OPINION

Planar cell polarity: one or two pathways?

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Abstract | In multicellular organisms, cells are polarized in the plane of the epithelial sheet, revealed in some cell types by oriented hairs or cilia. Many of the underlying genes have been identified in *Drosophila melanogaster* and are conserved in vertebrates. Here we dissect the logic of planar cell polarity (PCP). We review studies of genetic mosaics in adult flies — marked cells of different genotypes help us to understand how polarizing information is generated and how it passes from one cell to another. We argue that the prevailing opinion that planar polarity depends on a single genetic pathway is wrong and conclude that there are (at least) two independently acting processes. This conclusion has major consequences for the PCP field.

In tribute to Sydney Brenner, who was 80 on 13 January 2007:

> *'[At that time, (ca 1964)] we tried to decompose the complexity of higher organisms into a set of subsidiary problems […] There'd be problems of how cells move […] There'd be problems of how cells grow. There'd be problems of the polarity of the cells. Which in my mind is still the essential problem; in the sense that cells move in one direction and not in another, grow in one direction, or face the world from one side of themselves and not the other. How was all this polarity established?'*[1]

Animals are largely built of epithelia, the cells of which are specified by both scalars and vectors. The scalars are in the form of positional information that tells cells where they are located along the axes of the body; they use this information to decide where to differentiate, one from another, in order to build a pattern. But to construct a part of London, or to find one's way in a desert, plans or maps are not enough; one needs a compass or the sun for orientation. Likewise, to build a limb, individual cells need vectors to tell them in which direction to move, to divide and how to orient extensions, such as cilia, bristles or axons[2–5]. Multicellular organisms could not be built without vectors.

Over the past 110 years[6], many embryologists have clarified the mechanisms of positional information and defined morphogens, molecules that are released from localized sources to form gradients of concentration. The local concentration of the morphogen (the scalar) tells each cell its distance from the source. By contrast, relatively few researchers have studied vectors, partly because polarity is often hidden and imperceptible. Although a latent polarity can sometimes be revealed by experiments[7], some cell types openly display their polarity by the orientation of hairs or cilia, a property called planar cell polarity (PCP)[8–11].

PCP is being intensively studied — but there are so many genes, experiments and contrasting models that the field is perplexing, even for the insiders. Our purpose here is to reach the outsider by looking for a common logic of mechanism, rather than emphasising diverse outcomes. Because the insect integument is fundamentally a monolayer of cells that form oriented structures such as bristles, and because of 100 years of investment in its genetics, *Drosophila melanogaster* is the best model system for this purpose. However, the results from vertebrates, particularly from the molecular genetics of convergent extension, the stereocilia of the ear, mammalian hairs and the orientation of axon growth[12–16], argue that the mechanisms of PCP are strongly conserved, at least between flies and vertebrates. The PCP field has become dominated by the view that planar polarity is the outcome of one genetic pathway. Our recent analysis of the adult abdomen of the fly challenges this view; here we explain why this is so and the resulting consequences, and argue for a new way of looking at PCP.

Morphogens act upstream of PCP

A vector is not simply a product of some biochemical pathway (see REF. 17 for a discerning definition of polarity), it must be seen in an anatomical context — what matters is where a bristle points with respect to the body axis; for example, towards or away from the head. During development, localized determinants and oriented morphogen gradients determine the scale and orientation of body axes and PCP appears to be set up as a downstream consequence. For example, in the fly wing, a clone of cells that ectopically express only the morphogen Decapentaplegic (DPP) makes a new peak in the concentration gradient, and this induces a perfect winglet of the appropriate size, pattern and PCP[18]. Mutations in *wnt11* and *wnt5*, which encode signalling molecules, affect the orientation of cell movements in the zebrafish[19,20]. Although these molecules can produce changes in PCP, the experiments that show this do not establish that DPP and/or the Wnts are components of the PCP machinery itself, a fact that is often forgotten. To understand that machinery, one needs to define its components and work out what they do; the history of developmental biology argues that the best way to do this is via genetics[21,22], the "master science of biology."[1]

A functional assay tests the PCP genes

Most of the genes that are so far known to act in the mechanism of PCP were identified in *D. melanogaster*, and they fall into two groups. Mutations in genes of the first group not only change polarity,

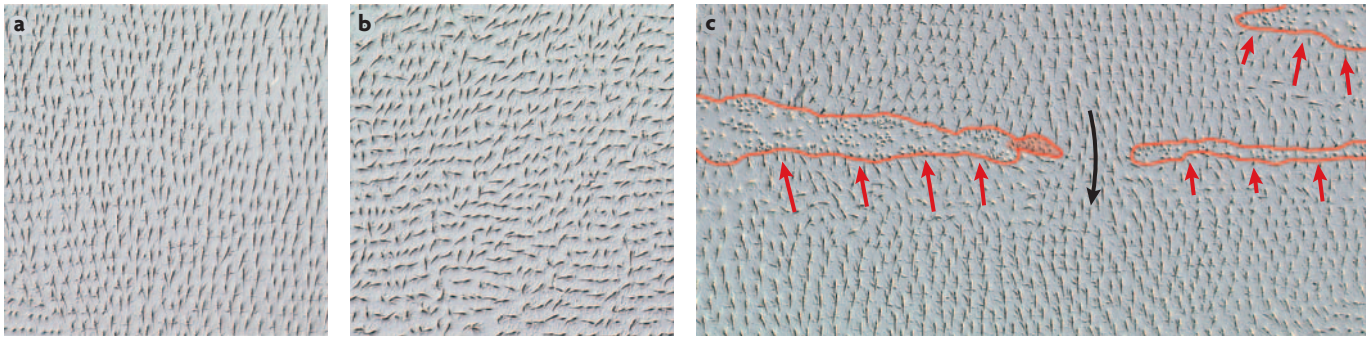

Figure 1 | **Planar cell polarity in the *Drosophila melanogaster* pleura.** **a** | In the wild type, the small cuticular hairs (several are produced by each cell) point posteriorly. **b** | In *fz*⁻ flies, the orientation is randomized. **c** | Three clones of *fz*⁻ cells in a wild-type fly, each outlined in red; the hairs that lie posterior to the *fz*⁻ cells, formed by about three rows of cells, mostly point anteriorwards (indicated by red arrows), but in the strait between two nearby clones polarity is normal (indicated by a black arrow). The *fz*⁻ cells are genetically marked, meaning that each cell in the clone secretes cuticle that is distinct and, in this case, each cell makes numerous hairs that point upwards (one such cell is filled in orange).

but also alter the shapes of wings and legs and can disturb growth. We limit our discussion to three members of this group: *fat (ft)*, *dachsous (ds)* and *four-jointed (fj)*. The second group includes mutations that disturb cell polarity but have little if any effect on pattern. To simplify, we discuss only genes that are central to the process: *dishevelled* (*dsh*), *frizzled (fz)* (FIG. 1), *prickle (pk)*, *Van Gogh* (*Vang*; also known as *strabismus* (*stbm*)) and *starry night* (*stan*; also known as *flamingo* (*fmi*)) (TABLE 1).

How do these genes organize PCP? To help answer this question, we introduce the fly abdomen into the current picture of PCP (which is presented in REF. 10). We rely on a functional assay that springs from the finding that clones of mutant cells alter the polarity of wild-type cells nearby[23,24]. The beauty of this assay is that the clone and its surround can be given different genotypes by the experimenter and, in the best systems, the polarity and genotype can be monitored cell by cell. We call the cells within the clone 'sending' cells because, for simplicity, here we focus on the information that is being passed from the clone to the 'receiving' cells that surround it (of course, information can also flow in the opposite direction). Now, take a small clone of cells that lack *fz*: in both the abdomen[25] and wing[24], the clone reverses the polarity of some nearby wild-type receiving cells so that all cells point inwards (FIG. 1). Clones of cells that overexpress *fz* reverse the polarity of some receiving cells so that all cells point outwards, away from the clone (FIG. 2a). It follows that information from sending cells makes receiving cells turn to point their hairs towards cells with a lower level of FZ and away from those with a higher level[26]; typically, this effect spreads several cells into the surround.

PCP: one or two pathways?

In the current literature, there is a consensus that the main genes (TABLE 1) act in a single pathway to build PCP (for example, REFS 10,27–29). An upper tier of proteins, encoded by *ds*, *ft* and *fj* (which we call the 'DS system'), is thought to polarize and regulate the activity of a lower tier, which consists of the FZ receptor and associated proteins such as VANG and STAN (which we call the 'STAN system'). The lower tier is then thought to interact with executive proteins that are involved in making the polarized structures (such as actin)[30]. This single-pathway hypothesis has not been established as fact, but has been reiterated so often that it is becoming perceived and presented as such (for example, REF. 31). We now offer four pieces of evidence that it is incorrect, at the very least in the abdomen, in which we have done our experiments.

First, the most persuasive piece of evidence: in the functional assay, excess DS, FT or FJ in the sending cells can repolarize the receiving cells even when all of the cells, sending and receiving, lack FZ, or STAN, or both[32] (FIGS 2b,3). Thus, the genes of the DS system can drive PCP in the complete absence of the STAN system.

Second, if there were two independent systems, blocking either should have a weaker effect than blocking both. In fact,

Table 1 | **The selected molecules in mice and fruitflies**

Fruitfly gene	Gene product or function	Mouse genes	References
fj (four-jointed)	Type II membrane protein, Golgi localization	*Fjx1*	52,59–62
ds (dachsous)	Atypical cadherin	*Dchs1*	33,35,52
ft (fat)	Atypical cadherin	*Fat-j*	29,34,40,52,63,64
stan (starry night, or flamingo)	Seven transmembrane-pass domains and cadherin repeats	*Celsr1*	43,65,66
fz (frizzled)	Seven transmembrane-pass domains, Wnt receptor	*Fz3, Fz6*	15,23,24,67
Vang (Van Gogh, or strabismus)	Two (at least) transmembrane-pass domains	*Vangl2*	68–70
pk (prickle)	LIM and PET domains	*Prickle1, Prickle2*	71–73
dsh (dishevelled)	DIX, PDZ and DEP domains	*Dvl2*	15,74–76

Figure 2 | **The functional assay.** Clones (represented by yellow boxes) are made in the *Drosophila melanogaster* abdomen of a genotype (the 'sending' cells) that might alter the polarity of hairs in the 'receiving' cells (each colour represents a different genetic background of receiving cells). All diagrams are oriented so that the anterior is towards the top. Black arrows indicate normal polarity, blue arrows indicate disturbed polarity and red arrows indicate reversed polarity. **a** | Cells that lack *frizzled* (fz^-) reverse the polarity of receiving cells that lie posterior to the clone, whereas overexpression of *fz* (*UAS.fz*) reverses the polarity of cells that lie anterior to the clone. The assays show that Starry night (STAN) is needed in both sending and receiving cells. **b** | The signal emanating from cells overexpressing *fat* (*UAS.ft*) acts independently of STAN. **c** | These three assays argue that Four-jointed (FJ) acts through FT. Clones overexpressing *four-jointed* (*UAS.fj*) reverse receiving cells that lie in front, like *UAS.ft* clones (shown in part **d**), but if FT is missing, as in a *UAS.fj* ft^- clone, it behaves like a ft^- clone (reversing the polarity of the receiving cells behind the clone). **d** | These seven assays plus the ft^- clones (shown in part **c**) show that Dachsous (DS) alone is sufficient in the sending cells to affect the polarity of the receiving cells (similar results show that FT alone is also sufficient[32]), but that both DS and FT are needed in the receiving cells.

in $stan^-$ or fz^- flies (in which the STAN system is broken), hair polarity in the abdomen is only slightly disturbed. Similarly, when *ds* is removed (to break the DS system), polarity is, again, little damaged. Yet, if both systems are broken at once (ds^- $stan^-$ flies) the orientation of both hairs and bristles is mostly randomized[32] (FIG. 4).

Third, ds^- cells provide a sensitized assay for activity of the STAN system: in the absence of *ds*, clones that either lack or overexpress *fz* cause receiving cells to repolarize over a longer range than similar clones in the wild type[28,32,33]. Also, sending cells in which the level of FZ is modestly altered, which would normally have no visible consequence, now change the polarity of ds^- (or ft^-) receiving cells[32]. Therefore, if raising the level of DS (or FT) in the sending cells were to alter FZ activity in those cells, as the single-pathway model might predict, then ds^- (or ft^-) receiving cells should show increased responsiveness. In fact, sending cells that express DS (or FT) have no effect at all on the polarity of ds^- (or ft^-) receiving cells[32] (FIG. 2d). It seems that neither DS nor FT affect the STAN system of sending cells.

Fourth, when manipulated in clones, the two systems are fundamentally different — they can even have opposite effects. Assays that deploy the DS system (for example, sending cells that overexpress *ft*) behave differently in the two compartments of the abdominal segment: in the anterior compartment, polarity is reversed in the receiving cells that lie in front of the clone whereas, in the posterior compartments, polarity is reversed in the receiving

Figure 3 | **A repolarizing clone in a *stan*⁻ fly.** The clone (outlined in red) consists of marked cells expressing a modified form of Dachsous (DS) in a mutant *starry night* (*stan*) background. In spite of the lack of the STAN system, the clone reverses the polarity of cells that lie posterior to it, and also organizes normal polarity in the cells that lie anterior to it (a clone of the same genotype is shown in Figure 2f of REF. 32).

cells that lie behind the clone[34]. By contrast, clones that affect the STAN system behave in the same way in the two compartments — clones that lack *fz* always reverse the polarity of cells that lie behind the clones[25].

Some of these arguments are more persuasive than others, but together they make a strong case that the DS and STAN systems are separate pathways that contribute to PCP by different mechanisms[34].

What is the counterevidence? What are the results that support the single-pathway model? One argument came from the eye, in which it was claimed that *ft*⁻ cells can bias the polarity of ommatidia in wild-type but not in *fz*⁻ flies[29] — however, the sample size was insufficient to draw this conclusion; moreover, appropriate controls (*ft*⁺ clones in *fz*⁻ eyes) were not provided. But it has been stated that, in the wing, *ft*⁻ clones do not repolarize cells in *fz*⁻ flies[28]; also, in *fz*⁻ abdominal pleura, the hairs are randomized and do not respond to clones that overexpress an active form of DS[32]. Alongside the contrary results on the dorsal abdomen, these findings might suggest that DS acts through FZ as part of a single pathway in some organs but not in others. However, we judge this to be unlikely, mainly because fundamental processes are normally conserved and used again and again, not only in different organs of one species but also between species. We prefer a simpler explanation, an example of a 'don't worry hypothesis'[1]: perhaps the PCP of eyes, wings and pleura of *fz*⁻ flies is too disturbed for cells to be able to respond to the DS signal. This explanation fits because, in *fz*⁻ flies, the eyes, wings and pleura are much more depolarized than the tergites. Under this particular don't worry hypothesis, the DS signal would be trying to impose a polarity on cells that are in disarray; it could be likened to looking for ripples caused by throwing a stone into a rough sea.

There is another way of regarding this central issue. Our experiments in the abdomen show that the DS system has an inherent capacity to change polarity without the STAN system. So, in different organs, even if the DS and STAN systems make contributions of different weight and in different ways, we would argue that both systems must have independent (and probably qualitatively distinct) inputs into the cell biology of the characteristic that we all pay attention to — the orientation of hairs and other indicators of PCP.

If we accept that there are two pathways, two new questions stand up and shout for answers: first, how does the DS system polarize cells, and second, what polarizes the STAN system?

How does the DS system generate PCP?

In a field of cells in wild-type flies, everyone agrees that there needs to be a biasing input to orient the DS system and, most likely, this is done by morphogen gradients that drive *ds* and/or *fj* transcription. Gradients of both DS and FJ have been inferred or observed in the eye, abdomen and perhaps in the wing[29,34–37], and the orientation of both gradients influences PCP[38]. Simon's work in the eye shows nicely that the two gradients are redundant: flattening of one is insufficient to disturb PCP, whereas flattening of both randomizes polarity. Reversing the FJ or the DS gradient can even turn the ommatidial polarity around[38]. The functional assay in the abdomen suggests that FJ acts mainly on FT (FIG. 2c) and therefore, both in the wild type and in Simon's experiments, we imagine that the FJ gradient generates a FT gradient of activity. Then, the mutually opposing gradients of FT and DS must orient individual cells — but how?

Previously, this issue was addressed by asking how FT might feed into FZ; we now think that this is the wrong question to ask. Instead, we have shown that the cadherin-family proteins DS and FT can polarize cells in the absence of FZ, so the right question is: how do they do it on their own? Important experiments[28,39,40] using antibodies against the two proteins suggested that, *in vivo*, DS and FT make *trans*-heterodimers that form bridges from one cell to another (FIG. 5e,f). Also, *in vitro*, DS and FT stabilize each other across intercellular boundaries and promote adhesion between cells[41,42]. These papers suggest that DS–FT heterodimers are agents in PCP, and we have therefore built a speculative model that employs DS–FT heterodimers and is derived from the functional assays (FIG. 6). When applied to the DS system, these assays show that, in order to change polarity of the receiving cells, either DS or FT is sufficient in the sending cells, but both proteins are essential in the receiving cells (FIG. 2d,e). The findings are clear and simple but the interpretation is not.

To build a model (FIG. 6), we imagined that, in any cell, the numbers of DS molecules that are engaged in *trans*-heterodimers (with FT molecules in adjacent cells) might differ between the anterior and posterior cell faces — an intracellular asymmetry that could serve to orient the cell. If so, an altered ratio of

DS and FT in the sending cell could affect the number or distribution of DS–FT *trans*-heterodimers in the receiving cells. Thus, when a sending cell contains, say, excess FT, these FT molecules draw DS molecules to the adjoining proximal face of the nearest receiving cell to form *trans*-heterodimers. DS then becomes redistributed within the receiving cell, taking molecules away from its distal face. That face would have relatively more FT and this would, in turn, draw excess DS to the facing membrane of the next cell, thereby propagating the original signal. Note that the sending cell need only contain too much, or too little, or none at all, of either DS or FT, but DS and FT are both needed in the receiving cell for polarization of the first receiving cell and for propagation to the next[32] (FIG. 6).

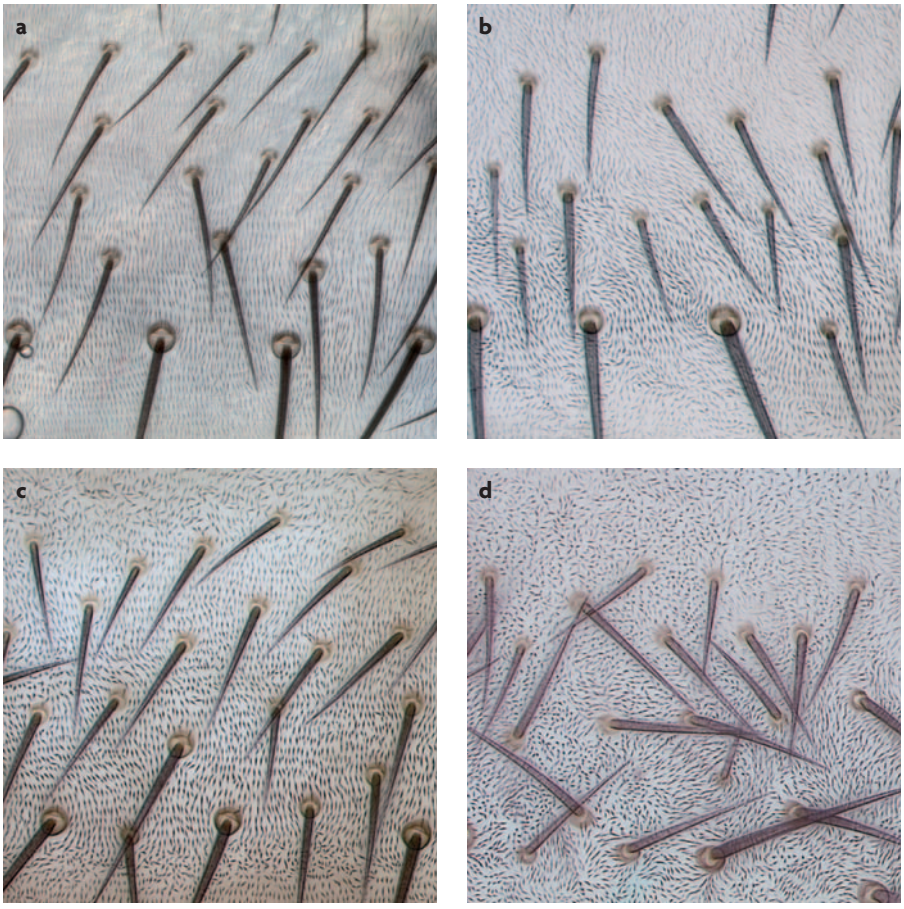

Figure 4 | **Cuticle from the dorsal abdomen of *Drosophila melanogaster*. a** | Wild-type cuticle. **b** | *ds*⁻ cuticle. **c** | *stan*⁻ cuticle. **d** | *ds*⁻ *stan*⁻ cuticle. Note the stronger randomization of hairs and bristles when both the Dachsous (DS) and Starry night (STAN) systems are broken (part **d**)[32].

How does the STAN system generate PCP?

Models derived from functional assays. There are many ideas about how FZ might mediate PCP[11]. To choose between these, we again rely on the functional assay in the abdomen. Looking at the main genes that have been implicated in the STAN system (TABLE 1), we ask: are they needed for function in the sending cell, the receiving cell or in both?

A clone that overexpresses *fz* reverses the polarity of receiving cells but, if STAN is lacking in either the sending or the receiving cells, no polarity change occurs (FIG. 2a). It seems that the signal passes across, or at least requires, intercellular bridges that are made by homodimers of STAN[25,43].

When the sending cells express *fz* in an otherwise *fz*⁻ background, the nearest *fz*⁻ receiving cell is repolarized — thus, a cell that completely lacks FZ protein can have an organized polarity, even a polarity that responds and changes[25]. This result is important because it helps rule out some models, many of which are built around FZ, and which suggest that this protein is an integral and essential part of PCP within each cell[11].

The assay shows that, if a difference in FZ activity between the clone and the surround is to be detectable, VANG is not required in the sending cells but is absolutely required in the receiving cells[25].

When the sending cells express *fz*, but both the sending and the receiving cells lack *pk*, the receiving cells are repolarized[25]. Therefore, PK is not needed for either sending or reception of the signal. This finding has been extended to the wing, in which the same conclusion was drawn for both PK and DSH[44]. Note that the proteins that are required for transmission of the signal (FZ, STAN and VANG) are transmembrane proteins, whereas those that are not required (PK and DSH) are cytoplasmic proteins.

From these data, we produced a model (FIG. 6) in which there is a gradient of FZ activity across the field, and the cells interact so that the level of FZ activity of any cell becomes modified towards an average of the levels of its neighbouring cells. To become polarized, a cell then compares the levels of FZ activity in neighbouring cells, using STAN, and points its hair towards the neighbour with the lowest value. This mathematical model is built with FZ, VANG and STAN[25]. But note, Sydney Brenner has a view of mathematical modelling: "One can do things in a very sophisticated mathematical way … but there is a difference between theories being correct and theories being true. Many theoreticians don't make that distinction, and, even though many theories are correct in the logical sense, they are untrue because they don't relate to the natural thing we're all interested in!"[1]

Models derived from asymmetrical protein localization. Currently, the most popular model of PCP is the 'Tree–Amonlirdviman model'[45,46], which is derived largely from a different set of data. The direction of the field was abruptly diverted when it was discovered that some PCP proteins are distributed asymmetrically in wing cells, at least during a short period before formation of the cell hairs[43,47–49]. For example, STAN accumulates on both the proximal and distal faces of cells (FIG. 5a), and FZ and DSH accumulate on the distal membranes[47,48] (FIG. 5c), whereas VANG and PK accumulate on the proximal membranes[46,50]. Using these facts, some assumptions and, later, a mathematical simulation that requires optimization of several parameters, the Tree–Amonlirdviman model was built to explain how localized protein interactions within and between cells might drive PCP[45,46] (FIG. 6).

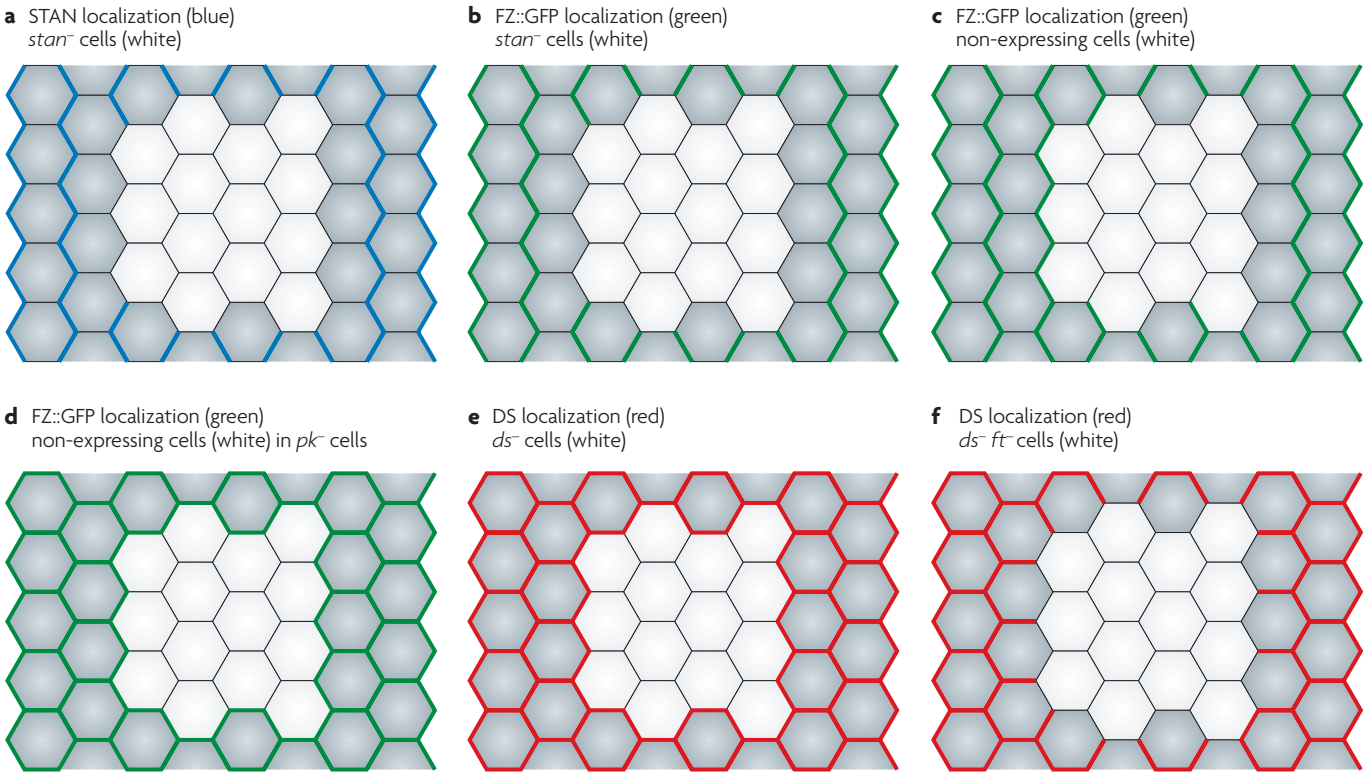

Figure 5 | **The localization of planar cell polarity (PCP) proteins in clones in the wing.** The PCP proteins can be located on particular faces of the cell membranes. Cells that lie anterior to the clone are shown towards the top of each figure, and cells that lie distal are shown towards the right. **a** | Starry night (STAN) accumulates mostly on the proximodistal faces of the cells and is not seen at all in the membrane unless STAN is present on both confronting cells[43]. **b** | Frizzled (FZ) is also seen mostly on the proximodistal faces of the cells and, if tagged with GFP, the clone shows that FZ does not go to the membrane without the STAN protein[48]. **c** | FZ is actually localized on the distal face of the cells; the white cells represent a clone of cells that lack the tagged FZ[48]. **d** | In cells that lack *prickle* (*pk*), FZ accumulates uniformly, with no asymmetry[50]. **e,f** | Evidence for DS–FT heterodimers. A comparison of both cases shows that DS protein only accumulates at the membrane when there is Fat (FT) protein on the facing membrane of the neighbouring cell[28].

However, the functional assays raise serious objections to this model. First, PK is a central component of the Tree–Amonlirdviman model, in which it is proposed to act, together with VANG, in an amplification step to localize FZ on one side of the cell. But, cells that lack PK lose the asymmetrical localization of VANG, FZ and STAN[44,46,50] (FIG. 5d), asymmetries that are essential to the model and around which the model was built. Nevertheless, *pk*⁻ cells can send, receive and propagate the FZ-dependent signal as well as, or better than, wild-type cells[25,44,51]. It follows that the asymmetrical accumulation of proteins is not required for the transfer of polarizing information from cell to cell, a conclusion that runs counter to the Tree–Amonlirdviman model.

Second, STAN is a key protein in PCP; functional assays show that STAN is essential in both sending and receiving cells. STAN is also required for FZ to accumulate normally on the membrane; in its absence, FZ is seen mainly in the cytoplasm (FIG. 5b)[48]. Yet, in the Tree–Amonlirdviman model, STAN is ignored.

Third, in the Tree–Amonlirdviman model, the polarity of a cell depends on and incorporates the asymmetrical distribution of FZ within that cell. Therefore, the model might have difficulty in explaining how a cell that lacks FZ can be repolarized, as we have observed[25].

Note that both models depend on interactions between neighbouring cells to consolidate initial, possibly small, differences in FZ activity. Both models posit local interactions between proteins, but with different elements and outcomes. The Tree–Amonlirdviman model has FZ and VANG interacting to change their distributions at or near the membrane. The result is a sharp differential of FZ in each cell, from one surface to the opposite surface, to make an intracellular gradient that orients the cell (FIG. 6a). Our model depends on interactions via intercellular homodimers of STAN that bring the level of FZ activity in one cell towards an average of the levels of its neighbours; this process initiates and propagates changes in polarity when the sending and receiving cells differ sufficiently in their levels of FZ activity. In the wild-type epithelium, we imagine a shallow intercellular gradient of FZ activity, with only small incremental differences in the scalar levels from one cell to the next, detected via the STAN bridges and polarizing each cell.

The functional assays also argue that the DS and STAN systems operate in logically distinct ways: in the STAN system, information about the level of FZ activity is conveyed by means of the STAN bridges, so that FZ in one cell behaves like a 'ligand', sending a message to VANG in the neighbouring cell, which acts like a 'receptor'. However, the DS system acts through a two-way interaction between DS and FT, with each functioning as both a ligand and a receptor.

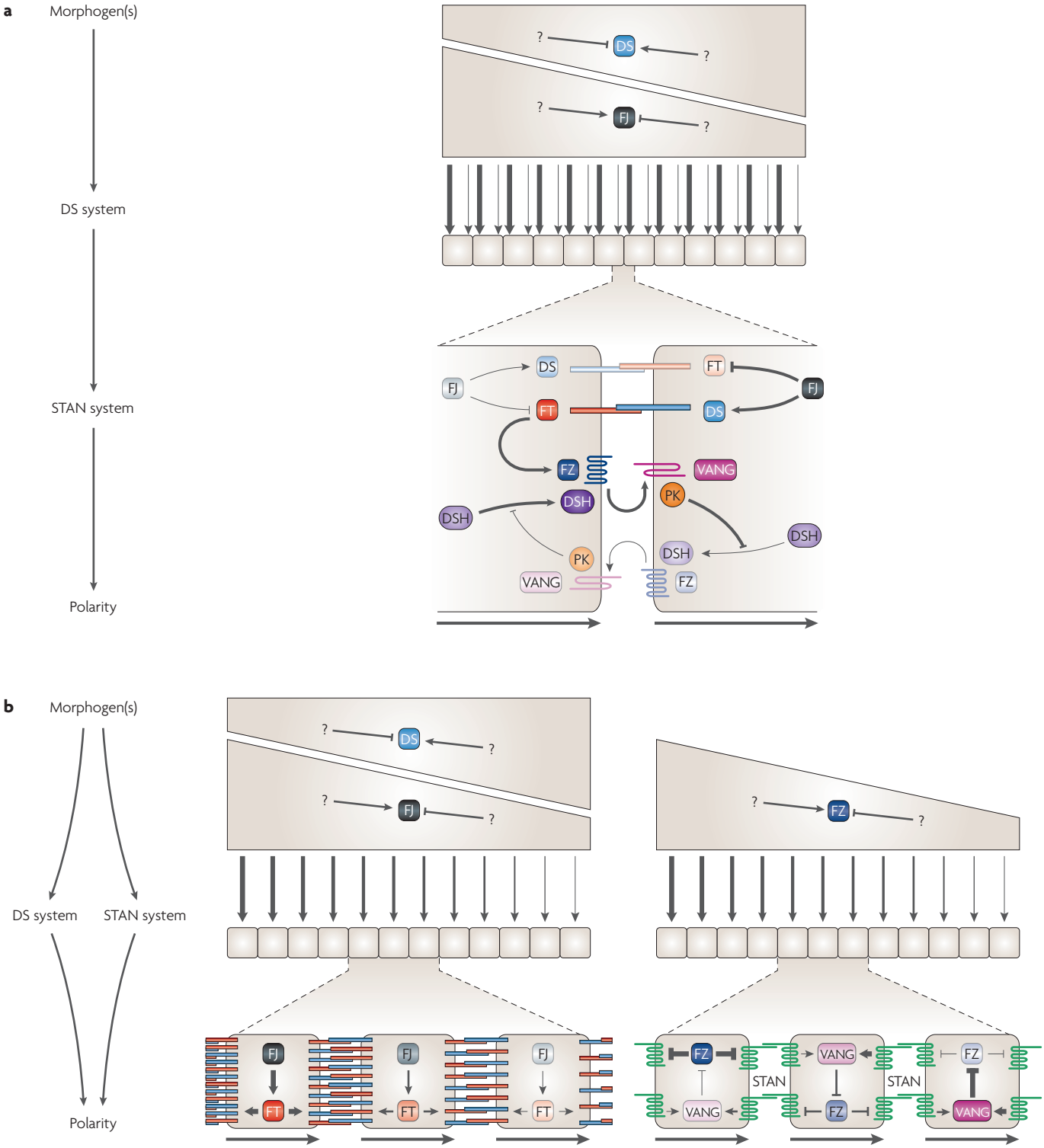

Figure 6 | **Two alternative models of planar cell polarity (PCP).** **a** | The single-pathway model. Morphogens drive gradients of Four-jointed (FJ) and Dachsous (DS) expression, which affects the DS system by a small differential input into the proximal and distal faces of the membrane of each cell. This would somehow influence the distribution of Dishevelled (DSH), Prickle (PK), Van Gogh (VANG) and Frizzled (FZ) in the membrane which, through a feedback loop, would be amplified to polarize the cells[28,45,46]. **b** | The two-pathway model. The DS system is shown on the left hand side of the figure. Here the morphogens drive gradients of Four-jointed (FJ) and Ds expression so that both Fat (FT) and DS become graded across the field of cells as a gradient of DS–FT heterodimers. The difference between the numbers of these on the anterior and posterior faces might polarize each cell. The evidence for this model comes from the functional assays described in the text[32]. The Starry night (STAN) system is shown on the right hand side of the figure. Here the morphogens might feed directly into FZ, setting up a FZ activity gradient across the whole field of cells. Using STAN, the level of FZ activity in neighbouring cells is compared, so that each cell becomes oriented to point towards the neighbour with the lowest FZ activity[25].

What biases the STAN system?

The various models might describe how the cells interact by means of the STAN system, but they do not tell us how the STAN system becomes oriented *in situ*; there must be some input, aligned with the body axis, which would feed into FZ activity and orient PCP. In the past, the consensus was that the DS system provides that input[10,17,25,28,29]. Indeed, Axelrod, Simon and colleagues[28,38,45] believe that the STAN system is oriented by vectors that are "…imposed through the agency…" of the pervasive gradients of FJ and/or DS[45] (FIG. 6), a view that we judge to be unsupported. Moreover, evidence against this view is given in the points of argument against a single pathway that we presented earlier. Instead of an effect via the DS system, we suggest that the morphogen gradients affect FZ activity more directly; in the abdomen, there is even evidence suggesting that Hedgehog (HH) might act on FZ via the receptor protein Patched (PTC)[32]. If this were true, HH would have at least two inputs into PCP, one through its effects on the transcription of both FJ and DS and a separate one, through FZ.

The next steps?

If our views are correct and generalizable, there are far-reaching consequences for the PCP field. Obviously, the question of whether there are one or two pathways is central and needs further tests in different organisms. Unfortunately, partly because we fly people have placed so much emphasis on the STAN system, particularly on FZ, little work has been done on the DS system in vertebrates. For example, there seem to be four Fat genes in mammals, of which *Fat-j* is the closest homologue of the *D. melanogaster* gene *ft*[52].

> "One can do things in a very sophisticated mathematical way … but there is a difference between theories being correct and theories being true. Many theoreticians don't make that distinction, and, even though many theories are correct in the logical sense, they are untrue because they don't relate to the natural thing we're all interested in!"[1]

There are two homologues of *ds*, but little is known about their functions and whether they are involved in PCP. If, in vertebrates, both systems were broken, would the PCP phenotypes in the stereocilia, in hair orientation and in convergent extension, be stronger? Another big question: if the two inputs from the two systems affect cell polarity independently, as we argue, then how are they integrated in the cell to fix the orientation of structures? And another: flies that lack both the DS and STAN systems develop well and almost emerge as adults from the pupal case. They even have some residual and consistent polarity in hairs and bristles, suggesting that there are yet other inputs into vectors and into PCP.

The excessive growth of *ft*$^-$ clones has suggested that PCP and the regulation of cell division might be linked. There certainly needs to be feedback from a growing organ to tell all cells when the final size has been reached to stop mitosis. In each axis, this feedback should depend on the dimension of the organ in that axis. But how could dimension be encoded and transmitted to single cells? Scientists who investigate the control of size have evidence that morphogen gradients are instrumental. But morphogens are generated from localized sources and spread out in decreasing concentration; it is not easy to see how they could directly control a pattern of growth that, typically, is evenly distributed over the tissue. However, as we have seen, morphogens do establish and orient the DS system. That system might therefore translate the uneven slope of a morphogen gradient into an even and possibly linear gradient, providing a constant differential between the faces of each cell (or between neighbouring cells). If so, a cell could get a measure of dimension (in the relevant axis) by comparing the difference in the scalar (perhaps the number of DS–FT heterodimers) across an individual cell or between cells. In this way, PCP gradients could encode information about dimension that would tell the cells when to stop dividing[53,54]. If these speculations were even partly true, they would have many repercussions. For example, they could focus attention on how morphogens affect growth via the machinery of the DS system, perhaps through the action of FT in the Hippo pathway[55–58] — and thus help us find out why hippopotami are so short in stature and so broad in girth.

The models shown in FIG. 6 are molecular and demand molecular tests. For the STAN system, it is important to know how to monitor FZ activity and see whether it varies across the cell and/or tissue (FIG. 6). Resolving this might require molecular probes to assay FZ PCP activity as distinct from FZ protein accumulation or its involvement in transducing Wingless (WG) signalling. We also need to know more about FT and DS, especially their interactions with each other and how their activities depend on FJ. The structures of FT and DS, which are massive proteins with many domains, need further analysis. Too little is known about their routes through the cell, their distributions on and off the plasma membrane and their binding partners inside and outside the cell. We do not understand how differences in the distribution of DS–FT heterodimers could orient cells and point the outgrowing hairs. Much remains to be done.

Glossary

Clones
Patches of clonally derived cells in an organism that have been engineered to be genetically distinct from surrounding cells (for example, a homozygous mutant clone in a heterozygous background).

Convergent extension
The process by which a sheet of cells changes shape by extending in one direction and narrowing — converging — in a direction at right angles to the extension.

Ommatidia
The elements of the compound eye of insects (in *Drosophila melanogaster*, the eye is formed from 800 ommatidia), each of which is an independent visual unit that contains eight photoreceptor cells, surrounded by four cone cells that secrete the lens, and seven pigment cells.

Stereocilium
A large, rigid, actin-filled microvillus on the apical surface of hair cells in the inner ear.

Tergites
Cuticular plates, one per segment, that bear oriented hairs and bristles that make up most of the dorsal abdomen of *Drosophila melanogaster* and other insects.

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doi:10.1038/nrg2125
Published online 12 June 2007

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Acknowledgements

We thank the Wellcome Trust and the Medical Research Council, UK, for support and D. Strutt for advice and encouragement. G.S. is a Howard Hughes Medical Institute Investigator.

Competing interests statement

The authors declare no competing financial interests.

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