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

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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 arista from inturned (in) and fuzzy (fy) mutants. On the wing mutations in these genes result in 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. 11). This wider distribution of F-actin typically resolved into two (or occasionally more) actin-filled laterals (Fig. 11,1K). 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 available for these mutants).
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 \textit{in} and \textit{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 \textit{in} and \textit{fy} adult wings and indeed we were able to find small numbers of branched hairs. The presence of branched \textit{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 \textit{in} function in lateral development using temperature shift experiments with the temperature sensitive allele \textit{in} \textit{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 1

\textbf{The arista phenotypes of frizzled pathway mutants.} Panels A – D show adult aristae from A – wild type, B – \textit{fz}, C – \textit{fy}, and D – mwh flies. The arrows in panels C and D point to split and/or multiplied 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 – \textit{fz} (30 hr apf) (arrows point to laterals forming at a central location in the cell), G – \textit{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 – \textit{fy} (28 hr apf) (arrows point to regions of actin accumulation), J – \textit{fy} (30 hr apf) (arrow point to cells with multiple independent laterals), K – \textit{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].
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 multiple 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

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**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.
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        |                                          |                             |
| fy            | 2.2 (1.2)                                | p < 0.0001 (Ore R)          |
| in            | 2.1 (0.74)                               | p < 0.0001 (Ore R)          |
| fy; in        | 2.3 (0.95)                               | p = 0.94 (fy), 0.80 (in)    |
| fz in         | 2.0 (1.07)                               | p = 0.67 (in)               |
| fy; stan      | 2.2 (0.75)                               | p = 0.90 (fy)               |
| fy; fz        | 2.4 (0.72)                               | 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) |

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 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 fluorescence 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(y); 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 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
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-

| Genotype | average position | sd  | n @ | P compared to Oregon R | P compared to frizzled |
|----------|------------------|-----|-----|------------------------|------------------------|
| Oregon R | 0.918            | 0.043 | 32  | 2 × 10⁻¹⁰              | 2 × 10⁻¹⁰               |
| fz       | 0.695            | 0.099 | 22  | 2 × 10⁻¹⁰              | 0.82                   |
| dsh      | 0.701            | 0.078 | 24  | 6.2 × 10⁻¹⁴            | 0.34                   |
| stan     | 0.734            | 0.121 | 13  | 1.3 × 10⁻⁴            | 3.5 × 10⁻⁶             |
| 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.
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 arista. 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 arista 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 arista 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 dsh1; 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.
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 pl 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-
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 placed 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.

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**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.
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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, Cardew 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 episthelial 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 D: Asymmetric localization of frizzled and the establishment of cell polarity in the Drosophila wing. Mol Cell 2001, 7:367-75
11. Feigun 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 polarity signaling. Genes Dev 2001, 15:1182-7
13. Shimada Y, Usui T, Yangawala S, Takeichi M, Uemura T: Asymmetric co-localization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. Curr Biol 2001, 11:859-63
14. Gubb D, García-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, 137:229-36
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, Feigun 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 D, 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 Drosophila tissue polarity gene. J Cell Biol 1996, 137:229-36
28. Jones KH, Liu J, Adler PN: Molecular analysis of EMS-induced frizzled mutations in Drosophila melanogaster. Genetics 1996, 142:205-15

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 lateral cells 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.