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Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in *Drosophila* wing formation

Dan Doherty, Georg Feger,[1] Susan Younger-Shepherd, Lily Yeh Jan, and Yuh Nung Jan[2]

Howard Hughes Medical Institute and the Departments of Physiology and Biochemistry, University of California, San Francisco, California 94143-0724 USA; [1]Gesellschaft für Analyse-Technik und Consulting mbH, 78467 Konstanz, Germany

Wing margin formation in *Drosophila* requires the Notch receptor and, in the dorsal compartment, one of its ligands, Serrate. We provide evidence that Delta, the other known ligand for Notch, is also essential for this process. *Delta* is required in ventral cells at the dorsal/ventral compartment boundary, where its expression is specifically elevated in second-instar wing discs during wing margin formation. Moreover, ectopic Delta expression induces *wingless*, *vestigial*, and *cut* and causes adult wing tissue outgrowth in the dorsal compartment. This effect is mediated by *Notch*, because loss of *Notch* activity suppresses Delta-induced ectopic wing outgrowth. Whereas ectopic expression of Notch or the truncated activated Notch induces *cut* in both dorsal and ventral compartments, ectopic Delta expression induces *cut* only in the dorsal compartment and ectopic Serrate induces *cut* only in the ventral compartment. These observations indicate that *Notch*-expressing cells in a given compartment have different responses to Delta and Serrate. We propose that Delta and Serrate function as compartment-specific signals in the wing disc, to activate Notch and induce downstream genes required for wing formation.

[*Key Words*: Delta; Notch; Serrate; *Drosophila*; wing patterning; compartment-specific signaling]

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Cell–cell communication is a fundamental process required for patterning and growth during *Drosophila* wing development (for review, see Whittle 1990; Blair 1995). The wing develops from a cluster of undifferentiated cells called the wing imaginal disc. The disc cells are set aside during embryogenesis, proliferate during larval development, and by late third larval instar, the disc has developed the basic pattern elements of the mature wing. Within the epithelial monolayer of the disc, dorsal and ventral compartment cells lie in two adjoining regions (Fig. 2C, below). During pupal development, the dorsal cells become apposed to the ventral cells as the disc folds and everts along the dorsal/ventral (D/V) boundary to form the mature wing blade that is comprised of two symmetrical cell layers. The D/V boundary becomes the wing margin, marked with sensory bristles along the anterior margin and large noninnervated hairs along the posterior margin.

The wing consists of four compartments (anterior, posterior, dorsal, and ventral) as defined by lineage restriction studies (Garcia-Bellido et al. 1973; Blair 1993). Tissue excision and transplantation experiments have demonstrated that juxtaposition of cells from different regions of developing appendages induces proliferation and intercalation of pattern elements (for review, see French et al. 1976). More recently, it has been proposed that interaction between cells with different compartmental identities is required for the normal growth and patterning of the wing and other appendages (Meinhardt 1983; Diaz-Benjumea and Cohen 1993; Tabata and Kornberg 1994).

A number of genes involved in signaling between dorsal and ventral compartment cells have been identified. Dorsal but not ventral cells express *apterous* (*ap*), a homeo domain transcription factor that is required for wing disc proliferation and formation of the wing margin (Bourgouin et al. 1992; Diaz-Benjumea and Cohen 1993; Williams et al. 1993). The *fringe* (*fng*) gene, which encodes a novel, putatively secreted molecule, is expressed in the dorsal compartment under the control of *ap* (Irvine and Wieschaus 1994). Juxtaposition of *fng*-expressing and *fng*-nonexpressing cells induce tissue outgrowth and the formation of wing margin structures, suggesting that the fng signal can only be received by cells that do not express *fng*. To explain that both fng^- and fng^+ cells at the borders of fng^- clones are transformed into wing margin, Irvine and Wieschaus (1994) proposed that *fng* is a dorsal to ventral signaling molecule that induces a reciprocal (ventral to dorsal) signal from the fng^- cells to the fng^+ cells. The existence of a reciprocal signal was also proposed by Williams et al. (1994) to explain the similar behavior of *ap* clones.

[2]Corresponding author.

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Candidates for the ventral to dorsal signal include *wingless* (*wg*), a member of the Wnt gene family, which encodes a secreted protein and is essential for wing development, as well as many other developmental processes in *Drosophila* (for review, see Klingensmith and Nusse 1994; Siegfried and Perrimon 1994). Loss-of-function *wg* mutations result in abnormal wing phenotypes, ranging from loss of wing margin bristles to complete absence of wing tissue, depending on the particular combination of alleles (Phillips and Whittle 1993; for review, see Lindsley and Zimm 1992). In second-instar wing discs, *wg* is expressed in the ventral compartment and may function to maintain the restriction of *ap* expression to the dorsal compartment (Williams et al. 1993). Both *wg* and *ap* functions are required for the expression of the *vestigial* (*vg*) enhancer *lacZ* reporter construct, the earliest molecular marker for the wing margin (Williams et al. 1994). Later, during third instar, *wg* expression is restricted to a stripe at the D/V boundary in cells that later form the wing margin. These results indicate that *wg* is required early for proliferation and/or patterning of the disc and later for formation of wing margin structures.

The *Notch* (*N*) signaling pathway is also essential for wing development. Loss-of-function mutations in *N* cause loss of wing tissue similar to that observed in *wg* mutants. *N* encodes a large transmembrane receptor necessary for communication in a number of developmental processes. During neurogenesis, the *N* gene product, in concert with its ligand Delta (Dl), functions to single out neural precursors from fields of neuroectodermal cells (for review, see Campos-Ortega 1988; Ghysen et al. 1993; Artavanis-Tsakonas et al. 1995). This process requires cell–cell communication among groups of cells, all of which can both send and receive signals (Heitzler and Simpson 1991). Strong genetic interactions between *N* and *wg* indicate that the two genes function in the same pathway during wing margin formation (Couso and Martinez Arias 1994; Hing et al. 1994). Couso and Martinez-Arias (1994) have proposed models in which *wg* acts upstream or parallel to *N* during wing margin development and may even be an N ligand; however, loss of *N* function on either side of the D/V boundary causes loss of *wg* expression, wing margin, and wing blade tissue in both compartments (de Celis and Garcia-Bellido 1994; Rulifson and Blair 1995), suggesting a function for *N* upstream of *wg*.

Kim et al. (1995) and Diaz-Benjumea and Cohen (1995) have proposed that Serrate (Ser), an N ligand with sequence similarity to Dl (Fleming et al. 1990; Rebay et al. 1991), functions as a dorsal to ventral signal downstream of *fng*. *Ser* expression is restricted to the dorsal compartment in the second-instar wing disc. Loss of *Ser* function in dorsal cells at the D/V boundary results in loss of wing margin, whereas ectopic expression of Ser in both the dorsal and ventral compartments induces adult wing tissue outgrowth and *wg* expression only in the ventral compartment (Speicher et al. 1994; Kim et al. 1995).

The mild wing notching observed with temperature-sensitive combinations of *Dl* alleles has implicated *Dl* in wing development, although its role has remained unclear (Parody and Muskavitch 1993). We demonstrate that *Dl* is required for wing development and can induce a number of genes required for wing formation including *wg*, *vg*, and *cut*. We propose that *Dl* encodes a ventral to dorsal signal, because *Dl* is required in ventral cells at the D/V boundary and ectopic Dl induces *cut* expression and adult wing outgrowth only in the dorsal compartment. In contrast, ectopic Ser induces *cut* expression only in the ventral compartment, indicating that *N*-expressing cells can have different responses to the two N ligands. These results suggest that *Dl* plays an equivalent but complementary role to *Ser* as a compartment-specific signal in the genetic program for wing margin development.

Results

Loss of Dl *function during wing development causes loss of wing tissue*

As part of the *N* signaling pathway, *Dl* plays an important role in several developmental processes (for review, see Muskavitch 1994). Using a temperature-sensitive allele of *Dl*, Parody and Muskavitch (1993) have shown that *Dl* is required during late second and early third instar for wing margin formation. Exposure of such *Dl* mutant flies to the restrictive temperature at this developmental stage leads to notching at the distal tip of the wing, similar to the phenotype seen in flies heterozygous for an *N* null mutation. To further analyze the role of *Dl* in wing development, we generated clones homozygous for a loss-of-function *Dl* allele, Dl^{rev10} (Heitzler and Simpson 1991), by X-irradiating second-instar larvae. We found that *Dl* is required for wing margin formation in the ventral but not the dorsal compartment. Ventral clones that abutted the D/V boundary caused gaps in the wing margin (Fig. 1E,F; Table 1), whereas clones that abutted the boundary from the dorsal side and those within the wing blade in either compartment did not cause gaps in the wing margin (Fig. 1C,D). The loss of wing margin phenotype is nearly identical to that caused by clones lacking *Ser* (Fig. 1B; Speicher et al. 1994; Diaz-Benjumea and Cohen 1995). Clones were identified using *forked* as a marker (Diaz-Benjumea and Cohen 1993) and by scoring hypertrophy of the wing veins caused by loss of *Dl* function (Fig. 1C,F; Parody and Muskavitch 1993). Loss of sensory bristles on the anterior wing margin and the noninnervated posterior wing margin hairs was caused by both dorsal and ventral clones that included the domains of cells that give rise to these cuticle structures. All clones, both dorsal and ventral, also caused hypertrophy of vein tissue when they overlapped the normal position of wing veins. The phenotypes in bristle differentiation and wing vein formation are consistent with the known functions for *Dl* (Parks and Muskavitch 1993; Parody and Muskavitch 1993). The absence of large portions of the wing in *Dl* mosaics indicates that *Dl* is required for formation of the wing margin as well as the proliferation and/or viability of wing blade cells. The

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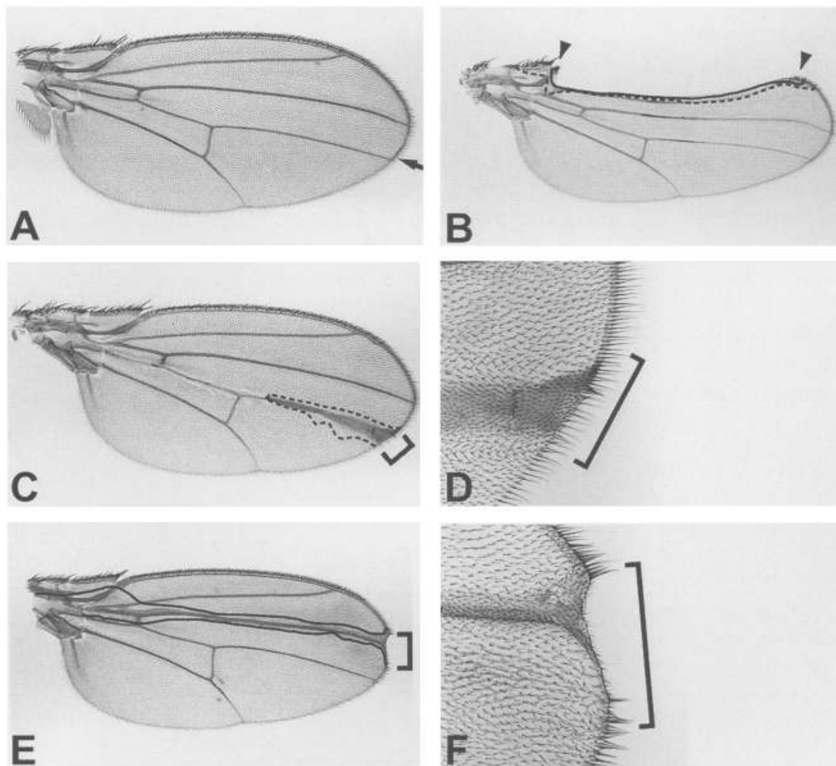

Figure 1. Ventral but not dorsal Dl^{rev10} clones that abut the D/V boundary cause loss of wing margin. All wings are oriented with anterior up, proximal to the *left*. Broken lines mark clone boundaries in the dorsal compartment, whereas solid lines mark clone boundaries in the ventral compartment. (*A*) Unirradiated control f^{36a}; Dl^{rev10} *e/bld cu* f^{+98B} wing. The A/P compartment boundary between veins 3 and 4 is marked by an arrow. (*B*) Wing with a Dl^{rev10} that crosses the D/V boundary; the anterior wing margin and a large part of the wing blade are missing. Black arrowheads mark groups of *forked ebony* bristles at the clone borders. (*C*) Wing with a Dl^{rev10} clone that abuts the D/V boundary from the dorsal side (bracket), whereas the dorsal marginal hairs are missing and the wing margin and blade are intact. (*D*) High-magnification view of *C*. (*E*) Wing with a Dl^{rev10} clone that abuts the D/V boundary from the ventral side (bracket); there is a gap in the wing margin and adjacent wing blade. (*F*) High-magnification view of *E*.

compartment-specific requirement for *Dl* is the first indication that *Dl* encodes a ventral to dorsal signal reciprocal to the dorsal to ventral *Ser* signal (Diaz-Benjumea and Cohen 1995; Kim et al. 1995).

Loss of Dl *function during wing development alters* wg *expression*

Because our mosaic studies indicated that *Dl* plays an important role in wing development, we used *wg* expression as a marker to examine how loss of *Dl* function in mitotic clones affected formation of the D/V boundary. *wg* is expressed in the ventral compartment of second-instar wing discs and then along the D/V boundary from early third instar onward (Baker 1988; Couso et al. 1993; Williams et al. 1993). Adult viable *wg* alleles cause loss of the entire wing and occasional wing to notum transformations (for review, see Lindsley and Zimm 1992); however, reduced *wg* activity during third instar causes loss of wing margin structures only (Phillips and Whittle 1993).

We generated homozygous Dl^{rev10} clones in second-instar wing discs and examined wg protein expression in these discs at late third instar using an anti-wg antibody (van den Heuvel et al. 1989). We identified clones by the absence of heat shock-induced expression of a nuclear Myc marker on the Dl^{+} third chromosome (see Materials and methods). wg expression was altered when mutant clones intersected the D/V boundary. In large clones, we consistently observed a reduction in wg ex-

Table 1. Dl *is required only in the ventral compartment for wing margin formation*

Location of clone[a]	Total clones[b]	Only dorsal bristles absent	Only ventral bristles absent	Dorsal and ventral bristles absent (no gap)	Gap in margin and blade
Dorsal	11	11	0	0	0
Ventral	10	0	2[c]	0	8
Dorsal and ventral	31	0	1[d]	2	28
Internal (dorsal or ventral)	14	0	0	0	0

[a]Clone location was determined based on the presence of *forked* hairs adjacent to the D/V boundary in the dorsal, ventral, or both compartments; *forked* hairs were confined to the wing blade in internal clones.
[b]Only wings with a *Dl* wing vein phenotype were analyzed; six wings with clones were unscorable owing to gross distortion of the wing tissue.
[c]Wings with clones that appear to abut the boundary because they eliminate ventral bristles.
[d]Wing with both dorsal and ventral *forked* hairs; wing margin and dorsal bristles unaffected.

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pression at the D/V boundary in homozygous Dl^{rev10} cells that were surrounded by other homozygous Dl^{rev10} cells (not shown). At the borders of narrower clones, in the homozygous Dl^{rev10} cells that were adjacent to heterozygous Dl^{rev10} cells, D/V boundary wg expression was elevated and ectopic wg expression extended into the ventral compartment (Fig. 2A,B). The separation between wg and Myc expression is attributable to the membrane association of wg and nuclear localization of Myc. Rarely, low levels of ectopic *wg* expression extended for short distances into the dorsal compartment along the inside border of clones. These results suggest that *Dl*, like *fng* (Irvine and Wieschaus 1994), can induce *wg* expression at the border between *Dl*-expressing and *Dl*-nonexpressing cells.

Dl *expression is elevated at the D/V boundary in second-instar wing discs*

The effect of Dl^- clones on *wg* expression raises the question of whether *Dl* plays an early role in setting up the D/V boundary; so we examined *Dl* expression in wing discs during second and third instar when proliferation and wing margin formation occur. To characterize *Dl* expression with respect to the dorsal and ventral compartments, we stained discs expressing an *ap–lacZ* enhancer trap, which marks cells of the dorsal compartment (Diaz-Benjumea and Cohen 1993), with antibodies to β-galactosidase and Dl (Kooh et al. 1993). The earliest patterned Dl protein expression appeared during mid-second-instar. The highest levels of Dl were centered

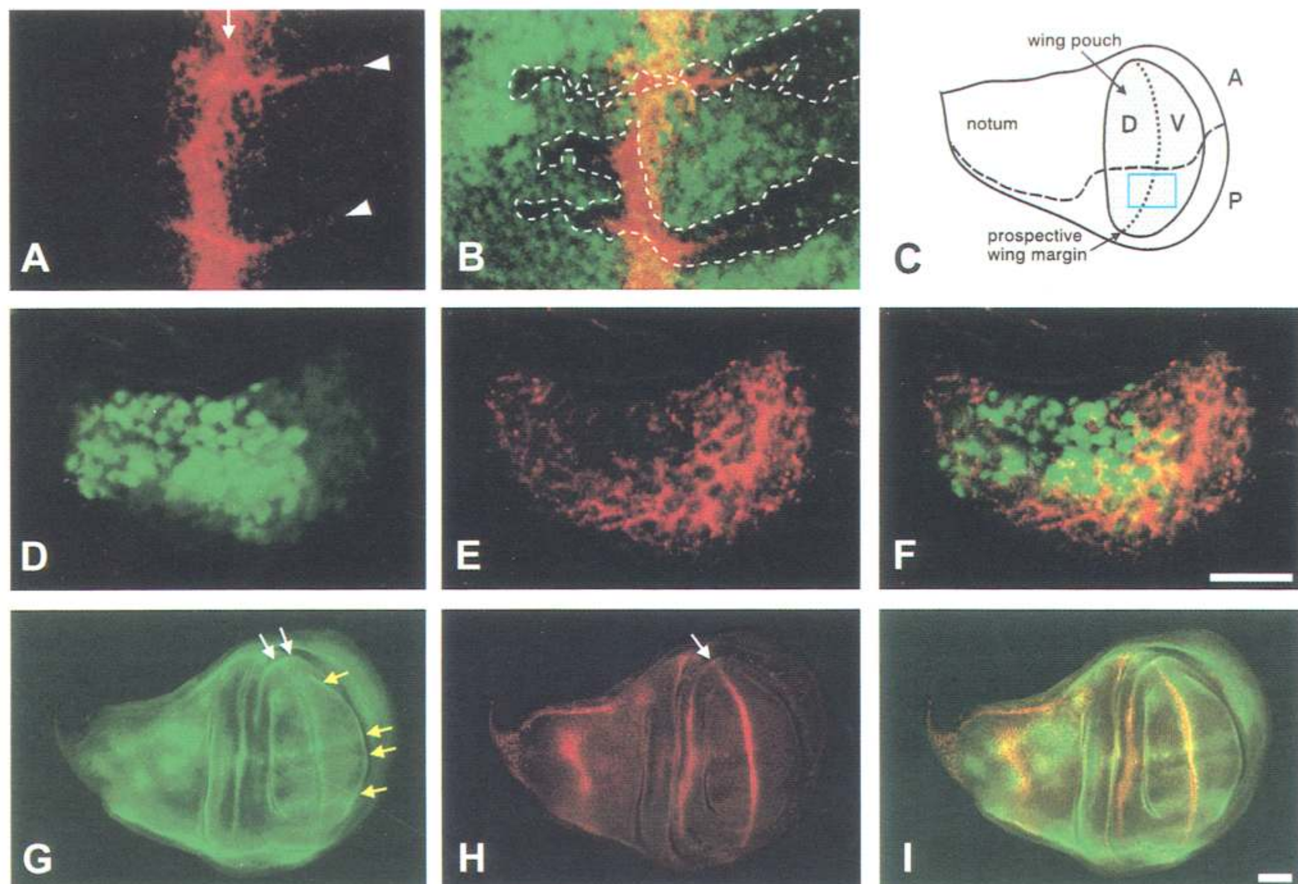

Figure 2. Dl^- clones and wild-type Dl expression in the wing disc. (*A,B*) Homozygous Dl^{rev10} clones in a third-instar wing disc (box in *C* marks the region of the disc in the images). (*A*) wg expression (red). The arrow marks the endogenous stripe of wg expression at the D/V boundary, whereas the arrowheads mark ectopic wg expression. (*B*) Overlay of *A* with the Myc epitope tag signal (green); the homozygous Dl^{rev10} clones (broken lines) are marked by the absence of Myc signal. Ectopic wg is present approximately one cell width from the nuclei of *myc*-expressing cells. (*C*) Fate map of wing disc (Bryant 1975) adapted from Diaz-Benjumea and Cohen (1993); the box marks the region shown in (*A,B*). (*D–F*) Dl protein and *ap–lacZ* expression in a mid-second-instar wing disc. Dorsal is to the *left*. Scale bar, 8 μm. (*D*) *ap–lacZ* (green) is expressed in the nuclei of dorsal compartment cells. (*E*) Dl protein (red) is expressed in the membranes of cells at the D/V boundary and mainly in the ventral compartment. (*F*) Overlay of *D* and *E*. (*G–I*) Dl and wg expression in a wild-type third-instar wing disc. In all wing disc figures anterior is up, dorsal is to the *left*. Scale bar, 50 μm. (*G*) Endogenous Dl (green) is expressed in two stripes of cells along the prospective wing margin (white arrows), the prospective wing veins (yellow arrows), and in proneural clusters. (*H*) Endogenous wg (red) is expressed in the prospective wing margin (white arrow) and in a band across the notum. (*I*) Overlay of *G* and *H*. The wg stripe is flanked by the Dl stripes.

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along the D/V boundary, as marked by the limit of the *ap–lacZ* domain (Fig. 2D–F). High levels of Dl protein were also present in the ventral compartment of the wing pouch, with lower levels in the dorsal compartment. Dl was absent from the region of the disc that forms the dorsal notum. In early third-instar wing discs there was a small amount of punctate Dl staining in a narrow stripe of cells at the D/V boundary (not shown). Finally, at late third instar, Dl expression was highest in two stripes flanking the *wg*-expressing cells at the D/V boundary, as well as in the prospective wing veins and proneural clusters (Kooh et al. 1993; Fig. 2G–I). The *Dl* expression pattern is consistent with *Dl* having an early role in setting up the wing margin and a later role in maintaining the wing margin and patterning the wing margin bristles.

Ectopic expression of Dl *results in abnormal outgrowth of dorsal wing tissue*

Having found that *Dl* is required for wing margin formation, we examined whether ectopic *Dl* expression could induce an ectopic wing margin or wing tissue outgrowth. Using the GAL4 system (Brand and Perrimon 1993), we ectopically expressed a UAS–*Dl* transgene in the wing disc with the *patched*-GAL4 enhancer trap line G559.1 (*ptc*G4) (Hinz et al. 1994). We refer to the combination of *ptc*G4 with UAS–*Dl* as ptcG4–Dl. *ptc*G4 is expressed strongly in a stripe along the anterior/posterior (A/P) border of the wing disc by mid third instar, with the highest level at the sharp posterior border and gradually lower levels toward the more irregular anterior border (see Materials and methods; Kim et al. 1995). The GAL4 system has been reported to give more extreme ectopic expression phenotypes at higher temperatures (Speicher et al. 1994). When raised at 29°C, all of our UAS–Dl lines were lethal in combination with *ptc*G4. At 22°C, one line (UAS–Dl^{30A1}) produced viable ptcG4–Dl adults with disrupted anterior cross veins. Shifting ptcG4–Dl^{30A1} larvae to 29°C for 24 hr during second instar resulted in adult flies with striking wing outgrowth. The abnormal wing tissue outgrowth occurred only on the dorsal side of the wing blade (Fig. 3A–C); patches of large bristles characteristic of the anterior double row or posterior wing margin were present at the distal tip of each outgrowth. We found even more extreme outgrowth in the wings of ptcG4–Dl^{30A1} pharate adults raised at 29°C throughout development. Other UAS–*Dl* insertions crossed to *ptc*G4 yielded pharate adults with extreme wing outgrowth even when raised at 22°C. These pharate adults also displayed severe defects in the legs, heads, nota, and male genitalia. Thus, ectopic expression of *Dl* can induce wing outgrowth and a new wing margin, as well as defects in other tissues.

Ectopic Dl *acts through* N

Dl has been shown to signal through the N receptor during neuronal precursor selection, and this signaling is sensitive to levels of both *N* and *Dl* (Vaessin et al. 1987; Heitzler and Simpson 1991). To determine whether ectopic Dl also utilizes the *N* signaling pathway for induction of wing tissue outgrowth, we asked whether outgrowth depended on wild-type *N* function. We used the N^{ts} mutation to reduce *N* activity; raising the temperature to 29°C for 24 hr during late second- and early third-instar larval development resulted in notching of the wing characteristic of the *N* mutant phenotype (Fig. 3D; Shellenbarger and Mohler 1978). Shifting second-instar ptcG4–Dl male larvae that carried the N^{ts} mutation to 29°C for 24 hr suppressed completely the wing outgrowth phenotype, whereas control ptcG4–Dl flies that were N^+ or heterozygous for N^{ts} displayed a strong outgrowth phenotype (Fig. 3C).

Further evidence that ectopic Dl functions through N is that ectopic N expression also induces hairs characteristic of the wing margin, similar to the phenotype of ptcG4–Dl flies raised at 25°C. We ectopically expressed wild-type UAS–*N* (gift from P. Simpson, Institute of Genetics and Molecular and Cellular Biology, Centre de Recherche National Scientifique (CNRS), Strasbourg, France) by crossing it to *ptc*G4 (ptcG4–N). ptcG4–N flies raised at 29°C throughout development had a short row of ectopic hairs along the fourth wing vein on both the dorsal and ventral sides of the wing blade, reminiscent of anterior double row or posterior wing margin hairs (Fig. 3E,F); these flies occasionally displayed mild wing tissue outgrowth (data not shown). Taken together, these data confirm that ectopic Dl acts through N, its known receptor, to induce abnormal wing outgrowth.

Ectopic expression of Dl *induces* wg, vg, *and* cut *expression*

Our mosaic studies indicated that *Dl* may be able to induce *wg* expression at the border between *Dl*-expressing and *Dl*-nonexpressing cells. To examine this possibility further, we looked for ectopic wg expression in third-instar ptcG4–Dl wing discs. wg was induced mainly along the posterior border of the ectopic Dl stripe and rarely at lower levels along the anterior border. In the dorsal compartment, wg was induced in the cells just inside and just outside the posterior border of the ptcG4–Dl stripe, whereas in the ventral compartment, *wg* was induced at a lower level and only outside the stripe (Fig. 4A–C). Induction of *wg* was not limited to lines that expressed GAL4 at the A/P boundary; lines driving UAS–*Dl* expression at other locations within the wing pouch also induced *wg* expression and disc tissue outgrowth but not adult wing outgrowth (data not shown). We did not observe *wg* induction outside the wing pouch, suggesting that there are region-specific factors that modulate the ability of Dl to induce *wg* expression.

In third-instar wing discs, ectopic Dl expression also induced *cut*, *vg*, *deadpan* (*dpn*), and *big brain* (*bib*) in cells along the *ptc*G4 stripe (Fig. 4D,E; additional data not shown). Whereas all four of these genes are normally expressed in the prospective wing margin (Williams et al. 1991; Bier et al. 1992; Blochlinger et al. 1993; D.

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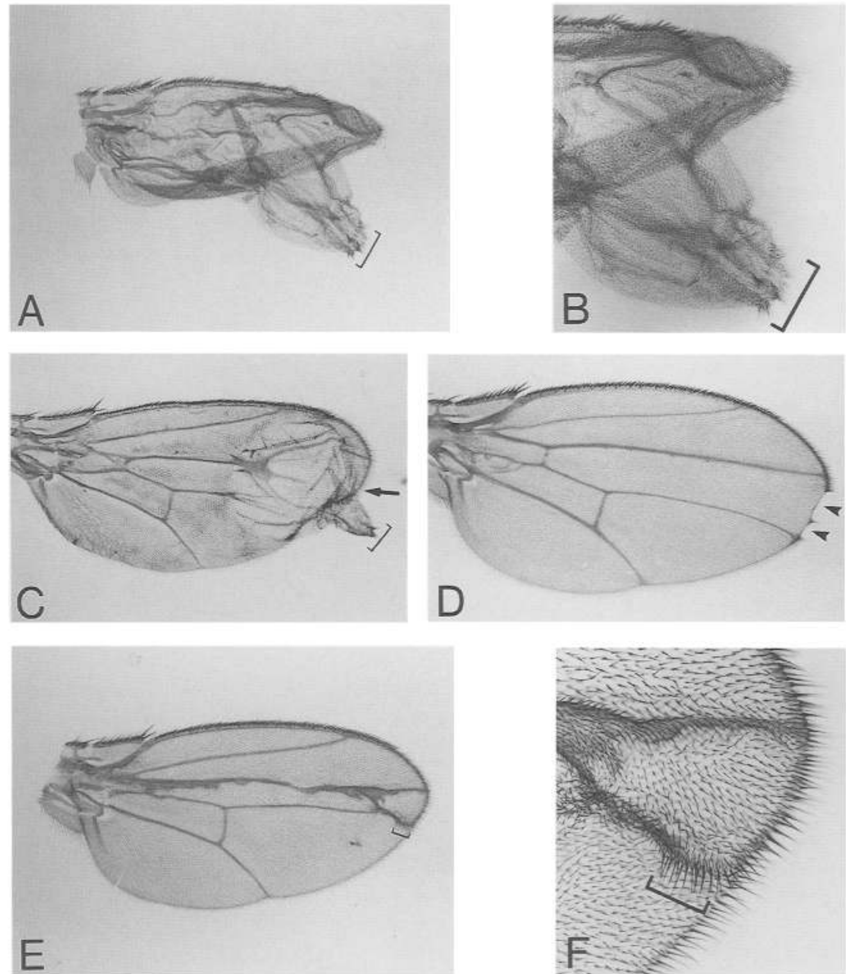

Figure 3. Ectopic wing margin formation and wing tissue outgrowth induced by ectopic Dl is suppressed by N^{ts}. (*A*) Wing from a ptcG4–Dl fly shifted to 29°C for 24 hr during the late second and early third larval instars. Wing outgrowth with hairs characteristic of the wing margin always occurs on the dorsal side of the wing (bracket). (*B*) High-magnification view of *A*, showing the hairs at the distal tip of the outgrowth. (*C*) Wing from a N^{ts}/yw; ptcG4/ + ; UAS–Dl/ + female shifted to 29°C for 24 hr during the late second and early third larval instars. Note the *Dl*-induced outgrowth with hairs characteristic of the wing margin at the tip (bracket). (*D*) Wing from a N^{ts}/Y; ptcG4/ + ; UAS-Dl/ + male shifted to 29°C for 24 hr during the late second and early third larval instars. The *Dl*-induced ectopic outgrowth is completely suppressed (cf. *C*). Notches in the distal wing (arrowheads) are due to the reduction in N activity (see text). (*E*) Wing from a ptcG4–N fly shifted to 29°C for all of the second and third larval instars. Ectopic margin-like hairs along vein 4 (bracket) occur on both the dorsal and ventral sides of the wing blade. (*F*) High-magnification view of *E*, showing ectopic hairs characteristic of the wing margin (bracket).

Doherty, in prep.), only *cut* and *vg* have wing phenotypes (for review, see Lindsley and Zimm 1992). There was a notable difference in the ectopic expression of *cut*, which was induced only in the dorsal compartment of the wing pouch (Fig. 4E; Table 2), whereas *vg*, *bib*, and *dpn*, like *wg*, were induced in both the dorsal and ventral compartments (Fig. 4A–D; data not shown). Furthermore, these discs were distorted by tissue overgrowth in both the dorsal and ventral compartments (cf. Fig. 4A–C with Fig. 2G–I); however, adult wing outgrowth was exclusively dorsal (Fig. 3A–C). Our observations indicate that many genes, including *wg*, *vg*, and *cut*, can be downstream targets of *Dl* in the wing disc and that *Dl* has different effects in the dorsal and ventral compartments.

Because ptcG4–Dl wing discs displayed such striking outgrowth, we asked whether compartmental organization was disrupted. We examined expression of *cubitus interuptus*, a gene expressed in the anterior compartment, *engrailed*, a gene expressed in the posterior compartment, and *ap–lacZ*, an enhancer trap expressed in the dorsal compartment, to determine whether ectopic Dl expression causes general reorganization of the disc. We found that despite the dramatic dorsal and ventral disc tissue outgrowth associated with ectopic Dl expression, both A/P and D/V compartmental organization appeared undisturbed (data not shown). Our results indicate that Dl is able to induce many of the aspects of the normal wing margin program including *wg*, *vg*, and *cut* expression, as well as wing tissue outgrowth and wing margin bristle formation.

Ectopic expression of N *induces* wg *expression*

We examined ptcG4–N wing discs to determine whether ectopic N expression induced *wg* and *cut* in a manner consistent with its role as a receptor for Dl. Immunohistochemical labeling of ptcG4–N third-instar wing discs with anti-N and anti-wg antibodies revealed induction of *wg* expression in a short stripe perpendicular to the D/V boundary that gives rise to the wild-type wing margin (white arrowheads in Fig. 4F). ptcG4–N induced *wg* and *cut* in many fewer cells than ptcG4–Dl. *wg* and *cut* induction occurred in both dorsal and ventral cells but only close to the prospective wing margin (white arrowheads in Fig. 4F,H). Whereas it is formally possible that the levels of *N* activity were not sufficiently high to induce *wg* along the entire ptcG4–N stripe, a more likely explanation is that the ectopically expressed N receptor

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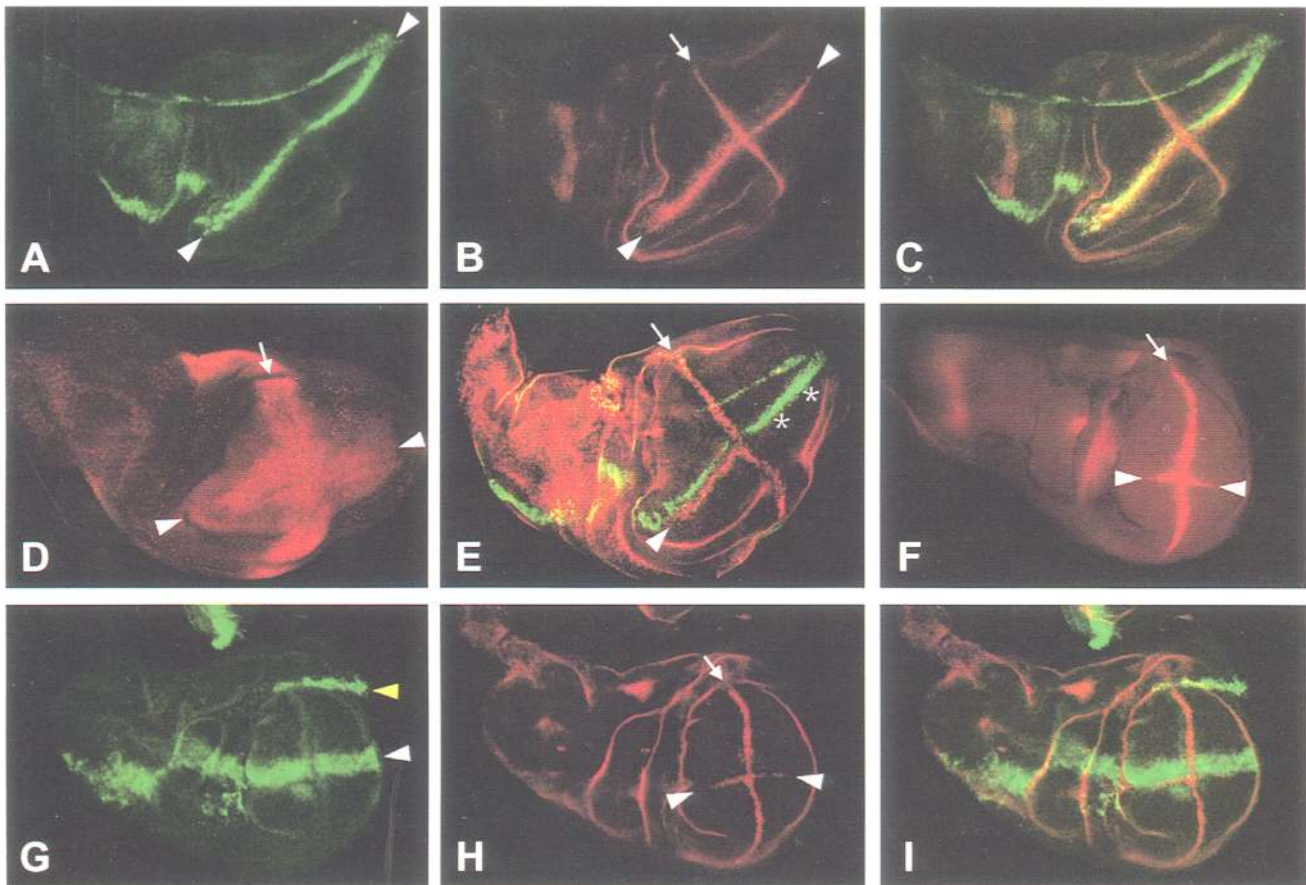

Figure 4. Ectopic Dl or N expression induces *wg*, *cut*, and *vg* in the third-instar wing disc. Anterior is up and dorsal is *left* in all images. (*A–E*) Dl, wg, vg, and cut expression in a ptcG4–Dl third-instar wing disc. Ectopic Dl induces *wg*, *vg*, and *cut* expression as well as overgrowth of the dorsal and ventral wing pouch. UAS–*Dl* was expressed using the *ptc*G4 enhancer trap in larvae raised at 29°C. (*A*) Dl (green) is expressed ectopically in a stripe along the A/P compartment boundary (white arrowheads). Endogenous Dl expression is barely visible flanking the D/V boundary at this contrast setting. (*B*) wg (red) is induced ectopically in a stripe along the A/P compartment boundary (white arrowheads). Endogenous wg expression is visible in the D/V compartment boundary cells (white arrow). (*C*) Overlay of *A* and *B*. (*D*) vg (red) is induced ectopically in a wide band centered on the A/P compartment boundary (white arrowheads). Endogenous vg expression is visible in a wide band of cells centered on the D/V boundary (white arrow). (*E*) cut (red) is induced ectopically in a stripe along the A/P compartment boundary only in the dorsal compartment (white arrowhead). Asterisks (*) mark the A/P boundary in the ventral compartment that is devoid of cut expression. (*F–I*) N, wg, and cut expression in a ptcG4–N third-instar wing disc. Ectopic N induces *wg* and *cut* expression. UAS–*N* was expressed using the ptcG4 enhancer trap in larvae raised at 29°C. (*F*) wg (red) is induced ectopically in cells along the A/P compartment boundary only near the prospective wing margin (white arrowheads). Ectopic wg is expressed in cells on both the dorsal and ventral sides of the wing margin. Endogenous wg expression is visible in the wild-type wing margin (white arrow). (*G*) N (green) is expressed ectopically in a stripe along the A/P compartment boundary (white arrowheads). The yellow arrowhead marks ptcG4–N expression in the peripodial membrane. (*H*) cut (red) is induced ectopically only in cells along the A/P compartment boundary near the wing margin (white arrowheads). Ectopic cut is expressed in cells on both the dorsal and ventral sides of the wing margin. Endogenous cut expression is visible in the wild-type wing margin in the same cells that express wg (white arrow). (*I*) Overlay of *G* and *H*. cut is expressed within the ptcG4–N stripe. There is only a slight overlap (yellow) because N is a transmembrane protein, whereas cut is nuclear.

was only activated in the cells that are in contact with cells near the wing margin that express Dl and Ser, known ligands for N (yellow arrow in Fig. 2G). In other words, endogenous Dl and/or Ser may activate the ectopically expressed N in the ptcG4–N stripe, inducing *wg* and *cut* expression.

wg *is induced at borders between* Dl *expressing and nonexpressing cells*

In ptcG4–Dl wing discs, Dl was ectopically expressed at a high level in a stripe several cells wide and endogenous *N* is expressed throughout the disc; however, *wg* was not induced throughout the ptcG4–Dl stripe. In the ventral compartment, *wg* was induced mainly in the cells adjacent to the posterior border of the stripe (Fig. 4A–C), indicating that *Dl*-expressing cells do not receive the Dl signal. In the dorsal compartment, *wg* was induced in cells along the posterior border of the ptcG4–Dl stripe, in cells outside as well as within the stripe. One explanation for *wg* induction within the stripe is that Dl induces

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Table 2. *Effects of ectopic Dl and Ser expression in the dorsal and ventral compartments*

	ptcG4–Dl	ptcG4–actN	ptcG4–Ser[a]
Dorsal			
gene expression			
wg	+ +	+ +	− (2)
vg	+ +	+ +	− (2)
cut	+ +	+ +	− (3)
disc growth	+ +	+ +	− (1) (2)
adult outgrowth	+ +	N.D.[a]	− (1) (2)
wing margin	+ +	N.D.[a]	− (1) (2)
Ventral			
gene expression			
wg	+	+ +	+ + (2)
vg	+ +	+ +	+ + (2)
cut	−	+ +	+ + (3)
disc outgrowth	+ +	+ +	+ + (1) (2)
adult outgrowth	−	N.D.[a]	+ + (1) (2)
wing margin	−	N.D.[a]	+ + (1) (2)

[a]Not determined because ptcG4–actN causes early pupal lethality.
[a](1) Speicher et al. (1994); (2) Kim et al. (1995); (3) D. Doherty and G. Feger (unpubl).

a reciprocal signal from the cells outside of the stripe (see Discussion). This reciprocal signal would be capable of inducing *wg* within the ptcG4–Dl stripe but not *cut*, as *cut* was expressed only in cells outside of the stripe (Fig. 4E). Restriction of *wg* induction to the posterior edge of the ptcG4–Dl stripe cannot be attributable to factors specific to the A/P boundary, because we also observed *wg* induction at the borders between Dl-expressing and -nonexpressing cells generated by GAL4 lines expressed at other locations.

One possible explanation for these observations is that Dl autonomously inhibits the ability of a cell to receive Dl signal from other cells. To determine whether increasing the level of N would allow Dl-expressing cells to receive Dl signal, we coexpressed N and Dl using *ptc*G4. *cut* was expressed throughout the width of the *ptc*G4 stripe in these discs (Fig. 5A–C), indicating that N was activated by Dl throughout the stripe. *cut* expression was still restricted to the dorsal compartment, as in ptcG4–Dl discs, but it was no longer induced in cells outside the *ptc*G4 stripe. It is possible that N expressed within the stripe binds most of the Dl within the stripe, preventing signaling to the adjacent cells that express N at a lower level. These results provide evidence that Dl within a cell can inhibit Dl signal reception by that same cell and that the ratio of Dl to N within a cell may determine its ability to both send and receive the Dl signal.

Dl *and* Ser *have different signaling abilities in the dorsal and ventral compartments*

Dorsal and ventral cells respond differently to ectopic Dl expression (Table 2). Dl induces higher levels of *wg* in the dorsal compartment than in the ventral compartment, and *cut* expression and adult wing outgrowth are restricted to the dorsal compartment. Even when high levels of Dl and N are coexpressed in the ventral compartment, *cut* expression and adult wing outgrowth are not induced in the ventral compartment (Fig. 5A–C; data not shown). Similarly, dorsal and ventral cells respond differently to ectopic Ser expression (Speicher et al. 1994; Kim et al. 1995; Table 2). Ser-induced *wg*, *cut*, and *vg* expression, as well as both disc and adult wing outgrowth, are restricted to the ventral compartment (Kim et al. 1995; Table 2; data not shown). Ser can partially substitute for Dl during neurogenesis in the embryo (Gu et al. 1995), and Dl and Ser have been shown to bind the same EGF repeat in N (Rebay et al. 1991); thus the Dl-induced *wg* and *vg* expression in the ventral compartment may indicate that Dl can partially substitute for Ser. Nonetheless, ectopically expressed Dl and Ser have strikingly different effects in the dorsal and ventral compartments.

Is the specificity attributable to compartmental differences in receptor–ligand interactions, or is it attributable to compartmental differences in the downstream response of the N pathway? To answer this question, we expressed a constitutively active truncated N protein (actN) using *ptc*G4. At 16°C, ptcG4–actN animals die as early pupae; however, third-instar wing discs displayed extreme outgrowth in both dorsal and ventral compartments, and *cut* was induced equally in the dorsal and ventral compartments (Fig. 5D–F). This result indicates that the factors responsible for compartment-specific N signaling act on or upstream of the N receptor.

Discussion

We have shown that *Dl* is required during *Drosophila* wing development as a ventral to dorsal signal. Mitotic clones lacking *Dl* that include cells on the ventral side of the D/V boundary cause loss of wing margin and blade tissue in both compartments, and Dl protein is elevated at the D/V compartment boundary of second-instar larval wing discs. Furthermore, ectopic Dl expression induces wing margin formation and wing tissue outgrowth. In light of these findings, we discuss the role of other genes in the *N* signaling pathway, the likely involvement of A/P boundary signaling components, and the requirement for a border between signaling and receiving cells at the D/V boundary during normal wing development. We also propose a model for *Dl* and *Ser* function during the early steps of wing margin formation in the second-instar wing disc.

The Dl–N *signaling pathway is required for wing formation*

The *Dl–N* pathway consists of a cassette of genes that functions to transmit signals between cells at many stages during development (Jan and Jan 1993; Artavanis-Tsakonas et al. 1995), and we have shown that Dl plays an essential role in wing development, probably by acti-

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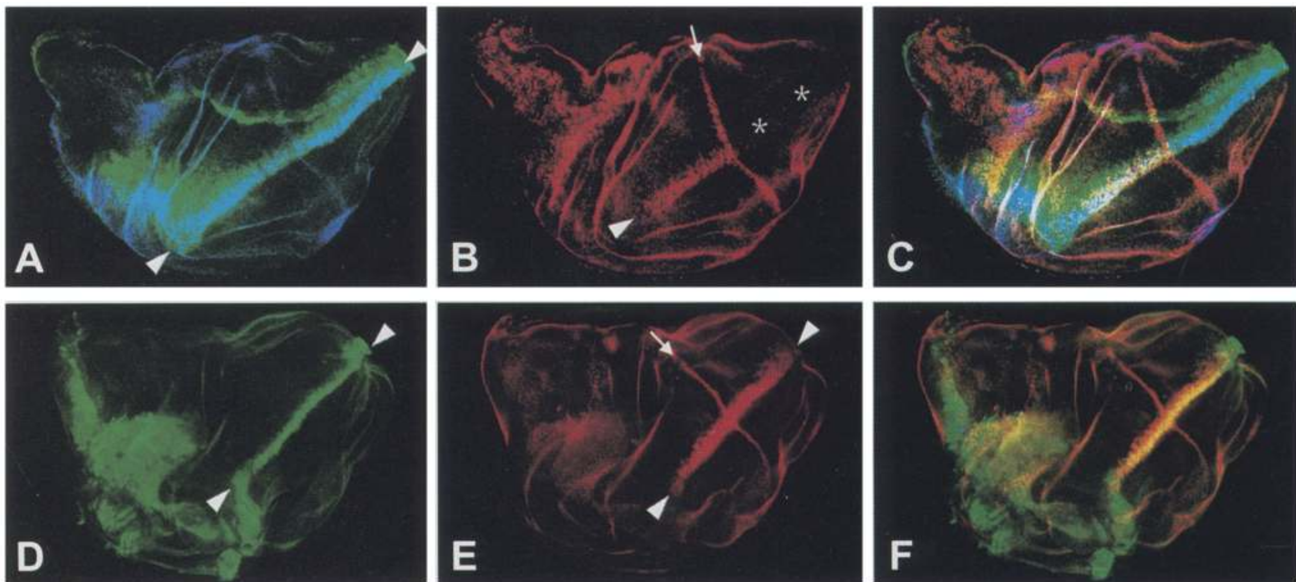

Figure 5. *cut* expression in ptcG4–Dl + N and ptcG4-activated N third-instar wing discs. (*A–C*) Dl, N, and cut expression in a ptcG4–Dl + N third-instar disc. Ectopic N and Dl together still induce cut expression only in the ventral compartment. UAS–*Dl* and UAS–*N* were expressed using the *ptc*G4 enhancer trap in larvae raised at 29°C. (*A*) Dl (blue) and N (green) are expressed ectopically in a stripe along the A/P compartment boundary (white arrowheads). (*B*) cut (red) is induced ectopically within the ptcG4 stripe only in the dorsal compartment (white arrowhead). Asterisks (*) mark the A/P boundary in the ventral compartment that is devoid of cut expression. (*C*) Overlay of *A* and *B*. Ectopic Dl, N, and cut expression overlap in the dorsal compartment (white signal) but not in the ventral compartment (blue signal). (*D–F*) N and cut expression in a ptcG4-activated N third-instar disc. Ectopic-activated N induces cut expression in the dorsal and ventral compartments. UAS-activated *N* was expressed using the *ptc*G4 enhancer trap in larvae raised at 16°C. (*D*) activated N (green) is expressed ectopically in a stripe along the A/P compartment boundary (white arrowheads). (*E*) cut (red) is induced ectopically within the *ptc*G4 stripe in both the dorsal and ventral compartments (white arrowheads). Endogenous cut expression is visible at the D/V boundary (white arrow). (*F*) Overlay of *D* and *E*. Ectopic-activated N and cut expression overlap in the dorsal and ventral compartments.

vating N . We propose that in addition to *N*, *Dl*, and *Ser*, other genes in the cassette are likely to be involved in wing development. *Suppressor of Hairless* [*Su(H)*] is downstream of *N* in the signaling pathway, and Su(H) protein has been shown to translocate from the cytoplasm to the nucleus upon activation of N by Dl in transfected S2 cells (Fortini and Artavanis-Tsakonas 1994). Consistent with the requirement for *N* in wing margin formation and growth, decreased *Su(H)* function results in a small third-instar wing pouch (Schweisguth and Posakony 1992) and very small adult wings, reminiscent of *vg* mutant wings (Ashburner 1982). *Su(H)* activity is antagonized by *Hairless* (*H*), which encodes a novel nuclear protein (Bang and Posakony 1992). Loss of *H* function leads to the opposite phenotype, an abnormally large wing pouch (Bang et al. 1991). Thus, it appears that multiple elements of the *Dl–N* signaling pathway operate during wing margin formation.

wg has been shown to interact with *N* during wing margin formation, indicating that these two genes function in the same pathway (Couso and Martinez-Arias 1994; Hing et al. 1994). Couso and Martinez-Arias (1994) proposed several molecular models to explain this interaction, favoring a model in which N functions as a receptor for wg. The observations that *N* is required for *wg* expression (Rulifson and Blair 1995) and that ectopic N and Dl induce *wg* demonstrate that *wg* is a downstream target of the *Dl–N* signaling pathway; however, we cannot eliminate the possibility that *wg* also functions upstream of *N* earlier during wing development.

Adult wing outgrowth requires factors expressed at the A/P compartment boundary

Our ectopic Dl expression experiments indicate that *Dl* can be sufficient to induce wing margin formation. We have strong evidence that the effects of ectopic Dl expression reflect the function of *Dl* during wild-type development. The UAS–*Dl* construct produces protein that is localized to the plasma membrane and cytoplasmic vesicles in a manner indistinguishable from wild-type Dl, and UAS–*Dl* rescues the *Dl* loss-of-function neurogenic phenotype in the embryo when driven by *hairy*–G4 (see Materials and methods). Furthermore, the ectopic expression phenotype is opposite to the loss-of-function phenotype and requires normal activity of N, the only known receptor for Dl.

We have found that ectopic expression of Dl near the A/P compartment boundary results in adult wing tissue outgrowth. Ectopic expression at other locations in the wing pouch causes ectopic gene expression and outgrowth of wing disc tissue but not adult wing tissue out-

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growth. Dl-induced adult outgrowth is always associated with wing margin structures, indicating that disc tissue overgrowth may resolve unless it is maintained by an established wing margin. Distal outgrowth caused by *fng*$^-$ clones also occurs only near the A/P boundary (Irvine and Wieschaus 1994). It is therefore likely that factors specific to the A/P border are required for induction of an ectopic wing margin and the resulting wing outgrowth. One candidate is *decapentaplegic* (*dpp*) that is expressed along the A/P border (Posakony et al. 1990). Ectopic *dpp* expression induces growth and pattern duplication in the wing (Capdevila et al. 1994). Moreover, overlapping expression of *wg* and *dpp* in the leg disc is required for proximodistal growth (Struhl and Basler 1993). Ectopic Dl-induced growth cannot be simply due to the ectopic expression of *wg* in *dpp*-expressing cells along the A/P boundary, because neither Dl-induced *wg* expression in the ventral compartment nor *ptc*G4-driven *wg* expression in both compartments is sufficient to cause adult wing outgrowth (Fig. 3A; E. Wilder, pers. comm.). It appears that the constellation of ectopic gene expression induced by ptcG4–Dl acts in concert with endogenous factors at the A/P compartment boundary to cause ectopic wing outgrowth. It will be interesting to determine how endogenous factors at the D/V and A/P boundaries interact to cause growth of the wing.

Dl can inhibit signal reception by N in the same cell

Ectopic Dl expression induces *wg* and *cut* only at the borders between Dl-expressing and -nonexpressing cells and not in all cells that express ectopic Dl. One possible explanation for this observation is that Dl inhibits N receptor activity when expressed within the same cell as N; Irvine and Wieschaus (1994) have proposed an analogous model for *fng* and its putative receptor. In our model, the ratio of Dl to N within a cell would determine its ability to receive a signal via N. Signaling would only occur when cells with a Dl/N ratio low enough to allow signal reception are juxtaposed to Dl-expressing cells. This model explains why there is decreased Dl signaling within the ptcG4–Dl stripe. We have tested three predictions of this model: (1) Signaling should be strongest between cells that express high levels of Dl and cells that express low levels of Dl. We observe maximal *wg* and *cut* induction immediately posterior to the ptcG4–Dl stripe, where cells expressing high Dl levels are juxtaposed to cells expressing low levels. (2) Increasing the level of N should relieve the Dl-mediated inhibition of N receptor activity. As expected, in discs expressing N and Dl under the control of *ptc*B4, *cut* is expressed throughout the width of the *ptc*G4 stripe. (3) Expressing high levels of Dl should mimic reduction in *N* function. This prediction is met by two paradoxical observations. Dl overexpression in the wing blade results in hypertrophy of wing veins (Fig. 3A–C), and Dl overexpression in the proneural cluster results in the development of extra sense organs; both of these phenotypes are similar to those caused by reduction in *N* or *Dl* activity (Parks and Muskavitch 1993; de Celis and Garcia-Bellido 1994; Fig. 1C–F; D. Doherty and G. Feger, unpubl.).

Dl could exert its inhibitory effect on N activity by directly interacting with N or by indirectly inhibiting N activity via other proteins. Alternatively, Fehon et al. (1990) proposed that Dl in receiving cells might interfere with N signaling by binding Dl ligand on the signaling cells. It should be noted that inhibition of signal reception by Dl does not appear to play a role in the early D/V patterning of the wing, because we did not detect a sharp border between *Dl*-expressing and -nonexpressing cells; however, later, the *wg* stripe is flanked on both sides by *Dl*-expressing cells, and signaling from *Dl*-expressing to -nonexpressing cells may be important.

A model for symmetrical gene activation at the D/V boundary

N activity is required on both sides of the D/V boundary for wing margin formation, whereas *Dl* and *Ser* are each required only on the ventral and dorsal sides, respectively. In addition, cells in the dorsal and ventral compartments respond differently to ectopically expressed Ser and Dl, whereas cells in both compartments respond equally to ectopically expressed activated N (Table 2). One possible explanation for the different activities of *Dl* and *Ser* could be their roles as compartment-specific signals. Bidirectional signaling between dorsal and ventral compartment cells has been invoked as a mechanism to generate the wing margin and symmetric growth of the wing (Irvine and Wieschaus 1994; Williams et al. 1994; Rulifson and Blair 1995). Compartment-specific signaling can be generated by spatial restriction of the ligand or by spatial restriction of the response. For example, *fng* and *Ser* are expressed only in the dorsal compartment of second-instar wing discs (Irvine and Wieschaus 1994; cited in Kim et al. 1995). Furthermore, *Ser* is ectopically induced at the borders of *fng*$^-$ clones in the dorsal compartment, and ectopic *fng* induces *Ser* in the ventral compartment; however, ectopic *Ser* does not induce *fng*, indicating that *Ser* functions downstream of *fng*. Irvine and Wieschaus (1994) have proposed that *fng* encodes a compartment-specific dorsal to ventral signal, and Kim et al. (1995) have shown that *fng*-expressing dorsal cells can recognize when they are adjacent to cells not expressing *fng* and respond by activating expression of *Ser*, which they propose encodes a dorsal to ventral signal. The existence of a reciprocal signal from ventral to dorsal cells is based on the observation that cells both inside and outside *fng*$^-$ or *ap*$^-$ clones are transformed into wing margin (Irvine and Wieschaus 1994; Williams et al. 1994). The N receptor is required for signaling in both directions, because loss of *N* function on one side of the D/V boundary eliminates *wg* expression and causes loss of wing tissue on both sides of the boundary (de Celis and Garcia-Bellido 1994; Rulifson and Blair 1995).

We propose that Dl acts as a ventral to dorsal signal that activates N to induce wing margin-specific genes during second-instar development. Dl displays three characteristics expected of such a signal: (1) *Dl* is re-

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quired in ventral cells at the D/V boundary for wing margin formation; (2) *Dl* is expressed at the D/V compartment boundary in second-instar discs; (3) ectopic Dl can induce ectopic wing margin formation and wing outgrowth, but only in the dorsal compartment. For the Dl signal, restriction of the response plays an important role in compartment specificity. For Ser, restriction of the response as well as restriction of the ligand is important, because *Ser* is expressed only in the dorsal compartment, and only ventral cells respond to ectopic Ser.

We propose the following model to explain the early steps of wing margin formation during the second larval instar (Fig. 6). Ser in the dorsal compartment induces *wg* expression and *Dl* expression or activity in ventral compartment cells. In turn, Dl in ventral compartment cells signals back to dorsal compartment cells via *N* to induce *wg* and to reinforce *Ser* expression or activity (Fig. 6). Preliminary results indicate that ectopic Dl expression induces *Ser* in the dorsal compartment of ptcG4–Dl discs (C. Micchelli, pers. comm.) and that ectopic Ser induces *Dl* in the ventral compartment of ptcG4–Ser discs (D. Doherty and G. Feger, unpubl.). Kim et al. (1995) have shown that *fng* acts upstream of *Ser* to activate *Ser* expression. The role of *fng* could be to initiate the positive feedback loop between *Dl* and *Ser* either by signaling from dorsal to ventral cells to activate *Dl* in the ventral cells, or by inhibiting the response to *Ser* and activating the response to *Dl* in dorsal cells creating a border for *Dl* and *Ser* signaling, or both. As wing development proceeds, the early pattern of *Dl* expression evolves into the third-instar pattern where *Dl* is expressed in two stripes flanking the *wg*-expressing cells at the D/V boundary. It is likely that the combined activities of *wg, Dl, Ser,* and other genes generate the later expression pattern. This

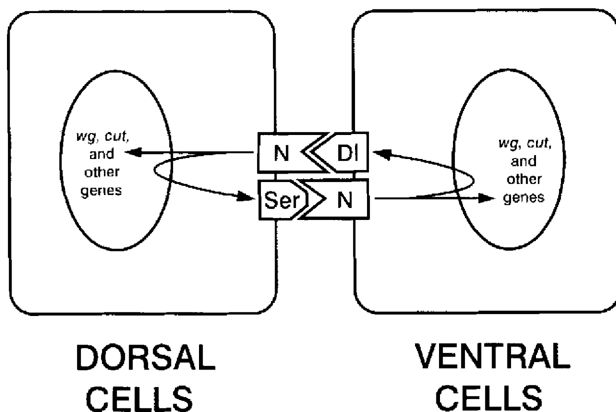

Figure 6. Model for symmetrical gene expression at the D/V boundary mediated by a positive feedback loop. Dl signals from ventral to dorsal cells via N to activate or maintain *Ser* and *wg* expression, whereas Ser signals from dorsal to ventral cells via N to activate or maintain *Dl* and *wg* expression. As wing development proceeds, the early pattern of *Dl* and *Ser* expression evolves into the third-instar pattern where *Dl* and *Ser* are expressed in two stripes flanking the *wg*-expressing cells at the D/V boundary. It is likely that the combined activities of *wg, Dl, Ser,* and other genes generate this later expression pattern.

system enables an intrinsically asymmetric boundary between *ap*-expressing and -nonexpressing cells to induce the symmetric patterns of growth and gene expression required to form the wing.

Conservation of mechanisms for axis formation and distal outgrowth in appendage development

How axes are specified is a universal problem during appendage development. Data from a variety of species suggest that there may be a limited number of molecular mechanisms for generating axes and other pattern information. For example, the *hedgehog* pathway is used to pattern different types of appendages in animals as evolutionarily divergent as the fly, chicken, and mouse (for review, see Perrimon 1995; Tabin 1995). The *Dl–N* signaling pathway described in this paper may also be used in vertebrate limbs. *Jagged*, a murine member of the *Dl/Ser* family, is expressed in developing limbs (Lindsell et al. 1995). *Wnt-7a*, a mouse homolog of *wg*, has been shown to function in D/V patterning of limbs (Parr and McMahon 1995; Riddle et al. 1995). It remains to be determined whether the *Dl–N/Ser–N* signaling pathways are used for limb axis formation throughout the animal kingdom.

Materials and methods

Clonal analysis

Adult mosaic clones of mutant *Dl* tissue were generated by X-irradiating second-instar larvae, as described by Diaz-Benjumea and Cohen (1993). To mark the Dl^+ chromosome, we used a $forked^+$ duplication on the third chromosome (distal to *Dl* at cytological map position 98B), kindly provided by F. Diaz-Benjumea and S. Cohen, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. This allowed us to identify *Dl forked* clones in flies hemizygous for f^{36a}. We used Dl^{rev10}, a loss-of-function *Dl* allele (Heitzler and Simpson 1991), which gave no detectable Dl protein in homozygous Dl^{rev10} embryos.

For analysis in wing discs, mitotic clones lacking *Dl* were generated using flipase-mediated mitotic recombination (Golic and Lindquist 1989; Golic 1991). We recombined Dl^{rev10} and *ebony*, a recessive mutation that darkens cuticular structures, onto a chromosome carrying p[FRT, neo, ry^+] at 82B (Xu and Rubin 1993). We crossed this chromosome into flies with a heat shock Myc-marked p[FRT, *neo*, ry^+] third chromosome and a heat shock flipase X chromosome and generated homozygous clones in wing discs and adult wings (see Xu and Rubin 1993). We identified *Dl* mutant clones by the absence of Myc expression in wing disc cells. Many wing discs with clones induced during second instar were extremely distorted, making them difficult to analyze.

Immunocytochemistry

Larvae were dissected in phosphate-buffered saline (PBS), fixed for 10 min in 4% formaldehyde and PEMS (0.1 M PIPES at pH 6.9, 1 mM EGTA, 2 mM $MgSO_4$), rinsed several times in PBT, blocked for 1 hr at room temperature with 2% normal goat serum, and incubated overnight at 4°C with the primary antibody. After several washes with PBT, fluorescent-labeled secondary antibodies (Jackson Laboratories, USA) were added for 1–2 hr at room temperature, washed with PBT, and mounted in

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glycerol/PBS/2% n-propylgallate. Samples were examined using a Bio-Rad MRC-600 confocal microscope. The following antibodies were used: rabbit anti-β-galactosidase (Cappel, USA) mouse anti-Dl mAb 202 (Kooh et al. 1993), rabbit anti-Vg (Williams et al. 1991), rat anti-cut (Blochlinger et al. 1988), rabbit anti-N (E. Giniger and Y.N. Jan, in prep.), rabbit anti-wg (van den Heuvel et al. 1989), mouse anti-en (Patel et al. 1989), rabbit anti-cubitus-interuptus (Schwartz et al. 1996), guinea pig anti-myc (G. Feger and Y.N. Jan, unpubl.). Confocal figures were assembled using Photoshop 3.0 (Adobe, USA) and Canvas 3.5.3 (Deneba, USA).

Ectopic expression of N *and* Dl

Targeted ectopic expression of *N* and *Dl* was accomplished using the GAL4 system (Brand and Perrimon 1993). UAS–*Dl* transgenic lines were generated by subcloning *Dl* cDNA 3.2 (Vaessin et al. 1987) into the pUAST vector and transformation into w^- flies by standard techniques. UAS-activated *N* lines were generated by subcloning the transmembrane and intracellular domain sequences of *N* fused to the *Dl* signal sequence into pUAST (E. Giniger, pers. comm.). To test for wild-type function of the UAS–*Dl* constructs we used hairy–GAL4-driven expression of UAS–Dl to rescue the neurogenic phenotype of Dl^{9P39} mutant embryos (D. Doherty and Y.N. Jan, in prep.). The UAS-activated *N* constructs displayed antineurogenic activity in embryos when expressed with hairy–GAL4, as expected for a constitutively active *N* construct (Lieber et al. 1993; Rebay et al. 1993; Struhl et al. 1993).

UAS–*Dl*, UAS-activated *N*, and UAS–*N* (gift from L. Seugnet, M. Haenlin, and P. Simpson, CNRS, Strasbourg, France) ectopic expression in imaginal discs was targeted using *ptc* GAL4 (Hinz et al. 1994) and other GAL4 enhancer trap lines. Homozygous GAL4 flies were crossed to UAS–*Dl/TM6B, Tb*, or homozygous UAS–*N* flies and grown at either 18°C, 22°C, or 29°C, as indicated. Larvae carrying both the GAL4 and the UAS insertions were identified on the basis of the Tb^+ phenotype.

The expression pattern of ptcGAL4 was examined by crossing it to a UAS–*lacZ* reporter line carrying a nuclear localized β-galactosidase under UAS control.

Genetics and temperature shifts

To test for N^{ts} suppression of the ptcG4–Dl adult phenotype, we crossed *w*, N^{ts}/*FM6*; *ptc* GAL4 females to *y w*; UAS–*Dl/TM6B, Tb* males in vials. We collected eggs in vials over a period of 24 hr (at 22°C), incubated the vials at 22°C until the larvae had developed to second instar, and then shifted the vials to 29°C for 24 hr. A range of abnormal wing outgrowth phenotypes was observed in both classes (*yw*/N^{ts} and *yw/FM6*) of ptc–Dl females, whereas none of the *w* N^{ts}/Y; ptc–Dl males had abnormal wing outgrowth.

All flies were grown on standard cornmeal–agar medium at room temperature unless otherwise noted. Mutations not specifically discussed here are described in Lindsley and Zimm (1992).

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Delta is a ventral to dorsal signal complementary to Serrate, another Notch ligand, in Drosophila wing formation.

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