View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Developmental Cell, Vol. 2, 525–535, May, 2002, Copyright ©2002 by Cell Press

Planar Signaling and Morphogenesis in *Drosophila*

Review

Paul N. Adler
Biology Department and
Cancer Center
University of Virginia
Charlottesville, Virginia 22903

The regulatory mechanisms governing the parallel alignment of hairs, bristles, and ommatidia in *Drosophila* have all served as model systems for studying planar signaling and tissue level morphogenesis. Polarity in all three systems is mediated by the serpentine receptor Frizzled and a number of additional gene products. The localized accumulation of these proteins within cells plays a key role in the development of planar polarity. A comparison of the function of these gene products in the different cell types suggests cell-specific modifications of the pathway.

Most eukaryotic cells show a clear and obvious polarity. The most common form is the apical/basal polarity found in epithelial cells. Many cells are also polarized within the plane of the tissue. This planar polarity (also called tissue polarity) is often obvious in the epidermis of animals. For example, the scales, bristles, and hairs of insects are typically aligned along major body axes (e.g., the proximal/distal axes of appendages). A similar common polarity is seen in the epidermis of vertebrates (e.g., the scales of fish and hair of mammals). Planar polarity falls at the interface between cellular and tissue level biology, and may provide a general model system for studying the ways in which the behavior of individual cells is modified to achieve larger scale morphogenesis. Recently, great progress has been made in discerning parts of the mechanisms that lead to tissue polarity in *Drosophila*. This includes finding that a number of tissue polarity proteins accumulate asymmetrically in cells and the identification of additional genes important for the process. In this review, I will focus on the role of intercellular signaling in tissue polarity and on the response of wing and eye cells to the polarizing signal.

The Cellular Basis for Planar Polarity in the *Drosophila* Epidermis

Early studies on the development of planar polarity used the epidermis of insects such as the milkweed bug *Oncopeltus* and led to the hypothesis that gradients of morphogens were involved (Lawrence, 1966). Starting in the 1980s, studies using *Drosophila* led to the identification of what is now called the *frizzled* pathway as a key for the development of tissue polarity (Adler and Lee, 2001; Gubb and Garcia-Bellido, 1982; Shulman et al., 1998; Strutt and Strutt, 1999; Vinson and Adler, 1987; Wong and Adler, 1993). Among the key members of this pathway are *frizzled* (*fz*), which encodes a serpentine receptor (Vinson et al., 1989), *dishevelled* (*dsh*), which encodes a cytoplasmic protein (Klingensmith et al., 1994; Theisen et al., 1994), *Van Gogh* (*Vang*; also known as *strabismus* [*stbm*]), which encodes a putative membrane protein (Taylor et al., 1998; Wolff and Rubin, 1998), and *starry night* (*stan*; also known as *flamingo* [*fmi*]), which encodes a serpentine membrane protein with cadherin domains (Chae et al., 1999; Usui et al., 1999; see Table 1).

In *Drosophila*, planar polarity has been studied in several different body regions and cell types (Figure 1). These include the wing, which is covered by an array of distally pointing hairs, the eye, which comprises more than 700 polarized ommatidia, and sensory bristles, which typically share the same orientation as hairs. In this review, we will focus primarily on planar polarity in the wing and eye. These cell types/developmental units differ in a number of interesting ways. The wing hair is part of a single cell, and each wing cell is polarized within the plane of the epithelium. The developing hairs, which contain F-actin and microtubules, are initiated at the distal-most part of the cell and grow outward from the cell leading to distal hair polarity (Wong and Adler, 1993). The genes of the *fz* pathway control hair polarity by restricting hair formation to the distal-most part of each wing cell (Wong and Adler, 1993). Bristles and ommatidia are multicellular, and for a multicellular structure, polarity can reflect the arrangement of cells in addition to or instead of individual cells being polarized. Each ommatidium consists of almost 20 cells. These include eight photoreceptor cells, pigment cells, corneal cells, and bristles. In any cross-section, seven photoreceptor cells are visible (R7 is located on top of R8) and arranged in a trapezoidal manner (Figure 1). Ommatidia on the dorsal and ventral sides of the eye show mirror image symmetry. The line that separates dorsal from ventral ommatidia is called the equator. As will be discussed in more depth below, the *fz* pathway regulates ommatidial polarity by controlling the R3/R4 cell fate decision, which specifies both ommatidia chirality and the subsequent rotation of the ommatidia as a unit (Strutt and Strutt, 1999; Zheng et al., 1995).

Recently, it has become clear that many of the same genes that function in planar polarity in the fly also function in vertebrate embryos during convergent extension (Heisenberg et al., 2000; Wallingford et al., 2001). The cell migration that leads to convergent extension is mediated by polarized protrusive activity of individual cells (Shih and Keller, 1992). Hence, this is another system where a *frizzled*-based pathway appears to function by polarizing individual cells.

Mutant Phenotypes of *fz* Pathway Genes Are Complex

Epidermal hairs on the wing all point distally. Mutations in *fz* pathway genes disrupt this, but they do not result in a complete randomization of wing hair polarity. For example, in a *fz* null mutant there are regions where the polarity is relatively normal, regions where it is close to random, and large regions where hairs have abnormal

Correspondence: pna@virginia.edu

Table 1. Genes Implicated in Planar Cell Polarity in *Drosophila*

Name	Type of Protein	Body Parts	Comments
frizzled (*fz*)	Serpentine receptor	H, B, O	Accumulates at distal edge of wing cells, both cell-autonomous and -nonautonomous alleles.
dishevelled (*dsh*)	PDZ	H, B, O	Accumulates at distal edge of wing cells.
starry night (*stan*)/*flamingo* (*fmi*)	Cadherin domains, serpentine receptor	H, B, O	Accumulates at proximal and distal edges of wing cells.
Van Gogh (*Vang*)/*strabismus* (*stbm*)	PDZ, membrane protein	H, B, O	Domineering nonautonomy complementary to *fz*.
prickle (*pk*)/*spiny legs* (*sple*)	LIM domain	H, B, O	Complex gene, proteins accumulate at proximal edge of wing cells.
diego (*dgo*)	WD domains	H, O	Accumulates at distal end of wing cells.
dachsous (*ds*)	Cadherin domains	H, B, O	Also alters body shape.
fat (*ft*)	Cadherin domains	H, B, O	Can result in formation of tumors.
four jointed (*fj*)	Secreted protein	H, ?, O	Expressed in a gradient, weak phenotype.
unpaired (*upd*)	JAK/STAT ligand	H, ?, O	Expressed in a gradient in eye.
hopscotch (*hop*)	Janus kinase	O	Regulated by *unpaired*, phosphorylates STAT.
Stat92E/marelle	Transcription factor	O	Transcription factor, regulated by JAK.
Notch (*N*)	Transmembrane receptor	O	Mediates lacteral inhibition, receptor for Dl.
Delta (*Dl*)	Ligand	O	N ligand.
wingless (*wg*)	Secreted Wnt ligand	O	Expressed in a gradient in eye.
fringe (*fng*)	Glycosyltransferase	O	Involved in equator formation in eye.
mirror (*mirr*)	Homeobox transcription factor	O	Expressed in dorsal half of eye.
inturned (*in*)	Weak PDZ homology	H, B, O wk	Both polarity and multiple hair cells on wing. Eye phenotype weak.
fuzzy (*fy*)	Novel	H, B	Phenotype similar to *in*.
fritz (*frtz*)	unknown	H, B	Phenotype similar to *in*.
multiple wing hairs (*mwh*)	unknown	H	Strong multiple hair cell phenotype.
RhoA	GTPase	H, O	Multiple hair cells, but no polarity phenotype on wing.
Rho kinase	RhoA effector	H, O	Multiple hair cells, no polarity phenotype on wing, ommatidial rotation.
Rac	GTPase	H, O	Multiple hair cells, based on expression of DN protein. Loss-of-function mutations show no phenotype.
Dcdc42	GTPase	H	Multiple hair cells, short hairs, based on expression of DN protein.
tricornered (*trc*)	Kinase	H	Clustered and split hairs.
furry (*fry*)	Large protein	H	Clustered and split hairs.
basket (*bsk*)	MAPK	H?, O?	Part of Jun N-terminal kinase pathway (JNK).
hemipterous (*hep*)	MAPKK	H?, O?	Part of Jun N-terminal kinase pathway (JNK).
misshapen (*msn*)	MAPKKKK	H, O	Part of Jun N-terminal kinase pathway (JNK).
jun	Transcription factor	O wk	Target of JNK pathway.

H, hair; B, bristle; O, ommatidia; ?, uncertain phenotype; wk, very weak phenotype.

polarity but where the polarity of neighboring hairs remains well aligned, resulting in distinctive swirling patterns (Adler et al., 1987; Gubb and Garcia-Bellido, 1982; Jones et al., 1996). This lack of randomness indicates that other systems must also function in the development of wing tissue polarity. As first noted by Gubb and Garcia-Bellido 20 years ago, the mutant patterns are stereotypic (Gubb and Garcia-Bellido, 1982). For example, hairs in the posterior region of a *fz* mutant wing routinely point toward the wing margin. Remarkably, the patterns are not only stereotypic for individual mutations but are similar for strong alleles of most tissue polarity genes (Adler et al., 1998). Thus, there are only subtle differences between the hair polarity phenotypes of *fz*, *stan/fmi*, *Vang/stbm*, *inturned* (*in*), *fuzzy* (*fy*), *fritz* (*frtz*), and *multiple wing hairs* (*mwh*), which suggests that these genes function in a common process. In contrast, there are two well-studied genes, *ds* and *pk*, where mutations result in a different polarity pattern; however, neither of these genes appears to be components of an independent pathway. Mutations in *ds* and *pk* appear to modulate, but not inactivate, the function of the *fz* pathway (Adler et al., 2000a, 1998).

Bristle polarity is also not random in *fz* pathway mutants (Gubb and Garcia-Bellido, 1982). For example, on both the thorax and abdomen, mutations in *in*, *fy*, and *frtz* cause bristles to point toward the midline (Collier and Gubb, 1997; Collier et al., 1997; Gubb and Garcia-Bellido, 1982). In the eye, however, the chirality and orientation of ommatidia appear to be random in *fz* mutants (Wehrli and Tomlinson, 1998; Zheng et al., 1995). It is unclear whether this represents a fundamental difference between planar polarity in the eye versus hairs and bristles.

How Is the Overall Orientation of Hairs, Bristles, and Ommatidia Specified?

Overall planar polarity in any body region could be organized by a discrete group of cells, perhaps by the secretion of a polarity morphogen. A gradient of a morphogen could provide a polarity vector to align cells (Figure 2; Lawrence, 1966). An alternative mechanism by which a specific group of cells could organize a large region is by initiating a signal that propagates across a tissue. There is evidence for both of these mechanisms in the specification of eye planar polarity. In the wing, there is no compelling evidence for either.

If there are special organizing cells, where would they be located and how could they be identified? In a simple

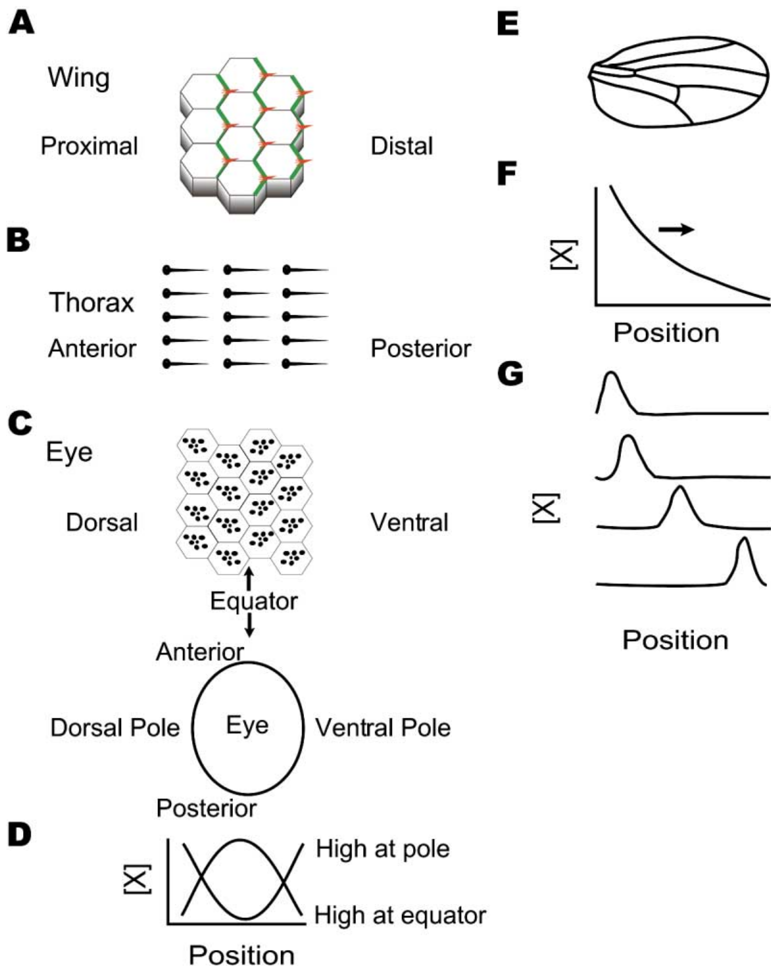


Figure 1. The *Drosophila* Epidermis Displays Global Polarity

Drawings of a group of wing cells (A), a group of thoracic sensory bristles (B), and a group of ommatidia (C) that span the equator. The wing cells have started to build hairs, and these are assembled at the distal-most vertex and grow distally (A). The green line represents the accumulation of Fz and Dsh. An ommatidium is comprised of about 20 cells. In the cartoon, only the rhabdomeres of the photoreceptor cells are shown. These are present in two chiral types that are found in mirror image symmetry on opposite sides of the equator. In (D) is a drawing of an eye and below, the concentration of morphogens with a peak at either the poles or equator. (E) shows a drawing of a wing and (F) is a representation of a global gradient of a polarity morphogen. The vector of this gradient could provide polarity information to cells. In (G) is a cartoon showing a signaling wave move across the wing. This is an alternative way to polarize a tissue.

model, the genes essential for the organizing function should show dramatic domineering nonautonomy (i.e., mutant cells would disrupt the polarity of wild-type neighbors) when a clone of mutant cells was located at the organizer but should have no phenotypic effect when located elsewhere. Many mutations have been described that alter planar polarity in the fly, and most have been studied in mosaic clones. Thus far, no gene has been reported in which a mutation fits the expectation for a gene involved in the localized production of a polarity morphogen on the wing.

A number of observations argue against wing polarity being organized by a special group of cells in the pupal wing. The most likely locations for an organizer are the distal tip or the distal wing margin. However, mutations that cause the loss of distal wing tissue do not alter

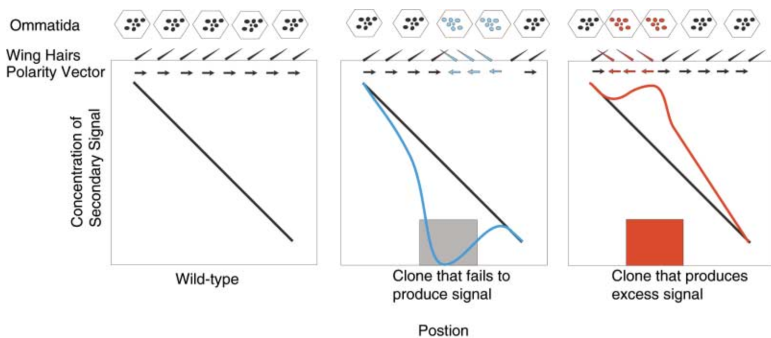


Figure 2. Models for Directional Domineering Nonautonomy

In the panel on the left is a wild-type tissue. A gradient of a locally acting polarity morphogen (secondary signal is present) is shown as a black line. The local vector of this gradient is shown in the small arrows. This polarizes the cells and leads to distally pointing wing hairs and ommatidia with the R3 cell in a polar position. In the middle panel, there is a clone of cells (in gray) that cannot synthesize the morphogen. This leads to a local valley in the concentration gradient (blue), which results in a change in the polarity vector and altered polarity of hairs and ommatidia distal to the clone. The hairs now appear as if attracted to the clone and the affected ommatidia appear to have reversed polarity. In the right panel is a clone of cells (red) that synthesizes too much of the morphogen. This leads to a local peak of the morphogen, which results in altered polarity of cells proximal to the clone which now appear as if repelled by the clone. The polarity of hairs or ommatidia inside of the clone is also altered on one of the two sides. This is a generalized version of models that have been proposed for both the wing and eye.

the polarity of remaining wing hairs (Gubb and Garcia-Bellido, 1982). For example, hairs on the small wing stump that remains in a *vestigial* mutant still point distally. Could this be due to polarity being established prior to the cell death that leads to the loss of distal wing? It is known that the temperature-sensitive period for *fz* is in the mid-early pupae during the 18 hr or so before hairs start to form (Adler et al., 1994). However, this is well after the time when distal tissue is lost in a *vg* mutation (Fristrom, 1968), indicating that *fz* function could not be part of a distal wing organizer. It is of course possible that a global signal would not involve genes identified as being part of the *fz* pathway in the wing. Another possible location for an organizer is the proximal wing or wing hinge, but surgical experiments have shown this to be unlikely (Adler et al., 2000a). For example, the hairs produced by cells of cultured pupal wing fragments still point distally (Turner and Adler, 1995), implying that no essential polarity signal is produced by proximal wing cells. Overall, the data argue that any organizer of wing tissue polarity must act prior to the pupal stage and suggests that *fz* pathway genes are not involved.

The ommatidia of the eye display planar polarity along two axes—anterior/posterior and pole/equator (Figure 1; Zheng et al., 1995). Eye development involves two orthogonal signals, which utilize two different mechanisms (Reifegerste et al., 1997; Wehrli and Tomlinson, 1998). Anterior/posterior (AP) patterning of ommatidia is linked to the morphogenetic furrow. The furrow is an indentation oriented along the AP axis that propagates across the eye disc. It is perhaps the best example of a signal that propagates across a tissue (Brennan and Moses, 2000; Chanut and Heberlein, 1995, 1997; Curtiss and Mlodzik, 2000; Dominguez and Hafen, 1997; Reifegerste and Moses, 1999; Strutt and Mlodzik, 1995). Owing to space considerations, I will not discuss this further.

The polar/equator polarity of ommatidia is specified by a multistep process and is connected to the formation of the equator at the dorsal midline (Reifegerste et al., 1997; Wehrli and Tomlinson, 1998). Two long-range signals have been identified as playing important roles. The *wg* gene is expressed at the polar edges of the eye disc prior to differentiation, and Wg protein is thought to form a diffusion gradient that is high at the poles and low at the equator (Figure 1; Reifegerste et al., 1997; Wehrli and Tomlinson, 1998). This leads to members of the Iroquois family of transcription factors (e.g., *mirror*) being expressed in the dorsal cells where they repress the expression of *fringe*, leading to *fng* only being expressed in ventral cells (Blair, 2000; Cavodeassi et al., 1999; Irvine, 1999; Maurel-Zaffran and Treisman, 2000). The juxtaposition of these two domains leads to the activation of Notch signaling and the formation of the equator. Induction of clones of cells that cannot respond to Wg (e.g., *arrow*$^{-}$) leads to the polarity reversals that are restricted to the equatorial side of the clone (Wehrli and Tomlinson, 1998). Such disruptions do not, however, result in regions of random polarity, so *wg* signaling/signal transduction is unlikely to be directly specifying eye tissue polarity. It has been hypothesized that the *wg* gradient leads to the production of a second signal (factor X or second factor) in a gradient fashion, which acts to specify ommatidial polarity (Wehrli and Tomlinson, 1998). This could be independent of *wg* function in regulating *mirror* expression and equator formation. A clone of cells unable to respond to *wg* would lead to a local decline in the concentration of X and a subsequent polarity reversal (Figure 2). The JAK/STAT pathway and its ligand Unpaired (Upd) have also been implicated in this or an equivalent process (Zeidler et al., 1999a, 1999b). Upd is expressed at a high concentration at the optic nerve stalk (on the DV midline) and diffuses poleward. It also functions in specifying the equator via regulating *fng* and *mirror* expression. Clones unable to transduce the Upd signal lead to polarity reversals on the poleward side of the clone (Zeidler et al., 1999b). Once again, it was hypothesized that this was due to a lack of production of a secondary signal. It is unclear whether there are one or two "second signals" and its/their identity. As will be discussed in more depth below, the readout of this second signal is thought to involve the *fz* gene and many of the genes that have been studied for their role in planar polarity in the wing (Wehrli and Tomlinson, 1998).

In the abdomen, it has been suggested that a *hedgehog* (*hh*) gradient results in a graded secondary signal that directly controls planar polarity (Lawrence et al., 1999). There is, however, some disagreement on the role of *hh* in regulating abdominal development (Kopp and Duncan, 2002).

The *four jointed* (*fj*) gene is expressed in a gradient fashion in both the eye and wing (Zeidler et al., 1999a, 2000). The Fj protein is thought to be secreted, making it a candidate for factor X (Villano and Katz, 1995). However, mutations in *fj* produce only modest polarity phenotypes, so if Fj is factor X it is likely to be redundant (Zeidler et al., 1999a, 2000). There are reasons to doubt that Fj functions as a Fz ligand, which is a prediction for factor X. In the eye, *fj* appears to regulate itself in a feedback loop. However, *fz* mutations do not alter *fj* expression (Zeidler et al., 1999a). How could a secreted ligand regulate its own gene expression without utilizing its receptor? Further, no genetic interactions were detected between *fj* and *fz* as might be expected if they were a ligand and receptor pair (Zeidler et al., 2000). A recent paper focused on the role of the atypical cadherins *dachsous* (*ds*) and *fat* (*ft*) suggests a new way to potentially explain the long-range organization of planar polarity in the eye (Yang et al., 2002). The authors report that *ft* promotes R3 development in a *fz*-dependent manner while *ds* promotes R4 development in an *ft*-dependent manner. *ft* is expressed uniformly in the eye but *ds* is expressed in a gradient with its high point at the pole (Yang et al., 2002). The authors propose that Ds inhibits Ft in a concentration-dependent manner, leading to a gradient of Ft activation with its high point at the equator. Ft is proposed to activate Fz, leading to a gradient of Fz activity that could function to specify the R3 and R4 cell fates and ommatidial polarity and chirality (discussed in more depth below). In addition, in mosaic ommatidia, *fj* promotes R3 development in a *ds*-dependent manner, suggesting that *fj* functions upstream of *ds* (Yang et al., 2002). It is possible that a combination of *ft, ds*, and *fj* functions as part of factor X.

Domineering Nonautonomy of *fz* and the Evidence for a Locally Acting Intercellular Signal

Evidence for a local intercellular polarity signal comes from the complementary domineering nonautonomy of *fz* (Vinson and Adler, 1987) and *Vang/stbm* (Taylor et al., 1998) clones on the wing. That is, the presence of a clone of *fz* or *Vang/stbm* mutant cells disrupts the polarity of some neighboring wild-type wing hairs. For a *fz* clone, neighboring hairs point as if attracted to the clone (Adler et al., 1997) and for *Vang/stbm* clone as if repelled by the clone (Adler et al., 2000a). Similar domineering nonautonomy for *fz* and *Vang/stbm* clones is also seen in the thorax and abdomen (Lee and Adler, 2002).

What is the cause of the domineering nonautonomy of *fz* and *Vang/stbm* clones? Two classes of hypotheses have been suggested. The first is that the presence of cells that lack *fz* (or *Vang/stbm*) function results in failure in the transmission or propagation of an intercellular signal along the proximal distal axis (Vinson and Adler, 1987). The second is that *fz* (or *Vang/stbm*) clone cells send either too little or too much of a locally acting (over as many as ten cell diameters) intercellular signal (Figures 2B and 2C; Adler et al., 2000a; Zheng et al., 1995). Several lines of evidence support the latter hypothesis, although the nature of the signal remains obscure. For example, if cells need *fz* and *Vang/stbm* function for the transmission or propagation of a signal, then the presence of cells should also be needed. However, ablation of wing cells did not produce an equivalent domineering effect on the polarity of surrounding cells (Adler et al., 2000a).

There are atypical *fz* alleles that behave cell autonomously (Jones et al., 1996; Vinson and Adler, 1987). They are functional for the cell-nonautonomous function of *fz* but defective for a cell-autonomous function. The mutant protein produced by these alleles fails to accumulate at the distal side of wing cells (as discussed below), implying that this localization is only required for the cell autonomous function of *fz* (Strutt, 2001b). It is possible that the cell-autonomous function could reflect Fz acting as a receptor for the locally acting signal, which could be the same as factor X in the eye. It is also worth noting that *dsh* acts cell autonomously (Klingensmith et al., 1994; Theisen et al., 1994), and thus is presumably not part of the pathway that gives rise to the domineering nonautonomy of *fz* or *Vang/stbm* clones. The Ca^{2+}-mediated *fz* pathway identified in vertebrate embryos is *dishevelled* independent (Slusarski et al., 1997), and a similar pathway could function in the wing.

A recent paper proposed an alternative mechanism for local signaling that leads to domineering nonautonomy (Tree et al., 2002). The model is based on the evidence for a feedback interaction between the accumulation of Fz and Dsh at the distal side of wing cells and the accumulation of Pk on the proximal side (discussed in more detail below). Disruption of this system could lead to propagation of an aberrant signal from a clone boundary. An attractive feature of this elegant model is that it uses a single signaling module to account for both the cell-autonomous and -nonautonomous function of *fz*. The model does not, however, so nicely explain the difference between the strong domineering nonautonomy of *fz* clones and the relative cell autonomy of *dsh*, *pk*, and *stan/fmi* clones (Adler et al., 2000a; Chae et al., 1999; Gubb et al., 1999; Usui et al., 1999; these proteins are all required for the asymmetric accumulation of the others). It also does not easily explain the cell-autonomous *fz* alleles described above.

There is some disagreement in the literature about the behavior of *fz* clones in the eye. Zheng et al. found the presence of a *fz* clone often disrupted the polarity of neighboring ommatidia on the polar but not equatorial side (Zheng et al., 1995). This implies a *fz*-dependent intercellular and interommatidial signal (Figure 2). In contrast, Tomlinson and Struhl found only evidence for very short-range nonautonomy (Tomlinson and Struhl, 1999). These groups used somewhat different approaches in analyzing clones and this may be the cause of the different results. However, this is an important point, as it speaks to the conservation of *frizzled* pathway function in different body regions. An additional interesting observation from these studies is that a *sev-fz* transgene, which expresses *fz* only in the R3, R4, and two mystery cells, provides almost complete rescue of a null *fz* allele. Thus, *fz* function is not needed in most eye disc cells, making it unlikely that it is required for cell-by-cell propagation of a polarity signal across the eye field. This conclusion is consistent with the results from the wing.

Polarizing Individual Wing Cells

The formation of a hair at the distal-most part of a wing cell implies that this region of the cell is special in some way (Wong and Adler, 1993). An important insight into the mechanism has come as the finding that a number of tissue polarity proteins accumulate asymmetrically in wing cells (Figure 3). The distal edge of wing cells is marked by the preferential accumulation of Fz and Dsh (Axelrod, 2001; Strutt, 2001b; Shimada et al., 2001) and the proximal edge by the accumulation of Pk/Sple (Tree et al., 2002). There is a preferential accumulation of Stan/Fmi at both the distal and proximal edges (Usui et al., 1999), and it is thought that Diego (Dgo) also accumulates on both sides (Feiguin et al., 2001). The function of each of these proteins appears to be essential for the asymmetric accumulation of the others. For Dsh and Fz, it has been shown that only active protein molecules accumulate asymmetrically, implying that this is an activity-dependent event (Axelrod, 2001; Strutt, 2001b). Activation-dependent clustering of the Fz receptor (Bhanot et al., 1996; Vinson et al., 1989) could serve to drive the distal localization of the putative complex, although so far no planar polarity ligand has been identified. A recent paper (Tree et al., 2002) has implicated an interaction between Pk and Dsh in mediating the development of asymmetric protein localization. In a $pk^{pk\text{-}sple}$ mutant, Dsh is recruited to the membrane (implying active Fz signaling) but it does not accumulate asymmetrically. Biochemical experiments found a direct physical interaction between Pk/Sple and Dsh, and in transfected cultured cells, Pk is able to block the Fz-dependent membrane localization of Dsh (Tree et al., 2002). This suggests that proximal Pk could locally block the membrane association of Dsh, and through this cause the loss of Fz from the proximal part of the cell. Analysis of *pk* clones argues that Pk on the proximal side of one cell promotes the accumulation of Dsh on the distal

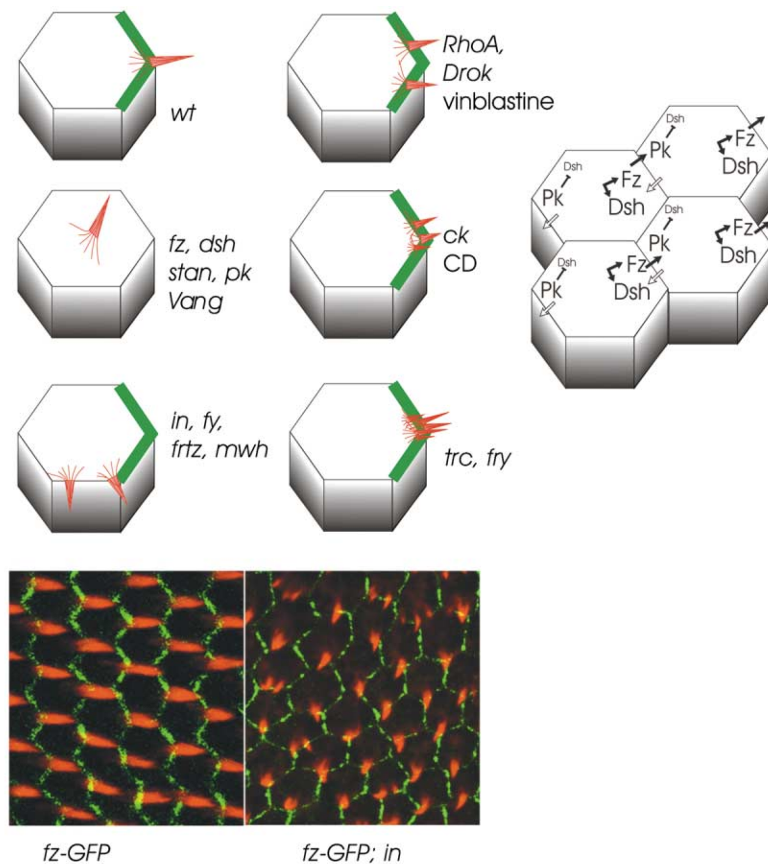


Figure 3. Mutant Phenotypes and the Distal Accumulation of Fz and Dsh

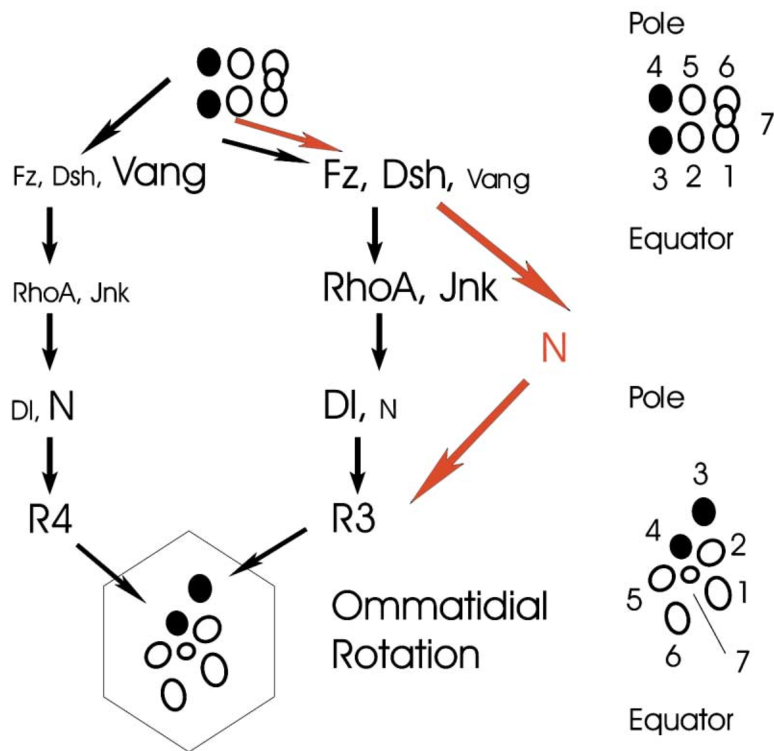
Shown is a typical wing cell for six different phenotypes. The small growing hair is shown in red as if stained for F-actin. The distal edge accumulation of Fz and Dsh is shown in green. In a wild-type cell, Fz and Dsh accumulate at the distal edge of the cell and the hair is assembled at the distal-most vertex. In a mutant for *fz*, *dsh*, *stan/fmi*, or a number of other genes, the hair often forms at a relatively central location on the apical surface. Hairs in cells of these genotypes are formed at a variety of locations along the cell periphery. In *fz*, *dsh*, *stan/fmi*, and *dgo* mutants, the distal accumulation of Fz and Dsh is lost. In a mutant for *in*, *fy*, etc., hairs form at a variety of locations along the cell periphery. In addition, many cells form multiple hairs. The distal edge accumulation of Fz and Dsh is not altered. In *Drok* and *RhoA* mutants and in cells where the microtubule cytoskeleton is disrupted by drugs such as vinblastine, multiple hairs are formed but these are all still pointing distally. In mutants for *ck* or in cells treated with antagonists of the actin cytoskeleton, multiple hairs of normal polarity are formed. These are typically more tightly clustered than those formed in a *RhoA* mutant. The hairs are also often split and shorter than normal. In mutants for *trc* or *fry*, an average of about five hairs is produced per cell. These hairs are tightly clustered and often split. Based on the generally good distal polarity of hairs in these three latter phenotypic groups, it is presumed that the distal accumulation of Fz and Dsh is not altered but this remains to be established. The micrographs at the bottom show the accumulation of Fz-GFP in wild-type and *in* mutant wings that are also stained to show the actin cytoskeleton. On the far right is a cartoon of four wing cells showing possible interactions between Fz, Dsh, and Pk/Sple (abbreviated Pk for simplicity). Fz and Dsh positively interact (solid arrows) to drive localization along the distal edge of the cell. They also promote the proximal accumulation of Pk on the distal neighbor cell. Pk could also promote the distal accumulation of Fz and Dsh on the proximal neighbor cell (outlined arrows). Pk would inhibit the accumulation of Dsh distally (solid inhibitor signal).

edge of the juxtaposed proximal neighbor. Taken together, this forms the basis of a potential feedback loop where Pk on the proximal side of the cell promotes accumulation of Dsh (and Fz) on the distal side of its proximal neighbor (Figure 3). The accumulation of Fz and Dsh at the distal side of a cell could promote the proximal accumulation of Pk on the distal neighbor. The cadherin domain containing Stan/Fmi protein also appears to play an important role in the process. It has been suggested that the cadherin domains allow Stan/Fmi protein molecules on the distal side of one cell to interact with Stan/Fmi protein molecules on the proximal edge of the neighboring cell, and in this way helps to stabilize the uneven accumulation of the protein (Usui et al., 1999).

It is notable that the accumulation of these proteins is not even along the distal edge of wing cells, and there is a higher level of accumulation along some parts of the distal edge than others (e.g., see micrographs of Fz-GFP on the bottom of Figure 3). If the distal edge accumulation is associated with a unique protein complex, one would predict that the uneven accumulation along the distal edge would be coordinate for all proteins that are part of the complex. Published studies have emphasized the coincidence of such regions, but in the published micrographs the coincidence is far from complete (Axelrod, 2001; Feiguin et al., 2001; Shimada et al., 2001; Strutt, 2001b). Despite the impressive recent progress, much remains to be learned as to how the distal edge protein complex is formed. Following the example of work on polarized cell divisions, it would be very helpful to have in vivo observations on the distal accumulation of these proteins (e.g., Bellaiche et al., 2001; Gho and Schweisguth, 1998; Roegiers et al., 2001).

How Does the Distal Accumulation of Fz Stimulate the Cytoskeleton?

Formation of a hair requires stimulation of the actin and microtubule cytoskeletons (Eaton et al., 1996; Turner and Adler, 1998). How is the Fz/Dsh distal mark transduced to the cytoskeleton? The function of several cell-autonomously acting genes including *inturned* (*in*), *fuzzy* (*fy*), *fritz* (*frtz*), and *multiple wing hairs* (*mwh*) appears to be essential for this (Collier and Gubb, 1997; Collier et al., 1997; Park et al., 1996; Wong and Adler, 1993). Distal accumulation of Fz and Dsh is not blocked in an *in* mutant, although hair formation is no longer restricted to this distal region (Axelrod, 2001; Shimada et al., 2001; Strutt, 2001b; Usui et al., 1999; Figure 3, bottom right). In addition, *in*, *fy*, and *mwh* are epistatic to both loss-



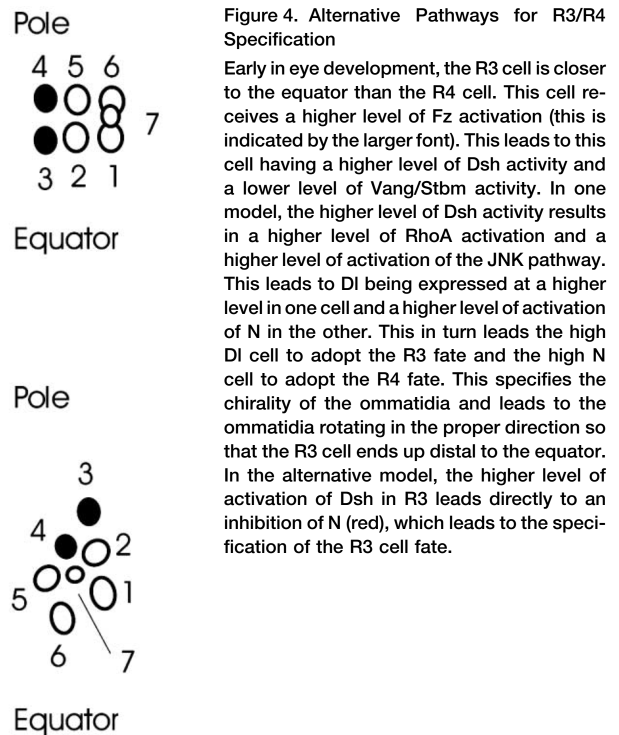


Figure 4. Alternative Pathways for R3/R4 Specification

Early in eye development, the R3 cell is closer to the equator than the R4 cell. This cell receives a higher level of Fz activation (this is indicated by the larger font). This leads to this cell having a higher level of Dsh activity and a lower level of Vang/Stbm activity. In one model, the higher level of Dsh activity results in a higher level of RhoA activation and a higher level of activation of the JNK pathway. This leads to Dl being expressed at a higher level in one cell and a higher level of activation of N in the other. This in turn leads the high Dl cell to adopt the R3 fate and the high N cell to adopt the R4 fate. This specifies the chirality of the ommatidia and leads to the ommatidia rotating in the proper direction so that the R3 cell ends up distal to the equator. In the alternative model, the higher level of activation of Dsh in R3 leads directly to an inhibition of N (red), which leads to the specification of the R3 cell fate.

and gain-of-function mutations in *fz*, *dsh*, *stan*, *pk*, and V*ang*/*stbm* (Lee and Adler, 2002; Wong and Adler, 1993). The mechanism of function of this group of proteins remains to be elucidated.

Refinement of the Region for Hair Assembly

Fz and Dsh proteins accumulate along the approximately one-third of the apical cell periphery of a typical wing cell (Figure 3). Formation of a hair takes place over a smaller part of the cell periphery, perhaps 1/15–1/10 of the distal edge of a wing cell (Figure 3). Thus, in wing cells, more than the distal side of the cell must be specified—that is, the location must be refined further. Consistent with the need for refinement, increasing the apical cell surface of wing cells leads to the formation of multiple hair cells (Adler et al., 2000b). The existing data point to the actin and microtubule cytoskeletons as being important for this process. Disruption of the microtubule cytoskeleton with vinblastine (VB) or colchicine results in multiple hair cells that still point distally (Figure 3; Turner and Adler, 1998). A large number of genes have been identified that can give a multiple hair cell phenotype, and many of these implicate the actin cytoskeleton. Mutations in *Drok* and *RhoA* lead to cells forming more than one hair, but of normal polarity (Figure 3; Strutt, 2001a; Strutt et al., 1997; Winter et al., 2001). Gene interactions argue that Drok is downstream of and activated by Fz. A likely target of Drok in wing cells is myosin II regulatory light chain. An activated form of this protein can rescue the multiple hair cell phenotype of Drok (Winter et al., 2001). Furthermore, mutations in *zipper* (myosin II) and *crinkled* (myosin VII) also result in multiple hair cells. In *ck*, the hairs are more tightly clustered and are also shorter than normal (Figure 3; Turner and Adler, 1998). Clustered or split hairs have also been found in mutants for an increasing number of genes including *capping protein* β (Hopmann et al., 1996), *trc* (Figure 3; Geng et al., 2000), *fry* (Cong et al., 2001), *slingshot* (Niwa et al., 2002), and as a consequence of the directed overexpression of wild-type *misshapen* (*msn*; a *ste-20* like kinase; Paricio et al., 1999), or a dominant-negative *cdc42* (Eaton et al., 1996). These mutations vary in the strength of the multiple hair cell phenotype, the tightness of the clustering, the frequency of split hairs, and whether hair morphology is altered in other ways. In addition, the *ck*-like phenotype can be phenocopied by the actin cytoskeleton antagonists cytochalasin D and latrunculin A (Figure 3; Geng et al., 2000; Turner and Adler, 1998). Taken together, these data suggest that the refinement of the region for hair morphogenesis may be a function of the activity of the actin cytoskeleton itself (i.e., it is a self-refining process). This would tightly link this part of wing planar cell polarity to the morphogenesis of the hair itself.

Specification of R3/R4 Cell Fate

The chirality of ommatidia is set when the R3 and R4 cell fates are determined (Figure 4). The cell that is closer to the equator adopts the R3 fate (Strutt and Strutt, 1999). This assignment appears to govern the subsequent direction of rotation of the ommatidia, leading to ommatidia of mirror image symmetry dorsally and ventrally (Figure 1). In *fz* and *dsh* mutants, this process is perturbed such that in some ommatidia, the cell closer to the equator chooses the R4 fate, leading to dorsal ommatidia on the ventral side of the equator and vice versa (Zheng et al., 1995). In addition, some ommatidia end up being symmetrical. Genetic mosaic studies have

shown that the relative activity of Fz in the R3/R4 cell pair determines which cell adopts the R3 fate. In mosaics where the boundary between *fz* wild-type and mutant cells runs between the R3 and R4 precursor cells, the wild-type cell adopts the R3 fate and the mutant one the R4 cell fate (Tomlinson and Struhl, 1999; Zheng et al., 1995). In a wild-type ommatidium, the cell closer to the equator is thought to adopt the R3 fate because it is exposed to a higher level of factor X, resulting in the preferential activation of Fz (Wehrli and Tomlinson, 1998). During the time when R3/R4 fate is specified, Fz and Dsh transiently accumulate on the R3 side of the R3/R4 border (Das et al., 2002; Strutt et al., 2002). Fmi also accumulates preferentially along the R3/R4 border (Das et al., 2002). This is proposed to lead, in a Dsh-dependent manner, to preferential activation of the *Notch* (*N*) pathway in the R4 cell (Cooper and Bray, 1999; Fanto and Mlodzik, 1999; Strutt et al., 2002) and stabilization of the fate decision by lateral inhibition. Consistent with this model, expression of an activated N protein in one member of the R3/R4 group results in that cell adopting the R4 fate and repressing the activity of N signal transduction in only one of these cells results in that cell adopting the R3 fate. Manipulation of *N* pathway function is effective in specifying R3/R4 cell fate even in a *fz* mutant background, indicating that N functions downstream of *fz* (Fanto and Mlodzik, 1999; Tomlinson and Struhl, 1999).

Two models have been proposed to account for the preferential activation of N in R4. A substantial body of work suggests that the activation of Fz in R3 leads to the activation of RhoA, Rac, and the JNK pathway, resulting in increased expression of the N ligand Delta in R3 (Figure 4; Boutros et al., 1998; Fanto et al., 2000; Paricio et al., 1999; Strutt et al., 1997; Weber et al., 2000). Many of the key experiments leading to this model have used the property that the overexpression of *fz* or *dsh* disrupts planar polarity in the eye (Strutt et al., 1997; Zheng et al., 1995). Caution is usually appropriate in evaluating such overexpression experiments, but in this case an extensive body of experiments supports the model.

Among the genes that have been suggested to function downstream of *fz* and *dsh* in the eye to mediate R3/R4 determination are *RhoA*, *Rac1*, and genes of the JNK (Jun N-terminal kinase) pathway (*bsk* [JNK], *hep* [JNKK], *msn* [Ste-20-like kinase], and *jun* (Boutros et al., 1998; Fanto et al., 2000; Paricio et al., 1999; Strutt et al., 1997; Weber et al., 2000). Mutations in some of these genes, such as *RhoA* and *msn*, produce planar polarity phenotypes (e.g., altered chirality, symmetrical ommatidia, and altered rotation) with high penetrance. Indeed, in genetic mosaics for *msn* where the clone boundary runs between R3 and R4, the *msn*$^+$ cell adopts the R3 cell fate (Paricio et al., 1999). This result mimics the result for equivalent experiments on *fz*. Loss-of-function mutations in some of the JNK pathway genes do not show a mutant phenotype (e.g., *bsk* and *hep*) or show one with only low penetrance (e.g., *jun*). This may be due to redundancy, although this has not been clearly established (Weber et al., 2000). In some cases (e.g., *jun*), expression of an activated mutant results in an expected mutant phenotype. For example, consistent with the hypothesis that *fz* signaling activates Dl expression in R3 through activating the JNK pathway (Figure 4), the expression of a constitutively activated form of Jun results in the activation of Dl expression in R4 and can result in putative R4 to R3 transformations or the formation of symmetrical ommatidia (Weber et al., 2000).

A recent paper has found that cells simultaneously mutant for loss-of-function mutations in the three *Drosophila Rac* genes do not have a planar polarity phenotype in the wing or eye (Hakeda-Suzuki et al., 2002). This suggests that the disruption of both eye and wing planar polarity by the expression of a dominant-negative Rac protein (Eaton et al., 1996; Fanto et al., 2000) may be due to a loss of specificity associated with overexpression and that Rac does not function in planar polarity.

An alternative model that does not rely on activation of *Delta* transcription to explain the preferential activation of N signal transduction in R4 has emerged in a recent paper (Strutt et al., 2002). These experiments used the expression of the *m*δ*0.5-lacz* construct as a reporter of N-dependent activation of *E(spl)* transcription in the R4 cell. Null alleles of *bsk* and *Djun* and a hypomorphic allele of *RhoA* failed to alter *m*δ*0.5* transcription. Similarly, directed expression of *Delta* or a dominant-negative RhoA or Rac1 protein also failed to alter *m*δ*0.5* transcription. The authors suggest that Dsh directly binds to and represses N activity, as is thought to be the case in other developmental contexts (Axelrod et al., 1996). This paper also reexamines the phenotype of *RhoA* mutants in the eye and found little evidence for an effect on R3/R4 specification. Many abnormal ommatidia were seen, but these appeared to be due to alterations in rotation or to a loss of photoreceptor cells and not to changes in chirality. This is similar to the phenotype of Drok in the eye (Winter et al., 2001). Further studies will be necessary to determine which model for planar polarity in the eye is closer to being correct.

Until recently, there was no indication as to how closely RhoA and Fz/Dsh might act in planar polarity. A newly described *Xenopus* FH domain-containing protein, Daam1, can bind to both Dsh and Rho and is required for Dsh-mediated activation of Rho (Habas et al., 2001), suggesting that these proteins act in a common complex. Blocking Daam1 function with morpholino oligomers resulted in defective gastrulation, consistent with Daam1 being required for Fz planar polarity signaling. There is a fly Daam1 homolog, but it has not been studied with respect to planar signaling.

The *Vang/stbm* gene is also required for R3/R4 specification and the correct rotation of ommatidia (Wolff and Rubin, 1998). Interestingly, in mosaic ommatidia where the clone boundary separates the R3 and R4 cells, the *Vang/stbm*$^+$ cell develops as R4 and the *Vang/stbm*$^-$ cell as R3. This is the opposite of the result for *fz* and is reminiscent of the complementary domineering nonautonomy of *fz* and *Vang/stbm* clones in the wing (Taylor et al., 1998). Consistent with the requirement for *Vang/stbm* in R4, Vang/Stbm accumulates asymmetrically on the R4 side of the R3/R4 cell boundary (Strutt et al., 2002).

The atypical cadherin encoded by *stan/fmi* is unusual in that it is required in both R3 and R4 (Das et al., 2002; Strutt et al., 2002). It is possible that *stan/fmi* is required

in a permissive way, as opposed to the instructive role the *frizzled* pathway plays in the eye.

Bristle Cell Polarity

The orientation of the cell divisions that give rise to the bristle sense organs on the thorax have been studied intensively (see e.g., Bellaiche et al., 2001; Gho et al., 1999; Gho and Schweisguth, 1998; Lu et al., 1999; Roegiers et al., 2001), and space considerations prevent a full review here. The lineage is initiated by the pI cell, which divides within the plane of the epithelium to give rise to the pIIa and pIIb cells (Gho et al., 1999; Gho and Schweisguth, 1998). For this division, the spindle is oriented along the anterior/posterior axis of the animal, and this is associated with the accumulation of Numb, Pon, Dlg, and Pins at the anterior cortex and Baz and DaPKC at the posterior cortex of the pI cell (Bellaiche et al., 2001; Roegiers et al., 2001). This requires the function of *fz* and a number of tissue polarity genes (*dsh* and *fmi/stan*). In the absence of *fz* function, asymmetric accumulation of Numb, Pon, Pins, and Baz is seen but the orientation of the crescent where they accumulate is altered. Instead of being oriented along the AP axis, it is random. It has been assumed that this misoriented spindle is the mechanism by which *fz* mutations result in altered bristle polarity. However, as noted by Lu and colleagues (Lu et al., 1999), there is no direct correlation between bristle and pI cell spindle orientation. Spindle orientation in a *fz* mutant is close to random while bristle orientation is much less so. It has been suggested that there are additional factors that help correct the final bristle orientation (Lu et al., 1999). The many elegant studies on this topic have largely focused on the regulation of the asymmetric oriented cell divisions and not on the polarity of the adult bristle, so it is possible that later events also play an important role in bristle planar polarity (Yang et al., 2002).

Concluding Remarks

It has been suggested that a core group of planar polarity genes function in the same way in all cell types (Shulman et al., 1998). This fits with current ideas about the use of gene modules for signaling and signal transduction. It is clear that many tissue polarity genes function in multiple body regions and cell types, but it is still unclear how conserved the mechanism is. A gene that encodes an essential component of a core system should have a strong mutant phenotype in all cell types (for our purposes wing hairs, bristles, and ommatidia.). By contrast, non-core genes would presumably function downstream and only have a polarity phenotype in a subset of tissues. Several *Drosophila* genes fit this definition of being a component of the core (*fz*, *dsh*, *ds*, *pk/sple*, *stan/fmi*, and *Vang/stbm*). For some genes (e.g., *RhoA*), the phenotype has not been adequately described in all three cell types, and other genes appear to be cell-type specific. For example, *dgo* has little or no bristle phenotype (Feiguin et al., 2001) and *in* only has a very weak eye polarity phenotype (Lee and Adler, 2002). The preferential accumulation of Fz, Dsh, Fmi, and Dgo along the distal/proximal edges of wing cells is a process that intuitively seems likely to be part of a core system (Axelrod, 2001; Feiguin et al., 2001; Lu et al., 1999; Shimada et al., 2001; Usui et al., 1999). However, Stan/Fmi is evenly distributed around the pI cell in the bristle lineage (Lu et al., 1999) and *dgo* is not required for the asymmetric localization of Fmi/Stan in the eye (Das et al., 2002). How can these observations be accommodated by the idea of a common core pathway in hair and bristle forming cells? One possibility is that the asymmetric distribution of these proteins is not part of the core planar cell polarity process. However, this would leave rather little in the core. It seems more likely that that while some molecular interactions will be conserved in different cell types (e.g., the recruitment of Dsh to the membrane by Fz), they will be modified in a highly context-dependent manner. Only further experiments will point to the correct interpretation.

Acknowledgments

This paper was supported by the NIGMS. I thank David Strutt, Marek Mlodzik, and Jeff Axelrod for providing papers prior to publication.

References

Adler, P., and Lee, H. (2001). Frizzled signaling and cell-cell interactions in planar polarity. Curr. Opin. Cell Biol. *13*, 635–640.

Adler, P.N., Charlton, J., and Vinson, C. (1987). Allelic variation at the frizzled locus of *Drosophila*. Dev. Genet. *8*, 99–119.

Adler, P.N., Charlton, J., Jones, K.H., and Liu, J. (1994). The cold-sensitive period for frizzled in the development of wing hair polarity ends prior to the start of hair morphogenesis. Mech. Dev. *46*, 101–107.

Adler, P.N., Krasnow, R.E., and Liu, J. (1997). Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels. Curr. Biol. *7*, 940–949.

Adler, P.N., Charlton, J., and Liu, J. (1998). Mutations in the cadherin superfamily member gene dachsous cause a tissue polarity phenotype by altering frizzled signaling. Development *125*, 959–968.

Adler, P., Taylor, J., and Charlton, J. (2000a). 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. *96*, 197–207.

Adler, P.N., Liu, J., and Charlton, J. (2000b). Cell size and the morphogenesis of wing hairs in *Drosophila*. Genesis *28*, 82–91.

Axelrod, J.D. (2001). Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. Genes Dev. *15*, 1182–1187.

Axelrod, J.D., Matsuno, K., Artavanis-Tsakonas, S., and Perrimon, N. (1996). Interaction between Wingless and Notch signaling pathways mediated by Dishevelled. Science *271*, 1826–1832.

Bellaiche, Y., Gho, M., Kaltschmidt, J., Brand, A., and Schweisguth, F. (2001). Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat. Cell Biol. *3*, 50–56.

Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. Nature *382*, 225–230.

Blair, S.S. (2000). Notch signaling: Fringe really is a glycosyltransferase. Curr. Biol. *10*, R608–R612.

Boutros, M., Paricio, N., Strutt, D.I., and Mlodzik, M. (1998). Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. Cell *94*, 109–118.

Brennan, C.A., and Moses, K. (2000). Determination of *Drosophila* photoreceptors: timing is everything. Cell. Mol. Life Sci. *57*, 195–214.

Cavodeassi, F., Diez Del Corral, R., Campuzano, S., and Dominguez, M. (1999). Compartments and organising boundaries in the *Drosophila* eye: the role of the homeodomain Iroquois proteins. Development *126*, 4933–4942.

Chae, J., Kim, M.J., Goo, J.H., Collier, S., Gubb, D., Charlton, J., Adler, P.N., and Park, W.J. (1999). The *Drosophila* tissue polarity gene starry night encodes a member of the protocadherin family. Development *126*, 5421–5429.

Chanut, F., and Heberlein, U. (1995). Role of the morphogenetic furrow in establishing polarity in the *Drosophila* eye. Development *121*, 4085–4094.

Chanut, F., and Heberlein, U. (1997). Role of decapentaplegic in initiation and progression of the morphogenetic furrow in the developing *Drosophila* retina. Development *124*, 559–567.

Collier, S., and Gubb, D. (1997). *Drosophila* tissue polarity requires the cell-autonomous activity of the fuzzy gene, which encodes a novel transmembrane protein. Development *124*, 4029–4037.

Collier, S., Tree, D., Johnson, G., and Adler, P.N. (1997). Characterization of the fuzzy gene product and a new tissue polarity gene fritz. Dros. Res. Conf. *38*, 147A.

Cong, J., Geng, W., He, B., Liu, J., Charlton, J., and Adler, P. (2001). The furry gene of *Drosophila* is important for maintaining the integrity of cellular extensions during morphogenesis. Development *128*, 2793–2802.

Cooper, M.T., and Bray, S.J. (1999). Frizzled regulation of Notch signalling polarizes cell fate in the *Drosophila* eye. Nature *397*, 526–530.

Curtiss, J., and Mlodzik, M. (2000). Morphogenetic furrow initiation and progression during eye development in *Drosophila*: the roles of decapentaplegic, hedgehog and eyes absent. Development *127*, 1325–1336.

Das, G., Reynolds-Kenneally, J., and Mlodzik, M. (2002). The atypical cadherin Flamingo links Frizzled and Notch signaling in planar polarity establishment in the *Drosophila* eye. Dev. Cell *2*, this issue, 655–666.

Dominguez, M., and Hafen, E. (1997). Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. Genes Dev. *11*, 3254–3264.

Eaton, S., Wepf, R., and Simons, K. (1996). Roles for Rac1 and Cdc42 in planar polarization and hair outgrowth in the wing of *Drosophila*. J. Cell Biol. *135*, 1277–1289.

Fanto, M., and Mlodzik, M. (1999). Asymmetric Notch activation specifies photoreceptors R3 and R4 and planar polarity in the *Drosophila* eye. Nature *397*, 523–526.

Fanto, M., Weber, U., Strutt, D.I., and Mlodzik, M. (2000). Nuclear signaling by Rac and Rho GTPases is required in the establishment of epithelial planar polarity in the *Drosophila* eye. Curr. Biol. *10*, 979–988.

Feiguin, F., Hannus, M., Mlodzik, M., and Eaton, S. (2001). The ankyrin repeat protein Diego mediates Frizzled-dependent planar polarization. Dev. Cell *1*, 93–101.

Fristrom, D. (1968). Cellular degeneration in wing development of the mutant vestigial of *Drosophila melanogaster*. J. Cell Biol. *39*, 488–491.

Geng, W., He, B., Wang, M., and Adler, P.N. (2000). The tricornered gene, which encodes the *Drosophila* NDR kinase is required to maintain the integrity of cellular extensions. Genetics *156*, 1817–1828.

Gho, M., and Schweisguth, F. (1998). Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature *393*, 178–181.

Gho, M., Bellaiche, Y., and Schweisguth, F. (1999). Revisiting the *Drosophila* microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. Development *126*, 3573–3584.

Gubb, D., and Garcia-Bellido, A. (1982). A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*. J. Embryol. Exp. Morphol. *68*, 37–57.

Gubb, D., Green, C., Huen, D., Coulson, D., Johnson, G., Tree, D., Collier, S., and Roote, J. (1999). The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in *Drosophila* imaginal discs. Genes Dev. *13*, 2315–2327.

Habas, R., Kato, Y., and He, X. (2001). Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel Formin homology protein Daam1. Cell *107*, 843–854.

Hakeda-Suzuki, S., Ng, J., Tzu, J., Dietzl, G., Sun, Y., Harms, M., Nardine, T., Luo, L., and Dickson, B.J. (2002). Rac function and regulation during *Drosophila* development. Nature *416*, 438–442.

Heisenberg, C.P., Tada, M., Rauch, G.J., Saude, L., Concha, M.L., Geisler, R., Stemple, D.L., Smith, J.C., and Wilson, S.W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. Nature *405*, 76–81.

Hopmann, R., Cooper, J.A., and Miller, K.G. (1996). Actin organization, bristle morphology, and viability are affected by actin capping protein mutations in *Drosophila*. J. Cell Biol. *133*, 1293–1305.

Irvine, K.D. (1999). Fringe, Notch, and making developmental boundaries. Curr. Opin. Genet. Dev. *9*, 434–441.

Jones, K.H., Liu, J., and Adler, P.N. (1996). Molecular analysis of EMS-induced frizzled mutations in *Drosophila melanogaster*. Genetics *142*, 205–215.

Klingensmith, J., Nusse, R., and Perrimon, N. (1994). The *Drosophila* segment polarity gene dishevelled encodes a novel protein required for response to the wingless signal. Genes Dev. *8*, 118–130.

Kopp, A., and Duncan, I. (2002). Anterioposterior patterning in adult abdominal segments of *Drosophila*. Dev. Biol. *242*, 15–30.

Lawrence, P. (1966). Gradients in the insect segment: the orientation of hairs in the milkweed bug *Oncopeltus fasciatus*. J. Exp. Biol. *44*, 452–458.

Lawrence, P.A., Casal, J., and Struhl, G. (1999). The hedgehog morphogen and gradients of cell affinity in the abdomen of *Drosophila*. Development *126*, 2441–2449.

Lee, H., and Adler, P.N. (2002). *inturned* and *fuzzy* are essential for the function of the *frizzled* pathway in the wing. Genetics, in press.

Lu, B., Usui, T., Uemura, T., Jan, L., and Jan, Y.N. (1999). Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in *Drosophila*. Curr. Biol. *9*, 1247–1250.

Maurel-Zaffran, C., and Treisman, J.E. (2000). pannier acts upstream of wingless to direct dorsal eye disc development in *Drosophila*. Development *127*, 1007–1016.

Niwa, R., Nagata-Ohashi, K., Takeichi, M., Mizuno, K., and Uemura, T. (2002). Control of actin reorganization by Slingshot, a family of phosphatases that dephosphorylate ADF/cofilin. Cell *108*, 233–246.

Paricio, N., Feiguin, F., Boutros, M., Eaton, S., and Mlodzik, M. (1999). The *Drosophila* STE20-like kinase misshapen is required downstream of the Frizzled receptor in planar polarity signaling. EMBO J. *18*, 4669–4678.

Park, W.J., Liu, J., Sharp, E.J., and Adler, P.N. (1996). The *Drosophila* tissue polarity gene inturned acts cell autonomously and encodes a novel protein. Development *122*, 961–969.

Reifegerste, R., and Moses, K. (1999). Genetics of epithelial polarity and pattern in the *Drosophila* retina. Bioessays *21*, 275–285.

Reifegerste, R., Ma, C., and Moses, K. (1997). A polarity field is established early in the development of the *Drosophila* compound eye. Mech. Dev. *68*, 69–79.

Roegiers, F., Younger-Shepherd, S., Jan, L.Y., and Jan, Y.N. (2001). Two types of asymmetric divisions in the *Drosophila* sensory organ precursor cell lineage. Nat. Cell Biol. *3*, 58–67.

Shih, J., and Keller, R. (1992). Patterns of cell motility in the organizer and dorsal mesoderm of *Xenopus laevis*. Development *116*, 915–930.

Shimada, Y., Usui, T., Yanagawa, S., Takeichi, M., and Uemura, T. (2001). Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. Curr. Biol. *11*, 859–863.

Shulman, J.M., Perrimon, N., and Axelrod, J.D. (1998). Frizzled signaling and the developmental control of cell polarity. Trends Genet. *14*, 452–458.

Slusarski, D.C., Corces, V.G., and Moon, R.T. (1997). Interaction of Wnt and a Frizzled homologue triggers G-protein-linked phosphatidylinositol signalling. Nature *390*, 410–413.

Strutt, D. (2001a). Planar polarity: getting ready to ROCK. Curr. Biol. *11*, R506–R509.

Strutt, D.I. (2001b). Asymmetric localization of frizzled and the establishment of cell polarity in the *Drosophila* wing. Mol. Cell *7*, 367–375.

Strutt, D.I., and Mlodzik, M. (1995). Ommatidial polarity in the *Drosophila* eye is determined by the direction of furrow progression and local interactions. Development *121*, 4247–4256.

Strutt, H., and Strutt, D. (1999). Polarity determination in the *Drosophila* eye. Curr. Opin. Genet. Dev. *9*, 442–446.

Strutt, D.I., Weber, U., and Mlodzik, M. (1997). The role of RhoA in tissue polarity and Frizzled signalling. Nature *387*, 292–295.

Strutt, D.I., Johnson, R., Cooper, K., and Bray, S. (2002). Asymmetric localization of Frizzled and the determination of Notch-dependent cell fate in the *Drosophila* eye. Curr. Biol., in press.

Taylor, J., Abramova, N., Charlton, J., and Adler, P.N. (1998). Van Gogh: a new *Drosophila* tissue polarity gene. Genetics *150*, 199–210.

Theisen, H., Purcell, J., Bennett, M., Kansagara, D., Syed, A., and Marsh, J.L. (1994). dishevelled is required during wingless signaling to establish both cell polarity and cell identity. Development *120*, 347–360.

Tomlinson, A., and Struhl, G. (1999). Decoding vectorial information from a gradient: sequential roles of the receptors Frizzled and Notch in establishing planar polarity in the *Drosophila* eye. Development *126*, 5725–5738.

Tree, D.R.P., Shulman, J.M., Rousset, R., Scott, M.P., Gubb, D., and Axelrod, J.D. (2002). Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. Cell *109*, 371–381.

Turner, C.M., and Adler, P.N. (1995). Morphogenesis of *Drosophila* pupal wings in vitro. Mech. Dev. *52*, 247–255.

Turner, C.M., and Adler, P.N. (1998). Distinct roles for the actin and microtubule cytoskeletons in the morphogenesis of epidermal hairs during wing development in *Drosophila*. Mech. Dev. *70*, 181–192.

Usui, T., Shima, Y., Shimada, Y., Hirano, S., Burgess, R.W., Schwarz, T.L., Takeichi, M., and Uemura, T. (1999). Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. Cell *98*, 585–595.

Villano, J.L., and Katz, F.N. (1995). Four-jointed is required for intermediate growth in the proximal-distal axis in *Drosophila*. Development *121*, 2767–2777.

Vinson, C.R., and Adler, P.N. (1987). Directional non-cell autonomy and the transmission of polarity information by the frizzled gene of *Drosophila*. Nature *329*, 549–551.

Vinson, C.R., Conover, S., and Adler, P.N. (1989). A *Drosophila* tissue polarity locus encodes a protein containing seven potential transmembrane domains. Nature *338*, 263–264.

Wallingford, J.B., Vogeli, K.M., and Harland, R.M. (2001). Regulation of convergent extension in *Xenopus* by Wnt5a and Frizzled-8 is independent of the canonical Wnt pathway. Int. J. Dev. Biol. *45*, 225–227.

Weber, U., Paricio, N., and Mlodzik, M. (2000). Jun mediates Frizzled-induced R3/R4 cell fate distinction and planar polarity determination in the *Drosophila* eye. Development *127*, 3619–3629.

Wehrli, M., and Tomlinson, A. (1998). Independent regulation of anterior/posterior and equatorial/polar polarity in the *Drosophila* eye. Development *125*, 1149–1159.

Winter, C.G., Wang, B., Ballew, A., Royou, A., Karess, R., Axelrod, J.D., and Luo, L. (2001). *Drosophila* rho-associated kinase (drok) links frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. Cell *105*, 81–91.

Wolff, T., and Rubin, G. (1998). Strabismus, a novel gene that regulates tissue polarity and cell fate decisions in *Drosophila*. Development *125*, 1149–1159.

Wong, L.L., and Adler, P.N. (1993). Tissue polarity genes of *Drosophila* regulate the subcellular location for prehair initiation in pupal wing cells. J. Cell Biol. *123*, 209–221.

Yang, C.H., Axelrod, J.D., and Simon, M.A. (2002). Regulation of Frizzled by Fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. Cell *108*, 675–688.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (1999a). The four-jointed gene is required in the *Drosophila* eye for ommatidial polarity specification. Curr. Biol. *9*, 1363–1372.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (1999b). Polarity determination in the *Drosophila* eye: a novel role for unpaired and JAK/STAT signaling. Genes Dev. *13*, 1342–1353.

Zeidler, M.P., Perrimon, N., and Strutt, D.I. (2000). Multiple roles for four-jointed in planar polarity and limb patterning. Dev. Biol. *228*, 181–196.

Zheng, L., Zhang, J., and Carthew, R.W. (1995). frizzled regulates mirror-symmetric pattern formation in the *Drosophila* eye. Development *121*, 3045–3055.