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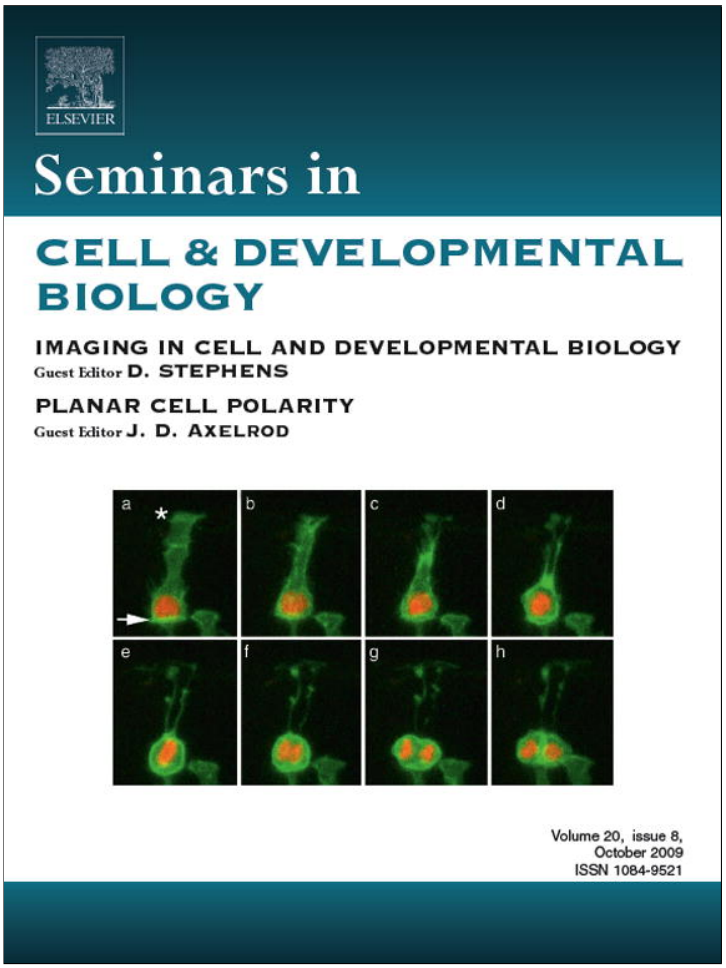

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Review

Progress and challenges in understanding planar cell polarity signaling

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ABSTRACT

During development, epithelial cells in some tissues acquire a polarity orthogonal to their apical–basal axis. This polarity, referred to as planar cell polarity (PCP), or tissue polarity, is essential for the normal physiological function of many epithelia. Early studies of PCP focused on insect epithelia (Lawrence, 1966 [1]), and the earliest genetic analyses were carried out in *Drosophila* (Held et al., 1986; Gubb and Garcia-Bellido, 1982 [2,3]). Indeed, most of our mechanistic understanding of PCP derives from the ongoing use of *Drosophila* as a model system. However, a range of medically important developmental defects and physiological processes are under the control of PCP mechanisms that appear to be at least partially conserved, driving considerable interest in studying PCP both in *Drosophila* and in vertebrate model systems. Here, I present a model of the PCP signaling mechanism based on studies in *Drosophila*. I highlight two areas in which our understanding is deficient, and which lead to current confusion in the literature. Future studies that shed light on these areas will substantially enhance our understanding of the fascinating yet challenging problem of understanding the mechanisms that generate PCP.

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Contents

1. Introduction—PCP in flies and vertebrates

PCP signaling controls the polarity of numerous epithelia in both *Drosophila* and vertebrates [1–4]. Furthermore, a number of non-epithelial morphological processes in vertebrates are controlled by vertebrate homologs of PCP genes, and appear to involve cell polarization, though the extent of mechanistic similarity is unclear [4]. In *Drosophila*, PCP has been studied primarily in four tissues, the wing, the abdomen, the eye, and the bristles of the notum, with some attention being paid to other tissues as well. In each case, two phenotypic features of PCP signaling are important. First, cells are observed to align with each other, thus organizing their polarities in a cooperative, domino-like fashion. This cooperative aspect of PCP signaling distinguishes it from polarization events occurring in isolated cells within chemotactic or other gradients. Second, this polarization is seen to occur in a specified orientation with respect to the tissue axes, indicating the existence of global signals that orient PCP in polarizing tissues.

In vertebrates, many features of the PCP signaling system identified in flies appear to be conserved, while additional features are implicated that are not present in flies [4–9]. In vertebrates, defects in PCP result in a range of developmental anomalies and diseases

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(reviewed in [5,8,10,11]). Perhaps best characterized among these, PCP is required for the correct orientation of sensory hair cells in the organ of Corti and in the vestibular epithelia, and defects result in deafness [12–22]. Other PCP related developmental defects in humans and in model organisms include open neural tube defects [20,23–28], polycystic kidneys [29–32], and conotruncal heart defects [33–35]. PCP is also believed to underlie the pathogenesis of idiopathic pulmonary hypertension [36,37] and the directed migration that occurs during invasion and metastasis of malignant cells [38–43]. Despite considerable progress in recent years, the molecular mechanisms of the PCP signaling modules, and the interactions between them are as yet insufficiently understood, thereby limiting the potentially substantial opportunities for therapeutic interventions for these disorders.

The goal of this review is to present a general overview and a model of the PCP signaling pathway in Drosophila, and to discuss several unresolved issues that will be the focus of future studies. Those issues include the relationship between distinct PCP signaling modules, and the existence and/or identity of an activation signal for PCP.

2. Organization of the PCP signaling mechanism

2.1. PCP outcomes in flies

Drosophila tissues use PCP in related but distinct ways. In the wing, each cell uses PCP information to position the assembly of a trichome ("hair"), that in wild type, emerges from the distal side of the cell and points distally (Fig. 1) [44]. Mutants either fail to choose a side, thus producing a hair from the center of the cell, or choose an incorrect side, resulting in an incorrectly oriented hair. The cells of the abdominal epithelium also produce a single, posteriorly oriented hair, and although the location of hair emergence has not been studied, it seems likely that polarization of hair growth is morphologically similar to that in the wing. The eye uses PCP-dependent polarity somewhat differently. Polarity in the eye results from the differentiation of the initially equipotent R3/R4 photoreceptor progenitors into an equatorial R3 and a polar R4 (see chapter by Strutt and Strutt, in this volume). Here, the key distinction is not between opposite sides of the same cell, but between adjacent sides of this pair of progenitor cells. A competition for

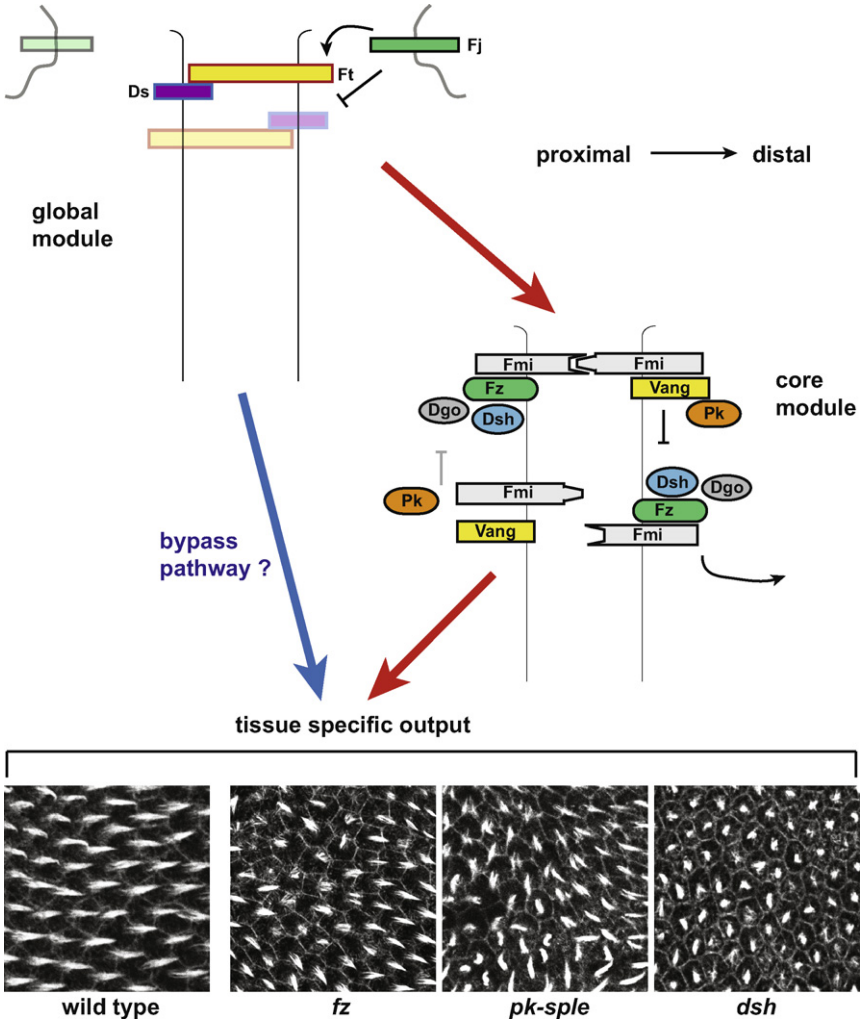

Fig. 1. A modular model of PCP signaling. The linear model (red arrows) posits that the global module acts upon the core module, which in turn acts on the tissue specific modules (examples of wing-specific outputs are shown), while the bypass model suggests that the global module provides a signal that can be interpreted independently of core module function. The global module produces a slight excess of Ft–Ds heterodimers oriented in one direction relative to the other. In the linear model, this produces, by an unknown mechanism, a signal that biases the direction of core module function. Core module components assemble asymmetric complexes, initially in either orientation. Mutual antagonism between the oppositely oriented complexes, together with a symmetry breaking signal from upstream, amplifies asymmetry by removing incorrectly oriented complexes, resulting in highly asymmetric localization. In a wild-type wing, this molecular asymmetry results in prehair assembly at the distal side of cells. Phalloidin images of wild-type and mutant wings are shown. In *fz* and *pk-sple* mutant wings, some prehairs assemble in the center of the cell, but others assemble at or near the periphery. In *dsh* mutant wings, prehairs invariably assemble in the center.

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Notch signaling activation between the R3/R4 pair becomes biased by the PCP signal at the intercellular junction so that the equatorial cell always expresses low Notch levels and becomes R3. PCP mutants lead either to incorrect R3/R4 fate decisions, or in some cases, indistinctly differentiated pairs of R3/R4 cells [45,46]. On the notum, PCP controls the orientation of an asymmetric cell division in the sensory organ precursor cells. pI cells differentiate within the epithelium and divide asymmetrically to produce an anterior pIIb daughter and a posterior pIIa daughter cell. The PCP pathway distinguishes anterior and posterior sides of the pI cell through asymmetric interactions with its anterior and posterior neighbors. The pI cell therefore seems to become polarized much like the surrounding epithelial cells do, but it uses this polarity to position cell fate determinants and the mitotic spindle prior to an asymmetric division. PCP mutants cause this division to be incorrectly oriented (see chapter by Segalen and Bellaiche, in this volume). Thus, in all of these events, PCP coordinates the polarization of cellular ensembles in epithelia, and orients that polarization with respect to the tissue axes.

2.2. A modular system controls PCP signaling

Genetic and molecular analyses in *Drosophila* have identified components of the PCP signaling mechanism, and have suggested that they may be divided into three modules (Fig. 1). It is relatively well established that these components function in distinct modules, but the relationship between the modules is controversial. Here, I will refer to them using names consistent with what I believe to be the best understanding of their relationship and function, but this will be discussed at length below. PCP in a given tissue involves modules including a *global* directional cue that links the direction of polarization to the tissue axes, a *core* module that amplifies and stabilizes subcellular asymmetry through the activity of a bistable feedback mechanism, and one of several distinct *tissue specific effector* modules that respond to the upstream modules to produce morphological asymmetry in individual tissues [47].

The global module is comprised of the atypical cadherins Fat (Ft) [48–52] and Dachsous (Ds) [49–54], and the golgi resident protein Four-jointed (Fj) [49,50,55,56], whose functions are to translate tissue-wide transcription gradients of two or more components into subcellular gradients. This module is characterized by mutant phenotypes in which cells still polarize and coordinate their polarity with neighboring cells, but often fail to align with the tissue axes. The core module consists of proteins that communicate at cell boundaries, recruiting one group to the distal side of cells, and the other to the proximal side, through the function of a poorly understood feedback mechanism [57,58]. The result is the molecular polarization of individual cells, as well as the coordinated polarization of neighboring cells, like dominoes, thus propagating polarity locally from cell to cell. The tissue specific effector modules respond to the upstream modules to execute morphological polarization [5,6,47]. For example, polarization of hair cells recruits tissue specific effectors to proximal and distal sides of the cell that control actin polymerization and bundling to produce a distal hair [59,60]. PCP is also executed by systems that control eye polarity, orientation of asymmetric cell divisions, and bristle/bract orientation.

2.3. Workings of the Ft/Ds/Fj global module: linking direction of PCP to the tissue axes

The discovery of functions for Ft/Ds/Fj in PCP provided an alternative to hypothetical diffusible factors as the signal providing a global directional cue in PCP signaling. The module is proposed to function by converting transcription gradients of Fj and Ds into subcellular asymmetries of Ds–Ft heterodimers [49,50]. Ft and Ds form heterodimers that are predicted to orient in either of two directions, and are observed in puncta in the marginal zone of all cell–cell boundaries. Fj is thought to act on both Ft and Ds, possibly as an ectokinase [61], to make Ft a stronger ligand, and Ds a weaker ligand, for the other. As Fj and Ds are expressed in gradients across tissues [49–52,62], the result is an excess of Ft–Ds heterodimers in one orientation relative to the other [50] (Fig. 1). The net result is to convert directional information contained in transcriptional gradients of Fj and Ds into subcellular gradients of Ds and Ft. These subcellular gradients are then proposed to signal to downstream components of the PCP pathway to regulate orientation of polarization according to the direction of the gradients by mechanisms that are not yet identified.

Fj and Ds gradients are observed in all polarized tissues, and are established early in development, when the tissues are small, most likely by expression of diffusible factors [49,62]. As the tissues grow, these gradients are predicted to become very shallow, but are likely to be maintained by feedback regulation. The steepness of these gradients has also been proposed to function as an indicator of tissue size to control growth [63]. There are, as yet, no data indicating how Fj, Ds and Ft might transmit polarity information to downstream elements of the PCP signaling pathway. Several proposals, and the data supporting them, are discussed below.

Despite the appeal of this simple model, puzzles remain. For example, the abdomen is composed of segments in which the Fj and Ds gradients alternate direction in anterior and posterior compartments, yet the polarity of epidermal hairs is uniform. Thus, the linkage between the output of this system and the responding elements must be reversed in anterior and posterior compartments. Another puzzle comes from the prediction that flattening the gradients of Fj and Ds should disrupt polarity. Indeed, this is observed in the eye [64]. However, in the same flies, polarity in the wings is only modestly disturbed, indicating that residual directional information from another source remains intact. Because *ft* mutant clones disrupt polarity, the other source apparently also relies on Ft function, and one model is that yet another graded signal feeds through Ft to polarize its activity.

2.4. Workings of the core PCP module: local cell–cell alignment

Proteins in the "core" signaling module include the earliest described PCP proteins, the serpentine receptor Frizzled (Fz) [65,66], the multi-domain protein Dishevelled (Dsh) [67,68], the Lim domain protein Prickle (Pk) [69], and the more recently identified 4-pass transmembrane protein Van Gogh (Vang; a.k.a. Strabismus/Stbm) [70,71], the Ankryin repeat protein Diego (Dgo) [72] and the and the seven-transmembrane atypical cadherin Flamingo (Fmi; a.k.a. Starry night/Stan) [73,74] (reviewed in [6]). In contrast to the global mutants, mutation of the core module proteins typically disrupts cell polarization, causing, in the wing for example, prehairs to grow at or nearer to the center of the cell rather than from a side, and disruption of the local correlation of cell polarities (Fig. 1). The core proteins localize to adherens junctions, just basal to the global proteins, and preceding morphological polarization, they adopt characteristic asymmetric subcellular localizations that predict the hair polarity pattern [58,72,74–77]. Largely based on mosaic analyses of clones of cells lacking or overexpressing individual components, it has been deduced that these proteins communicate at cell boundaries, recruiting one group (Fmi, Fz, Dsh, Dgo) to the distal side of cells, and the other (Fmi, Vang, Pk) to the proximal side. Through the function of a poorly understood feedback mechanism, these proteins generate a highly polarized arrangement [57,78]. Proximal group proteins recruit the distal group to the cell boundary of neighboring cells, and vice versa, and a poorly understood mutual exclusion mechanism promotes an all-or-none accumulation in one or the other orientation [57,76,78]. This module therefore behaves as a bistable switch, amplifying

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small asymmetries to produce strong asymmetry. See the chapter by Strutt and Strutt, in this volume for a thoughtful review of the asymmetric localization of these proteins and its role in PCP signaling.

A characteristic set of mutant phenotypes associated with core PCP components has driven the development of models for the function of the core module. Clones of cells mutant for PCP genes, or that overexpress various PCP proteins, display characteristic perturbations (or lack thereof) of cells in nearby wing tissue. This observation has provided a rich set of clues to the PCP signaling mechanism. For example, *fz* and *vang* mutant clones strongly perturb the polarity of prehairs in adjacent zones of non-mutant tissue, though in opposite directions [3,65,70,79]. This phenomenon is called domineering nonautonomy. Notably, it has proven to be beyond our abilities to intuit how specific molecular alterations in various models are predicted to affect the emergent tissue level polarity patterns, and mathematical modeling has been instrumental in using these phenotypes to better understand the signaling mechanisms [52,57,80–82]. While models based on diffusible factors were first proposed to explain these phenomena, these models have largely given way to local signaling models that were first hypothesized, with remarkable insight, even before the discovery of asymmetrically localized PCP proteins [70,83].

Though the molecular mechanisms underlying local PCP signaling are incompletely understood, several specific features bear some discussion here. First, the asymmetrically localized subcellular complexes, with Fz on the distal side and Vang on the proximal side of adjacent cells, communicate information bidirectionally between those cells [84,85]. This is perhaps most simply illustrated by the observation that cells on either side of the border between adjacent *vang* and *fz* mutant clones both strongly polarize, indicating that cells with only the Fz complex and cells with only the Vang complex can strongly polarize and be polarized by a neighboring cell [85]. Fmi homodimers are essential for this communication [52,74,76,84,85]. We have shown that rather than acting simply as a scaffold for complex assembly, information passes bidirectionally through the Fmi bridge that, although a homodimer, is functionally, and presumably structurally, asymmetric [84]. Second, it is evident that although in wild type, all of the core PCP components are required to achieve a fully asymmetric subcellular localization, they must be viewed as having distinct molecular functions, and disruption of individual functions may leave other activities intact. For example, Fz, Vang and Fmi are sufficient to mediate intercellular communication, while Dsh, Pk and Dgo functions are required for the feedback-mediated amplification of the asymmetry that develops at proximal–distal intercellular boundaries ([52,84,85]: chapter by Strutt and Strutt, in this volume). Furthermore, residual morphological polarization can be observed in tissues mutant for any component except for dsh^{null} [57,85,86], suggesting residual function in the absence of most components. While these findings constrain the set of potential models, the molecular mechanism for feedback-mediated mutual exclusion of oppositely oriented complexes is not known.

A major finding suggesting a second asymmetry breaking mechanism at the level of the core module was the discovery that Fz containing vesicles traffic distally along an apical microtubule web that is itself polarized, with an excess of plus ends at the distal sides of cells [87]. Little is known about what regulates this directed vesicular trafficking. The distal transport of Fz vesicles was proposed to be instrumental in establishing the asymmetric cortical domains of core PCP proteins, but it is also an obvious point at which a directional bias may feed into the core PCP mechanism.

The mechanism described for the core module is capable of breaking symmetry and coordinating polarity within a sheet of cells, but to consistently polarize in the correct direction, it must receive input that provides a directional signal. Despite considerable efforts, however, the point at which such a signal feeds into the core module is not known. The involvement of Fz in the core module led originally to models in which a Wnt, such as Wingless, might provide such a directional cue, but ample evidence now argues against a direct role for Wnts or other known secreted signaling factors in this process [84,88]. A clue to events upstream of core PCP protein asymmetry was the finding that Widerborst (Wdb), a regulatory subunit of protein phosphatase 2A, becomes asymmetrically localized and is required for asymmetric localization of the core PCP proteins, but does not require their activities for its own asymmetric localization [89]. Wdb therefore functions upstream of the core components, and interestingly, was seen to associate with an apical microtubule web that is further described below. The distal transport of Fz vesicles is an obvious point at which a directional bias may feed into the core PCP mechanism.

3. Conundrums

3.1. Relationship between the global and core modules

When the global PCP module was first identified, it was proposed to act upstream of the core module to orient its polarization with respect to the tissue axes, though it is not required for the core module to function per se [49,50]. This proposal was based on evidence from the eye and the wing. In the eye, mutations of the global system result in fully polarized R3/R4 ommatidia, but in a nearly randomized orientation [49]. In the wing, mutations result in a functional core mechanism that produces asymmetric localization of core PCP proteins, hair growth from the periphery, and creation of local alignment, but orientation with respect to the global tissue axes is lost [50]. In both tissues, manipulating the expression of global components causes a corresponding alteration of both core PCP protein localization and morphological polarization. Based on these observations, it appears that the global module provides a signal that orients the function of the core PCP module, although the specific nature of that signal is unknown. Since the core module seems to directly regulate downstream modules, at least in the wing [59,90], this leads to the suggestion of a simple linear arrangement of the three modules (Fig. 1).

This relationship has been challenged by additional observations that were interpreted to indicate that a signal from the global module acts directly on the tissue specific effector modules, thereby bypassing the core module (Fig. 1). The key experiments supporting a bypass pathway are the observations that *fmi* or *fz* mutant cells, in which the core module is impaired, can be repolarized by a neighboring clone overexpressing Ft or Ds (or a modified Ds lacking its cytoplasmic tail), at least in the abdomen [86]. These experiments make clear that cells can be polarized in the absence of Fmi or Fz. Based on the assumption that loss of Fmi renders the core PCP module entirely inactive, it would then be logical to conclude that the global module can bypass the core module to regulate morphological polarization [86,91]. Other arguments relying on this same assumption were also offered. At least two additional observations are suggestive of the possibility that the global module directly communicates with the tissue specific effector modules. First, cell divisions in the larval wing disc are oriented by the global components Ft and Ds, but these phenotypes are not affected by the core module [92]. Second, the orientation of the bristle-bract vector is strongly affected by global mutants, but only weakly affected by core mutants ([2] and our unpublished observations). It is unclear how related these systems are to the better-studied PCP models, but assuming similarity, both observations imply the existence of a bypass signal. Taken together, these observations raise the possibility that a signal from the global module bypasses the core module. Notably, from the Ft and Ds overexpression experiments, it was also concluded that if the global module acts on the tissue specific

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effector modules, then it must not act on the core module [86,91]; why these possibilities were argued to be mutually exclusive is unclear. Because the Ft and Ds overexpression experiments were performed in the abdomen and not the wing or eye, it is possible that the modules are connected differently in different tissues. However, it seems more likely that the overall organization of PCP in the two tissues is the same, and the remaining discussion is based on the premise of a common mechanism.

The conclusion that a global signal bypasses the core module rests on the assumption that the core module is completely inactive in *fmi* or *fz* mutant cells, yet careful examination of the evidence reveals instead that disruption of individual core components has distinct consequences. While both the distal Fz complex and the proximal Vang complex produce signals that can polarize cells [59], Dsh has a unique function in producing cellular asymmetry, and its activity may be regulated by both proximal and distal complexes. We have shown that while *fz*, *vang*, *fmi*, *pk* and *dgo* mutant wings have a strong tendency for prehairs to emerge from the center of cells [44], many cells are still somewhat polarized, with prehairs emerging at or near the periphery ([57] and unpublished observations). In contrast, prehairs in *dsh* mutant wings invariably emerge from the center of the cell [57] (Fig. 1). Similarly, propagation of polarity signals through *fmi*, *fz*, *vang*, *pk*, *dgo* and dsh^{1} tissue has been observed, but not through dsh^{null} tissue [85,86]. This indicates that at least a small amount of Dsh function is essential, and suggests that asymmetric subcellular localization of Dsh, or of another determinant that depends on Dsh function, is essential for cell polarization. Why should Dsh be uniquely required for polarization?

As discussed above, Fz containing vesicles have been observed to traverse the cell on a polarized microtubule cytoskeleton during the accumulation of asymmetric complexes, and these vesicles were said to also contain Dsh [87] (Fig. 2). The microtubules are intact in *fz* mutants [87], but are dependent on Wdb [89], suggesting that their polarization results from a signal upstream of the core proteins. The global protein Ft is required to somehow orient mitotic spindles [92], and is therefore a strong candidate for organizing this apical microtubule web. Thus, in the absence of stable accumulation of core complexes, either because *fmi*, *fz* or *vang* are mutant, or because asymmetry is not amplified in *pk* or *dgo* mutants, Dsh could, in principle, be transported distally on these microtubule arrays, but it would accumulate to only low levels because it would fail to be bound and stabilized by the distal complex. Consistent with this idea, tissues mutant for some core PCP components, but not dsh^{null}, have been noted to polarize prehairs without observable accumulation of asymmetric complexes. According to this view, transport of Dsh, or a determinant bound to Dsh, to produce even a subtle subcellular asymmetry, would be sufficient to produce morphological polarization. In the wild type, the remainder of the core complex would be involved in stabilizing this asymmetry, amplifying it, and coordinating it with neighboring cells. Dsh may itself be a part of the vesicle trafficking machinery. *C. elegans* and vertebrate Dsh associate with the clathrin AP-2 adapter, and Dsh is required for Fz internalization in canonical Wnt signaling [93]. Similarly, *Drosophila* Dsh is necessary for the production of Fz::GFP vesicles that are normally transported distally on the mircotubule web [87].

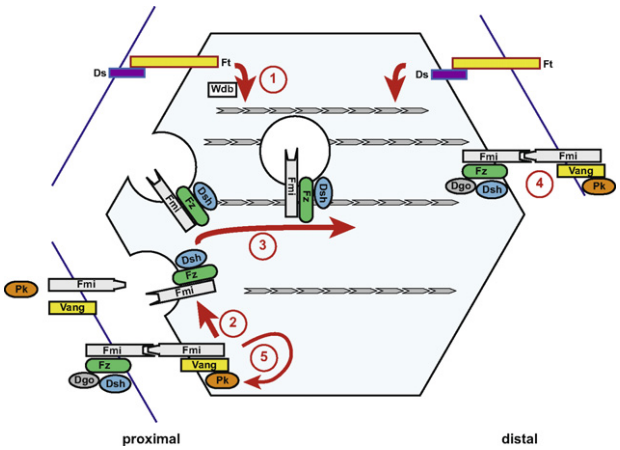

Fig. 2. Mechanistic model of PCP signaling. (1) Asymmetrically oriented Ft–Ds heterodimers, via an unknown mechanism requiring Wdb, orient the apical microtubule web with an excess of plus ends at the distal side of the cell. (2) Competition between oppositely oriented core complexes may induce the internalization of vesicles containing Fz, Fmi and Dsh. These vesicles form all around the cell, but are transported along microtubules toward the distal side of the cell (3). (4) Interaction between neighboring cells stabilizes distal Fz complexes and proximal Vang complexes, allows amplification, and aligns polarity between cells. In the absence of Fz, Fmi or other core components except Dsh, Dsh-containing vesicles could still accumulate toward the distal side of the cell, providing an asymmetry cue. (5) Recycling through an endosomal compartment may remove incorrectly assembled complexes.

Therefore, the ability of Ft or Ds overexpressing clones to polarize neighboring cells mutant for *fmi* or *fz* (or *vang*, *pk*, and *dgo*) does not demonstrate the necessity of a bypass pathway, nor does it rule out the possibility that the signal from Ft and Ds passes through the core module, but instead might indicate that these mutants fail to entirely disrupt the ability of the core module to respond by transporting Dsh to the distal side of the cell. The critical tests of this model would be a demonstration that the global module regulates directed Dsh-containing vesicle trafficking, a demonstration that asymmetrically localized Dsh is sufficient for morphological polarization, and the demonstration that dsh^{null} mutations block transmission of all polarity information from the global module to the tissue specific effector modules. Results of such experiments have yet to be reported.

To explain the Ft or Ds overexpression effect on neighboring cells, one must also understand how overexpression of these proteins affects neighboring cells. Because Ft and Ds make heterodimers that span neighboring cells, a simple explanation is that overexpression of one causes excess accumulation of the other at the wild-type clone border, thus biasing distribution nonautonomously. Furthermore, one might expect this effect to propagate for some distance through the neighboring tissue. This nonautonomy of Ft–Ds signaling has been proposed in the abdomen [86], and we have also provided evidence for nonautonomy of Ft–Ds signaling in the wing [82].

Whether or not a bypass pathway exists, an interesting possibility is that the global module is the descendant of an ancestral mechanism for establishing PCP, in which subtle asymmetry of Ft–Ds heterodimers produced PCP outputs. This system would be expected to function imperfectly, as it relies on subtle gradients over large domains, but it may persist in acting alone to polarize dividing cells in the wing, for example, where precision is not required. The core module might have been added later to amplify asymmetry and to provide robustness to the PCP response by locally coordinating polarization. Both might rely on asymmetric accumulation of Dsh, with the core complex making this function more reliable. Indeed, different tissues might rely on the two mechanisms to differing extents, potentially explaining why core mutants appear to maintain some polarity in the abdomen, but much less in the wing [91].

3.2. Fz activation

Models in which the global module is postulated to function in parallel but not in series with the core module require an alternative directional input to the core module. Since Fz protein levels are not observed in a graded pattern across tissues, these models have

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therefore typically invoked a graded "activation" of Fz in response to a gradient of an unidentified activating ligand (see for example [91,94]). Despite the failure to identify a secreted ligand for Fz in PCP signaling, Fz is still frequently suggested to be somehow "activated" in a gradient across polarizing tissues [84,88].

The molecular basis of Fz activation is not defined, but might include modifications such as phosphorylation. There is as yet no direct evidence for phosphorylation or other posttranslational modification of Fz in vivo, although at least one report has shown that aPKC can phosphorylate Fz in vitro [95]. Overexpression of non-phosphorylatable and phosphomimetic variants in vivo suggest the possibility that phosphorylation by aPKC may inactivate Fz, but this is yet to be rigorously shown, and neither Fz protein localization nor Dsh recruitment are affected in the variant proteins. Interestingly, this mode of regulation was not proposed to produce a gradient of activation, as required of a directional signal, but rather was proposed to limit activation to a subset of cells within each ommatidium of the eye. It is not clear that this mode of regulation would be relevant in other tissues.

The kinase CKIε/discs overgrown has been suggested to play a role in activation of PCP signaling in both vertebrates and in flies [96–98]. Hypomorphic loss-of-function allelic combinations produce polarity defects, and impair overexpression PCP phenotypes [97,98]. CKIε can phosphorylate Dsh, and a single target serine residue was identified [97], but a Dsh rescue construct mutated to alanine localizes correctly and rescues the *dsh*^{1} phenotype, suggesting that this phosphorylation is not necessary for Dsh function [98]. Indeed, a kinase-dead CKIε appears to function in PCP similarly to the wild type protein [97]. Therefore, while CKIε is somehow required for PCP signaling, its kinase function appears to be dispensable, and its point of action in the pathway is unclear. CKIε is thus not a likely candidate for a graded activator of PCP signaling.

The focus on Fz as a target for activation in PCP signaling undoubtedly derives from the knowledge that Fz proteins act as Wnt receptors. However, the lack of requirement for a Wnt or other known secreted ligand, and the evidence (albeit controversial) that a Fz construct lacking its extracellular Wnt binding domain can function in PCP signaling ([84,99]; Strutt and Strutt, in this volume), suggest that Fz may not act as a receptor in PCP signaling. Fmi and Vang are known to interact with Fmi and Fz on the adjacent cell, so another possibility is that Fmi and Vang somehow function as ligands for Fz. However, the one readout of Fz function that is known outside of intact PCP signaling is recruitment of Dsh, and since this occurs in cultured cells without added ligand or cell–cell contact [78], another possibility is that Fz function does not require any activation at all.

Interestingly, some investigators argue that information flows exclusively from Fz containing complexes to Vang containing complexes [52,99], making Fz more of a ligand than a receptor, while others suggest that information flows bidirectionally between the two ([84,85]; Strutt and Strutt, in this volume, and the discussion above). I suggest that binding interactions between components of the core complex may be sufficient to signal PCP simply by virtue of localizing determinants, and that "activation" in the traditional sense may not be required. If the system does need activation, it is conceivable that any of the components might be regulated. Furthermore, as discussed above, a very attractive though still untested hypothesis is that the directional signal comes not from activation of a core complex component, but from orientation of the microtubule network upon which Fz vesicles traffic.

4. Conclusions

PCP, originally recognized and studied in insects, has emerged in vertebrates as an important developmental mechanism, affecting multiple organs, tissues, and physiological processes. Genetic analyses in *Drosophila* continue to lead the way in dissecting the molecular mechanisms underlying PCP. Despite considerable progress, there remains much to be learned, and with the application of increasingly powerful approaches, the next few years hold the promise of substantial leaps in our knowledge of this fascinating system.

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