Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels

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Background: The *frizzled (fz)* gene of *Drosophila* encodes the founding member of the large family of receptors for the Wnt family of signaling molecules. It was originally studied in the adult epidermis, where it plays a key role in the generation of tissue polarity. Mutations in components of the *fz* signal transduction pathway disrupt tissue polarity; on the wing, hairs normally point distally but their polarity is altered by these mutations.

Results: We devised a method to induce a gradient of *fz* expression with the highest levels near the distal wing tip. The result was a large area of proximally pointing hairs in this region. This reversal of polarity was seen when *fz* expression was induced just before the start of hair morphogenesis when polarity is established, suggesting that the gradient of Fz protein acted fairly directly to reverse hair polarity. A similar induction of the *dishevelled* (*dsh*) gene, which acts cell autonomously and functions downstream of *fz* in the generation of tissue polarity, resulted in a distinct tissue polarity phenotype, but no reversal of polarity; this argues that *fz* signaling was required for polarity reversal. Furthermore, the finding that functional *dsh* was required for the reversal of polarity argues that the reversal requires normal *fz* signal transduction.

Conclusions: The data suggest that cells sense the level of Fz protein on neighboring cells and use this information in order to polarize themselves. A polarizing signal is transmitted from cells with higher Fz levels to cells with lower levels. Our observations enable us to propose a general mechanism to explain how Wnts polarize target cells.

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Background

The *frizzled (fz)* gene is required for the development of tissue polarity in the adult *Drosophila* epidermis [1–4]; *fz* encodes a transmembrane receptor [5] that has both cell-autonomous and non-cell-autonomous functions [6]. Although it has become clear that the protein products of the *fz* gene family can function as receptors for Wnt signaling molecules [7–16], the identity of the relevant Wnt in the context of *Drosophila* tissue polarity is uncertain. The product of the *dishevelled (dsh)* gene acts cell autonomously and is downstream of *fz* and required for the transduction of the *fz* signal [17–19]. Either loss-of-function or gain-of-function mutations in *fz* or *dsh* result in abnormal polarity of hairs, bristles and ommatidia ([1–4,17–21]; J. Schulman, J. Axelrod, N. Perrimon, personal communication). The mutant phenotype seen in the adult wing is reflected in the pupal wing on which the prehairs that give rise to the adult cuticular hairs are formed. In a wild-type wing, prehairs are formed in the vicinity of the distal-most vertex of the wing cells and extend from the cell in a distal direction [4]. This leads to distally pointing cuticular hairs. In tissue-polarity mutants, alternative subcellular locations are used for prehair initiation [4], resulting in prehairs that extend in abnormal directions and adult hairs of abnormal polarity.

It is unclear how *fz* signaling leads to all wing cells adopting the vicinity of the distal vertex as the site for prehair initiation. Genetic studies have provided evidence that planar signaling along the proximal–distal axis of the wing is important. Clones of *fz* mutant cells have a distal-domineering non-cell-autonomous effect: that is, wild-type cells distal to—and, in part, anterior and posterior to—but not proximal to such a clone display abnormal polarity [6]. To explain this, we proposed that the activity of *fz* is required for the transmission or propagation of an intercellular signal that moves along the proximal–distal axis of the wing and which polarizes cells [6]. The abnormal polarity of cells distal to a clone was ascribed to the failure of these cells to receive the signal.

In recent years, progress has been made in understanding the function of the genes that operate downstream of *fz* in the generation of tissue polarity [4,19,22], but little new insight has been obtained that can explain the distal-domineering non-cell-autonomous effect. One important observation is that overexpression of *fz* results in a tissue-polarity phenotype, and that this is due, in part, to a non-cell-autonomous effect [20]. Thus, the polarity of wild-type cells was found to be altered when they were

juxtaposed to cells overexpressing *fz*. This raises the possibility that the domineering non-cell-autonomous effects of *fz* mutant clones might be due to the juxtaposition of cells of widely different *fz* activity and not to a failure to receive a directional signal. To address this possibility, we have examined a number of cases where cells that have different Fz protein levels are juxtaposed. The results of these experiments suggest that cells can assess the level of Fz on neighboring cells and use this as a source of polarity information.

Results

The domineering non-cell-autonomous property of *fz* is 'attractive'

We examined *fz* mutant clones in adult wings and found that the wild-type hairs distal to—and, in part, anterior and posterior to—the clone displaying abnormal polarity [6] had a strong bias to point towards rather than away from the clone (Figure 1a). More than 95% of clones showed this behavior. We also examined clones in differentiating pupal wings and found the same behavior (Figure 1c). We refer to this as an 'attractive' non-cell-autonomous effect. Occasionally (<10% of clones) the attractive non-cell-autonomous effect was strong enough that wild-type hairs distal to the clone pointed directly towards the clone (see Figure 1c). We also noticed that the polarity of *fz* mutant clone cells at the proximal edge of the clone was usually rescued (Figure 1b); this may be analogous to the rescue of mosaic ommatidia at the equatorial border of *fz* clones in the eye [21].

Figure 1

All panels are oriented so that proximal is to the left and distal to the right. (a) The typical distal domineering non-cell-autonomous effect of a clone of cells mutant for *fz* and *multiple wing hair* (*mwh*) in an adult wing (the clone is outlined). Within the clone, cells produce multiple hairs due to the *mwh* mutation, which is epistatic to *fz* [4], and the hairs are of abnormal polarity. The wild-type hairs distal to the clone also show abnormal polarity and point more towards, rather than away from, the clone. Similar results have been obtained using a variety of *fz* alleles and other cuticular markers such as *starburst* (*strb*) and *tricorner* (*trc*). (b) A confocal image of the proximal side of a *fz* clone in a pupal wing that is marked by the loss of the N-myc plasma membrane epitope tag (*fz* cells of the clone do not show the red outline staining). The pupal wing was stained with an anti-N-myc antibody and rhodamine-conjugated anti-mouse secondary antibody. The cells were also stained with fluorescein-conjugated phalloidin (Molecular Probes), which stains the actin-filled prehair [4] green . In examining the micrographs, remember that prehairs grow out over neighboring cells [4]. Note the rescued polarity of the proximal cells within the *fz* clone (arrowhead); note also that several wild-type cells bordering the clone produce more than one hair (arrow). We saw a similar one-cell proximal non-cell-autonomous effect in our analysis of *inturned* (*in*) clones [41]. (c) Distal end of a *fz* clone in a pupal wing; not the same clone as is depicted in (b), but with the staining procedure as in (b). Note the wild-type (red) cells distal to the clone showing abnormal polarity with many hairs pointing directly towards the clone (arrowhead). Wild-type cells near the upper right side (arrow) show normal distal polarity. The size bar is 10 μm.

Inducing a gradient of gene expression in the pupal wing

In order to induce the localized expression of transgenes containing heat shock gene (*hs*) promoters [23], we applied a small drop of hot wax to the pupal cuticle. When the drop covered the entire pupal wing it induced the expression of an *hs–lacZ* gene [24] throughout the wing (data not shown). The smallest drops we could apply were larger than the pupal wing, but by appropriate placement of the drop, we could induce expression of *hs–lacZ* in only distal or only proximal wing cells (Figure 2). The intensity of induction of β-galactosidase appeared roughly similar after distal,

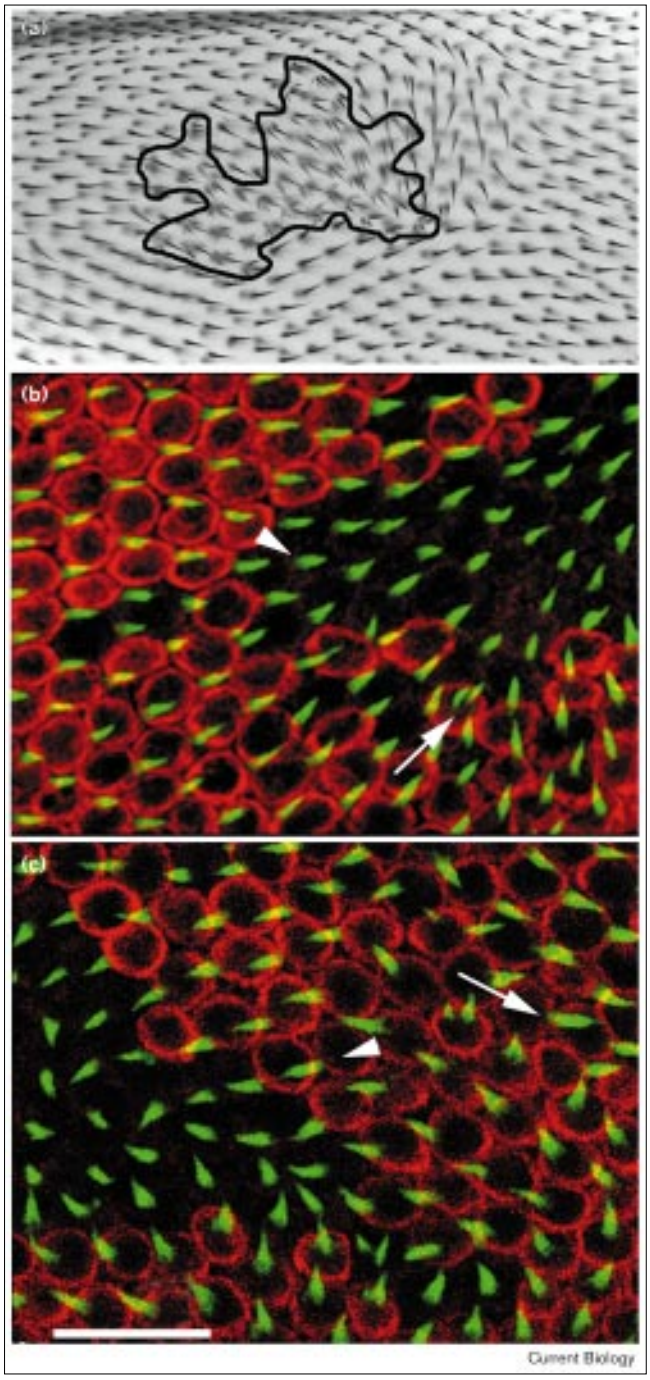
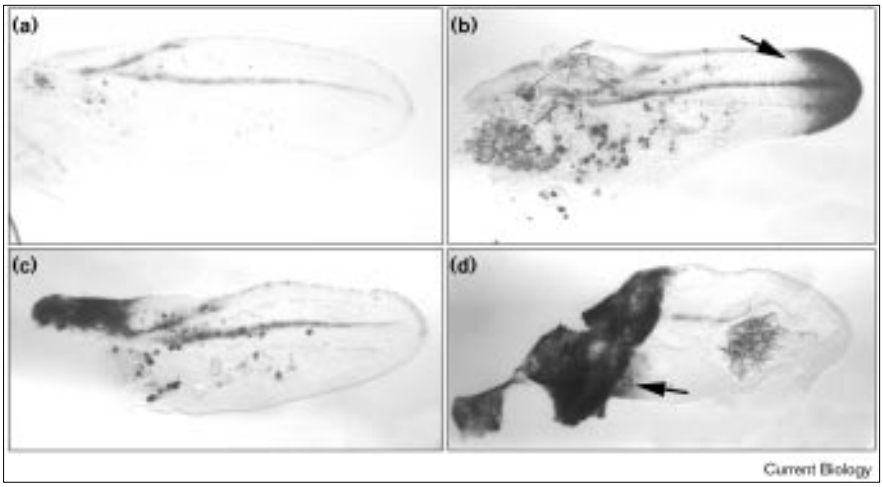


Figure 2



(a) Basal expression of the *hs–lacZ* transgene in a pupal wing in the absence of heat shock. This expression presumably is due to trapping of an enhancer near the transgene. Other panels show the lacZ staining that results from (b) distal waxing, (c) proximal waxing and (d) proximal-half waxing. Arrows point to regions showing a gradient of induced expression. All panels were from an experiment in which pupae were treated with wax 26 h after the white pupal stage (awp). The wings were fixed and stained 6 h later.

proximal or proximal-half 'waxing' (see Materials and methods). At the edges of the induced regions, a gradient of β-galactosidase expression appeared to be induced (Figure 2, arrows). The waxing of pupal wings only rarely (<10% of cases) caused lethality or resulted in a grossly abnormal wing, the latter being due to killing of wing cells. The waxing procedure often resulted in a 1–3 hour delay in prehair initiation (particularly when the waxing was done 6 hours or less prior to prehair initiation), but it did not have any effect on wing hair polarity or morphology [25].

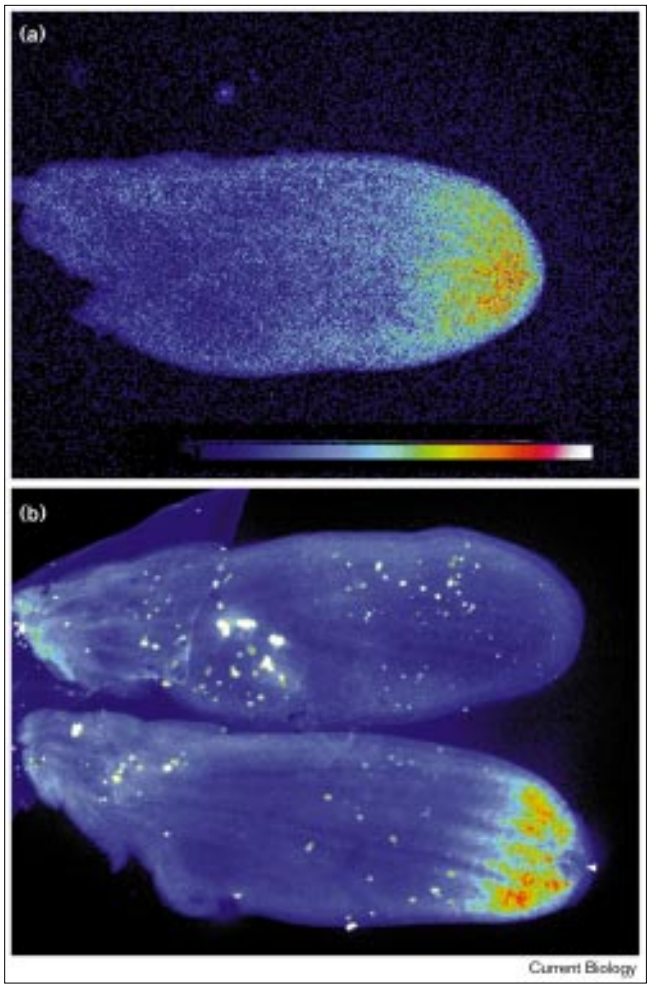
We next waxed *hs–fz* pupae and the stained the pupal wing using anti-Fz antibodies several hours later. [5]. We found a clear induction of *fz* expression after both distal (Figure 3) and proximal (data not shown) waxing. Once again, after distal waxing a gradient of expression was seen on the proximal side of the region of induced Fz protein (Figure 3). Variation was seen in the evenness of the induction along the anterior–posterior axis and in the steepness of the gradient. Some wings showed relatively even expression across this axis (Figure 3a) whereas others showed interdigitating 'fingers' of higher and lower expression (Figure 3b). In some wings, the induction was also uneven at the distal tip of the wing, with regions showing only slight induction (Figure 3b). We suspect that the local spatial variation seen in the induction of gene expression was due to variation in the position and folding of the wing in the pupa resulting in variable heating from the wax drop.

Consequences of induced *fz* expression

When we applied hot wax over entire *hs–fz* pupal wings, a strong tissue-polarity phenotype was induced across the wing (Figure 4b; compare with wild-type in Figure 4a). This observation is consistent with what is seen after heat-shock treatment of whole pupae [20]. The distal waxing of *hs–fz* pupae resulted in adult wings with remarkable large regions of reversed polarity (Figures 4d,4e,5,6 and Table 1). These regions overlapped with areas where we could see a gradient of *fz* expression in pupal wings. Similar results were obtained with different transgene-containing lines, suggesting that the phenotype is not related to the site of insertion of the transgene. In regions where hair polarity was proximal in adult wings, prehairs were formed in the vicinity of the proximal-most vertex of the pupal wing cells (Figure 6a), in contrast to their normal location in the vicinity of the distal-most vertex (Figure 6b) [4]. The length of the region showing reversed polarity varied in a smooth fashion from one wing to another, presumably reflecting differences in the effective induction of *fz* expression. Typically, we saw regions of reversed polarity that extended over 25–35 cells along the proximal–distal axis, although examples of more than 50 cells were not rare. At the proximal end of the region showing reversed polarity, there was a swirling pattern that separated regions of reversed and normal polarity (Figures 4–6). In some wings, interdigitating 'fingers' of regions showing reversed and normal polarity were seen (Figures 4g,5), perhaps reflecting the fingers of induced *fz* expression described above (Figure 3b).

Distal to the region of reversed polarity, a typical tissue-polarity phenotype was usually seen. In some wings, the phenotype was relatively mild in this region. These wings could be ones in which the induction of the *hs–fz* transgene was relatively weak distally (Figure 3b). When we examined pupal wings after distal waxing of *hs–fz* pupae, we sometimes found that a region of reversed polarity was located proximal to a region where prehair initiation had still not commenced, presumably due to the delay in differentiation associated with heating (Figure 6a). This shows that prehair initiation and morphogenesis do not

Figure 3



Pseudocolor confocal images of pupal wings stained with an anti-Fz antibody and an Oregon-Green anti-mouse secondary antibody (Molecular Probes). (a) A wing that displays a relatively smooth gradient of Fz induction after distal waxing. (b) A pupal wing with 'fingers' of higher and lower levels of Fz induction after distal waxing (bottom wing); this wing also shows a relatively low level of staining at the distal tip of the wing (arrowhead). The contralateral, untreated wing is shown above the treated wing.

need to be preceded by the differentiation of more distal cells, even though that is the normal sequence of events [4].

The region of reversed polarity seen in adult wings was larger on average than the region in pupal wings where we saw induced *fz* expression (as detected by antibody staining) (compare Figure 3a,b with Figures 4–6). In one experiment in which we stained pupal wings for actin and Fz protein after distal waxing, the region of reversed polarity was found to extend beyond where we could see Fz antibody staining. A likely explanation for this is that our antibody detection procedure was less sensitive than the cellular response to Fz; the sensitivity of the antibody has previously been found to be relatively low [5]. An alternative, although not mutually exclusive, hypothesis is that cells induced to express *fz* produced a signal that acted on distant cells to reorganize polarity.

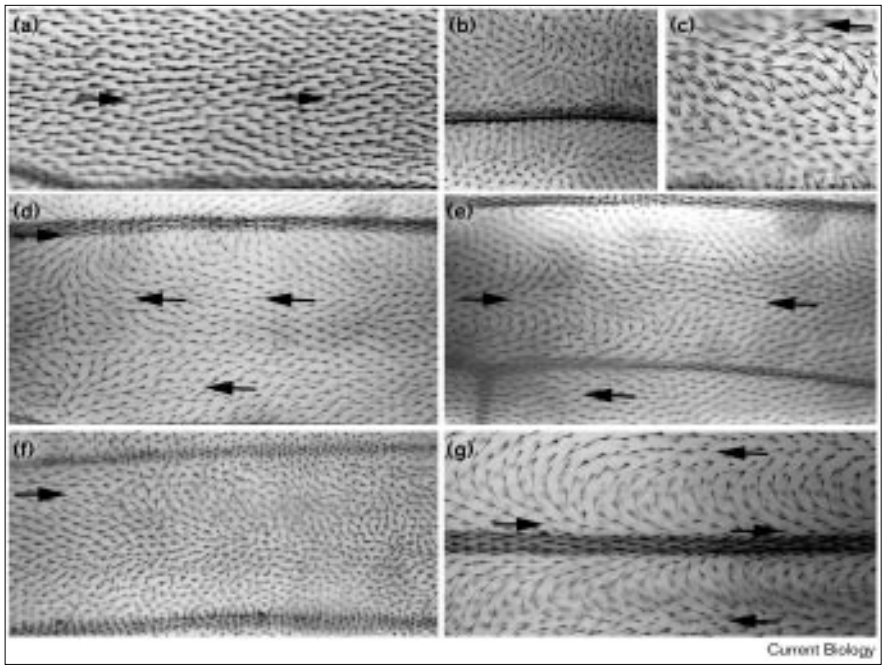
In most of our experiments we waxed wings 6–12 hours before prehair initiation (Table 1). Within this time frame we did not see any variation in the fraction of wings showing reversed polarity. Although we did not carefully quantify the size of the region of reversed polarity in these experiments, simple observations suggested that it was slightly larger on average when older pupae were treated. In several experiments, we applied the wax 0–2 hours prior to prehair initiation. This treatment also resulted in most wings having regions of reversed polarity (Table 1). The regions of reversed polarity induced following this treatment were similar in size to those seen when the wax was applied 6 hours prior to prehair initiation. Many of these wings also showed regions with a large number of double or triple hair cells (Figure 4c). This phenocopy of the *inturned* (*in*) group of tissue-polarity mutants was seen previously, when *hs–fz* (or *hs–dsh*) pupae were subjected to heat shock treatment 0–3 hours before prehair initiation [20]. We found previously that the sensitive period for induction of a gain-of-function tissue-polarity phenotype by *fz* overexpression ended at prehair initiation [20]. Consistent with this, we found that when wings were waxed 1–2 hours after prehair initiation no tissue-polarity phenotype was seen (Table 1).

In contrast to the dramatic phenotype found after distal waxing, little or no effect on hair polarity was seen after proximal or proximal-half waxing of *hs–fz* pupal wings (see Supplementary material provided with the internet version of this paper). At most, a very weak phenotype was seen (Figure 5). In no case did we see a phenotype that was equivalent to that seen after heat-shock treatment of whole *hs–fz* pupae. The reason for the minimal consequences of proximal overexpression of *fz* is unclear.

Induction of the gene *dsh*, which acts cell autonomously, does not produce a region of reversed polarity

The *fz* gene has both cell-autonomous and non-cell-autonomous functions [6]. To determine whether activation of the cell-autonomous function was sufficient to produce the regions of reversed polarity, we examined the effect of overexpression of *dsh*, which functions cell autonomously [17–18] and is downstream of, and required for, *fz* signal transduction in establishing tissue polarity [19]. The mutant phenotypes and gene dosage interactions argue that Fz activates Dsh [19]. We reasoned that if the cell-autonomous function of induced *fz* was responsible for the reversed polarity, then a similar induction of *dsh* should also induce a region of reversed polarity. When we applied hot wax over entire *hs–dsh* pupal wings, a strong tissue-polarity phenotype was induced, consistent

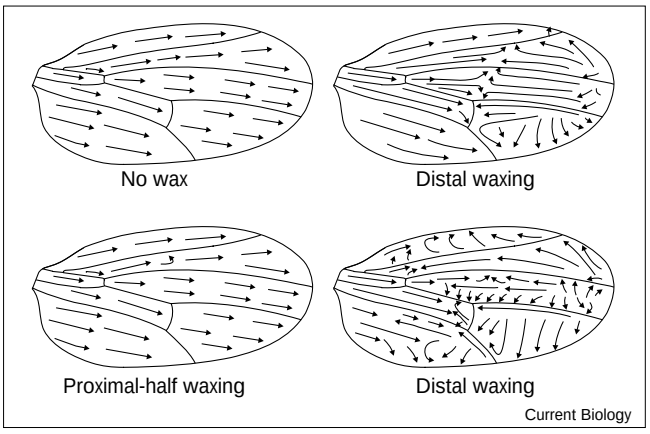
Figure 4



(a) A wild-type wing. In this and other panels, proximal is to the left and distal to the right and the arrows indicate the hair polarity in the region. (b) The tissue-polarity phenotype that results from whole-wing waxing of *hs–fz* 26 h awp pupae. (The presence of the wing vein in the center of the micrograph is normal and unrelated to the experiment.) (c) A wing from a *hs–fz; fz* pupa after distal waxing at 35.5 h awp. It shows many multiple hair cells (*in* phenotype) induced by *fz* overexpression just prior to prehair initiation [20]. The proximal polarity shows that the endogenous *fz* gene is not needed for the reversed-polarity phenotype. (d, e) Regions of reversed polarity that result from the distal waxing of *hs–fz* 30 h awp pupae. The region of swirling that separates the regions of reversed and normal polarity is near the left end of the panels. (f) The major polarity disruption after distal waxing of a 30 h awp *hs–dsh* pupa. The left end of the region shown does not show a strong phenotype. Note the lack of a region of reversed polarity in this wing. (g) At higher magnification, the interdigitating of regions of distal and proximal polarity as described in the text. Compare with Figure 3b and note the lower expression near the vein in Figure 3b and the lack of a phenotype in this region. The wings in panels (b,d,e,g) were from flies that are mutant at the *yellow* locus, resulting in wing hairs that are finer and of lower contrast.

with what is seen after heat-shock treatment of whole *hs–dsh* pupae (J. Axelrod, J. Schulman, N. Perrimon, personal communication). Distal waxing of *hs–dsh* pupal wings resulted in a strong tissue-polarity phenotype distally, but no regions of reversed polarity were seen (Figure 4f, Table 1). This argues that the reversed polarity seen after distal waxing of *hs–fz* pupal wings requires the non-cell-autonomous function of *fz*.

Figure 5



Drawings of the polarity patterns on the distal surface of individual wings. The drawings show examples of wings with no wax treatment or after distal waxing and proximal-half waxing; the example is typical of the weak phenotype seen with proximal-half waxing.

Induction of a gradient of *fz* expression by an alternative approach

As a further test of the consequences of an induced gradient of *fz* expression, we targeted *fz* expression to the distal part of the pupal wing using the *distal-less* (*dll*) promoter to drive expression of the *Gal4*-encoded transcriptional activator and a *fz* transgene driven by the upstream activator sequence (*UAS*) to which Gal4 binds [26]. In the distal part of the pupal wing *dll* is expressed in a graded fashion, with its high point near the distal tip ([27]; P.N.A., unpublished observations). Transgenic flies (*dll-Gal4*/+; *UAS-fz*/+) showed a region of reversed polarity near the distal end of the wing (Figure 7a). The region showing proximally oriented hairs is typically smaller than that seen with distal waxing, presumably because there is a smaller region, and perhaps lower level, of induced *fz* expression. We also expressed *fz* using the control of a *patched* (*ptc*)–*Gal4* driver, which drives expression of genes in a graded fashion in a band of anterior compartment cells along the anterior–posterior (AP) compartment boundary [28]. Inside the proximal part of the *ptc* expression domain, wing hairs of *ptc–Gal4*/+; *UAS–fz*/+ flies tended to point away from the high point of expression along the AP compartment boundary (Figure 7b).

Does the normal *fz* pathway function in producing the regions of reversed polarity?

To determine whether the normal *fz* signaling pathway was used in generating regions of reversed polarity, we repeated our waxing experiments in *hs–fz* pupae that were also mutant for the endogenous *fz*, *prickle* (*pk*) or *dsh* genes. As the basal expression of *fz* from a *hs–fz* transgene results in almost complete rescue of a null *fz* mutant [20], we predicted that a functional endogenous *fz* gene would not be needed for induction of the reversed polarity phenotype, and, indeed, that was what we found (Figure 4c, Table 1). Previous experiments have shown that *pk* is not required for the transduction of the *fz* signal [19], and we found it was also not required for the reversed-polarity pattern (Table 1). The *dsh* gene, which is downstream of *fz* and required for the transduction of the *fz* signal during normal tissue polarity establishment [19], was, however, found to be required (Table 1). Thus, reversed polarity resulting from a gradient of induced *fz* expression seems to involve genes that normally function downstream of *fz* during tissue polarity establishment.

Discussion

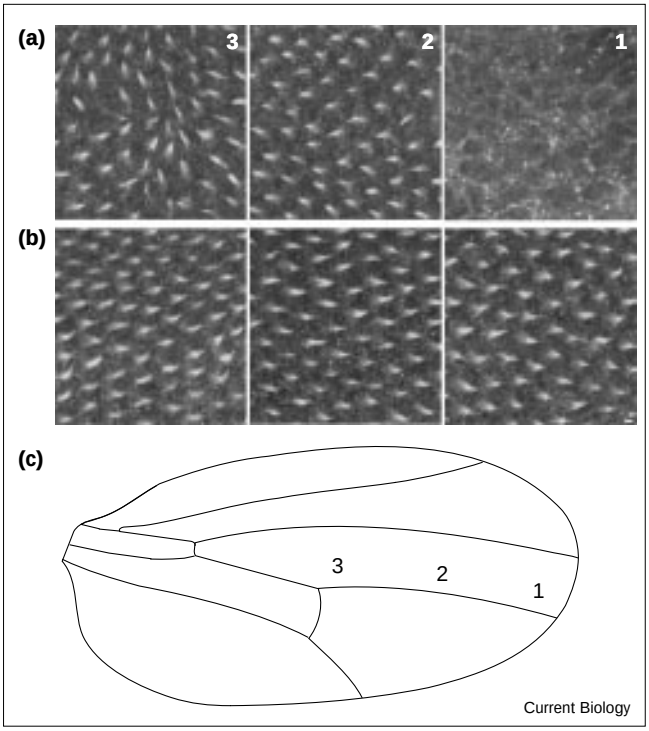
Local heating as a tool to study the consequences of gene expression in the epidermis

A variety of approaches have been used to apply heating locally to induce the expression of heat-shock-driven transgenes at the desired time and place [23,29–31]. Our use of hot wax to induce expression of genes at specific times in defined regions of the pupal epidermis is an example of a 'low-tech' approach to doing this. It has the advantage of not requiring expensive or specialized equipment and it should be adaptable to other situations. In developing the wax technique, we tried several other approaches, such as irradiation with the laser from a confocal microscope, but none gave as vigorous a response. One useful property of wax is that it has a high viscosity when melted and it begins to solidify at a temperature that is still hot enough to provide substantial heating to juxtaposed tissue.

A gradient of *fz* expression is not responsible for the normal wing polarity pattern

We found that when a gradient of *fz* expression is generated in the pupal wing, the hairs pointed down the gradient. This suggests that the normal distal polarity pattern of wing hairs could be due to a gradient of *fz* expression along the proximal–distal axis of the wing. For several reasons, we do not think this is the case, however. The observation that a null *fz* genotype can be rescued by a *hs–fz* transgene argues that the patterned transcription of *fz* is not essential for normal tissue polarity establishment [20]. Furthermore, no gradient of *fz* expression has been seen along the wing [5,32], and patterned expression of *fz* is not required for its function in eye development [21]. It is possible, however, that the relatively high level of *fz* expression in the proximal wing hinge (see [32] and Figure 1 therein) serves as a redundant cue in organizing wing polarity.

Figure 6

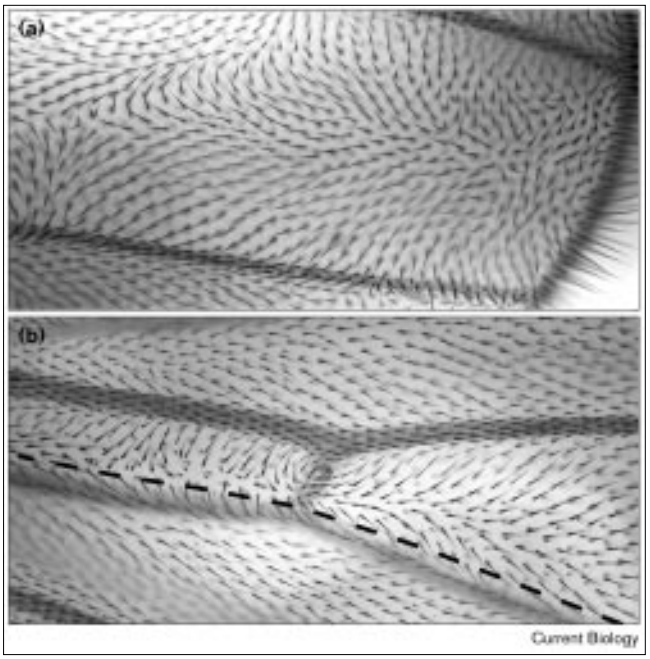


Confocal images of (a) an actin-stained, distal-waxed pupal wing (above) and (b) its contralateral control. (c) Drawing of a wing and the locations along the proximal–distal axis where the confocal images shown in (a) and (b) were taken. The distal-most image (location 1) shows a waxed wing with a strong delay in prehair initiation. Location 2 is a region of reversed polarity where there was no dramatic delay in prehair initiation; location 3 is the region where the normal and reversed polarities meet. Size bar is 1 μm.

Is the reversed polarity due to a direct or indirect effect of the induced Fz protein?

The induced gradient of *fz* expression could repolarize wing cells locally or at a distance. For example, wing cells might assess the level of Fz on their neighbors' surfaces and use this information to determine the site for hair morphogenesis and hence hair polarity. Alternatively, the induced Fz protein could induce the production of a second signal that polarizes distant cells. For example, in the eye an ectopic source of Hedgehog protein or the loss of protein kinase A function is able to induce the formation of an ectopic morphogenetic furrow that polarizes cells as it moves [33–36]. It is possible that the overexpression of Fz above a critical concentration induces wing cells to form an analogous signal that polarizes cells as it moves across the wing. We think that this latter (indirect effect) hypothesis is unlikely. If a propagating signal was involved

Figure 7



(a) The distal region of a *dll–GAL4/+*; *UAS–fz/+* fly wing. Note the region of proximally pointing hairs. (b) The wing region that displays a fz gain-of-function phenotype in *ptc–GAL4/+*; *UAS–fz/+* flies. A broken line shows the approximate position of the anterior–posterior (AP) compartment boundary.

in producing the region of reversed polarity, one would predict that, the earlier the *fz* expression was induced, the larger would be the region of reversed polarity, as the signal would have more time to propagate. This was not observed; indeed, the size of the region of reversed polarity appeared slightly larger when the wax was applied at later developmental times.

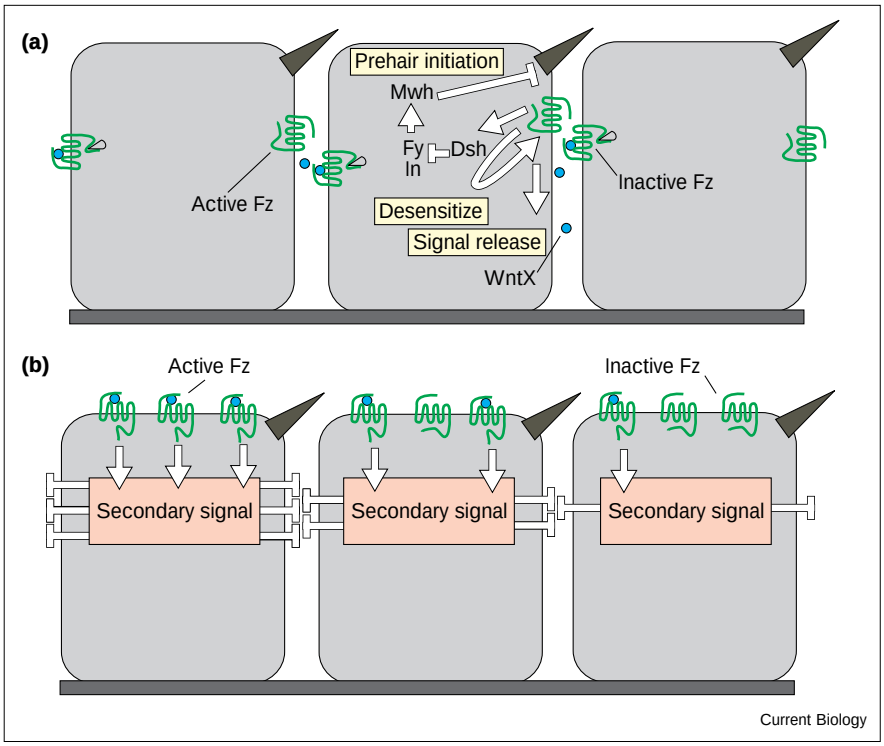
Models for direct action of induced Fz

The correspondence between the gradient of induced *fz* expression and the altered polarity of hairs suggests that the overexpressed Fz protein acted directly to polarize cells and to determine hair polarity. We suggest that, in our experiments, the Fz protein was activated in a ligand-independent manner, producing a gradient of Fz activity. We further suggest that, in these experiments, cells produced, and neighbors responded, to a local signal that was proportional to *fz* activity, and this enabled cells to sense the level of *fz* activity on neighboring cells.

Two models have been proposed to account for the role of *fz* in tissue polarity (see Figure 8 for modified versions of these models) [5,21]. We previously proposed a cell-by-cell signaling model in which the Fz protein is activated unevenly across cells [5]. The binding of ligand to Fz protein on the proximal side of a cell was suggested to activate two signal transduction pathways, one leading to prehair initiation and the other to the release of ligand to relay the signal, with both operating at the distal edge of the cell [5]. An alternative model was proposed by Carthew and colleagues from their work on *fz* in the eye [21]. They suggested that the Fz protein was activated by binding a gradient morphogen [37–39] and this resulted in the production of a secondary signal that polarized cells. Some observations are difficult to explain with either model. For example, it is difficult to explain the existence of cell-autonomous *fz* alleles that alter tissue polarity using the secondary-signal model [6]; it is also difficult to explain the observation that the polarity of cells at the proximal edge of clones is rescued (Figure 1b) using the cell-by-cell signaling model. The latter observation, however, may be due to an independent phenomenon. A striking feature of all tissue polarity mutations is the swirling hair pattern produced on the wing. In such wings, most hairs have an abnormal polarity that is similar to that of their neighbors, indicating that neighboring cells are interacting during morphogenesis. This is true both for genes such as *fz* and *Van Gogh* that act non-cell-autonomously, and for genes such as *dsh*, *in* and *mwh* that otherwise appear to act strictly cell autonomously ([1–3,6,17,18,40,41]; P.N.A., unpublished observations). We suggest that there is a *fz*-independent system that functions to help align neighboring cells and that this is responsible for the ubiquity of swirling. Such a system could be responsible for the rescue of the proximal cells in *fz* clones; but proof of its existence will require the isolation of mutations that inactivate it.

To accommodate our observation that hairs point from cells with higher Fz levels towards cells with lower Fz levels, our earlier cell-by-cell signaling model [5] needs to be modified. One modification invokes the idea that, during the establishment of tissue polarity, Wnt binding inactivates Fz (Figure 8a). Modifications that use an activating Wnt are also possible but require the signal to go in the other direction. We find the model depicted in Figure 8a to be attractive because all the interactions are local within the cell and the signal moves from proximal to distal in the wing. We prefer this direction of signaling, as mutations that remove the wing margin do not produce a tissue-polarity phenotype. The idea that a Wnt might inactivate Fz (in establishing tissue polarity) is consistent with the observation that overexpression of *wg* and *Dwnt3* inhibits the wing tissue-polarity phenotype caused by *fz* overexpression (J. Schulman, J. Axelrod and N. Perrimon, personal communication; P.N.A., unpublished observations). The lack of a wing tissue-polarity phenotype when these Wnts are overexpresssed alone (J. Schulman, J. Axelrod, N. Perrimon, personal communication; P.N.A., unpublished observations) could be due to the Fz protein being normally shielded from them by a specificity factor.

Figure 8



Models to explain the relationship between *fz* activity and polarity. (**a**) This model is one of several possible cell-by-cell signaling models. The binding of a Wnt to Fz on the proximal edge of the cell results in the inactivation of the receptor. At the distal edge of the cell, any Fz that is not bound to ligand activates signal transduction pathways that lead to prehair initiation (through activation of Dsh, which leads to local inactivation of the products of the *in, fuzzy* (*fy*) and *mwh* genes), release of ligand (to signal to the neighboring distal cell) and desensitization of Fz to ligand. All are predicted to occur at the distal edge of the cell. (**b**) A long range gradient of a Wnt results in a gradient of Fz activation due to a greater fraction of Fz binding to ligand in proximal cells than in distal cells. The activation of Fz results in the activation of a signal transduction pathway that causes the proportional production of a local signal (either tethered or locally diffusible) that polarizes cells and inhibits prehair initiation next to cells with higher Fz activity. The location of the various genes downstream of *fz* in this model is not clear.

Table 1

Distal waxing of pupal wings from *hs–fz* transgenic flies causes polarity reversals.

Genotype*	Time of treatment†	Number of wings with polarity reversal	Number with major effects but no reversal‡	Number with minor effects§	Number with no polarity effects
hs–fz (22)	12–6 h BHI	68†	3	1	3
hs–fz (22)	1–2 h AHI	0	0	1	8
hs–fz (2)	12–6 h BHI	16	4	0	0
hs–fz (2)	1–0 h BHI	13	4	1	2
hs–fz (3)	12–6 h BHI	46	9	3	2
hs–fz (3)	1–0 h BHI	12	3	1	4
hs–dsh	12–6 h BHI	0	17	1	1
hs–dsh	1–0 h BHI	0	6	0	0
dsh; hs–fz (3)	12–6 h BHI	0	10	0	0
pk cn; hs–fz (3)	12–6 h BHI	19	5	2	0
hs–fz (2); *fz*	12–6 h BHI	15	10	0	1
hs–fz (2); *fz*	2–0 h BHI	4	2	0	3

*Three different *hs–fz* transgene-containing chromosomes were used in these experiments. The *hs–fz* (2) line carries an insert on the second chromosome (named 30.9), and the *hs–fz* (3) line carries an insert on the third chromosome (named 73.7). The *hs–fz* (22) line carries the same transgene as the *hs–fz* (2) line and an additional second chromosome insert. The *dsh* allele was dsh^{1}, the *pk* genotype pk^{1}, and the *fz* genotype fz^{K21}/fz^{R54}. †The time of treatment relative to prehair initiation. BHI and AHI are, respectively, time before and after prehair initiation. ‡Major effects are equivalent to a *fz* or *dsh* mutation but with no region of well-organized reversed polarity. §Minor effects are equivalent to a weak allele of *fz*.

When *fz* is overexpressed, there might not be enough of the specificity factor to shield Fz from these Wnts.

The ability to assess the level of Fz activity of neighboring cells can explain other observations on tissue polarity

An attractive feature of the models described above is that they provide a mechanism to explain a number of previous observations on tissue polarity, including the distal domineering non-cell-autonomous effect of *fz*. Cells distal to a *fz* clone would find themselves juxtaposed to cells with no Fz activity and are predicted to respond by repolarizing towards the clone. The cell-by-cell model also predicts that cells proximal to a *fz* clone will not respond to the lack of Fz activity in the cells of the clone because the proximal edges of cells will normally have lower levels of Fz activity. Similarly, in the secondary signaling model, distal cells are predicted to produce less signal. Thus, these models provide an explanation for the domineering non-cell-autonomous effect of *fz* being distal-acting and 'attractive'.

Additional evidence supporting the idea that cells of high Fz activity signal to cells of lower activity comes from previous experiments in which we found that cells overexpressing *fz* could disrupt the polarity of clones of neighboring cells expressing normal *fz* levels [20]. The hairs produced by the normal cells were typically observed to point away from the clone border, that is, away from the cells expressing high levels of *fz*. This observation is consistent with the observations reported in this paper. Finally, the idea that the relative level of Fz activity between neighboring cells determines polarity is consistent with the model proposed by Zheng *et al*. to explain the function of *fz* in the eye [21]. Ommatidia that were mosaic for *fz* were examined, and it was found that when the ommatidia was mosaic for the R3 and R4 photoreceptor cells, the fz^+ cell almost always adopted the R3 cell fate. Thus, the decision as to whether a cell adopted the R3 or R4 fate appeared to be determined by the Fz activity of the cell, implying that these cells can assess the Fz activity of neighbors. Furthermore, the presence or absence of Fz protein in these cells also influenced the direction of rotation of the ommatidia. Although the cell biology of tissue polarity in the eye and the wing are quite different, the ability of cells to assess and to respond to differences in Fz activity of their neighbors may be at the crux of tissue-polarity establishment in both structures.

Conclusions

The induction of gradients of *fz* expression in the pupal wing resulted in the reorientation of wing hair polarity. Hairs pointed from cells of higher Fz levels towards cells of lower Fz levels. This correspondence between Fz level and polarity suggests that cells can assess the Fz level or activity of neighboring cells and use this to differentiate in a polarized way. The ability of cells to assess the Fz activity of neighboring cells, or the level of Fz on juxtaposed cell membranes, could provide the basis for a general mechanism by which cells are polarized by Wnts, the ligands that activate Fz [7,8,11,14–16].

Materials and methods

Genetic reagents

The *fz, dsh, pk, hs–fz, hs–dsh, hs–lacZ, hs–GAL4, strb* stocks have been described elsewhere [1,17,18,24,27,40,41]. The *UAS-fz* transgene contains the *fz* open reading frame [42] subcloned into the pUAST vector [27]. We thank Natasha Abramova for constructing the *UAS–fz* transgene and for generating the transgenic lines. The FRT, *hs–flp* and N-myc epitope marker stocks [43] used in the mitotic clone experiments and the *ptc–GAL4* and *dll–GAL4* driver lines were obtained from the *Drosophila* stock center at Indiana University.

Staining and mounting of samples

Adult wings were mounted in Euparal and observed under bright-field optics. LacZ, rhodamine–phalloidin and antibody staining were done by standard procedures. The 1C11 anti-Fz monoclonal antibody was used for the antibody staining as described previously [5].

Waxing

White prepupae were collected, washed in water, and then aged at either 25°C or 18°C. Pupal wing development is approximately twice as fast at 25°C as at 18°C. Prehair initiation starts at around 36 h after emergence of white prepupae at 25°C and at around 72 h at 18°C. For simplicity, all developmental times are converted to equivalent hours at 25°C. After aging, the pupae were attached ventral-side-up to a piece of double-stick tape on a microscope slide, and then a small (1–3μl) drop of hot wax (typically at approximately 120°C) was applied to the pupal case with a pipetter. The speed of the experimenter determines the optimal temperature of the wax. For distal waxing, the drop of wax was placed largely posterior to the wing and only overlapped the distal tip of the wing. For proximal waxing, the drop only overlapped the proximal 10–20% of the wing. For proximal-half waxing, the overlap was about a third to a half of the wing. On the basis of bristle loss on the abdomens of waxed *hs–dsh* pupae, it was clear that the effective heat induction of gene expression goes beyond the extent of the drop of wax.

Supplementary material

A supplementary table, Table S1, showing the results of proximal waxing of pupal wings from *hs–fz* flies is published with this paper on the internet.

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