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Mutations in the cadherin superfamily member gene *dachsous* cause a tissue polarity phenotype by altering *frizzled* signaling

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SUMMARY

The adult cuticular wing of *Drosophila* is covered by an array of distally pointing hairs that reveals the planar polarity of the wing. We report here that mutations in *dachsous* disrupt this regular pattern, and do so by affecting *frizzled* signaling. *dachsous* encodes a large membrane protein that contains many cadherin domains and *dachsous* mutations cause deformed body parts. We found that mutations in *dachsous* also result in a tissue polarity phenotype that at the cellular level is similar to *frizzled, dishevelled* and *prickle*, as many cells form a single hair of abnormal polarity. Although their cellular phenotype is similar to *frizzled, dishevelled* and *prickle*, *dachsous* mutant wings display a unique and distinctive abnormal hair polarity pattern including regions of reversed polarity. The development of this pattern requires the function of *frizzled* pathway genes suggesting that in a *dachsous* mutant the *frizzled* pathway is functioning - but in an abnormal way. Genetic experiments indicated that *dachsous* was not required for the intracellular transduction of the *frizzled* signal. However, we found that *dachsous* clones disrupted the polarity of neighboring wild-type cells suggesting the possibility that *dachsous* affected the intercellular signaling function of *frizzled*. Consistent with this hypothesis we found that *frizzled* clones in a *dachsous* mutant background displayed enhanced domineering non-autonomy, and that the anatomical direction of this domineering non-autonomy was altered in regions of *dachsous* wings that have abnormal hair polarity. The direction of this domineering nonautonomy was coincident with the direction of the abnormal hair polarity. We conclude that *dachsous* causes a tissue polarity phenotype because it alters the direction of *frizzled* signaling.

Key words: *dachsous*, Cadherin, *frizzled* signaling, Tissue polarity, *Drosophila*

INTRODUCTION

Cadherins comprise a group of transmembrane proteins that mediate Ca^{2+}-dependent cell adhesion (Geiger and Ayalon, 1992). They are located in adherens junctions and desmosomes. To function in adhesion, cadherins need to associate and form complexes with the actin cytoskeleton and the catenins (Nagafuchi and Takeichi, 1988). In addition to serving as mediators of adhesion the cadherin-catenin proteins are also involved in signaling pathways. In *Drosophila*, the *armadillo* (*arm*) gene encodes β-catenin (Peifer and Weischaus, 1990). This gene is an essential component of the *wingless* (*wg*) (*wg* is a member of the Wnt family) signaling pathway in *Drosophila* (Peifer et al., 1991). β-catenin is also involved in Wnt signaling in the *Xenopus* embryo (Gumbiner, 1995). In both of these cases there is evidence that the β-catenin signaling activity is independent of its role in cadherin-mediated cell adhesion (Heasman et al., 1994; Peifer et al., 1994), and involves the activation of the lef/TCF/pangolin transcription factor (van de Wetering et al., 1997; Brunner et al., 1997). However, since both activities take place in the same cell it would not be surprising if there was a linkage between signaling and adhesion (Gumbiner, 1995; Eaton and Cohen, 1996). Indeed, overexpression of cadherin can inhibit β-catenin signaling (Heasman et al., 1994; Funayama et al., 1995, Sanson et al., 1996).

The products of several *Drosophila* genes have been found to encode cadherin like proteins. Genetic studies indicate that two of these genes, *fat* (*ft*) and *dachsous* (*ds*), (Clark et al., 1995; Mahoney et al., 1991) have interesting roles in imaginal development. Consistent with the hypothesis that the proteins encoded by these genes have cadherin like activity, mutations in both *ds* and *ft* produce a number of phenotypes that can be interpreted as being due to effects on cell adhesion (Clark et al, 1994; Mahoney et al., 1991). However, both of these genes encode proteins that are substantially larger than the typical vertebrate cadherins or the *Drosophila* E-cadherin (Tepass et al., 1996; Uemura et al., 1996), raising the possibility that their function is not typical of the cadherin family.

The adult cuticle of *Drosophila* is decorated with large numbers of polarized structures. In any body regions these are typically aligned in parallel, giving the region a 'tissue polarity' (Adler, 1992; Gubb, 1993). For example, the adult wing is covered by a distally pointing array of hairs. Several groups have been investigating the genetic basis for the morphogenesis of tissue polarity (Adler, 1992; Gubb, 1993;

Strutt et al., 1997; Zheng et al., 1995). We have primarily used the wing as a model system. In the pupal wing each cell extends a microvillus like prehair from the apical surface of the cell (Wong and Adler, 1993). This prehair, which gives rise to the adult cuticular hair, is formed in the vicinity of the distal-most vertex of these polygonally shaped cells. The use of this subcellular location appears to be tightly linked to the distal polarity of the hair, as mutations identified because of altered hair polarity also result in an abnormal subcellular location for prehair initiation (Wong and Adler, 1993). The *frizzled (fz)* signaling/signal transduction pathway, which has both cell nonautonomous and cell autonomous functions, appears to control this process. We have recently carried out a screen for new wing tissue polarity mutations and among those recovered were mutations in both *ds* and *ft*.

As has been described previously (Clark et al., 1995) strong *ds* mutations result in adults with abnormally shaped legs and wings. We show here that *ds* mutations result in a wing tissue polarity phenotype that, based on its cellular phenotype (e.g. relatively few multiple hair cells),puts them in the *fz*-like phenotypic group of tissue polarity mutations (Wong and Adler, 1993; Krasnow and Adler, 1994). The *ds* mutant polarity pattern is however, unique among tissue polarity mutants. Most notably, in some regions hair polarity is reversed and hairs point proximally.

In genetic mosaics we found that *ds* displayed domineering cell nonautonomy and by constructing and examining double mutants we found that the function of *fz* tissue polarity pathway genes was required for the development of the unique *ds* polarity pattern. This requirement suggested the possibility that the *ds* polarity pattern resulted from abnormal activity of the *fz* pathway. The ability of *fz* overexpression to produce an *inturned* (*in*) like phenotype (Krasnow and Adler, 1994) was used as a test for the cell autonomous transduction of the *fz* signal. We found that *ds* mutations did not block the ability of *fz* overexpression to produce an *in*-like phenotype, and concluded that *ds*, unlike *dsh* was not required for the transduction of the *fz* signal (Krasnow et al., 1995). In contrast, we found that *ds* mutations caused abnormalities in *fz* intercellular signaling (i.e. the cell nonautonomous function of *fz*). We saw both an enhanced extent and an altered anatomical direction for the domineering non-autonomy of *fz* clones. The direction of the domineering nonautonomy was the same as the local polarity of hairs. This was seen both in regions with normal distal polarity and in regions with abnormal proximal polarity. It seems likely that the abnormal polarity seen in regions of *ds* wings is due to the abnormal anatomical direction of *fz* signaling in these regions. The data suggest that *ds* is involved in *fz* signaling and raise the possibility that *fz* signaling will be associated with adherens junctions.

MATERIALS AND METHODS

Fly culture and strains

Unless otherwise stated, flies were cultured at 25ºC. Many mutant and Deficiency containing stocks were obtained from the stock centers at Indiana University and Bowling Green State University. We have been carrying out a large-scale FLP/FRT screen (Xu and Rubin, 1993) to recover hair polarity mutations, which will be presented elsewhere (P. N. Adler, J. Charlton and J. Liu, in preparation). Briefly, FRT flies were mutagenized with EMS, crossed to *hs-flp; FRT* flies and clones induced in the F_1 progeny via heat shocking larvae. The adult F_1 flies were anesthetized under CO_2, and one wing was removed without killing the fly. The wing was examined under a compound microscope and flies where clones with altered hair polarity, number or morphology were saved and bred to recover the mutation. In these screens we recovered mutations in *ds* that prompted the experiments reported here.

Cytological procedures

Pupal wings were stained with a fluorescent phalloidin to stain the actin cytoskeleton and/or with flourescent antibodies and examined using a Molecular Dynamics confocal microscope as described previously (Wong and Adler, 1993; Park et al., 1994).

Generation of genetic mosaics

Mosaic clones were generated using the FLP/FRT system (Golic and Lindquist; Xu and Rubin, 1993). To mark *ds* clones we used recessive mutations in the *ck* gene. These mutations result in multiple, split and shortened hairs that typically lie at a greater angle to the wing blade than wild-type cells (Gubb et al., 1984; C. Turner and P. N. Adler unpublished).

In the experiments where we generated *fz* mutant clones in a *ds* mutant background we used the cell autonomously acting hair morphology mutation *starburst* (*strb*) (Park et al., 1996). Clones of *strb* produce hairs with a distinctive and easy to identify phenotype (although difficult to photograph). We classified *fz* clones for the extent of domineering nonautonomy they produced in the following

Table 1. *ds* is not required for the transduction of the *fz* signal

Genotype	*hs-fz*/+			ds^{UA071}/ds^{33k}; *hs-fz*/+			ds^{UAO71}/ds^{33k}		ds^{UAO71}/Df††	ds^{33K}/Df
Time of heat shock	None	1-3 hrs prior to PHI*	1-3 hrs after PHI	None	1-3 hrs prior to PHI	1-3 hrs after PHI	None	1-3 hrs prior PHI	None	None
Mean number of mhc†	0.2	73.5	0.0	3.7	71.2	4.7	7.7	12.2	5.8	4.5
Std. error	0.4	14.6	0.0	0.7	15.1	2.9	0.9	7.0	0.8	0.7
Number of wings scored	11	12	4	17	8	6	4	4	6	6
Different from no heat shock‡	NR§	Yes	No	NR	Yes	No	NR	Marginal¶	NR	No**

*PHI - prehair initiation.

†mhc - multiple hair cells (the number in the dorsal A cell was scored).

‡ - the ability of the induction of *fz* expression via heat shock was tested by comparing the number of multiple hair cells after a heat shock treatment with that seen with no heat shock using the Mann-Whitney test (Sigmastat). The groups with differences had P values of less than 0.001.

§NR - not relevant as this was the no treatment group.

¶The Mann-Whitney test gave a P value of between 0.01-0.05 a marginal result. Considering the small n we are reluctant to put any significance into the suggestion of a marginal difference.

**Here we compared ds^{UAO71}/Df and ds^{33K}/Df.

††Df means *Df(2L)ast2*.

way. Clones that caused surrounding wild-type cells, no more than 3 cell diameters from the border of the clone, to point no more than 45° from distal were classified as showing weak domineering nonautonomy. When the disruption was more pronounced (wild-type cells as many as 5 cells from the clone were affected and pointed as much as 90° from distal) the clones were classified as showing moderate nonautonomy. For clones showing strong nonautonomy surrounding wild-type cells as many as 10 cells from the border were affected and occasional hairs pointed as much as 180º from distal. For a clone to be classified as showing extreme nonautonomy there had to be a substantial region (>5 cells) that had well aligned reversed polarity.

Scoring of mutant wings

Wings were mounted in Euparal (Asco Labs) and examined under bright-field microscopy. As part of the analysis we often made drawings of individual wings that showed the abnormal polarity pattern of wing hairs on the dorsal surface of the wing (see Figs 3 and 7). The pattern shown is of an individual wing, however, at least five other wings of that genotype were examined to ensure that the wing drawn was typical for the genotype. We quantified the *ds* tissue polarity phenotype by estimating the fraction of a wing (or region of a wing) with abnormal polarity and by determining the fraction or number of multiple hair cells in specific regions of the wing as described previously (Wong and Adler, 1993; Krasnow and Adler, 1994).

RESULTS

ds mutations result in a tissue polarity phenotype on the wing

In a screen to identify new tissue polarity mutations we recovered a mutation (UAO71) which gave a *fz*-like phenotype in wing clones. Rare homozygous UAO71 adult flies showed shortened wings with misplaced and severely eroded cross veins (see Fig. 1D), a short fat body and severely distorted legs. We subsequently isolated 12 additional mutations in this gene (as judged by a failure to complement UA071) that gave a similar, albeit in most cases a less severe phenotype (Fig. 1). Most of our experiments have utilized the UAO71 allele. The mutation was mapped to cytogenetic location 21D1 on the basis of being uncovered by *Df(2L)al* (21B8-C1; 21C8D1) and *Df(2L)ast2* (21D1-2; 22B2,3) but not by *Df(2L)S3* (21D2,3; 22A1). The genetic location and phenotype suggested that UAO71 could be an allele of *ds*. This was confirmed by the failure of UAO71 to complement ds^{1} and ds^{33K} (a presumptive null allele which is associated with $In(2LR)bw^{v1}$; Clark et al., 1995). We henceforth refer to UA071 as ds^{UAO71}. The ds^{UA071}/ds^{33K} flies showed the same extreme phenotype we saw in the ds^{UA071} homozygotes and hemizygotes. We found that ds^{33k} /*Df(2L)al* and ds^{33k} /*Df(2L)ast2* flies had wing tissue polarity phenotypes that were similar to those of ds^{UA071} homozygotes and hemizygotes both with respect to the abnormal polarity pattern and the frequency of multiple hair cells produced (Table 1). These data indicate that ds^{UA071} is close to a null allele for wing tissue polarity and that the *ds* alleles we recovered are not unusual *ds* alleles. The ds^{UAO71}/ds^{1} flies did not show any major wing hair polarity phenotype, but did show the body shape phenotype associated with the ds^{1} allele (Clark et al., 1995). The ds^{1} allele appears not to be severe enough to routinely cause a tissue polarity phenotype on the wing. About half of the wings of either ds^{1}/ds^{UA071} or ds^{1}/ds^{33k} flies showed a very weak tissue polarity phenotype. This consisted of a couple of multiple hair

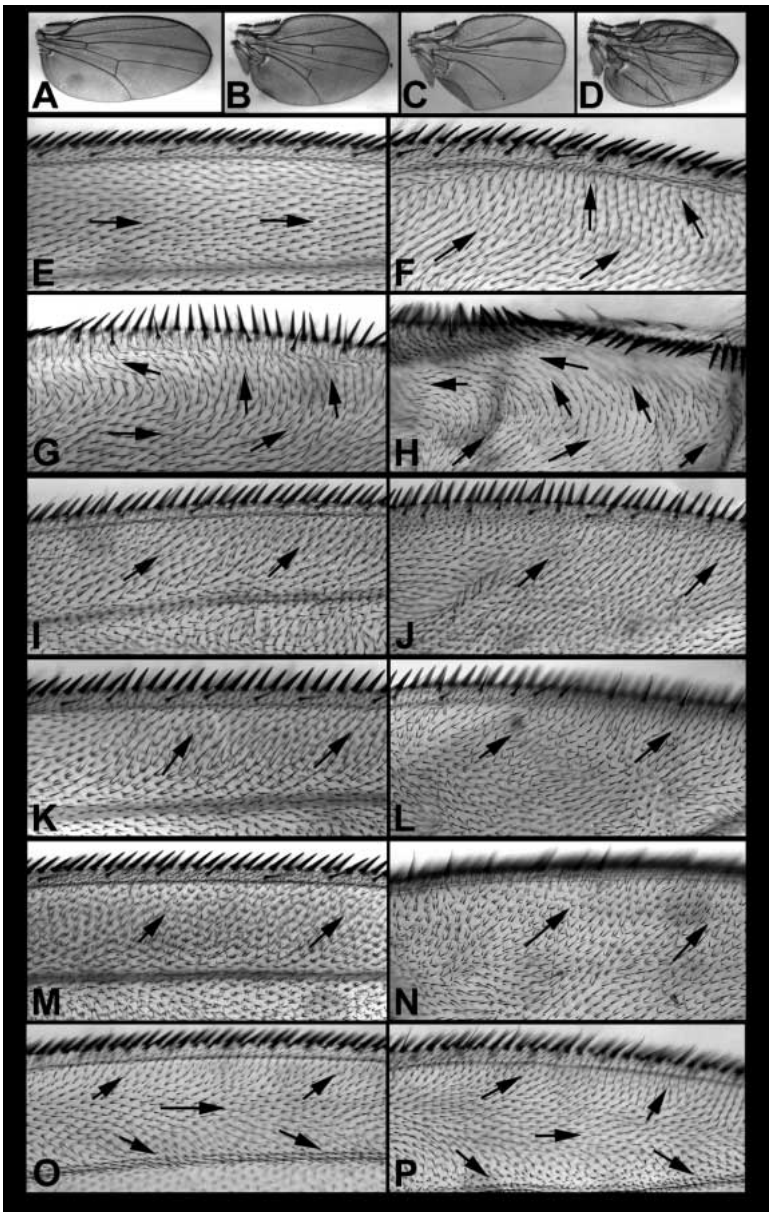

Fig. 1. Light micrographs of *Drosophila* wings. All wings are oriented with proximal to the left. (A) Oregon R, (B) ds^{YM31}, (C) ds^{YAC41}, (D) ds^{UAO71}/ds^{33k}. E-P show the dorsal surface along the anterior margin of the wing. (E) Oregon R, (F) ds^{YM31}, (G) ds^{YAC41}, (H) ds^{UAO71}/ds^{33k}, (I) fz^{R54}/fz^{K21} (a strong *fz* genotype), (J) ds^{UAO71}/ds^{33k}; fz^{R54}/fz^{K21}, (K) in^{IH56} (a strong *in* allele), (L) ds^{UAO71}/ds^{33k}; in^{IH56}, (M) mwh^{3} (a strong *mwh* allele), (N) ds^{UAO71}/ds^{33k}; mwh^{3}, (O) *hs-fz/+* (1-hour heat shock 24 hours awp formation), (P) ds^{UAO71}/ds^{33k}; *hs-fz/+* (1-hour heat shock 24 hours awp). Note that in all double mutants the wing vein placement abnormalities of *ds* were present, but hair polarity and number resemble the non-*ds* mutant. Arrows point in the local direction of hair polarity.

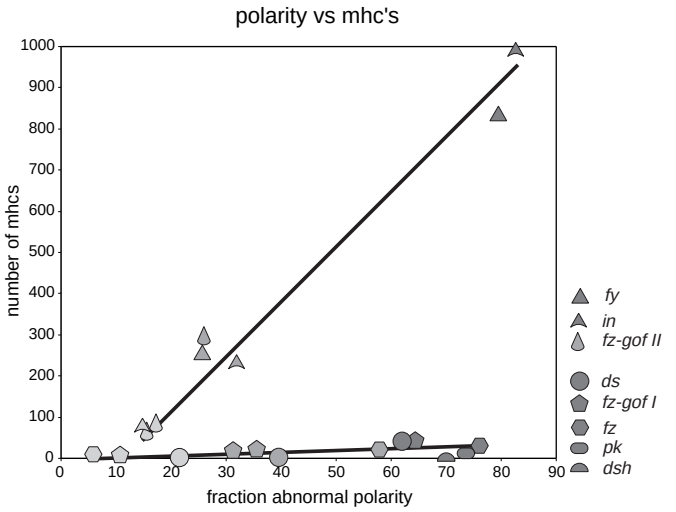

Fig. 2. Plot of the number of multiple hairs (in the dorsal C region) versus the fraction of the dorsal C region where hairs show abnormal polarity. Stronger alleles are indicated with darker shaded symbols. Different symbols indicate the gene (or condition) in question. From stronger to weaker, the genotypes for the different *fz* like genes were: fz^{R52}/fz^{D21}, fz^{HD21}/fz^{D21} (18°C), fz^{R53}, dsh^{1}, pk^{1}, ds^{UAO71}/ds^{33k}, ds^{YAC41}, ds^{YM31}, *hsfz*I (1 hour heat shock at 37°C at 30 hours awp), *hsfz*I (1 hour heat shock at 33ºC at 29 hours awp), *hsfz*I (1 hour heat shock at 37°C at 25 hours awp), *hsfz*I (1 hour heat shock at 31°C at 29 hours awp). For the *in* like genes the genotypes were in^{IH56}/*Df(3L)rdgC (*a deficiency for *in*), in^{II53}/*Df(in)* (at 29°C), in^{HC31}/*Df(in)* at 25°C, fy^{1}, fy^{JN12}/fy^{1}, *hsfz*I (1 hour heat shock at 37°C at 34 hours awp), *hsfz*I (1 hour heat shock at 33°C at 34 hours awp), *hsfz*I (1 hour heat shock at 31°C at 34 hours awp). The data for the *fz* and *in* alleles and the early and late *fz* gain-of-function points comes from Krasnow and Adler (1994).

cells and/or a small region of polarity disruption in the proximal part of the wing.

The cellular phenotype of *ds* places it in the *fz*-like group of genes

Previously we found we could distinguish between *fz*-like and *in*-like phenotypes by comparing the number of multiple hair cells as a function of the fraction of the wing showing altered polarity (Krasnow and Adler, 1994). We scored this phenotype for 3 *ds* genotypes of varying severity and found that they all fell in the *fz*-like group (Fig. 2). The phenotype of the *ds* mutants was however, more variable from one individual to another than is typical for other tissue polarity genes.

ds mutants display a unique wing hair polarity pattern

Mutations in tissue polarity genes do not result in a random pattern of hair polarity in most regions of the wing (Gubb and Garcia-Bellido, 1982; Wong and Adler, 1993). Rather, mutations result in gene-specific stereotypic patterns. Mutations in most tissue polarity genes (*e.g. fz, dishevelled (dsh), in, fuzzy (fy), Van Gogh, starry night, fritz, multiple wing hairs* (*mwh*) result in a similar (albeit not identical) abnormal polarity phenotype. For example, in wings from flies mutant for any of these genes, hairs in the E region of the wing point toward the posterior margin (Gubb and Garcia-Bellido, 1982; Wong and Adler, 1993; R. Krasnow and P. N. Adler, unpublished). We refer to this as the *fz*/*in* polarity pattern (Fig. 3). It appears to be the default pattern that results from the inactivation of the *fz* pathway. Mutations in *prickle (pk)* result in a distinctively different pattern. For example, in a *pk* wing cells in the E cell tend to point anteriorly (Fig. 3). We found that *ds* mutant wings also displayed stereotypic polarity patterns, although the pattern was dramatically different from any other tissue polarity mutants. Notably, in strong mutants there was a region at the anterior margin with a swirl of partially reversed polarity hairs (Figs 1, 3). In other regions in the anterior/central proximal part of the wing blade there were regions of completely reversed polarity (i.e. hairs that pointed proximally) (Figs 3, 4). Such regions of reversed polarity were separated from regions of relatively normal polarity via a region of swirling (Fig. 4). In weaker alleles these patterns were seen in a less extreme form (Figs 1, 3). As is typical for other tissue polarity genes, the polarity disruptions were more severe on the ventral than dorsal surface. In many *ds* wings the region of reversed polarity on the ventral surface was quite large (Fig. 4A,B). The novelty of the *ds* pattern argued that *ds* did not produce a polarity phenotype by inactivating the *fz* signaling/signal transduction pathway as do mutations in genes such as *fz*, *dsh* or *in*.

Pupal wing phenotype of *ds*

We observed the actin cytoskeleton in ds^{UAO71}/ds^{33K} pupal wings via phalloidin staining. As is the case in wild-type wings, prehair initiation began first in cells located distally and progressed proximally in a patchy pattern (Wong and Adler, 1993). In wing regions that did not show substantial polarity alterations prehairs were initiated in the vicinity of the distal vertex of the wing cells (this is what is seen in wild type; Wong and Adler, 1993). In most regions with abnormal polarity in *ds*

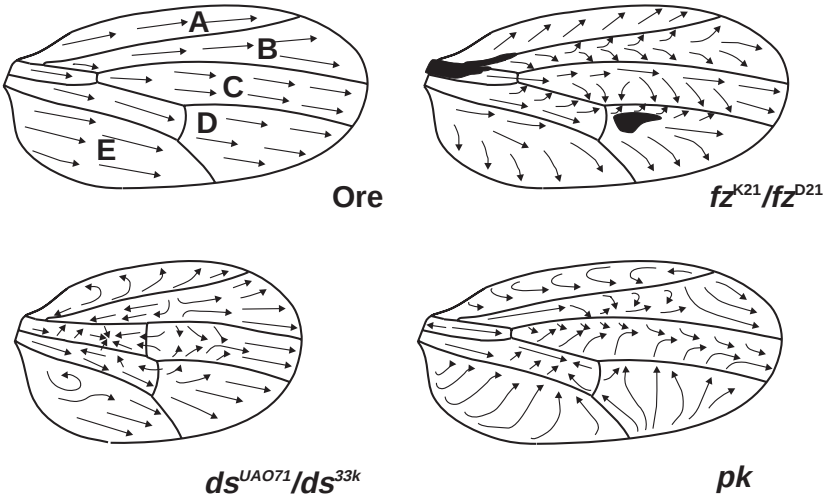

Fig. 3. Drawings of the wing hair polarity pattern on the dorsal surface of a typical mutant wing. The filled areas are regions where a polarity cannot be defined as neighboring hairs are not well aligned.

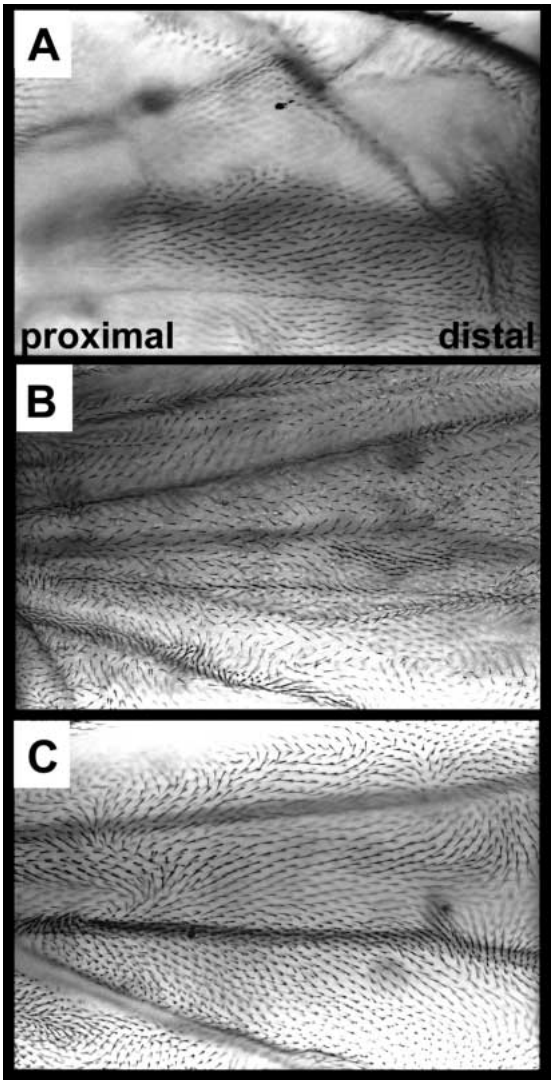

Fig. 4. Three examples of reversed hair polarity in ds^{UAO71}/ds^{33k} wings. (A,B) Ventral surface of the wing, and (C) the dorsal surface. In all cases proximal is to the left and distal to the right.

wings, prehairs initiated at an alternative location along the cell periphery. In the regions with reversed polarity, prehairs initiated in the vicinity of the proximal-most vertex (Fig. 5A). In regions where hair polarity was close to random and in regions at the center of swirls, prehairs were found to initiate in the central regions of the cells (Fig. 5A). This pattern is most similar to that seen previously for *pk* (Wong and Adler, 1993). Because of the well known interaction between catenins and cadherins we examined the distribution of the Armadillo (β-catenin) protein in wild-type and ds^{UAO71}/ds^{33K} pupal wings. No differences were seen, suggesting that *ds* does not play a key role in governing the subcellular localization of Armadillo.

fz pathway function is needed for the *ds* polarity pattern

We attempted to construct double mutants between *ds* and fz^{R54}/fz^{K21}, dsh^{1}, in^{IH56}, and mwh^{3}. The strong *ds* genotype (ds^{UAO71}/ds^{33K}) used results in low viability (only about half the expected number of adults emerged and most of these quickly got stuck in the food and died) and a severely distorted body shape. We did not recover any *dsh;* ds^{UAO71}/ds^{33K} adults. The double mutants between *ds* and *fz*, *in*, and *mwh* all showed the general body shape (e.g. wing, leg) and wing vein abnormalities of *ds*, but they all had a *fz/in*-like wing tissue polarity pattern (see Fig. 1). Further, in the *ds; in* and *ds; mwh* double mutants a large number of cells formed more than 1 hair as is seen in *in* and *mwh* (Gubb and Garcia-Bellido, 1982; Wong and Adler, 1993). The epistasis was not perfect in all aspects, however. For example, the number of hairs per cell in a *ds; mwh* double mutant was lower than in a *mwh* single mutant (2.68 vs 3.3). We concluded from these experiments that the function of the *fz* pathway genes was required for the unusual *ds* polarity pattern, which suggested that the *fz* signaling/signal transduction pathway was functioning, but in an abnormal way in *ds* mutants. Further evidence for an interaction between *fz* pathway genes and *ds* is that a weak *ds* mutant genotype (e.g. ds^{1}/ds^{33k}) acted as an enhancer of a hypomorphic *fz* allele (fz^{R53}) as it increased the fraction of the wing that showed abnormal polarity.

We also generated flies that were mutant for *ds* and which carried a *hs-fz* transgene (Krasnow and Adler, 1994). A single 1-hour heat shock at 24 hours after white prepupae (awp) formation suppressed the distinctive *ds* polarity phenotype (e.g. regions of reversed polarity) (Fig. 1O,P). The phenotype obtained resembled that seen in *hs-fz*/+ control flies (Krasnow and Adler, 1994). Other aspects of the *ds* mutant phenotype (e.g. appendage shape abnormalities, wing vein placement abnormalities) were not suppressed. We suspect that the *fz* overexpression phenotype results from the ligand independent activation of the *fz* signal transduction pathway. That *fz* overexpression suppressed the *ds* polarity phenotype is consistent with *fz* being downstream of *ds* in wing tissue polarity, although other data suggest the relationship between *ds* and *fz* is more complex.

ds displays domineering cell nonautonomy

Since the screen we used to isolate the *ds* alleles relied on seeing a mutant phenotype in clones it was clear that neighboring wild-type cells would not completely rescue the mutant phenotype of a *ds* clone. As a test of whether or not wild-type cells could rescue juxtaposed *ds* cells we induced *ds* and *ck* twin spots. We frequently saw cells with a *ck* phenotype next to cells showing altered polarity, indicating that juxtaposed wild-type cells need not rescue *ds* cells. Indeed, in some cases the *ck* cells had altered polarity suggesting that *ds* clones display the domineering cell nonautonomy characteristic of *fz* and *pk* (Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987). To determine if this was the case we generated *ds ck* clones. The clones larger than 16 cells appeared more oval and less extended than typical wing clones and they also often appeared to form a bulge on the wing surface. These phenotypes could be a manifestation of altered cell adhesion. We found a majority of clones (63/117) resulted in altered polarity of neighboring wild-type cells (Fig. 5B,C). This domineering nonautonomy was size dependent, and was the majority result only when the clones were relatively large (>100 cells). Both the frequency and the magnitude of the region of wild-type cells with altered polarity was smaller than we had seen in our earlier studies on *fz* (Vinson and Adler,

Table 2. *fz* nonautonomy is enhanced in *ds* mutant wings

Genotype of clone	Background genotype	No nonautonomy	Slight nonautonomy	Moderate nonautonomy	Strong nonautonomy	Extreme nonautonomy
fz strb	ds^{-} *	0†	0	4	5	15
fz strb	*wt*	0	6	13	2	0
fz trc	*wt*	1	7	10	4	0
mwh fz	*wt*	0	10	12	3	0

*The *ds* genotype was ds^{UA071}/ds^{33k}.
†Number of clones in this class.

1987; Jones et al., 1996). As we saw with *fz* nonautonomy, the affected wild-type hairs tended to point toward rather than away from the clone (Adler et al., 1997). In the case of *fz* there is a striking directionality to the domineering nonautonomy as neighboring wild-type cells distal (and anterior and posterior) but not proximal to the clone show altered polarity (Vinson and Adler, 1987). To determine if *ds* clones showed any directional bias to their non-autonomy we examined this for the 63 clones that displayed nonautonomy. We found 36 showed distal nonautonomy, 32 anterior nonautonomy, 30 posterior nonautonomy, and only 7 proximal nonautonomy. Thus, while *ds* nonautonomy is less biased in direction than *fz*, it is not random. That *ds* acts nonautonomously argues that if *ds* mutations produce a tissue polarity phenotype by altering the activity or function of the *fz* pathway it needs to affect the cell nonautonomous part of the pathway (i.e *fz* signaling).

ds is not required for the intracellular transduction of the *fz* signal

Loss-of-function mutations in the cell autonomously acting *in*-like genes results in many cells forming more than one hair (Wong and Adler, 1993; Adler et al., 1994). Thus, the *in*-like genes function as negative regulators of prehair initiation. Based on epistatic interactions, we previously suggested that Fz and Dsh acted upstream of In and Fy and inhibited the activity of these proteins in the vicinity of the distal vertex leading to prehair initiation at this location (Wong and Adler, 1993). The overexpression of *fz* a few hours prior to prehair initiation results in many cells forming more than one hair resembling a weak or moderate allele of *inturned* or *fuzzy* (Krasnow and Adler, 1994). We interpreted the induction of multiple hair cells by late *fz* overexpression as being due to the over activation of the *fz* signal transduction pathway leading to excess inhibition of In and Fy (Krasnow and Adler, 1994). Consistent with this hypothesis a similar phenotype is produced by the late overexpression of *dsh*, and the weak overexpression of *fz* acts as an enhancer of weak *in* and *fy* alleles (R. Krasnow and P. N. Adler, unpublished). We previously found that the ability of *fz* overexpression to produce this gain-of-function phenotype is blocked in a *dsh* mutant (but not a *pk* mutant). This argues that *dsh*, but not *pk*, is downstream of *fz* and required for *fz* signal transduction (Krasnow et al, 1995). When we overexpressed *fz* in a *ds* mutant wing we obtained an *in*-like phenotype (Table 1), leading us to conclude that *ds* is not required for *fz* signal transduction. It is notable that ds^{UA071}/ds^{33K}; *hs-fz*/+ flies that were not heat shocked showed a typical *ds* polarity pattern. In previous experiments we found that a *hs-fz* transgene provided enough function at 25°C (without any heat shocks) to provide substantial rescue of a null *fz* genotype (Krasnow and Adler, 1994). That we did not see any substantial rescue of the *ds* polarity pattern in these flies argues that *ds* does not produce a mutant polarity pattern by interfering with transcription of the *fz* gene.

ds alters the direction of *fz* intercellular signaling

We previously found that *fz* clones (for most alleles including null alleles) displayed distal domineering cell nonautonomy (Vinson and Adler, 1987). That is, wild-type cells distal (and

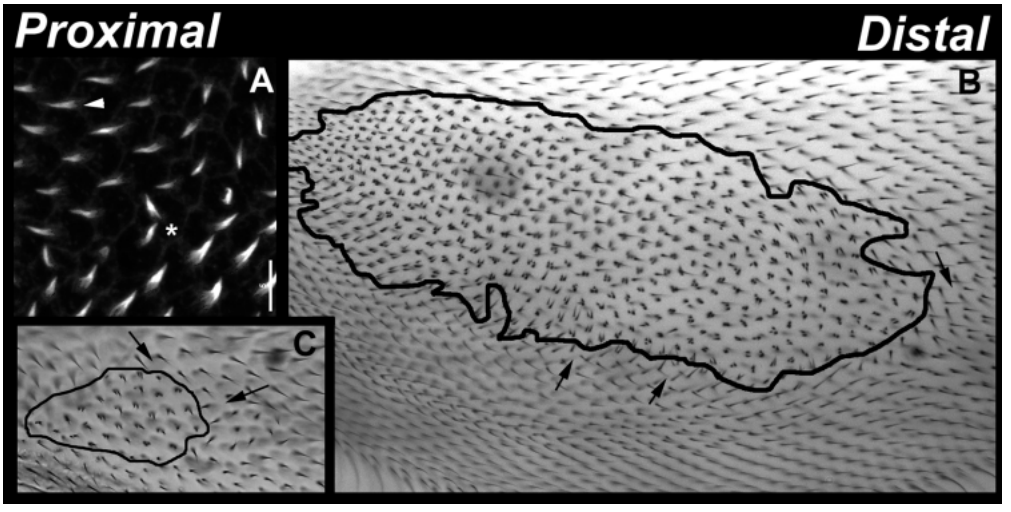

Fig. 5. (A) A micrograph of a ds^{UAO71}/ds^{33k} wing stained with rhodamine phalloidin and examined in the confocal microscope. The asterisk indicates a cell where a prehair is found in the center of the apical surface of the cell. Cells with prehairs that point proximally and are located at the proximal edge of the cell are obvious in the upper left side of the panel. An arrowhead points to one such cell. Scale bar is 5 μm. (B,C) ds^{UAO71} ck^{UAH21} clones. The clone boundaries are outlined. The arrows point to regions of wild-type cells with abnormal polarity. In all micrographs proximal is to the right and distal is to the left.

in part anterior and posterior) but not proximal to the clone showed abnormal polarity. We have used this property of *fz* clones as an assay for *fz* intercellular signaling (i.e the cell nonautonomous function of *fz*). To determine if *ds* was required for *fz* signaling we generated *fz strb* clones in ds^{UA071}/ds^{33K} wings. The interpretation of this experiment is more challenging than for an equivalent experiment in a wild-type wing. In a wild-type wing carrying a *fz* clone all hairs point distally, except those influenced by the *fz* clone. This is not the case in a *ds* wing, which has large regions with abnormal polarity. We first examined *fz* clones found in regions of *ds* wings where polarity is typically normal (i.e. distal). In such regions we scored 24 *fz strb* clones all of which showed distally directed domineering cell nonautonomy (Fig. 6A,B). Indeed, the domineering nonautonomy appeared much enhanced over that seen for *fz* clones in a wild-type wing (compare with Fig. 1 in Jones et al., 1996). In the *ds* background we saw substantial regions of hairs with completely reversed polarity distal to the clone. Indeed, in some cases (e.g. Fig. 6A) hairs on all sides of the clone pointed directly toward the clone boundary. We confirmed that *ds* resulted in enhanced domineering cell nonautonomy of *fz* clones by comparing the extent of nonautonomy (see methods for details) in *fz* clones induced in either wild-type or *ds* wings (Table 2). Thus, *ds* can be considered to be a negative regulator of *fz* domineering nonautonomy.

We next examined *fz strb* clones in regions of *ds* wings where hair polarity is typically proximal (Figs 6C, 7). In such regions of *ds* wings we found 9 *fz strb* clones. For all of these clones, hairs both proximal and distal to the clone pointed toward the clone (Figs 6C, 7). Hairs proximal to the clone showed distal polarity while hairs distal to these clones showed proximal polarity. We assume that hair polarity in these regions would have been proximal in the absence of the clone. Thus, the *fz* clone resulted in hairs proximal to the clone changing their polarity from proximal to distal (Fig. 7). Hence, these *fz* clones displayed proximal domineering nonautonomy. This argues that the normal distal direction of *fz* signaling (as defined by the direction of nonautonomy) was reversed in these regions of *ds* wings and was now proximal. We conclude that one of the consequences of *ds* mutations is that in regions of altered hair polarity (e.g. proximal polarity) the direction of *fz* signaling is similarly altered (i.e. we see proximal domineering nonautonomy).

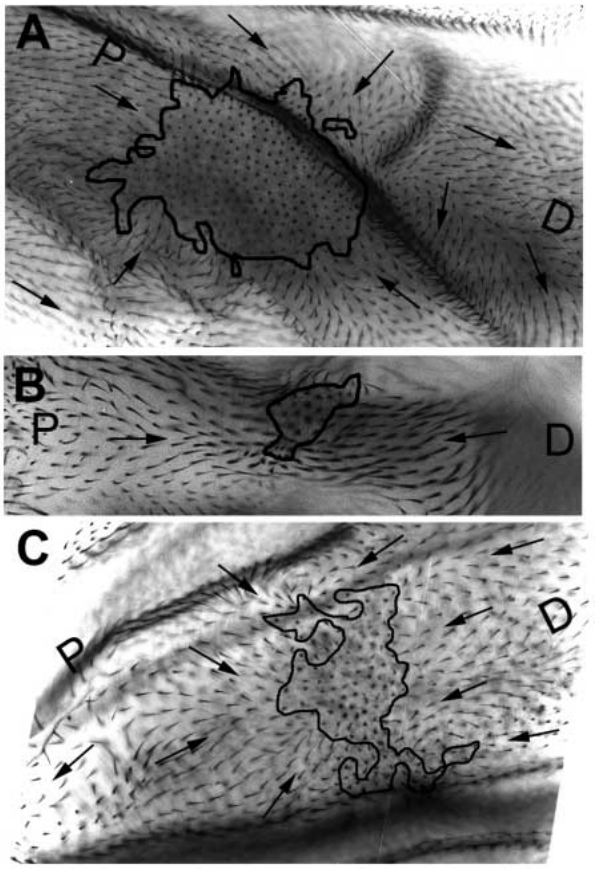

Fig. 6. *fz strb* clones in a ds^{UAO71}/ds^{33k} wing. The boundary of the clones is outlined. The arrows point in the direction of the hairs in that location. D is distal and P proximal. Note the extreme nonautonomy shown by all three clones. (A,B) Clones in regions that normally show distal polarity in *ds* wings. The nonautonomy here is distal, downstream and attractive domineering nonautonomy. (C) Hair polarity is typically proximal in this region on the ventral surface of a *ds* wing. This clone shows what appears to be proximal, downstream and attractive domineering nonautonomy. Due to this wing region not being flat we needed to assemble this panel using individual images from several different focal planes.

DISCUSSION

ds and cell adhesion

Cadherins are known to be mediators of Ca^{2+} mediated cell adhesion. The unusual structure of the Ds and Ft proteins raised the possibility that these proteins, which contain multiple cadherin motifs, might not function in cell adhesion. Finding that *ds* clones on the wing are biased towards an oval shape (minimizing contact with wild-type cells), and that the clones

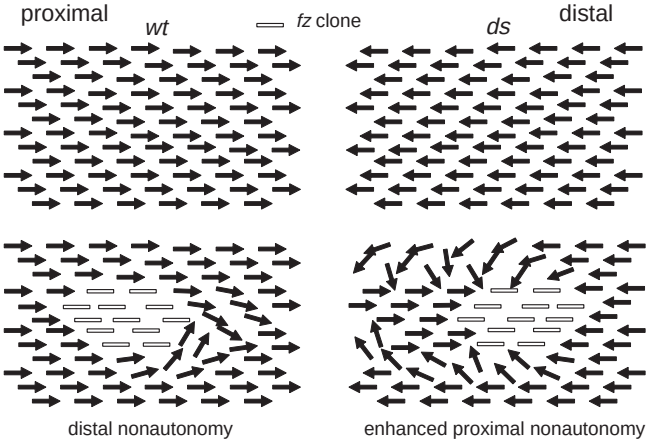

Fig. 7. Drawings of a wild-type wing and a region of a *ds* wing that shows proximal polarity. Distal is to the left and proximal to the right. The filled arrows represent hairs produced by cells that are wild type for *fz*; the open rectangles, cells mutant for *fz*. In the upper panels are wings with no *fz* clones, and the lower panels show the effects of *fz* clones in an otherwise wild-type or *ds* wing. Note that in the *ds* wing the anatomical direction of *fz* nonautonomy is now proximal and not distal as in wild type.

often bulged from the wing surface, supports the previous suggestions that *ds* functions in cell adhesion (Clark et al., 1995). In addition, we found that *ds* pupal wings were much more fragile than wild-type pupal wings, resulting in them frequently falling apart during manipulation. This also seems likely to be due to an adhesion defect. At least some of the tissue polarity phenotypes associated with *ds* however, resemble those seen in *fz* pathway mutants and seem unlikely to be due to an effect on cell adhesion. For example, the use of an altered subcellular location for prehair initiation seems unlikely to be due to altered cell adhesion. When we examined double mutants between *fz* pathway genes and *ds* we found they had the altered body shape of *ds*, but a tissue polarity phenotype that resembled the *fz* pathway mutation. That the body shape defects of *ds* are not suppressed in a fly that is doubly mutant for *fz* and *ds* indicates that the abnormal *fz* signaling seen in *ds* wings is not the cause of the body shape abnormalities. These observations do not rule out the possibility that the body shape defects cause the abnormalities in *fz* signaling, although we do not think this likely.

ds and tissue polarity on the wing

It is not terribly surprising that *ds* function is essential for the development of normal wing tissue polarity. Held and colleagues (1986) found that *ds* mutations disrupt the polarity of bristles on the legs and that they cause leg joint abnormalities that are similar to those produced by *fz* and *sple*. The overall morphological abnormalities caused by *ds* mutations are greater on the leg, but it appears that the effects on the wing and leg may be parallel.

ds mutations do not inactivate the *fz* pathway

Previous work has shown that the function of the *fz* pathway is essential for the formation of a wing with normal distally pointing hairs (Wong and Adler, 1993). This pathway is thought to be composed of both an intercellular signaling system and an intracellular signal transduction system (Vinson and Adler, 1987; Wong and Adler, 1993; Park et al., 1994). The cell autonomous function of this pathway leads to the restriction of prehair initiation to the vicinity of the distal vertex (Wong and Adler, 1993). Previous studies have argued that mutations in genes such as *dsh*, *in*, *fy* and *RhoA* produced a tissue polarity phenotype by interfering with the intracellular transduction of the *fz* signal (Wong and Adler, 1993; Krasnow et al., 1995; Strutt et al., 1997). We have found that *ds* is not required for the transduction of the *fz* signal and consistent with that conclusion it does not cause a polarity pattern that is typical of a lack of or reduction in *fz* pathway function.

ds causes a tissue polarity phenotype by altering the direction of fz signaling

We found that the correspondence between the direction of hair polarity (distal), the subcellular location for prehair initiation (in the vicinity of the distal vertex) (Wong and Adler, 1993) and the direction of *fz* domineering nonautonomy (distal) (Vinson and Adler, 1987) seen in wild-type wings was maintained in regions of *ds* wings with reversed polarity. In such wing regions proximally pointed hairs were formed in the vicinity of the proximal vertex, and *fz* clones displayed proximal domineering nonautonomy. This leads us to conclude that the cause of the abnormal hair polarity in *ds* wings is the abnormal direction of *fz* signaling and that *ds* cells in these regions are responding normally to an abnormal signal. This is consistent with the conclusion noted above that *fz* signal transduction does not require *ds* function. The hypothesis that *ds* causes a tissue polarity phenotype because it alters the direction of *fz* signaling also explains the need for *fz* pathway genes for the *ds* mutant polarity pattern, *ds* mutants having a *fz*-like cellular phenotype and the domineering nonautonomy of *ds*. It is also consistent with the ability of *fz* overexpression to suppress the *ds* polarity phenotype, since *fz* overexpression can 'suppress or override the wild-type polarity pattern' that arises from normal *fz* signaling.

In other experiments we have also found that in *prickle* mutant wings the abnormal direction of hair polarity predicts and is coincident with the abnormal direction of *fz* signaling (R.E. Krasnow and P.N. Adler, unpublished). We note that there is no gross abnormality in wing or body shape in *pk* mutants as there is in *ds* mutants, thus an abnormal direction of *fz* signaling does not rule out having a normal body shape. We think it is significant that these two tissue polarity mutants that do not produce a *fz/in* polarity pattern (see Fig. 3) both appear to cause a tissue polarity phenotype by altering the direction of *fz* signaling. We suggest that mutations that produce a *fz/in* like mutant polarity pattern will do so by inactivating the *fz* signaling/signal transduction pathway, and that mutations that produce different abnormal polarity patterns will do so by altering the direction of *fz* signaling.

Why does *ds* cause altered *fz* signaling?

Two types of models have been proposed to account for the role of Fz in tissue polarity and to explain the distal domineering nonautonomy of *fz* clones (Adler et al., 1997). The cell-by-cell signaling model suggests that the binding of ligand at one side of a cell leads to the Fz receptor becoming unevenly activated across the cell (Park et al., 1994). This leads to both prehair initiation and the relaying of the signal being localized at the distal edge of cells, which leads to hair polarity being coincident with the direction of signaling. This model does not specify how the overall polarity is established, but it seems likely that it is imposed by special 'boundary cells' located proximally. In this model the domineering distal nonautonomy of *fz* clones was ascribed to a failure of cells distal to the clone to receive the signal (Vinson and Adler, 1987). The secondary signal model suggests that the Fz receptor is activated in a gradient fashion along the proximal/distal axis of the wing by a long range gradient of a morphogen ligand (Zheng et al. 1995). Fz activation leads to the proportional production of a secondary signal, which acts more locally to polarize cells. In this model the nonautonomy of *fz* clones is due to a failure of clone cells to produce the secondary signal. A group of proximal cells presumably form the source for the gradient of Fz ligand. Either of these models can easily accommodate the observation that there are regions in a *ds* wing where the direction of *fz* signaling is reversed. For example, by hypothesizing that *ds* mutations result in a change in the fate of cells that then serve either as ectopic sources of the gradient morphogen or locations that initiate cell-by-cell signaling. Such a model might also be able to explain the altered wing shape and wing vein pattern as a consequence of the population of cells with altered cell fate. This hypothesis does not however, explain the need for *ds* function for tissue

polarity development in all regions of the wing as was shown by our analysis of *ds* clones. Nor does it explain the enhanced domineering nonautonomy of *fz* that we also observed in *ds* wings. We therefore prefer hypotheses where *ds* mutations produce their phenotypic effects by altering the function of the *fz* pathway in all regions of the wing. The effects of *ds* mutations on *fz* signaling are subtle and do not appear to be due to either a simple quantitative loss or gain of function. Perhaps *ds* mutations alter the efficiency, stability or propagation of a polarity signal in a way that leads to the system becoming destabilized. The regions of reversed polarity might be caused by minor perturbations in *fz* signaling being amplified and propagated in a *ds* mutant wing due to the decreased stability of the system.

It is possible that *fz* signaling takes place at the adherens junction and that *ds* mutations alter the structure or composition of the junction in a way that alters *fz* signaling. For example, *ds* could promote the assembly of a Fz receptor complex at the junction. An 'incomplete' complex formed in a *ds* mutant might be unstable leading to aberrant signaling. Alternatively, the effect of *ds* on *fz* signaling could be more indirect as is discussed below.

fz, Wnts and cadherins

It is now clear that the *fz* family of receptors recognize Wnt's as ligands (Bhanot et al., 1996). *wingless*, which encodes the prototypical Wnt in *Drosophila*, has important signaling functions in *Drosophila* development (Ingham,1996; Eaton and Cohen, 1996; Peifer, 1995). The *arm* gene, which encodes a β-catenin is essential for the transduction of the *wg* signal. In this case the role of *arm* is not linked to it's role in cell adhesion, but rather to it's role in signal transduction (Heasman et al., 1994; Peifer et al., 1994; Brunner et al., 1997; van de Wetering et al., 1997). Since no mutants in Wnt encoding genes have yet been found to have a *fz*-like wing tissue polarity phenotype the identity of the *fz* ligand for wing tissue polarity is unclear (but see Theisen et al., 1994). Thus far, only *dishevelled* has been shown, in a convincing way, to function in both the *wg* segment polarity and *fz* tissue polarity pathways (Krasnow et al., 1995; Klingensmith et al., 1994; Theisen et al., 1994). Indeed, experiments argue that *arm* does not function in tissue polarity (Strutt et al., 1997; Perrimon, 1996; Orsulic and Peifer, 1996; P. N. Adler, unpublished). Our data shows that an unusual cadherin like protein (Ds) is essential for the function of the *fz* tissue polarity pathway. Perhaps there is a novel Arm-like protein that functions in the *fz* pathway and which binds the Ds protein. Mutations in *ds* might affect the function of this pathway indirectly by increasing the free concentration of the putative Arm-like protein. A precedent for this comes from observations that the overexpression of E-cadherin (or portions of this protein) can have effects on *wg* signal transduction (Heasman et al., 1994; Funayama et al., 1995, Sanson et al., 1996).

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