhancers of a hypomorphic *fz* allele[47]. It was shown that the presence of faf is important for proper junctional localization of Fmi, but faf knockdown does not affect total protein levels, suggesting that the faf-mediated deubiquitylation of Fmi serves to regulate Fmi recycling back to membranes[48]. A related mechanism influences Dsh localization, with a Cullin-3–Diablo–Kelch ubiquitin ligase complex mediating the removal of Dsh from junctions without impacting overall Dsh levels[48].

Following ubiquitylation, PCP components can also be subject to proteasomal degradation (Fig. 2). For example, ubiquitylation and degradation of PK1 mediated by the before-mentioned DVL2, PAR6, and SMURF complex is essential for normal PCP signalling during mouse development[43]. In *D. melanogaster*, Vang appears to additionally play role in the ubiquitin-mediated proteosomal degradation of Pk[49]. Here, as well as in *X. laevis*, Pk overexpression results in an increase in junctional Vang, yet Vang overexpression counter intuitively results in diminished levels of junctional Pk[13,21,49]. Similarly, loss of Vang function can lead to higher overall Pk protein levels, which is likely the result of decreased Pk degradation[49]. This Vang-dependent degradation of farnesylated (that is, membrane associated) Pk is likely mediated through the ubiquitin ligase Cullin-1[28].

Global Alignment of PCP Patterning

While interactions between core PCP proteins and the regulated dynamics of their localization can coordinate PCP asymmetries between neighbouring cells and can propagate them throughout a tissue, global inputs into these patterns must govern their overall directionality with respect to the axis of an organ. Recent work has helped identify multiple, potentially overlapping influences on the global orientation of PCP, which apparently include expression gradients of *fj* and *ds* – the components of the "global" Ft–Ds–Fj PCP pathway, Wnt ligand gradients, and anisotropic tissue strain (Fig. 3).

Ft-Ds-Fj signalling

Ft and Ds are atypical protocadherins that hetereotypically interact across cellular junctions, and Fj is a transmembrane kinase that modulates Ft and Ds interactions[50]. These proteins have been shown to influence planar polarity (Box 2), and while it has remained contentious whether Ft-Ds-Fj and core PCP signalling are acting in parallel or in consecution[51,52], an intersect between these two pathways is becoming increasingly clear (at least in the fly). Gene expression gradients are a common mechanism of tissue patterning during development, and opposing gradients of *ds* and *fj* expression were observed along the axis of planar polarity in *D. melanogaster,* making these gradients attractive candidates for a global PCP-orienting cue[53,54]. In addition, these expression gradients result in the asymmetric localization of Ft and Ds to opposite sides of the cell, oriented similarly to core PCP asymmetry (Box 2). Indeed, while the core PCP pathway appears to retain function in mutant clones of Ft-Ds-Fj signalling components, the locally coordinated cells are not properly aligned along the global tissue axis[53,54]. The differential alignment of core PCP patterns in respect to the *ds-fj* gradient in different tissues challenged this notion. However, this could be explained by the fact that Pk exists in two isoforms, Prickle (Pk) and Spiny Legs (Sple), that only differ in the most N-terminal region of protein[55] and differentially interpret the Ft-Ds-Fj cues[39,56]; cells predominantly expressing Pk, such as those in the fly wing, asymmetrically localize Fmi–Vang–Pk on the same side of the cell as Ft, but in cells predominantly expressing Sple, Fmi-Vang-Sple localizes in the opposite orientation due to Sple interacting with Ds and Dachs[56,57] (Fig. 3a). Thus, as Fmi–Vang–Sple complexes localize with Ds and Dachs, Fmi–Fz–Dsh complexes then localize on the same side of the cell as Ft.

Box 2

Role of Fat, Dachsous, and Four-Jointed in planar polarity signalling

In addition to having roles in controlling tissue growth through the Hippo pathway, Fat (Ft)–Dachsous (Ds)–Four-jointed (Fj) signalling components participate in the planar polarization of the ommatidia in the eye, trichomes in the wing, and abdominal bristles of *Drosophila melanogaster*, similarly to the core planar cell polarity (PCP) pathway[4,136]. A vertebrate ortholog of Fj (Fjx1) and multiple homologs of Fat (Fat1-4) and Ds (Dchs1 and Dchs2) exist in vertebrates[137], and Fat4 and Dchs1, which have the highest homology to *D. melanogaster* Ft and Ds, are clearly important for PCP-mediated processes in mice, such as directionality of cell elongation and oriented cell

division[138–141].However, the molecular functions of Ft–Ds–Fj signalling components in vertebrates and how well they are conserved with their *D. melanogaster* counterparts are currently poorly understood.

In *D. melanogaster*, planar asymmetries in Ft and Ds localization can arise, likely owing to the opposing expression gradients of ds and fj[51,54,142]. Despite the fact that expression of ft appears uniform, the kinase activity of Fj (that forms a gradient), which may phosphorylate cadherin repeats of both Ft and Ds, can seemingly promote Ft binding to Ds[143–146] and inhibit Ds binding to Ft[146]. Controlling Ds binding to Ft and Ft binding to Ds is thought to lead to a graded distribution of binding affinity between these two proteins along the axis of differential ds and fj expression, whereby a cell with more Fj activity than its neighbor is going to have “stronger” Ft and “weaker” Ds in the sense of binding capacity, promoting Ft in the cell with higher Fj to bind with Ds in the cell with lower Fj (and thus “stronger” Ds). In the *D. melanogaster* wing, for example, the result is a higher proximal level of ds expression stabilizing Ds protein on the distal side of the cell, down the ds gradient[144,147] (Fig. 3a and 3b). Similar to the fashion in which core PCP proteins localize then, components of the Ft–Ds–Fj signalling pathway become asymmetrically distributed in cells of many planar-polarized tissues[105,147,148]. In more complex scenarios, such as in the *D. melanogaster* epidermis at later larval stages where large single cells can have multiple polarized structures and exchange neighbours, two sides of the same cell can be differentially polarized according the localization of Ft–Ds–Fj signalling components in the neighbours at any given time[149]. These findings suggest that although an uniform expression gradient has the potential to form a uniformly asymmetric distribution of Ft–Ds, it is the local, cell-to-cell sensing of asymmetries that in the end governs a distinct regional polarity. Whether expression gradients, localization asymmetries, or similar molecular functions for these proteins exist in vertebrates has yet to be determined.

One of the functional consequences of the differential interpretation of Ft–Ds–Fj through Pk and Sple is the orientation of microtubule polarity along Ft–Ds activity gradients. Microtubule plus ends are often oriented towards Ds in cells predominately expressing Pk and towards Ft in cells predominantly expressing Sple[38]. Using Ft–Ds–Fj polarity cues to orient microtubules results in a bias in the directional transport of core PCP signalling components[36,38], thereby providing a means of coupling these two signalling pathways[39,58]. However, detailed molecular mechanism through which these microtubules are oriented in response to the Ft–Ds gradient and whether this mode of orienting PCP is conserved in tissues other than the fly wing, as well as in other species, remains unclear.

Wnt ligand gradients

Along with gradients in gene expression, gradients of secreted ligands are also commonly employed in tissue patterning. There are a number of different Wnt proteins which can act as secreted ligands for Frizzled receptors, and the tendency for Wnts to be found in graded distributions along an axis of planar polarity makes them attractive candidates for global PCP orientation cues[59]. Indeed, a potentially instructive role for Wnt gradients in PCP pattern orientation is becoming increasingly apparent, with recent breakthroughs having

highlighted that various Wnt ligands can influence PCP patterning in different capacities and that some may act redundantly[60,61]. For example, both Wg and Wnt4 may be acting in the orientation of PCP complexes in the fly wing, where during early wing morphogenesis, the distal Fmi–Fz–Dsh complexes are oriented towards the expression gradients of these ligands emanating from the wing margin and the proximal Fmi-Vang-Pk complexes are oriented more towards the central regions of the wing blade[60] (Fig. 3b, top). Additionally, in the *X. laevis* ectoderm, Wnt5a, Wnt11, and Wnt11b have the capacity to orient Pk and Vang accumulations away from the source of ligand expression (Fig. 3c), whereas a closely related ligand, Wnt3a, elicits no such behaviour[61].

With this recently ascribed role for global PCP orientation, the mechanisms through which Wnt gradients could promote global orientation of PCP complexes remain not fully understood. Wnt4 and Wg seemingly inhibit Fz–Vang interactions between cultured fly S2 cells and can attenuate *fz* overexpression defects *in vivo*, which may indicate a role for Wnts in locally competing with Vang for Fz binding[60]. In mice, Wnt5a can promote the association of PCP-specific component VANGL2 into a signalling complex with ROR2, promoting VANGL2 phosphorylation, which has been shown to be required for planar-polarized rearrangements of osteoblasts that drive elongation of the limbs[44].

Mechanical Forces

When tissues change shape during growth and morphogenesis, or when fluid flows across a tissue surface, the anisotropic strains that are introduced can influence the development of PCP asymmetry and orientation of PCP patterns. Early reports of this phenomenon demonstrated the direct impact of fluid flow on the polarization of ciliary beating patterns in multicilated epithelia[62–64]. In addition, forces exerted throughout the embryonic epidermis of *X.laevis* during gastrulation, which is thought to initially establish the PCP axis[63], were found to measurably increase the polarized stability of core PCP complexes at cellular junctions, and these effects were shown to depend on microtubules, which can rearrange in response to high tissue strain[41] (Fig. 3d). This increase in the stability of Vangl2 at anteroposterior junctions can be measured even prior to the establishment of a clear PCP asymmetry[41], and it is interesting that the subsequent emergence of clear PCP asymmetry coincides with both rapid embryonic elongation and increasing strength of fluid flow[21] – events associated with directional mechanical strain.

Tissues that already exhibit PCP patterning can also shift their axis of localization orientation in response to morphogenetic forces. For example, in early pupal *D. melanogaster* wings, complexes of PCP proteins are oriented initially towards the wing margin (towards the source of Wg/Wnt4) as discussed previously; however, as hinge contraction drives rapid wing elongation, promoting large-scale cell shape changes and rearrangements, PCP complex orientation also seems to shift into alignment with proximal-distal axis of the developing wing[65] (Fig. 3a, bottom). This shift in patterning also coincides with an increase in the amount of protein that appears to be asymmetrically enrichment into planar-polarized accumulations seen at later pupal stages, indicative of larger and more stable complexes[11] (Fig. 3b bottom, longer vectors compared to Fig. 3b top). Similar behaviours are observed in the developing mouse epidermis, where again, PCP is aligned

according to tissue strains that are coincident with the remodelling of junctions and tissue shape changes[25]. Actin regulators such as WDR1 and CFL1 are important for maintaining cortical tension during these rearrangements, which when disrupted, result in the inability to establish robust PCP patterning[66] and in the impediment of coordinated cell movements[67] (see also Supplementary information S2 (Box)).

Directionally biased strain can clearly promote polarized patterning events, but molecular mechanisms of the force-influenced changes in PCP orientation and magnitude remain poorly characterized. Rab11-mediated trafficking has been shown to be involved in the delivery and recycling of PCP components to apicolateral junctions and can utilize some of the same actin regulators necessary to maintain cortical tension[11,66]. Interestingly, Rab11 and Ankrd6 appear to be planar-polarized together towards the central fold of the neural plate during neural tube closure and wound margins during wound healing, thus presumably having asymmetries that are oriented along the axis of tissue strain in these contexts[68]. Perturbing PCP signalling disrupts the localization of Rab11 towards the neural fold, whereas dominant-negative Rab11 disrupts actomyosin-driven cell shape changes and rearrangements, which are required for proper wound healing[68].

From molecular to functional asymmetry

The first description of PCP probably comes from Robert Hooke himself who discussed in his *Micrographia*[69] the “conical bristles, all whose ends pointed backwards” present on the body of insects. The robust readout of these bristles provided the foundation for genetic screens identifying PCP proteins that direct assembly of these structures through control of the actin cytoskeleton[2]. These structures also facilitated the use of mutant clones to explore signalling mechanisms, for example in the discovery of directional non-autonomy, which refers to directional non-autonomous effects of specific PCP mutations[70,71]. PCP proteins also control both polarized organization and asymmetric cell fate choices in *D. melanogaster* photoreceptors[72].

Following the work in flies, evolutionary conservation of PCP signalling in vertebrates was first revealed in the context of coordinated cell movements during gastrulation in *X. laevis* and zebrafish[73–75]. So-called convergent extension cell movements are most conspicuous for driving elongation of the embryonic body axis[76], but importantly, they also drive tissue elongation during organogenesis, for example in the kidney tubules[77]. While the asymmetric localization of PCP components is well-established in *D. melanogaster* wing, the control of core PCP localization during convergent extension remains poorly defined. However, predominant features of the system appear to be conserved, as PK and VANGL were described to localize anteriorly in cells undergoing convergent extension, while DVL was shown to localize posteriorly[78–82].

Another setting in which vertebrate PCP is essential is the oriented positioning of cilia within cells. First reported in the mouse inner ear, planar polarization of hair cells is organized by the asymmetric kinocilium positioning, which is preceded by asymmetric PCP patterning[83]. More recently, PCP signalling was also revealed to control planar polarization of motile ciliary beating in diverse ciliated cell types, and has been implicated in a wide

array of developmental events, including the orientation of cell division, the migration of facial motorneurons, and the planar orientation of hair follicles in the mammalian epidermis[84,85].

The mechanisms linking PCP to cell behaviour are most well defined in the context of wing hair positioning in *D. melanogaster*, and several reviews have been dedicated to this issue; we refer readers to that work[1–4]. Here, we will focus instead on two PCP-dependent processes in vertebrates, better understanding of which could have major clinical implications. We note here that our understanding of these mechanisms is only now beginning to emerge, so the following passages focus as much on what is not known as what is known.

Directional beating in multiciliated cells

Multiciliated cells (MCCs) bear dozens of directionally beating cilia that act in concert to drive fluid flow across the tissue (Fig. 4A). Such cells are critical for development and homeostasis of the airway, the central nervous system (CNS), and reproductive tracts[84] (Fig. 4B). Directional beating of MCCs has been studied on the amphibian epidermis for nearly 200 years[86], and not surprisingly, the first description of a role for PCP genes in these cells was in this context[63,87]. PCP signalling is now known to control polarized beating in all MCCs examined to date, including in the mammalian brain[64,88–90], airway[24], and oviduct[40,91]. Core PCP localization examined in these tissues reflects that seen in the fly wing; PK and VANGL occupy a domain complementary to that of DVL and FZ (Fig. 4A, C); however several additional cilia-associated proteins not found in *D. melanogaster* are required for normal core PCP protein localization in MCCs, including the myosin motor Myo1d[92], the novel protein Spag6 (Sperm-associated antigen 6), which localizes to the ciliary axoneme and is essential for rotational polarity[93], and c21orf59/Kurly, a novel cytoplasmic protein that physically interacts with the core PCP protein Dishevelled[94].

MCCs display multiple levels of planar polarization (Fig. 4D), a “rotational polarity” (organization of ciliary basal bodies within each cell) and distinct “tissue-level polarity (coordination of polarity between all cells across a tissue)[84,85]. Cell-autonomous defects in rotational polarity result from disruption of DVL or PK[21,87,89], but mild rotational with more striking non-autonomous, tissue-level polarity defects result from disruption of the transmembrane proteins VANGL, FZ, or CELSR[21,63,64,90]. Interestingly, in mouse ependymal cells, CELSR1 appears to coordinate tissue-level polarity by influencing microtubule patterning, whereas CELSR2 and CELSR3 appear instead to be required for the rotational polarity and organization of individual basal body patches through the control of actin[90].

Exactly how polarity information is communicated between PCP proteins and ciliary basal bodies is not yet known, but the cytoskeleton clearly plays a key role (see also Supplementary information S2 (Box)). Actin and microtubules comprise the cortical cytoskeleton and at the same time interact with accessory structures of the basal body, and both cytoskeletal elements are implicated in polarizing beating[84,85,90] (Fig. 4C). Indeed, planar-polarized apical microtubules are a conserved feature of MCCs in the mouse airway[24], and this is reminiscent of the microtubule arrangement observed in the *D.*

melanogaster wing cells discussed above[34–36]. It is important, then, that PCP proteins localize both to the cell cortex, where they are known to control cell polarity, and to the basal body, where they could act to control basal body polarity[21,24,64,87,89]. There is a growing roster of additional proteins asymmetrically localized to basal bodies, including ODF2, non-canonical tubulins, and Centrin2, that may additionally be involved[95–97], and among these, ODF2 is perhaps the most interesting, as mice lacking ODF2 display overt defects in the apical microtubule lattice[95]. Thus, these proteins provide excellent entry points for future studies exploring the interplay between PCP proteins, polarization of the cytoskeleton, and polarization of ciliary beating.

Convergent extension

Convergent extension was the first context in which vertebrate PCP signalling was studied[73–75], so it is surprising that it remains among the most poorly understood processes with respect to the instructive role of PCP. Convergent extension is driven by the mediolateral intercalation of cells, resulting in the subsequent narrowing and consequent elongation of the tissue in the anteroposterior axis (Fig. 5a, b). Two mechanisms have been evoked to explain force generation for cell movement during vertebrate mesenchymal convergent extension, though the two are not mutually exclusive. In one model, cellular protrusions at the mediolateral cell faces exert traction force on cell neighbors, driving intercalation by cell crawling[98]. In the second model, junctions between apposed anterior and posterior cell faces are actively contracted, drawing mediolaterally-attached neighbours toward one another[99] (Fig. 5a). Interestingly, PCP proteins can be implicated in both paradigms, as their manipulation disrupts both the stability and the polarity of mediolateral protrusions and the shrinkage of cell junctions to be biased towards those anteroposterior cell faces[75,99,100]. PCP-dependent stable cell protrusions and polarized junctional shrinking are also characteristics of cells undergoing convergent extension in vertebrate neural plate epithelia (Fig. 5b)[101,102], with slight differences arising due to differing tissue architecture and context.

Our understanding of the molecular biology of PCP during vertebrate convergent extension emerged from prior work in *D. melanogaster*, where PCP genes were shown to directly control actomyosin machinery via Rho GTPases and Rho Kinase[103,104]. Indeed, PCP-mediated convergent extension requires RhoA and Rho Kinase, as well as a host of other actin regulators such as the formin Daam1 and Rho GTPase Cdc42[84]. This conserved role for PCP in regulating actomyosin contraction makes it possible to assemble a preliminary model of how planar-polarized PCP protein localization directs collective cell movements (see also Supplementary information S2 (Box)).

Most evidence suggests that the PCP proteins form complementary asymmetric zones of localization on the anterior and posterior cell faces during convergent extension[78–82] (Fig. 5c). There is precedent for PCP asymmetries in the mechanical control of cell intercalation, as the atypical myosin Dachs, asymmetrically localized by Ds, has been shown to drive anisotropic junctional tension in the developing *D. melanogaster* thorax, promoting planar-polarized cell rearrangements[105]. With this in mind, it seems feasible that PCP proteins could directly recruit contractile actomyosin regulators and thereby drive junction shrinkage.

Indeed, live imaging has demonstrated pulses of actin, phosphorylation of myosin II, and enrichment of myosin regulators at sites of shrinking junctions in *X. laevis* gastrula mesenchyme and chick neural epithelial cells[99,101] (Fig. 5c).

Additional recent insights have come from studies of PTK7, which is a vertebrate-specific regulator of core PCP function[106]. PTK7 is required for convergent extension-associated cell behaviours during gastrulation and neurulation[100,106,107], but more recent work in the inner ear has revealed a mechanism linking PCP and PTK7 to Myosin II activation through Src kinase phosphorylation of myosin regulatory light chains[108,109]. While this preliminary model provides a parsimonious explanation of asymmetric PCP protein function during junction shrinking, it does not explain the effect of PCP proteins on formation of polarized membrane protrusions during convergent extension, which are also PTK7-dependent[102]. Among the possible mediators of this effect are septins, which have conserved functions in compartmentalizing cortical actomyosin during cytokinesis and are required for proper PCP function during convergent extension[99,110].

While significant strides have been made in our understanding of PCP in more static epithelial tissues (in particular in the fly), the mechanism of PCP signalling during convergent extension is only now coming into focus. This slow progress likely reflects the dynamic nature of PCP processes, which are more easily visualized in stable tissues. Unlike other epithelia, cells undergoing convergent extension constantly exchange their neighbors. Dynamic analyses of epithelial PCP protein localization with time-lapse imaging and probing localization stability utilizing photobleaching and photoconversion techniques are now providing deeper mechanistic insights into PCP signalling[11,21,40,41]; studies of PCP involvement in convergent extension should also benefit from the application of similar techniques.

Finally, mutations of *Vangl2* and *Celsr2* were found to elicit neural tube closure defects in mice[111,112]. Furthermore, time-lapse imaging in *X. laevis* revealed that PCP-related neural tube closure defects arise from a failure of convergent extension[75,113], and similar mechanisms were also documented in mice[114,115]. Therefore, better understanding of how PCP proteins direct cell behaviours during convergent extension can shed more light on the role of PCP gene mutations in human birth defects (Box 1).

Conclusions

PCP signalling is a ubiquitous mechanism of tissue patterning, with particular importance for animal development. Although significant strides have been made in understanding the establishment of PCP patterns and how they influence collective cell behaviours, much work remains to fully understand the instructive roles of PCP in morphogenesis and tissue organization. Importantly, many PCP effectors appear to be context-specific, especially in vertebrates, and the potential outcomes of establishing a directional PCP signal become increasingly complex in multifaceted tissues comprising multiple layers and cell types. Indeed, given the broad roles ascribed to PCP signalling in development in model animals, it is perhaps surprising that so few human disorders have yet been linked directly to mutation in PCP genes (Box 1). For example, despite their role in airway MCCs, no PCP genes have

yet been associated with diseases stemming from defective motile cilia (such as ciliary dyskinesias). Similarly, PCP dysfunction has been associated with cystic kidney disease in animal models, but there has been little evidence for PCP genes being involved in this condition in humans. One potential explanation is that more severe birth defects mask the subtler phenotypes. For example, some patients with neural tube closure defects associated with PCP gene mutations present with hydrocephalus[116–118], which could be a secondary consequence of neural tube defects or, alternatively, might be the manifestation of defects in MCC planar polarity in the brain[88]. Moreover, re-examination of patients with spina bifida with underlying PCP gene mutations recently revealed a high prevalence of congenital kidney defects that reflects those seen in PCP mutant mice[119]. These data highlight the complex nature of human birth defects and the potential role of PCP genes in their etiology. We consider this to be an essential area for future work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Endocytic Flux
The constant endocytosis and subsequent recycling of unstable protein components associated with the plasma membrane.

Clathrin
Vesicular coating component that plays an important structural role in mediating endocytosis of membrane-associated proteins.

Rab GTPase
Large family of small GTPases central to membrane trafficking regulation.

Dynamin
GTPase protein critical for driving membrane fission that helps facilitate endocytic events.

Polo-like kinase-1 (PLK1)
Serine/Threonine kinase that serves as an important cell cycle regulator, playing a notable role during the G2/M transition.

Signalling Protocadherins
Largest subgroup of cadherins with variable extracelluar domains and diverse cytoplasmic domains that distinguish their variety of potential functions from those of classical cadherins.

Mutant Clones

Groups of cells in a tissue that have perturbed gene expression and are surrounded by or interspersed between otherwise wild-type cells.

Neural plate
Sheet of neuroepithelial cells that undergo convergent extension, apical constriction, and regional folding to form the vertebrate neural tube.

Central fold
Midline of the neural plate that divides the left and right half, comprised of cells that apically constrict to facilitate neural plate folding and neural tube closure.

Kinocilium
Microtubule-based protrusion on the apical surface of hair cells in tissues of the inner ear that guides the polarized orientation of an actin-based stereociliary bundle.

Ependymal cells
Multiciliated cells the line the interface between the central nervous system and cerebrospinal fluid, which propel cerebrospinal flow through the brain ventricles and central spinal canal.

Basal body
Modified centriole and accessory proteins that serves as a specialized microtubule organizing center at the base of the cilium, which they nucleate.

Non-canonical tubulins
Tubulin superfamily members that are not conserved in all eukaryotes as alpha-, beta-, and gamma-tubulin are and that often function as centriole accessory structures.

Traction force
The forces exerted across the junction between two cells that help facilitate cell rearrangements and tissue morphogenesis.

Septins
Cytoskeletal components that upon binding to GTP can polymerize into ordered structures such as rings and filaments, which can function as scaffolds or diffusion barriers, for example.

Spina bifida
Neural tube defect associated with a regional, incomplete closure of the spinal chord and associated tissues.

Ommatidia
Optical units of insect compound eyes containing groups of polarized photoreceptive cells

Trichomes
Actin-based hairs found on each cell of the fly wing blade that emanate from the apical, distal side of the cells and point distally as well – properties which are controlled by PCP signalling.

Notum

The dorsal region of the thorax of a mature insect.

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Biographies

Mitchell Butler is a graduate student under the advisement of John Wallingford at UT Austin. His doctoral research reflects his profound interest in cell polarity and tissue morphogenesis during development and is primarily focused on studying various planar-polarized protein dynamics and cellular behaviours in the *Xenopus* embryo.

John Wallingford is a Professor of Molecular Biosciences at The University of Texas at Austin. He received his Ph.D. from UT Austin in 1998 and performed postdoctoral work at UC Berkeley with Richard Harland and at Caltech with Scott Fraser. His lab seeks to understand the mechanisms linking systems-level programs of gene expression and protein function to discrete cell biological processes in developing embryos with the ultimate aim of understanding the etiology of human developmental disorders. When not in the lab, he enjoys a fine gin, climbing large rocks, and shooting birds.

Key Points

- Planar Cell Polarity (PCP) is a polarity axis that organizes cells throughout the plane of the tissue. It is conserved in metazoans and is essential for proper development and tissue homeostasis.
- Asymmetric and mutually exclusive subcellular enrichments of key PCP proteins pattern cells in planar-polarized tissues. They also coordinate planar polarity between cells and control polarized behaviours through modulation of the cytoskeleton.
- PCP patterns develop gradually from an initially disordered state through dynamic trafficking and various feedback interactions that can influence protein localization and stability.
- PCP patterns seem to be globally oriented along a pre-defined axis in a given tissue . Notably, multiple mechanistic inputs may have differential influences on PCP patterning depending on developmental timing and tissue context, and may only partially overlap in different contexts.
- The morphogenetic events governed by PCP signalling are best understood in *Drosophila melanogaster*, where in particular the orientation of hairs and bristles on the fly body has served to unravel basic principles of PCP-dependent processes. Work in this model has helped to better understand equivalent mechanisms in vertebrates, particularly in the context of the orientation of fluid flow mediated by multiciliated cells and cell rearrangements during convergent extension.
- Mutations in PCP genes have been implicated in diverse human pathologies, and the body of evidence supporting involvement of PCP defects in human birth defects continues to grow at an increasing rate.

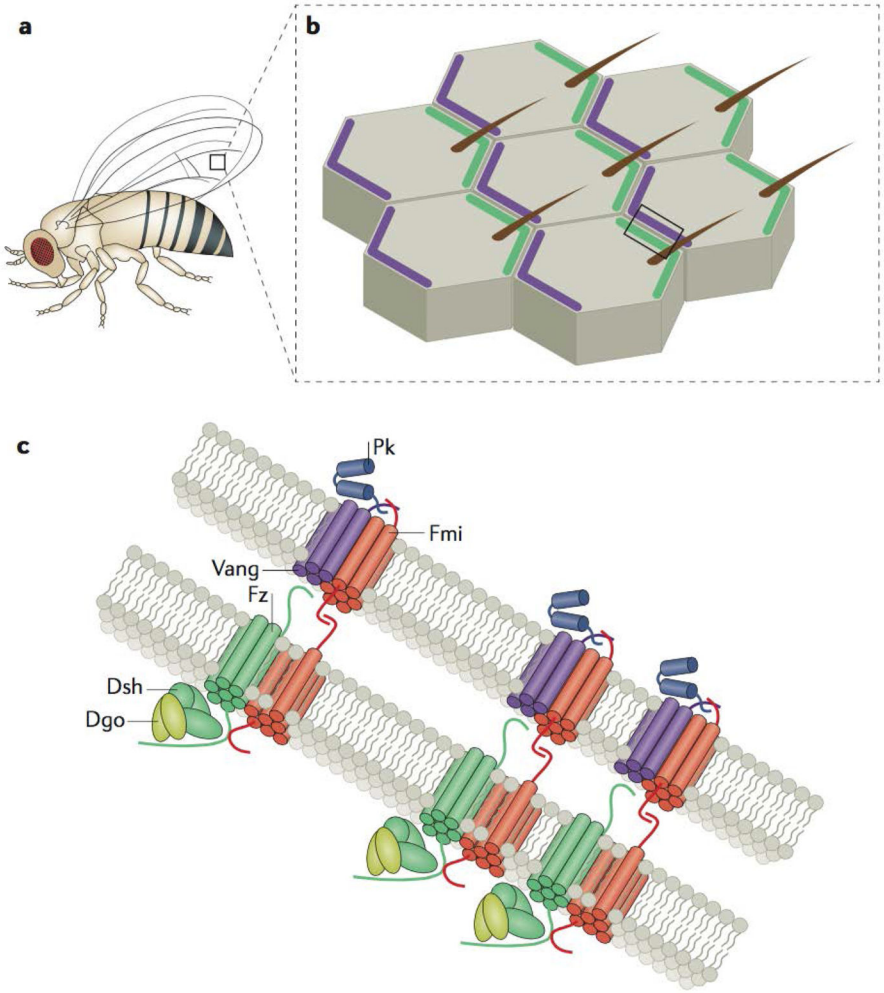

Figure 1. Asymmetric signalling complexes pattern planar polarity

a | *Drosophila melanogaster* has many planar-polarized external features, including actin-based, distally or posteriorly oriented hairs and bristles that cover the legs, wings, and notum, which serve as a robust readout of planar cell polarity signalling. The box drawn on the wing blade demarcates the region illustrated in (**b**). **b** | Cells of the *D. melanogaster* wing blade are asymmetrically patterned by proximal accumulations of Van Gogh (Vang) and Prickle (Pk) (both shown in purple) that are complementary to distal accumulations of Frizzled (Fz), Dishevelled (Dsh), and Diego (Dgo) (shown in in green). These patterns govern the distal positioning and orientation of a single actin-based trichome in each cell of the wing epithelium. The box drawn on the tissue demarcates the region illustrated in (**c**). **c** | Asymmetric PCP signalling components form junctional signalling complexes that are physically linked from cell to cell. Both Fz and Vang are transmembrane proteins that associate with physically linked Flamingo (Fmi) homodimers established between the opposing membranes at cell–cell junctions. Distal accumulations of Fmi and Fz are asymmetrically clustered through the activity of Dsh and Dgo, while proximal Fmi and Vang complexes are enriched by the activity of Pk. Note that the proteins as they are portrayed are

not necessarily to scale and merely approximations as there is no high-resolution protein structural data available to support them.

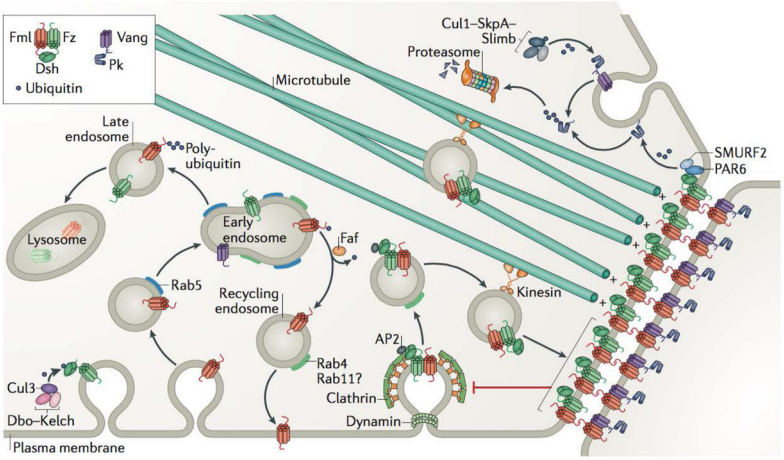

Figure 2. Graphical summary of endocytosis, trafficking, and degradation events that facilitate the dynamic patterning of planar cell polarity

Planar cell polarity (PCP) signalling components are resistant to endocytic flux when stably linked across junctions (red inhibitory arrow)[8,11]. The internalization of PCP components is partially dependent on dynamin and Clathrin-mediated endocytosis[27], and the interaction of membrane-associated Dishevelled (Dsh)[150] with Clathrin-associated adaptor protein AP-2 is essential for PCP signalling[29]. PCP protein internalization and trafficking are regulated through ubiquitylation. For example, the activity of a Cullin-3–Diablo–Kelch (Cul3–Dbo–Kelch) ubiquitin ligase complex promotes ubiquitylation and internalization – but not degradation – of Dsh, while fat facets (faf) deubiquitinase prevents the accumulation of Flamingo (Fmi) in Rab5-positive endosomes and promotes Fmi recycling to the membrane[48] – a process that appears to be mediated by Rab4 and potentially Rab11 for PCP proteins[8]. Although faf loss of function does not lead to a reduction in the overall levels of Fmi, inhibition of lysosomal maturation leads to the overaccumulation of Fmi as well as Frizzled (Fz) in endosomes, indicating that cellular levels of these components are, at least in part, regulated through lysosomal degradation[8]. Kinesin-dependent trafficking of Fmi, Fz and Dsh towards microtubule plus ends appears to direct these complexes to sites of junctional enrichment[36]. Prickle (Pk) appears to have a dual role in both clustering and stable Fmi–Van Gogh (Vang) complexes at junctions as well as in mediating the internalization of unstable Vang[28]. Proteasomal degradation also plays a role in regulating PCP components. For example, Vang promotes the proteasomal degradation of farnesylated (membrane associated) Pk[49], likely through poly-ubiquitylation mediated by a Cullin-1 (Cul1)–SkpA–Supernumerary limbs (Slimb) E3 ubiquitin ligase complex[28]. Similarly, vertebrate DVL2 (when phosphorylated in response to WNT5a stimulation) can associate with PAR6 and ubiquitin ligase SMURF2 resulting in poly-ubiquitylation of PK1, which leads to proteasomal degradation of PK1, thereby controlling PK1 localization and protein levels[43].

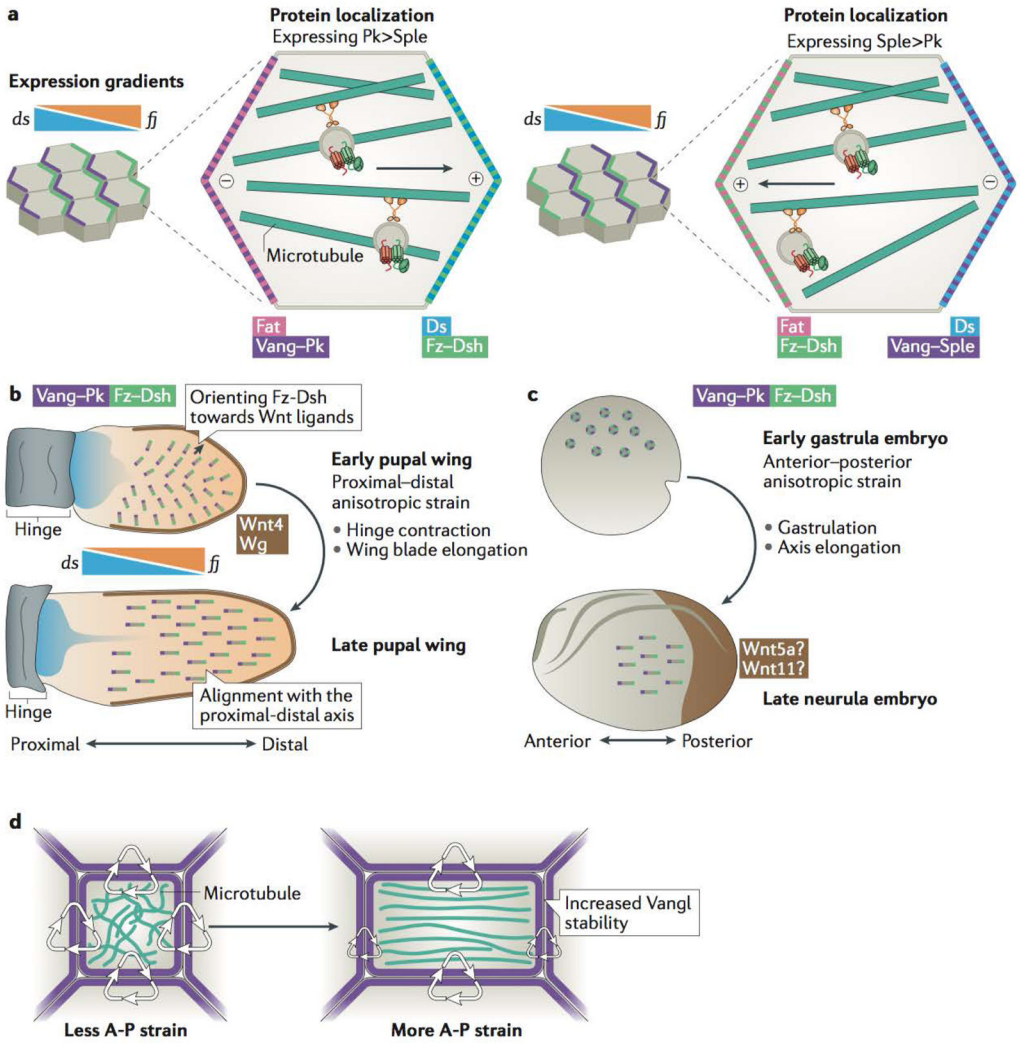

Figure 3. Multiple global inputs can collectively influence planar cell polarity orientation and stability across developing tissues

a | In *Drosophila melanogaster* core planar cell polarity (PCP) components organize according to Fat (Ft)–Dachsous (Ds) axis, established as a result of expression gradient of *dachsous* (*ds*) and the gradient of expression of kinase *four-jointed* (*fj*), which regulates the interactions between Ft and Ds and thereby contributes to the asymmetric distribution of these two proteins in the cell. Localization of PCP components with respect to Ft–Ds axis depends on the levels of expression of two Prickle isoforms: Prickle (Pk) and Spiny Legs (Sple)[38]. In cells expressing predominantly Pk, Vang–Pk localizes on the same side of the cell as Ft, but in cells expressing mostly Sple, Vang–Sple localizes together with Ds. This then regulates the directionality of microtubule-based transport in cells, as microtubule plus ends orient towards the Fz–Dsh-containing membranes. b | Top: The *D. melanogaster* wing blade exhibits a narrow source of Wnt ligands along the distal margin (brown) that may serve to orient PCP complex vectors (grey lines with Frizzled (Fz) and Dishevelled (Dsh) (green) localizing towards the wing margin opposite Vang Gogh (Vang) and Prickle (Pk) (purple) early in development (around 5-15 hours after puparium formation (hAPF))[60].

Broad and shallow expression gradients of *ds* and *fj* oppose one another along the proximal–distal axis of the developing wing and may influence the directionality of early PCP pattern[39,56,57] so that PCP is oriented in a radial fashion towards the edge of the wing. Bottom: Later in development (16–32 hAPF), during hinge contraction and rapid wing blade elongation, cell shape changes and cell rearrangement reorient microtubule polarity, leading to the coordinated alignment of asymmetric PCP patterns along the proximal–distal axis of the tissue (with Fz–Dsh oriented towards the distal edge of the tissue)[65]. Note the increased length of PCP vectors at the later stage that signifies an increase in the amount and stability of core PCP complexes at junctions c | Top: Prior to gastrulation in *Xenopus laevis*, no detectable PCP vector is observed (rings). Bottom: PCP axes (line vectors) are established in the embryonic ectoderm during gastrulation[62], as forces along the axis of elongation increase the cortical stability of Vang-like protein 2 (Vangl2)[41] (purple)(see also panel d). A posterior source of Wnt ligands (such as Wnt5a and Wnt11;brown) is likely involved by directing the anterior localization of Vangl2[61]. d | Anisotropic tissue strain orients microtubules along the axis of strain. Concomitantly, Vangl2 (purple) becomes more stable at cell–cell junctions along the axis of tissue strain, in a manner that has been shown to depend on microtubules[41].

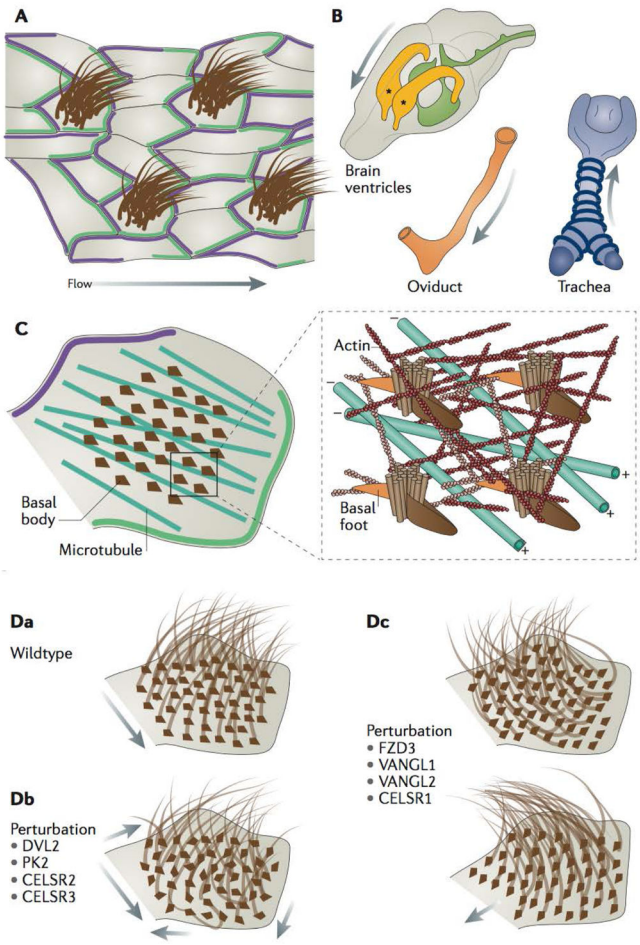

Figure 4. Planar cell polarity patterns direct the cytoskeletal organization and ciliary polarity of multiciliated cells

a | Illustration of a multiciliated epithelium with asymmetric localization of planar cell polarity (PCP) complexes (green and purple), which direct polarized fluid flow across the surface of the tissue along the axis of PCP asymmetry (arrow). **b** | PCP patterning directs the fluid flow in various vertebrate tissues: the rostral flow of cerebrospinal fluid in the lateral ventricles[64] (asterisks) of the brain, rostral mucociliary flow in the trachea[24], and medial flow in the oviduct of mice[91]. **c** | PCP signalling is essential for the organization of the apical cytoskeletal elements responsible for ciliary orientation in multiciliated cells (MCCs)[87,90]. An apical actin network (red) spaces basal bodies, whereas a subapical actin network (pink) links basal feet of one basal body to the neighboring basal body. These linkages to actin control rotational polarity, that ensures the uniform orientation of basal bodies, and thereby cilia in individual MCCs, whereas polarized apical microtubules orient groups of basal bodies along the axis of planar polarity[24,90], with the microtubule plus-ends oriented towards Frizzled and Dishevelled (FZD–DVL) accumulations (green)[24]. **d** | Conserved rotational polarity observed in wild-type MCCs (a), can be disorganized by perturbation of DVL2, PK2, and CELSR2 and 3[21,87,89,90], leading to largely disorganized cilia arrangements and disorganized ciliary beating and fluid flow (arrows) (b). Tissue-level polarity, the conservation of the uniform orientation of cilia across multiple MCCs, is

disrupted upon perturbation of FZD3, Vang-like 1 (VANGL1) and VANGL2, and CELSR1[21,63,90]; in this case different cells in the tissue can feature largely different orientation of their cilia. These defects can have considerable implications for the functions of MCCs in regulating fluid flow over tissues surfaces.

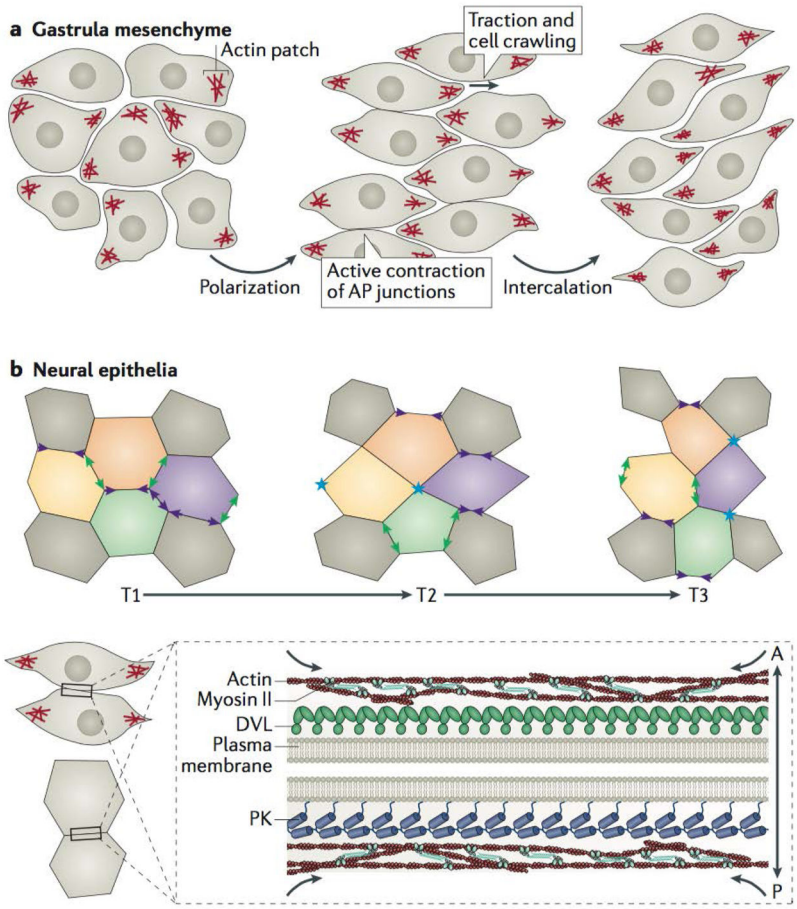

Figure 5. Planar cell polarity signalling directs polarized cell rearrangements during convergent extension

a | Mesenchymal cells during vertebrate gastrulation collectively elongate along the mediolateral axis, stabilize mediolateral actin-based protrusions, and mediolaterally intercalate to narrow and lengthen the tissue; all these processes require intact planar cell polarity (PCP) signalling[75,110]. **b** | Neural ectodermal cells mediolaterally intercalate by preferentially shrinking antero-posterior (AP) junctions (purple arrowheads), which first resolve (blue asterisks) and then elongate to form new medio-lateral (ML) junctions (green arrowheads). The stages of this intercalation behaviour are often referred to as “T” transitions, where T1 to T2 transitions involve the shrinking of the AP junction, whereas T2 to T3 transitions involve the lengthening of a new ML junction between two cells previously separated along the ML axis at T1. **c** | Core PCP components can be found asymmetrically enriched along AP junctions of cells undergoing convergent extension in vertebrates, with Prickle (PK) typically localizing to the anterior cell faces and Dishevelled (DVL) along the posterior cell faces[79–82]. In the context of convergent extension, localization of PCP components at AP junctions coincides with the localization of filamentous actin enrichments and phosphorylated non-muscle Myosin II, which comprises the contractile actomyosin machinery that shrinks AP junctions[81,99,101,102]; there is evidence that PCP components are involved in regulating this contractility[108,109].