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Planar Polarity: Converting a Morphogen Gradient into Cellular Polarity

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Abstract

Epithelial cells are polarized within the apico-basal and planar axes. The latter—planar cell polarity—requires long-range regulation of orientation as well as short-range, cell-to-cell realignment through feedback loops. New insights into the long-range, gradient-type regulation reveal how a kinase translates the morphogen gradient input into cellular orientation.

Epithelial cells are polarized within the apico-basal axis and within the plane of the epithelium, the latter being termed planar cell polarity (PCP). Each cell within the sheet is itself polarized, for instance in the proximo-distal axis of the *Drosophila* wing, and this polarity is coordinated with the overall polarity of the tissue. In the case of the fly wing, polarity is manifested through the individual actin-rich wing hairs that point distally (Figure 1A). PCP is generated through heterophilic interactions of two sets of transmembrane proteins: the Frizzled–Vang–Flamingo system (Fz–Vang–Fmi), also called the ‘core’ pathway; and the Fat–Dachsous (Ft–Ds) system (Figure 1B,C) [1]. In both cases, asymmetric intermolecular complexes form with one component being localized to the distal side of a cell and interacting with its partner on the proximal side of the apposing cell: Fz with Vang, and Ds with Ft, respectively. The long-range co-ordination across a whole tissue of cell–cell asymmetrical protein distribution and intracellular PCP manifestation has only recently begun to be addressed. In a recent article published in *eLife*, Hale *et al*. [2] set out to demonstrate how the graded phosphorylation of Ft and Ds by the Golgi-resident kinase Four-jointed (Fj) regulates the Ft–Ds interaction and the planar-polarized accumulation of Ft–Ds complexes across the entire wing.

Ft and Ds are large proto-cadherins that coordinate planar polarity and — through an as yet unknown mechanism — link polarized membrane complexes to the cytoskeleton [3]. Fj is a kinase that is active in the Golgi and phosphorylates the extracellular cadherin domains of both Ft and Ds [4]. *In vitro* and cell culture studies have shown that phosphorylation by Fj has opposing effects on Ft and Ds: phosphorylated Ds has reduced affinity for Ft and phosphorylated Ft has increased affinity for Ds [5,6]. On a tissue level, Ds and Fj are expressed in reciprocal gradients within the developing wing such that, in the presumptive proximal area, Ds is high and Fj is low, and at the distal area the situation is reversed (Figure 1C). On a cellular level, Ds accumulates on the distal side and Ft on the proximal side of any

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given cell. Exactly how the complementary expression patterns of Ds and Fj expression cause asymmetric accumulation of Ft–Ds within a cell is unclear, although previous studies suggested that there is a gradient of Ft–Ds dimer formation across the tissue [7]. In their recent work, Hale *et al*. [2] used a combination of *in vivo* imaging and computational methods to demonstrate that the Ft–Ds binding gradient can be explained by the graded activity of Fj and that this is sufficient to propagate the polarization of complexes across the whole tissue.

Based on previous data from cell culture and overexpression studies, the authors used computational modelling to generate predictions about the stability of Ft–Ds complexes that they then tested *in vivo*. As a measure of stability, they monitored fluorescence recovery after photobleaching (FRAP), the basic idea being that the proteins in stable complexes are less mobile and so when they are bleached there is less interchange with unbleached proteins from elsewhere in the cell, and so the fluorescence signal does not return to its pre-bleach level: the greater the mobility and therefore the greater the level of protein exchange, the higher the level of recovery of the fluorescence signal. The authors elegantly use endogenously tagged Ft and Ds proteins to be sure to investigate how Fj functions in vivo, rather than interpreting an artificial expression system with all its caveats [2]. Analysis of endogenous protein dynamics revealed a stable pool of Ft and Ds present in puncta at cell junctions, as well as a more dynamic pool that is also found at junctions. The effect of Fj on Ft and Ds binding affinities was then analyzed and it was found that, in a *fj* mutant background, Ft–Ds complexes are less stable. Thus, the effect of Fj on Ft is dominant and Fj has a net positive effect on Ft–Ds binding. Expression of mutant forms of Ft or Ds in which the Fj phosphorylation sites were mutated confirmed this result, with the Ds mutant being more stable and the Ft mutant less so. One thing that remains unclear is how Fj exerts a differential effect on Ft and Ds. Fj phosphorylates analogous serine residues within sub-regions of the cadherin repeats of both Ft and Ds. As this phosphorylation inhibits the binding affinity of Ds for Ft but has the opposite effect on Ft, it is possible that phosphorylation induces a conformational change in the extracellular region that differentially alters the affinity of Ft and Ds for each other.

An interesting point that follows on from previous data and is clarified here is how relatively small the protein asymmetries can be, so feedback mechanisms and additional inputs must presumably be required to reliably reproduce the Ft–Ds polarity both within each cell and from animal to animal. For instance, Fj activity resulted in only a two-fold increase in Ds asymmetry across an individual cell [2], and previous reports suggested that the changes in polarity that result from manipulating Ds expression can only be propagated over the distance of a few cells [8,9]. Coupled with this is the fact that, although *fj* clones can reorient the polarity of a tissue near clone borders, the *fj* null phenotype shows basically wild-type planar polarity [10], and uniform (i.e. non-graded) expression of Ds in a *ds* or *ds*, *fj* mutant background can rescue polarity in the wing [11,12]. Despite its clear effect on Ds–Ft binding, Fj appears to be somewhat redundant with other positive-feedback mechanisms that must help to reinforce small asymmetries and lead to the robustness seen in PCP at the tissue level. One such mechanism may be the amplification of Ft–Ds polarity by the ubiquitin

ligase FbxL7, which co-operates with Ft in localizing Ds and the downstream component Dachs to the opposite side of the cell [13,14].

The core pathway is better understood in terms of how feedback mechanisms can amplify the initial asymmetry and result in asymmetries of Fz and Vang that are significantly higher than two-fold across a given cell. The Wnt ligands Wg and dWnt4 serve as a polarizing cue by binding to the Fz extracellular domain and modulating Fz–Vang intercellular interactions [15]. Wg and dWnt4 are expressed at the presumptive distal area of the wing, and thus, in a given cell, Vang localizes to the proximal side where it is better able to bind to Fz on the distal side of the neighboring cell (Figure 1B). Fmi–Vang complexes can recruit the intracellular effector Prickle (Pk) and Fmi–Fz complexes recruit Dishevelled and Diego (Dsh and Dgo); interactions between Pk, Dsh and Dgo then reinforce the initial Fz–Vang asymmetry [16].

How could these polarity systems be coordinated across whole tissues? Genetic data strongly argue that the Ds–Ft and Fz–Vang systems are independent and act in parallel [1], despite early suggestions that Ds–Ft signaling occurs upstream of the core system. However, recent results have started to suggest that the two systems are more closely linked. Hale *et al*. [2] add to the body of data showing that the gradients of Ds and Fj transcription are sufficient to propagate Ft–Ds asymmetry in the wing and eye, but this raises the question of which factors are responsible for the graded expression. One such input is the morphogen Wg, which acts via canonical Wnt signaling to regulate gradient-type transcription of Ds and Fj [10,12]. Thus, in an intertwining of the two PCP systems, Wg is a polarizing cue for both pathways, it impacts the core PCP factors by direct binding to Fz (thereby modulating the Fz–Vang interaction) and it regulates the Ds–Ft system transcriptionally via canonical signaling (including the transcriptional gradient of Fj expression!). So, in other words, Wg (and dWnt4) are the long-range regulators of PCP. The connections between the two systems probably occur at multiple levels. For example, Ds–Ft activity orients microtubules and thus participates in Fz and Dsh trafficking to the distal side of a cell to reinforce initial polarity [17,18]. Also, Pk isoforms have recently been shown to couple Ds–Ft to the core system at late stages of PCP reinforcement by ‘interpreting’ the Ds–Ft gradient to orient microtubules [19,20]. The new work from Hale *et al*. [2], together with other recent studies, therefore reveals that the Ds–Ft and Fz–Vang PCP systems seem to be much more intertwined than originally assumed and, importantly, that they are both ‘oriented’ by Wnt signals.

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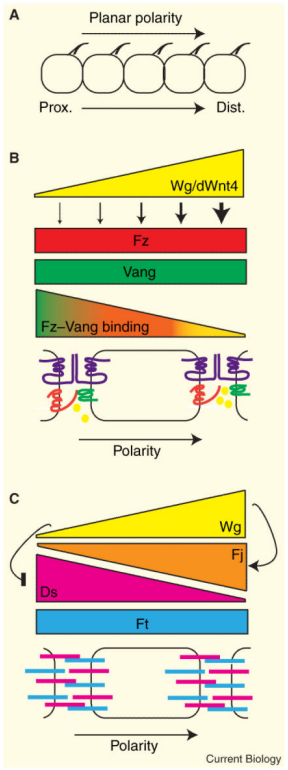

Figure 1. Schematics of long-range planar cell polarity regulation

(A) Schematic of planar cell polarity in the *Drosophila* adult wing. Each epithelial cell produces an actin-rich hair that points distally. (B) Top: Long-range patterning of the 'core' pathway. Frizzled (Fz) and Van Gogh (Vang) show uniform, non-graded expression, but their ligand–receptor interaction is modulated by the Wg/dWnt4 gradient (direct binding of Wnts to Fz; represented by straight arrows) leading to a polarized field. Bottom: The cell senses the direction of the Wnt gradient by the relative amount of Wnt available to Fz setting up the axis of Fz–Vang binding. Flamingo (purple) is also critical for the Fz–Vang interaction. (C) Top: Long-range patterning of the Fat–Dachsous (Ft–Ds) pathway is dependent on the graded expression of Ds and Four-jointed (Fj); Ft expression is not graded. Wg acts via the canonical Wnt pathway to regulate graded Fj and Ds transcription (curved arrows represent transcriptional activation or repression of the Wg targets). Bottom: The cell senses the gradient of Ft and Ds phosphorylation with Ds accumulating on the distal side of the cell and forming intercellular complexes with Ft on the proximal side of the neighboring cell.