Development 133, 2315-2324 (2006) doi:10.1242/dev.02401

Separating the adhesive and signaling functions of the Fat and Dachsous protocadherins

Hitoshi Matakatsu and Seth S. Blair*

The protocadherins Fat (Ft) and Dachsous (Ds) are required for several processes in the development of *Drosophila*, including controlling growth of imaginal discs, planar cell polarity (PCP) and the proximodistal patterning of appendages. Ft and Ds bind in a preferentially heterophilic fashion, and Ds is expressed in distinct patterns along the axes of polarity. It has thus been suggested that Ft and Ds serve not as adhesion molecules, but as receptor and ligand in a poorly understood signaling pathway. To test this hypothesis, we performed a structure-function analysis of Ft and Ds, separating their adhesive and signaling functions. We found that the extracellular domain of Ft is not required for its activity in growth, PCP and proximodistal patterning. Thus, ligand binding is not necessary for Ft activity. By contrast, the extracellular domain of Ds is necessary and sufficient to mediate its effects on PCP, consistent with the model that Ds acts as a ligand during PCP. However, we also provide evidence that Ds can regulate growth independently of Ft, and that the intracellular domain of Ds can affect proximodistal patterning, both suggestive of functions independent of binding Ft. Finally, we show that *ft* mutants or a dominant-negative Ft construct can affect disc growth without changes in the expression of *wingless* and Wingless target genes.

KEY WORDS: Overgrowth, Junction, Four-jointed, Dachs, Frizzled, Grunge, Atrophin

INTRODUCTION

The *Drosophila* proteins Fat (Ft) and Dachsous (Ds) are required in several processes during the development of imaginal disc tissues. First, they help regulate the growth of discs. Removal of Ft and, to a lesser extent, Ds, induces a disc overgrowth phenotype, caused by both an increase in the rate of cell division and a failure to properly arrest disc growth at the end of larval development (Bryant et al., 1988; Clark et al., 1995; Garoia et al., 2000; Garoia et al., 2005; Rodriguez, 2004). Second, both Ft and Ds are required for the normal establishment of planar cell polarity (PCP) in the eye, wing and abdomen (Adler et al., 1998; Casal et al., 2002; Ma et al., 2003; Matakatsu and Blair, 2004; Rawls et al., 2002; Simon, 2004; Strutt and Strutt, 2002; Yang et al., 2002). Here, Ft and Ds can reorient the subcellular polarization of the 'core' planar polarity proteins that occurs during pupal stages (reviewed by Fanto and McNeil, 2004; Uemura and Shimada, 2003). Finally, Ft and Ds are required for proper proximodistal patterning of some appendages; reduced function causes shape changes and foreshortening in the blade and hinge of the wing, and the loss or fusion of tarsal segments from the leg (Bryant et al., 1988; Clark et al., 1995). Some *ds* alleles can affect PCP without affecting growth, and proximodistal defects can occur without affecting growth or PCP, as in homozygous flies lacking Four-jointed (Fj), a protein thought to alter Ft or Ds function (Brodsky and Steller, 1996; Ma et al., 2003; Strutt et al., 2004; Strutt and Strutt, 2002; Villano and Katz, 1995; Waddington, 1940; Zeidler et al., 2000).

As Ft and Ds are protocadherins (Clark et al., 1995; Mahoney et al., 1991), differences in cell adhesion have been invoked to explain the mutant phenotypes. When homozygous mutant clones are generated in wild-type discs, they round up and form unusually smooth boundaries with their neighbors, consistent with a change in adhesion (Adler et al., 1998; Garoia et al., 2000). The role of cell adhesion and junctional proteins in growth control in vertebrate cells is well known, and in *Drosophila*, failures in junctional proteins such as Discs large 1 can lead to a disc overgrowth phenotype (reviewed by Bilder, 2004; Hajra and Fearon, 2002; Johnston and Gallant, 2002). It is also easy to imagine that failures in adhesion could lead to changes in cell-cell communication, such as the signals thought to be mediated by the core PCP proteins to transmit polarity information from cell to cell (see Amonlirdviman et al., 2005), or the unknown cues that result in proper proximodistal patterning of the wing and leg.

However, several lines of evidence have suggested an alternative hypothesis, that Ft and Ds act partially or wholly as receptor and ligand, respectively, in a poorly understood signaling pathway (reviewed by Saburi and McNeill, 2005). Ft and Ds are unusual among members of the cadherin family, as they bind each other in a preferentially heterophilic fashion. Each is necessary to stabilize the other on the cell surface in imaginal discs (Ma et al., 2003; Strutt and Strutt, 2002). Overexpression of one leads to excess accumulation of the other in vivo; in vitro, Ds-expressing cells preferentially aggregate with Ft-expressing cells (Matakatsu and Blair, 2004) (this study).

Ft is expressed in a largely uniform fashion (Garoia et al., 2000; Ma et al., 2003). Ds, however, has spatially restricted domains and gradients of expression in the eye, wing, leg and abdomen, suggesting that it might provide positional cues for PCP; indeed, artificial boundaries and gradients of Ds can reorient PCP (Adler et al., 1998; Casal et al., 2002; Ma et al., 2003; Matakatsu and Blair, 2004; Rawls et al., 2002; Simon, 2004; Strutt and Strutt, 2002; Yang et al., 2002). Genetic epistasis experiments examining PCP in the eye suggest that Ft acts downstream of Ds (Yang et al., 2002). Thus, Ds may alter the activity of Ft, thereby regulating the polarity of cells along the axis of graded Ds expression (Fanto et al., 2003; Ma et al., 2003; Yang et al., 2002). By extension, Ds may similarly regulate Ft activity during growth control and proximodistal patterning (Clark et al., 1995).

Such signaling may be mediated by the intracellular domains of Ft or Ds. The extracellular domain of Ft contains 34 cadherin repeats, followed by five EGF-like and two laminin A-G domains;

Department of Zoology, University of Wisconsin, 250 North Mills Street, Madison, WI 53706, USA.

*Author for correspondence (e-mail: ssblair@wisc.edu)

Accepted 27 March 2006

the extracellular domain of Ds contains 27 cadherin repeats (Fig. 1) (Clark et al., 1995; Mahoney et al., 1991) (reviewed by Tepass, 1999). Several Ft-like and Ds-like protocadherins have been identified in vertebrates and a second 'Fat-like' protocadherin has been identified in *Drosophila* (also called Fat2), based on their similar arrangement of extracellular domains (reviewed by Tanoue and Takeichi, 2005). Intriguingly, a subset of these, the vertebrate proteins Fat4 (also called Fat-J), dachsous 1 (also called protocadherin 16) and dachsous 2, are also similar to Ft and Ds in their intracellular domains (Hong et al., 2004; Nakajima et al., 2001). Moreover, the intracellular domain of Ft can bind to the *Drosophila* Atrophin protein Grunge, and *grunge* mutants have PCP defects (Fanto et al., 2003).

But while suggestive, the evidence for signaling via Ds or Ft in PCP is not definitive. The role of Grunge is uncertain (see Discussion), and many of these results could be explained by spatially regulated changes in cell adhesion. Moreover, most of the evidence for signaling has focused on PCP, and different mechanisms might underlie growth control and proximodistal patterning.

Therefore, we have taken a structure-function approach, asking whether the various activities of Ft and Ds are mediated by their extracellular or intracellular domains. For this, we concentrated especially on two well-studied phenotypes: the strong overgrowth of *ft* mutant discs, and the effects of *ft* and *ds* on hair polarity in the wing and abdomen. Our results (summarized in Table 1) show that a form of Ft lacking almost the entire extracellular domain retains nearly wild-type activity in growth control and PCP, while a form of Ds that lacks the intracellular domain retained nearly wild-type activity in PCP. This is consistent with the model Ft has a receptor-like function mediated by its intracellular domain, while Ds has a ligand-like function in PCP. However, we will also present evidence suggesting that Ds can act independently of Ft in growth control, and that the intracellular domain of Ds has some activity in the proximodistal patterning of the wing. Because each assay raises separate issues, and may rely on divergent biological mechanisms, we have separated our presentation of the data below depending on the assay used.

MATERIALS AND METHODS

Molecular biology

UAS-ft (Matakatsu and Blair, 2004) contains the entire coding sequence from the Ft-RA prediction (FlyBase). Nucleotide positions below are from *ft-RA*. For *UAS-ftΔECD*, a fragment from nucleotide 1 to 412 was amplified by PCR and fused with a fragment from nucleotide 13704 to the stop codon; the amino acid sequence at the fusion is QPT^{137}-C^{4569}RGD. The deletion thus begins within the first cadherin domain and ends three amino acids N-terminal to the transmembrane domain predicted by TMHMM (Krogh et al., 2001). For *UAS-ftΔICD*, a fragment from nucleotide 13858 to the stop codon was replaced with HA tag sequence, yielding a C-terminal amino acid sequence of KQEKIG^{4619}-YPYDVPDYAstop (HA tag underlined). The deletion thus begins 11 amino acids C-terminal to the transmembrane domain, removing the regions with high homology to the intracellular domain of vertebrate Fat4.

UAS-ds (Matakatsu and Blair, 2004) contains the entire region coding for GenBank Accession Numbers AAA79329 and Q24292, including 20 amino acids N-terminal of the SQA-AS signal cleavage site predicted by SignalP v.3.0 (Bendtsen et al., 2004), but lacks the additional N-terminal 53 amino acids of the Ds-RA prediction (Flybase). Nucleotide positions below are from GenBank Accession Number L08811.2 and amino acid positions are from Ds-RA (FlyBase). For *UAS-dsΔECD*, a fragment from nucleotide 955 to 1225 was ligated with a fragment from nucleotide 10033 to the stop codon; the amino acid sequence at the fusion is RETR^{143}-G^{3080}TNL. The deletion thus begins N-terminal to the cadherin repeats and ends 17 amino acids N-terminal to the predicted transmembrane domain. For *UAS-dsΔICD*, a DNA fragment was amplified lacking nucleotides 10198 to the stop codon, yielding a C-terminal amino acid sequence of VKPHL^{3134}stop. The deletion, thus, begins 14 amino acids C-terminal to the transmembrane domain, removing the regions with high homology to the intracellular domains of vertebrate dachsous 1 and 2.

All constructs were confirmed by sequencing and cloned between the *Not*I (5′) and *Kpn*I (3′) sites of pUAST. Detailed information for DNA constructs is available upon request.

To determine molecular lesions for $ft^{G\text{-}rv}$ and ft^{fd}, genomic DNA from heterozygote adults and homozygote larvae were used as template for PCR and the resultant products were directly sequenced.

Mutant alleles and fly strains

$ft^{G\text{-}rv}$ and ft^{fd} are lethal alleles (Bryant et al., 1988). *ft* clones were generated in *y w hs-Flp*; $ft^{G\text{-}rv}$ FRT^{40A}/y^{+} FRT^{40A} or *y w hs-Flp*; ft^{fd} FRT^{40A}/*ubi-GFP* FRT^{40A} larvae. ft^{18} (ft^{k07918}) is a semi-lethal allele. ds^{05142} (Bloomington Stock Center) and ds^{UAO71} are strong alleles that lack detectable cell surface anti-Ds staining (Adler et al., 1998; Matakatsu and Blair, 2004) (data not shown). ds^{UAO71} $ft^{G\text{-}rv}$ has been described previously (Ma et al., 2003). ds^{gal4} was isolated by mobilizing the P element in *30A-gal4*, and is a strong *ds* allele.

Misexpression experiments used *actin5C* (*act*)-*gal4*, *AyGal4*, *daughterless* (*da*)-*gal4*, *engrailed* (*en*)-*gal4*; *tubulin* (*tub*)-*gal4* and *UAS-GFP* (Bloomington Stock Center); *spalt* (*sal*)-*gal4*, *hedgehog* (*hh*)-*gal4*, *apterous* (*ap*)-*gal4*, *UAS-ds* and *UAS-ft* (Matakatsu and Blair, 2004); and the *UAS* stocks generated above.

In vitro studies

Transfection of S2 cells and cell aggregation assays were as described previously (Matakatsu and Blair, 2004).

Immunostaining and westerns

Fixation, anti-Ds and anti-Ft staining and visualization in vivo were as described previously (Matakatsu and Blair, 2004), with the exception that EGTA was added to the Brower fix buffer when used for nuclear antigens. We used the following additional primary antibodies: mouse anti-Engrailed 4F11 (1:10) (Patel et al., 1989), rabbit anti-Vestigial (1:500) (Williams et al., 1991), rabbit anti-Distal-less (1:200) (Panganiban et al., 1995), rat anti-DE-cadherin (1:20) (Oda et al., 1994) (Development Studies Hybridoma Bank) or goat anti-DE-cadherin (1:500) (Santa Cruz), and rat anti-HA (1:100) (Roche) or rabbit anti-HA (1:200) (Santa Cruz).

For western blots, protein was extracted from S2 cells or larval discs and CNS using sample buffer, run on 3-8% Tris-Acetate gels (Invitrogen), and transferred to PVDF membranes (Millipore) in transfer buffer (48 mM Tris, 390 mM glycine, 0.1% SDS, 10% methanol). Blots were stained with anti-Ft, anti-Ds (see above) or anti-HA (Santa Cruz), followed by appropriate HRP-linked secondary antisera (Jackson) and the Super Signal detection kit (Pierce).

RESULTS

The extracellular domains of Ft and Ds are sufficient and necessary for binding and stabilization at the cell surface

We made two deletion constructs for both Ft and Ds, removing most of the extracellular (FtΔECD, DsΔECD) or intracellular (FtΔICD, DsΔICD) domains (Fig. 1; details in Materials and methods). Because the available anti-Ft antiserum was generated to the intracellular domain (Yang et al., 2002), we used a version of FtΔICD with a C-terminal HA tag.

To check the stability and subcellular localization of these deleted proteins, we expressed these in imaginal discs and S2 cells using the Gal4-UAS system (Brand and Perrimon, 1993). For those that could be directly compared by antibody staining (all but FtΔICD), levels of expression were equivalent, and well above endogenous anti-Ft or anti-Ds levels in wing discs (see Fig. S1 in

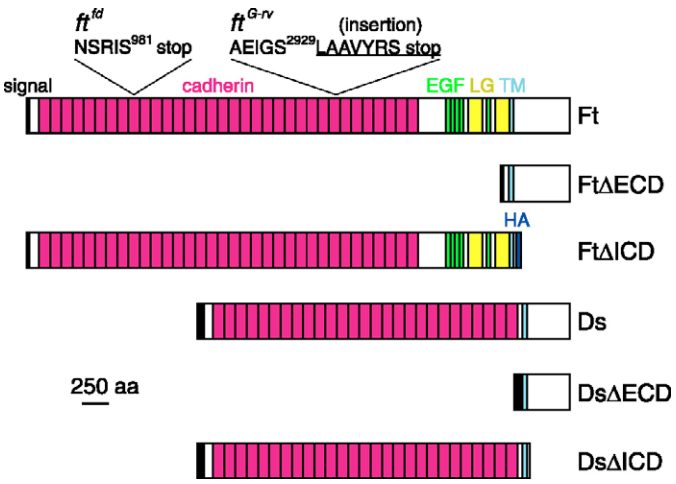

Fig. 1. Structures of full-length and deleted Ft and Ds proteins. Shown are the cleaved signal sequences (black), cadherin domains (purple), EGF-like domains (green), Laminin-G domains (yellow-green), transmembrane domains (light blue) and the HA tag (dark blue). Sites mutated in ft^{fd} and $ft^{G\text{-}rv}$ are also shown. ft^{fd} contains a stop codon in the 9th cadherin domain. $ft^{G\text{-}rv}$ contains a *mdg3* retrotransposon insertion (the beginning of its sequence is underlined) that introduces a stop codon in the 27th cadherin domain; it also contains additional rearrangements in the first intron and a previously described insertion in the 33rd cadherin domain (not shown) (Mahoney et al., 1991).

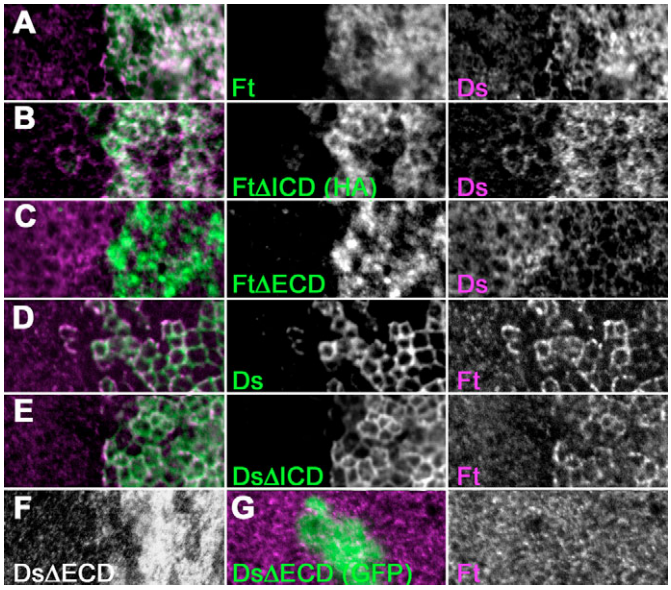

Fig. 2. Expression of full-length and deleted *ft* and *ds* constructs. (A-F) Expression of full-length and deleted *ft* and *ds* constructs in the posterior compartments (right) of $ft^{G\text{-}rv}/ft^{fd}$ (A-C), ds^{05142} (D,E) or wild type (F) wing imaginal discs with *en-gal4*. (**A**) *UAS-ft*. (**B**) *UAS-ftΔICD*. In both A and B, Ft or the HA-tagged FtΔICD (green and center panels) is concentrated at the cell cortex, and leads to stronger anti-Ds staining (purple and right panels) in the region of misexpression. (**C**) *UAS-ftΔECD*; anti-Ft staining (green and center panel) is diffuse and not concentrated at the cell cortex, and does not lead to stronger anti-Ds staining (purple and right panel) in the region of misexpression. There appears to be a slight decrease in Ds levels. (**D**) *UAS-ds*. (**E**) *UAS-dsΔICD*. In both D and E, anti-Ds staining (green and center panels) is concentrated at the cell cortex, and leads to stronger anti-Ft staining (purple and right panel) in the region of misexpression. (**F**) *UAS-dsΔECD*; anti-Ds staining (white) is diffuse and not concentrated at the cell cortex. (**G**) *AyGal4* FLPout clone expressing *UAS-dsΔECD* and *UAS-GFP* (green) in a wild-type wing imaginal disc. The clone does not lead to stronger anti-Ft staining (purple and right panel).

the supplementary material). However, the ΔICD and ΔECD proteins had different subcellular localizations. Both FtΔICD and DsΔICD were largely localized at the cell cortex (Fig. 2B,E), similar to what we observed previously with full-length Ft and Ds (Fig. 2A,D) (Matakatsu and Blair, 2004). By contrast, the FtΔECD and DsΔECD proteins were diffusely localized, except for a few vesicle-like structures, and only very low levels of protein were located at the cell cortex (Fig. 2C,F; data not shown). This is reminiscent of the mislocalization of wild-type Ft and Ds that occurs in vivo after the loss of their Ds- or Ft-binding partners (Ma et al., 2003; Strutt and Strutt, 2002), and thus may represent a failure in the stabilization of the ΔECD proteins at the cell surface by binding to endogenous Ds or Ft.

We therefore next checked whether the deleted proteins retained the ability shown by full-length Ft or Ds to stabilize endogenous cell surface Ds or Ft, respectively, after misexpression in wing discs (Fig. 2A,D) (Matakatsu and Blair, 2004). FtΔICD and DsΔICD constructs did retain this ability (Fig. 2B,E). However, misexpression of the FtΔECD or DsΔECD constructs in wing discs did not result in the stabilization of endogenous Ft or Ds, respectively (Fig. 2C,G).

To confirm that removal of the extracellular domains prevented the binding of FtΔECD to Ds and DsΔECD to Ft, we checked the ability of these constructs to mediate heterophilic cell aggregation in vitro. S2 cells co-transfected or separately transfected with *ft* and *ds* aggregate (Fig. 3B,F) (Matakatsu and Blair, 2004). Cells co-transfected with *dsΔICD* and *ft* also aggregated (Fig. 3C), as did cells separately transfected with *ftΔICD* and *ds* (Fig. 3G). Thus, the deletions of the intracellular domains of Ft or Ds did not obviously impair their ability to bind each other. By contrast, S2 cells co-transfected with *dsΔECD* and *ft* or *ftΔECD* and *ds* did not aggregate (Fig. 3D,E).

The intracellular domain of Ft is sufficient for viability and growth control

Strong *ft* alleles, such as the combination $ft^{G\text{-}rv}/ft^{fd}$, show extensive overgrowth of imaginal disc tissue at late third instar (Fig. 4B, Fig. 5B; see Fig. S2B,I in the supplementary material) and are lethal during pupal stages (Bryant et al., 1988). Both $ft^{G\text{-}rv}$ and ft^{fd} remove detectable anti-Ft staining in discs (Ma et al., 2003) (data not shown), but the molecular defects in $ft^{G\text{-}rv}$ and ft^{fd} had not previously been determined. We therefore sequenced both mutants. Both ft^{fd} and $ft^{G\text{-}rv}$ contain stop codons predicted to truncate the proteins within the cadherin domains (Fig. 1). As this is prior to the transmembrane and intracellular domains, $ft^{G\text{-}rv}$ and ft^{fd} should be null for any adhesive or receptor function; it remains possible that the truncated proteins retain some activity as ligands for other proteins.

UAS-ft can rescue both the disc overgrowth and pupal lethality of $ft^{G\text{-}rv}/ft^{fd}$ when driven using *act-gal4* (Fig. 4C) or *da-gal4* (see Fig. S2C,J in the supplementary material) (Matakatsu and Blair, 2004). Similarly, expression of *UAS-ft* in the posterior of $ft^{G\text{-}rv}/ft^{fd}$ discs using *en-gal4* rescued overgrowth in a region-autonomous fashion; this assay has the advantage that the degree of rescue can be assessed in a single disc (Fig. 5C).

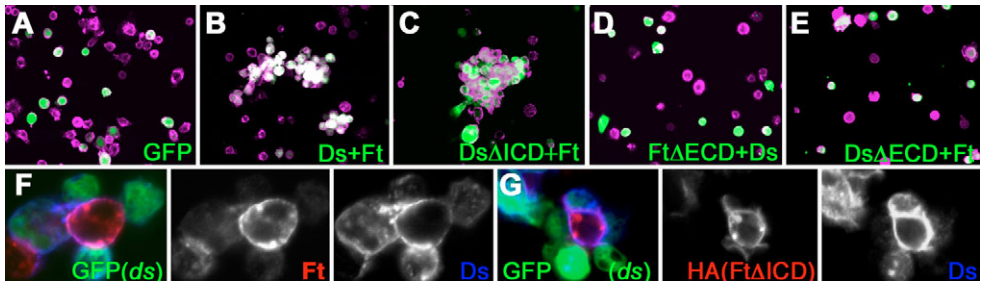

Fig. 3. Aggregation of S2 cells induced by transfection with full-length and deleted *ft* and *ds* constructs. Expression in cells was induced by co-transfection with *act-gal4*. Cells in A-E were counterstained with rhodamine-labeled phalloidin (purple). (**A**) Control cells transfected with *UAS-GFP* (green) did not aggregate. (**B**,**C**) Cells co-transfected with *UAS-ft, UAS-GFP* and either *UAS-ds* (B) or *UAS-dsΔICD* (C) aggregated. (**D**,**E**) Cells co-transfected with *UAS-GFP* and either *UAS-ftΔECD* and *UAS-ds* (D) or *UAS-dsΔECD* and *UAS-ft* (E) did not aggregate. (**F**,**G**) Mixture of cells transfected with *UAS-ds* and *UAS-GFP* (green), and cells transfected with either *UAS-ft* (F, red) or *UAS-ft ΔICD* (G, red) aggregated. Ft (anti-Ft, red and center panel) or FtΔICD (anti-HA, red and center panel) concentrated with Ds (blue and right panels) at the sites of cell contact.

We therefore compared the abilities of the deleted Ft constructs to rescue lethality and overgrowth. Surprisingly, *UAS-ftΔECD* was nearly as effective as *UAS-ft* in rescuing $ft^{G\text{-}rv}/ft^{fd}$ disc overgrowth when misexpressed using either *act-gal4* (Fig. 4D), *da-gal4* (see Fig. S2D,K in the supplementary material) or *en-gal4* (Fig. 5D), and rescued lethality with either *act-gal4* or *da-gal4*. As shown above, FtΔECD cannot bind Ds and fails to accumulate at high levels at the cell surface, and thus should lack any adhesive function. Nonetheless, FtΔECD is sufficient for growth control at the levels of expression being driven in these experiments. This strongly suggests that the ability of Ft to control growth is mediated, not via any putative adhesive function, but through its intracellular domain.

FtΔICD has dominant-negative effects on growth control

We showed above that FtΔICD can bind to and stabilize Ds in vivo and in vitro. However, in contrast to *UAS-ftΔECD*, *UAS-ftΔICD* was unable to rescue either the disc overgrowth or pupal lethality of $ft^{G\text{-}rv}/ft^{fd}$ when driven using *act-gal4* (Fig. 4E, Fig. 5E). Thus, binding to Ds is not sufficient to confer wild-type Ft activity in the absence of the intracellular domain. This failure cannot simply be attributed to low levels of the FtΔICD protein, as FtΔICD had a dominant-negative effect on growth control, opposite to the effects of *UAS-ft* or *UAS-ftΔECD*. The overgrowth normally observed in $ft^{G\text{-}rv}/ft^{fd}$ discs was markedly enhanced by expressing *UAS-ft ΔICD* with either *act-gal4* or *da-gal4* [compare Fig. 4B with Fig. 4E and Fig. S2B,I with Fig. S2E,L (supplementary material)]. Misexpression of *UAS-ftΔICD* in otherwise wild-type wing discs also caused a range of overgrowth phenotypes; the effect was moderate with *hh-gal4*, but very strong with the stronger *ap-gal4* driver (Fig. 6B-D). Overgrowth was largely or wholly limited to the region of misexpression, as the region lacking misexpression was a nearly normal size (e.g. ventral with the dorsally expressed *ap-gal4*; compare Fig. 6A with 6B). This dominant-negative effect apparently depends on the existence of an intact transmembrane domain, as misexpression of a Ft construct lacking both the transmembrane and intracellular domains did not cause significant overgrowth or enhance the overgrowth observed in $ft^{G\text{-}rv}/ft^{fd}$ discs; it also did not stabilize Ds in vivo and in vitro (H.M., unpublished).

It is unlikely that FtΔICD is acting only by interfering with the activity of endogenous Ft, as FtΔICD can enhance overgrowth in $ft^{G\text{-}rv}/ft^{fd}$ discs. Nonetheless, such interference could contribute to the phenotype; for example, FtΔICD might titrate out some factor required for the normal stability or localization of endogenous Ft. We therefore examined the effects of FtΔICD on endogenous Ft, using an antiserum that was generated against the intracellular domain of Ft (Yang et al., 2002) and that does not cross-react with FtΔICD (Fig. 6E). We found that the levels of endogenous Ft on the cell surface were actually increased in the region of FtΔICD misexpression (Fig. 6F). As the levels of endogenous *ft* mRNA were unchanged by FtΔICD misexpression (data not shown), endogenous Ft protein is probably being stabilized by FtΔICD, although without providing enough Ft activity to suppress overgrowth. This stabilization is surprising given the lack of any evidence for homophilic binding in 'trans' between cells expressing Ft (Stutt and Strutt, 2002; Ma et al., 2003; Matakatsu and Blair, 2004), and we cannot detect aggregation in vitro between cells expressing full-length Ft and FtΔICD (data not shown). However, it is possible that stabilization results from 'cis' dimerization between the Ft and FtΔICD expressed on the surface of a single cell, as has been proposed to occur between other cadherins (e.g. Takeda et al., 1999).

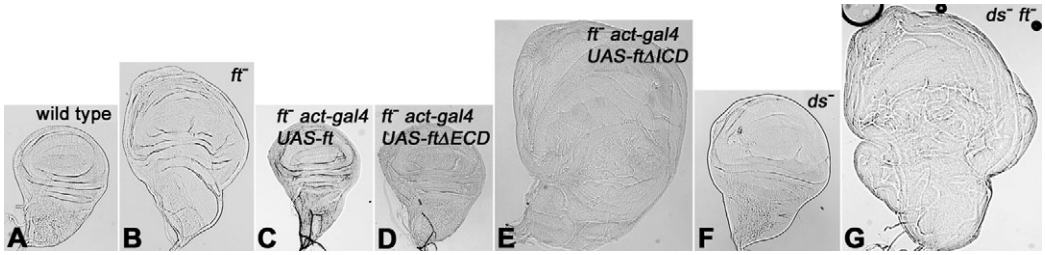

Fig. 4. Comparison of overgrowth of wing imaginal discs. Typical wing discs for each genotype are shown at the same magnification. ft^- is $ft^{G\text{-}rv}/ft^{fd}$, ds^- is ds^{UAO71}, and $ds^-\ ft^-$ is $ds^{UAO71}\ ft^{G\text{-}rv}/ds^{UAO71}ft^{fd}$. (**A**) Wild type. (**B**) Overgrowth in ft^-. (**C**) Rescue of ft^- overgrowth with *UAS-ft* and *act-gal4*. (**D**) Rescue of ft^- overgrowth with *UAS-ftΔECD* and *act-gal4*. (**E**) Potentiation of ft^- overgrowth with *UAS-ftΔICD* and *act-gal4*. (**F**) Mild overgrowth in ds^-. (**G**) Potentiation of ft^- overgrowth by ds^-.

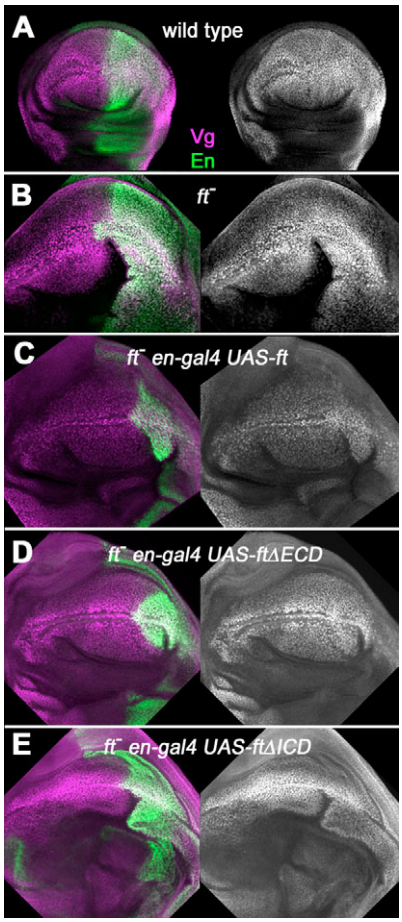

Fig. 5. Assay for rescue of $ft^{G\text{-}rv}/ft^{fd}$ overgrowth in posterior of imaginal wing discs. *en-gal4* (anti-En, green) was used to drive expressions of full-length and deleted *ft* constructs. All are shown at the same magnification. (**A**) Wild type. (**B**). Overgrowth of anterior and posterior wing pouch in $ft^{G\text{-}rv}/ft^{fd}$. (**C**,**D**) Rescue of overgrowth in posterior wing pouch by *UAS-ft* (C) or *UAS-ftΔECD* (D). (**E**) Failure to rescue overgrowth in posterior wing pouch with *UAS-ftΔICD*. (A-E) There is no effect on the expression of the wing blade Wg target Vg (purple and right panels).

As FtΔICD can bind and stabilize Ds (Fig. 2B, Fig. 3G, Fig. 6E), another explanation for the overgrowth is that FtΔICD binds to Ds in a way that prevents full Ds activity. Again, this is unlikely to be the sole mechanism, as the overgrowth phenotype induced by FtΔICD (Fig. 6B) can be much stronger than that of strong *ds* mutants (see below). Nonetheless, we will show below that removal of *ds* also enhances the overgrowth observed in $ft^{G\text{-}rv}/ft^{fd}$ discs (Fig. 4G, see Fig. S2G,N in the supplementary material) in a manner similar to FtΔICD misexpression. Thus, FtΔICD may bind to and block the activities of both Ds and Ft (see Discussion).

ft mutants and FtΔICD can disrupt growth without affecting *wg* or Wg targets

Recent studies have suggested that reducing the function of Ft and Ds can lead to modulation of Wg signaling, especially in the prospective hinge region of the wing disc, and expand the inner (distal) ring of *wg* expression in the hinge (Cho and Irvine, 2004; Rodriguez, 2004). Moreover, it has recently been reported that *ft* overexpression inhibits the expression Distal-less (Dll) and the 'quadrant' enhancer of *vestigial* (*vg-QE*), and that expression of the *vg-QE* is heightened in ft^{-} clones (Jaiswal et al., 2006). As expression of *vg* and *Dll* is stimulated by Wg signaling (Blair, 1994; Neumann and Cohen, 1997), these results raise the possibility that gains in *wg* or Wg signal transduction underlie some portion of the disc overgrowth phenotype.

However, although we find that ft^{fd} clones occasionally lead to distortions and apparent expansions in anti-Vg and anti-Dll staining, most clones, even those with obvious overgrowth, showed no obvious change in staining (see Fig. S3A-D in the supplementary material). To test this in another way, we examined the expression of Vg and Dll in two assays. In the first, we examined $ft^{G\text{-}rv}/ft^{fd}$ discs in which overgrowth had been rescued in the posterior using *en-gal4* and *UAS-ft* or *UAS-ftΔECD* (Fig. 5). In the second we compared the expression of Dll in wild-type regions with regions in which overgrowth was induced by posterior misexpression of *UAS-ftΔICD*, using *hh-gal4* (Fig. 6C). We did not observe convincing increases in the expression of Vg or Dll in regions of overgrowth (Fig. 5C,D; Fig. 6C). Posterior expression of *UAS-ftΔICD* also did not obviously increase the width of the distal ring of *wg-lacZ* expression in the hinge region when compared with adjacent anterior cells (Fig. 6D), in contrast to the expansions observed in *ft* mutant clones (Cho and Irvine, 2004). Thus, gains in the expression of *wg* or Wg targets were not reliable correlates of overgrowth.

It was also reported that Wg signaling stimulates and Ft represses the transcription of the *Drosophila* E-cadherin (DE-cadherin, also called Shotgun) (Jaiswal et al., 2006). We misexpressed *UAS-ftΔICD* with the strong, dorsal-specific *ap-gal4* driver, and observed a slight decrease in anti-DE-cadherin staining in the dorsal region (Fig. 6G). This is similar to what was reported with *UAS-ft* (Jaiswal et al., 2006), despite the opposite effects of full length Ft and FtΔICD on growth control. Thus, overgrowth does not reliably correlate with gains in DE-cadherin.

Ds contributes to growth control independently of the intracellular domain of Ft

Although weaker *ds* alleles do not induce obvious overgrowth phenotypes, strong *ds* mutations can induce mild overgrowth phenotypes (e.g. ds^{05142} or ds^{UAO71}; Fig. 4F, see Fig. S2F,M in the supplementary material) (see also Rodriguez, 2004). This raises the possibility that Ds regulates growth partly or wholly by binding to Ft and regulating the activity of its intracellular domain. Alternatively, it may be that Ds regulates growth independently of Ft.

Unfortunately, the mildness and variability of the *ds* mutant overgrowth made it difficult to test these alternatives using deleted Ds constructs. Instead, we tested the epistatic relationship between *ds* and *ft* in growth control, reasoning that if Ds regulated disc growth solely by regulating the activity of the intracellular domain of Ft, then removing *ds* should have no additional effect in the $ft^{G\text{-}rv}/ft^{fd}$ mutant background. As noted above, we observed a marked enhancement of the disc overgrowth phenotype in ds^{UAO71} $ft^{G\text{-}rv}/ds^{UAO71}$ ft^{fd} wing and eye-antennal discs (Fig. 4G, see Fig. S2G,N in the supplementary material). Thus, Ds must have some growth control activity that is not mediated by the intracellular domain of Ft.

The intracellular domain of Ft is sufficient for PCP

Uniform expression of *UAS-ft* with *act-gal4* or *da-gal4* (data not shown) not only rescued the viability of $ft^{G\text{-}rv}/ft^{fd}$ flies, but produced wings with largely normal PCP in distal regions; defects were

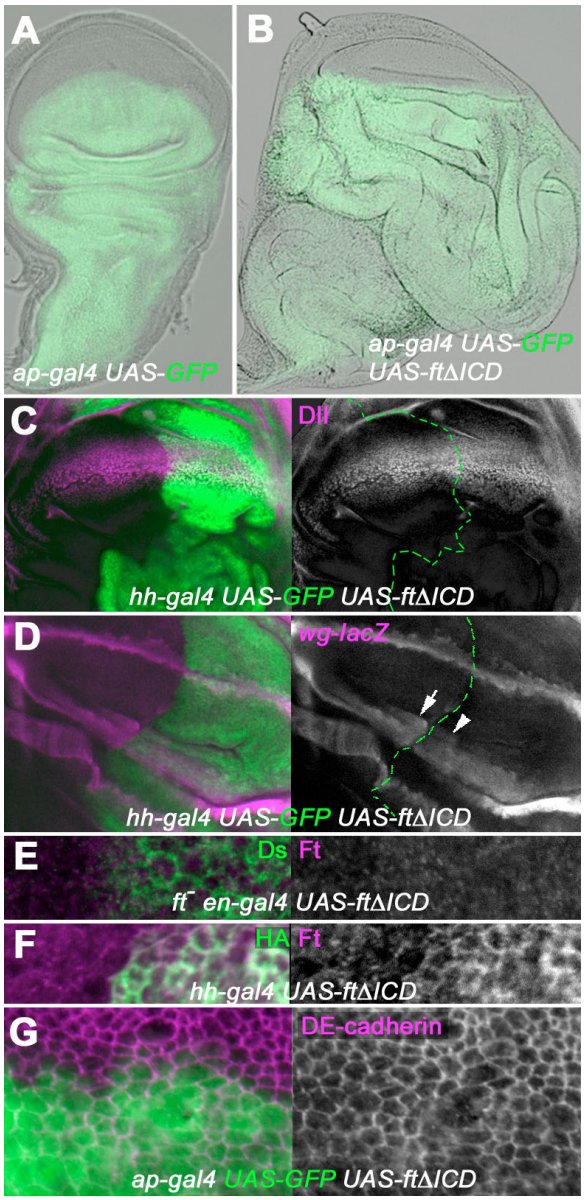

Fig. 6. Overgrowth phenotype induced by expression of *UAS-ftΔICD* in wild-type wing discs. (**A**) Control *ap-gal4 UAS-GFP* wing disc showing dorsal region of expression (green). (**B**) Overgrowth induced in dorsal cells by *ap-gal4 UAS-GFP UAS-ftΔICD*. Ventral regions lacking expression in A and B are similar in size. (**C**,**D**) Overgrowth induced by *hh-gal4 UAS-GFP UAS-ftΔICD* in posterior compartment (green) of wing pouch and prospective hinge region. There is similar expression of the wing pouch Wg target Dll (purple and right panel) in anterior and posterior cells in C. The distal ring of *wg-lacZ* expression (purple and right panel) is similar in width in cells inside (arrowhead) and just anterior to (arrow) the region of misexpression in D. (**E**,**F**) Expression of *UAS-ftΔICD* in the posterior of wing imaginal discs using *en-gal4* (E) or *hh-gal4* (F). Discs were stained using an antiserum generated against the intracellular domain of Ft (Yang et al., 2002). (E) Expression in $ft^{G\text{-}rv}/ft^{fd}$ discs. The region of misexpression was identified by the stabilization of Ds (green, left panel); the anti-Ft (purple and right panel) did not crossreact with FtΔICD. (F) Expression in wild-type discs. Endogenous Ft (purple and right panel) was stabilized in the region of FtΔICD expression, identified by the HA tag on FtΔICD (green, left panel). (**G**) Anti-DE-cadherin (purple and right panel) staining after dorsal expression of *UAS-GFP* (green) and *UAS-ftΔICD* using *ap-gal4*. Shg levels were slightly decreased dorsally.

largely limited to the region proximal and anterior to the ACV (Fig. 7D, see Fig. S4I,J in the supplementary material). Nearly identical results were obtained using *act-gal4* and *UAS-ftΔECD* (Fig. 7E, see Fig. S4K,L in the supplementary material). The normal PCP in the distal wing probably constitutes rescue of the mutant state. Although $ft^{G\text{-}rv}/ft^{fd}$ flies do not survive to produce adult wings, and the morphology of the mutant wings is too disrupted to assess PCP at pupal or pharate stages, homozygous $ft^{G\text{-}rv}$ or ft^{fd} clones disrupt PCP in a central region (Ma et al., 2003; Strutt and Strutt, 2002) that extends into the distal wing (distal L2-L3 intervein; Fig. 7C). The weaker viable ft^{18} mutation also produces PCP defects that extend distal to the PCV (Fig. 7B).

For an additional test of the rescue of *ft* mutant PCP defects, we turned to a different system, the abdomen of the adult fly. $ft^{G\text{-}rv}/ft^{fd}$ abdomens have fairly normal morphology and can be examined at pharate stages; these had characteristic swirls of hairs that had lost their normally posterior polarity (Fig. 7J) (Casal et al., 2002). This phenotype was partially rescued by misexpression of *UAS-ft* (Fig. 7K) or *UAS-ftΔECD* (Fig. 7L) using *act-gal4*. Thus, a form of Ft that cannot bind Ds can substantially rescue PCP.

The severe overgrowth and early larval-pupal lethality induced by *UAS-ftΔICD* in $ft^{G\text{-}rv}/ft^{fd}$ flies prevented us from assessing the rescuing ability of FtΔICD in either the wing or abdomen PCP assays. Therefore, as a final test of the PCP activities of deleted Ft constructs, we made use of the fact that expression of *UAS-ft* can disrupt PCP in wild-type wings (Matakatsu and Blair, 2004). Expression of *UAS-ft, UAS-ftΔECD* or *UAS-ftΔICD* with *act-gal4* all caused mild perturbation of PCP in the wing (Fig. 7F-H, see Fig. S4C-H in the supplementary material). Similar effects were observed in abdomens (data not shown). As this assay does not allow us to distinguish whether the defects were being caused by gains or losses in Ft activity, we do not know whether FtΔICD is having the same dominant-negative effects on PCP that it has on growth control.

The extracellular domain of Ds is sufficient for wing PCP

Strong *ds* mutants, such as ds^{05142}, survive and produce adult wings with widespread, characteristic PCP defects (Fig. 8B) (Adler et al., 1998). Uniform misexpression of *UAS-ds* with *tub-gal4* can rescue the PCP defect in all but the most proximal regions of the wing blade (Fig. 8C) (Matakatsu and Blair, 2004), and expression using *ds-gal4* partially rescued PCP in the proximal wing (Fig. S5D). Nearly identical rescue of PCP was obtained using *UAS-dsΔICD* and *tub-gal4* (Fig. 8D) or *ds-gal4* (see Fig. S5E in the supplementary material). Thus, the extracellular domain of Ds is sufficient to drive normal PCP. By contrast, driving UAS-*dsΔECD* using *ds-gal4* did not obviously rescue the PCP phenotype (see Fig. S5F in the supplementary material) (driving *UAS-dsΔECD* in ds^{05142} mutants using *tub-gal4* caused lethality before PCP could be assessed). These results are consistent with the model that Ds acts chiefly as a ligand for Ft in PCP.

To further compare the effects of DsΔICD and DsΔECD on PCP, we drove expression in wild type flies. We showed previously that wing hairs reorient away from regions of high *ds* misexpression [e.g. after misexpression in a gradient orthogonal to the proximodistal axis of the wing using *sal-gal4* (Fig. 8J)] distally (using *dll-gal4*) or posteriorly (using *en-gal4*) (Matakatsu and Blair, 2004). Driving misexpression of *UAS-dsΔICD* with *sal-gal4*, *dll-gal4* or *en-gal4* also caused hairs to point away from the region of misexpression (Fig. 8K and data not shown). By contrast, patterned misexpression of *UAS-dsΔECD* did not cause any change in wing PCP (*en-gal4*, Fig. 8H; *sal-gal4*, Fig. 8L; *dll-gal4*, *ap-gal4*, *tub-gal4*; data not

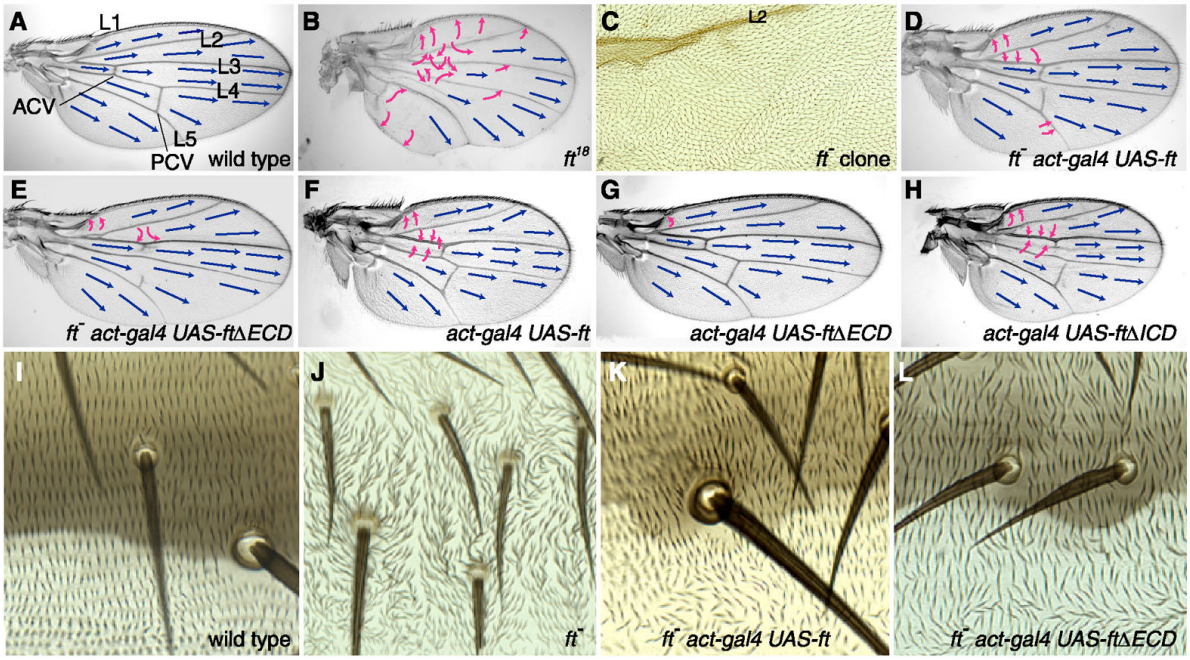

Fig. 7. Effects of full-length and deleted *ft* constructs on PCP and crossvein spacing. (A-H) Polarity of hairs in adult wings; normal polarity is indicated by blue arrows and abnormal polarity by red arrows. Crossvein spacing is reduced by all constructs. (**A**) Wild type; positions of the longitudinal (L1-L5) and crossveins (ACV, PCV) are marked. (**B**) Defects in viable ft^{18} mutant. (**C**) Defects induced by large homozygous $ft^{G\text{-}rv}$ clone (y^-) in the distal region of the wing blade between L2 and L3. The clone occupies the entire region shown. (**D**,**E**) Apparent partial rescue of PCP defects of $ft^{G\text{-}rv}/ft^{fd}$ wing with *act-gal4* and *UAS-ft* (D) or *UAS-ftΔECD* (E). (F-H) Proximal PCP defects induced in wild-type wing with *act-gal4* and *UAS-ft* (**F**), *UAS-ftΔECD* (**G**) or *UAS-ftΔICD* (**H**). Defects were milder with *UAS-ftΔECD*. (I-L) Polarity of abdominal hairs. (**I**) Wild type. (**J**) Disruption in $ft^{G\text{-}rv}/ft^{fd}$. (**K**,**L**) Partial rescue of $ft^{G\text{-}rv}/ft^{fd}$ by *act-gal4* and *UAS-ft* (K) or *UAS-ftΔECD* (L).

shown). Thus, the intracellular domain of Ds does not have any detectable activity in PCP in the absence of the extracellular domain, despite being driven at levels in excess of the endogenous protein (see Fig. S1E in the supplementary material). Again, this is consistent with the model that Ds acts chiefly as a ligand in this process.

The intracellular domain of Ds has biological activity

Although DsΔECD showed no activity in PCP, it did show biological activity in a third phenotype common to *ft* and *ds* mutants: the alteration of proximodistal wing blade patterning. Adult wings from *ds* and weaker *ft* alleles are foreshortened along the proximodistal axis of the wing blade, as indicated by the abnormally close proximity between the anterior crossvein (ACV) and the posterior crossvein (PCV); with stronger alleles, regions of one or both crossveins are lost (Fig. 7B, Fig. 8B). We have as yet been unable to rescue the crossvein spacing defects of *ft* or *ds* mutants using full-length Ft or Ds (Fig. 7D, Fig. 8C; data not shown). In fact, overexpression of full-length Ft or Ds in wild-type flies produced a crossvein spacing defect that resembled the mutant phenotype (Fig. 7F, Fig. 8E). This suggests that the normal spacing between the crossveins requires a precise level or pattern of Ft and Ds that we were unable to reproduce. A similar situation occurs with Fj, a distally expressed protein that probably modulates the activity of Ds or Ft (Ma et al., 2003; Strutt and Strutt, 2002; Strutt et al., 2004). Both losses and gains in *fj* reduce crossvein spacing (Zeidler et al., 2000), and the *fj* crossvein spacing defect is rescued only when *fj* transgene transcription is driven directly by cloned *fj* enhancers (Strutt et al., 2004).

Although we cannot use the crossvein spacing defect to distinguish between gains and losses in Ft or Ds function, it is an extremely sensitive, and thus useful, indicator of the perturbation of Ft or Ds function. All four of the Ft and Ds deletion constructs induced crossvein spacing defects (Fig. 7G,H, Fig. 8F-H). This included *UAS-dsΔECD*. Although misexpression with *tub-gal4* caused a milder crossvein defect than observed with the other Ds constructs (Fig. 8G), strong defects were observed with drivers such as *en-gal4* (Fig. 8H).

DISCUSSION

Chief amongst our findings (summarized in Table 1) is that Ft activity is not simply a byproduct of changes in cell-cell adhesion. The FtΔECD construct lacks almost the entire extracellular domain and cannot bind or stabilize Ds in vitro or in vivo. Nonetheless, it can rescue the lethality, overgrowth and PCP defects of *ft* alleles that should be null for any adhesive or receptor function, and in a wild-type background can disrupt proximodistal patterning. This suggests that the intracellular domain of Ft can act in the absence of binding between endogenous Ft and Ds, or indeed between Ft and any other extracellular ligand, as long as sufficient levels are expressed.

Conversely, we found that a form of Ft lacking the intracellular domain (FtΔICD) failed to rescue overgrowth in *ft* mutants. In fact, this form acted as a strong dominant negative, inducing overgrowth of wild-type and *ft* mutant imaginal discs. This occurred despite the ability of FtΔICD to stabilize endogenous cell surface Ds and Ft, raising the possibility that FtΔICD binds to Ds and Ft is a way that blocks their activities. We cannot, however, rule out the possibility that FtΔICD alters the activity of some additional, unknown player.

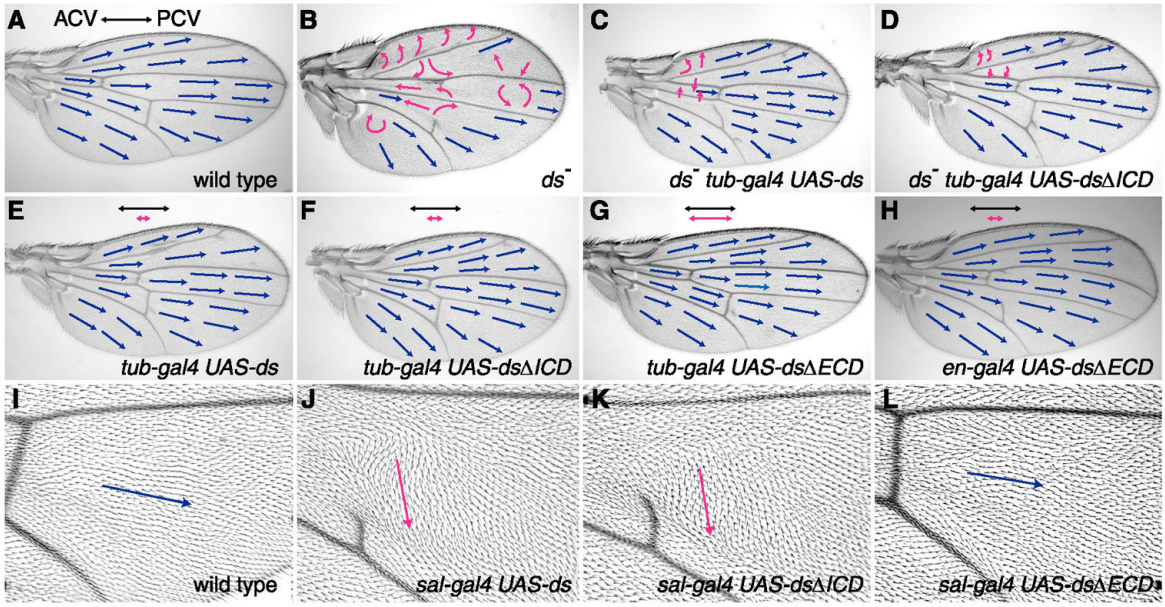

Fig. 8. Effects of full-length and deleted *ds* constructs on wing PCP and crossvein spacing. Normal polarity is indicated by blue arrows and abnormal polarity by red arrows. Proximodistal crossvein spacing in wild type (A) is indicated by a horizontal black double arrow, and is compared with abnormal spacing (red double arrow) in E-H. (**A**) Wild type. (**B**) ds^{05142}. (**C**,**D**) Partial rescue of ds^{05142} PCP defects, especially in distal wing blade, by *tub-gal4* and *UAS-ds* (C) or *UAS-dsΔICD* (D). (E-G) Expression of *UAS-ds* (**E**), *UAS-dsΔICD* (**F**) or *UAS-dsΔECD* (**G**) with *tub-gal4* did not induce PCP defects, but reduced crossvein spacing. (**H**) The crossvein spacing defect induced by *UAS-dsΔECD* was stronger using *en-gal4*, but there were no PCP defects at the sharp boundary of misexpression between L3 and L4. (I-L) PCP in the posterior wing. (**I**) Wild type. (**J**,**K**) Expression of *UAS-ds* (J) or *UAS-dsΔICD* (K) in the central region of the wing using *sal-gal4* results in the orientation of hairs away from the region of misexpression. (**L**) *sal-gal4* and *UAS-dsΔECD* has no effect on PCP.

Although lethality prevented us from determining whether FtΔICD could rescue *ft* mutant PCP defects, expression of FtΔICD in wild-type wings also disrupted PCP. These PCP defects were weaker than those observed in *ft* mutants, suggesting that FtΔICD might have stronger effects on growth control than PCP.

In contrast to Ft, the extracellular domain of Ds was sufficient for its effects on PCP. The DsΔICD construct lacks almost the entire intracellular domain, but nonetheless can rescue the PCP defects of strong *ds* mutants and disrupt PCP in wild-type wings. The DsΔECD construct, however, cannot bind or stabilize Ft and cannot rescue *ds* mutant PCP defects or influence PCP in wild-type wings. Our results thus support the hypothesis that in PCP Ds acts chiefly as a ligand for Ft, modulating its activity.

Nonetheless, we cannot rule out the possibility that the intracellular domain of Ds has some PCP activity within the context of the whole protein, and the conservation of large regions of the Ds intracellular domain in its vertebrate homologs dachsous 1 and dachsous 2 suggests that Ds may have activity beyond that of a ligand. Thus, it is intriguing that expression of DsΔECD can disrupt another *ds*-sensitive phenotype, crossvein spacing in wild-type wings. As crossvein spacing defects can result from either gains or losses in Ds or Ft function, it is possible that this defect is caused by disrupting the function of endogenous Ds, and thus the ability of that Ds to signal via Ft. However, DsΔECD did not cause any obvious change in the levels of endogenous Ds (see Fig. S1 in the supplementary material). Moreover, loss of Ds normally causes visible destabilization of cell surface Ft (Ma et al., 2003; Strutt and Strutt, 2002), and we did not see any changes in Ft levels in cells misexpressing DsΔECD (Fig. 2G).

ds mutations can also enhance the overgrowth observed in mutants that lack the intracellular domain of Ft, indicating that in overgrowth, Ds activity is not completely dependent on regulating the activity of the intracellular domain of Ft. In this respect, overgrowth differs from PCP, as *ft* mutants and *ds ft* double mutants produce identical PCP phenotypes (Ma et al., 2003). Our result

Table 1. Summary of phenotypes

	Rescues ft^{-} lethality and overgrowth	Rescues ft^{-} PCP defects	Rescues ds^{-} PCP defects	Induces overgrowth in wild type	Induces crossvein spacing defects in wild type	Induces PCP defects in wild type
Ft full length	Yes	Yes	–	No	Yes	Yes
FtΔECD	Yes	Yes	–	No	Yes, weaker	Yes, weak
FtΔICD	DN	Lethal	–	Yes	Yes	Yes
Ds full length	–		Yes	No	Yes	Yes
DsΔECD	–		No	No	Yes, weaker	No
DsΔICD	–		Yes	No	Yes	Yes

–, not determined; DN, enhanced ft^{-} overgrowth; lethal, preventing assay of PCP.

could be explained if Ds regulates growth via its intracellular domain. Alternatively, Ds may be acting as an extracellular ligand for a binding partner other than Ft.

Mechanisms of Ft and Ds signaling

Our results support the hypothesis the Ft signals via its intracellular domain in growth control, PCP and proximodistal patterning. Similarly, it is likely that the intracellular domain of Ds contributes to proximodistal patterning and perhaps growth control. The conservation of long stretches of the intracellular domain of Ft and Ds in the vertebrate homologs Fat4, dachsous 1 and dachsous 2 also suggests that there is conserved binding to intracellular factors.

There are no known binding partners for the intracellular domain of Ds or dachsous-like proteins. The intracellular domain of *Drosophila* Ft also lacks the ENA-VASP binding sites that mediate at least some of the function of vertebrate Fat1 in vitro (Moeller et al., 2004; Tanoue and Takeichi, 2004; Tanoue and Takeichi, 2005). The intracellular domain of *Drosophila* Ft can bind the atrophin Grunge, and genetic evidence suggests a link between Grunge and PCP (Fanto et al., 2003). However, it is not yet clear if Grunge acts downstream of Ft, nor is it clear how atrophins, which act as transcriptional co-repressors (Erkner et al., 2002; Zhang et al., 2002; Zoltewicz et al., 2004), could polarize cells. *grunge* mutants also do not apparently reproduce the effects of *ft* mutants on disc growth (Fanto et al., 2003; Zhang et al., 2002) or on *wg* expression in the prospective wing hinge (Cho and Irvine, 2004).

Some evidence suggests that Ds and Ft regulate growth and patterning by altering either the expression of *wg* in the prospective wing hinge or the response to Wg signaling (Cho and Irvine, 2004; Rodriguez, 2004; Jaiswal et al., 2006). However, our results make it unlikely that this can explain all but a small part of the overgrowth phenotype. The overgrowth induced by *ft* mutations or FtΔICD occurred without any consistent change in the expression of Wg target genes Dll or Vg, or in the expression of *wg*. Moreover, FtΔICD induced overgrowth in the entire wing disc, but whereas increased Wg signaling can induce overgrowth in the hinge (Neumann and Cohen, 1996), in the prospective wing blade Wg signaling reduces growth (Johnston and Sanders, 2003). Our results are consistent with the failure of mutants in the Wg signaling pathway to modify the *ft* overgrowth phenotype (Garoia et al., 2000; Garoia et al., 2005; Resino and Garcia-Bellido, 2004).

A recent study has suggested a possible link between overgrowth and Ras signaling; mild reductions in Ras function that have little effect on the growth of wild-type cells can block the overgrowth observed in *ft* mutant clones (Garoia et al., 2005). It remains to be seen whether Ft can actually affect Ras signaling, or whether this represents the convergence of the two pathways on a shared target.

Orienting PCP in the wing

Because Ds is expressed in an apparently graded fashion along the axes of polarity, it was suggested that Ds provides a global cue that orients PCP in the eye, wing and abdomen (Casal et al., 2002; Ma et al., 2003; Yang et al., 2002). But whereas patterned Ds misexpression is sufficient to reorient PCP, and patterned Ds expression does appear to be necessary for normal PCP in the eye, in the wing uniform Ds expression is able to rescue most of the *ds* mutant PCP defects (Matakatsu and Blair, 2004; Simon, 2004). This suggests that most of the PCP defects in *ds* mutant wings are caused, not by a change in the spatial regulation of Ds-Ft signaling, but rather by the loss of a basal level of signaling required for the proper activity of some other polarizing cue. These results left open the possibility that Ft activity is being spatially regulated by an extracellular ligand other than Ds. However, we show here that *ft* mutant PCP defects can be substantially rescued by uniform expression of FtΔECD, a form of Ft that cannot bind Ds, or probably any other ligand.

There is, however, a region in the proximal wing where we were unable to rescue PCP defects with uniform expression of either Ds (Matakatsu and Blair, 2004), Ft, or FtΔECD. This is also the region of the wing where there is a boundary or sharp gradient between proximal regions with high and distal regions with low *ds* expression (Matakatsu and Blair, 2004) (see Fig. S5A,B in the supplementary material). Thus, it remains possible that Ds and Ft activities are permissive in much of the wing but, in the proximal wing, spatially instructive. The different sensitivities of different regions to changes in Ds and Ft may reflect localized differences in the strength of other partially redundant polarizing cues.

We thank Drs P. Adler, J. Axelrod, P. Bryant, S. Carroll, S. Hayashi, Y. Hiromi, H. McNeill, M. Simon, D. Strutt and T. Uemura; the Developmental Studies Hybridoma Bank and the Bloomington *Drosophila* Stock center for fly stocks, DNA and antibodies; Dr J. Pawley for use of his confocal microscope; and Dr M. Tanaka-Matakatsu for discussions. This work was supported by grants from NIH (R01-NS028202) and NSF (IBN-0416586).

Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/133/12/2315/DC1

References

Adler, P. N., Charlton, J. and Liu, J. (1998). Mutations in the cadherin superfamily member gene *dachsous* cause a tissue polarity phenotype by altering *frizzled* signaling. *Development* **125**, 959-968.

Amonlirdviman, K., Khare, N. A., Tree, D. R., Chen, W. S., Axelrod, J. D. and Tomlin, C. J. (2005). Mathematical modeling of planar cell polarity to understand domineering nonautonomy. *Science* **307**, 423-426.

Bendtsen, J. D., Nielsen, H., von Heijne, G. and Brunak, S. (2004). Improved prediction of signal peptides: SignalP 3.0. *J. Mol. Biol.* **340**, 783-795.

Bilder, D. (2004). Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. *Genes Dev.* **18**, 1909-1925.

Blair, S. S. (1994). A role for the segment polarity gene *shaggy-zeste white 3* in the specification of regional identity in the developing wing of *Drosophila*. *Dev. Biol.* **162**, 229-244.

Brand, A. H. and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415.

Brodsky, M. H. and Steller, H. (1996). Positional information along the dorsal-ventral axis of the *Drosophila* eye: graded expression of the four-jointed gene. *Dev. Biol.* **173**, 428-446.

Bryant, P. J., Huettner, B., Held, L. I., Jr, Ryerse, J. and Szidonya, J. (1988). Mutations at the *fat* locus interfere with cell proliferation control and epithelial morphogenesis in *Drosophila*. *Dev. Biol.* **129**, 541-554.

Casal, J., Struhl, G. and Lawrence, P. A. (2002). Developmental compartments and planar polarity in *Drosophila*. *Curr. Biol.* **12**, 1189-1198.

Cho, E. and Irvine, K. D. (2004). Action of *fat*, *four-jointed*, *dachsous* and *dachs* in distal-to-proximal wing signaling. *Development* **131**, 4489-4500.

Clark, H. F., Brentrup, D., Schneitz, K., Bieber, A., Goodman, C. and Noll, M. (1995). Dachsous encodes a member of the cadherin superfamily that controls imaginal disc morphogenesis in *Drosophila*. *Genes Dev.* **9**, 1530-1542.

Erkner, A., Roure, A., Charroux, B., Delaage, M., Holway, N., Core, N., Vola, C., Angelats, C., Pages, F., Fasano, L. et al. (2002). Grunge, related to human Atrophin-like proteins, has multiple functions in *Drosophila* development. *Development* **129**, 1119-1129.

Fanto, M. and McNeill, H. (2004). Planar polarity from flies to vertebrates. *J. Cell. Sci.* **117**, 527-533.

Fanto, M., Clayton, L., Meredith, J., Hardiman, K., Charroux, B., Kerridge, S. and McNeill, H. (2003). The tumor-suppressor and cell adhesion molecule Fat controls planar polarity via physical interactions with Atrophin, a transcriptional co-repressor. *Development* **130**, 763-774.

Garoia, F., Guerra, D., Pezzoli, M. C., Lopez-Varea, A., Cavicchi, S. and Garcia-Bellido, A. (2000). Cell behaviour of *Drosophila fat* cadherin mutations in wing development. *Mech. Dev.* **94**, 95-109.

Garoia, F., Grifoni, D., Trotta, V., Guerra, D., Pezzoli, M. C. and Cavicchi, S. (2005). The tumor suppressor gene *fat* modulates the EGFR-mediated proliferation control in the imaginal tissues of *Drosophila melanogaster*. *Mech. Dev.* **122**, 175-187.

Hajra, K. M. and Fearon, E. R. (2002). Cadherin and catenin alterations in human cancer. *Genes Chromosomes Cancer* **34**, 255-268.
Hong, J. C., Ivanov, N. V., Hodor, P., Xia, M., Wei, N., Blevins, R., Gerhold, D., Borodovsky, M. and Liu, Y. (2004). Identification of new human cadherin genes using a combination of protein motif search and gene finding methods. *J. Mol. Biol.* **337**, 307-317.
Jaiswal, M., Agrawal, N. and Sinha, P. (2006). Fat and Wingless signaling oppositely regulate epithelial cell-cell adhesion and distal wing development in *Drosophila*. *Development* **133**, 925-935.
Johnston, L. A. and Gallant, P. (2002). Control of growth and organ size in *Drosophila*. *BioEssays* **24**, 54-64.
Johnston, L. A. and Sanders, A. L. (2003). Wingless promotes cell survival but constrains growth during *Drosophila* wing development. *Nat. Cell Biol.* **5**, 827-833.
Krogh, A., Larsson, B., von Heijne, G. and Sonnhammer. E. L. L. (2001). Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. *J. Mol. Biol.* **305**, 567-580.
Ma, D., Yang, C. H., McNeill, H., Simon, M. A. and Axelrod, J. D. (2003). Fidelity in planar cell polarity signalling. *Nature* **421**, 543-547.
Mahoney, P. A., Weber, U., Onofrechuk, P., Biessmann, H., Bryant, P. J. and Goodman, C. S. (1991). The *fat* tumor suppressor gene in *Drosophila* encodes a novel member of the cadherin gene superfamily. *Cell* **67**, 853-868.
Matakatsu, H. and Blair, S. S. (2004). Interactions between Fat and Dachsous and the regulation of planar cell polarity in the *Drosophila* wing. *Development* **131**, 3785-3794.
Moeller, M. J., Soofi, A., Braun, G. S., Li, X., Watzl, C., Kriz, W. and Holzman, L. B. (2004). Protocadherin FAT1 binds Ena/VASP proteins and is necessary for actin dynamics and cell polarization. *EMBO J.* **23**, 3769-3779.
Nakajima, D., Nakayama, M., Kikuno, R., Hirosawa, M., Nagase, T. and Ohara, O. (2001). Identification of three novel non-classical cadherin genes through comprehensive analysis of large cDNAs. *Brain Res. Mol. Brain Res.* **94**, 85-95.
Neumann, C. J. and Cohen, S. M. (1996). Distinct mitogenic and cell fate specification functions of wingless in different regions of the wing. *Development* **122**, 1781-1789.
Neumann, C. J. and Cohen, S. M. (1997). Long-range action of Wingless organizes the dorsal-ventral axis of the *Drosophila* wing. *Development* **124**, 871-880.
Oda, H., Uemura, T., Harada, Y., Iwai, Y. and Takeichi, M. (1994). A *Drosophila* homolog of Cadherin associated with Armadillo and essential for embryonic cell-cell adhesion. *Dev. Biol.* **165**, 716-726.
Panganiban, G., Sebring, A., Nagy, L. and Carroll, S. (1995). The development of crustacean limbs and the evolution of arthropods. *Science* **270**, 1363-1366.
Patel, N. H., Martin-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S. (1989). Expression of Engrailed proteins in arthropods, annelids, and chordates. *Cell* **58**, 955-968.
Rawls, A. S., Guinto, J. B. and Wolff, T. (2002). The cadherins Fat and Dachsous regulate dorsal/ventral signaling in the *Drosophila* eye. *Curr. Biol.* **12**, 1021-1026.
Resino, J. and Garcia-Bellido, A. (2004). *Drosophila* genetic variants that change cell size and rate of proliferation affect cell communication and hence patterning. *Mech. Dev.* **121**, 351-364.
Rodriguez, I. (2004). The *dachsous* gene, a member of the cadherin family, is required for Wg-dependent pattern formation in the *Drosophila* wing disc. *Development* **131**, 3195-3206.
Saburi, S. and McNeill, H. (2005). Organising cells into tissues: new roles for cell adhesion molecules in planar cell polarity. *Curr. Opin. Cell Biol.* **17**, 482-488.
Simon, M. A. (2004). Planar cell polarity in the *Drosophila* eye is directed by graded Four-jointed and Dachsous expression. *Development* **131**, 6175-6184.
Strutt, H. and Strutt, D. (2002). Nonautonomous planar polarity patterning in *Drosophila*: dishevelled-independent functions of frizzled. *Dev. Cell* **3**, 851-863.
Strutt, H., Mundy, J., Hofstra, K. and Strutt, D. (2004). Cleavage and secretion is not required for Four-jointed function in Drosophila patterning. *Development* **131**, 881-890.
Takeda, H., Shimoyama, Y., Nagafuchi, A. and Hirohashi, S. (1999). E-cadherin functions as a cis-dimer at the cell-cell adhesive interface in vivo. *Nat. Struct. Biol.* **6**, 310-312.
Tanoue, T. and Takeichi, M. (2004). Mammalian Fat1 cadherin regulates actin dynamics and cell-cell contact. *J. Cell Biol.* **165**, 517-528.
Tanoue, T. and Takeichi, M. (2005). New insights into Fat cadherins. *J. Cell Sci.* **118**, 2347-2353.
Tepass, U. (1999). Genetic analysis of cadherin function in animal morphogenesis. *Curr. Opin. Cell Biol.* **11**, 540-548.
Uemura, T. and Shimada, Y. (2003). Breaking cellular symmetry along planar axes in *Drosophila* and vertebrate. *J. Biochem.* **134**, 625-630.
Villano, J. L. and Katz, F. N. (1995). four-jointed is required for intermediate growth in the proximal-distal axis in *Drosophila*. *Development* **121**, 2767-2777.
Waddington, C. H. (1940). The genetic control of wing development in *Drosophila*. *J. Genet.* **41**, 75-139.
Williams, J. A., Bell, J. B. and Carroll, S. B. (1991). Control of *Drosophila* wing and haltere development by the nuclear vestigial gene product. *Genes Dev.* **5**, 2481-2495.
Yang, C. H., Axelrod, J. D. and Simon, M. A. (2002). Regulation of Frizzled by Fat-like cadherins during planar polarity signaling in the Drosophila compound eye. *Cell* **108**, 675-688.
Zeidler, M. P., Perrimon, N. and Strutt, D. I. (2000). Multiple roles for *four-jointed* in planar polarity and limb patterning. *Dev. Biol.* **228**, 181-196.
Zhang, S., Xu, L., Lee, J. and Xu, T. (2002). *Drosophila* Atrophin homolog functions as a transcriptional corepressor in multiple developmental processes. *Cell* **108**, 45-56.
Zoltewicz, J. S., Stewart, N. J., Leung, R. and Peterson, A. S. (2004). Atrophin 2 recruits histone deacetylase and is required for the function of multiple signaling centers during mouse embryogenesis. *Development* **131**, 3-14.