

Cell flow and tissue polarity patterns

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Planar tissue polarity is a fundamental feature of many epithelia. Large-scale cell polarity patterns govern the orientation of external structures such as hairs and cilia. Tissue polarity patterns arise from the collective organization of cells, which are polarized individually. Such cell and tissue polarities are reflected in anisotropic distributions of proteins of the planar cell polarity (PCP) pathway. Here we give an overview on recent progress in understanding how large-scale patterns of tissue polarity are controlled. We highlight the role of active mechanical events in the organization of polarity patterns during the development of the pupal fly wing. Patterns of cell flow are generated by mechanical stresses exerted on the tissue as well as by oriented cell divisions and neighbor exchanges. We discuss how the resulting tissue shear controls polarity orientation. We argue that the often-observed alignment of PCP either parallel or perpendicular to the long axis of developing tissues is a characteristic consequence of shear-induced polarity alignment. This principle allows for the versatile and robust generation of polarity patterns in tissues.

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Introduction

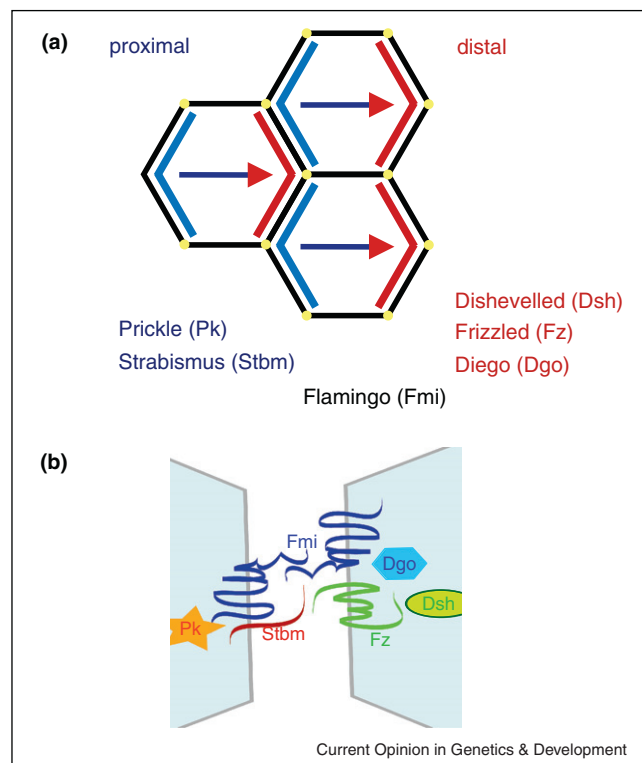
An important property of many epithelial tissues is the ability to build and to align external structures such as hairs, cilia, and stereocilia. This feature is conserved in many different phyla, and is controlled by the planar cell polarity (PCP) pathway. The PCP pathway coordinates the pattern of tissue anisotropies over large distances and aligns them with tissue shape [1–4]. The mechanisms underlying planar polarization have been most extensively studied in *Drosophila* — in particular in the wing, where the PCP pathway specifies the uniform distal orientation of wing hairs. Proteins of the PCP pathway

localize to *adherens* junctions, where they form asymmetric complexes that connect neighboring cells. In the *Drosophila* wing, they are found on the proximal and distal cell interfaces. PCP complexes consist of several different transmembrane and peripherally associated proteins. The seven-pass transmembrane Cadherin Flamingo (Fmi) mediates homophilic interactions that link neighboring cells, but it interacts with different partners on each side of the cell contact. In one cell, Fmi assembles complexes containing Frizzled (Fz), a seven-pass transmembrane protein that can act as a receptor for Wnts, and two peripherally associated proteins, Dishevelled and Diego. In the neighboring cell, Fmi recruits the transmembrane protein Strabismus, and the peripherally associated protein Prickle/Spiny leg (see Figure 1). The asymmetric localization of PCP complexes of different polarities to opposite sides of each cell defines the direction of PCP, and specifies the orientation in which hairs or cilia will form. The orientation of PCP is well correlated between neighboring cells and also exhibits large-scale order in tissue. These large-scale PCP patterns allow precise and reproducible alignment of hairs or cilia with respect to the tissue axes.

Much progress has been made toward understanding the intracellular polarization of PCP proteins and the local coupling of polarity between neighboring cells. However, the cues that orient global PCP patterns are less well understood. The formation of asymmetric PCP complexes and local polarity alignment between cells is self-organized by feedback loops that operate both within and between cells [5–9]. PCP proteins engaged in asymmetric complexes across cell boundaries are more stable to endocytosis than free PCP proteins [10,11••]. Thus, PCP proteins of one type (red in Figure 1a) tend to recruit and stabilize their complement (blue) across cell boundaries. Conversely, PCP proteins of one type discourage the accumulation of the other type nearby in the same cell. This may depend in part on the ability of the peripherally associated PCP proteins to cluster PCP complexes of the same polarity [11••]. These interactions, and the inherent asymmetry of PCP complexes that form across cell boundaries, couple the polarity of one cell to that of its neighbors. Local self-organization of PCP molecules accounts well for the domineering nonautonomy exhibited by clones of cells mutant for individual PCP proteins [5]. These clones perturb the planar polarity of wild type tissue, and do so on only one side of the clone [12].

A fundamental problem is how such local interactions can give rise to patterns of cell polarity that are aligned over large distances and what cues set the overall directions of

Figure 1



Schematic representation of planar cell polarity (PCP). PCP proteins form asymmetric complexes on cell junctions between neighboring cells. The distribution of these complexes defines the direction of cell polarity of individual cells. **(a)** Proximal and distally localized PCP proteins in the tissue. **(b)** Asymmetric complex bridging between two cell membranes.

tissue polarity. The difficulty of using only local interactions to establish PCP patterns that are aligned over long distances has been highlighted using several distinct theoretical approaches. The self-organization of PCP molecules in cells and between cells by feedback loops has been described by a dynamic model for the PCP network in cells [5]. A minimal model involving two antagonistic PCP components was proposed which could account for local emergence of cell polarity and alignment of polarity by neighboring cells [13[•]]. Such simplified models have been used to study the effects of fluctuations and the role of external cues to generate ordered patterns with controlled orientation. Finally, vertex models can describe cell packing geometries and the dynamic reorganization of the adherens junction network during tissue growth. Such models therefore provide a framework to describe the interplay of PCP distributions in cells with cellular rearrangements in a tissue [14^{••},15,16]. Despite their different theoretical bases, all of these approaches have demonstrated that local rules are not sufficient for the global alignment of PCP starting from random polarity in large groups of cells — rather, these systems become trapped in locally stable states with swirling-type polarity

defects. Introduction of small global biases, acting over the entire system, can produce uniform polarity — but the nature of such an orienting cue *in vivo* is unclear.

One popular idea has been that long-range or global cues, such as graded concentration profiles of signaling molecules in the tissue, help to align cell polarity over large distances. The fact that Fz can act as a receptor for Wingless (Wg) suggested the possibility that a Wg gradient might orient polarity. However, Wg is expressed along the entire presumptive wing margin throughout wing development, whereas PCP domains and wing hairs point distally in the late pupal wing — not toward the wing margin [14^{••}]. Another candidate is a pathway involving the unconventional cadherins Fat and Dachsous (Ds) (for reviews see [1,6,17]). Mutations in either Fat or Ds do cause misorientation of PCP complexes and wing hairs; they also disturb the amount and orientation of growth in the developing wing [18,19]. Fat, which is expressed uniformly throughout the wing, can form either homophilic complexes or heterophilic complexes with Ds. Ds expression is strongest in the proximal wing, which led to the idea that intracellular asymmetries in the distribution of heterophilic complexes might directly bias PCP orientation in each cell [5,20]. One problem with this model is that uniform Ds expression can rescue normal planar polarity [14^{••},21]. Furthermore, the activity of Fat and Ds is required much earlier than proximal–distal polarization of PCP domains was thought to occur [21,22].

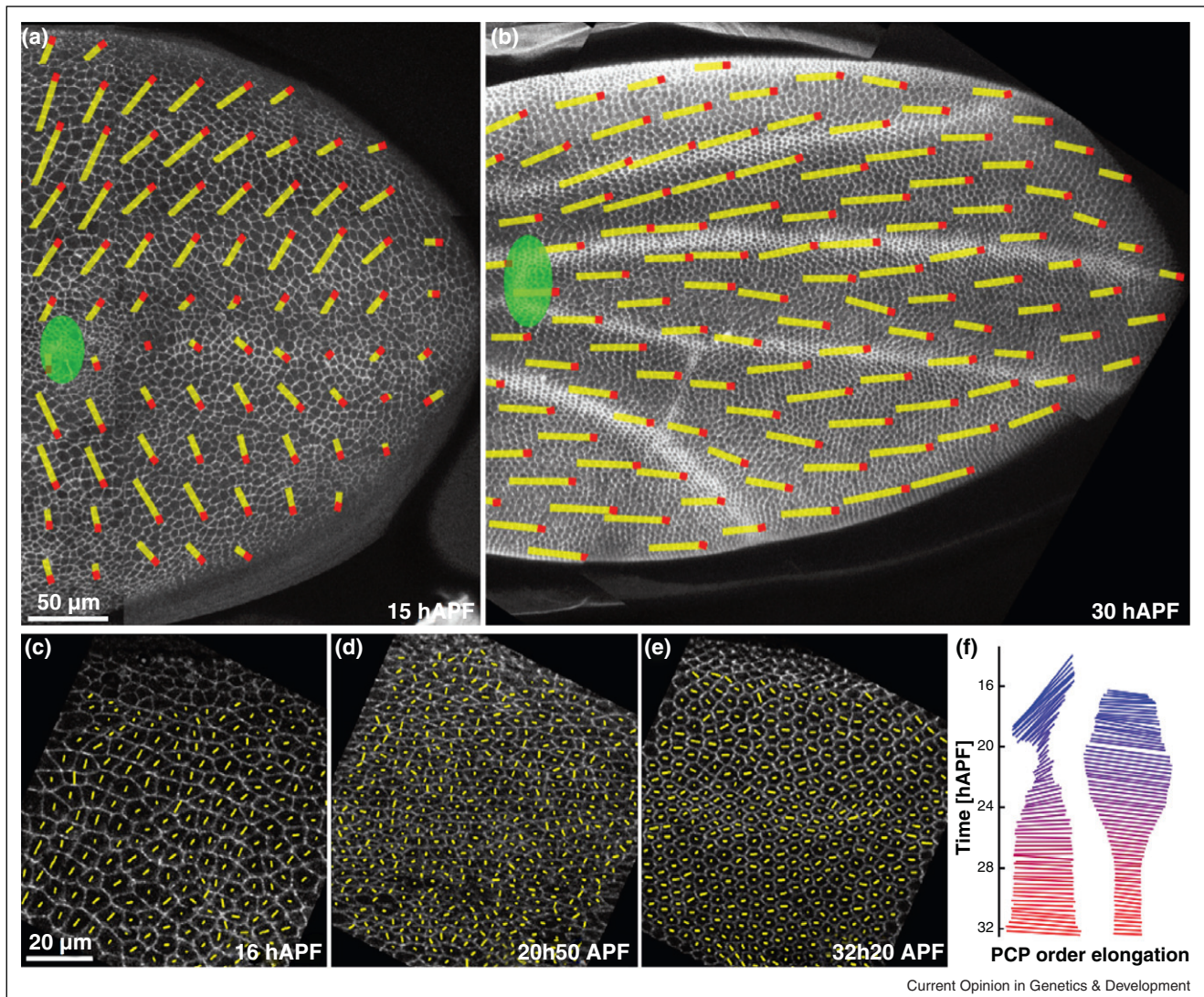
The global pattern of PCP in the wing changes during development

Key to identifying the cues that orient the global pattern of PCP is an understanding of how and when these patterns emerge. At first, it was thought that the proximal–distal polarization of PCP proteins seen in the pupal wing was established from a previously nonpolar state shortly before hairs actually emerge (between 18 and 26 hours after puparium formation (apf)). Recently, however, we used automated image analysis to quantify intracellular PCP protein distribution during time-lapse imaging. We used this information to define an axis and magnitude of polarity for each cell, and quantify the evolution of the global pattern of PCP over time. We found that large-scale patterns of PCP emerge much earlier during wing development, and that they reorient dynamically. At early pupal stages, the global pattern of PCP orients such that Fz domains face the wing margin — starting at about 16 hours apf, the global PCP pattern reorients to face distally, see Figure 2 and [14^{••}]. The almost nonpolar state that exists around 18 hours is actually a transient intermediate.

Tissue remodeling guides reorientation of planar polarity

The reorientation of PCP toward the proximal–distal axis of the wing correlates with a dramatic morphogenetic

Figure 2



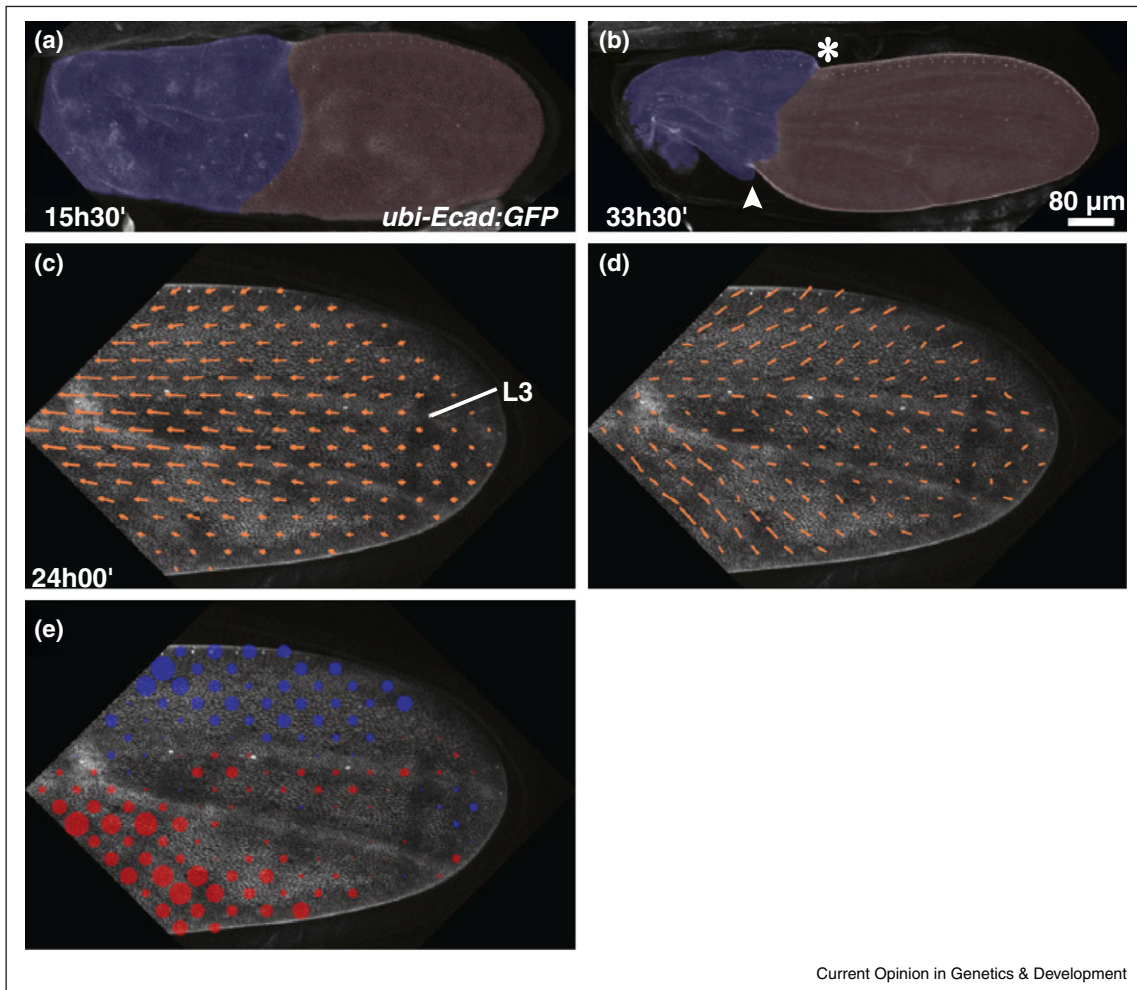
Quantification of planar cell polarity in the pupal fly wing at different times after puparium formation (APF). **(a)** At 15 hAPF, a characteristic early pattern of planar cell polarity exists. The bar indicates the axis and the strength of polarity, the red dot indicates the orientation of Fz-containing domains. **(b)** At 30 hAPF, just before hair outgrowth, the polarity pattern has reorganized with polarity pointing distally. **(c)–(f)** Quantified cell polarity of individual cells at different times. **(f)** Reorientation of the average polarity of the anterior half of the wing as a function of time reveals the dynamic reorientation of polarity.

reorganization of the wing epithelium that begins in pupae about 16 hours apf. At this time, cells in the proximal wing hinge contract, inducing an anisotropic pattern of mechanical stress in the wing blade. This leads to precisely choreographed patterns of cell flows that elongate the wing blade along its proximal–distal axis, see Figure 3a and [14^{••}]. We have analyzed this active tissue remodeling on different scales. The coarse grained patterns of cell flow velocities can be quantified from sequences of microscopy images. From such cell flow patterns the associated patterns of tissue shear and local rotation can be extracted, see Figure 3. Analysis on

smaller scale, based on quantification of junctional network rearrangements, reveals that these flow patterns emerge both from oriented cell divisions, and from cell elongation that is relaxed by oriented neighbor exchanges.

The simultaneous dynamics of PCP reorientation and active tissue remodeling suggests that these processes are linked, and that cell flows might directly reorient PCP. In support of this idea, severing the hinge from the blade leads to altered cell flow patterns that disturb polarity reorientation and the resulting global PCP pattern.

Figure 3



During pupal development, the network of cell junctions is dynamically remodeled and cell flows appear. **(a)** and **(b)** Wing hinge and wing blade at two different times APF reveals hinge contraction. Two morphological landmarks, the allula (arrowhead) and the costa (*) are indicated. **(c)** Cell flow field at 14 hAPF. From this cell flow field the patterns of shear **(d)** and rotation **(e)** can be determined. Blue dots indicate clockwise rotation, red dots counterclockwise rotation.

Furthermore, *ds* mutants exhibit a reduced anisotropy of cell elongation and of cell division during wing blade remodeling. This supports the idea that disturbed morphogenesis could contribute to the polarity defects observed in these mutants.

Tissue shear can reorient polarity either parallel or perpendicular to the shear axis

It is known from physics of complex fluids that shear deformations generally reorient anisotropies — a well-studied example is the reorientation of liquid crystals in anisotropic flow fields [23]. These effects can be described most elegantly in hydrodynamic continuum descriptions where the polarity in a fluid aligns either parallel or perpendicular to the axis defined by shear — the axis in which the system extends. The general behaviors of this reorientation can be captured by the value of a

single parameter ν . If ν is negative, polarity aligns parallel to the shear axis, if ν is positive it aligns perpendicular to it. The observation that cell polarity in the wing aligns with the long axis, which is also the axis of shear, suggests that PCP reorientation in the wing corresponds to the case of negative ν .

Of course tissues are not simple fluids and continuum descriptions may not be appropriate. We have therefore developed a vertex model for cell mechanics, which includes variables on the cell bonds that describe levels of PCP proteins [14**]. We can study the reorientation of PCP under shear introduced by different mechanisms. How do the different cellular mechanisms underlying tissue shear influence the polarity axis? Simulations of the vertex model suggest that the effect of shear on the PCP axis can differ depending on either the type of shear

generated or the kinetics of relaxation of the PCP system. External forces generate tissue shear by causing cell elongation, oriented cell divisions and neighbor exchanges, which can each affect the polarity axis. Cell elongation may influence PCP through its effects on microtubule orientation [24^{**}]. Simulations of the vertex model suggest that the average cell division axis can orient the PCP pattern either perpendicular or parallel to the division axis, depending for example on the relative rates of cell division and PCP relaxation. These two possibilities correspond to positive and negative signs of the parameter ν , which describes polarity reorientation due to shear in the hydrodynamic limit. Finally, for the parameter ranges explored in the vertex model, oriented cell rearrangements generated by external stress typically orient PCP perpendicular to the shear axis, that is they are characterized by a positive value of ν , unless there is an effect of cell elongation on PCP orientation.

In the wing, PCP responds to a combination of these different types of cellular processes that give rise to tissue shear. In normal wings, the net effect of this response is alignment of PCP parallel to the shear axis, characterized by a negative sign of ν . However, the sign of ν can change in principle when the mechanisms that produce shear are altered. For example, the shear observed in the wing blade when it is severed from the hinge is produced by different combinations of cell elongation, cell divisions, and cell rearrangements than those occurring in intact wings. Interestingly, the modified polarity pattern caused by severing can only be quantitatively accounted for if ν is positive.

The establishment of early patterns of planar polarity in the wing

Our work has shown that tissue polarity in the wing is reoriented by cell flows during pupal stages, but how is the earlier, margin-oriented polarity pattern established? Simulations show that it is difficult to establish uniform patterns of PCP starting with large fields of cells. However, our simulations in the vertex model suggest a novel solution to this problem: globally oriented polarity can be easily generated in small groups of cells and maintained during growth [14^{**}]. PCP domains in early pupal wings point toward the wing margin, a source of Wnts such as Wg and Wnt4. Fz is a receptor for Wg and the pattern is consistent with the idea that Wg may help to orient the global pattern of PCP. Wg is expressed at the DV boundary (the future wing margin) starting at the second to third instar transition, when the wing pouch first begins to grow [25]. Thus, it would be available to orient PCP domains while the wing epithelium was still small. Consistent with this idea, earlier studies suggested that global patterns of PCP already exist in growing larval wing discs [26], although their orientation has not yet been precisely defined.

A second mechanism that could influence the evolution of the PCP pattern during growth of the wing disc is growth itself. Vertex model simulations show that the axis of PCP is influenced by the average axis of cell division in a tissue. Clonal analysis of the larval wing disc shows that the pattern of growth is anisotropic and highly reproducible. Fat and Ds are involved in the control of growth patterns in the wing [18,19], and also influence the pattern of planar polarity — consistent with the idea that oriented growth could be a factor for establishing the global polarity of PCP domains.

Strategies to create large-scale polar order in different tissues

The PCP system is used in a wide range of species and tissues. In most cases the cell polarity defined by PCP molecules is directed either parallel or perpendicular to the axis of tissue morphogenesis, defined as the long axis of the tissue. Examples of polarization parallel to the axis of morphogenesis include the fly wing and leg, where PCP patterns guide hair orientation along the proximal–distal axis. Similarly, in the zebrafish lateral line organ, sensory hair cell bundles align parallel to the direction of elongation and migration of the primordium. Indeed, the direction of primordium migration specifies hair bundle orientation [27]. In contrast, in the mammalian cochlea, PCP domains and resulting sensory hair cell polarity are aligned perpendicular to the long axis of the tissue. This is also the case in the abdomen of the fly. The abdominal segments are generated by the growth of histoblast nests, which expand dorso-ventrally [28]. However, PCP in the abdomen is oriented perpendicular to the direction of morphogenesis, along the anterior–posterior axis.

These instances of polarity orientation perpendicular to the tissue axis could be the consequence of the reorientation of PCP under shear if these tissues were characterized by a positive sign of the effective parameter ν . Our observations of wounded wings suggest that the sign of ν can indeed change. One might speculate that the different cellular processes that generate tissue elongation in these different examples could be responsible for the different signs of ν . Simulations in the vertex model also suggest that the sign of ν can vary if the rate at which PCP relaxes changes with respect to the shear rate. Such a change might be easily achieved by altering molecular trafficking rates, diffusion rates, or binding affinities of PCP proteins. More complex polarity patterns could be generated by the combined effects of cell flows and locally acting polarity cues. This represents a highly flexible mechanism to generate new patterns of planar polarity during evolution. To function properly, hairs and cilia must be precisely aligned with tissue shape. Coupling planar polarization to the mechanisms underlying tissue morphogenesis is an elegant solution to this problem.

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