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Cell biology of planar polarity transmission in the *Drosophila* wing

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Abstract

The coordination of epithelial planar polarization is a critical step in the formation of well-ordered tissues. The process has been extensively studied in *Drosophila*, where genetic analysis has identified a set of "tissue polarity" genes that serve to coordinate planar polarity of cells in the developing wings, bristles and eyes. In the last several years, it has emerged that six of these genes encode junctional proteins. In the wing epithelium, these proteins undergo a polarized redistribution, forming separate proximal and distal cortical domains within each cell. The mechanisms that mediate cortical polarization and cue its direction have been the subject of intense investigation. Cuing the orientation of cortical polarization appears to depend on the atypical Cadherins Fat and Dachsous, although these proteins do not become polarized themselves, nor do they colocalize with components of polarized cortical domains. Interestingly, these Cadherins also act at earlier developmental stages to polarize tissue growth along the proximal–distal axis and it will be interesting to see whether these processes are mechanistically related. Once the axis of polarization is determined, cortical polarity seems to be propagated, at least locally, by a cascade of direct cell-cell interactions mediated by the proximal and distal domains. The cell biological mechanisms leading to polarization are still unclear, but the process depends on the control of Protein Phosphatase 2A activity by its regulatory subunit, Widerborst. Interestingly, Widerborst is found on a planar web of microtubules with connections to apical junctions, suggesting that these microtubules may have an important function in polarizing the cortex.

Keywords: Cell biology; Planar polarization; *Drosophila melanogaster*

Understanding the control of tissue shape, size and organization is a central problem at the interface of cell and developmental biology. During tissue development, cells must integrate signals controlling the amount and direction of growth with the final differentiative signals that determine specific cellular structures and functions (Eaton, 1997). For example, ciliated epithelial cells of the oviduct or the airway must form a tube of a particular size and shape, and then orient ciliary beating along its long axis (Boisvieux-Ulrich and Sandoz, 1991). The development of a coordinated intracellular polarity along the planar epithelial axis has been called tissue or planar polarization. The vertebrate cochlea is another example of a tissue in which the final differentiation of planar polarity must be properly coordinated with the shape of the tissue; staircases of actin-filled stereocilia on the apical surface of each sensory hair cell are aligned so as to maximize their sensitivity to displacement of the overlying tectorial membrane (Tilney and Saunders, 1983; Tilney et al., 1986). In insects, the mechanosensory bristles and hairs that decorate the cuticle are constructed by ectodermal epithelial cells and are uniformly polarized along the different body axes. On the limbs, these structures are oriented along the long, proximal–distal axis in which the majority of cell growth has occurred. How do cells properly coordinate their planar polarity in the context of these tissues?

For many years, the fruitfly *Drosophila melanogaster* has provided a genetic model organism with which to address both the mechanisms of growth control and of tissue polarization (Adler, 2002; Gubb and Garcia-Bellido, 1982). The recent application of more cell biological approaches, in combination with genetics, has led to rapid development in our understanding of these processes. In particular, great progress has been made in understanding how cells communicate locally to coordinate their polarity within the plane of the epithelium (Axelrod, 2001; Feiguin et al., 2001; Shimada et al., 2001; Strutt, 2001; Tree et al., 2002b; Usui et al., 1999). The nature of the global cues that initiate polarization and determine its axis have been much more difficult to understand, however. Intriguingly, several recent reports have raised the possibility that these global cues may

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be linked to the mechanisms that control the size and shape of developing tissues (Adler et al., 1998; Casal et al., 2002; Ma et al., 2003; Strutt and Strutt, 2002; Yang et al., 2002; Zeidler et al., 2000). This review will examine our current thinking about planar polarization in the wing epithelium of *Drosophila*.

1. Introduction

To understand the tissue in which planar polarization is occurring, it is necessary to briefly outline its growth and development. Many of the ectodermal cells that will form the adult fly are set aside late during embryonic development and eventually form a series of separate epithelial sacs called 'imaginal discs'. The imaginal discs are patterned and grow during larval life. As the disc epithelium proliferates, the additional tissue is constrained in epithelial folds that form in a characteristic pattern in each imaginal disc. After the larva pupariates, the discs unfold and assume the final shape of the different adult structures (e.g. legs, wings, etc.) that they will create (see Fig. 1A). The dorsal mesothoracic disc, which will give rise to the wing and notum, is derived from about 40 cells; by the time the larva pupariates, the disc consists of more than 50,000, about half of which are contained in a single large fold called the wing pouch. Growth in the developing wing pouch region of the disc is not isometric; instead, cells come to lie preferentially along the proximal–distal axis of the future wing. Since mitotic spindles observed in fixed tissue do not appear to lie preferentially along the proximal–distal axis, it is thought that subsequent rearrangement, perhaps based on adhesive differences, influences the relative positioning of daughter cells (Milan et al., 1996a,b; Resino et al., 2002). Growth polarization is thought to be important in determining the final shape of the wing, which is longer along the proximal–distal than along the anterior–posterior axis (Resino et al., 2002).

At pupariation, the wing pouch epithelium flattens, everts from the plane of the disc, and folds itself into an epithelial bilayer that is shaped like the future adult wing. After 2–3 more rounds of cell division, the wing epithelium secretes cuticle from its apical surface. This cuticle is shed about 17 h after puparium formation (APP) and forms a thin, waxy sac around what is now called the pupal wing (Fristrom and Fristrom, 1975). At about 32 h APP, each pupal wing epithelial cell begins to construct a single distally oriented wing hair (Fig. 1A). These structures are apical membrane outgrowths that are supported by polarized rearrangements of both actin and microtubules (Eaton et al., 1995; Wong and Adler, 1993). How is the distal orientation of wing hairs controlled?

A priori, it is clear that polarization in the plane must rely on different kinds of cues than are used to determine epithelial apical–basal polarity. In the latter case, different environmental interactions on the apical, basal and lateral sides of the cell are available to direct the axis of polarization (Eaton and Simons, 1995). The source and nature of planar signals is not obvious. Speculation has centered on two opposing types of models. One model entails the localized secretion of one or more 'polarization ligands' that form concentration gradients; in this case, each cell would interpret a small difference in concentration

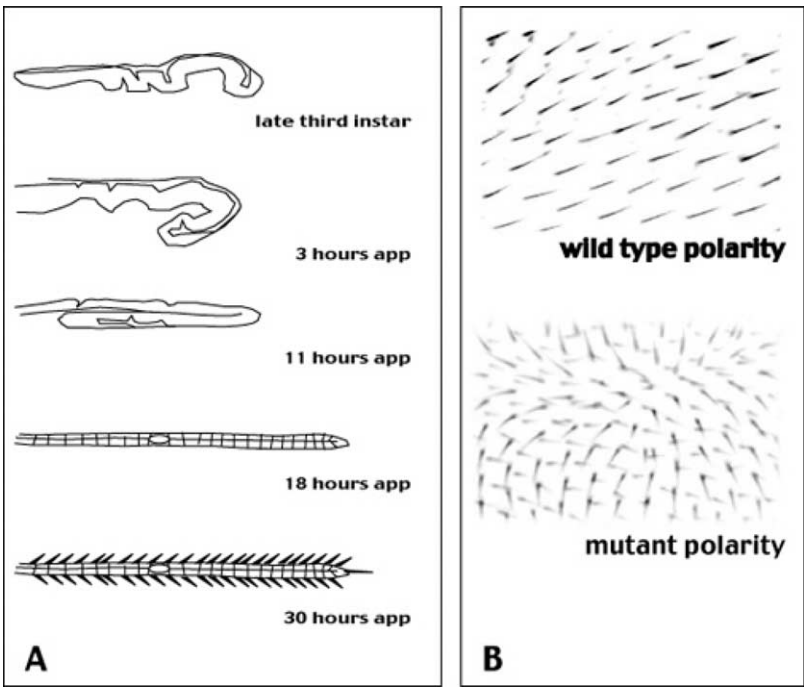

Fig. 1. Morphogenesis of the pupal wing. (A) The morphogenetic movements giving rise to the pupal wing are outlined. APP, after puparium formation. (B) Hair orientation on a wild type wing, and on a wing with planar polarity defects typical of the core group tissue polarity genes.

Table 1
Protein components of proximal and distal cortical domains

	Sequence features	Localization	Activity
Frizzled	Seven transmembrane domains	Distal	Induces long-range, non-autonomous polarization of cortical domains
Dishevelled	PDZ domain, DEP domain, DIX domain	Distal	Moves to cortex in response to Frizzled signalling
Prickle-Spiney leg	LIM domain	Proximal	Prevents Frizzled and Dishevelled accumulation at the cortex
Flamingo	Seven transmembrane domains, Cadherin repeats	Distal and proximal	Homophilic adhesion
Diego	Ankyrin repeats	Distal and/or proximal	Clusters Flamingo

along its proximal–distal axis and translate it into planar polarity. Alternatively, the mechanism could depend on the relay of polarity information from cell to cell. In this case, one or more locally acting signals would polarize one cell, which would in turn generate a signal to polarize its neighbor and so on. Recent advances in our understanding of the cell biology of this process suggest that planar polarization in the wing depends at least partially on a signal relay mechanism.

2. Relay of planar polarization by cortical interactions

Genetic analysis in *Drosophila* has identified a 'core' group of tissue polarity genes, so called because they affect polarization of many structures including the omatidia of the eye, and the bristles and hairs that cover the body. In the wing, core group mutants display characteristic whorls of hairs in which local coordination between cells is maintained, but global polarity is lost (Gubb, 1993). Over the last 4 years, it has emerged that this class of genes encodes proteins that localize to apical junctions. Strikingly, between 6 and 12 h before hair formation, these proteins polarize their distribution along the proximal–distal axis of each cell, forming separate proximal and distal 'cortical domains' at the junctional region (Adler, 2002; Adler and Lee, 2001; Strutt, 2002; Tree et al., 2002a). The process appears to be highly cooperative in that elimination of any one of the protein components interferes with the polarization of all of them. One very exciting property of these domains is that cortical polarization in one cell can induce cortical polarization in adjacent cells, suggesting that the proteins involved could form part of a signal relay mechanism. These core tissue polarity proteins (Frizzled, Dishevelled, Strabismus/Van Gogh, Prickle-Spiney leg, Flamingo/Starry night and Diego) comprise an assortment of transmembrane and membrane-associated molecules with only partially characterized functions (see Table 1 and Fig. 2).

Proximal and distal cortical domains contain both shared and specific components. Flamingo (Usui et al., 1999), also known as Starry night (Chae et al., 1999), is a seven-pass transmembrane Cadherin that is found on both sides of the cell; here it engages in homophilic adhesive interactions that couple the proximal domain of one cell to the distal domain of the next. These interactions are critical for polarization and, in the absence of Flamingo, none of the other core group proteins is found at the junctional region (Axelrod, 2001; Feiguin et al., 2001; Shimada et al., 2001; Strutt, 2001; Usui et al., 1999). Two of the molecules that move to the distal domain are Frizzled (Strutt, 2001), a seven-pass transmembrane protein, and Dishevelled (Axelrod, 2001; Shimada et al., 2001). Earlier in disc development, Frizzled signals in response to Wingless through Dishevelled via the well-studied 'canonical' pathway to activate transcription of target genes via TCF. The partners of Frizzled and Dishevelled in planar cell polarity (PCP) signaling are clearly different; there is no evidence that Wingless or any other Wnt family protein is required to activate Frizzled during planar polarization, and Dishevelled appears to operate independently of its canonical pathway partners. On the proximal side of the cell are Prickle-Spiney leg (Tree et al., 2002b) and Strabismus, also known as Van Gogh (Bastock et al., 2003; Taylor et al., 1997; Wolff and Rubin, 1998). Strabismus is a putative transmembrane protein whereas Prickle-Spiney leg

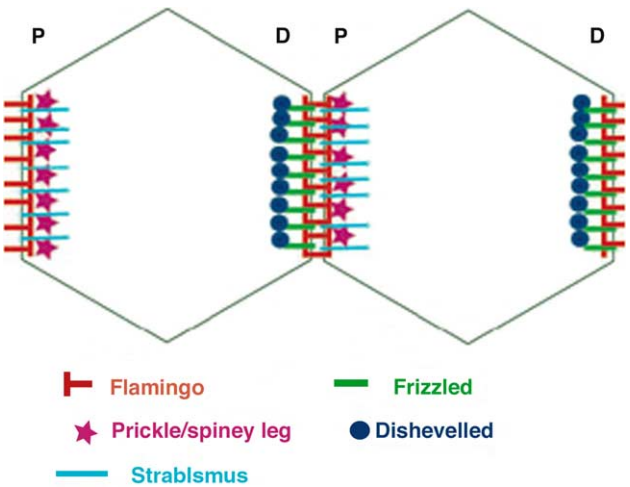

Fig. 2. Localization of proteins comprising the proximal and distal cortical domains. The identity of the proteins is indicated. P, proximal; D, distal.

contains LIM domains, which might be used for protein–protein interactions (Gubb et al., 1999; Tree et al., 2002b). Diego localizes to proximal–distal boundaries, although it is not yet clear whether it is present on one or both sides of the cell. It moves to the cortex in response to Frizzled signaling where it colocalizes tightly with other core group proteins. Diego appears to promote the clustering of other cortical protein with itself, and in its absence polarization is inefficient and inaccurate (Feiguin et al., 2001).

The idea that a signal relay mechanism might contribute to planar polarization was first suggested by one intriguing property of Frizzled mutant cells; clones of cells missing Frizzled activity cause a long-range disruption of hair polarity in distal wild type tissue, a phenomenon referred to as 'domineering non-autonomy' (Vinson and Adler, 1987). Subsequent experiments showing an opposite effect of Frizzled over-expression made it clear that hairs tended to point towards regions of low Frizzled activity and away from regions of high Frizzled activity (Adler et al., 1997). Interestingly, altering Strabismus/Van Gogh activity causes long-range polarity disruption opposite to those of Frizzled; hairs tend to point away from cells with high levels of Strabismus (Taylor et al., 1997). Mutations in other core group genes alter polarity in mutant cells, but have only limited effects on adjacent wild type tissue. Studies of the distribution of these core group proteins under both wild type and mutant conditions have shown that the long range hair repolarization caused by Frizzled mutant cells is presaged by long range, coherent alterations in cortical domain polarity (Axelrod, 2001; Feiguin et al., 2001; Shimada et al., 2001; Strutt, 2001; Tree et al., 2002b; Usui et al., 1999).

Recent data suggest that local disturbances in the polarity of cortical domains caused by Frizzled mutant cells are propagated to surrounding tissue by direct cellular interactions that depend on the properties of proximal and distal cortical proteins themselves. Tree et al. have shown that Pk-Sple prefers to accumulate at boundaries that contact cells expressing Frizzled and Dishevelled. Furthermore, Pk-Sple appears to discourage cortical accumulation of Dishevelled, at least in tissue culture cells (Tree et al., 2002b). Interestingly, Dsh appears to interact physically with both Pk-Sple and Strabismus, despite the fact that these proteins are normally found on opposite cell boundaries in the steady state (Bastock et al., 2003; Tree et al., 2002b). This raises the possibility that the turnover of these proteins is high and that transient interactions are critical in generating a polarized steady-state distribution.

Based on these data, Tree et al. (2002a,b) have proposed the existence of a feedback loop that operates across proximal–distal cell contact sites. Distal Frizzled and Dishevelled in one cell recruit Pk-Sple to the proximal side of the adjacent cell—Pk-Sple in turn excludes Dishevelled from the proximal cortex (see Fig. 3B). This loop would serve to amplify small initial differences in proximal–distal protein composition. Such a mechanism would ensure that a proximal cortical domain always formed next to a distal domain in an adjacent cell. Furthermore, it would mean that localized Frizzled over-expression would promote 'distalness' in the over-expressing cells and induce the formation of proximal cortex where they contact adjacent cells. Conversely, Frizzled mutant cells that could not form a distal domain might encourage their neighbors to form distal cortex where they contacted the mutant clone.

This helps explain how Frizzled perturbation repolarizes directly adjacent membranes, but how does repolarization propagate to more distant cells? This would require that Pk-mediated exclusion of distal components from the proximal cortex would result in their enrichment on the distal side of the same cell—a not unreasonable possibility. If this were so, then cortical polarity might be transmitted from cell to cell indefinitely. Could long range propagation of cortical polarity from cell to cell be sufficient to polarize the entire wing in response to just a few localized signals, obviating the need for a graded distribution of a polarizing signal?

3. Are graded polarization ligands required?

There are several problems with this model that seem to argue for the necessity of some sort of secreted protein to mediate longer-range polarization. First, only some Frizzled mutants (including nulls) exert non-autonomous effects on surrounding cell polarity; others disrupt the polarity of mutant cells without interfering with that of their neighbors. Strutt et al. have shown that at least one of these purely autonomous Frizzled alleles encodes a protein that does not become polarized to the distal side of the cell (Strutt and Strutt, 2002). This suggests that polarization of Frizzled protein in these cells is not required for the polarization of their neighbors. Furthermore, Frizzled is not found at all at the apical junctions of cells that are missing Flamingo, nevertheless Flamingo clones do not exert long-range effects on the polarity of their neighbors (Strutt, 2001). So it would seem that neither unpolarized Frizzled nor the total absence of Frizzled at cell boundaries prevents polarization of neighboring cells. At first glance, both of these observations are inconsistent with a model in which polarity is transmitted purely by a cascade of cortical interactions. An alternative proposition has been that the long range effects of Frizzled null mutant clones are due to disruption in the secretion of a 'polarization ligand'. According to this model, those Frizzled mutants that act purely autonomously would retain the ability to secrete this ligand. To date, however, no good candidates for such a ligand have been identified. Although Frizzled family members normally act as receptors for Wnt-type molecules, none of the *Drosophila wnt* mutants tested have any effects on tissue polarization (Cohen et al., 2002; Kozopas et al., 1998). Could the behavior of *flamingo* mutant and autonomous *frizzled*

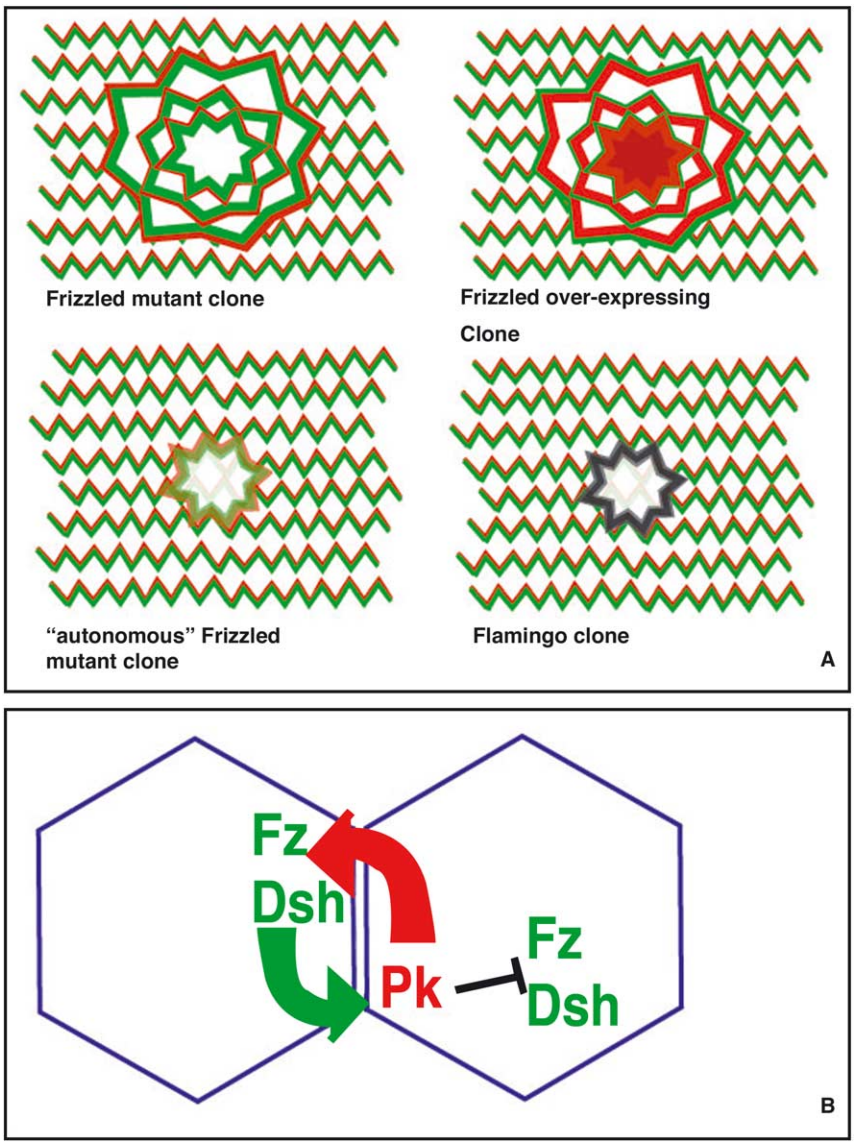

Fig. 3. Propagation of cortical polarity. (A) Outline of cortical disturbances produced by *frizzled* null, *frizzled* autonomous, *frizzled* over-expressing and *flamingo* mutant clones. Red indicates distal cortical domains, and green indicates proximal cortical domains. The black boundaries of *flamingo* clones indicates that they have neither proximal nor distal character. (B) Outline of the interactions between Pk-Sple and Dsh across proximal–distal boundaries.

mutant clones be explained in the context of a signal relay mechanism after all?

Probably a key factor in determining whether a mutant clone exerts domineering non-autonomy is whether its ability to perturb cortical polarity is stronger or weaker than competing normal signals from surrounding tissue. The crucial difference between *frizzled* null mutant cells and *flamingo* mutant cells (with respect to their ability to exert domineering non-autonomy) is that, although *flamingo* mutant cells are missing Frizzled at the cortex, they are also unable to localize any of the other core tissue polarity proteins there (Feiguin et al., 2001; Tree et al., 2002b; Usui et al., 1999). This means that no feedback loop could operate at the boundary of *flamingo* mutant clones to induce repolarization of wild type cortical domains and normal cortical signals from surrounding tissue would prevail in polarizing the cells adjacent to the clone (see Fig. 3A). This idea is consistent with the observation that even *frizzled* null cells do not exert non-autonomous effects on their neighbors in a *flamingo* mutant background (Chae et al., 1999). In contrast, Pk-Sple is uniformly mislocalized around the cortex of *frizzled* mutant cells (Tree et al., 2002b) and might actively induce Dsh accumulation in adjacent wild type cells, perturbing polarity of the surrounding tissue (see Fig. 3A).

In the case of autonomous *frizzled* mutant clones, it is possible that interaction with wild type cortical domains might predominate in polarizing cells that lie adjacent to the clone if the autonomous mutants were weak but not null (see Fig. 3A). Consistent with this idea, recent data suggest that even the long-range disturbances in cortical polarity caused by Frizzled over-expression are limited in their

extent by competing wild type signals. Under appropriate mutant conditions that eliminate these signals, the cortical repolarization induced by Frizzled over-expression can propagate throughout the wing (Ma et al., 2003). Taken together, recent data suggest that propagation of cortical polarity from cell to cell might operate quite efficiently in the absence of any long range secreted polarizing ligand.

Although cascades of cortical interactions might propagate and coordinate polarity efficiently, they cannot by themselves explain the reproducibility of distal hair orientation in the wing. One or more signals must be required to initiate these cascades and bias their direction. Good candidates for the molecules that generate these signals would be those proteins that are required for normal cortical polarization, but which do not themselves localize to proximal–distal cortical domains. The atypical Cadherins Fat and Dachsous, and Widerborst, a B′ regulatory subunit of Protein phosphatase 2A, fall into this category.

3.1. Fat, dachsous and four-jointed link cortical polarization to the axis of growth

Recent work from several labs has suggested that Fat, Dachsous and Four-jointed may collaborate to cue the axis of cortical polarization shortly after puparium formation. Interestingly, these three proteins also collaborate at earlier stages of wing development to control growth along the proximal–distal axis. Fat and Dachsous are large atypical Cadherins. The third, Four-jointed, is a type II membrane protein that, at least under some circumstances, can be cleaved and released. Viable mutations in each of these three genes give rise to flies with defects in the ratio of proximal–distal to anterior–posterior growth (Bryant et al., 1988; Clark et al., 1995; Villano and Katz, 1995; Zeidler et al., 2000, see Fig. 4A). Normally, growth in the developing wing blade is biased along its proximal–distal axis—this is reflected in the elongated shape of marked clones of wild type cells in the imaginal disc (Milan et al., 1996b). Preferential positioning of daughter cells along the proximal–distal axis is not believed to be the result of spindle orientation, but rather to result from subsequent rearrangement—perhaps as a result of adhesive differences along the axis. The defects caused by *fat*, *dachsous* and *four-jointed* mutations suggest that they are involved in regulating these growth differences. Mutation of *fat* causes disc overgrowth and delayed pupariation; wings and legs made by flies homozygous for viable *fat* alleles are display overgrowth that is mostly in the anterior–posterior direction (i.e. perpendicular to the proximal–distal axis) (Garoia et al., 2000). Similar defects in the ratio of anterior–posterior to proximal–distal growth are apparent in *dachsous* and *four-jointed* mutants (Adler et al., 1998; Clark et al., 1995; Villano and Katz, 1995; Zeidler et al., 2000). Furthermore, the pattern in which these genes are expressed, both during growth of the wing epithelium and at later pupal stages, is non-uniform along the proximal–distal axis.

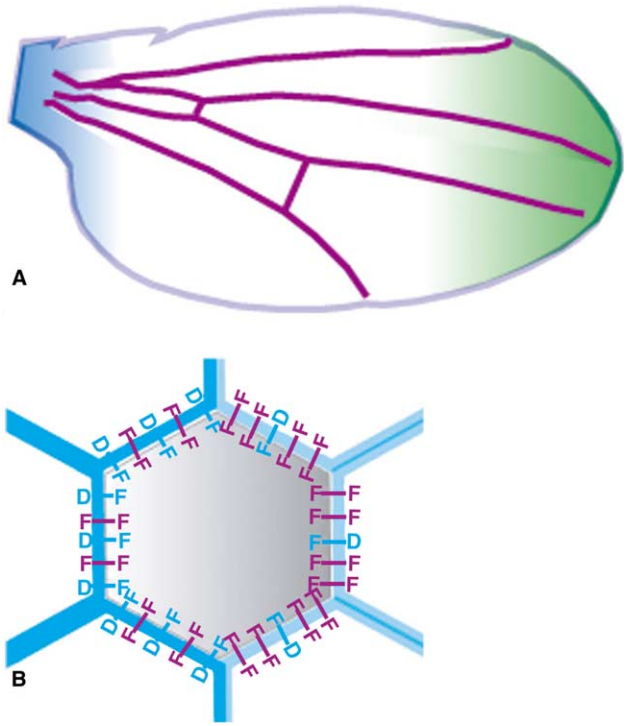

Fig. 4. Control of proximal–distal polarity by Fat, Dachsous and Four-jointed. (A) Dachsous expression (blue) is higher in the hinge region of the wing, whereas Four-jointed (green) is highest distally. Fat expression is uniform throughout. (B) Different levels of Dachsous protein between cells might lead to asymmetric Fat:Dachsous interactions within cells. The darker blue cells on the left express higher levels of Dachsous that the lighter blue cells on the right. Although Fat is uniformly distributed around the cortex of the central cell, it engages in different types of interactions on the right and left sides of the cell.

Dachsous expression is particularly strong in the dorsal hinge region, whereas Four-jointed is most highly expressed distally (Ma et al., 2003; Strutt and Strutt, 2002; Zeidler et al., 2000). Fat protein is present more uniformly throughout, being slightly upregulated at the dorsal–ventral compartment boundary at least during larval stages (Strutt and Strutt, 2002). Several years ago, genetic experiments indicated that Dachsous acted upstream of Frizzled in controlling planar polarization in the wing (Adler et al., 1998). More recently, analysis of the effects of the Fat/Dachsous/Four-jointed cassette on cortical polarization has supported and extended this idea.

Several lines of evidence suggest that the Fat Dachsous Four-jointed cassette acts upstream of cortical polarization to cue its orientation. The cortical domain proteins colocalize extensively and their polarization is highly interdependent; the absence of any one perturbs polarization of the rest. In contrast, cortical domains in *fat* or *dachsous* mutant tissue polarize efficiently, but along the wrong axis (Ma et al., 2003; Strutt and Strutt, 2002). The distribution of Fat and Dachsous does not become polarized along the proximal–distal axis of the cell and, not surprisingly, is unaffected by mutations in the cortical domain proteins (Ma et al., 2003; Strutt and Strutt, 2002). An upstream role for Dachsous is further

supported by elegant timed rescue experiments (Strutt and Strutt, 2002) which suggest that Frizzled acts in two distinct temporal phases; starting shortly after pupariation and ending shortly before cortical polarization begins, Frizzled is able to receive positional cues from Dachsous that help to orient the proximal–distal axis. If Frizzled activity is missing during this time, but provided subsequently, the resulting wings resemble *dachsous* mutant wings; cortical polarization occurs, but in an abnormal orientation. If Frizzled is also withheld during the time when cortical polarization normally occurs, the other cortical proteins do not polarize and the resulting wings of course display the typical Frizzled loss of function phenotype.

Dachsous is most highly expressed in proximal regions of the wing, Four-jointed is expressed distally, whereas Fat is expressed throughout the wing blade. How might this information be converted into intracellular polarity? Clonal analysis indicates that Fat and Dachsous are able to form heterodimers and that Four-jointed modulates the efficiency of this process (Ma et al., 2003; Strutt and Strutt, 2002). Although the overall distribution of Fat within a cell is apparently not polar, it is easy to imagine that the proportion of Fat molecules engaged in heterotypic interactions with Dachsous might be different on the proximal versus the distal side of the cell, at least in some regions of the wing (see Fig. 4B). Possibly, this proximal–distal asymmetry in Fat:Dachsous interaction could be used to bias Frizzled activity or localization to one side of the cell. Axelrod and his colleagues have pointed out that even quite minimal polarity differences at this stage might be amplified, propagated, and stabilized by subsequent proximal–distal cortical interactions.

The nature of the intracellular asymmetry generated by Fat, Dachsous and Four-jointed and its molecular connection to Frizzled remain unclear. Direct protein–protein interactions between Frizzled and these molecules seem unlikely because they localize at different apical–basal levels within the cell. While Frizzled and the cortical domain proteins lie at the level of adherens junctions, Fat and Dachsous are located more apically—at the apical marginal zone (Ma et al., 2003). Just how indirect are their effects on the polarity of Frizzled signalling? At one extreme, one might consider whether Frizzled activity could be influenced as an indirect consequence of growth polarity itself, since the prepupal mitoses tend to produce daughter cells that are aligned along the proximal–distal axis (Milan et al., 1996a). In this respect, it is interesting that the critical time at which Frizzled must read the Dachsous signal is during prepupal stages when cell division has not yet ceased. Alternatively, Fat, Dachsous and Four-jointed might control tissue polarity independently from growth polarity. At the very least, placing the two processes under common control may provide a tight correlation between the shape of the wing and its planar polarity. Correlating tissue shape and polarity is obviously of very general importance; it would not be very useful to polarize ciliary beating perpendicular to the long axis of the oviduct for example.

4. Cell biology of cortical polarization

A large gap remains in our understanding of the cell biology of cortical polarization in response to Fat, Dachsous and Four-jointed. Clues to this process may come from analyzing the function of other genes that act during this time to promote cortical polarity. One such gene is *widerborst* (Hannus et al., 2002). When Widerborst activity is perturbed, the cortical domain proteins accumulate uniformly at a higher than normal levels all around the cortex. This is in contrast to the phenotypes produced by mutating genes that encode components of the cortical domains themselves; mutations in these genes prevent polarization but do not seem to cause over-accumulation—if anything the reverse is true. Although Widerborst is required for cortical polarization, it is not a component of the cortical domains; instead it is found on the distal side of a web of microtubules that lies at the level of apical junctions within the plane of the epithelium. Its distal localization occurs before cortical polarization, and is dynamically rearranged during prepupal stages—the same time that Frizzled is interpreting the cues provided by Dachsous. This raises the possibility that Widerborst is a component of this early machinery. What could its role be? Widerborst encodes a B′ regulatory subunit of Protein Phosphatase 2A (PP2a) and presumably acts to target the catalytic subunit to specific intracellular locations and substrates. Its localization to the distal side of the planar microtubule web suggests that the Widerborst target might be located there. One universal function of microtubules is to provide tracks for the movement of intracellular components including both endocytic and secretory vesicles. Although the planar microtubule web does not display any obvious structural polarity at this time, asymmetric Widerborst localization suggests that the microtubules may be functionally polarized. One possibility worth investigating is that Widerborst biases vesicular trafficking along the proximal–distal axis of the cell. Small polarized differences in the rate of Frizzled delivery, endocytosis, or recycling might then be amplified and stabilized by the cortical polarization feedback loop.

In the last several years, studying the subcellular localization of the proteins encoded by tissue polarity genes and their redistribution under different mutant conditions has produced major advances in our understanding of the mechanism underlying planar polarization. In particular, the identification of proximal and distal cortical domains and our emerging understanding of their ability to propagate polarity from cell to cell have helped to explain some of the fascinating properties of this polarization system. Major gaps in our knowledge remain, however. The cell biological mechanism used to polarize the cortical domains is still a mystery. Does it involve polarized vesicular trafficking? Is there a role for cytoskeletal linkage? The identity and nature of the protein–protein interactions that

occur between components of the cortical domains is still underdescribed. Progress in the future will depend on the continued application of cell biological and biochemical techniques to this remarkable developmental problem.

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