Published in final edited form as:

Nat Rev Genet. 2007 July ; 8(7): 555–563. doi:10.1038/nrg2125.

Planar cell polarity: one or two pathways?

Peter A Lawrence^{*,‡,¶}, Gary Struhl[§], and José Casal^{*}

^{*}Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom.

[‡]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH. United Kingdom.

[§]Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, 701 West 168th Street, New York, NY 10032, USA

Abstract

In multicellular organisms, cells are polarised in the plane of the epithelial sheet, demonstrated in some cell types by oriented hairs or cilia. Many of the genes involved have been identified in *Drosophila* 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 are confronted, the aim being to understand how polarising 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 there are (at least) two independently acting processes. This conclusion has major consequences for the PCP field.

Animals are largely built of epithelia whose cells are specified by both scalars and vectors. The scalars are in the form of positional information that tells cells where they are located on 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 as well as how to orient extensions, such as cilia, bristles or axons²⁻⁵. Multicellular organisms could not be built without vectors.

Over the last 110 years⁶, many embryologists have clarified the mechanisms of positional information and defined morphogens, molecules that are released from localised 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 experiments⁷, a minority of 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 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

[¶]Author for correspondence (pal@mrc-lmb.cam.ac.uk).

In tribute to Sydney Brenner who was 80 on January 13th, 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.

form oriented structures such as bristles, and because of 100 years of investment in its genetics, *Drosophila* is the best model system. 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 growth¹²⁻¹⁶ 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 and here we try to explain why this is so and what the consequences are. We argue for a new way of looking at PCP.

Morphogens act upstream of PCP

A vector is not a simple product of some biochemical pathway (see¹⁷ 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, localised 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 overexpressing only the morphogen Dpp makes a new peak in the concentration gradient and this induces a perfect winglet of the appropriate size, pattern *and* PCP¹⁸. Mutations in the signalling molecules Wnt11 and Wnt 5 affect the orientation of cell movements in the zebra fish^{19, 20}. Even though these molecules can produce changes in PCP, the experiments 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 so far known to act in the mechanism of PCP were identified in *Drosophila* and they fall into two groups: Mutations in genes of the first group not only change polarity but also alter the shapes of wings and legs and disturb growth. We limit discussion to *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: these are *dishevelled (dsh)*, *frizzled (fz)* (Fig 1), *prickle (pk)*, *Van Gogh/strabismus (Vang/stbm)* and *starry night/flamingo (stan/fmi)* (Table 1).

How do these genes organise PCP? To help answer this question, we introduce the fly abdomen into the current picture of PCP (for this picture, see¹⁰). We rely on a *functional* assay that springs from the finding that clones of mutant cells alter the polarity of wildtype 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 of the clone **sending** cells because, for simplicity and for this review, we focus on the information that is being passed from the clone to the **receiving** cells surrounding it (of course, information may also go in the opposite direction). Now, take a small clone of cells lacking the *fz* gene: in both abdomen²⁵ and wing²⁴, the clone reverses the polarity of some nearby wildtype receiving cells so that all cells point inwards (Fig 1). Clones overexpressing *fz* reverse 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 level²⁶; 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 e.g.10, 27-29. An upper tier of proteins, encoded by the Ds, Ft and Fz genes (which we call the “Ds system”) is thought to polarise and regulate the activity of a lower tier, consisting 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 involved in making the polarised structures³⁰ (such as actin). This single-pathway hypothesis is not fact but has been reiterated so often that it is becoming perceived and presented as such e.g.³¹. We now offer four pieces of evidence that it is incorrect, at the very least in the abdomen where we have done our experiments:

First, the most persuasive piece of evidence: in the functional assay, excess Ds, Ft or Fz in the sending cells can repolarise receiving cells even when *all* the cells, sending and receiving, lack Fz, or Stan, or both³² (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, 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 randomised³² (Fig 4).

Third, *ds*⁻ cells provide a sensitised assay for activity of the Stan system: in the absence of Ds, clones that lack or overexpress Fz repolarise receiving cells over a longer range than similar clones in the wildtype; also sending cells with modest alterations in the level of Fz that would normally have no visible consequence, now change the polarity of *ds*⁻ (or *ft*⁻) receiving cells^{28, 32, 33}. 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 be very responsive. In fact, sending cells that express Ds (or Ft) have no effect at all on the polarity of *ds*⁻ (or *ft*⁻) receiving cells³². It seems that neither Ds nor Ft affect the Stan system of the sending cells.

Fourth, when manipulated in clones, the two systems are fundamentally different, they can even have effects of opposite sign. Assays deploying the Ds system (for example, sending cells that overexpress *ft*) behave differently in the two compartments of the abdominal segment; reversing the polarity of receiving cells in front of the clone in the anterior compartments and behind in the posterior compartments³⁴. By contrast, clones affecting the Stan system behave in the same way in the two compartments — clones lacking *fz* always reverse the polarity of cells behind the clones²⁵.

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 contributing to PCP by different mechanisms³⁴.

What is the counterevidence? What are the results supporting the single pathway model? One argument came from the eye, where it was claimed that *ft*⁻ cells can bias the polarity of ommatidia in wild type but not in *fz*⁻ flies²⁹ — however the sample size was insufficient to draw this conclusion and, also, appropriate controls were not provided (*ft*⁺ clones in *fz*⁻ eyes). But, in the wing, it has been stated that *ft*⁻ clones do not repolarise cells in *fz*⁻ flies²⁸ and also, in *fz*⁻ abdominal pleura, the hairs are randomised and do not respond to clones overexpressing an active form of Ds³². Put with 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 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? An explanation that fits, because, in *fz*⁻ flies, the eyes, wings and pleura are much more depolarised than the tergites. Under this particular “don’t worry hypothesis”, the Ds signal would be trying to impose a polarity on cells in disarray; it might be like looking for ripples caused by throwing a stone into a rough sea.

There is another way of regarding this central issue: our experiments on the abdomen show that the Ds system has an inherent capacity to change polarity without the Stan system. Thus, 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 what we all pay attention to — the orientation of hairs and other indicators of PCP.

If we accept there are two pathways, two new questions stand up and shout for answers: **First**, how does the Ds system polarise cells? **Second**, what polarises the Stan system?

How does the Ds system generate PCP?

In a field of cells in the wildtype, everyone agrees that there needs to be a biasing input to orient the Ds system and, most likely, this is done by morphogen gradients driving *ds* and/or *fj* transcription. Gradients of both Ds and Fj have been inferred and/or seen in the eye, abdomen and, perhaps, in the wing^{29, 34-37} and the orientation of both gradients shown to influence PCP³⁸. Simon’s work in the eye shows nicely that the two gradients are redundant; flattening of one is insufficient to disturb PCP, while flattening of both produces randomisation of polarity. Reversing the Fj gradient can even turn the ommatidial polarity around³⁸. The functional assay in the abdomen suggests that Fj acts mainly on Ft (Fig 2c) and therefore, both in the wildtype 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 last question was addressed by asking *how* Ft might feed into Fz, which we now think is the wrong question. Instead, we have shown that the cadherin-family proteins, Ds and Ft can polarise cells without 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 stabilise 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 based on 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 transheterodimers (with Ft molecules in adjacent cells) might differ between the anterior and posterior faces; an intracellular asymmetry that could 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 of either Ds or Ft, but Ds and Ft are both needed in the receiving cell, for polarisation of the first receiving cell **and** for propagation to the next³² (Fig 6).

How does the Stan system generate PCP?

Models based on functional assays

There are many ideas about how Fz might mediate PCP¹¹. To choose between these, we again rely on the functional assay in the abdomen. Looking at the main genes implicated in the Stan system (Table 1), we ask are they needed for function in the sending cell, the receiving cell or in both?

1. A clone overexpressing *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 appears that the signal passes across, or at least requires, intercellular bridges made by homodimers of Stan (see^{25, 43}).
2. When the sending cells express *fz* in an otherwise *fz*⁻ background, the nearest *fz*⁻ receiving cell is repolarised — thus a cell that completely lacks Fz protein can have an organised polarity, and even a polarity that responds and changes²⁵. This result is important because it helps rule out some models; many of which are built around the Fz protein, making it an integral part of PCP **within** each cell¹¹.
3. The assay shows that, for detection of a difference in Fz activity between the clone and the surround, Vang is absolutely required in the receiving but not the sending cells²⁵.
4. When the sending cells express *fz*, but both the sending and receiving cells lack *pk*, the receiving cells are repolarised²⁵. Thus, Pk is not needed for either sending or reception of the signal. This finding has been extended to the wing, where the same conclusion was also drawn for both Pk and Dsh⁴⁴. Note that the proteins needed for transmission of the signal (Fz, Stan and Vang) are transmembrane, while those not so required (Pk and Dsh) are cytoplasmic.

From this 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 polarised, 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 Stan²⁵.

“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 based on asymmetric protein localisation

The currently most popular model of PCP, the “Tree/Amonlirdviman model”^{45, 46}, is based largely on 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 prior to formation of the cell hairs^{43, 47-49}. For example, Stan accumulates on both the proximal and distal faces of cells (Fig 5a), Fz and Dsh accumulate

on the distal membranes^{47, 48} (Fig 5c) while Vang and Pk accumulate on the proximal membranes^{46, 50}. Using these facts, some assumptions, and, later, a mathematical simulation requiring optimisation of several parameters, the Tree/Amonlirdviman model was built to explain how localised protein interactions within and between cells might drive PCP^{45, 46} see Fig 6).

However, the functional assays raise serious objections to this model:

First, Pk is a central component of the Tree/Amonlirdviman model where it acts, with Vang, in an amplification step to localise Fz on one side of the cell. Moreover, cells lacking Pk lose the asymmetric localisation of Vang, Fz and Stan^{44, 46, 50} (Fig 5d), asymmetries that are essential to the model and were used to build it. Yet, pk^- cells can receive and propagate the Fz-dependent signal as well as, or better than, wildtype cells^{25, 44, 51}. These results argue that the asymmetric accumulation of proteins is not the primary engine by which a cell acquires polarity to accord with its neighbours. Instead it might be an outcome of the intercellular gradient of Fz activity, acting to stabilise nascent polarity. This conjecture fits with repolarisation occurring more freely in pk^- cells.

Second, Stan is a key protein in PCP; the functional assays show Stan to be 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 the Tree/Amonlirdviman model ignores it.

Third, in the Tree/Amonlirdviman model, the polarity of a cell depends on and incorporates the asymmetric distribution of Fz within that cell. The model therefore might have difficulty in explaining how a cell lacking Fz can be repolarised as we have observed²⁵.

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 (arrows in Fig. 5a). 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 cells and receiving cells differ sufficiently in their levels of Fz activity. In the wildtype 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 polarising 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, each one acting as both a ligand and as a receptor.

What biases the Stan system?

The various models may illustrate 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 needs to be some input, aligned with the body axis, that 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 “imposed through the agency” of the pervasive gradients of Fj and/or Ds⁴⁵ (Fig 6), a view

we judge to be unsupported. Moreover, evidence against this view is given in the points of argument against a single pathway 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 might act on Fz via the receptor protein Patched32. If this were true, Hedgehog would have at least two inputs into PCP, one via its effects on the transcription of both Fj and Ds and a separate one, via Fz.

The next steps?

If our views are correct and generalisable there are far-reaching consequences for the PCP field. Obviously, the question of 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 appear to be four Fat genes in mammals, of which *fat-j* is the closest homologue of the *Drosophila* *fz52*. There are two homologues of *ds* but little is known of 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 lacking 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 shown by *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 investigating the control of size have evidence that morphogen gradients are instrumental. But morphogens are generated from localised 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 of 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 pathway⁵⁵⁻⁵⁸— 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 and to monitor when Fz activity is distributed across the cell and across the tissue (Fig. 6). Resolving this may require molecular probes to assay Fz PCP activity, as distinct from Fz protein accumulation or its involvement in transducing Wingless. Also, we 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, 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. There is much to do.

References

1. Brenner, S. My Life in Science. BioMed Central; 2001.
2. Drubin, D. Cell Polarity. Hames, B.; Glover, D., editors. Oxford University Press; Oxford: 2000.
3. Ghosh M, Schweisguth F. Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. *Nature*. 1998; 393:178–81. [PubMed: 9603522]
4. Lawrence PA. Development and determination of hairs and bristles in the milkweed bug, *Oncopeltus fasciatus* (Lygaeidae, Hemiptera). *J. Cell Science*. 1966; 1:475–498. [PubMed: 5956722]
5. Wang Y, Thekdi N, Smallwood PM, Macke JP, Nathans J. Frizzled-3 is required for the development of major fiber tracts in the rostral CNS. *J Neurosci*. 2002; 22:8563–73. [PubMed: 12351730]
6. Morgan TH. Regeneration in *Allolobophora fætida*. *Archives für Entwicklungsmechanik der Organismen*. 1897; 5:570–586.
7. Lecuit T, Samanta R, Wieschaus E. *slam* encodes a developmental regulator of polarized membrane growth during cleavage of the *Drosophila* embryo. *Dev Cell*. 2002; 2:425–36. [PubMed: 11970893]
8. Adler PN. Planar signaling and morphogenesis in *Drosophila*. *Dev Cell*. 2002; 2:525–35. [PubMed: 12015961]
9. Nübler-Jung K, Bonitz R, Sonnenschein M. Cell polarity during wound healing in an insect epidermis. *Development*. 1987; 100:163–170. [PubMed: 3652965]
10. Seifert JR, Mlodzik M. Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. *Nat Rev Genet*. 2007; 8:126–38. [PubMed: 17230199]
11. Strutt D. Frizzled signalling and cell polarisation in *Drosophila* and vertebrates. *Development*. 2003; 130:4501–13. [PubMed: 12925579]
12. Fanto M, McNeill H. Planar polarity from flies to vertebrates. *J Cell Sci*. 2004; 117:527–33. [PubMed: 14730010]
13. Klein TJ, Mlodzik M. Planar Cell Polarization: An Emerging Model Points in the Right Direction. *Annu Rev Cell Dev Biol*. 2005; 23:23.
14. Wallingford JB. Planar cell polarity, ciliogenesis and neural tube defects. *Hum Mol Genet*. 2006; 15 Spec No 2:R227–34. [PubMed: 16987888]
15. Wang Y, Guo N, Nathans J. The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J Neurosci*. 2006; 26:2147–56. [PubMed: 16495441]
16. Wang Y, Nathans J. Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development*. 2007; 134:647–58. [PubMed: 17259302]
17. Lewis J, Davies A. Planar cell polarity in the inner ear: how do hair cells acquire their oriented structure? *J Neurobiol*. 2002; 53:190–201. [PubMed: 12382275]
18. Zecca M, Basler K, Struhl G. Sequential organizing activities of *engrailed*, *hedgehog* and *decapentaplegic* in the *Drosophila* wing. *Development*. 1995; 121:2265–2278. [PubMed: 7671794]
19. Heisenberg CP, et al. Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature*. 2000; 405:76–81. [PubMed: 10811221]
20. Kilian B, et al. The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation. *Mech Dev*. 2003; 120:467–76. [PubMed: 12676324]
21. Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature*. 1980; 287:795–801. [PubMed: 6776413]
22. Lewis EB. A gene complex controlling segmentation in *Drosophila*. *Nature*. 1978; 276:565–70. [PubMed: 103000]
23. Gubb D, Garcia-Bellido A. A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*. *J Embryol Exp Morphol*. 1982; 68:37–57. [PubMed: 6809878]

24. Vinson CR, Adler PN. Directional non-cell autonomy and the transmission of polarity information by the *frizzled* gene of *Drosophila*. *Nature*. 1987; 329:549–51. [PubMed: 3116434]
25. Lawrence PA, Casal J, Struhl G. Cell interactions and planar polarity in the abdominal epidermis of *Drosophila*. *Development*. 2004; 131:4651–64. [PubMed: 15329345]
26. Adler PN, Krasnow RE, Liu J. Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels. *Curr Biol*. 1997; 7:940–9. [PubMed: 9382848]
27. Karner C, Wharton KA Jr, Carroll TJ. Planar cell polarity and vertebrate organogenesis. *Semin Cell Dev Biol*. 2006; 17:194–203. [PubMed: 16839790]
28. Ma D, Yang CH, McNeill H, Simon MA, Axelrod JD. Fidelity in planar cell polarity signalling. *Nature*. 2003
29. Yang C, Axelrod JD, Simon MA. Regulation of Frizzled by Fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. *Cell*. 2002; 108:675–88. [PubMed: 11893338]
30. Wong LL, Adler PN. Tissue polarity genes of *Drosophila* regulate the subcellular location for prehair initiation in pupal wing cells. *J Cell Biol*. 1993; 123:209–21. [PubMed: 8408199]
31. Deans MR, et al. Asymmetric distribution of Prickle-like 2 reveals an early underlying polarization of vestibular sensory epithelia in the inner ear. *J Neurosci*. 2007; 27:3139–47. [PubMed: 17376975]
32. Casal J, Lawrence PA, Struhl G. Two separate molecular systems, *Dachsous*/Fat and *Starry night*/Frizzled, act independently to confer planar cell polarity. *Development*. 2006; 133:4561–72. [PubMed: 17075008]
33. Adler PN, Charlton J, Liu J. Mutations in the cadherin superfamily member gene *dachsous* cause a tissue polarity phenotype by altering *frizzled* signaling. *Development*. 1998; 125:959–68. [PubMed: 9449678]
34. Casal J, Struhl G, Lawrence PA. Developmental compartments and planar polarity in *Drosophila*. *Curr Biol*. 2002; 12:1189–98. [PubMed: 12176328]
35. Clark HF, et al. *Dachsous* encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in *Drosophila*. *Genes Dev*. 1995; 9:1530–42. [PubMed: 7601355]
36. Zeidler MP, Perrimon N, Strutt DI. The *four-jointed* gene is required in the *Drosophila* eye for ommatidial polarity specification. *Curr Biol*. 1999; 9:1363–72. [PubMed: 10607560]
37. Zeidler MP, Perrimon N, Strutt DI. Multiple roles for *four-jointed* in planar polarity and limb patterning. *Dev Biol*. 2000; 228:181–96. [PubMed: 11112323]
38. Simon MA. Planar cell polarity in the *Drosophila* eye is directed by graded *Four-jointed* and *Dachsous* expression. *Development*. 2004; 131:6175–84. [PubMed: 15548581]
39. Mao Y, et al. *Dachs*: an unconventional myosin that functions downstream of *Fat* to regulate growth, affinity and gene expression in *Drosophila*. *Development*. 2006; 133:2539–51. [PubMed: 16735478]
40. Strutt H, Strutt D. Nonautonomous planar polarity patterning in *Drosophila*: dishevelled-independent functions of *frizzled*. *Dev Cell*. 2002; 3:851–63. [PubMed: 12479810]
41. Matakatsu H, Blair SS. Interactions between *Fat* and *Dachsous* and the regulation of planar cell polarity in the *Drosophila* wing. *Development*. 2004
42. Matakatsu H, Blair SS. Separating the adhesive and signaling functions of the *Fat* and *Dachsous* protocadherins. *Development*. 2006
43. Usui T, et al. *Flamingo*, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of *Frizzled*. *Cell*. 1999; 98:585–95. [PubMed: 10490098]
44. Strutt D, Strutt H. Differential activities of the core planar polarity proteins during *Drosophila* wing patterning. *Dev Biol*. 2007; 302:181–94. [PubMed: 17045581]
45. Amonlirdviman K, et al. Mathematical modeling of planar cell polarity to understand domineering nonautonomy. *Science*. 2005; 307:423–6. [PubMed: 15662015]
46. Tree DR, et al. *Prickle* mediates feedback amplification to generate asymmetric planar cell polarity signaling. *Cell*. 2002; 109:371–81. [PubMed: 12015986]
47. Axelrod JD. Unipolar membrane association of *Dishevelled* mediates *Frizzled* planar cell polarity signaling. *Genes Dev*. 2001; 15:1182–7. [PubMed: 11358862]

48. Strutt DI. Asymmetric localization of Frizzled and the establishment of cell polarity in the *Drosophila* wing. *Mol Cell*. 2001; 7:367–75. [PubMed: 11239465]
49. Strutt DI. The asymmetric subcellular localisation of components of the planar polarity pathway. *Semin Cell Dev Biol*. 2002; 13:225–31. [PubMed: 12137731]
50. Bastock R, Strutt H, Strutt D. Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. *Development*. 2003; 130:3007–14. [PubMed: 12756182]
51. Adler PN, Taylor J, Charlton J. The domineering non-autonomy of frizzled and van Gogh clones in the *Drosophila* wing is a consequence of a disruption in local signaling. *Mech Dev*. 2000; 96:197–207. [PubMed: 10960784]
52. Rock R, Schrauth S, Gessler M. Expression of mouse *dchs1*, *fjx1*, and *fat-j* suggests conservation of the planar cell polarity pathway identified in *Drosophila*. *Dev Dyn*. 2005; 234:747–55. [PubMed: 16059920]
53. Day SJ, Lawrence PA. Measuring dimensions: the regulation of size and shape. *Development*. 2000; 127:2977–2987. [PubMed: 10862736]
54. Lawrence PA. Last hideout of the unknown? *Nature*. 2004; 429:247. [PubMed: 15152230]
55. Cho E, et al. Delineation of a Fat tumor suppressor pathway. *Nat Genet*. 2006; 38:1142–50. [PubMed: 16980976]
56. Bennett FC, Harvey KF. Fat cadherin modulates organ size in *Drosophila* via the Salvador/Warts/Hippo signaling pathway. *Curr Biol*. 2006; 16:2101–10. [PubMed: 17045801]
57. Silva E, Tsatskis Y, Gardano L, Tapon N, McNeill H. The tumor-suppressor gene *fat* controls tissue growth upstream of expanded in the hippo signaling pathway. *Curr Biol*. 2006; 16:2081–9. [PubMed: 16996266]
58. Willecke M, et al. The fat cadherin acts through the hippo tumor-suppressor pathway to regulate tissue size. *Curr Biol*. 2006; 16:2090–100. [PubMed: 16996265]
59. Villano JL, Katz FN. *four-jointed* is required for intermediate growth in the proximal-distal axis in *Drosophila*. *Development*. 1995; 121:2767–77. [PubMed: 7555705]
60. Brodsky MH, Steller H. Positional information along the dorsal-ventral axis of the *Drosophila* eye: graded expression of the *four-jointed* gene. *Dev Biol*. 1996; 173:428–46. [PubMed: 8606003]
61. Strutt H, Mundy J, Hofstra K, Strutt D. Cleavage and secretion is not required for Four-jointed function in *Drosophila* patterning. *Development*. 2004; 131:881–90. [PubMed: 14757640]
62. Ashery-Padan R, Alvarez-Bolado G, Klamt B, Gessler M, Gruss P. *Fjx1*, the murine homologue of the *Drosophila four-jointed* gene, codes for a putative secreted protein expressed in restricted domains of the developing and adult brain. *Mech Dev*. 1999; 80:213–7. [PubMed: 10072791]
63. Mahoney PA, et al. The *fat* tumor suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily. *Cell*. 1991; 67:853–68. [PubMed: 1959133]
64. Rawls AS, Guinto JB, Wolff T. The Cadherins Fat and Dachous Regulate Dorsal/Ventral Signaling in the *Drosophila* Eye. *Curr Biol*. 2002; 12:1021–1026. [PubMed: 12123577]
65. Chae J, et al. The *Drosophila* tissue polarity gene *starry night* encodes a member of the protocadherin family. *Development*. 1999; 126:5421–9. [PubMed: 10556066]
66. Curtin JA, et al. Mutation of *Celsr1* disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr Biol*. 2003; 13:1129–33. [PubMed: 12842012]
67. Vinson CR, Conover S, Adler PN. A *Drosophila* tissue polarity locus encodes a protein containing seven potential transmembrane domains. *Nature*. 1989; 338:263–4. [PubMed: 2493583]
68. Wolff T, Rubin GM. Strabismus, a novel gene that regulates tissue polarity and cell fate decisions in *Drosophila*. *Development*. 1998; 125:1149–59. [PubMed: 9463361]
69. Taylor J, Abramova N, Charlton J, Adler PN. *Van Gogh*: a new *Drosophila* tissue polarity gene. *Genetics*. 1998; 150:199–210. [PubMed: 9725839]
70. Montcouquiol M, et al. Asymmetric localization of Vangl2 and Fz3 indicate novel mechanisms for planar cell polarity in mammals. *J Neurosci*. 2006; 26:5265–75. [PubMed: 16687519]
71. Gubb D, et al. The balance between isoforms of the prickly LIM domain protein is critical for planar polarity in *Drosophila* imaginal discs. *Genes Dev*. 1999; 13:2315–27. [PubMed: 10485852]

72. Katoh M, Katoh M. Identification and characterization of human PRICKLE1 and PRICKLE2 genes as well as mouse Prickle1 and Prickle2 genes homologous to *Drosophila* tissue polarity gene prickler. *Int J Mol Med*. 2003; 11:249–56. [PubMed: 12525887]
73. Bekman E, Henrique D. Embryonic expression of three mouse genes with homology to the *Drosophila melanogaster* prickler gene. *Mech Dev*. 2002; 119(Suppl 1):S77–81. [PubMed: 14516664]
74. Theisen H, et al. dishevelled is required during wingless signaling to establish both cell polarity and cell identity. *Development*. 1994; 120:347–60. [PubMed: 8149913]
75. Klingensmith J, Nusse R, Perrimon N. The *Drosophila* segment polarity gene dishevelled encodes a novel protein required for response to the wingless signal. *Genes Dev*. 1994; 8:118–30. [PubMed: 8288125]
76. Krasnow RE, Wong LL, Adler PN. Dishevelled is a component of the frizzled signaling pathway in *Drosophila*. *Development*. 1995; 121:4095–102. [PubMed: 8575310]

Figure 1. Planar cell polarity in the *Drosophila* pleura

- a.** In the wildtype, the small cuticular hairs (several are produced by each cell) point posteriorly.
- b.** In *fz*⁻ flies, the orientation is randomised.
- c.** Three clones of *fz*⁻ cells in a wildtype fly, each outlined in red; the hairs posterior to the *fz*⁻ cells mostly point anteriorwards (red arrows) but in the strait between two nearby clones, polarity is normal (black arrow) The *fz*⁻ cells (one is filled in orange to show its extent) are genetically marked so that each makes numerous hairs that point upwards.

Figure 2. The functional assay

Clones (blank) are made in the *Drosophila* abdomen of one genotype (the “sending” cells) that may alter the polarity of hairs in the “receiving” cells (grey background). Anterior to the top. Black arrows, normal polarity; blue arrows, disturbed; red arrows, reversed polarity.

a. *ft⁻* cells reverse the polarity of receiving cells posterior to the clone, while overexpression of *ft* (*UAS.ft*) reverses the polarity anterior to the clone. The assays show that Stan is needed in both sending and receiving cells.

b. The signal emanating from *UAS.ft* cells acts independently of Stan.

c. These three assays argue that Fj acts through Ft. *UAS.fj* clones reverse receiving cells in front, like *UAS.ft* clones (**d.**) but if Ft is missing, as in a *UAS.fj, ft⁻* clone, it behaves like a *ft⁻* clone (which reverse receiving cells behind the clone).

d. These 7 assays plus the *ft⁻* clones (**c.**) show that Ds alone is sufficient in the sending cells to affect polarity of the receiving cells (similar results show that Ft alone is also sufficient³²), but that both Ds and Ft are needed in the receiving cells.

Figure 3. A repolarising clone in a *stan*⁻ fly

The clone is illustrated as in Fig 1, and consists of marked cells expressing a modified form of Ds in a fly mutant for the *stan* gene. In spite of the lack of the Stan system, the clone reverses the polarity of cells behind it, and also organises normal polarity in the cells in front of it (a clone of the same genotype is shown in Fig 2F of 32)

Figure 4. Cuticle from the dorsal abdomen of *Drosophila*

a. wildtype cuticle **b.** *ds*⁻ **c.** *stan*⁻ **d.** *ds*⁻ *stan*⁻. Note the stronger randomisation of hairs and bristles when both systems are broken³².

Figure 5. The localisation of PCP proteins in clones in the wing

The proteins may be located on particular faces of the cell membranes (protein shown in red). Anterior to the top, distal to the right.

- a. Stan accumulates mostly on the proximodistal faces of the cells and is not seen at all in the membrane unless Stan protein is present on both confronting cells⁴³.
- b. Fz protein 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 protein⁴⁸.
- c. Fz is actually localised on the distal face of the cells; the white cells are a clone of cells that lack the tagged Fz⁴⁸.
- d. In cells lacking Pk, Fz accumulates uniformly, with no asymmetry⁵⁰.
- e, f. Evidence for Ds-Ft heterodimers. A comparison of both cases shows that Ds protein only accumulates at the membrane when there is Ft protein on the facing membrane of the neighbouring cell²⁸.

Figure 6. Alternative models of PCP

a. The single pathway model. Morphogens drive gradients of Fj and Ds expression that affects the Ds system by a small differential input into the proximal and distal faces of the membrane of each cell. This would influence, somehow, the distribution of the Dsh, Pk, Vang and Fz in the membrane which, through a feedback loop, would be amplified to polarise the cells^{45, 46}.

b. The two pathway model. On the left, the Ds system is shown; here the morphogens drive gradients of Fj and Ds expression so that both 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 polarise each cell. The evidence for this model comes from the functional assays, see text³².

On the right, the Stan system is shown. 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 of neighbouring cells is compared, so that each cell becomes oriented to point towards the neighbour with the lowest Fz activity²⁵.

Table 1

The selected molecules in mice and flies.

Drosophila Genes		Mouse Genes	References
<i>fj</i> (<i>four-jointed</i>)	Type II membrane protein/ Golgi localisation	fjx1	52, 59-62
<i>ds</i> (<i>dachsous</i>)	Atypical cadherin	dchs1	33, 35, 52
<i>ft</i> (<i>fat</i>)	Atypical cadherin	fat-j	29, 34, 40, 52, 63, 64
<i>stan</i> (<i>starry night/ flamingo</i>)	Seven transmembrane-pass domains and cadherin repeats	Celsr1	43, 65, 66
<i>fz</i> (<i>frizzled</i>)	Seven transmembrane-pass domain Wnt receptor	Fz3, Fz6	15, 23, 24, 67
<i>Vang</i> (<i>Van Gogh/ strabismus</i>)	2 (at least) transmembrane-pass domains	Vangl2	68-70
<i>pk</i> (<i>prickle</i>)	LIM and PET domains	Prickle1, Prickle2	71-73
<i>dsh</i> (<i>dishevelled</i>)	DIX, PDZ and DEP domains	Dvl2	15, 74-76