The Function of the *frizzled* Pathway in the Drosophila Wing Is Dependent on *inturned* and *fuzzy*

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Manuscript received November 9, 2001
Accepted for publication January 30, 2002

ABSTRACT

The Drosophila epidermis is characterized by a dramatic planar or tissue polarity. The *frizzled* pathway has been shown to be a key regulator of planar polarity for hairs on the wing, ommatidia in the eye, and sensory bristles on the notum. We have investigated the genetic relationships between putative *frizzled* pathway downstream genes *inturned*, *fuzzy*, and *multiple wing hairs* (*inturned*-like genes) and upstream genes such as *frizzled*, *prickle*, and *starry night* (*frizzled*-like genes). Previous data showed that the *inturned*-like genes were epistatic to the *frizzled*-like genes when the entire wing was mutant. We extended those experiments and examined the behavior of *frizzled* clones in mutant wings. We found the domineering nonautonomy of *frizzled* clones was not altered when the clone cells were simultaneously mutant for *inturned*, *multiple wing hairs*, or *dishevelled* but it was blocked when the entire wing was mutant for *inturned*, *fuzzy*, *multiple wing hairs*, or *dishevelled*. Thus, for the domineering nonautonomy phenotype of *frizzled*, *inturned* and *multiple wing hairs* are needed in the responding cells but not in the clone itself. Expressing a number of *frizzled* pathway genes in a gradient across part of the wing repolarizes wing cells in that region. We found *inturned*, *fuzzy*, and *multiple wing hairs* were required for a gradient of *frizzled*, *starry night*, *prickle*, or *spiny-legs* expression to repolarize wing cells. These data argue that *inturned*, *fuzzy*, and *multiple wing hairs* are downstream components of the *frizzled* pathway. To further probe the relationship between the *frizzled*-like and *inturned*-like genes we determined the consequences of altering the activity of *frizzled*-like genes in wings that carried weak alleles of *inturned* or *fuzzy*. Interestingly, both increasing and decreasing the activity of *frizzled* and other upstream genes enhanced the phenotypes of hypomorphic *inturned* and *fuzzy* mutants. We also examined the relationship between the *frizzled*-like and *inturned*-like genes in other regions of the fly. For some body regions and cell types (*e.g.*, abdomen) the *inturned*-like genes were epistatic to the *frizzled*-like genes, but in other body regions (*e.g.*, eye) that was not the case. Thus, the genetic control of tissue polarity is body region specific.

THE adult Drosophila epidermis secretes a cuticle with a rich morphology and dramatic planar polarity (also known as tissue polarity). A number of different cuticular structures share this polarity. Cells that secrete epidermal hairs cover much of the body. These noninnervated structures typically point distally on appendages and posteriorly on the trunk. Hair development has principally been studied on the wing. Sensory bristles are found in most regions of the fly. These multicell sense organs contain a prominent shaft that usually points distally on appendages and posteriorly on the trunk. Planar polarity is also manifested in the eye by the arrangement of photoreceptor cells in ommatidia.

Mutations in a number of genes disrupt the polarity of all three of these cellular structures, which has led to the suggestion that a common genetic pathway regulates polarity in all parts of the epidermis. Among the genes where mutations affect all three cell types are *frizzled* (*fz*), *starry night* [*stan*; aka *flamingo* (*fmi*)], *dishevelled* (*dsh*), and *Van Gogh* [*Vang*; aka *strabismus* (*stbm*); Gubb and Garcia-Bellido 1982; Adler *et al.* 1987; Wong and Adler 1993; Klingensmith *et al.* 1994; Theisen *et al.* 1994; Zheng *et al.* 1995; Taylor *et al.* 1998; Wolff and Rubin 1998; Usui *et al.* 1999]. Mutations in other genes disrupt tissue polarity in one or two of these cell types. For example, mutations in *inturned* (*in*), *fuzzy* (*fy*), and *fritz* (*frtz*) affect hairs and bristles (Gubb and Garcia-Bellido 1982; Wong and Adler 1993; Park *et al.* 1996; Collier and Gubb 1997; Collier *et al.* 1997), mutations in *diego* (*dgo*) affect hairs and ommatidia (Feiguin *et al.* 2001), and mutations in *multiple wing hairs* (*mwh*) affect only hairs (Gubb and Garcia-Bellido 1982). One interpretation of these observations is that genes that affect all three cell types are part of a functional core group of genes, while genes that affect only one or two cell types are part of the cell type-specific interpretation of the common pathway (Adler 1992; Shulman *et al.* 1998). There are, however, some difficulties with this interpretation.

The cellular basis for the development of planar po-

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larity is different in all three of these cell types. The distal polarity of wing hairs is a consequence of wing cells forming hairs at the distal-most part of the cell. Mutations that alter hair polarity do so by altering the subcellular location for hair initiation (Wong and Adler 1993). The polarity of sensory bristles is a consequence of the orientation of mitotic spindles in the differentiative cell divisions in the sense organ lineage. Mutations that alter bristle polarity have been found to alter the orientation of the sensory precursor cell (PI) division (Gho and Schweisguth 1998; Gho *et al.* 1999). The chirality of ommatidia is dependent on which of two cells adopt the R3 *vs.* R4 cell identity (Zheng *et al.* 1995; Wherli and Tomlinson 1998). This subsequently determines the orientation of ommatidial rotation. Mutations that alter ommatidial polarity disrupt the R3 *vs.* R4 cell fate decision.

The *fz* pathway has both cell autonomous and cell nonautonomous functions in the specification of wing hair polarity (Vinson and Adler 1987). Clones of cells mutant for *fz* or *Vang* typically alter the polarity of wild-type hairs that surround the clones. In the case of *fz* the wild-type hairs appear to be attracted to the clone while for *Vang* the wild-type hairs appear to be repulsed by the clone (Taylor *et al.* 1998). This led to the suggestion that *fz* clones fail to produce a polarity signal while *Vang* clones produce too much of the signal (or vice versa; Adler *et al.* 2000a). However, little is known about the nature of the putative signal. Downstream are a group of genes that function cell autonomously and encode proteins that accumulate asymmetrically in wing cells. Fz (which is a serpentine receptor; Vinson *et al.* 1989) and Dsh (which is a PDZ domain containing protein; Axelrod *et al.* 1998; Boutros *et al.* 1998) accumulate at the distal side of the cell and Fmi/Stan (which is a protocadherin; Chae *et al.* 1999; Usui *et al.* 1999) and Dgo (which contains ankyrin repeats; Feiguin *et al.* 2001) appear to accumulate at both the distal and proximal sides (Usui *et al.* 1999; Axelrod 2001; Feiguin *et al.* 2001; Shimada *et al.* 2001; Strutt 2001). The function of all of these genes is a corequirement for the asymmetric localization of others. This asymmetric accumulation could provide a cortical mark to specify the location for hair initiation. However, there is some doubt that this cortical mark is a general mechanism as *dgo* does not produce a tissue polarity phenotype in bristles (Feiguin *et al.* 2001) and Fmi/Stan does not accumulate asymmetrically in the PI cell in the bristle lineage (Lu *et al.* 1999).

Mutations in a number of genes, including *in* and *fy*, result in both polarity abnormalities and many cells forming more than one hair (we refer to these genes as the *in*-like genes). Earlier studies showed that loss-of-function mutations in *fy*, *in*, and *mwh* are epistatic to loss-of-function mutations in *fz*, *dsh*, and *Vang* (Wong and Adler 1993; Taylor *et al.* 1998). This was interpreted as *in*, *fy*, and *mwh* being downstream of *fz* and *dsh*. Consistent with this are observations showing that the asymmetric accumulation of Fz, Dsh, and Fmi is not altered in *in*, *fy*, or *mwh* mutants (Usui *et al.* 1999; Axelrod 2001; Feiguin *et al.* 2001; Shimada *et al.* 2001; Strutt 2001). In this article we report a series of experiments where we have further probed the relationship between *fz*, *dsh*, *prickle* (*pk*), *spiny-legs* (*sple*), and *stan/fmi* and *in*, *fy*, and *mwh* in the wing. We have also examined the relationship between *fz* and *in* and *fy* in other body regions. Results from several different experimental paradigms supported the hypotheses that *in* and *fy* function downstream of *fz*, *dsh*, *stan*, *pk*, and *sple* in the wing and that they are required for the transduction of the *fz* signal to regulate hair morphogenesis and hence the actin and microtubule cytoskeletons. We further found that the situation is quite complex when we consider other body regions. In the thorax and abdomen the relationship between *fz* and *in* and *fy* appeared similar to that in the wing. However, the situation was different in the eye and the leg, where *in* and *fy* were not epistatic to *fz*.

MATERIALS AND METHODS

Fly culture and strains: Flies were grown on standard media at 25° or 18°. Many of the mutations used in this article were isolated in this lab. These included mutations in *in*, *fz*, *fy*, *mwh*, *pk*, and *strb*. Other mutant, deficiency, or *GAL4* enhancer trap stocks were obtained from the stock center at Indiana University. Unless noted, all mutations used were phenotypic nulls or in the case of dsh^{1} the strongest viable allele. *UAS-dsh* was obtained from J. Axelrod, *UAS-stan/fmi* was obtained from the T. Uemura lab, and *UAS-pk* and *UAS-sple* were from D. Gubb. Several of the genes used in this article have multiple names. We use *Vang* instead of *stbm* and *stan* instead of *fmi*. This usage is in line with their FlyBase listings.

Mitotic clone analyses: Loss-of-function clones of *fz*, *dsh*, *in*, and *mwh* were generated using FLP/FRT-based mitotic recombination (Xu and Rubin 1993). *tricornered* (*trc*), *starburst* (*strb*), and *forked* (f^{36}) were used to mark the clones in the wing, abdomen, and thorax. Clones were induced by heat shock for 1 hr at 37° at 2–4 days after egg lay (AEL). The genotypes in which we examined clones are listed below. In experiments not described in depth in results we examined the ability to induce FLP/FRT-mediated mitotic crossing over for two different chromosome arms in the same wing cell lineage. In these experiments we used *ultrahairA* (on 2L; Adler *et al.* 2000b) and *tricornered* (on 3L; Geng *et al.* 2000). These mutations produce very different cellular phenotypes so it was quite easy to identify the doubly mutant cells that had an additive phenotype. In our original experiments we used two heat shocks but we found that this was not necessary. The frequency of clones where recombination took place on both arms simultaneously (as compared to the frequency of clones where recombination took place on one arm) was much higher than random. We found in these experiments that FRT40 and FRT42 resulted in a frequency of mitotic clones much higher than that of FRT80 (Xu and Rubin 1993). This was consistent with our past experience with these chromosomes. On the basis of clone frequency and size we estimate that approximately 1/100 wing cells were recombinant for *ultA* (FRT40) and 1/1000 for *trc* (FTR80). Among the *trc* clones, 30% (18/60) were also mutant for *ultA*. This is 30

times more frequent than would be expected if exchanges on the two chromosomes were independent. Our interpretation of this result is that only a subpopulation of cells is competent for crossing over and the frequency per FRT site for these cells is quite high. We note that the induction of recombination on two separate chromosome arms has been described previously (Selva and Perrimon 2000).

fz trc in clones: *w hsflp; fz^{P21} trc^{1} in^{IH56} FRT80/FRT80*

fz mwh strb clones: *w hsflp; mwh^{1} fz^{R52} $strb^{1}$ FRT80/FRT80*

dsh forked; fz strb clones: *dsh^{1} f^{36a} FRT18/FRT18; hsflp/+; fz^{R52} $strb^{1}$ FRT80/FRT80*

fz trc in clones in an *inturned* wing: *w hsflp; fz^{P21} trc^{1} in^{IH56} FRT80/in^{IH56} FRT80*

fz mwh strb clones in a *mwh* wing: *w hsflp; mwh^{1} fz^{R52} $strb^{1}$ FRT80/mwh^{1} FRT80*

fz strb clones in a *dsh* wing: *dsh^{1}/Y; hsflp/+; fz^{R52} $strb^{1}$ FRT80/FRT80*

fz strb clones in a *fy* wing: *w hsflp; fy^{2}; fz^{R52} $strb^{1}$ FRT80/FRT80*

Waxing experiments: To induce a gradient of expression of polarity genes from heat-shock-regulated transgenes (*hs-fz*, *hs-dsh*, and *hs-in*), hot wax was applied to the distal tip of the wing of developing pupae 24–28 hr after prepupa formation (Adler *et al.* 1997).

Mounting of the wing and other body parts: Adult wings were mounted in euparal after dehydration in 100% ethanol. Abdomens, thoraces, and legs were mounted in Gary's Magic Mount after dehydration in 100% ethanol. Mounted samples were examined under the bright field microscope. Their images were taken with Spot digital camera (Diagnostics) and processed in the Adobe Photoshop 5.0.

Scoring of multiple hair phenotypes: Unless otherwise noted, the area with 100 cells (20 × 5 cells) in the "C" region centered above the posterior cross vein was selected for counting the number or fraction of the cells with multiple hairs.

Injection of microtubule antagonists into pupae: Vinblastine and colchicine were injected into pupae as described previously (Geng *et al.* 2000).

RESULTS

***inturned* and *fuzzy* are needed in cells receiving and responding to a *frizzled* dependent intercellular signal:** The cell nonautonomous function of *fz* is seen when clones of cells mutant for *fz* are generated in the wing (Vinson and Adler 1987). The normal distal polarity of wild-type hairs located distal to the clone is altered so that these hairs appear to be attracted to the clone (Adler *et al.* 1997). This seems likely to be due to the clone cells failing to produce an intercellular signal (or to producing excess signal). To determine if genes such as *in* were required in the clone cells for the production of the domineering nonautonomy we induced and examined *fz trc in* mutant clones (*trc* was used to mark the clone cells; Geng *et al.* 2000). Twelve of 14 clones displayed the domineering nonautonomy characteristic of *fz* clones (Figure 1, c–e). This led us to conclude that *in* was not required for *fz* clone cells to produce domineering nonautonomy. Previously we found that *mwh fz* clones also displayed typical *fz* domineering nonautonomy, but in those experiments there was no independent marker to identify clone cells (Vinson and Adler 1987). We therefore reexamined this by inducing *mwh fz strb* clones (*strb* was used to mark the clone; Park *et al.* 1996). All 17 of the *mwh fz strb* clones examined displayed typical *fz* domineering nonautonomy (Figure 1g). Thus, like *in*, *mwh* is not required for *fz* clone cells to produce domineering nonautonomy.

To determine if *dsh* was required for *fz* clone cells to produce domineering nonautonomy we wanted to generate clones that were doubly mutant for *dsh f* and *fz strb* by the simultaneous induction of FLP/FRT-based crossing over on the X chromosome and on 3L. To determine if this was feasible we carried out a test experiment using *ultA* (Adler *et al.* 2000b) and *trc* (Geng *et al.* 2000), which provide easily distinguishable phenotypes (Figure 1, n–p). We found that the simultaneous induction of cells where there were recombination events on both arms was more frequent than random (these experiments are described in more detail in materials and methods). We were able to identify the *dsh; fz* doubly mutant clone cells by their hair morphology (*f; strb* hairs are short, thin, and bent, while *f* hairs are bent and *strb* hairs are very short, frequently split, or multipled and thin). As was the case for the *fz in* and *mwh fz* clones the domineering nonautonomy of *fz* was not blocked by the cell also being mutant for *dsh* (11/11 clones; Figure 1i).

To determine if *in* was required for cells to respond to the presence of mutant *fz* cells we induced *fz trc in* clones in a *fz trc in/in* wing. We could identify the clone due to the hair morphology phenotype of *trc*. While *in* mutations result in altered hair polarity, over most of the wing the *in* mutant pattern is consistent enough to enable us to determine if the abnormal intercellular signal expected from the *fz trc* clone resulted in domineering nonautonomy. The *fz trc* clones did not alter the polarity of the surrounding cells (11/11 clones; Figure 2a). Thus, we concluded that *in* was required for cells to detect or respond to *fz* clone cells. Similar results were obtained when we induced *fz* clones in wings mutant for *dsh* (5/5 clones; Figure 2e), *fy* (22/22), and *mwh* (4/5 clones; Figure 2c). Hence we concluded that these genes were also essential for cells to detect or respond to *fz* clone cells. Previous experiments had found mutations in *stan* and *Vang* also blocked or suppressed the domineering nonautonomy of *fz* (Taylor *et al.* 1998; Chae *et al.* 1999). The results for these six genes stand in contrast to the findings for *pk* and *ds*, which did not block the domineering nonautonomy of *fz* clones, although mutations in *pk* and *ds* did alter the anatomical direction of the domineering nonautonomy (Adler *et al.* 1998, 2000a).

The domineering nonautonomy of *fz* clones suggests that cells can assess the level of *fz* activity of neighboring cells and respond to this. When a gradient of *fz* expression is induced, hair polarity is directed to point from

cells of higher levels toward cells of lower levels (Adler *et al.* 1997). To determine if the activity of *in*, *fy*, and *mwh* was required for cells to respond to a gradient of *fz* expression we induced such a gradient in a wing that was mutant for any of these genes. We found no effect on hair polarity of directing a gradient of *fz* expression in wings mutant for *in* (Figure 3, A–C), *fy* (Figure 3, D–F), or *mwh* (data not shown). We concluded that these genes were required for cells to respond to altered levels of *fz* expression, suggesting that they functioned downstream of *fz* for planar polarity in the wing. We note that previous experiments showed that mutations in *dsh*, *stan*, and *Vang* similarly blocked the ability of cells to respond to a gradient of *fz* expression (Adler *et al.* 1997; Taylor *et al.* 1998; Chae *et al.* 1999).

***inturned* and *fuzzy* are needed for cells to respond to *prickle*, *spiny-legs*, and *starry night*:** To determine the relative positions of *in*, *fy*, and *mwh* with respect to several other *fz*-like tissue polarity genes we examined their ability to block the gain-of-function phenotypes that result from the directed expression of *stan/fmi*, *pk*, and *sple*. We used *ptc-GAL4 to* overexpress *pk*. This results in a band of expression in the C region of the wing, with a decreasing gradient of expression away from the center of the *ptc* expression domain (Adler *et al.* 1997; Usui *et al.* 1999). This results in hairs pointing toward the midline in the C region of the wing (Gubb *et al.* 1999). This phenotype was blocked by mutations in *in* (Figure 3, G–I), *fy*, and *mwh*. The resulting wings (*e.g.*, *ptc-GAL4/UAS-pk; in/in*) showed no effects from the directed expression of *pk*. We used two different *GAL4* drivers (*ms1096-GAL4* and *act-GAL4*) to direct the expression of *UAS-sple*. Both of these drivers result in relatively even expression across the wing (although in *ms1096* the level in the dorsal cell layer is higher than that in the ventral cell layer). This results in a reversal of hair polarity over much of the wing (Figure 3J) that resembles that seen in pk^{D} (Adler *et al.* 2000a). Once again we found that mutations in *in*, *fy* (Figure 3, J–L), and *mwh* were able to block this gain-of-function phenotype. We used the *omb-GAL4* driver to drive expression of *UAS-stan*. In the wing disc *omb-GAL4* drives expression in a band located centrally along the anterior/posterior axis of the wing. However, in the pupal wing the expression pattern is more complicated during the time for tissue polarity development. During this time in the distal region of the wing we see a series of alternating bands of expression and no expression. Driving expres-

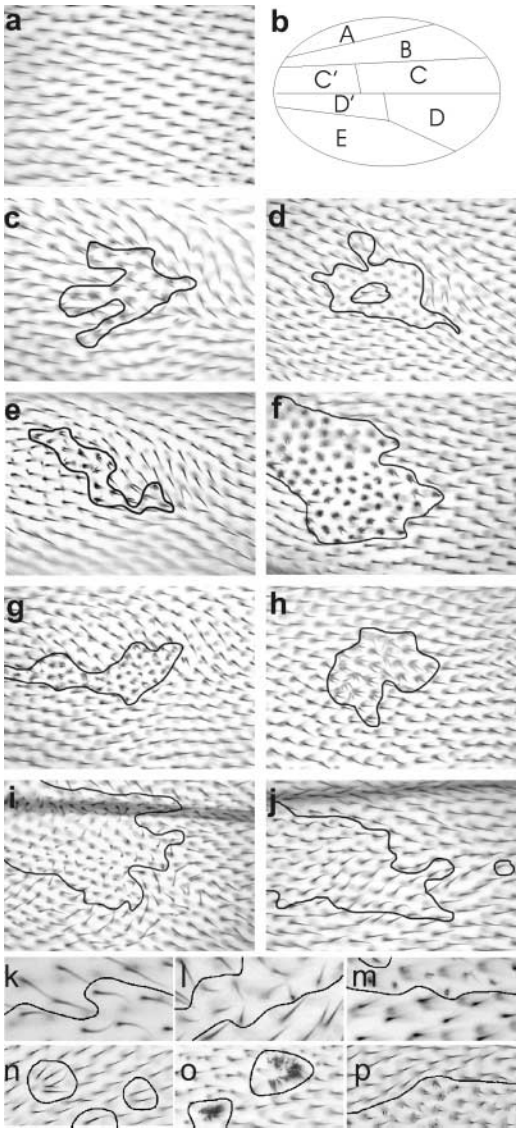

Figure 1.—*in*, *mwh*, and *dsh* are not required for *fz* clone cells to alter the polarity of neighbors. (a) Wild-type wing. Note that every wing cell forms a single hair that points distally. (b) A diagram of the wing regions (A, B, C, C′, D, D′, and E) divided by wing veins. The domineering nonautonomy of *fz* clones marked by *trc* (c) or *strb* (d; clones are outlined) alters the polarity of wild-type hairs located distal to the clone. (e) A *fz in* double mutant clone marked by *trc* shows the domineering nonautonomy typical of *fz* clones. (f) An *in* mutant clone marked by *trc* acts cell autonomously. (g) A *fz mwh* double mutant clone marked by *strb* displays the typical *fz* domineering nonautonomy. (h) A *mwh* clone acts cell autonomously. (i) A *dsh; fz* double mutant clone marked by *f* and *strb* produces domineering nonautonomy. (j) A *dsh* clone marked by *forked* acts cell autonomously. (k) A higher magnification view of a *dsh f* clone. (l) A higher magnification of a *dsh f; fz strb* clone. (m) A higher magnification view of a *fz strb* clone. Note in these higher magnification views the more severe phenotype (smaller and clustered or split) *fz strb* hairs compared to the *dsh f; fz strb* doubly mutant cells. *f* appears to partially suppress the extreme hair phenotype of *strb*. (n) An *ultA* clone. (o) A pair of *ultA; trc* doubly mutant cells. (p) A *trc* clone. Note the larger size of the polyploid *ultA* cells compared to the wild-type cells (multiple hairs produced by *ultA* cells are as long as or longer than the single hairs formed by wild type). Note the remarkable phenotype of the polyploid *ultA; trc* cells. All wings are positioned with proximal to the left, distal to the right, anterior up, and posterior down. The clonal boundaries are marked with a line according to the presence of the wing cell marker phenotype. Clones of >10 cells were selected to determine the nonautonomy.

sion of *stan* using *omb-GAL4* leads to a series of bands of polarity reversals consistent with the previous results of Usui *et al.* (1999) who found that polarity was oriented from cells of low toward cells of high *stan* expression. Once again we found that mutations in *in* (Figure 3, M–O), *fy*, and *mwh* were able to block the consequences of overexpressing *stan*. These data argue that *in*, *fy*, and *mwh* functioned downstream of *fz*, *pk*, *sple*, and *stan* in tissue polarity.

Driving expression of *dsh* using the *ptc-GAL4* driver results in the formation of multiple hair cells in the proximal part of the *ptc* expression domain (Figure 4, A and D). In addition, ectopic bristles are also often found, presumably due to the role of *dsh* in canonical *wg* signaling (Axelrod *et al.* 1996). When we examined *UAS-dsh +/+ ptc-GAL4; in/in* flies we found an additive multiple hair cell phenotype (*i.e.*, stronger than either single phenotype) in the proximal part of the *ptc* domain (Figure 4C) and the presence of ectopic bristles. Equivalent results were found in *UAS-dsh fy; fy ptc-GAL4* wings (Figure 4F). The formation of the ectopic bristles was not surprising, since this is likely an effect of *dsh* on canonical *wg* signaling and *in* and *fy* are not thought to play a role in *wg* signaling. The additive multiple hair cell phenotype was unexpected and argues either that *in* and *fy* are not downstream of *dsh* in the *fz* pathway or that when overexpressed, Dsh can bypass the requirement for *in* and *fy* function.

The overexpression of *fz* just prior to hair initiation results in a multiple hair cell phenotype that resembles that of *in* and *fy* (this is sometimes referred to as the late *fz* gain of function; Krasnow and Adler 1994). When we overexpressed *fz* just prior to hair initiation in pupae that were mutant for a null allele of either *in* or *fy* we found an increased number of multiple hair cells (Table 1). Thus, for the late *fz* gain of function, as for the overexpression of *dsh*, either *in* and *fy* are not required for *fz* signal transduction or when *fz* is overexpressed late the requirement for *in* and *fy* can be bypassed.

The relationship between *in*, *fy*, and the *fz*-like genes: To test whether the *frizzled*-like genes could be regulating the *inturned*-like genes negatively or positively, we examined the effects of both decreased and increased *fz*-like gene activity on hypomorphic alleles of *in* and *fy*. In these experiments we took advantage of the much higher number of multiple hair cells found in *in* and *fy* mutants compared to *fz*-like mutants. In all cases examined we found that mutations (null or hypomorphic) in *fz*, *pk*, *Vang*, *stan*, and *dsh* acted as strong enhancers of a weak *in* or *fy* phenotype as assayed by the frequency of multiple hair cells (Figure 5; Table 2). These data are consistent with the *fz*-like genes acting as positive regulators of *in* and *fy*.

If there were a simple positive relationship between the *fz*-like genes and *in* and *fy* then we would expect that the overexpression of *fz*-like genes should suppress

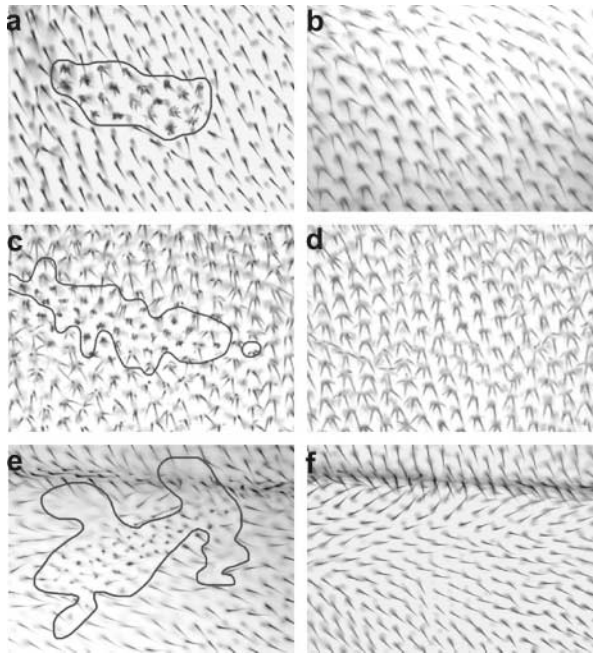

Figure 2.—*in*, *mwh*, and *dsh* are required in cells to receive or respond to altered *fz* activity of neighboring cells. (a) The presence of a clone of fz^{P21} *trc* cells in an in^{IH56}/Df wing fails to alter the hair polarity of the neighboring cells. (b) For comparison with a, we show the same region (E region) of an in^{IH56}/Df mutant wing without a clone. (c) The presence of a fz^{R52} *strb* clone in a *mwh* background does not alter the polarity of neighboring cells. (d) For comparison with c, we show the same region (D region) of a *mwh* wing without a clone. (e) The presence of a fz^{R52} *strb* clone in a *dsh* background does not alter the polarity of neighboring cells. (f) For comparison with e we show the same region (D region) of a *dsh* wing without a clone. All wings are positioned with proximal to the left, distal to the right, anterior up, and posterior down. Clones with >10 cells were chosen to score for the nonautonomy.

hypomorphic alleles of *in* and *fy*. To test this we overexpressed *fz* from a *hs-fz* transgene and found that this enhanced the multiple hair cell phenotypes of weak in^{II53} and fy^{JN12} alleles. These results argued that *fz* antagonized the activity of *in* and *fy* (Table 3). We similarly tested the genetic relationship between *pk*, *sple*, and *fmi* and weak alleles of *in* and *fy*. Overexpression of *sple* and *stan* from *ms1096-GAL4* (or *act-GAL4*)*; UAS-sple* and *omb-GAL4; UAS-stan*, respectively, in hypomorphic mutant backgrounds of *in* and *fy* resulted in an increase in the multiple hair cell phenotypes in the region of the wing where expression was driven by the *GAL4* enhancer trap (Figure 3, J, Q, T, M, R, and U). A similar enhancement was not seen for the overexpression of *pk* from *ptc-GAL4 UAS-pk* (Figure 3, G, P, and S). These results suggested that *sple* and *stan* antagonized the activity of the *in* and *fy*. Notably the gain-of-function polarity phenotypes of overexpressed *pk*, *sple*, and *stan* were blocked even with weak alleles of *in* and *fy*, confirming that these genes are required for the function of the *fz* pathway. Taken with the data described above it is clear that the *fz*-like genes do not act as simple positive or negative regulators of *in* and *fy*. The interaction is reminiscent of the obser-

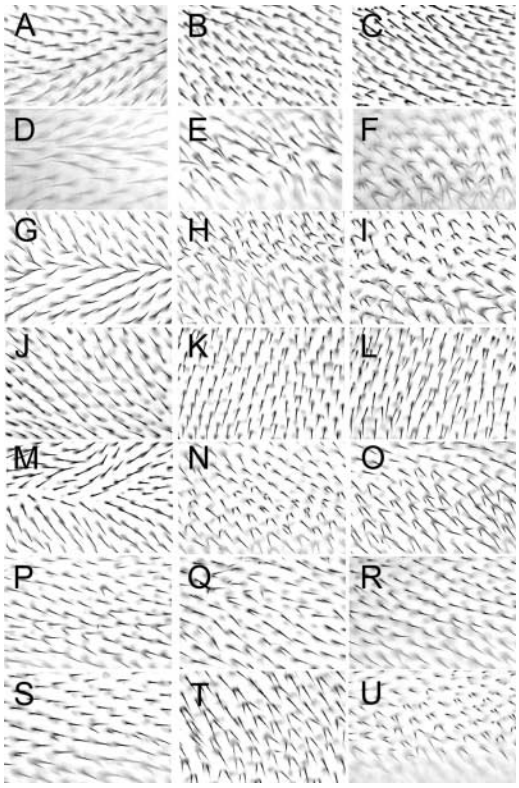

FIGURE 3.—The repolarization of hairs by a gradient of *fz* expression is blocked in wings mutant for *in* or *fy*. (A) Driving expression of *UAS-fz* with *dll-GAL4* induces a gradient of *frizzled* expression decreasing away from the distal part of the wing, causing wing hairs to point proximally (ADLER *et al.* 1997). (B) In an in^1 wing, hairs point slightly posteriorly in the C region of the wing. (C) *UAS-fz / dll-GAL4*; *in*. The reversal of hair polarity is blocked. (D) The gradient of *fz* expression induced by "hot waxing" on the distal tip of the wing of the *hs-fz* pupa causes wing hairs to point down the *fz* gradient, resulting in the proximal polarity (ADLER *et al.* 1997). (E) Hairs in the fy^2 mutant wing point in the posterior direction in the C region of the wing. (F) The reversed polarity induced by hot waxing is blocked by mutations in *fy*. Note that the wings shown in D–F were mutant at the *yellow* locus and this leads to the wing hairs being of low contrast. (G) Driving expression of *pk* using *ptc-GAL4* causes hairs to point toward the midline in the C region of the wing. (H) Mutations in *in* result in hairs pointing posteriorly in this part of the C region of the wing. (I) Wings from *UAS-pk/ptc-GAL4;* in^1 flies show the *in* phenotype. (J) Driving expression of *UAS-sple* using *act-GAL4* results in proximal hair polarity over much of the wing. (K) In fy^2 mutants, hairs point posteriorly in the E region of the wing. (L) In a fy^2*; UAS-sple/act-GAL4* wing the *UAS-sple/act-GAL4* gain-of-function phenotype is blocked. (M) Driving expression of *stan* using *omb-GAL4* causes wing hairs to point away from the midline in the C region of the wing. (N) In an in^1 mutant, hairs point posteriorly in the C region. (O) In an *omb-GAL4/+; UAS-stan;* in^1 mutant the *omb-GAL4/+; UAS-stan* gain of function is blocked. (P) fy^{JN12}/fy^2. A wing with a weak *fy* phenotype produces occasional multiple hair cells. (Q) in^{HC31}/Df. A wing mutant for a weak *in* allele displays occasional multiple hair cells. (R) fy^{JN12}/fy^2. A weak *fy* phenotype in this region produces occasional multiple hair cells. (S) fy^{JN12}*/ptc-GAL4* fy^2*;UAS-pk*. The multiple hair cell phenotype of a weak *fy* wing is not enhanced by overexpression of *pk* (compare to P). (T) *UAS-sple/ act-GAL4;* in^{HC31}/Df. The multiple hair cell phenotype of the weak *in* genotype is enhanced by overexpression of *sple* (compare to Q). (U) *omb-GAL4;* fy^{JN12}/fy^2*; UAS-stan*. Overexpression of *stan* by *omb-GAL4* induces an increase in the number of the multiple hair cells in a weak *fy* mutant (compare to R).

vations that similar phenotypes result from either overexpression or a lack of function of *fz*-like genes (KRASNOW and ADLER 1994; AXELROD *et al.* 1998; BOUTROS *et al.* 1998; GUBB *et al.* 1999; USUI *et al.* 1999).

Overexpression of *dsh* from *UAS-dsh; ptc-GAL4* at 18° in weak *in* and *fy* backgrounds produced severe multiple hair cell phenotypes that were more severe than null *in* and *fy* alleles (data not shown). These results are reminiscent of what we saw with the null *in* and *fy* alleles.

As a further test of the dose relationship between the *fz*-like and *in*-like genes we examined the consequence of overexpressing both a *fz*-like and an *in*-like gene simultaneously. We first confirmed that the overexpression of *in* or *fy* did not produce a wing phenotype (PARK *et al.* 1996; COLLIER and GUBB 1997). When we coordinately overexpressed *fz* and *in* using the waxing protocol to generate a distal-to-proximal expression gradient we found an enhanced reversed polarity phenotype. Both the frequency of finding regions of polarity reversal (41/73 for *hs-fz*, 0/11 for *hs-in*, and 22/24 for *hs-fz; hs-in*) and the size of the region appeared to be enhanced. In an additional set of experiments we found that the simultaneous overexpression of *fz* and *dsh* using the waxing procedure also resulted in an enhanced reversed polarity phenotype (0/17 for *hs-dsh*, 61/69 for *hs-fz; hs-dsh*).

***in*, *fy*, and *mwh*:** Double mutants of *in*, *fy*, and *frtz* with *mwh* display a *mwh* phenotype (WONG and ADLER 1993; COLLIER *et al.* 1997). In generating such flies we noticed that *in* and *fy* flies that carry one mutant *mwh* gene had an enhanced multiple hair cell phenotype. We examined this in some detail and found it to be a consistent result for seven different *in* alleles at both 18° and 29°. We further found that an increase in *mwh* gene dose (the presence of a duplication) weakly suppressed the *in* multiple hair cell phenotype (Table 4). Since the dominant enhancement by *mwh* is seen for null alleles of *in* and *fy*, the enhanced phenotypes cannot be from *mwh* serving as an upstream activator of *in* or *fy*. The data are consistent with *in* and *fy* acting to increase the activity of *mwh*. This is also consistent with previous observations that argued that *mwh* was epistatic to *in* and *fy* (WONG and ADLER 1993).

The microtubule cytoskeleton and *in* and *fy* function: The disruption of the microtubule cytoskeleton by treatment with vinblastine or colchicine results in many wing cells forming more than one hair (TURNER and ADLER 1998). At the level of the individual cell these multiple hair cells resemble those found in *in* and *fy* mutants as the hairs are formed at the cell periphery and appear to be the result of independent initiation events. The altered polarity seen in *in* and *fy* mutant cells is not induced by vinblastine or colchicine. Immunostaining

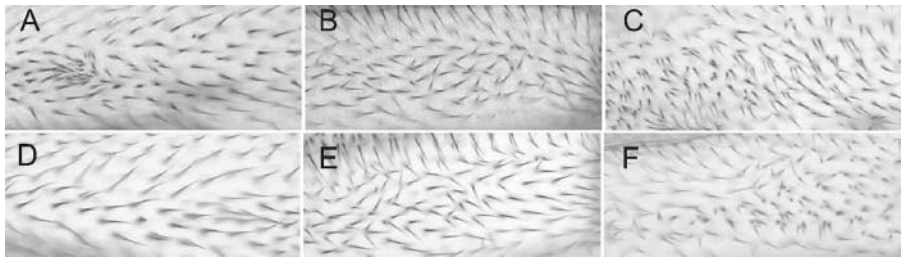

FIGURE 4.—The multiple hair phenotype of overexpressed *dsh* is enhanced in wings mutant for *fuzzy* and *inturned*. Driving expression of *UAS-dsh* using *ptc-GAL4* leads to lethality when flies are raised at 25° but adults frequently eclose when raised at 18°. We find occasional multiple hair cells in the C region of the wing on such flies. This phenotype shows a little variation in different genetic backgrounds (A and D). In addition, the overexpression of *dsh* sometimes leads to patches of smaller cells. Mutations in *fy* (fy^2; B) and *in* (in^{IH56}/Df; E) cause some cells in this region to form multiple hairs. The multiple hair cell phenotype of overexpressed *dsh* is additive or synergistic with the multiple hair cell phenotypes of *fy* (C) and *in* (F).

has not revealed any defects in microtubule organization in *in* or *fy* mutants (P. N. ADLER, unpublished results). Thus, it seems unlikely that the *in* and *fy* phenotypes are due to disrupting the microtubule cytoskeleton and in this way indirectly affecting hair morphogenesis. However, it seemed possible that the disruption of the microtubule cytoskeleton could be inducing multiple hairs by interfering with *in* and/or *fy* function. We reasoned that if that was the case then treatment of *in* or *fy* null mutant wing cells with vinblastine or colchicine would not produce a stronger phenotype than the *in* or *fy* null cells by themselves. We injected vinblastine into *in* or *fy* mutant pupae and found an additive response. That is, injected *in* or *fy* mutant cells produced a stronger multiple hair cell phenotype than did untreated mutant cells or cells from injected wild-type pupae (Figure 6). This result shows that the microtubule disruption phenotype cannot be due to a simple interference with *in/fy* function and argues that the microtubule cytoskeleton has a function that is independent of the *fz* pathway. The additive response in this experiment stands in sharp contrast with the lack of additivity in double mutants of *in*, *fy*, *frtz*, and *mwh*.

***fz*, *in*, and *fy* on the abdomen:** Mutations in most *fz* pathway genes produce planar polarity phenotypes in many body regions (GUBB and GARCIA-BELLIDO 1982; ADLER 1992). On the abdomen *fz* mutants produce altered bristle polarity, with most bristles now pointing at least partially toward the midline and not posteriorly as in wild type (GUBB and GARCIA-BELLIDO 1982). Abdominal hair polarity is also altered in *fz* mutants. The polarity phenotype seen in the abdomen of *in* and *fy* mutants is in general more severe than that of *fz* (GUBB and GARCIA-BELLIDO 1982). The tendency of bristles to point toward the midline is enhanced and, in addition to the hair polarity phenotype, most if not all hair-forming cells form more hairs than in wild type (wild-type abdominal cells often form 4–5 hairs). As is the case in the wing, *in* and *fy* are epistatic to *fz* as double mutants resemble *in* and *fy* and not *fz*. Wild-type abdominal cells located posteriorly to clones of *fz strb* cells (*strb* is a hair and bristle morphology marker) have their polarity altered and appear as if attracted to the clone (Figure 7). Similarly, wild-type cells located anteriorly to clones of *pwn Vang* cells have their polarity altered and appear as if repulsed by the clone (data not shown). Thus, the situation on the abdomen appears quite similar to the wing with *fz* and *Vang* showing complementary

TABLE 1

The overexpression of *frizzled* enhances null alleles of *inturned* and *fuzzy*

Genotype	Fraction of multiple hair cells[a]
in^1	0.67 (0.06)
hs-fz (II[b]) in^1	0.88 (0.04)
hs-fz (II)	0.11 (0.10)
fy^2	0.68 (0.08)
fy^2; *hs-fz* (III)	0.85 (0.09)
hs-fz (III)	0.24 (0.21)

[a] The fraction of multiple hair cells was scored in a 20 × 5 box of cells in the C region just anterior to the posterior cross vein.

[b] *hs-fz* inserts on either the second (II) or third (III) chromosome were used.

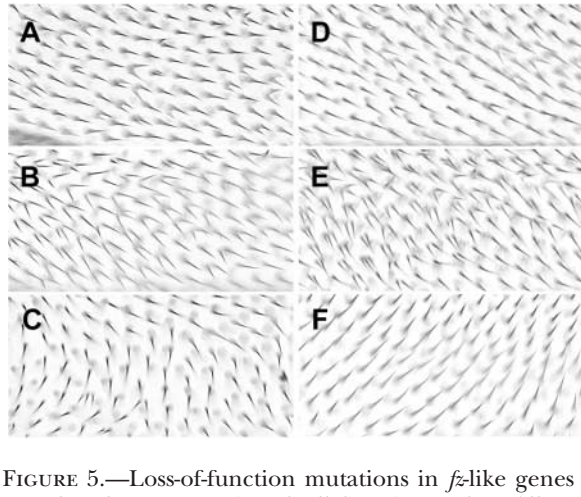

FIGURE 5.—Loss-of-function mutations in *fz*-like genes enhance the phenotypes of weak alleles of *in* and *fy*. All micrographs are from the C region of the wing just anterior to the posterior cross vein. This is the region we used for scoring the fraction of multiple hair cells for Table 2. (A) fy^{JN12}/fy^1. (B) fy^{JN12}/fy^1; fz^{R54}/fz^{K21}. (C) fz^{R54}/fz^{K21}. (D) in^{II53}; (E) pk^1; in^{II53}. (F) pk^1. Note the enhancement of the *in* and *fy* multiple hair cell phenotypes by *fz* and *pk*.

TABLE 2

Enhancement of weak *inturned* and *fuzzy* phenotypes by mutations in *frizzled* and other upstream genes

Sample	Genotype	Temp.	Fraction of mhc[a]	Significant difference from[b]
1	in^{II53}	25°	0.13 (0.06)	
2	pk^{1}	25°	0.01 (0.01)	
3	dsh^{1}	25°	0.01 (0.07)	
4	fz^{R53}	25°	0.01 (0.01)	
5	dsh^{1}; in^{II53}	25°	0.55 (0.07)	1, 3
6	pk^{1}; in^{II53}	25°	0.64 (0.07)	1, 2
7	fz^{R53} in^{II53}	25°	0.54 (0.09)	1, 4
8	in^{II53}/Df	25°	0.34 (0.12)	
9	$Vang^{TBS42}$	25°	0.35 (0.06)	
10	$Vang^{TBS42}$; in^{II53}/Df	25°	0.76 (0.08)	8, 9
11	in^{HC31}/Df	25°	0.19 (0.05)	
12	$stan^{3}$	25°	0.03 (0.005)	
13	$stan^{3}$; in^{HC31}/Df	25°	0.72 (0.13)	11, 12
14	in^{II53}	18°	0.013 (0.01)	
15	fz^{HD21}	18°	0.01 (0.02)	
16	fz^{HD21} in^{II53}	18°	0.62 (0.02)	14, 15
17	fy^{JN11}	25°	0.002 (0.004)	
18	fy^{JN11}; fz^{R53}	25°	0.15 (0.02)	4, 17
19	fy^{JN12}/fy^{2}	25°	0.34 (0.10)	
20	fz^{R52}/fz^{K21}	25°	0.02 (0.01)	
21	fy^{JN12}/fy^{2}; fz^{R52}/fz^{K21}	25°	0.71 (0.07)	19, 20
22	fy^{JN12}	25°	0.04 (0.04)	
23	fy^{JN12}; fz^{R53}	25°	0.42 (0.07)	4, 22

[a] The fraction of multiple hair cells was scored in a 20 × 5 cell box in the C region of the wing just anterior to the posterior cross vein. The standard deviation is given in parentheses. We have used this region previously (Wong and Adler 1993). Six to 12 wings were scored for each genotype.

[b] A *t*-test was used to compare samples. All significant differences had *P* values of <0.001.

domineering nonautonomy in both body regions. To determine if *in* was required for the domineering nonautonomy of *fz* clones we examined *fz trc in* clones in the abdomen. As we had seen in the wing, the domineering nonautonomy of *fz* in the abdomen was not blocked by cells also being mutant for *in* (Figure 7, J and K). We were unable to test if *in* was required in the responding abdomen cells as the hair phenotype of *in* mutants was too severe to allow us to assess if there was a nonautonomous effect of a *fz* clone.

TABLE 3

Enhancement of the multiple hair cell phenotype of hypomorphic alleles of *in* and *fy* by overexpression of *fz*

Genotype	Average no. of multiple hair cells in dorsal C region[a]
in^{II53}/in^{1}	82.8 (32.9)
hs-fz (II)	97.1 (59.7)
hs-fz (II); in^{II53}/in^{1}	235.1 (98.5)
fy^{JN12}	17.5 (9.6)
hs-fz (III)	23.5 (30.0)
fy^{JN12}; *hs-fz* (III)	386.0 (84.2)

[a] The dorsal C region was used to score the number of multiple hair cells. Seven or eight wings were scored for each treatment. The standard deviation is given in parentheses.

***fz, in,* and *fy* on the thorax:** *fz* mutations cause altered bristle and hair polarity on the thorax. A similar phenotype is seen in *in* and *fy* mutants, although the bristles with altered polarity most commonly point toward the midline in these mutants and the *fy* phenotype seems somewhat weaker. Over much of the notum in *in* and *fy* mutants hairs show altered polarity but in contrast to the wing and abdomen multiple hair cells are rare. As was seen on the abdomen both *fz* and *Vang* clones show complementary domineering nonautonomy in the thorax (data not shown). We generated *fz trc in* cells in the thorax and found they still showed the posteriorly directed domineering nonautonomy of *fz* clones (data not shown). Thus, as was seen in the wing and abdomen, *in* function is not required for the domineering nonautonomy of *fz* clones. Once again we were unable to determine if a *fz* clone produced a nonautonomous effect in an *in* mutant background.

In our examination of *in*, *fy*, and *fz* thoraces we identified two phenotypes where *fz* clearly differed from *in* and *fy*. On the wild-type scutellum hairs point posteriorly. This pattern is disrupted in *fz* mutants resulting in a complicated pattern. In *in* and *fy* mutants the hairs on the scutellum point anteriorly (*i.e.*, polarity is reversed compared to wild type; data not shown). In *fy; fz* and *fz in* double mutants the *in/fy* polarity pattern is seen.

TABLE 4

Enhancement of the multiple hair cell phenotypes of *in* and *fy* by the reduction of *mwh* dose

Genotype	Fraction of multiple hair cells[a]
$in^1/Df(in)$	0.73 (0.06)
mwh $in^1/+$ *Df(in)*	0.84 (0.03)
Dp(mwh) $in^1/Df(in)$	0.70 (0.07)
Dp(mwh) in^1*/mwh Df(in)*	0.75 (0.06)
$in^{II53}/Df(in)$	0.22 (0.11)
mwh $in^{II53}/+$ *Df(in)*	0.38 (0.11)
fy^2	0.79 (0.05)
fy^2*; mwh/+*	0.91 (0.06)

in^1 and fy^2 are null alleles of *inturned* and *fuzzy*, respectively, and in^{II53} is a weak *inturned* allele.

[a] The fraction of multiple hair cells was scored in a 20 × 5 cell box in the C region of the wing just anterior to the posterior cross vein. The standard deviation is given in parentheses. We have used this region previously (Wong and Adler 1993). Five to 15 wings were scored for each genotype.

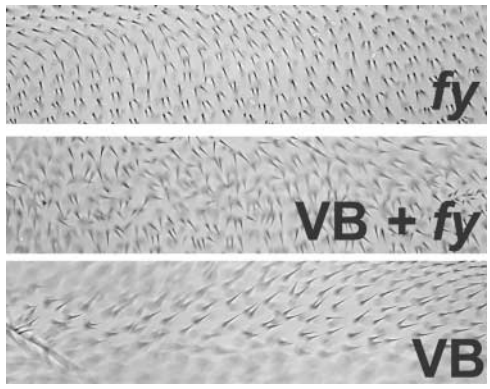

Figure 6.—Vinblastine treatment enhances the multiple hair cell phenotype of a *fy* null mutant. Null mutations in *fuzzy* result in wing cells forming multiple hairs with abnormal polarity (top). Wild-type pupae injected with vinblastine (VB) produce multiple hair cells in the wing (bottom) without polarity defects. When the *fuzzy* null mutants are treated with vinblastine, they display an enhanced multiple hair cell phenotype (middle).

A second *in/fy* phenotype not seen in *fz* mutants is the routine presence of several split thoracic microchaetae (Figure 7I). The split shaft is similar to that seen in *tricornered* and *furry* mutants (Geng *et al.* 2000; Cong *et al.* 2001) and does not appear to be due to a shaft duplication (unless the duplicated shafts fuse during morphogenesis). Such bristles are not routinely found in *fz* mutants. In *fy; fz* and *fz in* double mutants we routinely found such split bristles. Thus, *in* and *fy* are epistatic to *fz* for these two thoracic phenotypes.

***fz, in,* and *fy* on the leg:** In the leg, *fz* mutants (and *Vang* and *dsh* mutants) show several phenotypes (Gubb and Garcia-Bellido 1982; Held *et al.* 1986). Hair polarity is disrupted with many hairs no longer pointing distally. Occasional bristle cells also show abnormal polarity. This is most notable when the bract (a thick hair-like structure), which in a wild-type leg is always located proximal to the bristle, is found in an alternate location (*e.g.*, distal to the bristle). While this is not common, at least one (and usually several) mispositioned bracts can be found on essentially all *fz* legs (Figure 7F). Mutations in *fz* also have a dramatic phenotype in leg joints (particularly in tarsal segments; Figure 7B). The segments are typically incomplete (missing parts) and the joints (and/or parts of joints and segments) are found duplicated with a plane of mirror image symmetry perpendicular to the proximal distal axis (Held *et al.* 1986). We also frequently observed bulging blebs of cuticle. In contrast, in an *in* or *fy* mutant a somewhat different set of phenotypes are found. Hair polarity is abnormal, but compared to *fz* many more multiple hair cells are found on *in* and *fy* legs (Figure 7G). Occasional bristles with abnormal polarity are seen, but we typically do not see bristles with mispositioned bracts. The bracts are, however, often multipled in *in*, *mwh*, and *fy* (this phenotype is weaker in *fy* than the other mutants and is typically strongest in the tarsal segments; Figure 7G). Joint duplications are rare, and indeed few if any shape defects are seen in *fy* and *mwh* legs. On *in* legs we often see internal cuticular spheres, which may represent a mild manifestation of the joint phenotype. Rarely, we see duplicated joints. In *fy; fz* and *fz in* double mutants we find the joint defects (Figure 7D) and mispositioned bracts characteristic of *fz* and the multipled bracts characteristic of *in* and *fy* (Figure 7H). Thus, in the leg the phenotypes are additive.

***fz* and *in* in the eye:** Mutations in *fz* produce a rough eye phenotype that results from alterations in the regular arrangements of ommatidia. Ommatidia come in two chiral types defined by the arrangement of photoreceptor cells and, in any normal eye, each type is restricted to either the dorsal or the ventral half of the eye. Several defects are seen in *fz* mutants. These include incorrect chiral types, incorrect rotation, and symmetrical ommatidia (Zheng *et al.* 1995). *in*, *fy*, and *mwh* do not have rough eyes, although a few aberrant ommatidia are found in essentially all *in* eyes. In *in* flies we have seen examples of incorrect chiral type, incorrect rotation, symmetrical ommatidia, and ommatidia with an incorrect number of photoreceptor cells (data not shown). In *fz in* and *fy; fz* double mutants the eyes are rough and display the high number of abnormal ommatidia seen in *fz*. Thus, for the eye phenotype *fz* is epistatic to *in*.

***fz* and wing eversion:** Some *fz* flies have a deformed wing that is short, fat, and often kinked proximally (Figure 8, D–F). This is seen in all strong and null *fz* alleles we have examined. We have found that this is due to a defect in wing disc eversion. In a normal pupa the wing everts so that it extends posteriorly and ventrally (Figure 8B). In a *fz* mutant many pupae contain wings that extend anteriorly and sometimes dorsally (Figure 8A). A substantial fraction of such pupae fail to emerge from

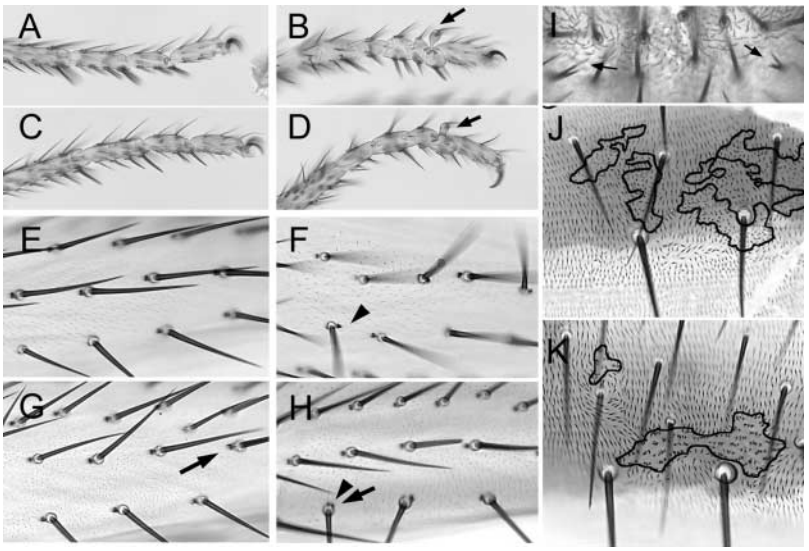

Figure 7.—*fz* and *in* in the abdomen, thorax, and leg. A–D show tarsal segments of wild type (A), *fz* (B), *in*^{1} (C), and *fz in* (D). The arrow points to a cuticular bleb seen in *fz* mutants. E–H show part of the femur of wild-type (E), *fz* (F), *in* (G), and *fz in* (H) flies. The arrows point to duplicated bracts and the arrowheads to bracts that are located incorrectly with respect to the proximal distal axis of the leg and the associated bristle. I shows part of a thorax from a living *in* fly. The arrows point to split bristles. J and K show *fz strb* and *fz trc in* clones, respectively, in the abdomen (outlined). Note in both cases the domineering nonautonomy of *fz* can be seen in the abnormal polarity of hairs located distal to the clone.

the pupal case due to the wing getting caught on the pupal case or the flies emerge with one torn wing. We suggest this is due to difficulty of the miseverted wing sliding out of the pupal case. The frequency of this phenotype is sensitive to genetic background and in individual backgrounds we have seen from 3 to 43% of wings from strong *fz* mutants affected. The wing eversion phenotype is rescued by the basal expression (25°) of a *hs-fz* transgene, as is the case for the wing hair polarity phenotype (Krasnow and Adler 1994). This phenotype is also seen occasionally in other tissue polarity mutants such as *pk*. The phenotype is rare in cell autonomous *fz* alleles (<1%) suggesting it is due to a defect in the nonautonomous function of *fz*. Flies with the miseverted wings are rarely seen (<1%) in flies mutant for *in*, *fy*, or *mwh*. In *fy; fz* and *fz in* double mutants we saw the low frequency of miseverted wings characteristic of *in* and *fy*. Hence for this phenotype *in* and *fy* appeared to be epistatic to *fz*.

DISCUSSION

***in* and *fy* and their role in wing development:** The hypothesis that *in*, *fy*, and *mwh* functioned downstream of *fz* and *dsh* originally came from the observation that mutations in *in*, *fy*, and *mwh* were epistatic to mutations in *fz* and *dsh* (Wong and Adler 1993). Consistent with this hypothesis it has recently been determined that the distal accumulation of Fz, Dsh, Fmi, and Dgo is not altered in *in*, *fy*, or *mwh* mutants (Usui *et al.* 1999; Axelrod 2001; Feiguin *et al.* 2001; Shimada *et al.* 2001; Strutt 2001). In the experiments we reported here we found that *in*, *fy*, and *mwh* were required for cells to either detect or respond to the lack of *fz* activity in a clone of neighboring mutant cells. We also found that the function of *in* and *fy* was required for cells to respond to a directed gradient of *fz*, *stan*, *pk*, or *sple* expression. Thus, using both of these experimental approaches we found that *in* and *fy* were required for the function of the *fz* pathway. The simplest interpretation of this is that *in* and *fy* are essential downstream components of the *fz* pathway in the wing. An alternative explanation is that *in* and *fy* function in a parallel pathway and that the function of the *fz* pathway is dependent on this putative *in* pathway. There were a couple of surprising exceptional results. The overexpression of *dsh* or the late overexpression of *fz* induces the formation of multiple hair cells (Krasnow and Adler 1994) and we found these phenotypes were additive with an *in* or *fy* mutation. This could indicate that there are multiple pathways downstream of *fz* and *dsh* in the wing and that one of them does not require *in* or *fy* function. We think it more likely that this is an example of overexpression bypassing a normal requirement for downstream proteins. We suggest that In and Fy normally function as adapters to allow distally accumulated Fz and Dsh to stimulate the cytoskeleton and induce hair initiation in the correct part of the cell. When Fz or Dsh is overexpressed just prior to hair initiation the high cellular concentration might allow these proteins to bypass the need for In and Fy and to interact with and hyperactivate the hair initiation machinery.

In an *in* or *fy* mutant not only is the site of hair initiation uncoupled from the distal accumulation of Fz, Dsh, Stan, and Dgo, but many wing cells now form multiple hairs. This appears to be a failure in a refinement process that in a wild-type cell leads to hairs initiating near the distal-most point in the cell and not all along the distal edge of the cell where Fz, Dsh, Stan, and Dgo accumulate. It is interesting that the region over which hairs form in an *in* or *fy* mutant is roughly equivalent in size to the region where hairs form in a *Rho kinase* mutant (Winter *et al.* 2001) or after disruption of the microtubule cytoskeleton (Turner and Adler 1998). This suggests that In/Fy may function in the activation of Drok that leads to the refinement of the area for hair initiation (Winter *et al.* 2001).

The observation that *in* and *fy* were epistatic to *fz* and

the frequent multiple hair cells in an *in* or *fy* wing led to the hypothesis that *in* and *fy* encoded negative regulators of hair initiation and that the activity of *fz* inactivated these negative regulators at the distal-most part of the cell leading to hairs initiating there (Wong and Adler 1993). The ability of the late overexpression of *fz* to produce an *in*-like phenotype (Krasnow and Adler 1994) was consistent with *fz* being a negative regulator of *in* and *fy*. In this article we looked more directly at the dose relationship between *fz*-like genes and *in*-like genes and our results show that our earlier model was incorrect and too simplistic. We found that both increases and decreases in *fz*-like gene activity enhanced the phenotypes of weak *in* and *fy* alleles. It is notable that both loss- and gain-of-function mutations in the *fz*-like genes can result in similar wing phenotypes (Krasnow and Adler 1994; Axelrod *et al.* 1998; Boutros *et al.* 1998; Gubb *et al.* 1999; Usui *et al.* 1999). Thus, one interpretation of these experiments is that the gain-of-function mutations in the *fz*-like genes are actually producing a loss of function. This would allow one to argue that the *fz*-like genes act as simple positive effectors of *in* and *fy*. This is possible; however, there are observations that a gain-of-function mutation in *fz* is not equivalent to a loss of function. For example, a hypomorphic *fz* wing phenotype is enhanced by decreased *dsh* function while a *fz* gain-of-function phenotype is suppressed by decreased *dsh* dose (Krasnow *et al.* 1995). We think the results described in this article are consistent with the hypothesis that In and Fy have two functions in hair morphogenesis. One is to transduce the *fz* signal to the cytoskeleton so that a hair forms in the distal-most part of the cell. This function would be dependent on the localized accumulation of Fz, Dsh, Fmi, and Dgo along the distal side of the cell (Axelrod 2001; Feiguin *et al.* 2001; Strutt 2001) and hence would be disrupted by either gain- or loss-of-function mutations in the *fz*-like genes. However, there is no reason to expect the disruption of this function to result in an enhancement of the multiple hair cell phenotypes of *in* and *fy*. The second function of *in* and *fy* would be to ensure a single hair is made. One possible mechanism for this could be to activate the hypothesized refinement process. In a wild-type cell this function would not require the function of the *fz*-like genes and could explain the strong multihair phenotype of *in* and *fy* mutants that is not seen in *fz*. How could such a model for In/Fy function explain the enhancement of the multihair phenotype of weak *in* and *fy* alleles by alterations in *fz* activity? One possibility is that a cooperative interaction between these functions might be revealed in the sensitized genetic background of a hypomorphic mutation.

The mechanism that leads to the domineering nonautonomy of *fz* and *Vang* clones is poorly understood. An attractive hypothesis is that such clones lead to either a lack of an intercellular signal or an excess of the signal (Taylor *et al.* 1998; Adler *et al.* 2000a). In the

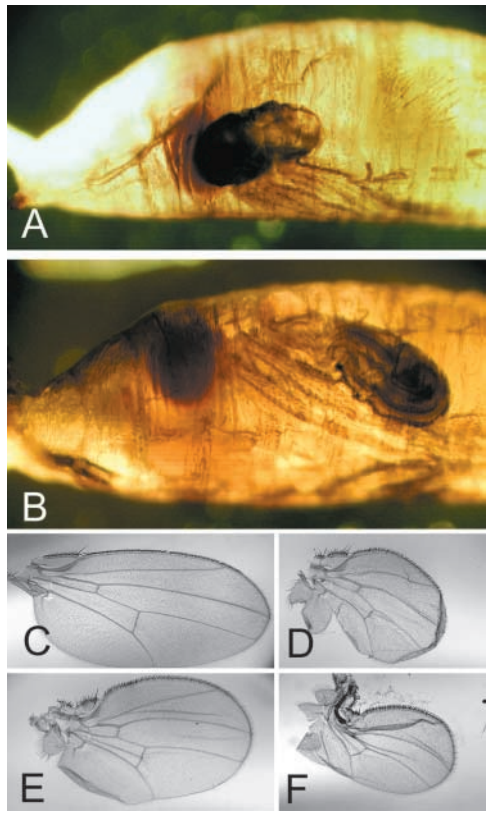

Figure 8.—The miseversion phenotype of *fz*. A shows a *fz* pupa with a miseverted wing. B shows a wild-type pupa for comparison. Note how the wing in A is pointing anteriorly and partially covers up the eye. The miseversion results in adult wings of abnormal shape. Examples of these are shown in D–F. Compare these to the wild-type wing in C.

experiments reported here we found that *in*, *mwh*, and *dsh* are not required in the cells that compose a *fz* clone for the clone to produce domineering nonautonomy. This was not a surprising result as all three of these genes act cell autonomously (Klingensmith *et al.* 1994; Theisen *et al.* 1994; Park *et al.* 1996). The result does emphasize that there is a *fz* function in tissue polarity that does not require the function of *dsh*, *in*, or *mwh*. It is possible that the cell nonautonomous function of *fz* in the wing involves a Ca^{2+}-mediated *fz* pathway identified in vertebrates that *dsh* is not a part of (Winkbauer *et al.* 2001).

Tissue and cell type-specific variation and what it says about a core pathway: The similar complementary domineering nonautonomy of *fz* and *Vang* clones in the wing (Vinson and Adler 1987; Taylor *et al.* 1998), abdomen, notum, and eye (Zheng *et al.* 1995; Wolff and Rubin 1998) argues for a common mechanism for *fz*-dependent intercellular signaling. There are also similarities in the cell autonomous function of the *fz* pathway in different body regions, but there are also differences. In the wing the polarized accumulation of Fz, Dsh, Stan, and Dgo is the earliest known cell autonomous step (Usui *et al.* 1999; Axelrod 2001; Feiguin *et al.* 2001; Shimada *et al.* 2001). There are reasons to doubt that

this is the case in other cell types. *dgo*, which is an essential component of the *fz* pathway in the wing, does not produce a bristle polarity phenotype (Feiguin *et al.* 2001) and Stan does not accumulate asymmetrically in the bristle cell lineage (Lu *et al.* 1999).

The relationship between *fz* and *in* and *fy* appears quite similar in the wing, abdomen, and thorax. In all three body regions, mutations in *in* and *fy* are epistatic to mutations in *fz*. There is also a great deal of similarity between the phenotypic consequences of most *fz* pathway mutations in these regions. For *in* and *fy* the phenotypes appear equivalent in the wing and abdomen as they result in both altered hair and bristle polarity and in many cells forming more hairs than normal. In the thorax *in* and *fy* mutations also cause altered bristle and hair polarity; however, over much of the notum most epidermal cells form only a single hair. This might be due to differences in the cell biology of notum cells compared to wing and abdomen cells. The Fz protein preferentially accumulates at the posterior/anterior edges of notum cells (P. N. Adler, unpublished results), much as it does along the distal/proximal boundaries of wing cells (Strutt 2001) and it is likely that in the notum it also serves to mark the side of the cell where a hair will form. However, the notum cells have a smaller apical surface than do wing cells so the need to further refine the region for hair initiation to ensure that a single hair is formed is likely reduced (Adler *et al.* 2000b). Thus, *in* and *fy* might still function to refine the region for hair initiation in notum, but this function may be unimportant in these cells. If one considers only the wing, notum, and abdomen, the idea of a common planar polarity pathway that includes *fz* and the *in*-like genes is attractive. However, there are problems with this hypothesis. In the eye, *in* and *fy* have either a weak phenotype or no phenotype, and double mutants show the *fz* phenotype. In the eye it is possible that *in* and *fy* either are redundant or have little or no function. In the leg the situation is more complicated. Mutations in both *in* and *fz* have dramatic phenotypic effects but these are not equivalent. The duplicated joint phenotype that is so dramatic in *fz* mutants is present weakly in *in* and appears to be missing in *fy* and *mwh*. In considering the bract cells we find two different phenotypes. In *fz* mutants we find mispositioned bracts, while in *in*, *fy*, and *mwh* we find multipled bracts. We suspect the multipled bracts are analogous to the multiple hair cells seen in other body regions. Thus, the bract cell is one where *fz* and *in* both have important functions, but they are apparently in independent processes.

The wing eversion phenotype and its possible relationship to convergent extension phenotypes in vertebrate embryos: In vertebrate embryos a *fz*-based planar polarity pathway regulates convergent extension (Heisenberg *et al.* 2000; Tada and Smith 2000; Wallingford *et al.* 2000; Wallingford and Harland 2001). Thus far there has been no evidence for a *fz*-based planar pathway having an analogous role in Drosophila. The eversion defect seen in *fz* mutant wings may be such an example. This is another phenotype where *in* and *fy* appear to be epistatic to *fz*. The observations are somewhat puzzling as the defect in *fz* appears to be rescued by a mutation in *in* or *fy*. Thus, it is not clear that a simple inactivation of the *fz* pathway is responsible for the eversion phenotype. Perhaps for this phenotype there are multiple inputs into the *fz* pathway upstream of *in* and *fy* and the eversion defect is due to an imbalance in the input to *in* and *fy*. Blocking the pathway at *in* or *fy* would eliminate the function of the pathway completely and suppress miseversion.

The cellular mechanisms involved in the eversion of the wing are only poorly understood, but it is possible that convergent extension plays a role (Condic *et al.* 1991). It is also possible that the joint defects seen in *fz* mutants have a similar basis. *In vivo* observations on everting wings and joint morphogenesis would make an important contribution toward determining if convergent extension plays a role in these morphogenetic events. The development of *in vivo* imaging approaches for Drosophila pupae makes such experiments possible.

We thank Paul Skogland and Kevin Lease for their comments on the manuscript. We thank Randi Krasnow, Chris Turner, and Simon Collier for help during the early phases of the research reported here. This work was supported by a grant from the National Institutes of Health (GM-37136).

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Communicating editor: T. W. Cline