

Chapter 5. The Ft/Ds/ Fj cassette, trans-membrane coupling and soap-bubble packing.

In addition to the core PCP functions, three genes that disrupt polarity patterns have been studied extensively in the fly: *fat* (*ft*), *dachsous* (*ds*) and *four-jointed* (*ff*). Mutants of these genes show misaligned hairs and misshapen limbs: with legs becoming shorter and wings rounder. None of these genes encode diffusible morphogens, but two of the three are expressed in opposing gradients in the wing and eye-antennal discs. The *ft* and *ds* genes encode transmembrane cadherins, while *ff* encodes a kinase that phosphorylates their extracellular domains. Intensive study of this *ft/ds/ff* cassette has led to somewhat contradictory interpretations, but PCP signalling is certainly affected^{1 2 3 4}. Intriguingly, although Ft and Ds bind each other in a 1:1 stoichiometric ratio, Ft is uniformly expressed; it is the expression of Ds and Fj that is graded. Binding affinity is therefore dependent on the Fj kinase activity, which is localised to the Golgi apparatus and active during the secretion pathway⁵. The heterotypic Ds/Ft complex is stabilised at adjacent cell boundaries², without which both cadherins are rapidly degraded. Although *ft/ds/ff* mutants show gross patterning defects, complementing *ds* and *ff* mutations with uniformly expressed proteins gives a fairly complete rescue of their mutant phenotypes, see critical discussion in⁶. However, *ff* LOF mutants show growth defects near the middle of the (Pr/Dist) length of the wing blade, and the middle tarsal segment⁷. A major function of the *ft/ds/ff* cassette may be to anchor cortical scaffold proteins and facilitate polarised signal transduction. In this context, the *ft/ds/ff* cassette regulates preferential, plus-end directed microtubule growth⁸. Whatever the detailed mechanisms, the *ft/ds/ff* cassette may modify MVB uptake, particularly at morphogenetic field boundaries. Notably, the preferential orientation of mitotic spindles along the D/V margin of the larval wing disc is lost in *ds* mutants⁹. In vertebrates, the murine *ft4* gene acts as a tumour suppressor, with the knockout of *ft4* resulting in mis-oriented cell divisions and polycystic kidney disease¹⁰. Taken together, these results support a link between mechanical stress, spindle polarity and cellular proliferation. The transmission of mechanical stress prior to mitotic spindle assembly may require directed microtubule growth from the periplasmic cytoskeletal/membrane interface. Such coupling occurs at via APC2 (Anaphase promoting complex-2) and Armadillo (Arm), both of which form structural components of junctional complexes¹¹.

Paradoxically, while the mutant PCP patterns are inconsistent with a morphogen diffusion gradient, they provide strong evidence for an underlying Pr/Dist signal, which is reversed in left and right wings. Without such a reversal, the distal whorl in *pk* mutants (Fig. 6) would be anticlockwise in both wings. All left wings might have the same complex pattern, but this should not be a mirror-image of the right-wing pattern. By contrast, the whorls on the D and V surfaces of *pk* wings are reversed, so that viewed from above their opposed (back-to-back) surfaces show clockwise whorls in left wings, and anticlockwise whorls in right wings.

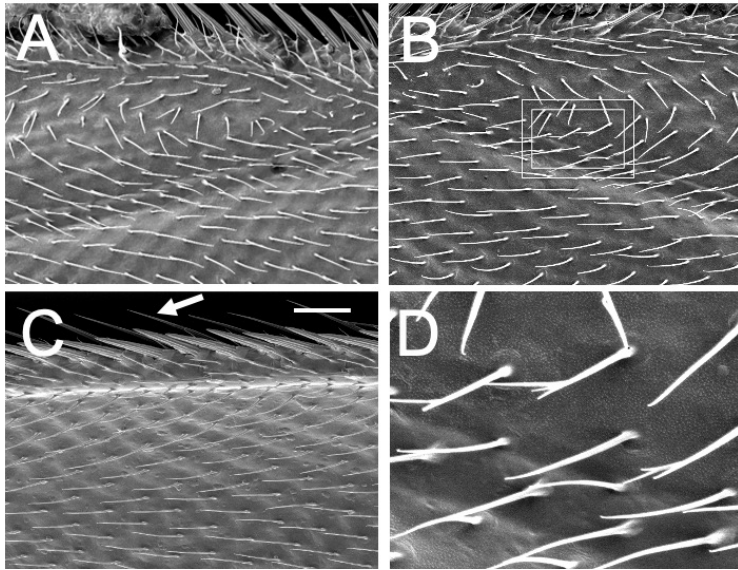


Fig. 6. SEM image pk^{pk} , D wing surface. A. L and B. R pk^{pk30} near tip of vein 2. C. Wild-type anterior wing margin, white bar = 20 microns. Note row of thin bristles extending from the V surface, white arrow. D. pk^{pk30} , enlarged region of B. The tapered, cylindrical hair shafts remain straight, in alignment with topological disclinations, but may show kinks and splayed tips. By implication, polarity is not cell-autonomous but may alter across the surface of an individual cell.

The mirror-symmetrical L/R wing patterns imply that a Pr/Dist polarising signal may be superimposed on tessellated cellular alignment. A more relevant model might be soap-bubble packing discontinuities^{12 13 14} Fig. 7. In the limiting case of a soap bubble monolayer, each bubble would have rectilinear lateral interfaces, with hexagonal top and bottom surfaces. Similarly, uniform contraction of cellular interfaces would favour hexagonal packing within an epithelial sheet.

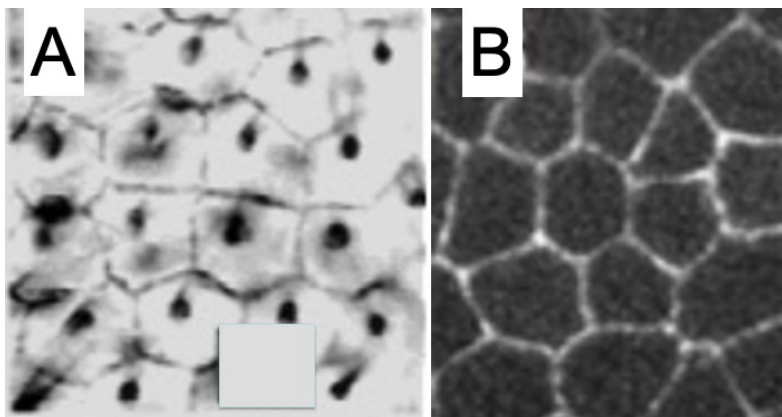


Fig. 7. Soap bubble stacking flaws. A. Four central pentagonal cells, with cruciform boundaries, surrounded by minimally distorted hexagonal cells, in $m; pk^{pk}$ wing, from Gubb et al. 1999. B. A single hexagonal cell is surrounded by ring of 7 distorted polygons, in the eye-antennal disc, from Zobel et al. 2015. In the adult ommatidium, the R7 photoreceptor is shifted below the R8, surrounded by the R1-R6 photoreceptors. In the pupal wing disc, junctional complexes may localise to hexagonal cell vertices, although an additional (Pr/Dist) polarising signal would be required restrict hair initiation to the distal

vertex. Such a Pr/Dist polarising signal may be transmitted across the wing blade as a metachronal wave of contraction during terminal PCP signalling, see ¹⁵. However, even if cellular interfaces are aligned with respect to the straight A/P boundary, distorted cells must be present along the curved D/V margin and, occasionally, within the wing blade. In wild-type wings, such occasional tessellation defects do not disrupt the Pr/Dist alignment of hairs. However, the swirling hair patterns of *pk^{pk}* mutants resemble the topological stacking flaws in mantid oothecal liquid crystalline proteins (Figs. 4, 5, 6). The occasional twin hairs in *pk^{pk}* mutants tend to have different orientations and be separated across the Ap cell surface. Furthermore, where topological disclinations are sharply curved the hair shafts do not bend; instead, straight lengths of the shaft track the disclinations with sudden kinks (Fig. 6D). Under these conditions, the planar polarity of extruded hairs may alter across the apical surface of an individual cell. Similarly, the polarity of larval denticles alters across the surface of wild-type abdominal cells ¹⁶.

Summary:

The *ft/ds/fj* cassette regulates epithelial growth and mitotic spindle orientation, with mechanical coupling of epithelial cell interfaces. Thus, bilaterally symmetrical morphogenetic signals may be transmitted, despite signal transduction being through chiral cell-surface receptors. A Pr > Dist wave of Ap cell-surface contraction aligns hexagonal cells across the wing blade, with occasional distorted interfaces. During normal development, hair shaft growth is initiated from the distal vertex of hexagonal wing cells. However, the alignment of hairs in PCP mutants may change across the surface of individual cells, consistent with altered transmission of a polarising signal. The *ft/ds/fj* cassette may regulate cell adhesion and spindle orientation during growth, while PCP genes direct cytoskeletal remodelling during terminal differentiation.

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