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Drosophila wing development in the absence of dorsal identity

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SUMMARY

The developing wing disc of *Drosophila* is divided into distinct lineage-restricted compartments along both the anterior/posterior (A/P) and dorsal/ventral (D/V) axes. At compartment boundaries, morphogenic signals pattern the disc epithelium and direct appropriate outgrowth and differentiation of adult wing structures. The mechanisms by which affinity boundaries are established and maintained, however, are not completely understood. Compartment-specific adhesive differences and inter-compartment signaling have both been implicated in this process. The selector gene *apterous* (*ap*) is expressed in dorsal cells of the wing disc and is essential for D/V compartmentalization, wing margin formation, wing outgrowth and dorsal-specific wing structures. To better understand the mechanisms of Ap function and compartment formation, we have rescued aspects of the *ap* mutant phenotype with genes known to be downstream of Ap. We show that Fringe (Fng), a secreted protein involved in modulation of Notch signaling, is sufficient to rescue D/V compartmentalization, margin formation and wing outgrowth when appropriately expressed in an *ap* mutant background. When Fng and α_{PS1}, a dorsally expressed integrin subunit, are co-expressed, a nearly normal-looking wing is generated. However, these wings are entirely of ventral identity. Our results demonstrate that a number of wing development features, including D/V compartmentalization and wing vein formation, can occur independently of dorsal identity and that inter-compartmental signaling, refined by Fng, plays the crucial role in maintaining the D/V affinity boundary. In addition, it is clear that key functions of the *ap* selector gene are mediated by only a small number of downstream effectors.

Key words: *Drosophila*, *apterous*, *fringe*, PS1, Compartmentalization

INTRODUCTION

During development, the *Drosophila* wing imaginal disc is transformed from a simple epithelial sheet into a complex adult wing structure. This transformation relies on selector genes (Garcia-Bellido, 1975) that act within the developmental field to differentiate cell types and direct specific morphogenic processes. Compartmental cell lineage restrictions, along both the anterior/posterior (A/P) and dorsal/ventral (D/V) axes of the wing disc, are early indications of cellular differentiation and selector gene function (Garcia-Bellido et al., 1973; Morata and Lawrence, 1975). As development proceeds, borders between compartments serve as organizing centers, patterning the disc epithelium and directing outgrowth of wing tissue.

Two transcription factors, Engrailed (En) and Apterous (Ap) serve as selector genes for the posterior and dorsal compartments, respectively, and regulate genetic cascades essential for cellular compartmentalization and wing morphogenesis. En is expressed in the posterior half of the wing disc beginning during embryogenesis (Kornberg et al., 1985). En cells produce the secreted molecule Hedgehog (Hh) (Tabata et al., 1992; Zecca et al., 1995), and Hh signaling to adjacent anterior cells is required for A/P compartmentalization (Blair and Ralston, 1997; Rodriguez and Basler, 1997; Dahmann and Basler, 2000). In addition, Hh induces Decapentaplegic (Dpp) expression in a narrow band of anterior cells (Basler and Struhl, 1994; Zecca et al., 1995). Dpp acts downstream of En and Hh as a morphogen, determining cell fates along the A/P axis and directing wing outgrowth (Zecca et al., 1995; Kim et al., 1996; Nellen et al., 1996). Therefore, *en* is the selector gene for the posterior compartment and directs signaling events that participate in many aspects of wing development.

In much the same way, *ap* acts as the selector gene for the dorsal compartment. Ap expression in the dorsal wing disc begins during the second larval instar (Cohen et al., 1992; Diaz-Benjumea and Cohen, 1993; Williams et al., 1993), and at this stage establishes the D/V affinity boundary (Blair et al., 1994). Activation of effectors downstream of Ap, including Fringe (Fng) and Serrate (Ser) (Irvine and Wieschaus, 1994; Bachmann and Knust, 1998), result in Notch activation specifically at the D/V boundary (Fleming et al., 1997; Panin et al., 1997). Both Fng and Notch have been shown to play key roles in D/V compartmentalization (Micchelli and Blair, 1999; Rauskolb et al., 1999). Notch signaling induces Wingless (Wg) expression at the D/V boundary (Diaz-Benjumea and Cohen, 1995), which patterns the D/V axis and directs wing outgrowth (Zecca et al., 1996; Neumann and Cohen, 1997). Finally, dorsal-

specific sensory cells along the wing margin require Ap for their proper differentiation (Diaz-Benjumea and Cohen, 1993). In this way Ap and En together establish the early coordinate system of the developing wing disc, activate signaling processes essential for wing outgrowth, and direct cell fate decisions appropriate for their respective compartments.

One controversy in the wing development field centers on the mechanisms by which selector genes define compartment boundaries. Early experiments suggested that adhesive differences between cells of adjacent compartments prevent cell mixing (Garcia-Bellido, 1975). Reaggregation experiments demonstrated that imaginal disc cells have different adhesive properties (Fausto-Sterling and Hsieh, 1987), and that these adhesive differences are specified cell autonomously as a result of selector gene function (Garcia-Bellido, 1968; Garcia-Bellido and Santamaria, 1972). Recently, however, there is increasing evidence to suggest that signaling between compartments plays an important role in boundary formation. Disruptions in either the Hh or Notch signaling pathways result in wing disc compartmentalization defects (Blair and Ralston, 1997; Rodriguez and Basler, 1997; Micchelli and Blair, 1999; Rauskolb et al., 1999). An emerging view is that these two mechanisms (intercompartmental signaling and compartment-specific adhesive differences) are both mediated by selector gene function, and both participate in regionalization of the wing disc epithelium (Dahmann and Basler, 2000).

To better understand the mechanisms of compartment formation and selector gene function, we have focused on the LIM homeodomain transcription factor Apterous. Using the GAL4 system (Brand and Perrimon, 1993), we have attempted to rescue aspects of the *ap* mutant phenotype with molecules known to be downstream of Ap function. In the absence of Ap, we show that the secreted molecule Fng is sufficient to restore D/V compartmentalization, wing margin formation and wing outgrowth of the developing wing disc. When co-expressed with α_{PS1}, an integrin subunit, morphologically normal-looking wings are generated, although they are composed entirely of ventral cell types. These results demonstrate that many aspects of wing development, including D/V compartmentalization and wing vein patterning, occur independently of dorsal identity, and that inter-compartmental signaling, refined by Fng, plays the crucial role in maintaining the D/V affinity boundary. In addition, our results suggest that while Ap may indeed regulate many genes to confer dorsal identity, in the generation of the overall morphology of the wing, Ap regulates only a small number of downstream effectors.

MATERIALS AND METHODS

Fly strains and genetics

For independent genotyping of larval discs and pupal wings, lines were balanced over the marked chromosome *CyO, wingless-lacZ*. ap^{rK568} (Cohen et al., 1992), ap^{GAL4} (Calleja et al., 1996), *UAS-fng* (Kim et al., 1995), ap^{ts78j} (Wilson, 1981) and *UAS-*α_{PS1} (Brabant et al., 1996) have been described previously. All crosses were carried out at 25°C.

Immunohistochemistry and dissections

X-Gal staining was performed as described previously (Klambt et al., 1991) on wing discs fixed in 4% paraformaldehyde for 15 minutes, or adult wings from newly eclosed flies briefly immersed in 50% glutaraldehyde. White pupae were collected and allowed to develop for 30 hours at 25°C. Following dissection, the pupal wings were fixed in 4% paraformaldehyde for 20 minutes. Subsequent HRP immunostaining was performed as described previously (Callahan and Thomas, 1994). Adult wings were dissected in isopropanol and mounted in Canada Balsam (Sigma). Fluorescent immunochemistry was performed as described previously (O'Keefe et al., 1998). Antibody dilutions were: monoclonal α-Delta 1:10, monoclonal α-En 1:10, monoclonal α-Wg 1:50, rabbit α-β-Gal 1:10,000 (Cappel). Preparations labeled with fluorescence were imaged using a Zeiss LSM 510 confocal microscope and images compiled with Adobe Photoshop.

RESULTS

The *ap* mutant phenotype

The insertional mutation ap^{rK568} is a strong mutant allele of *ap* and expresses *lacZ* in Ap cells (Diaz-Benjumea and Cohen, 1993). In ap^{rK568}/+ wing discs, β-galactosidase (β-gal) activity is confined to the dorsal compartment of the wing disc, where Ap is normally expressed (Fig. 1A), forming a sharp boundary with ventral cells (Fig. 2B). The wings of ap^{rK568}/+ adult flies are indistinguishable from wild type (Fig. 2A). ap^{GAL4} is another strong mutant allele of *ap*, and expresses the yeast transcriptional activator GAL4 in Ap cells (Calleja et al., 1996; O'Keefe et al., 1998). Since there is no significant autoregulation of the *ap* locus, cells of dorsal origin in the wing

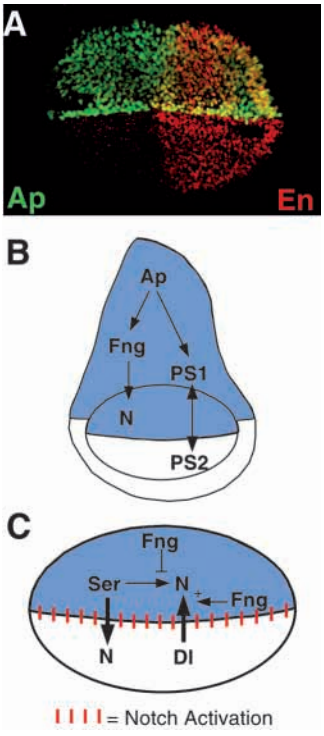

Fig. 1. *apterous* (*ap*) expression and function in the wing disc. ap^{rK568} is an insertional allele of *ap* that results in β-gal expression in Ap cells. (A) Wing pouch of late third instar wing disc (genotype ap^{rK568}/+) stained for β-gal (green) and Engrailed (En; red). Dorsal is upwards, anterior towards the left. Ap cells (marked by β-gal) are confined to the dorsal compartment and En protein defines the posterior compartment. Thus, the wing pouch is divided into four quadrants. (B) A late third instar wing disc. Dorsal is upwards. Both the secreted molecule Fng, and the α_{PS1} integrin subunit lie downstream of *ap* function in the dorsal compartment of the wing disc. Fng binds the transmembrane receptor Notch (N) and alters the responsiveness of Notch to its ligands, while PS1 cooperates with PS2 (a ventrally expressed integrin) to mediate adhesion between the two wing surfaces later in development. (C) Fng activity restricts N signaling to the D/V boundary. Fng blocks Ser activation of N cell autonomously, but potentiates Delta (Dl) signaling. In this way, Ser signals only to ventral cells, and Dl (expressed highest ventrally) signals to dorsal cells, resulting in a band of N activation straddling the D/V boundary (red lines).

disc maintain both *lacZ* and GAL4 expression in ap^{rK568}/ap^{GAL4} mutant individuals. In contrast to $ap^{rK568}/+$ discs, the D/V compartment boundary of ap^{rK568}/ap^{GAL4} wing discs is highly irregular (Fig. 2D). Dorsal and ventral cells are intermixed, indicating a loss of D/V compartmentalization. The resulting adult wings are almost completely eliminated and show no evidence of a wing margin (Fig. 2C). These phenotypes can be completely rescued by resupplying Ap from a *UAS-ap* transgene under the control of ap^{GAL4} (O'Keefe et al., 1998). Thus, ap^{GAL4}/ap^{rK568} flies provide an effective means for testing the specific roles of genes downstream of Ap by expressing them in the absence of Ap function.

Fringe function

Within the developing wing disc, Ap expression commences in the second larval instar (Diaz-Benjumea and Cohen, 1993; Williams et al., 1993) and functions to create and maintain the D/V affinity boundary (Blair et al., 1994). Fng is a secreted molecule also expressed in the dorsal compartment of the wing disc, beginning in the second instar. Fng expression depends on Ap function, suggesting that the *fng* gene may be directly regulated by Ap (Irvine and Wieschaus, 1994). It is well established that Fng plays a crucial role in wing margin formation and wing outgrowth through modulation of the Notch pathway (Fleming et al., 1997; Panin et al., 1997). Fng functions as a glycosyltransferase enzyme, interacting directly with Notch in the Golgi and altering the binding and responsiveness of Notch to its ligands (Yuan et al., 1997; Bruckner et al., 2000; de Celis and Bray, 2000; Ju et al., 2000). At the D/V boundary, two Notch ligands are expressed: Ser in dorsal cells (Thomas et al., 1991; Bachmann and Knust, 1998) and Delta (Dl) at highest concentrations ventrally (Doherty et al., 1996; Klein and Arias, 1998). Fng cell autonomously affects Notch signaling, rendering dorsal cells both refractory to Ser and sensitive to Dl (Panin et al., 1997). In this way, Ser signals only to ventral cells, and Dl signals to dorsal cells, resulting in a broad band of Notch activation that straddles the D/V boundary (Fig. 1C). Notch activation leads to Wingless, Vestigial and Cut expression, which in turn specify the wing margin and pattern cell fates along the D/V axis (Couso et al., 1995; Diaz-Benjumea and Cohen, 1995; Doherty et al., 1996; de Celis and Bray, 1997).

Fringe is sufficient to rescue D/V compartmentalization and wing outgrowth

To further test the role that Fng plays in wing development, we expressed Fng in *ap* mutant cells and asked if it was capable of rescuing aspects of the *ap* mutant wing phenotype. In ap^{rK568}/ap^{GAL4}*; UAS-fng/+* wing discs, a sharp boundary between dorsal and ventral cells is restored, indicating that Fng is capable of rescuing the *ap* D/V compartmentalization defects (Fig. 2F). No violations of the compartment boundary were observed in 15 of 18 discs examined. Adult flies of this genotype have wings of roughly normal size with a clearly demarcated wing margin. However, the dorsal and ventral wing surfaces are not adhered to one another, resulting in a balloon-like structure (Fig. 2E). Staining these adult wings for β-gal activity demonstrated that there is no mixing between dorsal and ventral cells, and that the D/V boundary precisely aligns with the wing margin (Fig. 2I). Thus, when specifically expressed in dorsal cells of *ap* mutant wing discs, Fng is sufficient to generate the D/V affinity boundary, wing margin and outgrowth. However, closer inspection

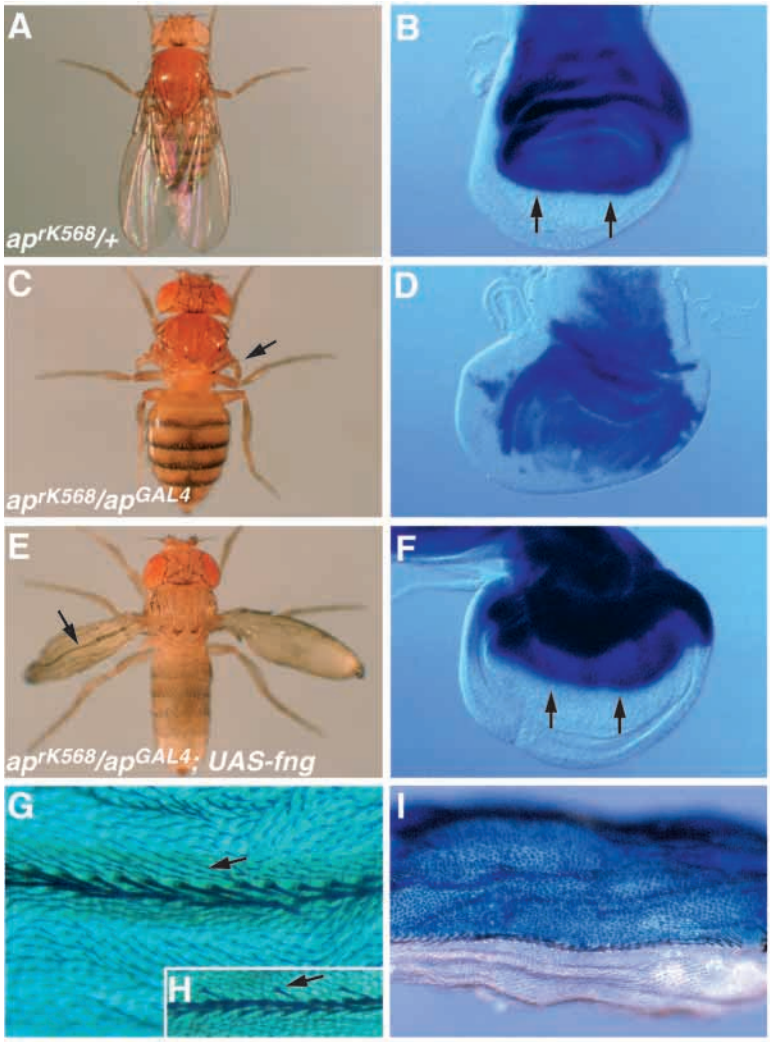

Fig. 2. Fng is sufficient for D/V compartmentalization of the wing disc, wing margin formation and wing outgrowth. (A-F) Pairs of panels showing adult flies (A,C,E) or third instar wing discs (B,D,F) of the given genotype. Wing discs were stained for β-gal activity; dorsal is upwards. In flies heterozygous for ap^{rK568} the adult wings are normal (A), and a sharp border exists between the dorsal and ventral cells of the wing disc (B). Arrows point to the D/V boundary. In ap^{rK568}/ap^{GAL4} mutant individuals, the wings are almost completely eliminated (arrow in C) and there is mixing between the dorsal and ventral cells of the wing disc (D). Resupplying Fng specifically in *ap* mutant cells of the wing disc generates wing outgrowth and a distinct margin (arrow), but the dorsal and ventral surfaces are unfused (E). In the wing disc, the border between dorsal and ventral cells is restored (arrows in F). (G) Higher magnification of the Fng-rescued wing margin. The anterior margin consists entirely of ventral bristle types. Arrow indicates missing dorsal-specific sensory bristles normally found in wild type (arrow in H). Staining the adult Fng-rescued wing for β-gal activity reveals the sharp border between dorsal and ventral cells aligns precisely with the wing margin (I).

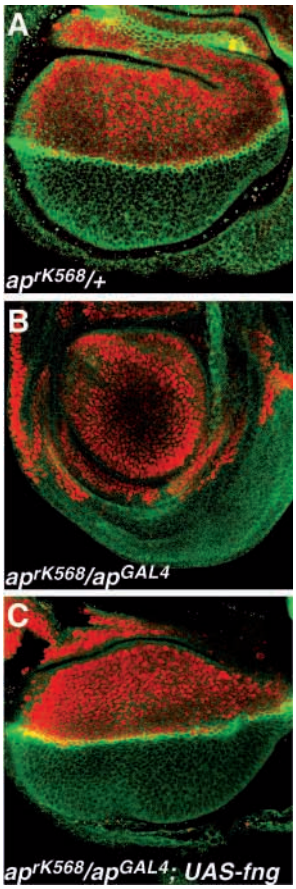

Fig. 3. Fng rescues Wingless expression at the D/V boundary. (A-C) High magnification views of late third instar wing pouches stained for β-gal (red) to mark the dorsal Ap cells, and Wingless (green). Dorsal is upwards. (A) In $ap^{rK568}/+$ discs a straight border exists between dorsal and ventral cells, and a stripe of Wingless expression is positioned at the D/V boundary. (B) In *ap* mutant wing discs (genotype: ap^{rK568}/ap^{GAL4}) dorsal and ventral cells intermingle, and there is no Wg expression in the wing pouch. (C) Resupplying Fng expression in dorsal cells of an *ap* mutant wing disc (genotype: ap^{rK568}/ap^{GAL4}; *UAS-fng/+*) rescues compartmentalization and Wg expression at the D/V boundary.

revealed that the Fng-rescued wing margin is ventralized. Dorsal-specific sensory bristles along the anterior margin are completely missing and cells on both sides of the margin secrete ventral bristle types (Fig. 2G,H). Thus, the D/V affinity boundary can be established independently of dorsal compartment identity.

Molecular markers confirm that Fng rescues D/V compartmentalization, and positions a stripe of Wg expression at the D/V boundary. In wild-type wing discs, Notch activation is confined to the border between Ap-expressing and non-Ap-expressing cells. Notch activation induces a band of Wg expression symmetrically distributed across the D/V boundary (Fig. 3A). In *ap* mutants, there is no D/V compartment boundary, and subsequently, no stripe of Wg expression (Fig. 3B). Expressing Fng in *ap* mutant cells, rescues the *ap* compartmentalization phenotype and restores Wg expression to the D/V boundary (Fig. 3C).

A previous study by Milan and Cohen (1999) concluded that Fng was insufficient to rescue the *ap* compartmentalization phenotype. Based on this report, we repeated our experiment using their genetic reagents. Similar to their findings, we observed wing margin defects in adult flies when rescuing the *ap* phenotype with *EP(3)3082*, an EP insertion that allows GAL4-dependent induction of *fng* (genotype: ap^{rK568}/ap^{GAL4}; *EP(3)3082/+*) (data not shown). However, when we introduced the *UAS-fng* line used in our study into their genetic background (genotype: ap^{UGO35}/ap^{GAL4}; *UAS-fng/+*), we observed no wing margin defects (data not shown). This strongly suggests that the opposing results of the two studies are due to differences in the level of expression between the *UAS-fng* and *EP(3)3082* lines, where *UAS-fng* expresses higher level of Fng than *EP(3)3082*, and is thus capable of rescuing the compartmentalization phenotype associated with either *ap* allelic combination.

α_{PS1} functions to fuse the dorsal and ventral wing surfaces

Fng rescues the *ap* wing phenotype to a significant extent, but fails to restore completely a normal-looking wing, suggesting that other genes controlling wing morphogenesis lie downstream of Ap. In an attempt to rescue the balloon-like phenotype characteristic of the Fng-rescued *ap* mutant wings, we co-expressed the position-specific (PS) α-integrin subunit α_{PS1}. For these experiments we used the *ap* mutant combination ap^{ts78j}/ap^{GAL4} that at 25ºC, like ap^{rK568}/ap^{GAL4}, results in a nearly complete loss of wings that can be rescued to a balloon-like structure by Fng. The PS1 and PS2 integrins consist of specific α-integrin subunits (α_{PS1} or α_{PS2}) dimerized to a common β-integrin subunit (Leptin et al., 1987). In the developing wing disc PS1 and PS2 are expressed in the dorsal and ventral compartments, respectively (Brower et al., 1984), and are required for the fusion of the dorsal and ventral wing surfaces (Brower and Jaffe, 1989; Brabant et al., 1996), but not for D/V compartmentalization (Brower and Jaffe, 1989). α_{PS1}, encoded by the gene *multiple edematous wings* (Brower et al., 1995), is specifically upregulated when Ap is misexpressed ventrally, placing it downstream of Ap function (Blair et al., 1994). When expressed in an *ap* mutant background under the control of ap^{GAL4}, α_{PS1} alone does not rescue any aspect of the *ap* wing phenotype; the wing disc D/V boundary and adult wing structure of these individuals are indistinguishable from *ap* mutants (data not shown). However, when α_{PS1} is co-expressed with Fng in an *ap* mutant background, morphologically wild-type-looking wings are generated (Fig. 4). General features including the overall size and shape, apposition of the two surfaces, position of the wing margin and pattern of wing veins are remarkably normal.

The Fng+α_{PS1}-rescued wings consist entirely of ventral cell types

Closer inspection of the Fng+α_{PS1}-rescued wings revealed that although their overall morphology is wild type in appearance, by a number of criteria they consist entirely of ventral cell types. First, in wild-type wings, the anterior wing margin has sensory bristles specific to both the dorsal and ventral wing surfaces (Fig. 4E). Fng+α_{PS1}-rescued wing margins lack the dorsal-specific bristle types and instead have ventral bristle types on both surfaces (Fig. 4F). Second, Fng+α_{PS1}-rescued wings have many more alula bristles (Fig. 4H), which in wild type are derived from only the ventral surface (Milan and Cohen, 1999; Fig. 4G). Third, a mechanosensory campaniform sensilla that is normally located only on the ventral surface of L3 is duplicated and present on both surfaces of the Fng+α_{PS1}-rescued wing (compare Fig. 4I with Fig. 4J). Finally, the morphology of the wing veins indicates that they are entirely of ventral identity. In wild type, each wing vein is composed of structurally asymmetric dorsal and ventral components that are precisely aligned. Veins consist of one major (convex) and one minor (concave) component, causing them to bulge either in a dorsal

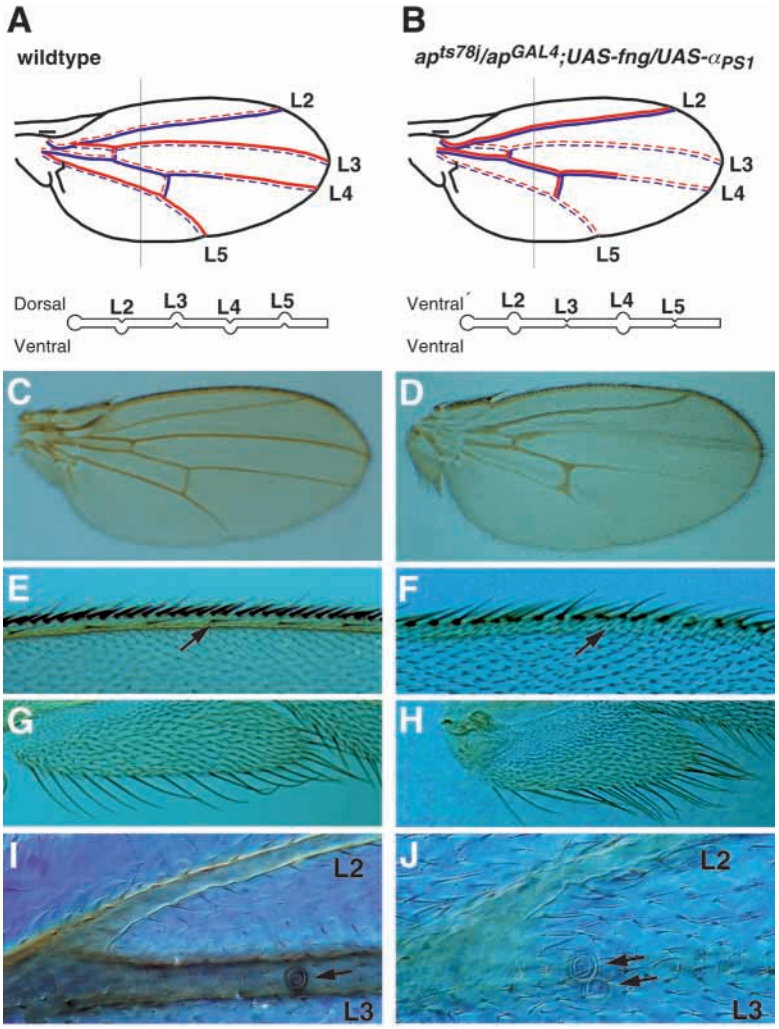

Fig. 4. Co-expression of Fng and α_{PS1} in *ap* mutants generates a ventralized wing. Wild type (A,C,E,G,I) or *ap* mutants expressing both Fng and α_{PS1} from UAS transgenes (B,D,F,H,J) (genotype ap^{ts78j}/ap^{GAL4}; *UAS-fng/UAS-*α_{PS1}). (A) The wild-type wing shown in C. The major longitudinal veins are labeled L2-L5. Each wing vein is composed of both a dorsal and ventral component. Dorsal components are labeled red; ventral are blue. Veins consist of one major (convex) and one minor (concave) component causing each vein to bulge either in a dorsal or ventral direction. Major vein components are indicated by unbroken lines while minor components are indicated by broken lines. L3, L5 and distal L4 bulge dorsally, while L2 and proximal L4 bulge ventrally. Cross-section shows the corrugation pattern of the proximal wing. (B) The Fng+α_{PS1}-rescued wing shown in D. Veins differentiate normally, but are entirely of ventral identity. Cross-section illustrates the double-ventral vein corrugation pattern. (E,F) Comparison of a wild-type wing margin (E) and a Fng+α_{PS1}-rescued margin in which bristles that differentiate on the dorsal surface have a ventral morphology (F). Arrow points to a dorsal-specific bristle in E, which is missing in the ventralized Fng+α_{PS1}-rescued wings (F). (G,H) Comparison of a wild-type alula, which has bristles only on the ventral surface, and a Fng+α_{PS1}-rescued alula (H), which has many more bristles, indicating two ventral surfaces. (I,J) A mechanosensory campaniform sensilla located on the ventral surface of L3 in wild-type (I) is present on both surfaces of Fng+α_{PS1}-rescued wings (J).

or ventral direction. Veins L3, L5 and distal L4 bulge dorsally, while L2, and proximal L4 bulge ventrally, resulting in a stereotypical pattern of corrugation for both the dorsal and ventral wing surfaces (Garcia-Bellido, 1977; Fig. 4A). In Fng+α_{PS1}-rescued wings, the veins show a ventral corrugation pattern on both surfaces of the wing (Fig. 4B,D). Veins that would normally have a major component on the ventral surface are composed of major components on both surfaces, and veins normally having a minor component on the ventral surface are composed of minor components on both surfaces. Collectively, these results indicate that despite their wild type overall morphology, Fng+α_{PS1}-rescued wings are entirely of ventral identity.

Wing veins differentiate independently from a dorsal-specific signal

It is generally thought that specific signals from dorsal cells are required for ventral vein development (Garcia-Bellido, 1977; Garcia-Bellido and de Celis, 1992; Milan et al., 1997). The normal vein pattern of Fng+α_{PS1}-rescued ventralized wings clearly demonstrates that differentiation of ventral veins does not require a compartment-specific signal from the dorsal surface. To determine whether earlier aspects of vein differentiation might be altered in the Fng+α_{PS1}-rescued wings, we examined Dl expression in pupal wings (Fig. 5). Dl is normally required for wing vein refinement, a process by which lateral inhibitory mechanisms gradually restrict vein competence during pupal stages. By 30 hours of pupal development, Dl is expressed specifically in the central provein cells, which will give rise to the differentiated vein (de Celis et al., 1997; Huppert et al., 1997). Staining of wild-type and Fng+α_{PS1}-rescued *ap* mutant pupal wings for Dl revealed no differences, indicating that vein refinement and alignment occur normally in the ventralized wings.

DISCUSSION

D/V compartmentalization is independent of dorsal identity

It has been proposed that lineage restrictions in the wing disc result from differential adhesive properties of cells in adjacent compartments (Garcia-Bellido, 1975; Lawrence and Morata, 1976). For example, in vitro reaggregation experiments have demonstrated that prospective wing-blade cells preferentially adhere to one another and sort away from notum cells (Fausto-Sterling and Hsieh, 1987). Differences in affinity such as these are thought to be acquired autonomously as a consequence of

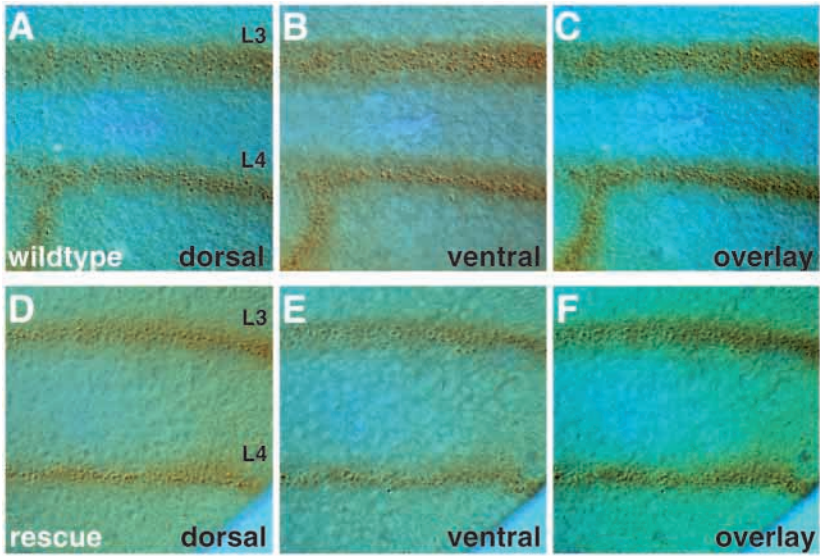

Fig. 5. Wing vein refinement and alignment do not require D/V signaling. 30 hour pupal wings stained for Delta (Dl). High magnification views of developing veins L3 and L4 of a wild-type pupal wing (A-C) and of a Fng+α_{PS1}-rescued pupal wing (D-F) of the genotype *ap^{ts78j}/ap^{GAL4}; UAS-fng/UAS-α_{PS1}*. Dl staining is present both on the dorsal (A,D) and ventral (B,E) wing surfaces. Overlaying the two images reveals a precise alignment of Dl staining between the two surfaces in both wild-type (C) and Fng+α_{PS1}-rescued (F) wings. Anterior is upwards, proximal towards the left.

compartment-specific identity and selector gene function. At the A/P boundary, En is expressed in posterior cells, and in vivo reaggregation experiments have demonstrated that posterior cells normally do not intermingle with anterior cells (Garcia-Bellido, 1966). *en* mutant posterior cells alter their aggregation behavior and display a positive affinity for anterior cells, suggesting that En regulates cell adhesion molecules essential for A/P compartmentalization (Garcia-Bellido and Santamaria, 1972). *en* mutant cells also differentiate anterior cell types, suggesting a link between posterior identity and posterior affinity.

Alternatively, it has been proposed that intercompartmental signaling maintains affinity boundaries. Posterior En cells secrete the molecule Hh, which signals across the A/P boundary to anterior cells (Tabata et al., 1992; Zecca et al., 1995). The response to Hh requires the receptor Smoothened (Smo) and the transcription factor Cubitus interruptus (Ci). Mutations in both *smo* (Blair and Ralston, 1997; Rodriguez and Basler, 1997) and *ci* (Dahmann and Basler, 2000) disrupt the A/P compartment boundary, demonstrating that Hh signaling between compartments is essential for segregation of anterior and posterior wing disc cells. Along the D/V axis, intercompartmental signaling results in Notch activation specifically at the D/V boundary (Fleming et al., 1997; Panin et al., 1997). Mutations in either *fng* (which modulates Notch signaling) or *Notch* disrupt D/V compartmentalization (Micchelli and Blair, 1999; Rauskolb et al., 1999), and again illustrate the importance of signaling in the compartmentalization process.

Our results suggest that intercompartmental signaling is sufficient to maintain the D/V affinity boundary. In the absence of dorsal identity, we can rescue compartmental defects associated with *ap* mutant wing discs with the molecule Fng. This argues that signaling between compartments mediated by Fng and Notch, and not the autonomous acquisition of compartment-specific affinity as an aspect of cell identity, plays the crucial role in D/V compartmentalization. Consistent with this are previous findings that both *fng* and *Notch* mutant clones generated in the dorsal compartment do not respect the D/V boundary, despite the fact that they likely retain dorsal identity (Micchelli and Blair, 1999; Rauskolb et al., 1999).

While the *ap* alleles used in this study are not molecularly-defined nulls, these allelic combinations (*ap^{rK568}/ap^{GAL4}* and *ap^{ts78j}/ap^{GAL4}* at 25°C) clearly reduce Ap function sufficiently to eliminate dorsal identity. Based on both sensory bristle and wing vein morphologies, the Fng and Fng+α_{PS1}-rescued wings consist entirely of ventral cell types. Although we cannot exclude the possibility that these *ap* allelic combinations might maintain small degrees of dorsal-specific affinity (independent of dorsal identity), the mutant phenotypes indicate that any adhesive differences are clearly not sufficient to maintain D/V compartmentalization (Fig. 2D, data not shown).

Cell autonomous differentiation of ventral veins

Prospective wing vein cells are identifiable in late third instar wing discs by molecular markers such as *rhomboid* (Sturtevant et al., 1993). Wing disc eversion results in apposition of dorsal and ventral vein components, and interplanar signaling between the dorsal and ventral wing surfaces has been shown to play a crucial role in wing vein differentiation. Clonal analysis has demonstrated that mutations that disrupt or alter vein formation, frequently have non-autonomous effects on the opposite surface, and that these effects are particularly dramatic when the genetic clone lies on the dorsal surface (Garcia-Bellido, 1977; Garcia-Bellido and de Celis, 1992; Milan et al., 1997). These results suggest a dorsal-specific signal that induces differentiation of ventral veins. However, when forced to differentiate without interplanar signaling, vein structures are capable of forming on both surfaces, although these veins are defective in terms of refinement and their pattern of corrugation (Milan et al., 1997).

In the Fng+α_{PS1}-rescued wing there is no dorsal identity and, therefore, no dorsal-specific signal directing ventral vein differentiation. Despite this abnormality, vein components on both surfaces differentiate appropriately based on their A/P and proximal/distal position in the wing, albeit entirely of ventral identity. This demonstrates that wing vein refinement, alignment and pattern of corrugation can occur independently

of dorsal cell types. Although interplanar signaling is certainly essential for proper wing vein differentiation, it is clear that a dorsal-to-ventral signal is not required, and that ventral cell types autonomously contain all the information necessary for wing vein development.

Selector gene function

An emerging view of selector gene function is that these genes may regulate large numbers of effector genes involved in particular morphogenetic processes. For example, in the differentiation of *Drosophila* haltere from wing, the transcription factor Ultrabithorax regulates genes at many levels of the wing patterning genetic cascade (Weatherbee et al., 1998). So too, the selector homeoproteins Even-skipped and Fushi tarazu (Ftz) have been shown to regulate either directly or indirectly most genes during embryogenesis (Liang and Biggin, 1998). However, fusion of the VP16 activation domain to Ftz has suggested that Ftz binds to and regulates only a small number of target genes (Nasiadka et al., 2000). It is therefore an unanswered question as to whether the number of genes regulated by selectors is large or small.

In the absence of normal *ap* selector gene function, the expression of only two downstream effectors is sufficient to rescue wing structures to a remarkable degree. This result suggests that the compartment-specific selector gene *ap* regulates only a small number of target genes during wing development. It will be interesting to determine whether selector genes with broader scopes of activities function in a similar manner. Selectors which control the formation of entire structures (such as *eyeless*) or entire body regions (such as the Hox genes) presumably sit at the top of larger genetic hierarchies than *ap*, and may control larger sets of target genes to fulfill their developmental roles.

Finally, although *ap* regulates only a small number of downstream effectors to generate the overall morphology of the wing, it may indeed regulate many genes to confer dorsal identity. It is tempting to speculate, however, that Ap may regulate only one additional gene, *Dorsal wing*, in order to specify dorsal cell fate in the wing. Loss-of-function mutations in the *Drosophila Dorsal wing* locus result in dorsal-to-ventral transformations in the wing blade, and ventral misexpression of *Dorsal wing* produces ectopic dorsal structures (Tiong et al., 1995). While the gene corresponding to this phenotype has yet to be characterized, *Dorsal wing* likely forms a crucial component of Ap-dependent wing developmental processes.

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