BMC Developmental Biology

Research article

The *frizzled* pathway regulates the development of arista laterals.

Biao He[1,2] and Paul N Adler*[1]

Address: [1]Biology Department and Cancer Center, Gilmer Hall, University of Virginia, Charlottesville, VA 22903, USA and [2]Thoracic Oncology Laboratory, UCSF Cancer Center, University of California, San Francisco, CA 94115, USA

E-mail: Biao He - bh3h@hotmail.com; Paul N Adler* - pna@virginia.edu

*Corresponding author

Published: 3 May 2002

BMC Developmental Biology 2002, **2**:7

Received: 25 March 2002
Accepted: 3 May 2002

This article is available from: http://www.biomedcentral.com/1471-213X/2/7

Abstract

Background: The *frizzled* pathway in Drosophila has been studied intensively for its role in the development of planar polarity in wing hairs, thoracic bristles and ommatidia. Selected cells in the arista (the terminal segment of the antenna) elaborate a lateral projection that shares characteristics with both hairs and bristles.

Results: We found that mutations in putative downstream members of the *frizzled* pathway, such as *inturned* resulted in multipled and split laterals, but no obvious polarity defects. Mutations in upstream genes such as *frizzled* did not show an adult arista phenotype. When we examined lateral development in the mutants we found that, as is the case on the wing for hair development the *frizzled* pathway regulated the subcellular location for lateral initiation. However, on the arista an altered location for lateral initiation did not result in altered polarity, as did an altered site for hair initiation. The regulation of lateral development involved the preferential accumulation of Frizzled protein at the distal edges of lateral cells much as is seen on the wing. In contrast to the situation in wing cells, in arista cells the location for lateral initiation was close to but did not overlap the region of preferential Frizzled accumulation.

Conclusion: Our data indicates that a modified version of the *frizzled* pathway regulates arista development. We conclude that the lack of a polarity defect in mutant aristae is likely to be a consequence of inherent differences in the cell biology of wing hair and lateral forming cells.

Background

The adult cuticular surface of insects is covered with hairs, sensory bristles, ridges, scales and other structures. In most body regions individual cuticular structures are aligned in the plane of the epithelium. The basis for the development of this planar or tissue polarity has principally been studied in Drosophila [1–4] where it is under the control of the *frizzled* (*fz*) pathway [5]. Many Drosophila epidermal cells produce cuticular hairs that are outgrowths from the apical surface of pupal epidermal cells. The development of epidermal hairs has principally been studied on the wing where each pupal cell produces a single distally pointing hair. The *fz* pathway controls hair polarity by directing the location for hair initiation [5]. In a wild type fly hairs are formed at the distal most vertex of the wing cells and extend distally. Mutations in *fz* pathway genes result in altered sites for hair initiation, which results in altered hair polarity and in some cases altered hair number [5]. Sensory bristles are found in all regions of the adult epidermis. Bristles are polarized within the plane of the epidermis and in most body region neighbouring bristles are aligned in parallel and point in the

same direction as neighbouring hairs. The *fz* pathway controls bristle polarity by regulating the orientation of the spindle for the first differentiative division of the sensory organ precursor cell [6]. The ommatidia of the compound eye also display a dramatic planar polarity that is under the control of the *fz* pathway. In this tissue the *fz* pathway controls polarity by regulating the R3/R4 cell fate decision [7].

The arista is the terminal segment of the antenna and it is comprised of a central core of epidermal cells, some of which elaborate a single lateral extension [8]. The laterals and the cells that form them share a number of characteristics of both hairs and bristles. The laterals are longer than many bristles, but thinner. At the ultrastructural level developing laterals resemble bristles and not hairs [8]. They contain a series of large bundles of F-actin that are juxtaposed to the plasma membrane. In addition, microtubules are distributed throughout the central region of the lateral. The developing laterals resemble hairs and not bristle in that they are formed at the apical surface of epithelial cells and they are not innervated.

Mutations in the genes of the *fz* pathway result in altered hair and bristle polarity and in some cases large numbers of cells that produce multiple hairs. To determine if the *fz* pathway played a role in arista development we examined the aristae of various *fz* pathway mutants [1,5]. We found that *multiple wing hairs* (*mwh*), which results in an average of almost 4 hairs per cell on the wing also resulted in the formation of multipled and branched laterals. However unlike the situation on the wing, where *mwh* results in altered hair polarity, on the arista we did not see any evidence of altered lateral polarity. We also examined aristae from *inturned* (*in*) and *fuzzy* (*fy*) mutants. On the wing mutations in these genes result in altered hair polarity and in many cells forming two or three hairs [5]. On mutant aristae we found that *in* and *fy* caused multipled and branched laterals, but as was the case for *mwh* we did not see any evidence for altered polarity. We did not detect any mutant phenotype in adult aristae of *fz*, *Van Gogh* (*Vang*), *dishevelled* (*dsh*), *starry night* (*stan*) also known as - *Flamingo* (*fmi*) or *prickle* (*pk*) mutants [1]. It was surprising that upstream members of the *fz* pathway did not show a mutant phenotype while downstream members did. We therefore examined the situation further. In developing wild type pupal aristae laterals formed near but not at the distal edge of aristae central core cells [8]. When we examined *fz, dsh* and*stan* mutants we noted that lateral initiation was no longer restricted to the distal region of central core cells. Although these laterals formed at an unusual subcellular location this did not produce a noticeable phenotype in the adult. The altered location for lateral initiation in *fz* and other mutants was similar to their phenotype in the wing [5]. Prior to hair initiation in wing cells Fz, Dsh, Fmi and Dgo accumulate on the distal side of wing cells (Stan and Dgo are also thought to accumulate on the proximal side) [9–13]. To see if this could be the case for lateral development we examined the distribution of Fz in developing arista using a Fz-GFP encoding transgene gene. We found that Fz-GFP preferentially accumulated on the distal side of arista central core cells much as it does in wing cells. In addition, we found that this asymmetric localization was lost in *dsh* mutants but retained in *in* and *fy* mutants as is the case in wing cells. As is the case in wing cells the distal accumulation of Fz did not appear to influence lateral development in an *in* or *fy* mutant. Overall the data argue that the *fz* pathway functions in a similar but modified way to regulate lateral morphogenesis as compared to wing hair morphogenesis. We suggest that the absence of a noticeable adult phenotype in *fz* aristae while there is a dramatic phenotype on the wing is due to inherent differences in the cell biology of the relevant cells.

Results

The arista phenotype of inturned and fuzzy

On the wing, mutations in *inturned* (*in*) and *fuzzy* (*fy*) result in both altered hair polarity and in many cells forming more than one hair [5,14–16]. In the arista mutations in these genes do not have an obvious polarity phenotype but they do result in the production of multipled and split laterals (Fig. 1C). We found about two lateral ends per lateral producing cell (a wild type unbranched lateral has one end per lateral producing cell) (Table 1). Most of these cases were due to independent initiation events in the same central core cell (≅ 50 %) or to a split near the proximal end of the lateral (≅ 30 %). In phalloidin stained preparations of *in* and *fy* mutant heads we saw that early in lateral morphogenesis F-actin accumulated over a larger fraction of the distal part of the cell than in wild type (Fig. 1I). This wider distribution of F-actin typically resolved into two (or occasionally more) actin-filled laterals (Fig. 1J,1K). On occasion we saw laterals in *fy* and *in* mutants that were branched distally (Fig. 1K). In a wild type arista the lateral initiates close to, but not at the distal most boundary of the cell. On average the lateral is located at 90% of the way toward the distal edge of the cell (Table 2). In *in* and *fy* mutants the proximal most lateral was located about 65% of the way toward the distal edge of the cell. This was significantly different from that in wild type (Table 2). When we made a series of timed observations of individual laterals *in pupae* we found that most cells formed multiple independent laterals at a similar time. We also saw examples where laterals split during outgrowth (Fig. 2). The splitting was seen at a variety of stages, from early in lateral outgrowth to as much as 8 hours or more after lateral initiation. We did not detect any delay in lateral initiation or any notable change in the rate of lateral elongation in these mutants ([17](data not

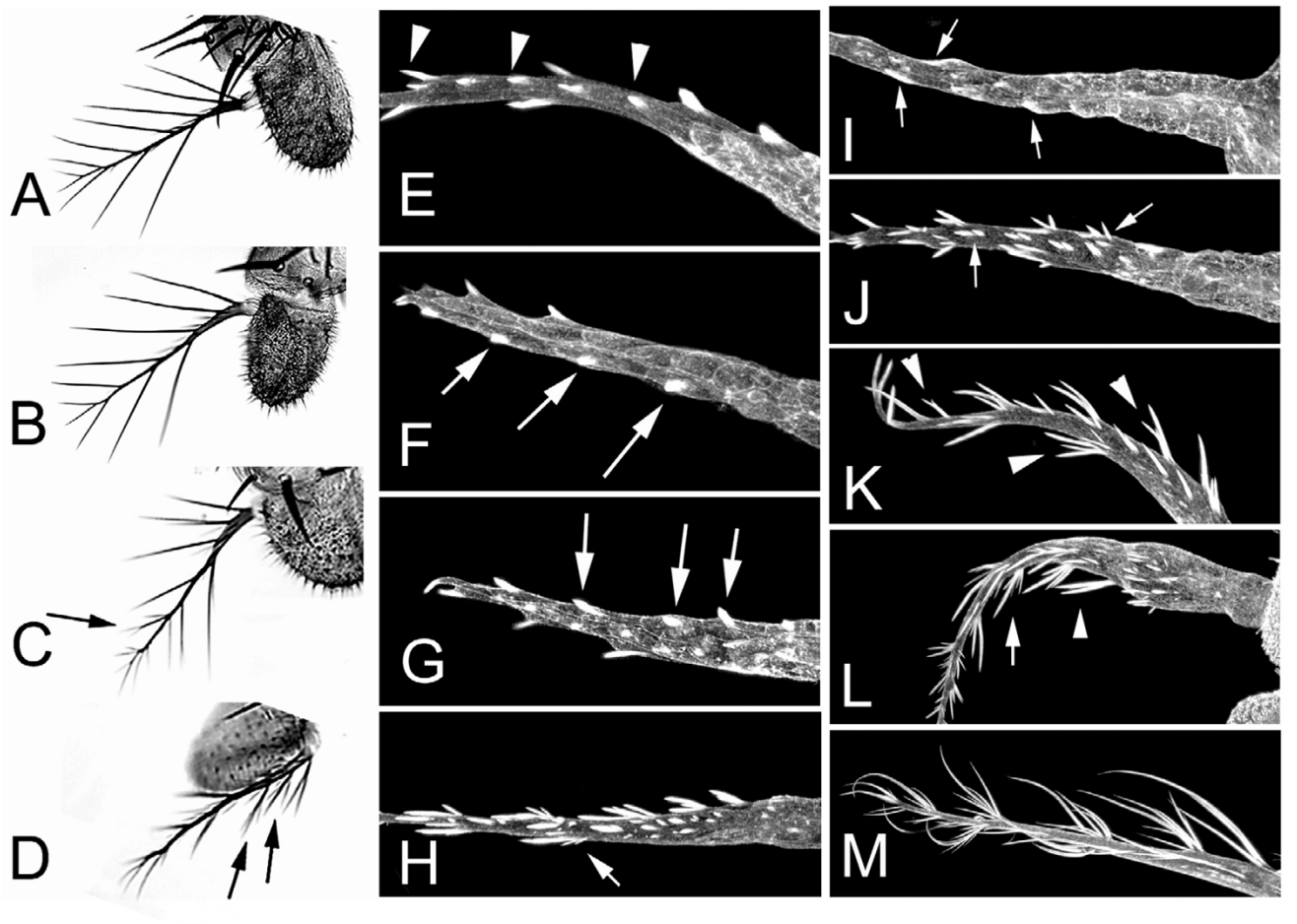

Figure 1
The arista phenotypes of *frizzled* pathway mutants. Panels A – D show adult aristae from A – wild type, B – fz, C – fy, and D – mwh flies. The arrows in panels C and D point to split and/or multipled laterals. Panels E – M are confocal images of Alexa 588 phalloidin (Molecular Probes) stained aristae. Panel E is wild type (30 hr apf (after prepupae formation)(arrowheads point to laterals forming near the distal edge of the cell), F – fz (30 hr apf) (arrows point to laterals forming at a central location in the cell), G – dsh (30 hr apf)(arrows as in F), H – mwh (31 hr apf)(arrow points to a cell that is forming multiple independent laterals), I – fy (28 hr apf) (arrows point to regions of actin accumulation), J – fy (30 hr apf) (arrow point to cells with multiple independent laterals), K – fy (36 hr apf) (arrow heads point to branched laterals), L – mwh (36 hr apf) (arrow points to a cell with multiple independent laterals and the arrowhead points to a branched lateral) and M – mwh 42 hr apf. Pupae were fixed and stained using standard procedures [8].

shown)). As split laterals extended there was an increase both in the distance from the proximal end of the lateral to the branch-point (Fig. 2B,2C) and from the branch-point to each of the distal tips. This is similar to what we have seen with other mutations [18,19]. That mutations in *in* and *fy* cause both independent lateral initiations and the splitting of single laterals during outgrowth was unexpected, as the multiple wing hair phenotype produced by these mutations has been interpreted as an effect only on hair initiation [5]. This result led us to reexamine *in* and *fy* adult wings and indeed we were able to find small numbers of branched hairs. The presence of branched *fy* hairs was reported previously [15]. Thus the phenotypic difference between the effects on laterals and hairs appears quantitative and not qualitative.

We next attempted to determine the critical time for *in* function in lateral development using temperature shift experiments with the temperature sensitive allele in^{II53}[20]. On the wing this allele results in a large phenotypic difference when pupae grown at the restrictive versus permissive temperatures. We found that the phenotypic difference was not so dramatic on the laterals, as at the restrictive condition approximately 55% of laterals were

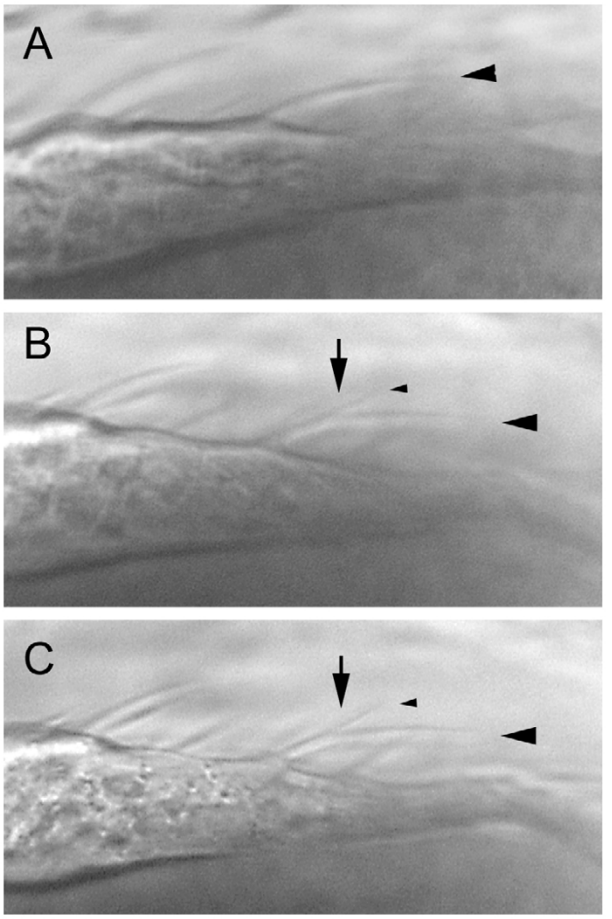

Figure 2
The splitting of a lateral in an *inturned* mutant. Shown are images of an *in* lateral at 36 hr (A), 40 hr (B) and 44 hr (C). The large arrowheads point to the distal tip of the unbranched lateral (A) and the tip of the longest arm of the branched lateral (B,C). The small arrowhead points to the tip of the shorter arm in the branched lateral. The arrow points to the branch-point.

split or multipled while at the permissive condition about 35% were split or multipled. This difference was still large enough to use for the shift experiments. Lateral growth takes place over more than 18 hours at 29°C and more than 30 hours at 18°C giving us a wide window for shift experiments [8]. In shift experiments we found that the crucial time for *in* function in lateral development was around the time of lateral initiation (26 hours at 29°C and 52 hrs at 18°C) (Fig. 3). Notably, temperature shifts in either direction even shortly after the start of lateral outgrowth had only minor effects on the phenotype. These results are similar to those previously obtained on the wing [20]. It is worth noting that *in* laterals will sometimes split 8 hours or more after the end of the *in* temperature sensitive period. We suggest that this is an example where the consequences of a mutation are not manifested until some hours after the time of action of the gene.

On the wing single and double mutant combinations of *in* and *fy* result in a similar phenotype [5]. This lack of additivity has been interpreted as evidence that these genes function and are required in a common process. We compared the multipled lateral phenotypes of *in* and *fy* single mutants and *fy; in* double mutants and found no difference consistent with *in* and *fy* functioning in the same process in lateral development (Table 1). In the wing *in* and *fy* are epistatic to mutations in *fz*, *dishevelled* (*dsh*), *prickle* (*pk*), *Van Gogh* (*Vang*) (aka *strabismus*) and *starry night* (*stan*) (aka *flamingo*) [5,21]. This was also the case in the arista (Table 1).

The arista phenotype of mutiple wing hairs

In the wing *multiple wing hairs* (*mwh*) results in similar polarity disruptions and a stronger multiple hair cell phenotype than *in* or *fy*[5,14]. *mwh* aristae had almost 5 lateral ends per cell (Table 1) (Fig. 1D). The laterals were also substantially shorter than wild type (40–45% of normal length). Thus, in the arista laterals as in wing hairs *mwh* has a substantially stronger phenotype than *in* or *fy*. At early stages in *mwh* lateral morphogenesis we saw multiple locations within individual cells where F-actin accumulated (Fig. 1H). At later stages these appeared as multiple independent laterals produced by individual cells (Figure 1L,1M). These conclusions were confirmed by time-lapse observations ([17](data not shown)). In addition, we saw examples where a lateral split during outgrowth ([17](data not shown)). There was no alteration in the time of lateral initiation in *mwh* although we did see a decrease in the rate of lateral elongation that was consistent with the short final length of *mwh* laterals ([17](data not shown)). The short length and slow growth may simply be a consequence of the extreme multiple lateral phenotype as laterals that were only slightly branched were of relatively normal length. In the wing all double mutant combinations of *mwh* and other *fz* pathway genes result in a *mwh* like phenotype [5]. For the lateral phenotype we found that *mwh fz* double mutants resembled *mwh*, as is the case in the wing (Table 1). However, *mwh in* and *fy; mwh* laterals produced an intermediate phenotype in terms of the mean number of lateral ends per cell (Table 1). Thus, the relationship between *in* and *fy* and *mwh* appears different in the wing and arista, presumably reflecting differences in the genetic circuitry in these two tissues.

The arista phenotype of fz mutants

We did not see any obvious mutant phenotype in aristae from *fz, dsh, pk, Vang* or *stan* flies (Fig. 1B). In the wing, mutations in *fz* and these other *fz* like genes result in hairs

Table 1: The adult arista phenotypes in single and selected double mutants.

Genotype	Number of lateral ends per cell mean (sd)	P value compared to (t-test)
Ore R	1	
fz	1	
dsh	1	
pk	1	
Vang	1	
stan	1	
pk; fz	1	
fy	2.2 (1.2)	p < 0.0001 (*Ore R*)
in	2.1 (.74)	p < 0.0001 (*Ore R*)
fy; in	2.3 (.95)	p = 0.94 (*fy*), 0.80 (*in*)
fz in	2.0 (1.07)	p = 0.67 (*in*)
fy;stan	2.2 (.75)	p = 0.90 (*fy*)
fy;fz	2.4 (.72)	p = 0.29 (*fy*)
mwh	4.5 (1.44)	p < 0.0001 (*Ore R*)
mwh fz	4.7 (1.78)	p = 0.68 (*mwh*)
mwh in	3.2 (1.3)	p = 0.002 (*in*), 0.0005 (*mwh*)
fy; mwh	3.4 (1.07)	p = 0.0001 (*fy*), 0.007 (*mwh*)

with abnormal polarity due to a failure to restrict prehair initiation to the distal most part of the cell [5]. Only infrequently does this result in a cell forming more than one hair. When we examined the development of *fz* mutant laterals by phalloidin staining we found that lateral initiation was not as restricted to the distal part of cells as is the case in wild type (Fig. 1F) (Table 2). Rather laterals formed at a wide range of locations along the proximal distal axis of the extended central core cells. For *fz, dsh* (Fig. 1G) and *stan* (data not shown) the lateral formed at about 70% of the way toward the distal edge of the cell, which was significantly different from that of wild type. This was slightly more distal than we saw for *in* and *fy* but not significantly so (Table 2). For *pk* and *Vang* mutants the average location for lateral initation was slightly less distal than in wild type (≅ 85% vs 90%) and this difference was only marginally significant. The position for lateral initiation in *pk* and *Vang* was clearly different from that of *fz*.

Fz accumulates asymmetrically in arista cells

The initiation of wing hairs in the vicinity of the distal most vertex of wing cells is preceded by the preferential accumulation of Fz protein along the distal side of wing cells [10]. To determine if the distal initiation of laterals was also associated with the preferential accumulation of Fz we examined aristae that expressed Fz-GFP under the control of the armadillo promoter. This construct is expressed at a low enough rate so that the subcellular localization of the Fz protein is still preferentially found along the distal edge of wing cells (Strutt, 2001). We found that Fz protein also accumulated preferentially along the distal/proximal edges of arista central core cells (Fig. 4). This could be detected both before and during lateral outgrowth. The ratio of the intensity of GFP flourescence on the distal versus lateral sides of the cells ranged from 1.7 to 2.4 in different aristae preparations. This is similar to the ratio for Fz-GFP that we have seen in wing cells. The emerging laterals were found close to but not overlapping this region. From our experiments we could not distinguish between preferential location along the distal edge, proximal edge or both. Based on analogy with the wing situation we suggest it at the distal edge of the cells. In occasional cells that bulged out from the central core this appeared to be the case. The distal accumulation of Fz-GFP could be detected until late in lateral development. At this time the cells become highly elongated along the proximal/distal axis and it becomes difficult to assess what the distal edge is (Fig 4).

In the wing the accumulation of Fz at the distal edge of the cell requires the function of *dsh*[10]. We examined aristae from *dsh*^{1}; *arm-fz-GFP* pupae and found that the preferential distal accumulation of Fz was lost (Fig. 4). The ratio of GFP fluorescence on the distal versus laterals sides of cells ranged from 1.2 – 0.9. Thus, as in the wing the distal accumulation of Fz in arista cells is dependent on *dsh* function. In the wing Dsh accumulates preferentially along the distal edge of wing cells [12,13]. We found that Dsh-GFP also accumulated along the distal edge of arista cells (Fig. 4).

In the wing the distal accumulation of Fz is not dependent on the function of *in* or *fy*, although in *in* and *fy* mutants the distal accumulation of Fz is no longer able to restrict hair formation to the distal edge of the cell [10]. We examined the distribution of Fz in *arm-fz-GFP; in* and *fy; arm-fz-GFP* aristae. The preferential distal accumulation of Fz was still present in these arista cells (Fig. 4) (we obtained distal to lateral edge ratios of 1.7 – 2.0), although the *in* and *fy* mutant lateral phenotype was obvious. Thus, *fz, in* and *fy* appear to have a similar relationship in the arista and wing.

There are a number of striking similarities in the morphogenesis of hairs, laterals and bristles [8]. Data presented here for laterals and previously for wing hairs [10] show that the asymmetric accumulation of Fz is prominent in these terminally differentiating cells. Given these observations we might expect that this would also be true for bristles. The bristle sense organ is a multicellular structure with the cells organized in a 3-dimensional arrangement making it difficult to observe Fz localization in all of these cells. The socket cell that surrounds the growing shaft remains in the plane of the adult epidermis and is easy to observe in whole mount preparations of pupal epidermis. The socket cell is also polarized in the same direction as

Table 2: *frizzled* pathway mutations and the position of lateral formation

Genotype	average position#	sd	n@	P* compared to Oregon R	P compared to *frizzled*
Oregon R	0.918	0.043	32		2×10^{-10}
fz	0.695	0.099	22	2×10^{-10}	
dsh	0.701	0.078	24	6.2×10^{-14}	0.82
stan	0.734	0.121	13	1.3×10^{-4}	0.34
pk	0.870	0.058	10	3.5×10^{-2}	1×10^{-6}
Vang	0.855	0.087	18	1×10^{-2}	3.5×10^{-6}
in	0.664	0.136	12	3.9×10^{-5}	0.50
fy	0.641	0.098	12	4.2×10^{-7}	0.14

the position of the distal edge of the lateral is given as the fractional distance of the cell along the proximal distal axis. @ number of laterals scored. * P values from t-test

the shaft as it is higher on one side than the other. We examined the accumulation of Fz-GFP in differentiating socket cells and found that it was asymmetrically distributed both early and late in bristle differentiation (Fig. 5). In most cells that we observed it appeared to accumulate on both the proximal and distal (dorsal/ventral etc.) edges of the socket cells. We cannot be certain from these observations if it was accumulating in the socket cell or in the neighboring epidermal cells. The level of Fz-GFP appeared higher than in typical epidermal cells suggesting that we are looking at Fz-GFP present in the socket cell. The ratio of GFP fluorescence at high to low regions was similar (2.4 – 1.6) to what we obtained for wing and arista cells. It was reported previously that Fmi/Stan protein accumulated to a higher level in the PI cell that forms the bristle lineage [22]. Previous experiments have shown that Fz regulates orientation of the spindle in the division of the PI cell of the bristle sense organ lineage and it has been thought that this is the way that *fz* regulated bristle polarity [4,6]. Our observations suggest that *fz* may also play a later role in specifying bristle polarity that is analogous to its role in regulating hair and lateral development.

Discussion

In wing cells the distal assembly of the hair is preceded by the preferential accumulation of Fz, Dsh, Fmi/Stan, and Diego along the distal edge of the cell [9,11–13]. This provides a cortical mark that is thought to specify the proximal-distal axis of the cells. In these cells the region for hair initiation overlaps, but is only a small part of the region where these proteins accumulate. It is unclear how the cytoskeleton is activated in this restricted region. One possibility is that a Fz receptor protein complex directly interacts with and activates proteins that stimulate the cytoskeleton to produce a hair (Fig. 6). In the arista we also see a preferential accumulation of Fz protein at the distal/proximal edges of cells and this likely serves a similar function as a cortical mark. It is worth noting however, that the location for lateral outgrowth does not directly overlap with the location for Fz accumulation (Fig. 4,7). Although the lateral is formed in the distal region of the cell it is not juxtaposed to the junctional complex or lateral cell periphery where Fz accumulates. Rather laterals in wild type flies form on the apical surface of arista cells (Fig. 7) [8]. Thus, in arista cells it is unlikely that there is any direct connection between Fz receptor complex proteins that accumulate distally and the actin cytoskeleton of the laterals. More likely in this cell type the distal accumulation of Fz and other proteins activates a signal transduction pathway that activates the cytoskeleton through a diffusible signal (Fig. 6). The JNK pathway, which has been implicated in Fz signal transduction in the eye is a candidate pathway to be involved in this ([23–25]). We note that a diffusible signal could be part of the mechanism that is used to restrict hair initiation to a smaller part of wing cells than the region where Fz accumulates.

Based on the precedent from wing cells it is at first glance surprising that an alteration in the site for lateral initiation does not produce obvious phenotypic consequences [5]. Pupal wing cells are relatively isotropic in terms of their shape in the planar direction. The assembly of the hair at the cell periphery puts it in close proximity to the junctional complex perhaps allowing for direct interactions between the cortical and hair cytoskeletons [5]. This could provide orientation guidance for the growing hair. In a *fz* mutant, where hair formation no longer takes place at the cell periphery this guidance would be lost (Fig. 7). In an *in* mutant, where hair formation takes place at alternative locations on the cell periphery, it would be incorrect (Fig. 7). Lateral forming cells are elongated in the proximal distal direction (Fig. 7) and laterals grow out at a large angle to the apical surface of the central core cells. In this way wild type laterals resemble *fz* mutant wing cells [5]. Thus, early in their development laterals are not close to the junctional complex or the prominent cortical cytoskele-

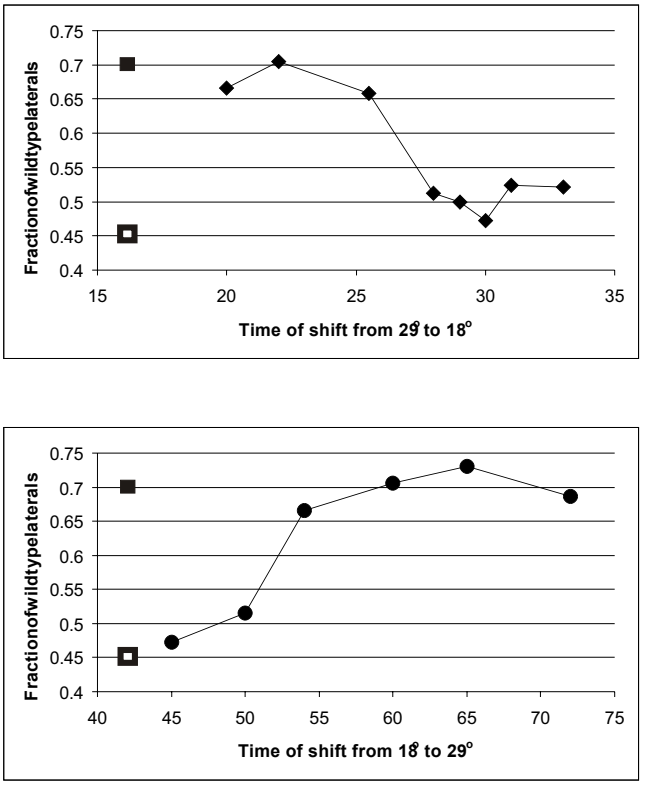

Figure 3
The temperature sensitive period for *inturned*. Shown is the fraction of normal laterals (i.e. not multipled or branched) after various treatments. At least 20 laterals were scored for each point (in most cases more than 40 laterals). The filled box is for pupae kept at 29°C and the open box for pupae kept at 18°C. The time of the shift is in hours after white prepupae formation. Laterals form at about 26 hrs at 29°C and 52 hrs at 18°C. There is some variability as distal laterals form earlier than proximal ones.

ton at the cell periphery and are unlikely to be getting any orientation guidance in this way. In a *fz* mutant lateral the location along the proximal distal axis of the cell is changed but there is no disruption of a close connection to the cortical cytoskeleton as there is in hair cells. In the mutant there is no change in the dorsal/ventral position for lateral outgrowth or the angle of the outgrowth to the apical surface. In part this may be due to limitations inherent in a relatively large lateral growing out of a long thin cell. It is also worth considering that the elongated shape of the lateral forming cells becomes much more extreme as development proceeds (Fig 4) [8]. This brings the laterals close to the cell edges that are parallel to the proximal distal axis of the central core and the junctional complexes at these edges could provide orientation guidance.

Previous studies on *in* and *fy* function in regulating wing hair development have focused on their role in regulating the site for hair initiation [5,20]. Observations reported in this paper show that multiple laterals associated with *in* and *fy* mutants can arise either from independent initiation events or from the splitting of an elongating lateral. The splitting of laterals could be due to a need for *in* and *fy* function during the outgrowth process or to a need for *in* and *fy* function at initiation with the consequences only being manifested at a later time. We carried out a set of temperature shift experiments to distinguish between these possibilities. These shift experiments showed that the essential time for *in* function in lateral development is around the time of lateral initiation. This is similar to what we previously found for *in* function in wing hair development [20]. It is interesting that the consequences of a lack of *in* function in laterals are manifested some hours after the end of the *in* temperature sensitive period. We suggest that in an *in* mutant there is a subtle defect in a cytoskeleton-organizing center that promotes lateral outgrowth. The defect is not severe enough to block or dramatically slow lateral outgrowth. In some cells it leads to the formation of multiple initiation sites and in others to an unstable lateral that is subject to splitting. A similar phenomenon could explain the formation of occasional branched wing hairs in *in*, *fy* and *mwh* wings. It could also explain differences in the hair, lateral and bristle phenotypes of *tricornered* (*trc*) and *furry* (*fry*) mutants [19,26]. In such mutants bristles and laterals are often branched. In contrast, *trc* and *fry* wing cells have a very dramatic multiple hair cells phenotype (average more than 5 hairs per cell). The multiple hairs are found primarily in clusters of independent hairs with only occasional branched hairs. We suggest that there is a common defect in *trc* and *fry* bristles, laterals and hairs. Differences in the phenotypes would be due to inherent differences in the sensitivity of these cell types to the defective organizing centers. The smaller hairs would be more sensitive to the defects leading to "spitting" during the initial stages of growth, which would result in what appears to be a cluster of independent hairs. The larger structures (laterals and bristles) could be less sensitive so that the consequences of the abnormal organizing center would only be manifested later in morphogenesis if at all.

Previous observations had indicated that the *fz* pathway functioned in the development of planar polarity in hairs, bristles and ommatidia [5–7,14]. Consistent with much current thinking on signal transduction pathways it has been suggested that there is a core group of *fz* pathway of genes that function similarly in different cell types [2]. Downstream genes would function to interpret the signal from the core group of genes in a cell type specific way. As attractive as this idea is a number of observations suggest that much of the *fz* pathway is modified in cell type specific ways. For example, the asymmetric accumulation of Fmi/Stan in wing cells is essential for marking the distal

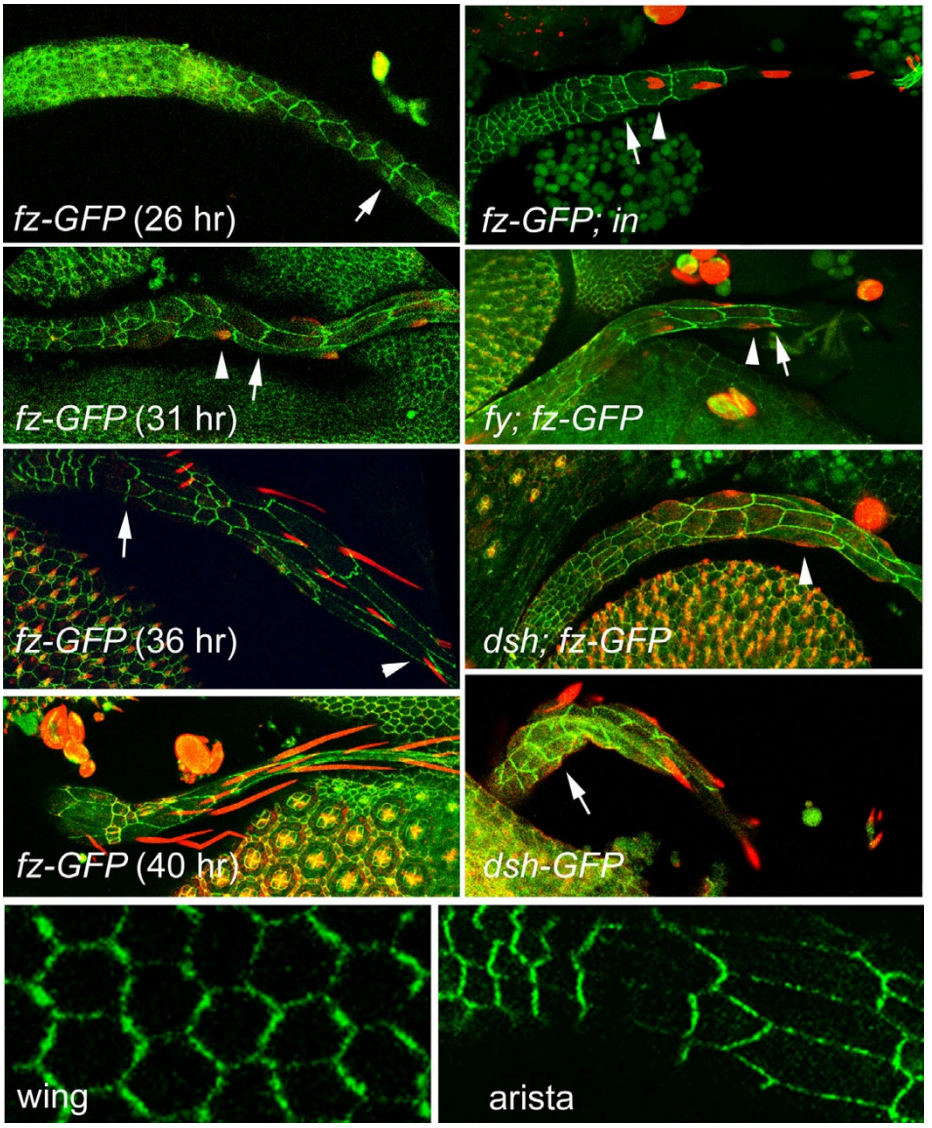

Figure 4

Confocal images of Fz-GFP and actin in aristae. All panels are projections from confocal stacks and are from Alexa 568 phalloidin stained *arm-fz-GFP* containing pupae. GFP is in green and phalloidin in red. The four panels on the right show aristae at progressively more advanced developmental stages. All of these animals are wild type except for carrying the *arm-fz-GFP* transgene. Note that Fz accumulated preferentially on the distal sides of cells (arrows). In the oldest arista (40 hr) the more distal central core cells have become far more elongated and in these it is difficult to assess if Fz is still present preferentially along the distal edge. Even in the proximal cells in this arista the preferential distal accumulation appears less prominent. Particularly in 31 and 36 hr aristae the actin-stained laterals are located in the distal part of the cell, but do not directly overlap the region where Fz-GFP has accumulated. The distal accumulation of Fz-GFP is also seen in cells that do not form laterals. In the *arm-fz-GFP* ; *in* arista we still see the preferential accumulation of Fz-GFP at the distal borders of cells. Note however that lateral formation is not restricted to the distal part of the cell and that one cell in this image (see arrowhead) is forming two closely juxtaposed laterals. Once again the arrow points to small non-lateral producing cells where Fz-GFP accumulated along the distal/proximal boundary of the cells. In the *fy; arm-fz-GFP* the situation is similar to the *arm-fz-GFP* ; *in* arista. In the dsh^{1}; *arm-fz-GFP* arista the preferential distal localization of Fz-GFP is lost. Actin is accumulating at a central location in the lateral producing cells. Note the formation of laterals is no longer restricted to the distal region of the cells in the *in*, *fy* or *dsh* mutants (marked by arrowheads). We also find that Dsh-GFP accumulates preferentially at the distal side of cells. The bottom two panels are higher magnification images of Fz-GFP in wing and arista cells. Note the preferential accumulation of Fz-GFP at the distal edges and that it is not uniform. Both images are from cells that where hairs or laterals are partly developed. The arista image is from the 36 hr arista panel in this figure.

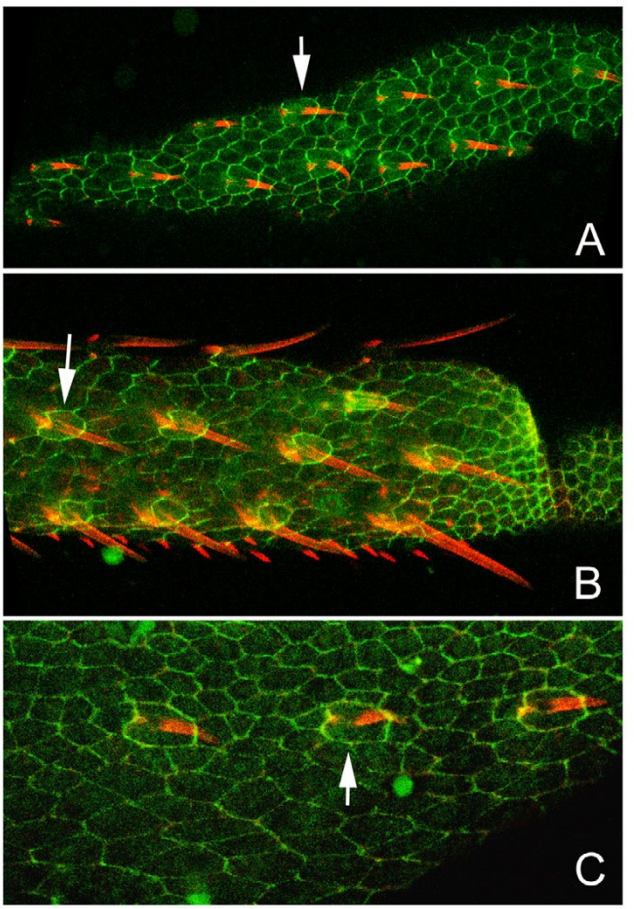

Figure 5
The Fz-GFP protein accumulates at the distal and proximal edges of socket cells. Shown in panel A and B are pupal legs from *arm-fz-GFP* pupae that were stained with Alexa-568 phalloidin. The actin stained bristle shafts can be seen growing up through the socket cells, which remain in the plane of the epidermis. Socket cells (arrows) are larger than the neighbouring epidermal cells, are indented on the proximal side and stain more brightly for Fz-GFP. Note the asymmetric accumulation of Fz-GFP in the socket cells. Fz-GFP accumulates preferentially at the distal/proximal boundaries of both the leg epidermal and socket cells. Panel C shows a region from a pupal head. The preferential accumulation of Fz-GFP on opposite sides of the socket cells is obvious here as well. The uneven accumulation of Fz-GFP is less noticeable in the head epithelial cells than on the leg or wing, although it can still usually be detected.

edge of wing cells [9]. However, Fmi/Stan does not accumulate asymmetrically in sensory organ precursor cell that gives rise to the bristle sense organ although the orientation of the pI cell spindle is altered in *fmi/stan* mutants [22]. Our observations on the arista suggest that the genetic circuitry of the *fz* pathway is modified in a different way in this cell type. The most notable differences were the lack of a substantial phenotype in *pk* and *Vang* and that *mwh* is not epistatic to *in* and *fy* as it the case on the wing.

Conclusions

In this paper we provide evidence that a modified version of the *frizzled* pathway regulates the development of arista laterals. As is the case for the wing the asymmetrical accumulation of Fz protein appears to produce a cortical mark that is used to control where the cytoskeleton is activated to produce a lateral.

Materials and methods

Fly strains

Fruit flies were grown on standard media. Many of the mutations were isolated in this lab and described previously [5,20,27,28]. Several were obtained from the Drosophila stock center at Indiana University. D. Strutt kindly provided flies carrying the *arm-fz-GFP* transgene [10] and J. Axelrod kindly provided flies carrying a *dsh-GFP* transgene (this construct uses the endogenous *dsh* promoter) [12]. Temperature shift experiments were done as previously [20].

Cytological procedures

Standard techniques were used. In most staining experiments pupae were fixed in phosphate buffered saline supplemented with 4% paraformaldehyde. Actin staining was done as described previously [8] using Alexa 568 phalloidin (Molecular Probes). For experiments where we visualized GFP we made an effort to use the minimum amount of fixation compatible with maintaining the morphology of the aristae.

Microscopy

Confocal images were obtained using a BioRad confocal microscope at the Keck Center for Biological Imaging at the University of Virginia. Other images were obtained using a Spot digital camera (National Diagnostics) on a Zeiss Axioskop microscope using bright field optics. Length measurements were made using either the software provided with the Spot camera or by using the ruler tool in Adobe Photoshop. To measure the intensity of GFP-fluorescence we took maximal projections of optical sections and used the Psion port of the NIH Image software to analyze the data. The tif images were inverted and the intensity plotted along an 8 pixel line drawn across the relevant cell boundary. The background was estimated by the lowest reading along the line and this was subtracted from the highest reading to obtain an intensity measurement. The same procedure was done for a line drawn along a "low" intensity cell border. Multiple cells were scored (usually 10) from the same projection, averages obtained used to provide an intensity ratio. Microsoft Excel was used to analyze the data (calculating means, stand-

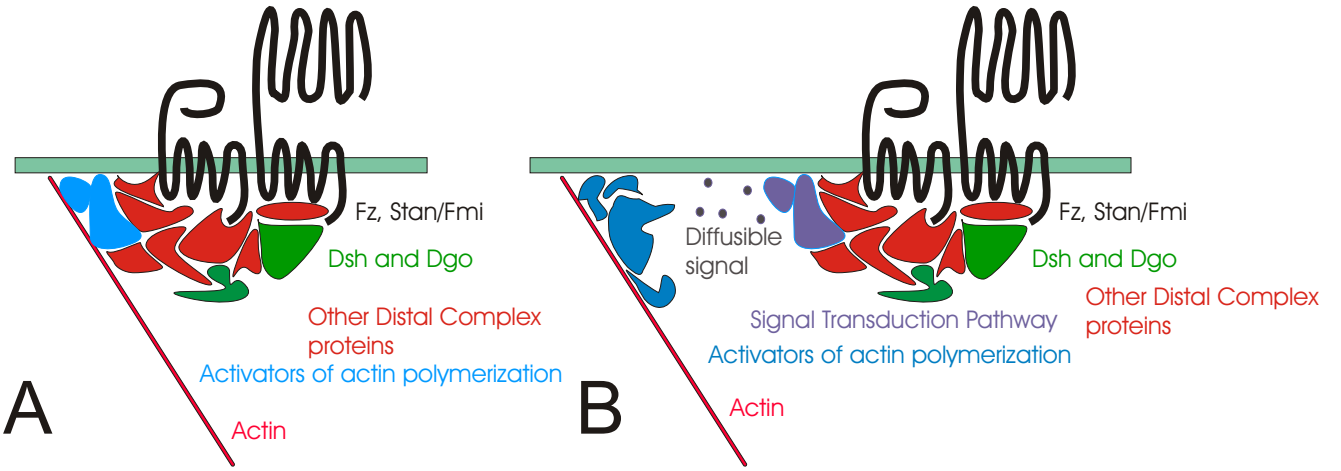

Figure 6
Models for Fz mediated activation of the cytoskeleton. Shown two different models for how the distal accumulation of Fz and other proteins could serve to activate the cytoskeleton to produce a hair or lateral. In panel A a Fz receptor complex accumulates at the distal side of the cell. The complex includes transmembrane proteins (such as Fz – filled black), known (such as Dsh – filled green) and unknown (filled red) cytoplasmic proteins. This receptor complex interacts with activators of the cytoskeleton (filled blue) leading to the production of a hair in the region where Fz preferentially accumulates. In panel B is an alternative model that can account for our results with laterals. As in A, a Fz receptor complex accumulates along the distal edge of the cell. This complex activates a signal transduction pathway (filled purple), which leads to the production of a diffusible signal. At some distance away this signal stimulates activators of the actin cytoskeleton promoting the formation of a lateral.

ard deviation, and plotting). To measure the location along the proximal/distal axes of arista cells we took micrographs of actin stained aristae where the lateral was small (less than 1/3 the length of the cell) and measured the distance from the proximal to distal sides of the cell. We also measured the distance from the proximal side to the distal edge of the base of the growing lateral where it was connected to the central core. The ratio of these two values is used as a measure of the location for lateral initiation.

Scoring of arista phenotypes
Adult aristae were mounted in Gary's Magic Mountant and examined under bright field microscope.

In vivo imaging of the arista laterals
Carefully aged pupae were washed and air-dried. In early experiments they were then attached to standard microscope slides with double-stick tape or to shallow well slides using super glue. In both preparations the animals were placed with their ventral side up. Pupal cases were opened anteriorly. At various time points they were examined by microscope under bright field optics in a drop of water using a water immersion objective (40×). After observation water was removed from the slides and the pupae were then kept in a humid chamber at 25°C for further development and observations. Most pupae tolerated the treatment, reached the pharate adult stage and were morphologically normal. More recently we built a chamber using rubber spacers, double stick tape and a cover-slip. The pupae were placed on the double stick tape ventral side up and the pupal case opened. A small amount of 10% gelatin, 10% glycerol was place on the anterior end of the pupae and between the opened pupae and the cover slip. Although the optics are not quite as good as with our earlier approaches, the chamber approach is less time consuming and can be used for automated time lapse.

Statistical analysis
Excel was used in the quantitative analysis of phenotypes. We used t-test and Z test in different experiments for comparing different genotypes.

Authors' contributions
BH carried out the scanning EM studies, many of the in vivo observations, the phenotypic analysis of adult and pupal aristae and helped with writing the paper. PNA carried out some of the in vivo observations, many of the studies on Fz-GFP and did much of the writing.

All authors read and approved the final manuscript.

Acknowledgements
This research was supported by a grant from the NIGMS to PNA.

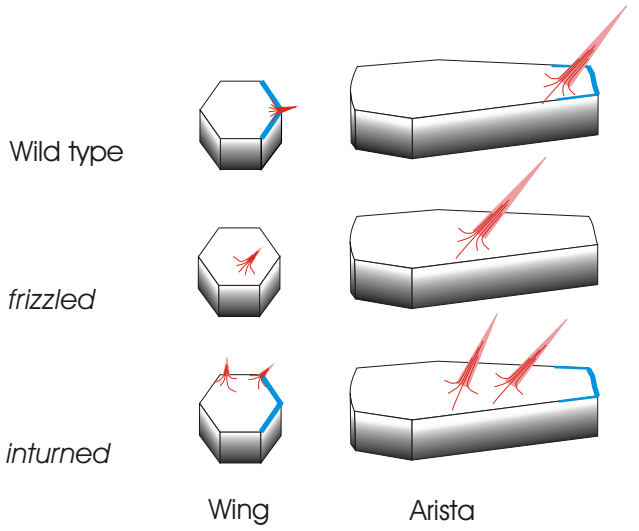

Figure 7
Wing and lateral cells from wild type, *frizzled* and *inturned*. The region where Fz-GFP preferentially accumulates is shown in blue. The actin filled hairs and laterals are in red with specific actin filaments shown in darker red. The alterations in the location for hair and lateral initiation in mutants are shown.

We thank David Strutt for kindly providing the *arm fz-GFP* transgenic flies and Jeff Axelrod for the *dsh-GFP* flies. We thank Job Taylor for his helpful comments on the manuscript.

References

1. Adler P, Lee H: **Frizzled signaling and cell-cell interactions in planar polarity.** *Curr Opin Cell Biol* 2001, **13:**635-640
2. Shulman JM, Perrimon N, Axelrod JD: **Frizzled signaling and the developmental control of cell polarity.** *Trends Genet* 1998, **14:**452-8
3. Strutt H, Strutt D: **Polarity determination in the Drosophila eye.** *Curr Opin Genet Dev* 1999, **9:**442-6
4. Adler PN, Taylor J: **Asymmetric cell division: Plane but not simple.** *Curr Biol* 2001, **11:**R233-6
5. Wong LL, Adler PN: **Tissue polarity genes of Drosophila regulate the subcellular location for prehair initiation in pupal wing cells.** *J Cell Biol* 1993, **123:**209-21
6. Gho M, Schweisguth F: **Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in Drosophila.** *Nature* 1998, **393:**178-81
7. Zheng L, Zhang J, Carthew RW: **frizzled regulates mirror-symmetric pattern formation in the Drosophila eye.** *Development* 1995, **121:**3045-55
8. He B, Adler PN: **Cellular mechanisms in the development of the epithelial arista.** *Mechanisms of Development* 2001
9. Usui T, Shima Y, Shimada Y, Hirano S, Burgess RW, Schwarz TL, Takeichi M, Uemura T: **Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled.** *Cell* 1999, **98:**585-95
10. Strutt DI: **Asymmetric localization of frizzled and the establishment of cell polarity in the Drosophila wing.** *Mol Cell* 2001, **7:**367-75
11. Feiguin F, Hannus M, Mlodzik M, Eaton S: **The ankyrin repeat protein Diego mediates Frizzled-dependent planar polarization.** *Dev Cell* 2001, **1:**93-101
12. Axelrod JD: **Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling.** *Genes Dev* 2001, **15:**1182-7
13. Shimada Y, Usui T, Yanagawa S, Takeichi M, Uemura T: **Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization.** *Curr Biol* 2001, **11:**859-63
14. Gubb D, Garcia-Bellido A: **A genetic analysis of the determination of cuticular polarity during development in Drosophila melanogaster.** *J Embryol Exp Morphol* 1982, **68:**37-57
15. Collier S, Gubb D: **Drosophila tissue polarity requires the cell-autonomous activity of the fuzzy gene, which encodes a novel transmembrane protein.** *Development* 1997, **124:**4029-4037
16. Park WJ, Liu J, Sharp EJ, Adler PN: **The Drosophila tissue polarity gene inturned acts cell autonomously and encodes a novel protein.** *Development* 1996, **122:**961-9
17. He B: *Genetic Control of Cell Extensions. Charlottesville: University of Virginia;* 2001
18. He B, Adler PN: **Genetic control of lateral morphogenesis.** *Development, genes and evolution* 2002
19. Cong J, Geng W, He B, Liu J, Charlton J, Adler P: **The furry gene of Drosophila is important for maintaining the integrity of cellular extensions during morphogenesis.** *Development* 2001, **128:**2793-2802
20. Adler PN, Charlton J, Park WJ: **The Drosophila tissue polarity gene inturned functions prior to wing hair morphogenesis in the regulation of hair polarity and number.** *Genetics* 1994, **137:**829-36
21. Taylor J, Abramova N, Charlton J, Adler PN: **Van Gogh: a new Drosophila tissue polarity gene.** *Genetics* 1998, **150:**199-210
22. Lu B, Usui T, Uemura T, Jan L, Jan YN: **Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in Drosophila.** *Curr Biol* 1999, **9:**1247-50
23. Weber U, Paricio N, Mlodzik M: **Jun mediates Frizzled-induced R3/R4 cell fate distinction and planar polarity determination in the Drosophila eye.** *Development* 2000, **127:**3619-29
24. Paricio N, Feiguin F, Boutros M, Eaton S, Mlodzik M: **The Drosophila STE20-like kinase misshapen is required downstream of the Frizzled receptor in planar polarity signaling.** *Embo J* 1999, **18:**4669-78
25. Boutros M, Paricio N, Strutt DI, Mlodzik M: **Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling.** *Cell* 1998, **94:**109-18
26. Geng W, He B, Wang M, Adler PN: **The tricornered gene, which encodes the Drosophila NDR kinase is required to maintain the integrity of cellular extensions.** *Genetics* 2000, **156:**1817-1828
27. Adler PN, Charlton J, Vinson C: **Allelic variation at the frizzled locus of Drosophila.** *Dev. Genet.* 1987, **8:**99-119
28. Jones KH, Liu J, Adler PN: **Molecular analysis of EMS-induced frizzled mutations in Drosophila melanogaster.** *Genetics* 1996, **142:**205-15