

PRIMER

Planar cell polarity: two genetic systems use one mechanism to read gradients

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ABSTRACT

Our aim in this short Primer is to explain the principles of planar cell polarity (PCP) in animal development. The literature in this small field is complex and specialized, but we have extracted a simple and central story from it. We explain our hypothesis that polarity, initially cued by the direction of slope of a multicellular gradient, is interpreted at the cellular level so that each cell becomes molecularly polarised. The mechanism involves a comparison between a cell and its neighbours. To achieve this comparison there are (at least) two disparate and independent molecular systems, each depending on molecular bridges that span between neighbouring cells. Even though the two systems are made up of different molecules, we argue that both systems function in a logically equivalent way.

KEY WORDS: Dachshous, Fat, Four-jointed, Frizzled, Starry-night, Van Gogh

'Polarity is in my mind the essential problem. We have to explain why one part of a cell becomes different from another part.'

Sydney Brenner (1994)

What is planar cell polarity and why is it important?

Imagine how tricky it would be to build a house without knowing how to orient the components. So it is with building embryos; as they construct organs, cells need access to information about polarity. Since embryos are largely made from epithelia, cells must define not only what is up and down (apicobasal polarity), but also sense their orientation (e.g. which way is anterior?) within the plane of the epithelial sheet. This latter kind of polarity is called planar cell polarity or PCP (Nübler-Jung, 1979). Research on PCP began in the middle of the last century with transplantation experiments (Wigglesworth, 1940; Piepho, 1955; Locke, 1959; Lawrence, 1966; Stumpf, 1966; Nübler-Jung, 1979), but nowadays includes standard genetics, molecular biology and genetic engineering. Even so, understanding PCP has gained relatively little attention from embryologists, especially if you compare that with the huge effort to work out how cells read their position in the embryo. Certainly, cells need to know where they are in a growing organ in order to determine what to build, but they also need to know in which direction to do things. Limbs must grow out in the right orientation, cells must define which way to migrate, to project axons or orient the active beat of cilia. It is commonplace to find that key genes and

mechanisms of development are conserved widely, and this is also the case with PCP; indeed, there is persuasive evidence that homologous genes and mechanisms operate in both flies and vertebrates. For example, precise orientation of the stereocilia within the vertebrate ear is vital for hearing and balance, but it is analysis of oriented structures in the fruitfly that have hinted at how this is achieved (Jones and Chen, 2007).

Our aim in this short Primer is to describe the surprising and elegant mechanism that evolution has fashioned to orient cells precisely, to build PCP. Since most knowledge has been gained from work on insects, particularly *Drosophila*, we stay there. The fruit fly has proved to be the best model system, not only because of our ability to identify and study fly genes. The key to understanding how PCP works is analysis of interactions between neighbouring cells of different genotypes. In *Drosophila*, the methods for making such genetic mosaics during development are unsurpassed. In order to make the big picture simple and accessible, we describe largely one part of the fly (the abdomen). However, we propose that the rules and logic we explain are universal and apply to other parts of the fly, and also to other multicellular organisms – even though the polarity outputs may vary. For simplicity, we include only a selection of references, but see other reviews for different model systems, more detail and a variety of opinion (Klein and Mlodzik, 2005; Lawrence et al., 2007; McNeill, 2010; Bayly and Axelrod, 2011; Eaton and Julicher, 2011; Goodrich and Strutt, 2011; Devenport, 2014; Butler and Wallingford, 2017).

Drawing an arrow

First let's define the field. PCP is contextual; cells need access to a vector that relates to the organism or to an organ being built. Where is the head, where is distal? Thus, PCP is not a mechanism for making something; it is a device to orient an arrow. A comparable image could be a compass needle reading a magnetic field; if so, what, in a sheet of cells, corresponds to the magnetic field? Long ago, several workers (Locke, 1959; Lawrence, 1966; Stumpf, 1966) proposed, on the basis of experimental results, that both positional information and PCP are specified by the scalar values and slopes of morphogen gradients, perhaps concentration gradients of secreted molecules. Versions of this hypothesis have survived up to now. Later, when these morphogen gradients were identified (and they do consist of secreted molecules such as Dpp, Hedgehog or Wnts) they were found to drive the transcription of secondary gradients of other molecules, rather than to act directly on PCP. Examples of these other molecules are Dachshous (Ds) and Frizzled (Fz), neither of which is secreted. As you will see, we believe it is the slopes of these secondary gradients that draw the arrow that specifies PCP (Struhl et al., 1997). Quantitation of these secondary gradients is still awaited. But, even so, their existence is supported by observations on the distribution of the gradient molecules themselves or by imaging their patterns of expression (Zeidler et al., 1999, 2000; Casal et al., 2002; Yang et al., 2002; Ma et al., 2003). We have

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evidence that Hh signalling is directly responsible for the expression of components of these secondary gradients (Casal et al., 2006). And since Hh acts as graded morphogen, transcription of these PCP components should vary with the level of signalling received. Nevertheless, our lack of knowledge about the molecular concentrations of the PCP gradients need not impede us here, we can simply envisage gradients of ‘activity’, without specifying precisely how they are generated (but see legend to Fig. 1).

PCP does not usually show itself; so it is very helpful that some epithelia of *Drosophila* have oriented structures such as cell hairs or bristles that reveal planar polarity. PCP is coordinated; typically, fields of cells show a consistency of orientation that suggests some pervasive control. There are two related questions to address: first, who reads the gradient, a single cell or a group of cells? You will see below that the answer is a mixture of the two: a cell acts with its immediate neighbours to read the gradient. Second, how is the gradient read?

How is the gradient read?

In the fly, we know of two different machines that read gradients to define PCP; each is made up of a different set of molecules. They are referred to as the Dachous/Fat (Ds/Ft) and Starry night/Frizzled (Stan/Fz) systems. We have argued that these two machines work in similar ways and use the same overall logic (Struhl et al., 2012), as follows.

There is an ‘activity’ gradient of a molecule (G) in the field of cells that is aligned in the anteroposterior axis. In principle, this activity gradient could simply be a gradient in the concentration of G. However, in practice, the shape and scale of the gradient will also depend on the distribution of other molecules that modulate G’s activity. The gradient is maintained by cell interaction as the cells increase in number. ‘PCP bridges’ that span between one cell membrane and its neighbour are the keys to this process; each bridge consists of an asymmetric dimer made of specialised protein molecules. Each dimer has one monomer made and located in one cell, and the other made and located in the abutting cell, with the two extracellular domains of the proteins forming the intercellular span. The deployment of PCP bridges in each cell is a direct consequence

of the slope of the gradient of G, and this is achieved because the orientation and distribution of the bridges in one cell is the outcome of a difference in the activity of G in its immediate anterior neighbour when compared with that of its immediate posterior neighbour. In its simplest and evolutionarily oldest form, the model might have incorporated only G and made homodimers whose asymmetric distribution would have been driven by the gradient of expression. But in Fig. 1 we show a derived form of the model in which each bridge consists of one molecule of G joined to a molecule of the partner of G.

Thus, consider any particular cell *a*: if the activity of G is higher in the cell anterior to *a* than the one posterior to it, more molecules of the partner of G settle in the anterior membrane of cell *a*, while more molecules of G accumulate in its posterior membrane. It is important that these molecules become stabilised in the cell membrane of one cell only when they are bound to partner molecules in the next cell. This mechanism might only produce small differences across the cell but these could be amplified to make the system more robust.

Then cell *a* can read the gradient and draw the arrow ‘simply’ by comparing the amounts of G and/or the partner of G lodged in its two membranes (although we think that this is what each cell does, it is not clear how this comparison is made, but see later). It follows automatically from this setup that a change in the distribution of bridge components in one cell will directly affect the distribution and orientation of bridges in the next cell, and also to the one beyond, propagating polarity further (Fig. 1, Fig. 2C).

Now we will describe the two systems, discussing only the three molecules that are essential to each reading machine. Other molecules (e.g. Dishevelled, Prickle, Diego and Dachs) have been implicated but, in our opinion, are best set aside for the time being because their roles are unclear. Also, they are dispensable to understanding the central mechanism of PCP.

The Dachous/Fat system

In the Ds/Ft system, three interacting proteins build the gradient-reading machine. Ds is equivalent to protein G as described above;

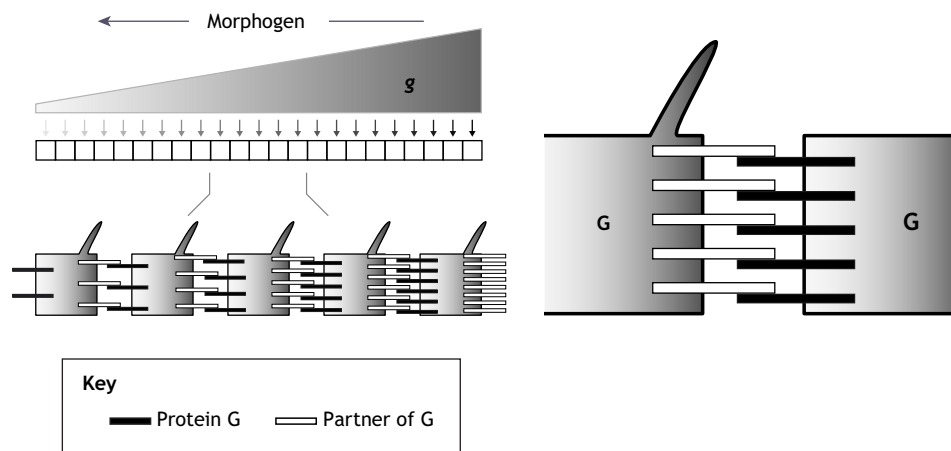


Fig. 1. A general model for the gradient-reading mechanism. This model shows the setup of the PCP bridges with respect to a gradient of expression of the gene *g* (upper left); each PCP bridge consists of a protein, G, located at a cell membrane linked to partner of G (lower left), a protein located at the membrane of a neighbouring cell. We oversimplify: in reality intercellular boundaries will be bridged by heterodimers in opposite polarities, and in this and subsequent figures we show only the net configuration of heterodimers. For PCP in general, it is not clear if what matters is the intracellular distribution of protein complexes per se or their asymmetric activity – indeed, these two features may not be linked. This diagram imagines the distribution and polarities of bridges to be biased by an activity gradient of G (in this case the activity of G in each cell is a direct consequence of its expression level). G and partner of G are stable at the surface only when part of an intercellular bridge. Polarity is read out as an oriented hair that is placed posteriorly in the cell. The model as shown is specific to the anterior compartment of the abdominal segment of the fly; the deployment of genes and morphogens can be different in the posterior compartment and in the wing, but the same principles apply. In all figures anterior is towards the left.

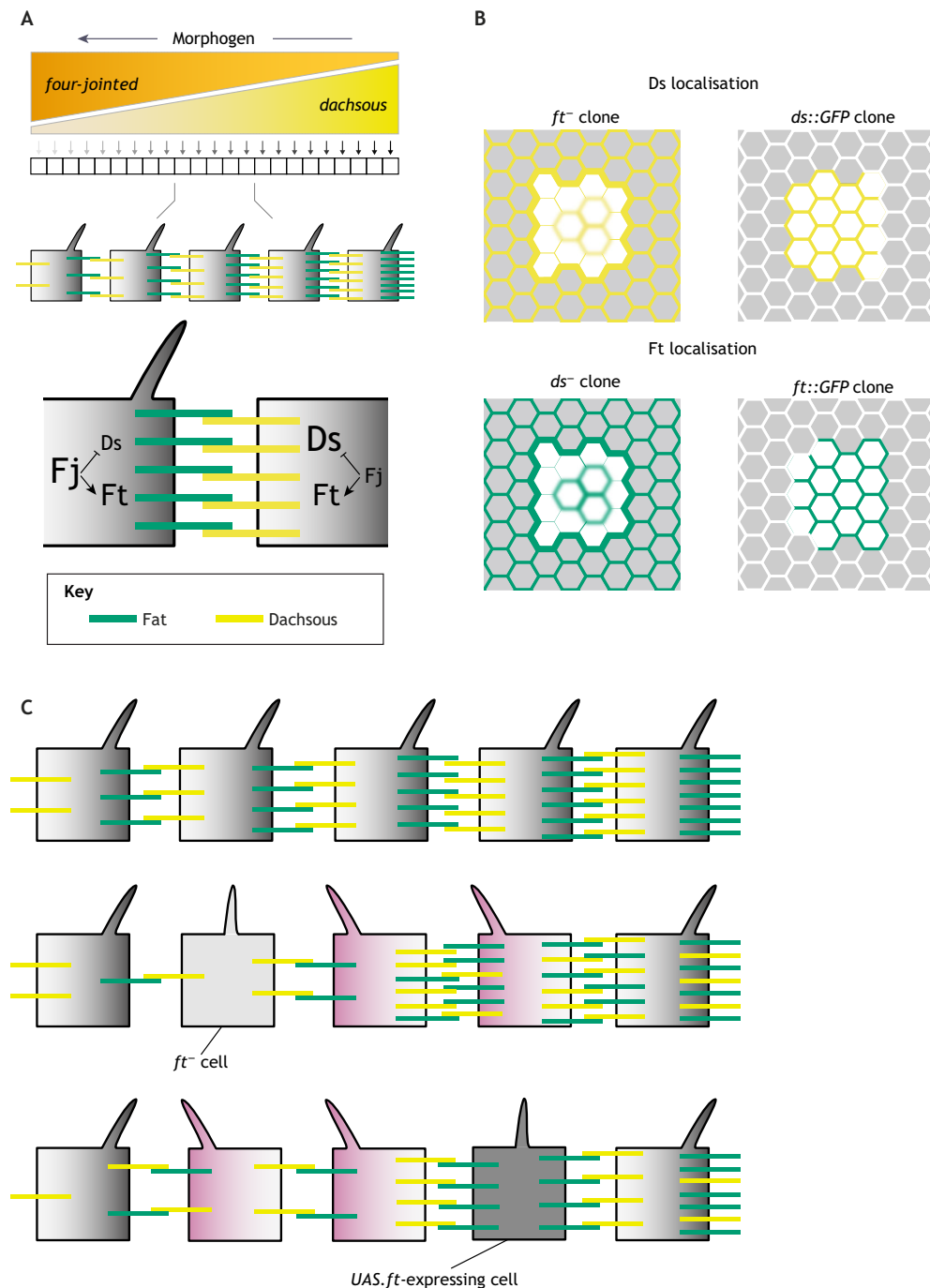


Fig. 2. The Dachsaus/Fat system. (A) Gradients of expression of the *four-jointed* (*fj*) and *dachsous* (*ds*) genes, driven by morphogens such as Hedgehog, are translated into the asymmetric distribution of Ds/Ft bridges within each cell and coordinate polarity of all the cells. Fj interacts with Ds and Ft, as shown, resulting in a gradient of Ds activity across the compartment, highest at the posterior. To fix the polarity of a cell, the numbers of bridges in its anterior and posterior membranes are compared. The cell's posterior membrane has more Ft, the anterior more Ds: consequently, the hair made by each cell points to the neighbour that presents the most Ds. (B) Clones of cells of various genotypes show that Ds and Ft link to form heterodimeric bridges from cell to cell *in vivo*. The top row shows the localisation of Ds. The cells surrounding the clones are wild type. Consider a *ft⁻* clone; obviously there is no Ft within the clone; however, Ds within the clone can form dimers with Ft outside it and thus Ds is seen to localise strongly at the clone perimeter, leaving very little free unpaired Ds in the outer cells. Deeper inside the clone there is some free Ds. The equivalent logic applies to the localisation of Ft around the *ds⁻* clone shown in the bottom row. The second column shows clones that express tagged forms of Ds or Ft in wild-type flies; the localisation of Ds (actually *ds::GFP*) is as expected and is found to be predominantly on the anterior face of a cell; Ft (actually *ft::GFP*) is found predominantly on the posterior face (after Ma et al., 2003; Brittle et al., 2012). (C) Top: the Ds/Ft gradient in a wild-type tissue. Hairs in each cell point towards the neighbour presenting the most Ds. Middle: a *ft⁻* cell can present only Ds to its neighbour, and its posterior neighbour will therefore have only Ft on the facing membrane, changing its polarity. This will lead to a surplus of Ds on that neighbour cell's posterior membrane, which will attract relatively more Ft from the next neighbour cell, thus propagating the reversal of polarity (cells with reversed polarity are shown in magenta). Bottom: a cell with excess Ft will present large numbers of Ft molecules to its neighbours, attracting Ds to the facing membranes, making the neighbours strongly asymmetric and changing the polarity of one neighbour as shown. The surplus of Ft on that neighbour's anterior membrane will propagate the reversal of polarity to the next cell (Casal et al., 2006).

its pertinent ‘activity’ is its propensity to bind its partner molecule, Fat (Ft), in the neighbouring cell. Ds and Ft are large atypical cadherin molecules (Mahoney et al., 1991; Clark et al., 1995); it is thought that Ds is distributed in reflected gradients in the anteroposterior axis of the abdomen (Casal et al., 2002). A molecule of Ds in one cell and one of Ft in the neighbouring cell can form a heterodimeric bridge; the two extracellular domains binding to each other (Matakatsu and Blair, 2004) and thereby stabilising the molecules in the membrane of each cell (Ma et al., 2003). The third protein, Four-jointed (Fj), is also graded (Zeidler et al., 2000; Casal et al., 2002) and there is evidence that it modifies the activities of both Ds and Ft, making the machine more robust (Brittle et al., 2010; Simon et al., 2010; Hale et al., 2015). The overall distribution of Ft is unknown, although it is not thought to be distributed in a gradient. This system is summarised in Fig. 2. In our example, the epidermis of the abdomen, the activity gradient of Ds rises to a peak towards the back of each segment. Thus, a cell’s posterior neighbour presents more Ds than its anterior neighbour. Consequently, each cell will have more Ft on its posterior face than on its anterior face (Casal et al., 2006; Brittle et al., 2012). The readout of this asymmetry is concrete: hairs point posteriorly – up the Ds gradient.

Many experiments have helped us understand how the Ds/Ft system works. A useful approach is to generate, in the developing fly, a clone of marked cells of a different genotype from the background and to consider the localisation of the two proteins at the cellular interface. If a clone of cells lacking Ds is made within a field of wild-type cells, then Ds in a neighbouring wild-type cell can join with the Ft present in the clone to make heterodimers. Likewise, if a clone of cells lacking Ft is made within a field of wild-type cells then Ft from a neighbouring cell binds to the perimeter of that clone (Fig. 2B). But when Ds and Ft are missing in a clone of cells, neither Ft nor Ds from the neighbouring cells accumulate at the edge of the clone (Ma et al., 2003).

Now consider the bridges and their effects on polarity. A cell lacking all Ft and able to present only Ds to its wild-type neighbours reverses the polarity of the neighbour immediately behind so that it now points its hair anteriorly and towards the *ft* cell (Fig. 2C). Consistently, therefore, a cell expressing extra Ft changes the polarity of its neighbours so that all the hairs point away from that cell. This cell presents mostly Ft to both its neighbours, so each of these has more Ds (and proportionally less Ft) on the membrane abutting the cell than on the membrane opposite (Fig. 2C). These experiments show that the orientation of a cell, as displayed by the pointing hair, is defined by a comparison between neighbours. The cell points its hair away from the neighbouring cell that has the most Ft and towards the neighbouring cell that has most Ds (Casal et al., 2006).

These experiments also show that the clone can change polarity not only of those cells in direct contact but, in addition, those beyond that neighbour – a propagation of polarity that is a direct consequence of the mechanism, without any further embellishment. Consider the cell neighbouring a clone that has excess Ft, much or most of the Ds protein in this cell is attracted to the membrane abutting the clone, depleting it from the cell membrane on the opposite side; that membrane now presents mostly Ft to its next neighbour and this tends to change also the neighbour’s polarity (Fig. 2C). Thus, polarity changes can be propagated from cell to cell. How far they propagate will depend on many factors; one would be the degree of difference in Ft or Ds activity between the clone and its immediate neighbours – the larger the difference, the further the propagation. Another would be the stability of the extant polarity of the cells near the clone and this might depend on

the number of Ft and Ds molecules already forming PCP bridges between those cells.

It is important to remember that, as we see it, the cell is assessing the activity of Ft (or Ds), as demonstrated by its ability to bind to Ds (or Ft). That assessment is not necessarily a direct readout of concentration; it could depend also on other things, such as accessibility of, or affinity for, the partner molecule. This is where the third molecule, Fj, comes in. A clone overexpressing Fj acts like a cell with more Ft and/or less Ds (Casal et al., 2002). However, if Ft and Ds are removed from a clone overexpressing Fj then there is no polarising effect, showing that Fj acts only via Ds and Ft (Fig. 3A; Casal et al., 2006). Experiments, both *in vitro* and *in vivo*, suggest that Fj is a Golgi-resident kinase molecule (Strutt et al., 2004; Ishikawa et al., 2008) that phosphorylates the mutually interacting parts of both Ds and Ft, such that phosphorylated Ds has a lower affinity for Ft, while phosphorylated Ft shows increased propensity to bind to Ds (Brittle et al., 2010; Simon et al., 2010). One simple experiment argues that Fj makes the system more stable, more difficult to change. If a clone of cells that contains extra Ft is made in a wild-type background, it alters the polarity of cells within about two or three cell diameters. The same clone in a fly that otherwise lacks Fj has a massive effect, with altered polarity spreading up to 10 cell diameters (Fig. 3B; Casal et al., 2006). Hale and colleagues (2015) have suggested how Fj makes the Ds/Ft system more robust.

Up to now we have explained how a gradient can be ‘read’, i.e. translated into the differential distribution of bridges within the membranes of individual cells. But we have not explained how whole cells becomes polarised.

The mechanism of reading facing membranes: an insight from multipolar cells

Thus, in order to orient itself and form a hair that points the right way, a cell needs to compare the amounts and polarities of the Ds-Ft heterodimers on its different faces. How might it do this? The answer is still unclear but there is a relevant hypothesis that concerns microtubules: over limited times and in specific regions of the wing imaginal discs, and also in parts of the adult abdomen, it was suggested that the Ds/Ft system might orient microtubules that could then direct intracellular traffic of other PCP components (Harumoto et al., 2010; Sharp and Axelrod, 2016). However, it is difficult to apply this hypothesis generally because, in other parts of the wing and abdomen, there is no correlation between microtubule orientation and PCP (Harumoto et al., 2010; Sharp and Axelrod, 2016).

Other useful information comes from the *Drosophila* larva: in the larval epidermis, PCP depends almost entirely on the Ds/Ft system (with little or no input from the Stan/Fz system). The principles worked out for the Ds/Ft system in the adult apply to the larva, even though the pattern is made up of relatively few cells. In the larva each epidermal cell is responsible for the formation of several denticles. Individual rows of denticles point forwards or backwards according to their position. This pattern appears to depend on two stripes of specialised cells that express high levels of Fj and are therefore low in Ds and high in Ft activity; consequently, denticles adjacent to each of these stripes point outwards from them: the rows in front pointing forwards; and the rows behind backwards (Fig. 4A; Saavedra et al., 2016).

In the larva, it is surprising and informative that, occasionally, in the wild-type, individual cells are multipolar; one region of a cell points its denticles forwards and another region of the same cell points its denticles backwards. This is due to the multipolar cell having two different neighbours on its posterior side. One part of the

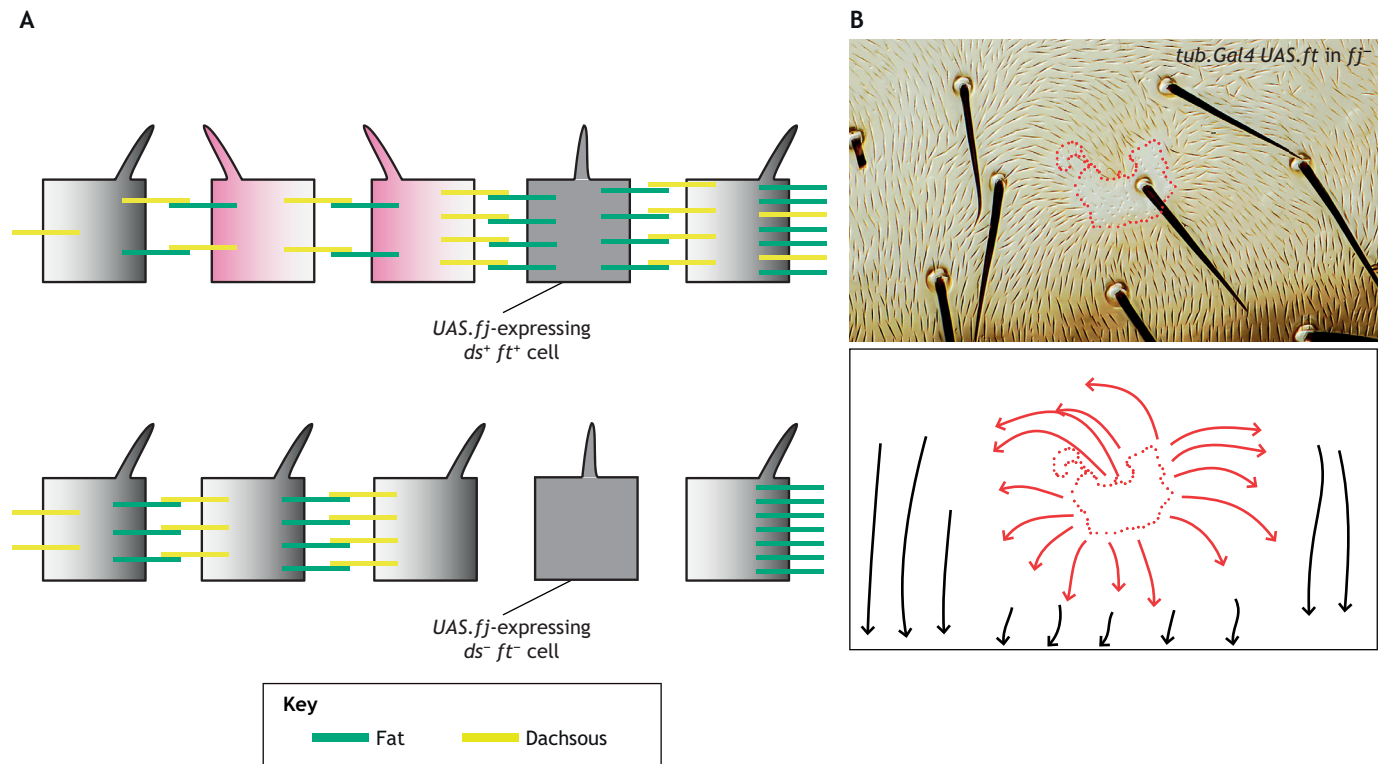


Fig. 3. Fj acts via Ds and Ft. (A) Top: when extra Fj is present in a wild-type cell (*UAS.fj*) it acts like a cell overexpressing *ft* and repolarises cells anterior to it as shown. Bottom: overexpression of *fj* in cells that lack Ds and Ft has no effect because no PCP bridges can be formed. Neighbouring cells are polarised normally by other wild-type cells around (Casal et al., 2006). (B) A clone overexpressing *ft* in a *fj⁻* fly. The clone, outlined by red dots, is genetically marked so that, instead of proper hairs, each of its 10 cells makes tiny protrusions of cuticle. The polarity of many cells surrounding the clone is affected: their hairs point away from the clone. The orientation of the hairs is indicated below.

multipolar cell abuts posteriorly a similar cell to itself, while the other part abuts a specialised cell (T2) that makes a large amount of Fj and therefore has high Ft activity (Fig. 4A; Rovira et al., 2015). To explain this phenotype, we imagine a channel or a conduit that carries information allowing limited regions of facing membranes to be compared. This exchange of information must be confined or channelled within the cell, as otherwise information from one region would spread and interfere with the adjacent part of the same cell contemporaneously making its separate comparison. This clearly does not happen. However, we do not know the nature of these hypothetical conduits of information.

The Starry night/Frizzled system

Like the Ds/Ft system, the Stan/Fz system is based on only three key molecules. The Stan molecule (also known as Flamingo) is a chimaeric protein with an extracellular domain that, like Ds and Ft, includes cadherin repeats; it contains also a seven-pass transmembrane domain receptor (Usui et al., 1999). Two Stan molecules, one from each cell, form a homodimer that bridges between neighbouring cells (Usui et al., 1999). Fz is a transmembrane receptor protein that is perhaps better known as a Wnt receptor (Bhanot et al., 1996). But regarding its function in PCP, we think that the Hedgehog signalling pathway (Casal et al., 2006) ensures that the activity of Fz is graded in the anteroposterior axis of the segment (Fig. 5A and legend; Lawrence et al., 2004). Evidence for this gradient is indirect: first, the experiments of Adler et al. (1997) provide *in vivo* evidence that hairs can be reoriented by an induced gradient of *fz* expression in the wing – they point down that induced gradient. Second, the behaviour of cells near to clones in the abdomen that lack or overexpress Fz suggest that hairs point from

cells with more Fz activity to cells with less, i.e. down a Fz gradient (Lawrence et al., 2004). These findings allow us to picture the wild-type gradient of Fz activity in the abdomen: because all the cell hairs in each abdominal segment point posteriorly, the gradient should be monotonic and extend to all the hairy parts of the segment. Therefore, it should peak at the front of the anterior compartment and decline to near the back of the posterior compartment. One would expect the Fz gradient to be reiterated from segment to segment.

Fz combines with Stan to make a complex molecule Fz.Stan in one cell that prefers to associate with a free Stan molecule (unattached to Fz) in the next cell and form an asymmetric PCP bridge: Fz.Stan-Stan (Lawrence et al., 2004; Strutt and Strutt, 2008; Struhl et al., 2012). These bridges behave in an equivalent way to Ds-Ft bridges in that each cadherin molecule anchors its partner in the membrane of the adjacent cell (Strutt and Strutt, 2008). Consequently, the amount of Stan anchored to one cell membrane is a measure of the amount of Fz.Stan in the abutting cell; using these readouts, each cell can compare the activity of Fz in its anterior neighbour with that of its posterior neighbour (compare Figs 5A and 2A). The third molecule, Van Gogh (Vang) is a multipass transmembrane protein (Taylor et al., 1998; Wolff and Rubin, 1998) without any other obvious domains. Vang can be viewed as functionally equivalent to Fj in that it increases the propensity of Stan to bind to Fz.Stan. Thus, Vang's presence in one cell helps drive the formation and the stabilisation of Fz.Stan in the membrane of the adjacent cell – indeed it does this strongly because, without Vang in one cell, very little Fz is found at the opposing membrane of the next cell (Bastock et al., 2003; Strutt and Strutt, 2008). In spite of this strong effect, Vang is not essential for the formation of Fz.Stan-Stan bridges; just as the Ds-Ft PCP bridges can convey polarity to a

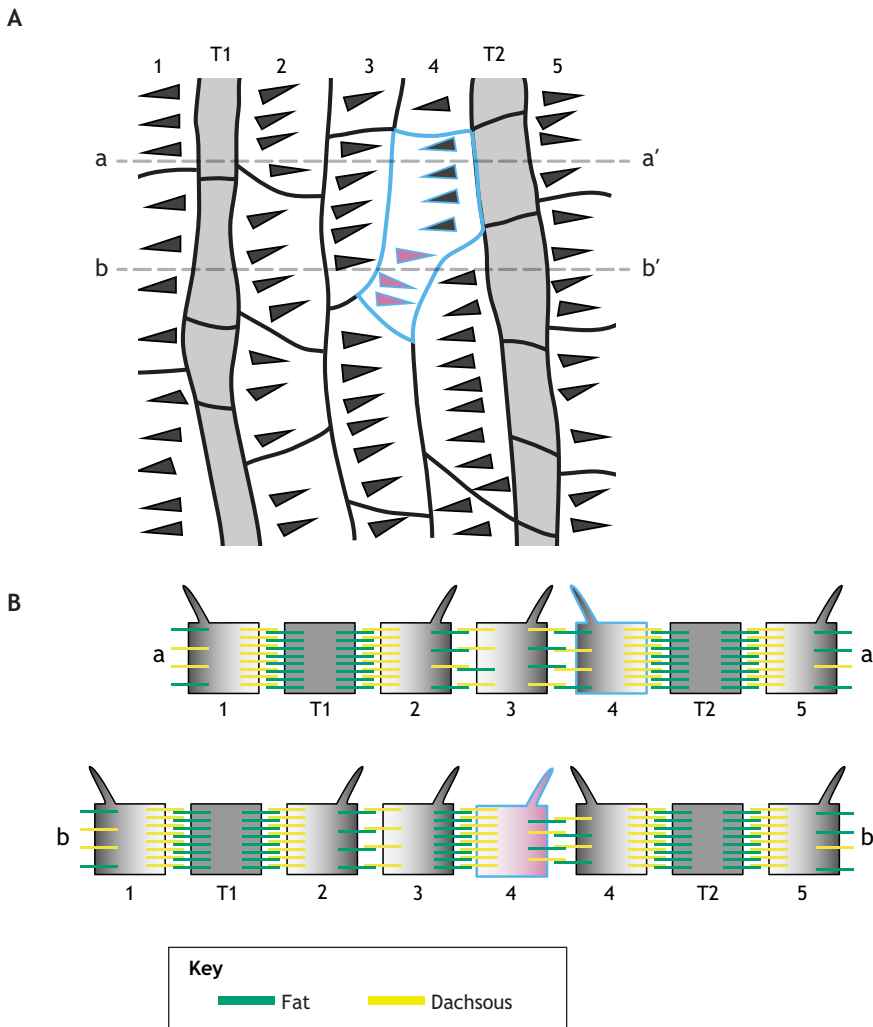


Fig. 4. A multipolar cell in the *Drosophila* larva. (A) Each cell makes several denticles and is outlined. The standard five rows of denticles are indicated (2, 3 and 5 point backwards; 1 and 4 point forwards). Two rows of muscle attachment cells, T1 and T2 (grey), contain high levels of Fj and therefore high Ft and low Ds activity (Saavedra et al., 2014). They present an excess of Ft to adjacent cells and thereby polarise neighbours as shown. Blue outlines a multipolar cell and its denticles; pink highlights the region of the cell where polarity has been reversed. Pink is also used to indicate reversal of polarity in other figures. (B) Cross-sections between a and a' and b and b' of the multipolar cell are illustrated below. These multipolar cells tell us unequivocally that polarity of a cell can be determined locally within the cell and that PCP can be subcellular (Rovira et al., 2015).

neighbour cell without Fj, so can the Fz.Stan-Stan bridges function without Vang (Struhl et al., 2012). Neither Fj nor Vang can function in the absence of their respective PCP bridges: overexpressing Fj in *ds⁻ ft⁻* clones or overexpressing Vang in *stan⁻* clones has no effect on the polarity of cells nearby (Casal et al., 2006; Struhl et al., 2012).

As with Ds and Ft (Fig. 2B), there is a mutual dependency in accumulation of Vang and Fz proteins – as shown by antibodies or tagged proteins (Fig. 5B). Clones also behave equivalently in the two systems: clones that contain either no Fz or a large amount induce the same polarity changes as clones that lack or overexpress Ft. In both systems, the polarity effects are due to changing the orientation and numbers of PCP bridges that link the clones to the abutting wild-type cells. Thus, in both cases, overexpression of Ft or Fz causes polarity reversal in the cell's anterior neighbour while its posterior neighbour's polarity is not altered – and is actually reinforced (Fig. 5C, compare with 2C).

Note how, in clones, overexpression of one component of a bridge gives the same phenotype as loss of expression of its partner (Fig. 6) – thus overexpression of Ds (or Vang) gives the same phenotype as loss of Ft (or Fz). This symmetry is found because, in both systems, the behaviour of the PCP bridges is equivalent: a cell containing an excess of Ft (or Fz) presents predominantly Ft (or Fz.Stan) to the neighbouring cells and thus attracts Ds (or Stan plus Vang) to the abutting membranes to form the Ft-Ds (or Fz.Stan-Stan) intercellular bridges. In both systems, the PCP bridges alone are sufficient to

initiate polarity without the supporting molecules, Fj or Vang (Struhl et al., 2012), but, in both systems, these supporting molecules make the PCP systems more stable and effective.

Looking at the Stan/Fz system with different types of clones reveals the working of this machine in more detail (Fig. 7). As we have seen, the PCP bridges allow the comparison of Fz activity between neighbouring cells. Stan is necessary in both abutting cells to build functional bridges (Lawrence et al., 2004; Chen et al., 2008). By contrast, Fz is not essential in both cells: if a cell has Fz activity and its neighbour has none, there will still be a difference in Fz activity between these two cells that can and does polarise both of them. Thus, a clone of cells that overexpresses Fz, made in a fly that lacks Fz, strongly polarises neighbouring cells to point outwards from the clone (Lawrence et al., 2004). However, and this is important, only the abutting cell is affected and polarity is not propagated further. This is because, even though Stan is present in all cells, that abutting cell, lacking Fz, cannot build Fz.Stan-Stan bridges connecting it with the next cell further into the clone. Thus, propagation cannot function in the complete absence of Fz (or Stan), illustrating again that these two molecules are indispensable components of the PCP bridges (Fig. 7; Struhl et al., 2012).

The outcome is different when a clone of cells overexpresses Fz in a fly that lacks Vang. Here, the neighbouring cell is polarised to point away from the clone, but now polarisation propagates further beyond the first cell to affect several more

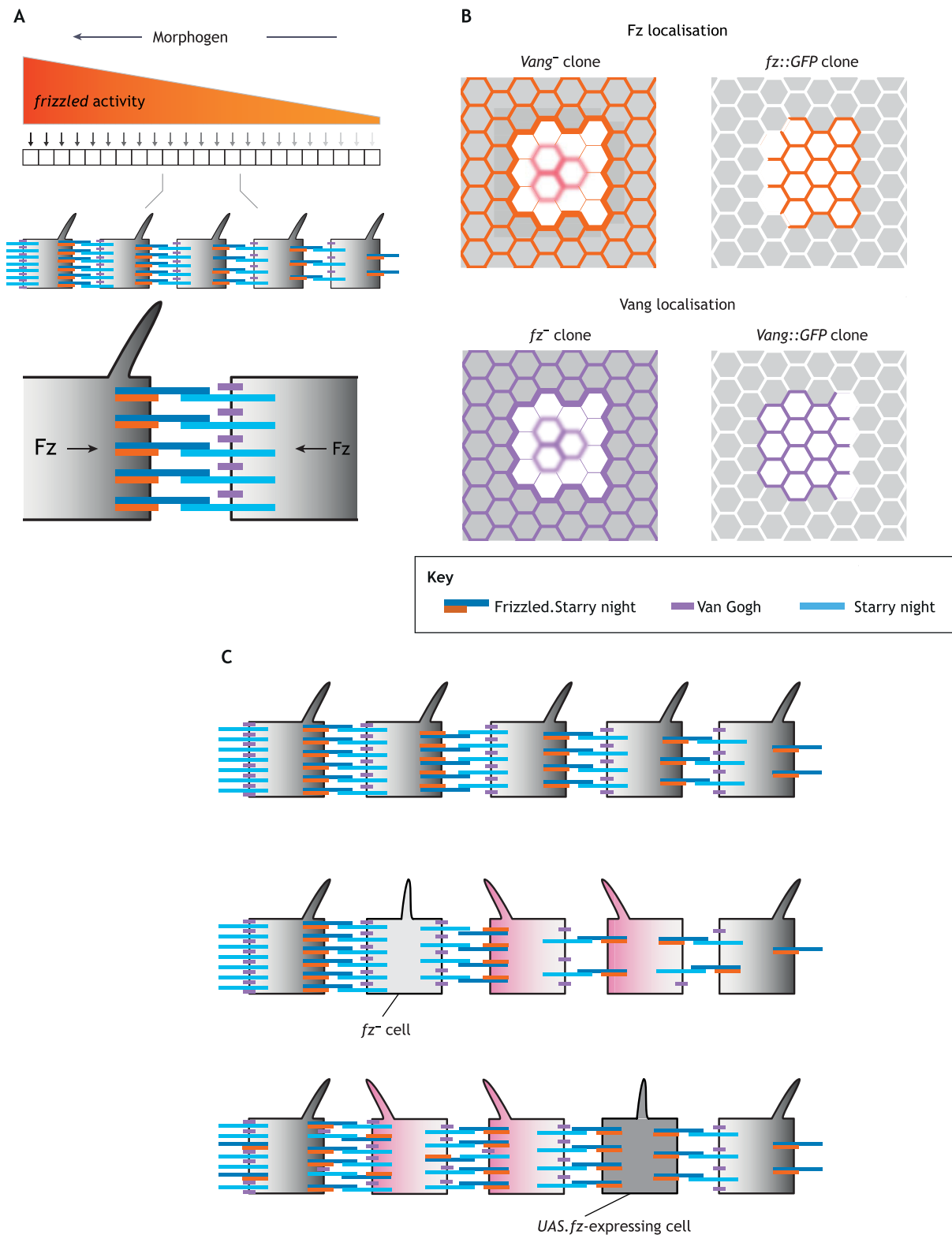


Fig. 5. The Starry night/Frizzled system. (A) We propose that a gradient of Frizzled (Fz) activity is translated into the distribution of Fz.Stan-Stan bridges and thence, perhaps via amplification, to the coordinated polarity of cells. Each cell has more Stan on its anterior membrane and more Fz-Stan on its posterior membrane. Van Gogh (Vang) promotes the formation of these bridges and accumulates on the Starry night (Stan) side of the intercellular bridge. The overall distribution of Vang in the segment is unknown, although it is not thought to form a gradient. Within each cell, the deployment of bridges in the anterior and posterior membranes is compared and consequently each hair points down the gradient of Fz activity, i.e. towards the neighbour that has least Fz activity. (B) Analysis of mutant clones and clones expressing tagged forms shows how asymmetrical bridges form. The upper row shows the localisation of Fz; the bottom row shows the localisation of Vang. A similar logic to that used in Fig. 2B helps us define the complementary localisations of Fz and Vang (Strutt, 2001; Bastock et al., 2003). (C) This figure is to be compared with Fig. 2C. Only the proteins are different, the logic is the same.

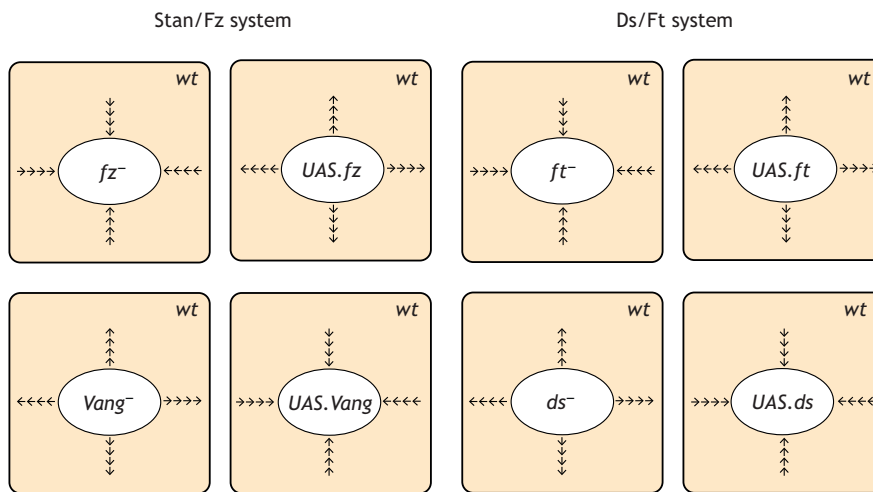


Fig. 6. A comparison of the two PCP systems. The effects of clones overexpressing (UAS) or lacking those elements mainly responsible for the asymmetry in the bridges (Ds and Ft; Fz and Vang) in wild-type flies are equivalent for the two systems. The range of the effects on polarity (in approximate number of cells) and in Fig. 7 is indicated by the number of arrows.

cells. It follows that propagation of polarity does not require Vang (Fig. 7; Strutt and Warrington, 2008; Struhl et al., 2012). Clones that overexpress Vang or Stan in flies that lack Vang polarise nearby cells (Fig. 7); this occurs because the elevated Stan activity in a cell at the edge of the clone can form many bridges with Fz.Stan in the next cell, altering the overall distribution and orientation of the Fz.Stan-Stan bridges around the clone and thereby cell polarity.

Downstream from the bridges

Other molecules and processes are necessary to build on the polarity cues made by those six molecules we have highlighted. For example, in the Ds/Ft system, one of the first molecules to respond to the nascent polarity is a myosin-related protein, Dachs (D), which becomes localised on the side of the cell where there is most Ds and least Ft (Mao et al., 2006, 2011). Clones of cells containing excess Ds (Saavedra et al., 2016), or excess Ft (P.A.L. and J.C., unpublished), fail to affect the polarity of cells that lack D, proving that D is essential for receiving and/or responding to a change in the distribution of Ds/Ft bridges.

The *prickle* gene has long been thought to be a crucial element in the Stan/Fz system and this view has persisted in spite of the

contradictory finding that the Stan/Fz system does not need it for the intercellular signalling of polarity or the propagation of polarity (Adler et al., 2000; Lawrence et al., 2004; Strutt and Strutt, 2008). Further complicating this story, there is evidence that *prickle* is engaged with the Ds/Ft system: proteins encoded by the *prickle* gene are required to interpret or ‘rectify’ polarising instructions generated by that system (Lawrence et al., 2004; Ayukawa et al., 2014; Ambegaonkar and Irvine, 2015; Casal et al., 2018).

There are several other molecules involved in PCP and some of these are also asymmetrically localised in the cell (e.g. Diego and Dishevelled); however, neither is essential for the intercellular communication of polarity (Strutt and Strutt, 2007). Nevertheless, we would like to understand the functions of these proteins.

Outstanding questions

Using diverse studies of the two PCP systems, we have built two homologous models to explain how intercellular gradients are translated into the polarities of cells. But many questions remain. One is how can cells maintain their polarity as organs grow? During mitosis, cells lose their planar polarity

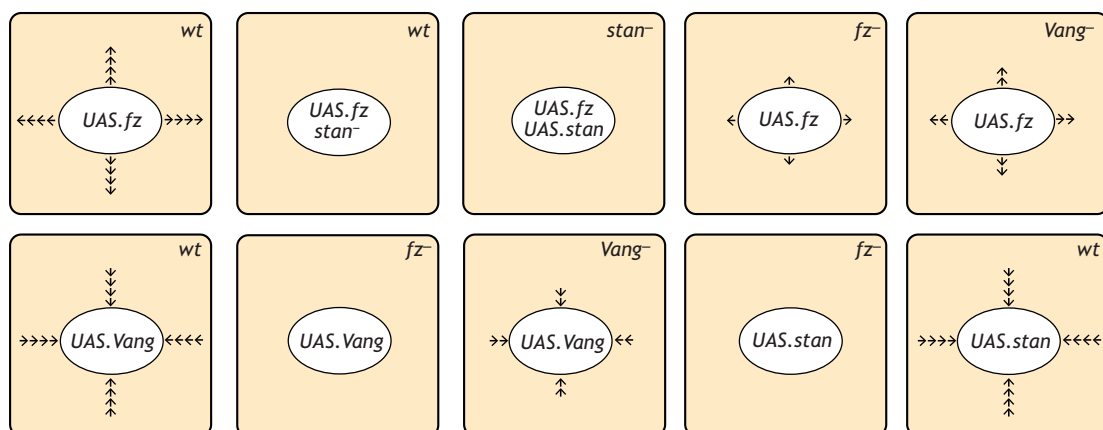


Fig. 7. The logic of the Stan/Fz system. The effects of these clones on the polarity of the surrounding cells, taken together, argue that PCP in one cell depends on a comparison between the levels of Fz present in its two neighbouring cells. In addition, that polarity signalling depends on Stan being present in both abutting cells and on Fz being in at least one of two adjacent cells. The results also argue that Vang assists polarisation and/or propagation, but is not essential; however, in its absence propagation is reduced. These results suggest that the constitution of the PCP bridges can be biased by overexpression of Stan or Vang.

(Devenport et al., 2011); but, if our models are correct, the daughter cells will renew their PCP from neighbouring cells via the bridges.

However, the models are unproven: for example, the evidence for gradients is still circumstantial and lacks detail. Over the whole field of cells, say over an abdominal segment, what are the ranges of concentration? How do the presumed activity gradients depend on interacting molecules (such as Fj and Vang)? We still do not fully understand how the gradients are initiated early in development and maintained during growth. Regarding the comparisons made between the two facing membranes of a cell, how large does the molecular difference need to be to drive polarisation of that cell? In the case of the Ds/Ft system, the maximum difference observed is near twofold (Brittle et al., 2012), but it may be smaller in other instances. What are the mechanisms that are used to make these comparisons; are microtubules or other intracellular elements involved? How are the comparisons transformed into oriented accumulations of actin and other proteins, and, maybe, polarised membrane trafficking? There are indications from *Drosophila* that this intracellular process is complex: for example, in the embryo, there are actin prehairsts that do not have the same orientation as the cuticular hairs that are made later (Dickinson and Thatcher, 1997; Saavedra et al., 2016). In the larva, the actin prehairsts originate at one cell boundary but move across the cell's apical surface as they mature (Saavedra et al., 2016).

How do the two systems work together to produce polarity? The evidence that the Ds/Ft and Stan/Fz systems can act independently is strong and has been discussed elsewhere [Lawrence et al., 2007; though see Axelrod (2009) for a different opinion]. Working out the contribution played by each system to the wild-type pattern will not be easy because both systems contribute to the same output – the orientation of cells as signalled by, for example, hairs on the abdomen or ommatidial asymmetry in the eye. Yet the cell biology of polarity is sophisticated: each hair is not simply oriented like a weathercock that responds to the net force generated by two independent but equivalent winds. Indeed, the two systems are not equivalent, they may act on PCP at different times and probably have qualitatively different outputs; this is suggested by the differing phenotypes of mutations. For example, *stan⁻* wings and abdomens, apart from misoriented hairs and bristles, appear normal. By contrast, abdomens, wings and legs lacking Ds not only have whorly polarity but are bloated and misshapen, due, at least in part, to the mislocalisation of Dachs (Ambegaonkar et al., 2012; Lawrence and Casal, 2013).

PCP is vital to the organisation of development; polarity must be precisely controlled in the embryo or organ, and it must be stable during manyfold growth. Such requirements are formidable but for the two molecular systems of PCP, evolution has fashioned essentially the same mechanistic solution. We argue that both systems are able to interpret the slope of a gradient and, in both, this function depends on molecular bridges that span from one cell to the next. Since the most crucial PCP genes are conserved as far as vertebrates and since there is no counterevidence that we know of, it seems most likely that these central mechanisms are also conserved.

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Competing interests

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