Planar Cell Polarity: The Frizzled homophobic signal

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Abstract

The cell's capacity to integrate and respond to spatial information is a crucial feature of morphogenesis and development. The Planar Cell Polarity (PCP) pathway is a signaling mechanism, widely conserved across metazoans, providing spatial orientation along the plane of an epithelium in morphogenic processes ranging from insect wing patterning to mammalian cochleae. Although the core genes involved in the PCP pathway have been molecularly identified in the 1990s, the PCP signaling mechanism remains controversial. In this article I discuss the main players and previous models of PCP signaling reported in the literature, and propose a new model. According to it PCP is established through an homophobic signal by transmembrane protein Frizzled (Fz): 1) a Fz signal in one cell repeals Fz itself in the adjacent cell, thereby generating symmetry breaking; 2) the instructive PCP signal is conveyed through Fz interaction with atypical cadherin Flamingo (Fmi). More broadly, homophobic signaling may represent a novel mechanism for cell-cell signaling of spatial information through modulation of cell adhesion rather than canonical ligand-receptor binding.

Keywords: Tissue polarity; Drosophila; epithelia; morphogenesis; cell adhesion

Background and stage set

In a variety of epithelia, across metazoans, cells are polarized along the plane of the tissue - *i.e.* perpendicular to apical-basal axis - leading to asymmetric and coordinated localization of sub-cellular structures. For instance hairs (called tricomes) in the insect wing surface are aligned and positioned distally [1]; similarly cilia in the mammalian cochlea are aligned and oriented towards the abneural side of the epithelium [2]. This phenomenon is referred to as "Planar Cell Polarity" (henceforth PCP) or "Tissue Polarity" (Figure 1) and is controlled by a conserved set of genes [3]. How epithelial cells communicate and coordinate their planar polarity has been studied for over thirty years, nonetheless it remains a matter of debate.

In *Drosophila melanogaster* wings and body cuticles each cell presents on the distal side of its apical surface, one actin rich hair - the tricome. In flies tricomes across the tissue are oriented in the same

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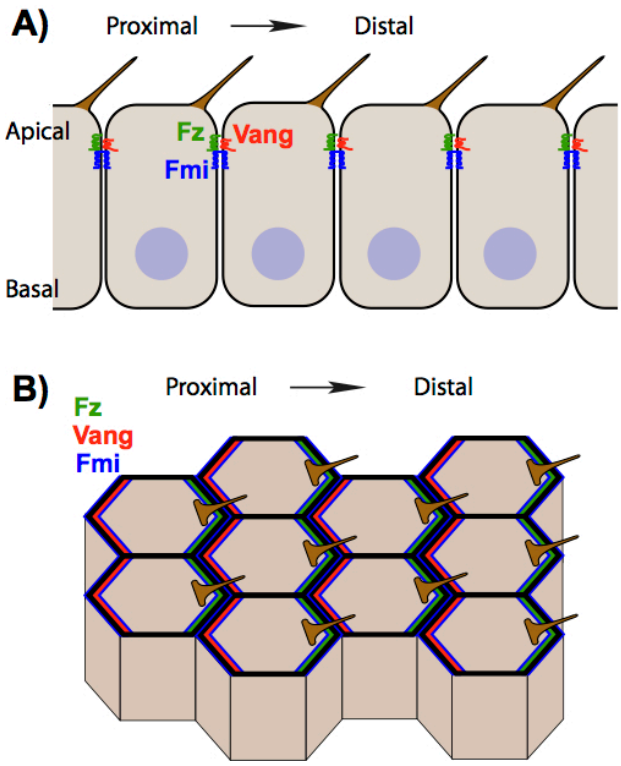

Figure 1 - Schematic representations of Planar Cell Polarity in the *Drosophila* wing epithelium (A - sagital view; B - top view). Fz protein (green) is localized in the distal apical cortex, Vang (red) in the proximal distal cortex and Fmi (blue) is present at both proximal and distal cortexes. Intercellular Fmi-Fmi homophilic interactions take place at the cell-cell contact. Tricomes are represented in brown, nuclei in light blue.

direction, along the plane of the epithelia. Tricome orientation in the *Drosophila* wing is perhaps the most thoroughly studied example of Planar Cell Polarity PCP, and remains a paradigm of choice (reviewed in [3]; Figure 1). However, the first insight in PCP was provided by the pioneering work on the milkeed bug *Oncopeltus fasciatus*, by Lawrence and Shelton. They showed that the retina's ommatidia were not only polarized and oriented in the same direction, but more importantly, that planar polarity was oriented through non-cell autonomous signaling [4]. Shortly after, Gubb and Garcia-Bellido isolated the first *Drosophila* mutants with abnormal tissue polarity, namely tricome orientation in the wing, starting the era of PCP genetics in the fly [1]. Further analysis of PCP-mutants established *Drosophila*, and particularly the fly wing, as a model of choice to study PCP [5, 6] (reviewed in [7, 8]).

Far from being an insect peculiarity, PCP has been shown to be essential for providing spatial information on broad range of developmental processes across metazoans. The genes involved in PCP (discussed bellow) were found to be conserved (reviewed in [9]). Homologues of the *Drosophila* PCP genes have been shown to regulate spatial orientation during asymmetric division of neural progenitors, patterning of the cochlea sensory cells and hair follicle positioning in mouse, as well as gastrulation movements in zebrafish or bone growth in chick, to name a few [2,10-14].

The characters

The molecular identification of the genes carrying the PCP mutations, and therefore the proteins they encode, uncovered the core components of PCP signaling: Frizzled (Fz) is a seven-pass transmembrane protein [6]; Van Gogh (Vang), also known as Strabismus (Stbm) is a four-pass transmembrane protein [15]; Flamingo (Fmi), also known as Starry night (Stan), is an atypical cadherin [16, 17]; Dishevelled (Dsh) is a DEP domain containing protein [18]; Diego is an ankrin repeats protein [19]; Prickle (Pk), also known as Spinny

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legs, contains three LIM and one PET domains [20].Fz and Vang, being transmembrane proteins, are likely involved in cell to cell signaling. Flamingo is an atypical cadherin, drawing a connection between cell adhesion and PCP signaling. Dsh, Pk and Dgo on the other hand, likely act intracellularly.

One other pathway, involving the atypical cadherins Fat and Daschous, and the one ectokinase Four-jointed, participates in PCP (reviewed in [21]). How this pathway fits in with the Fz pathway is still a matter of debate. The relationship between the core PCP pathway and the Fat/Daschous pathway, however, has no impact on the model proposed here, hence the Fat/Daschous/Four-jointed pathway will not be further discussed.

The Plot

Many questions remain to be answered: how do the core PCP pathway signals to establish planar polarity? What is the molecular mechanism involved? Who is the ligand and who is the receptor (if any)? How is the signal transduced? There is a significant amount of work published over the years addressing these questions, however, the challenge is to reconcile the plethora of data in one model.

The *Drosophila* wing model allowed provided the tools to perform phenotypic analysis of mutant somatic clones, which proved instrumental. In this approach a clonal group of mutant cells is generated within a wild-type (WT) tissue, and observe the effect on PCP of the cells at the interface between mutant and wild-type tissues. Such clone border analysis is quintessential to understand PCP signaling. With these types of experiments one can find two types of PCP mutants: those who disrupt planar polarity in the neighboring wild-type cells, and those who don't (Figure 2). The former is the case for *fmi(-)*, *fz(-)* and *vang(-)*, in the later category we find *dsh(-)*, *pk(-)* and *dgo(-)*. This distinguishes between the genes required for PCP signal sending from genes required solely for PCP signal response. *fz(-)* and *vang(-)* somatic clones disrupt the orientation of planar polarity in adjacent wild-type cells - the so-called "domineering nonautonomy" phenotype [5, 22]. This effect extends for several rows of cells into the wild-type tissue (Figure 2A and 2B). The tricomes in the wild-type tissue are oriented towards the *fz(-)* mutant clones and away from *vang(-)* mutant clones. On the other hand, *fmi(-)* somatic clones display a domineering nonautonomy only in a minority clones, and with a short-range effect, *i.e.* typically only the wild-type cells immediately adjacent to the clone border are affected [17] (Figure 2C). These data suggest that *fmi*, *fz* and *vang* are all required for PCP signal sending, however differences in mutant phenotypes point to distinct roles. By contrast, any one of *dsh(-)*, *pk(-)* or *dgo(-)* somatic clones mutant for display a strictly cell autonomous phenotype, PCP is disrupted within the mutant tissue but not in neighboring wild-type cells [23](Figure 2D). These data

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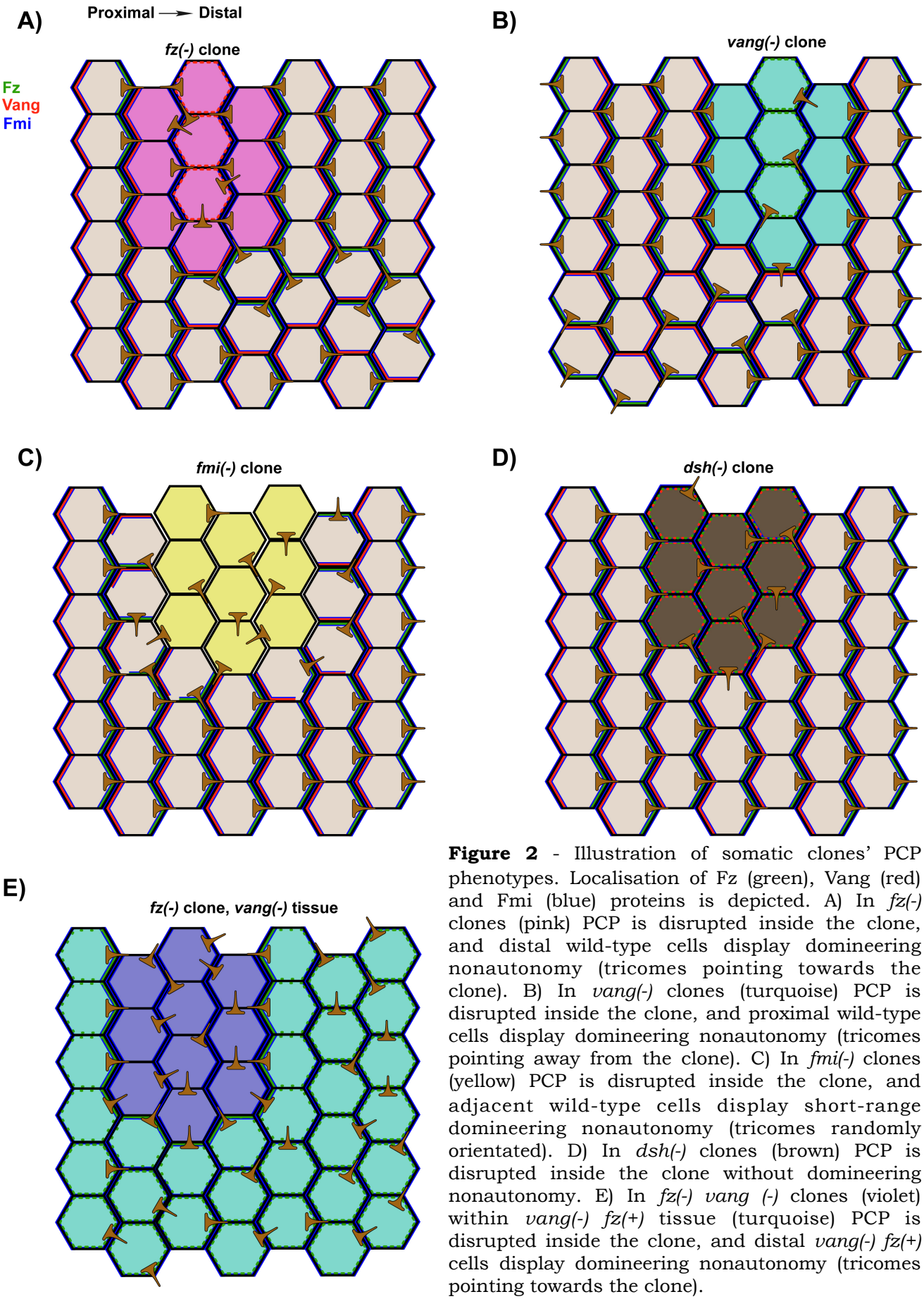

Figure 2 - Illustration of somatic clones' PCP phenotypes. Localisation of Fz (green), Vang (red) and Fmi (blue) proteins is depicted. A) In *fz(-)* clones (pink) PCP is disrupted inside the clone, and distal wild-type cells display domineering nonautonomy (tricomes pointing towards the clone). B) In *vang(-)* clones (turquoise) PCP is disrupted inside the clone, and proximal wild-type cells display domineering nonautonomy (tricomes pointing away from the clone). C) In *fmi(-)* clones (yellow) PCP is disrupted inside the clone, and adjacent wild-type cells display short-range domineering nonautonomy (tricomes randomly orientated). D) In *dsh(-)* clones (brown) PCP is disrupted inside the clone without domineering nonautonomy. E) In *fz(-) vang (-)* clones (violet) within *vang(-) fz(+)* tissue (turquoise) PCP is disrupted inside the clone, and distal *vang(-) fz(+)* cells display domineering nonautonomy (tricomes pointing towards the clone).

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indicates that *dsh*, *pk* and *dgo* are required for PCP response, but not for PCP signal sending.

Epistasis analysis further reveals the roles of *fz*, *stbm* and *fmi* in signal sending. Contrasting with single mutants, *vang(-) fz(-)* double mutant clones display a modest domineering nonautonomy, if any, resembling *fmi(-)* clones [23, 24]. This shows that the *fz(-)* domineering nonautonomy depends on Vang function, and reciprocally, *vang(-)* domineering nonautonomy depends on Fz function, and presumably in the absence of both Fz and Vang, there is no PCP signal. In addition *fmi(-)* is epistatic to both *fz(-)* and *stbm(-)*: *fmi(-) fz(-)* as well as *vang(-) fmi(-)* double mutant clones lack a strong domineering nonautonomy. This epistasis shows that both Fz and Vang require Fmi to signal non-cell autonomously. Finally, there is a noteworthy difference between *fz* and *vang*: the domineering effect of *vang(-)* clones occurs only at the proximal clone boundary, while *fz(-)* clones domineering nonautonomy is detected only at the distal boundary [5, 22]. This difference suggests that Vang signaling activity is localized at the proximal cell membrane and Fz activity at the distal cell membrane, which consistent with the reported localization of these proteins (Figure 1).

Regarding protein localization, most core PCP pathway proteins are asymmetric along the plane of the epithelium consistent with a role in planar polarity (see Figure 1). Vang and Fz proteins localize asymmetrically and opposite to one another at the apical cortex of the wing epithelial cells; Vang localizes to the proximal cell surface while Fz localizes to the distal cell surface [25, 26]. Pk localizes asymmetrically to proximal cortex, like Stbm, while Dsh and Dgo localize asymmetrically to the distal cortex, like Fz [27-30] Fmi protein, as one could expect from a cadherin, bridges cell-cell contacts through homophilic interaction [16], and is enriched both at the proximal and at the distal cell-cell contacts [16, 31].

The core PCP proteins are mutually dependent for their asymmetric localization. In a *vang(-)* mutant tissue Fz is still recruited to the apical cortex but its asymmetry is disrupted, and reciprocally in a *fz(-)* tissue Vang asymmetry is disrupted, but not its apical and cortical localization [25, 26](Figure 2A,B). Localization of Vang and Fz requires the atypical cadherin Fmi both cell autonomously and nonautonomously. In *fmi(-)* mutant cells neither Vang nor Fz are recruited to the cell cortex, and in wild-type cells at the clone border both Vang and Fz are recruited to the cortex but fail to localize to the surface adjacent to *fmi(-)* cells [24, 25] (Figure 2C). Importantly Fmi depends on Fz and Vang - somewhat redundantly - to localize at the cortex, while in *fz(-)* and *vang(-)* single mutants Fmi localization is not affected, in *vang(-) fz(-)* double mutants Fmi is mislocalized [32]. Asymmetric localization of Vang and Fz is also disrupted in *dsh(-)* and *pk(-)* mutant cells in the wing [25, 26] (Figure 2D). The same has been shown for *dgo(-)* rhabdomeres, and

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presumably holds true for wing cells as well [30, 33]. Therefore *dsh*, *pk* and *dgo* gene products participate intracellularly in the establishment of asymmetric and opposite domains of Vang and Fz. Confirming this hypothesis, it has been shown that Dsh and Dgo act downstream of Fz receptor to localize Vang at the proximal cortex, while Pk acts downstream of Vang to localize Fz distally [27, 29, 30, 34].

vang(-) and *fz(-)* somatic clones also display domineering nonautonomy at the molecular level. In wild-type cells neighboring *vang(-)* clones Vang is preferentially recruited to the cortex contacting the *vang(-)* cells, and Fz is preferentially recruited to the distal cell cortex [24-26] (Figure 2B). Reciprocally *fz(-)* clones promote Fz recruitment in neighboring wild-type cells at the cortex contacting the *fz(-)* clone while Vang is enriched opposing cell cortex [24, 26] (Figure 2A). This coherence between the domineering nonautonomy on Fz and Vang protein localization and tricome orientation shows that localization of the PCP core proteins is the causal mechanism of Planar Cell Polarity. Consistent with the tricome orientation phenotypes, the domineering nonautonomy of *fmi(-)* on protein localization is strikingly different from either *fz(-)* or *vang(-)*, as wild-type neighboring cells fail to recruit PCP core proteins altogether to the cell-cell contact, including Fmi itself [19, 23, 24, 26-28, 31, 32]. Fmi is thus instrumental for PCP signaling, being required both for Fz-signal sending and Vang-signal sending [24, 35]. While Fmi localizes at the apical cortex, with no sign of proximal-distal asymmetry, Chen et al demonstrate Fmi interacts physically with Fz, and suggest that Fmi associate with either Vang or Fz to form distinct complexes at opposite poles of the cell [24]. Further highlighting the interplay between PCP and cell adhesion, mutants in the core PCP genes affect cell adhesion in the wing [36]. In a wild-type wing epithelium cells adopt a quasi-hexagonal shape late in wing development, reflecting a tight and homogenous cell adhesion throughout the tissue. Mutants in core PCP genes display more irregular cell packing, compared to WT. Not only cell adhesion participates in PCP signaling, PCP core genes also play a role in cell adhesion.

The usual suspects...

Drawn from the data published, discussed above, different hypotheses have been proposed to explain signaling by the core PCP pathway, that can be grouped in two main models (reviewed in[37]):

1) Fz levels model: Planar orientation is established by levels of Fz activity, directionality is given from high levels of Fz to low levels of Fz (reviewed in [8]). Higher levels of Fz signal positively to Vang at the proximal cortex of neighboring distal cells, and reciprocally Vang to Fz, while cell-autonomously Vang and Fz promote one another's localization at opposing poles. This signaling process is amplified by a positive feed-back loop (reviewed in [38]). This model predicts that Fz binds to Vang

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extracellularly - for which some evidence exists [33]. It also predicts that Fmi acts as a scaffold to recruit Vang and Fz to the membrane, and thus has a positive yet permissive role in PCP signaling (reviewed in [39]). This model can be reconciled with a long-standing hypothesis of a Fz gradient acting throughout the tissue [7, 8].

2) Vector model: The PCP signal is relayed from cell to cell, through the cell adhesion complexes. Vang localization at the proximal cortex promotes the localization of Fz in the distal cortex of the neighboring cell, while intracellularly Fz and Vang localize at opposite to each other whereof propagating the PCP signal. Fmi plays an instrumental role in the vector model, given it was shown to provide the instructive PCP signal [24, 32]. Fmi is thought to bind homophilically, bridging cell-cell contacts, yet this cell bridging Fmi-Fmi interaction is functionally asymmetric: Fz-Fmi interaction on the proximal cell promotes nonautonomously Vang recruitment to the cortex at the distal cell [16, 24, 32] (reviewed in [40, 41]). In the vector model, PCP signal is tightly linked to cell adhesion.

Both proposed models present unsolved inconsistencies.

...and their alibis

One conundrum that neither model can explain is the capacity of Fz to signal in absence of Vang. In *Drosophila* abdominal cuticles, when *fz(-)* clones are generated within a *vang(-)* tissue, the *vang(-) fz(+)* cells orient in response to the *vang(-) fz(-)* clone border [42] (Figure 2E), demonstrating that PCP signaling can occur even when *vang* is absent from both signal sending and receiving cell. A similar observation was made in the fly notum, where the sensory organ precursor (SOP) cell divides asymmetrically oriented in response to PCP. *vang(-) fz(+)* SOPs orient in response to the *vang(-) fz(-)* clone border [43]. In similar experiments in the fly wing, tricome orientation was not reported, however Fmi is strongly enriched at the clone border in *vang(-) fz(+)* cells adjacent to *vang(-) fz(-)* cells [32]. By contrast, on the other side of the border there is no sign of *vang(-) fz(-)* cells responding to *vang(-) fz(+)* clones, in either abdominal cuticle tricomes, SOP asymmetric division or Fmi localization in the wing epithelium [32, 42, 43]. Thus, in the absence of both Vang and Fz the cell cannot respond to PCP signaling from either *vang(+) fz(-)* or *vang(-) fz(+)* tissue. Furthermore *vang(-) fz(-)* similarly do not respond to wild-type tissue [24]. Neither model - Fz levels, nor vectorial signal - explains the response of *vang(-) fz(+)* cells to *vang(-) fz(-)* clone border, as both assume that Vang and Fz are absolutely required for PCP signaling.

One other unresolved issue is the lack of domineering nonautonomy of mutant clones *dsh(-)*, *dgo(-)* and *pk(-)*. To be more accurate, the puzzle resides in the contrast with *vang(-)* and *fz(-)* clones, that do display the domineering nonautonomy phenotype (reviewed in [44]). Given the absence of domineering phenotype in *vang(-) fz(-)*

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double mutant clones, one can conclude that the domineering effect in *fz(-)* clones is due to Vang mislocalization, as Fz mislocalization is responsible for the domineering effect in *vang(-)* clones. As mentioned above, both Fz and Vang protein localization are dependent on *dsh(-)*, *dgo(-)* and *pk(-)*. Assuming Fz and Vang function in a canonical ligand/receptor fashion, the mislocalization of Fz and/or Vang in *dsh(-)*, *dgo(-)* or *pk(-)* clones should disrupt planar polarity in the neighboring wild-type cells, which is not the case. Importantly, mathematical modeling can account for the lack of domineering effect by *dsh(-)* and *pk(-)* mutant tissues, under the premise that Vang and Fz can still signal to the adjacent cells [45]. Nonetheless the underlying biological mechanism remains elusive. In summary, the current models cannot fully account for the difference in domineering effect by *fz(-)* and *vang(-)* on one side and *dsh(-)*, *dgo(-)* and *pk(-)* on the other.

How does it work then? Fz signals in the absence of Vang.

As mention above, one unresolved issue is how cells in a *fz(-)* somatic clones within a *vang(-)* tissue respond to the clone border (Figure 2E). How can *vang(-) fz(-)* cells signal to orient the planar polarity of *vang(-) fz(+)* cells? Actually they can't. According to the new model I put forward here the *vang(-) fz(+)* cell's response to the *vang(-) fz(-)* clone border (*i.e.* the Fz signal in the absence of Vang) can be accounted for by a Fz homophobic signal among *vang(-) fz(+)* cells. Recruitment of Fz to the membrane on one cell repels Fz itself from the membrane in the adjacent cell. Hence, given the absence of Fz repulsion in the *vang(-) fz(-)* cells, the Fz homophobic signal among the *vang(-) fz(+)* cells orients their planar polarity relative to the clone border (Figure 3A). The clone border is permissive in positioning the planar orientation of the cell precisely because of the absence of a negative Fz signaling. This hypothesis implies that Fz accumulates at the border between *vang(-) fz(+)* and *vang(-) fz(-)* cells, which is the case (David Strutt, personal communication). In summary, in the absence of Vang, Fz homophobic signal is sufficient to polarize the cell along the plane of the tissue.

Fmi was previously shown to provide the instructive signal for cell-to-cell planar polarization and to directly bind Fz [24], thus Fz homophobic signal must be mediated by Fmi. It has been shown that Fmi relays PCP signal through asymmetric yet homophilic Fmi-Fmi binding [24]. This is consistent with homophobic signal model: Fz binding to Fmi in the signal-sending cell represses Fmi-Fz interaction in the adjacent signal-receiving cell, thus providing directionality to PCP (Figure 4). This Fz homophobic signal favors Vang-Fmi interaction in the signal-receiving cell instead of Fmi-Fz interaction. Finally, the short range and sporadic domineering effect of *fmi(-)*, can be explained by the lack of Fmi homophilic intercellular bridge at the clone border, rather by misplaced Fz/

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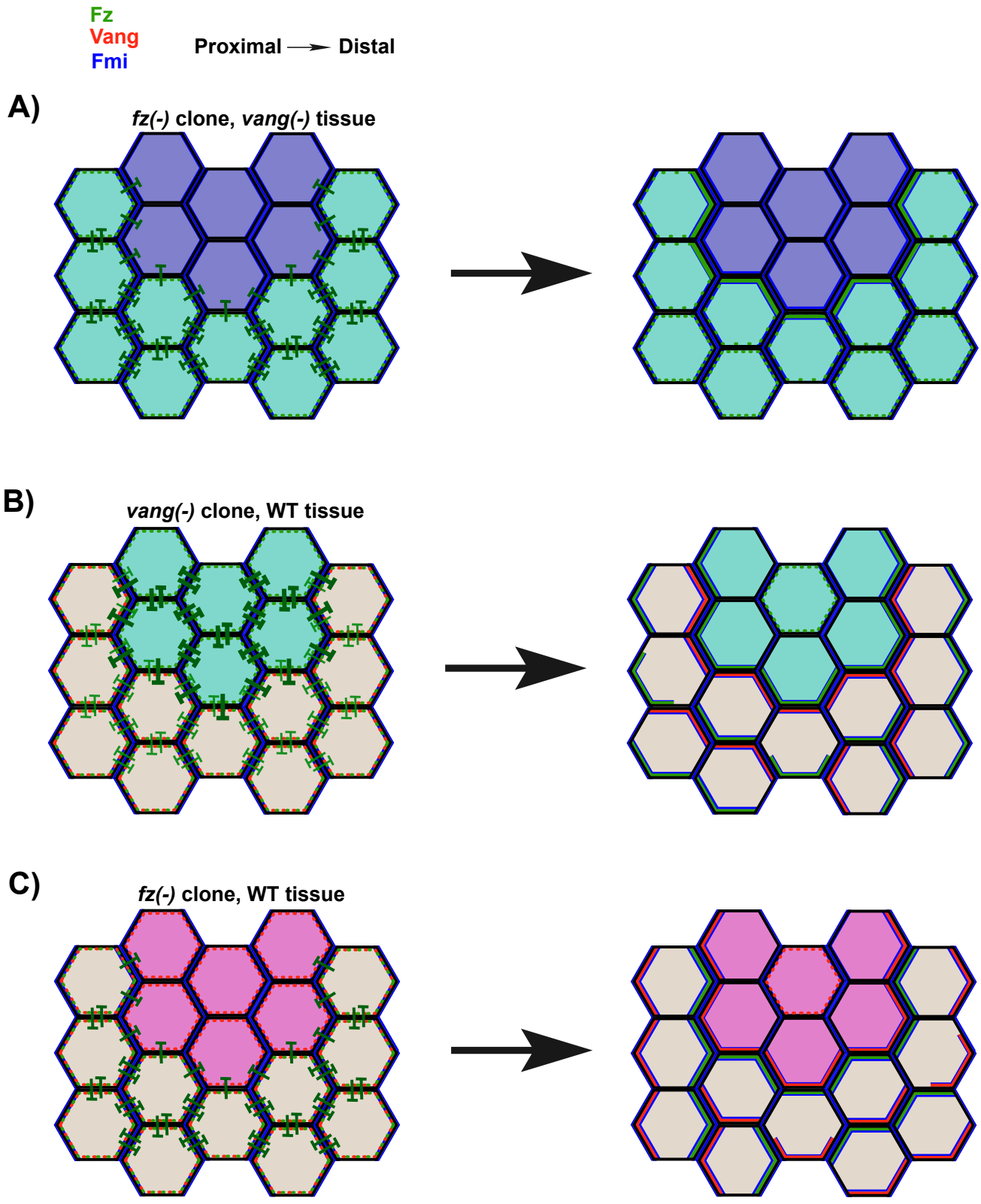

Figure 3 - PCP establishment early (left) and late (right) in somatic mutant clone borders. Localisation of Fz (green), Vang (red) and Fmi (blue) proteins is depicted. A) Early, Fz homophobic signal is uniform among *vang(-) fz(+)* (turquoise) cells whereas at the border with *fz(-) vang (-)* clones (violet) it's unidirectional, hence in *vang(-) fz(+)* cells Fz accumulates at the border (late) and cells orient towards the *fz(-) vang (-)* clone. B) In *vang(-)* clones (violet) adjacent to wild-type tissue (beige) Fz homophobic signal from *vang(-)* cells is stronger (early) hence Fz accumulates on the *vang (-)* side while Vang accumulates on the wild-type side (late) driving wild-type cells to orient away from the clone. C) *fz (-)* cells (pink) adjacent to wild-type tissue (beige) lack Fz homophobic signal (early) hence Fz accumulates in the wild-type side of the clone border while Vang accumulates in the *fz (-)* side (late) driving orientation of wild-type cells towards the clone.

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Vang signaling as previously suggested. Fmi in the wild-type cells cannot bind Fmi in *fmi(-)* cells, therefore neither Vang nor Fz are recruited to that cell contact [24] disrupting planar orientation in wild-type cells adjacent to *fmi(-)* cells. Given Fmi is essential for Fz signal, *fmi(-)* cells do not send homophobic signal, preventing the domineering effect to extend further than one cell row into the wild-type tissue.

The domineering nonautonomy...

Another unresolved issue, as discussed above, is the contrast between the *fz(-)* and *vang(-)* domineering nonautonomy, and the lack of in *dsh(-)*, *pk(-)* and *dgo(-)* mutant clones. The difference between the two types of mutants is that in *dsh(-)*, *pk(-)* and *dgo(-)* both Fz and Vang proteins are still present. This makes the difference if Fz and Vang are in competition for Fmi binding [46]. While Fz and Vang compete intracellularly for Fmi binding, Fz-Fmi binding at the membrane in the signal sending cell promotes Vang-Fmi interaction nonautonomously in the signal receiving cell - by repelling Fz (Figure 4). This generates a “tug of war like” mechanism, consistent with the bistable switch proposed for PCP signaling [28]. This combination with the Fz homophobic signal and competition between Fz and Vang for Fmi binding can explain domineering nonautonomy.

In a *vang(-)* cell Fz has no competition for Fmi binding, therefore at the clone boundary Fz-Fmi will

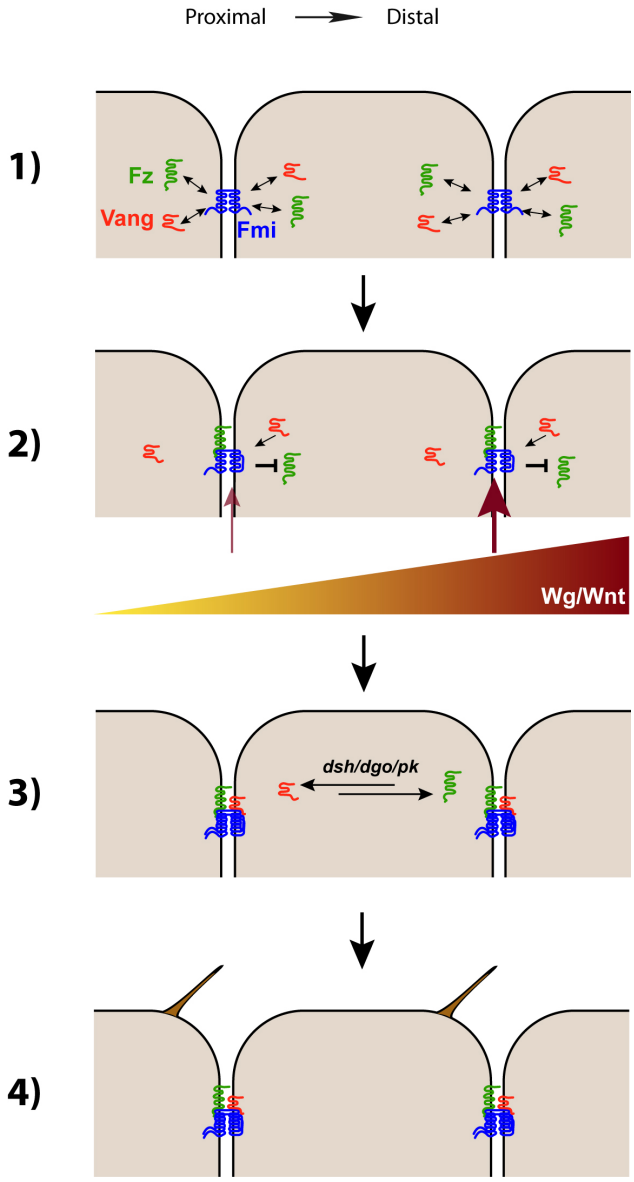

Figure 4 - Diagram presenting the Fz homophobic signal model in sequential steps; protein localisations are depicted for Fz (green), Vang (red) and Fmi (blue). 1) Extracellular Fmi-Fmi homophilic interaction occurs at the cell-cell apical contacts, Fz and Vang compete for Fmi binding intracellularly; 2) Fz binding to Fmi in one cell represses Fmi binding to Fz in the adjacent cell through a putative nonautonomous modification of Fmi in the signal receiving cell, this modification enables Vang binding to Fmi without Fz competition. An extracellular signal such as a Wg/Wnt gradient may bias PCP towards Fz distal localization; 3) Dsh/Pk/Dgo cell-autonomously drive Fz and Vang localizations to opposing poles of the cell reinforcing PCP and increasing Fmi levels at the membrane; 4) PCP is established with Vang proximal localization, Fz distal localization and the tricome (brown) emerging at the distal apical surface of the cell.

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repel Fz from the adjacent wild-type cell membrane (Figure 3B). As a result the bistable switch is tilted towards Fz-Fmi in the *vang(-)* side of clone border and Vang-Fmi on the wild-type side. The "tug of war" is therefore completely unbalanced driving the domineering effect of *vang(-)* clones on the proximal side of the clone border. The *fz(-)* domineering nonautonomy is explained by the Fz homophobic signal among the wild-type cells. Initially Fz homophobic signal is even throughout the tissue, except at the border with the *fz(-)* mutant cells, where the lack of negative signal drives Fz accumulation at the clone border (Figure 3C). Within the *fz(-)* clone Vang has no competition for Fmi binding. The "tug of war" is pushed towards accumulation of Fz-Fmi in wild-type cells and Vang-Fmi in *fz(-)* cells. This leads to the domineering nonautonomy on the distal side of *fz(-)* clones. Furthermore, this model predicts that in *vang(-)* cells adjacent to a *fz(-)* cells the former will be oriented towards the clone border, and the later away from the clone border, consistent with published observations [23].

...and the lack of

Within a *dsh(-)* somatic clone (or *dgo(-)*, or *pk(-)*) both Fz and Vang compete for Fmi binding, and initalle Fz homophobic signal is even throughout the tissue - inside and outside the mutant clone. Therefore, at the border with wild-type tissue, there still occurs a "balanced tug of war", in which case prevails the robust wild-type configuration of the PCP components, explaining the lack of domineering effect in *dsh(-)*. The same argument holds true for *pk(-)* or *dgo(-)* mutants. Importantly, in this model Fz and Vang do not function as canonical ligand and receptor, but it is rather the competition between Fz and Vang for Fmi binding, and the resulting asymmetric cell adhesion that provides the PCP signal.

So, if they're not required for Fz signaling, why do *dsh(-)*, *pk(-)* and *dgo(-)* display PCP defects? Dsh, Pk and Dgo are not required for signal sending, meaning they are not required for Fz or Vang to bind Fmi. They are, however, required for intracellular mutual exclusion of Fz and Vang, given in *dsh(-)*, *pk(-)* and *dgo(-)* Fz and Vang are no longer at opposing poles of the cell [23, 26-31]. This means that their role is to move the system from the single molecule level to the sub-cellular level. While one moiety of Fmi binds one moiety of Fz another neighboring moiety of Fmi could bind Vang. According to the model presented here, this doesn't happen because Dsh/Dgo/Pk prevent it. While Fz and Vang are competing for Fmi binding Dsh/Dgo/Pk act to exclude Vang in the vicinity of Fz-Fmi and conversely exclude Fz from the vicinity of Vang-Fmi. In other words, Dsh/Dgo/Pk drive a second level of tug of war between Fz and Vang, this one being cell-autonomous. This spread of Fz or Vang binding to Fmi from the single molecule to the sub-cellular level leads to proper asymmetric localization of Fz and Vang at opposing poles of the cell. This role is consistent with the cell autonomous phenotypes observed in

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dsh(-), *pk(-)* and *dgo(-)* mutants[23, 26-31].

The domineering phenotypes of *fz(-)* and *vang(-)* and the cell autonomy of *dgo(-)*, *pk(-)* and *dgo(-)*, are consistent with PCP signaling through modulation of cell adhesion, but are inconsistent with the view that Vang and Fz function in a canonical ligand/receptor fashion. One important implication of this model is that PCP should be tightly to adhesion properties of the wing epithelial cells, which has been shown to be the case [36, 47].

Is Vangogh homophobic as well?

A long lasting issue is whether the PCP signal by the core proteins is bidirectional. According to the homophobic signal model proposed here Fz favors Vang-Fmi interaction nonautonomously. If the signal were bidirectional a reciprocal Vang homophobic signal favoring Fz-Fmi interaction should occur too. It is conceivable that Vang binding to Fmi leads to the reciprocal nonautonomous modification of Fmi caused by Fz, thus repressing Vang-Fmi interaction in the adjacent cell. In other words Vang should be homophobic as well as Fz. If that were the case, Vang protein should accumulate at the clone border of *vang(-)* clones within a *fz(-)* tissue, due to repressive signal among *vang(+) fz(-)* cells. To my knowledge this direct test to the hypothesis has not been reported, however there are two results indirectly suggesting that it is not the case: Fmi does not accumulate at *vang(-)* clone border within a *fz(-)* tissue [32], contrasting with *vang(-) fz(+)* adjacent to *vang(-) fz(-)*; and in the abdomen *vang(+) fz(-)* cells do not orient their polarity in response to *vang(-) fz(-)* cells [42].

Most likely Vang does not signal in the absence of Fz, strongly suggesting signaling through the core PCP pathway is not bidirectional. The homophobic signal seems to be a one way street.

The Molecular mechanism

The most plausible molecular mechanism for a Fmi mediated Fz homophobic signal is through a nonautonomous Fmi conformational change and/or post translational modification (*e.g.* phosphorylation). Fz binding could triggers an asymmetric modification on the Fmi-Fmi dimer precluding Fz-Fmi binding in the adjacent cell while allowing Vang-Fmi interaction (Figure 4). This hypothesis is consistent with the proposed mode of action of the Fmi instructive signal [24].

Chen and colleagues proposed that in order to convey PCP signal Fmi adopts two distinct forms, V-Fmi and F-Fmi, depending on whether it associates with Vang or Fz respectively [24]. In the Fz homophobic model Fmi must also occur in two distinct forms, however it does so in a Fz-on or a Fz-off form: what differentiates the two forms is whether Fz can bind Fmi or not. Vang, by contrast can bind to either of the two Fmi forms (Figure 4). Unbound Fmi at the cell cortex is in a Fz-on state, thus Fz and Vang compete for its occupancy. Upon binding to Fz,

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the Fz-Fmi triggers the Fmi modification in the adjacent cell, that will adopt the Fz-off form, thus repelling Fz and allowing Vang to bind.

It is noteworthy that Fz recruitment to the cell cortex, even in the absence of Vang leads to increased Fmi levels at the cell-cell contact [32] (Figures 2E and 3A). This shows that while Fmi at the cell membrane is required to recruit Fz, Fz recruitment further increases Fmi at the cell cortex, suggesting that Fz binding changes the stoichiometry of the Fmi-Fmi intercellular complexes. Moreover an increase in the number of Fmi subunits in the Fz-Fmi-Fmi—Vang complexes may be itself part of the Fmi modification underlying PCP signal. Supporting this idea, the overexpression of Fmi was shown to promote Vang recruitment nonautonomously [24].

In order to test whether this molecular mechanism of PCP signaling it is essential to perform a structural characterization of the Fz-Fmi-Fmi-Vang complexes.

Fz and Vang: to bind or not to bind?

One hypothesis frequently evoked in the literature, is that physical interaction between Fz and Vang plays a role in relaying PCP signal [8, 48]. This interaction would occur extracellularly at the cell-cell interface, consistent with the transmembrane nature of Vang and Fz proteins. According to the Fz homophobic model, PCP signal can occur without an extracellular interaction between Fz and Vang.

Wu and Mlodzik provide evidence that a Fz-Vang physical interaction occurs [33], however two main criticisms can be drawn. 1) their experimental design does not allow to distinguish whether Fz/Vang interaction occurs in trans or in cis. 2) their experiments were carried in cultured cells rather than in a tissue where PCP signal is known to occur, and the observed interaction could be due to culture artifact.

It is still conceivable however, that a Vang/Fz extracellular interaction co-exists with Vang and Fz homophobic signal mediated by Fmi. The competition between Fz and Vang for Fmi binding, could be reinforced by Vang and Fz binding extracellularly. However Chen et al argue that the predicted structures of the extracellular domains of both Vang and Fz are incompatible with an interaction bridging across the predicted intercellular gap of 250Å at adherens junctions [24]. The authors further demonstrate that Fz deleted of its extracellular Cistein Rich Domain rescues *fz(-)* phenotypes [24].

The putative Fz-Vang interaction is not excluded by the Fz homophobic hypothesis, it might play an accessory role in reinforcing PCP signaling. However Fz-Vang extracellular binding it is not absolutely required for PCP, neither is it sufficient.

Wg/Wnt (and other) gradient(s)

Although both signaling through Fz-Vang direct interaction in trans and signaling through a Wnt/Wg gradient are plausible, the two models seem difficult to reconcile. If Fz-Vang

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interaction takes place extracellularly it occurs both at the proximal and distal sides of the cell, it is unclear how a gradient of a Fz ligand could generate asymmetry. Would a Wnt/Wg act to stabilize Fz-Vang binding or to compete with Vang for Fz binding? Either way a gradient could produce different levels of response in different cells depending on Wnt/Wg levels, but not an asymmetry within an individual cell.

Such a gradient could, nonetheless, play a role in another yet unresolved PCP issue: the proximal-distal orientation. The bistable switch can explain Fz and Vang domains opposite to each other are established, and how planar polarity can propagate throughout the tissue. However, it is not sufficient to explain why Vang is localized proximally and Fz distally. One needs to evoke a spatial cue capable of biasing the bistable switch along the proximal distal axis, to establish the specific proximal-distal orientation observed. One possibility often discussed in the literature is the existence of a gradient along the proximal distal axis that signals through Fz to orient PCP (reviewed *e.g.* in [7, 37, 41]). Wnt/Wg are sound candidate molecules for providing the gradient signal orienting PCP, given they are Fz ligands. Indeed Wu et al provide compelling evidence that Wg and Wnt4 secreted from the distal wing margin affect PCP orientation (redundantly), consistent with the gradient hypothesis [49]. Nonetheless, the crucial test to the gradient hypothesis - that uniform expression of Wg/Wnt throughout the wing disrupts PCP orientation - has not been reported.

The Fz homophobic signal model is compatible with a Wg/Wnt gradient provided that Fz bound to its ligand stabilizes Fz-Fmi interaction. Given Wg/Wnt levels are higher at the distal side of the cell, Fz-Fmi interaction will be favored relative to Vang-Fmi interaction thus biasing the bistable switch towards Fz localization at the cell surface with higher Wnt/Wg levels, *i.e.* distally. Even though Wnt/Wg form a gradient, the difference in Wnt/Wg across one cell length may be too small to tilt the switch. The long range PCP orientation by a Wnt/Wg gradient is best understood considering the tissue level. All cells in the field are exposed to the Wnt/Wg gradient, thus a small bias towards distal Fz, combined with the cell-to-cell relaying of PCP signal through Fz homophobic signal leading to proper PCP orientation across the tissue. Thus the Fz homophobic signal model provides a means to integrate the vector model with the gradient model to establish PCP across the wing epithelium (Fig 4).

Gradients of Daschous (Ds) and Fourjointed (Fj) have also been proposed to orient planar polarity in the wing (reviewed in [50]), although Ds uniform expression rescues the PCP defect of *ds(-)* [51] demonstrating that Ds gradient *per se* is not required for PCP (to my knowledge the equivalent experiment with Fj has not been reported). Nonetheless the Fz homophobic signal can be reconciled with the proximal-distal bias being established by any gradient provided

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that the said gradient affects the stability of the Fz-Fmi interaction. Stabilization of Fz-Fmi interaction distally, or destabilization proximally, suffice to bias PCP towards distal Fz localization hence tricome orientation. One implication of this integration of a gradient with a homophobic signal is that in *vang(-)* mutants there should be an overall bias towards distal Fz localization and tricome orientation, despite the swirl phenotypes. To my knowledge this prediction has not been tested.

The proximal-distal bias can conceivably be provided by means other than a gradient. One alternative possibility is a local signal from cells at the edge of the wing, either proximal or distal. The orientation could than be relayed from cell to cell through Fz-Fmi-Fmi-Vang bistable switch in a domino effect fashion. A Fz ligand would be good candidate for providing the initial bias, although such ligand remains elusive. Another simple - however speculative - hypothesis is that the initial bias can be provided by a single row of cells in the distal edge of the wing expressing Vang-Fmi but not Fz (or Fz-Fmi but nor Vang in proximal cells), much like the domineering nonautonomy.

Impressionism and PCP

One prominent feature of PCP mutants is that tricomes are oriented approximately in the same direction in neighboring cells [52, 53] - not randomly as one might expect from a loss of PCP signaling - resulting in swirly patterns reminiscent impressionistic paintings, hence gene names like Vangogh or Starrynight. This phenotype suggests that in PCP mutants cells are still capable of influencing their neighbors orientation, however PCP is imprecise and less robust than in wild-type. This is accounted for by the Fz homophobic signal hypothesis as long as Fz and Fmi are is still active, the prediction is that Fz is still capable of signaling through Fmi in the absence of other PCP signaling components. Notwithstanding, an important implication of the model, given Fmi provides the instructive signal, is that in a *fmi (0)* - null - mutant background no PCP signal occurs, hence planar orientation of one cell should be random relative to its neighbors. The same prediction is made for *fz (0) vang (0)* double mutants, as no PCP signal should be produced in this genetic background either. Wether a *fz (0)* single mutants would behave like *fmi (0)* or like the other PCP mutants depends on wether Vang also produces a homophobic signal or not, presumably *fz (0)* should be like *fmi (0)*. This defines two fundamentally different types of PCP mutants, thus one would expect two classes of PCP potentially distinguishable phenotypes: a stochastic tricome orientation in *fmi(0)* (and maybe *fz(0)*) mutants as well as *vang(0) fz(0)* double mutants, contrasting with the "impressionistic" phenotype of other PCP mutants. To my knowledge, no such difference is reported in the literature. It has been proposed, according to mathematical models, that stochastic tricome orientation can produce "impressionistic" patterns

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[52]. It is therefore plausible that both inaccurate PCP signal and actual lack of PCP signal would produce the similar patterns, explaining why phenotypic differences between the two types of mutants could have been missed if they are at all detectable. Alternatively, appropriate quantitative and statistical methods to describe planar cell orientation could provide means to discriminate between the two - stochastic and "impressionist" - phenotypes, something that the qualitative descriptions reported in the literature may have overlooked.

Conclusion

In summary the Fz homophobic model for PCP signaling (Figure 4) described here proposes the following: 1) The primary signal conveying directionality to PCP is a nonautonomous homophobic signaling by Frizzled (Fz): Fz protein at the cell junction repels Fz itself from the cortex of adjacent cells. 2) Atypical cadherin Flamingo (Fmi) provides the instructive signal through interaction with both Fz and Vangogh (Vang). 3) Fz and Vang compete for Fmi binding intracellularly at the cell cortex leading to a "tug of war" type mechanism. 4) Disheveled (Dsh), Prickle (Pk) and Diego (Dgo) are not required for PCP signal *per se*. They function cell autonomously to establish intracellular Fz and Vang mutual exclusion, driving their localization at opposing sides of the cell cortex. Importantly a recent article reports theoretical modelling of PCP where one scenario consistent with the Fz homophobic model is tested and meets the criteria for viable PCP signaling [54].

The Fz homophobic signal model provides a framework for integration of cell polarization and spatial information in the absence of a canonical ligand to receptor signaling pathway. This type of mechanism can potentially be used by any cell adhesion molecules, and may be widespread beyond the core PCP pathway.

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