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Temporal regulation of Apterous activity during development of the *Drosophila* wing

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SUMMARY

Dorsoventral axis formation in the *Drosophila* wing depends on the activity of the selector gene *apterous*. Although selector genes are usually thought of as binary developmental switches, we find that Apterous activity is negatively regulated during wing development by its target gene *dLMO*. Apterous-dependent expression of *Serrate* and *fringe* in dorsal cells leads to the restricted activation of Notch along the dorsoventral compartment boundary. We present evidence that the ability of cells to participate in this Apterous-dependent cell-interaction is under spatial and temporal control. Apterous-dependent expression of *dLMO* causes downregulation of *Serrate* and *fringe* and allows expression of *Delta* in dorsal cells. This limits the time window during which dorsoventral cell interactions can lead to localized activation of Notch and induction of the dorsoventral organizer. Overactivation of Apterous in the absence of dLMO leads to overexpression of Serrate, reduced expression of Delta and concomitant defects in differentiation and cell survival in the wing primordium. Thus, downregulation of Apterous activity is needed to allow normal wing development.

Key words: Selector gene, Cell interaction, *dLMO*, *serrate*, *fringe*, *Delta*, *Notch*, *Drosophila*

INTRODUCTION

The limbs of *Drosophila* are subdivided into compartments by the restricted expression of selector genes, which confer compartment-specific cell identity. Selector genes encode transcription factors and are thought to regulate genes required for cell-type-specific differentiation as well as genes that control cell interactions between compartments. For example, the signaling proteins Hedgehog and Serrate are expressed in a compartment-specific manner and mediate short-range interactions between adjacent compartments that induce expression of the long-range signaling molecules Wingless (Wg) and Decapentaplegic (Dpp) along the compartment boundaries (reviewed in Blair, 1995; Brook et al., 1996; Dahmann and Basler, 1999; Strigini and Cohen, 1999).

The selector gene *apterous* (*ap*) subdivides the wing imaginal disc into dorsal and ventral compartments (Diaz-Benjumea and Cohen, 1993; Blair, 1994; Williams et al., 1994). *ap* encodes a LIM-homeodomain transcription factor (Ap, Cohen et al., 1992). Ap activity depends on formation of a higher order complex, in which two molecules of Ap are bridged by a dimer of its cofactor, the LIM-domain binding protein dLDB/Chip (Fernandez-Funez et al., 1998; O'Keefe et al., 1998; Milán and Cohen, 1999a; van Meyel et al., 1999). Ap activity is also regulated by a competitive inhibitor encoded by the *dLMO/Beadex* gene (Milán et al., 1998; Shoresch et al., 1998; Zeng et al., 1998).

Ap activity confers dorsal fate and induces the expression of Serrate, a Notch ligand, in dorsal cells (Diaz-Benjumea and Cohen, 1995; Kim et al., 1995; de Celis et al., 1996; Doherty et al., 1996; Bachmann and Knust, 1998). At early stages, Serrate signals via Notch to induce Wg and Delta in nearby ventral cells. Delta, another Notch ligand, signals back to induce Wg and maintain Serrate in dorsal cells. Ap also induces Fringe expression in dorsal cells. Fringe serves to polarize activation of Notch by Delta and Serrate at the dorsoventral (DV) boundary. Fringe is thought to modulate the sensitivity of dorsal cells to activation of Notch by its ligands (Johnston et al., 1997; Panin et al., 1997; Fleming et al., 1997). Fringe-expressing cells are more sensitive to Delta than ventral cells and are refractory to Serrate. This limits activation of Notch to cells in close proximity to the DV boundary and therefore to tightly localized expression of Wg and Cut (de Celis et al., 1996; Neumann and Cohen, 1996; de Celis and Bray, 1997; Micchelli et al., 1997).

Two additional mechanisms contribute to limiting Wg expression to cells immediately adjacent to the DV boundary. Wg signaling regulates Notch activity via Dishevelled, which binds to Notch and reduces its activity (Axelrod et al., 1996). In addition, the POU domain-protein Nubbin limits the range over which Notch activation induces Wg in the wing pouch (Neumann and Cohen, 1998). Together with the polarizing activity of Fringe, these mechanisms limit Wg expression to cells abutting the DV boundary. Thereafter a different set of regulatory interactions takes over to maintain *wg* and *cut* expression at the boundary (de Celis and Bray, 1997; Micchelli et al., 1997). Wg signaling induces expression of Serrate and

Delta in nearby dorsal and ventral cells. In this context Serrate and Delta have two functions: they cooperate to maintain Wg and Cut expression in cells at the DV boundary and also repress Notch signaling in the cells in which they are expressed. The feedback loop between Wg and the Notch ligands serves to maintain expression of these genes in their symmetric DV expression domains, independent of further asymmetric signaling across the DV boundary.

The flow of cell interactions in the DV patterning system suggests that Apterous activity may only be required transiently to trigger the cascade of interactions between D and V cells. Early in wing development, Ap triggers the restricted activation of Notch along the DV boundary by inducing *Serrate* and *Fringe* and by repressing *Delta* expression in the dorsal compartment. At around the same time, Ap induces the expression of dLMO which serves as a competitive inhibitor of Ap complex formation (Milán et al., 1998; Milán and Cohen, 1999a). By inducing its own inhibitor, Ap limits the time during which it can activate *Serrate* and *fringe* in dorsal cells and restrict *Delta* to ventral cells. Consequently, the initially dorsal/ventral asymmetric expression of these genes is transient and each adopts a secondary expression domain that is independently controlled. The transition between Ap-dependent and Ap-independent modes of regulation is needed to allow a change in *Serrate* and *Delta* expression from a mutually restricted expression at early stages to a symmetrical expression at both sides of the DV boundary in late third instar wing discs. Prolonged activation of Ap in dorsal cells perturbs patterning, cell proliferation and cell survival in the wing primordium.

MATERIALS AND METHODS

Drosophila strains

hdp^{R590} and *MS1096* are *dLMO* hypomorphic mutations described in Milán et al. (1998) and Milán and Cohen (1999a). MS1096 is a GAL4 P-element insertion at the dLMO locus. hdp^{R590} was generated by imprecise excision of MS1096, such that dLMO protein expression is strongly reduced. GAL4 is expressed normally in the dLMO pattern in hdp^{R590}. We refer to this mutant as $dLMO^{GAL4}$. *UAS-dLMO* is described in Milán et al. (1998). $apterous^{GAL4}$ is described in Calleja et al. (1996). ap^{UGO35} is described in Cohen et al. (1992). $Serrate\text{-}lacZ^{9.1}$ is described in Bachmann and Knust (1998). l(3)rG554 (Bloomington stock P2109) is an enhancer trap insertion at the *fringe* locus and is allelic to $fringe^{80}$ (a null allele of *fringe*). This allele is referred to as $fringe^{P2109}$. *UAS-fringe* corresponds to *EP(3)3082* which is inserted at the *fringe* locus (Milán and Cohen, 1999b). $fringe^{UZ35}$ and $fringe^{13}$ are described in Irvine and Wieschaus (1994). *UAS-Ser* is described in Speicher et al. (1994). $Chip^{e55}$ is described in Morcillo et al. (1997). *UAS-ApΔHD* is described in O'Keefe et al. (1998). *UAS-ChAp* is described in Milán and Cohen (1999a). Dl^{6B37} is a *Dl* hypomorphic mutation described in Doherty et al. (1996).

Antibodies

Sources of antibodies used: rabbit anti-Serrate (Thomas et al., 1991); mouse anti-Wg (Brook and Cohen, 1996); mouse anti-dLMO (Milán et al., 1998); Rabbit anti-β-galactosidase (Cappell); mouse anti-Delta (Doherty et al., 1996). TUNEL staining was performed as in Milán et al. (1997).

Genotypes of larvae used for genetic mosaic analysis

Chip mutant clones

hs-FLP (I); FRT42 $Chip^{e55}$/ FRT42 arm-lacZ. Clones were marked by the loss of β-galactosidase expression in the discs.

hs-FLP f^{36a}; FRT42 $Chip^{e55}$/ FRT42 $P(f^{+})$. Clones were marked in the adult cuticle by loss of the *forked*$^{+}$ transgene on the $Chip^{+}$ chromosome.

Chip mutant clones in a *dLMO* loss-of-function background

hdp^{R590}/Y; FRT42 $Chip^{e55}$/ FRT42 arm-lacZ; hs-FLP (III). Clones were marked by the loss of β-galactosidase expression in the discs.

Chip mutant clones expressing Ser in the dorsal compartment

hs-FLP (I)/$dLMO^{GAL4}$; FRT42 $Chip^{e55}$/ FRT42 armadillo-lacZ; UAS-Ser/+. Clones were marked by the absence of Serrate protein expression. $dLMO^{GAL4}$ expression depends on Ap activity, so clones of *chip* mutant cells lose GAL4 activity and lose Serrate expression. This allows Serrate expression in cells surrounding the clone, but removes Serrate within the clone. Serrate overexpression has a dominant-negative effect on Notch signaling, so it is necessary to remove Serrate within the clone to assay whether providing Serrate in the surrounding cells is sufficient to induce Wg in the clones.

fringe mutant clones

hs-FLP (I); $fringe^{13}$ FRT80/ arm-lacZ FRT80 larvae. Clones were marked by the loss of β-galactosidase expression in the discs.

hs-FLP (I); mwh $fringe^{13}$ FRT80/ arm-lacZ FRT80 larvae. Clones were marked by the *mwh* mutation.

dLMO overexpressing clones

hs-FLP (I)/Actin>CD2>Gal4; UAS-dLMO. Clones were marked by dLMO protein expression in the discs.

dLMO mutant clones

hdp^{R590} FRT18/arm-lacZ FRT18; hs-FLP (II). Clones were marked by the loss of β-galactosidase expression in the discs.

f^{36a} hdp^{R590} FRT18/FRT18; hs-FLP (II). Clones were marked by the *forked* mutation in the adult cuticle.

Minute^{+} dLMO mutant clones

f^{36a} hdp^{R590} FRT18/WG1296-lacZ M(1)15D FRT18; hs-FLP (III)

Clones were marked by the *forked* mutation in the adult cuticle and by loss of β-galactosidase expression in the discs.

RESULTS

Ap-dependent and Ap-independent expression of *Serrate* and *fringe*

Serrate and *fringe* are initially induced in the dorsal compartment of the wing disc during second instar under control of Apterous (Irvine and Wieschaus, 1994; Speicher et al., 1994; Diaz-Benjumea and Cohen, 1995; Bachmann and Knust, 1998). Later in development they exhibit more complex expression patterns that do not show an obvious correlation with DV compartments (Fig. 1A,D). *lacZ*-reporter genes have been identified that reflect the early dorsal expression of *Serrate* and *fringe* (Fig. 1B,C). $fringe^{UZ35}$ is a *lacZ*-containing P-element enhancer trap at the *fringe* locus (Irvine and Wieschaus, 1994). $fringe^{UZ35}$ is expressed in the dorsal compartment of the wing disc throughout development (Fig. 1C). $fringe^{UZ35}$ does not resolve into the mature pattern of *fringe* expression, perhaps because the insertion contains 35 kb of the Ubx locus and may confer Polycomb-dependent stabilization on the early expression pattern of the reporter gene. The $fringe^{P2109}$ *lacZ* enhancer-trap line accurately reflects the developmental changes in *fringe* expression (Fig. 1D). $Ser\text{-}lacZ^{9.1}$ is a P-element construct in which an Ap-

dependent imaginal disc enhancer from the *Serrate* locus directs *lacZ* expression (Bachmann and Knust, 1998). *Ser-lacZ*$^{9.1}$ faithfully reflects the changes in Ap-dependent expression of *Serrate* throughout development (see below).

To ask whether the late expression patterns of *Serrate* and *fringe* depend on continued activity of Ap, we examined their expression in *apterous* mutant wing discs. Wing discs mutant for *apterous* fail to induce Wg expression at the DV boundary and the presumptive wing pouch fails to grow. In *ap*^{GAL4}/*ap*^{UGO35} mutant larvae, the requirement for Ap activity can be circumvented using *ap*^{GAL4} to force activation of the Notch signaling pathway at the DV boundary (Milán and Cohen, 1999b). Despite the lack of Ap function in *ap*^{GAL4}/*ap*^{UGO35}*; UAS-fringe* larvae, forced expression of *fringe* in the dorsal compartment restores Wg expression and growth of the wing pouch (Fig. 1E-H). In this genetic combination *Ser-lacZ*$^{9.1}$ and *fringe*^{UZ35} are not expressed, reflecting the low level of Ap activity from early stages of wing development (Fig. 1E,G). However, Serrate protein and *fringe*^{P2109} are expressed in essentially normal late patterns (Fig. 1F,H). These results indicate that late expression of Serrate in the presumptive wing veins and adjacent to the DV compartment boundary is independent of Ap activity and does not require prior Serrate expression under Ap control. Similarly, late expression of *fringe* in the quadrant pattern does not require prior Ap activity. There is a subtle difference between the pattern of *fringe*^{P2109} expression in wild-type and in the absence of Ap activity. In addition to its obvious broad expression in A, P and D, V quadrants of the wing pouch, *fringe*^{P2109} is also expressed at a low level in dorsal cells near the DV boundary. This expression is lost in the *ap* mutant (arrow, Fig. 1D, compare with 1H), indicating that this aspect of *fringe*^{P2109} expression is Ap dependent. This low-level dorsal expression is partly obscured by the stronger expression of *fringe* in the quadrant pattern, but is functionally important (see below).

Ap-dependent expression of *Ser* and *fng* is downregulated by dLMO

The shift from early, Ap-dependent, regulation to Ap-independent regulation of *Serrate* and *fringe* could be explained in two ways. Ap activity levels might be downregulated during development. Alternatively, *Serrate* and *fringe* might become insensitive to regulation by Ap. To distinguish between these possibilities, we used two different genetic methods to regulate Ap activity levels in the wing disc. (1) dLMO acts as a competitive inhibitor of Ap-dLDB complex formation and downregulates Ap activity (Milán et al., 1998; Milán and Cohen, 1999a). Reduced dLMO activity causes an increase in Ap activity, leading to a failure to downregulate the early-acting *Ser-lacZ*$^{9.1}$ enhancer in the dorsal wing pouch and to a reduced wing pouch (compare Fig. 1I with 1B). Overactivation of Ap in the *dLMO* mutant wing disc can be counteracted by expression of a dominant negative form of Apterous, which lacks the homeodomain (Milán and Cohen, 1999a). This resulted in a more normal pattern of *Ser-lacZ*$^{9.1}$ expression and to restoration of the size of the wing pouch (Fig. 1J). Likewise, the early dorsal expression of *fringe*^{P2109} failed to undergo normal modulation in the *dLMO* mutant wing disc, so that expression levels remain abnormally high in the dorsal compartment (Fig. 1K). *fringe*^{P2109} expression was also restored to normal by expression of dominant negative Apterous under *dLMO*^{GAL4} control (data not shown). (2) ChAp is a constitutively active form of Ap that is not subject to inhibition by dLMO (Milán and Cohen, 1999a; van Meyel et al., 1999). Serrate expression is elevated in the dorsal compartment of discs expressing ChAp (Fig. 1L). Together these observations indicate that *Serrate* and *fringe* remain sensitive to regulation by Ap as the disc matures. Thus, the developmental changes in their expression are likely to reflect changes in Ap activity levels, rather than changes in the intrinsic sensitivity of the target genes.

Temporal and spatial changes in the requirement for Ap activity in the developing wing

In addition to inducing the DV organizer, Ap has been implicated in specification of dorsal cell fate and regulation of PS integrin expression (Diaz-Benjumea and Cohen, 1993; Blair et al., 1994). PS integrin is required to maintain adhesion between the dorsal and ventral surfaces of the wing. To define more precisely the times at which Ap activity is required for these different functions, we examined clones of cells mutant for *Chip*, the cofactor required for Ap activity (Fernandez-Funez et al., 1998). *Chip* mutant clones were induced in second instar, and in early and mid-third instar and the resulting phenotypes were analyzed in adult wings (Fig. 2) and in wing discs (Fig. 3). Dorsal clones generated in second instar induced ectopic wing margin structures along their borders and induced outgrowth of the dorsal compartment, as reported previously (Fig. 2A). Wg was ectopically expressed in both mutant and neighboring wild-type cells (Fig. 3A, inset). Clones induced in early third instar resulted in ectopic margin tissue and ectopic Wg expression in clones located close to the endogenous DV boundary in the dorsal pouch (arrows Figs 2B, 3B). Ventral clones did not induce ectopic Wg expression. Clones located more proximally in the dorsal compartment produced blisters and a dorsal to ventral change in cell identity but did not induce ectopic margin tissue or ectopic Wg expression (arrowheads Figs 2B, 3B). Blistering was presumably due to loss of integrin expression in the clones. Thus the consequences of removing Ap activity differ with the position of the clone.

The observation that *Chip* mutant clones induced in early third instar produced different phenotypes in different positions would be difficult to explain in terms of Chip expression, which is uniform throughout the wing disc (Morcillo et al., 1997; Fernandez-Funez, et al., 1998). Nonetheless, it is possible that Chip activity could be lost earlier in proximal clones than in distal clones. To ask whether blocking Ap activity by a different mechanism would produce the same phenotypes, we made clones of cells expressing the Ap-antagonist dLMO under control of the actin promoter using the flip-out GAL4 system (Pignoni and Zipursky, 1997; hsFLP/act5C>CD2>GAL4; UAS-dLMO). Clones of dLMO-expressing cells induced in second instar produced phenotypes comparable to those produced by removing *ap* or *Chip* activity at this stage. Wg was ectopically expressed at the border between dLMO-expressing and non-expressing cells and large outgrowths of the dorsal wing were observed (Fig. 3D). Clones of dLMO-expressing cells induced in early third instar induced ectopic Wg when located close to the DV boundary (Fig. 3E), as described for *Chip* mutant clones (Fig. 3B). Ventral dLMO-expressing clones had no effect at any stage.

Chip mutant clones induced in mid third instar did not induce ectopic Wg expression (Fig. 3C) or induce ectopic wing

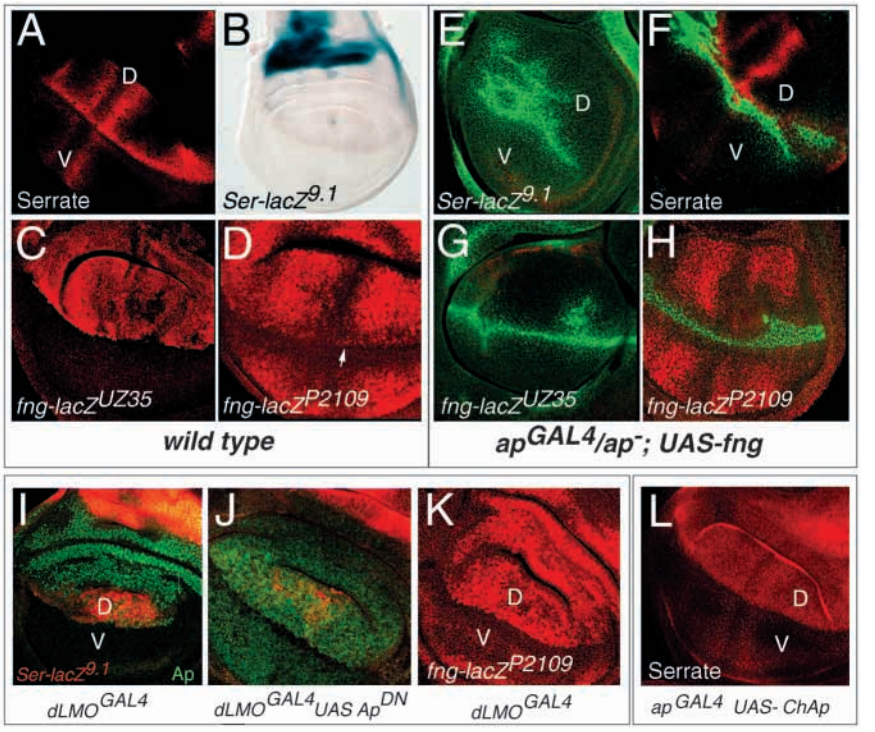

Fig. 1. Ap-dependent and Ap-independent expression of *Serrate* and *fringe.* (A) Serrate protein expression in the wing pouch of a wild-type third instar wing disc. D and V indicate dorsal and ventral compartments. (B) *Ser-lacZ*$^{9.1}$ expression in a wild-type wing disc visualized by histochemical staining for *lacZ* activity. (C,D) *fringe*^{UZ35} and *fringe*^{P2109} expression in the wing pouch of wild-type third instar wing discs visualized by antibody to β-Gal protein. The arrow indicates Ap-dependent expression of the reporter gene in cells dorsally-adjacent to the DV boundary. This expression overlaps with Wg (not shown). (E-H) *ap*^{GAL4}/*ap*^{UGO35}; *UAS-fringe* mutant wing discs labeled to visualize Wg protein (green) and Serrate or βGAL proteins (red). (E) *Ser-lacZ*$^{9.1}$ expression is absent from the D compartment (compare with B). (F) Serrate protein is expressed in the D compartment. (G) *fringe*^{UZ35} expression is absent from the D compartment (compare with C). (H) *fringe*^{P2109} is expressed in the D compartment, except that the low level expression in dorsal cells near the Wg stripe is missing (see arrow in D). (I) *dLMO* loss-of-function mutant wing disc (*hdp*^{R590}). *Ser-lacZ*$^{9.1}$ expression (red) was elevated in the dorsal wing pouch. The dorsal compartment was reduced in size (marked by Apterous protein expression in green). (J) *dLMO* mutant wing disc expressing a dominant negative form of Ap (ApΔHD). *Ser-lacZ*$^{9.1}$ expression (red) was reduced and the wing pouch was of normal size. Note that ApΔHD was expressed in D and V cells under *dLMO*^{GAL4} control (see Materials and Methods). (K) *fringeP2109* expression was elevated in the dorsal wing pouch in a *dLMO* mutant disc. (L) Serrate protein expression is elevated in the dorsal wing pouch in ap^{GAL4} UAS-ChAp discs.

margin differentiation (Fig. 2C). This could be because Ap has no function at this stage or because perdurance of Chip protein masks the effects of producing mutant clones at this late stage. Two pieces of evidence argue against the perdurance explanation. First, *Chip* mutant clones induced at this stage showed DV cell identity changes (Fig. 2E). Second, dLMO-expressing clones induced at 96 hours also fail to induce ectopic Wg (Fig. 3F).

Thus, the same phenotypes were observed whether Ap function was compromised by removing its cofactor Chip, or by expressing the antagonist dLMO. These observations suggest that removing Ap activity has different consequences at different stages and at different positions in the developing wing disc. Ap is required at both early and late stages for dorsal fate specification and for proper adhesion between D and V surfaces of the wing (inferred from blister formation). However, the ability of Ap to induce activation of the Notch pathway at the interface between cells that are functionally Ap^{+} and cells that are functionally Ap^{-} is gradually lost beginning in early third instar. Cells near the DV boundary appear to retain the ability to induce Wg expression in neighboring Ap^{-} cells for longer than cells at a distance from the boundary. This could reflect an autonomous change in the level of Ap activity in cells as a function of their position, or it could reflect an underlying difference that changes the interaction between cells in which Ap is active and cells in which Ap is inactive.

Differential requirements for Ap activity are due to changes in Serrate expression

To ask whether the spatial differences in the effect of removing Ap activity can be attributed to differential sensitivity to signals coming from the surrounding cells, we examined the effects of producing *fringe* mutant clones. Cells mutant for *fringe* become sensitive to stimulation by Serrate expressed on neighboring wild-type cells and respond by expressing Wg (Irvine and Wieschaus, 1994; Kim et al., 1995; Panin et al., 1997; Fleming et al., 1997). *fringe* mutant clones produced phenotypes comparable to *chip* mutant clones or clones expressing dLMO at all stages. In second instar discs *fringe* mutant clones induced ectopic Wg expression and caused bifurcation of the wing pouch (Fig. 3G), resulting in ectopic wing margin tissue and outgrowth of the dorsal compartment (Fig. 4A). One difference from *ap* or *chip* mutant clones is that ventral *fringe* mutant clones caused a phenotype. Clones located near the second longitudinal vein autonomously induced an ectopic vein (Fig. 4C). *fringe* mutant clones induced in early third instar discs show the same spatial difference in Wg expression as *chip* mutant clones or clones expressing dLMO. Clones close to the DV boundary ectopically expressed Wg (Fig. 3H) and induced wing margin structures (arrows, Fig. 4B) whereas clones more distant from the boundary did not (Fig. 3H, arrowheads Fig. 4B). *fringe* mutant clones induced in mid-third instar did not cause ectopic Wg expression and did not show any phenotype in the adult wing (data not shown).

These observations raised the possibility that Serrate expression might be too low to activate the Notch pathway in cells far from the DV boundary when Fringe activity was removed in early third instar. To address this, we examined the behavior of *chip* mutant clones in wing discs where Serrate

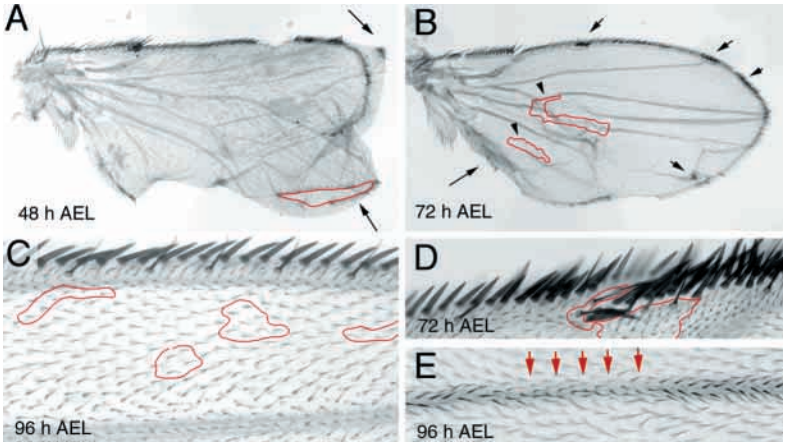

Fig. 2. Wing phenotypes caused by removing Apterous activity at different developmental stages. Cuticle preparations of adult wings carrying *Chip*^{e55} mutant clones. Clones were induced in *hs-FLP* f^{36a}*; FRT42 Chip*^{e55}*/FRT42 P(f*$^{+}$*)* larvae by heat-shock treatment. Mutant cells are marked by loss of the *forked*$^{+}$ transgene on the *Chip*$^{+}$ chromosome. (A) Dorsal clones induced in early second instar (48 hours after egg laying, 48 h AEL) induce ectopic wing margin tissue and cause outgrowth of the wing (arrows). Ventral clones produce no phenotype (not shown). (B) Dorsal clones induced in early third instar (72 h AEL) at or near the endogenous margin induce ectopic wing margin tissue (arrows). Clones at a distance from the margin induce blistering, without inducing ectopic margin (arrowheads). (C) Clones induced in mid third instar (96 h AEL) do not induce ectopic margin. Clones are marked with *forked* (mutant cells are encircled). (D) High magnification of a *Chip*^{e55} mutant clone induced at 72 h AEL at the anterior wing margin in the D compartment. Mutant cells (marked by *forked*) differentiate ventral margin identity. (E) Clone induced at 96 h AEL and located in the fifth longitudinal vein. Vein differentiation is asymmetric on dorsal and ventral surfaces of the wing. Vein 5 is thick and corrugated on the dorsal surface and thin on the ventral surface. Note the reduced vein thickness and the absence of corrugation within the clone. The contours of the clones are drawn in red with the exception of the clone shown in E which is demarcated by red arrows.

expression was maintained in the dorsal compartment under control of the GAL4 driver *MS1096* (which is inserted at *dLMO*, see methods for details). The experimental design depends on the fact that *chip* mutant clones lose dLMO expression (Fig. 5A) and therefore lose *MS1096*-dependent expression of Serrate, which would otherwise autonomously block Notch signaling (see de Celis and Bray, 1997; Micchelli et al., 1997). Consequently, Wg was expressed in *chip* mutant clones when Serrate expression was maintained in nearby cells (Fig. 5B). These clones were associated with bifurcations and outgrowth of the dorsal wing pouch. In discs where Serrate was not maintained artificially, comparable bifurcations could only be recovered when clones were induced earlier in development. Interestingly, only proximally located *chip* mutant clones showed ectopic Wg expression under these conditions. Clones located in the central part of the wing pouch did not induce Wg, despite the presence of Serrate in the surrounding cells. This is likely due to Ap-independent expression of Fringe in the wing pouch (see Fig. 1D).

dLMO is required in dorsal cells to reduce Serrate levels

Our results suggest that the spatial difference in the effects of removing Ap activity reflect an underlying difference in

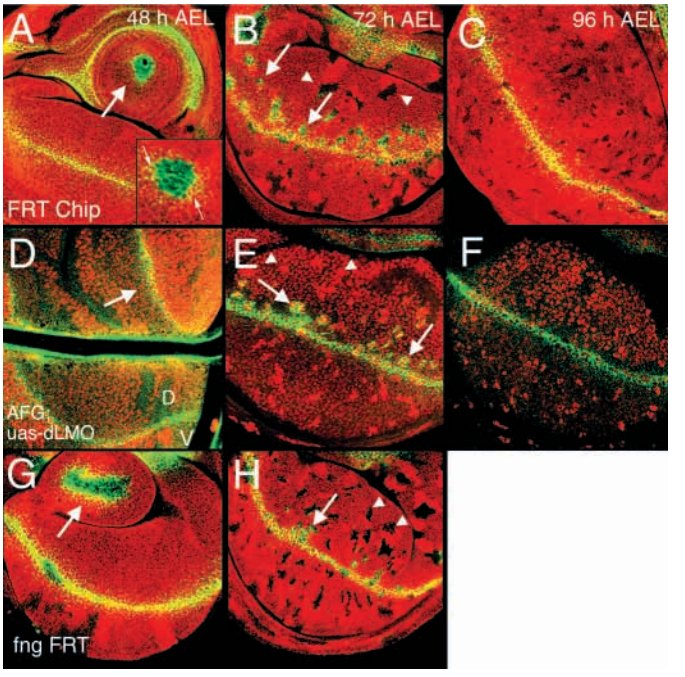

Fig. 3. Spatial differences in cell interactions caused by removing Apterous activity or Fringe at different developmental stages. (A-C) *Chip*^{e55} mutant clones in the wing pouch labeled by the absence of β-galactosidase expression (in red). (A) Clone induced in second instar (at 48 h AEL). Wg protein (green) is ectopically expressed along the clone border (arrow). The clone has caused a bifurcation and outgrowth in the dorsal wing pouch. Inset shows a proximal clone induced at 48 h AEL showing both wild-type and mutant cells expressing Wg. (B) Clones induced in early third instar (at 72 h AEL). Wg protein (green) is ectopically expressed along the clone borders of distal clones (arrows) but not in proximal ones (arrowheads). (C) Clones induced in mid third instar (at 96 h AEL). Wg protein (green) is not ectopically expressed along the clone borders. (D-F) dLMO overexpressing clones in the wing pouch visualized by the higher level of dLMO protein (in red). (D) Clone induced in second instar (at 48 h AEL). Wg protein (green) is ectopically expressed along the clone border (arrow). (E) Clones induced in early third instar (at 72 h AEL). Wg protein (green) is ectopically expressed along the clone borders of distal clones (arrows) but not in proximal ones (arrowheads). (F) Clones induced in mid third instar (at 96 h AEL). Wg protein (green) is not ectopically expressed along the clone borders. (G,H) *fng*^{13} mutant clones in the wing pouch labeled by the absence of β-galactosidase expression (in red). (G) Clone induced in second instar (at 48 h AEL). Wg protein (green) is ectopically expressed along the clone border (arrow). (H) Clones induced in early third instar (at 72 h AEL). Wg protein (green) is ectopically expressed along the clone borders of distal clones (arrows) but not in proximal ones (arrowheads).

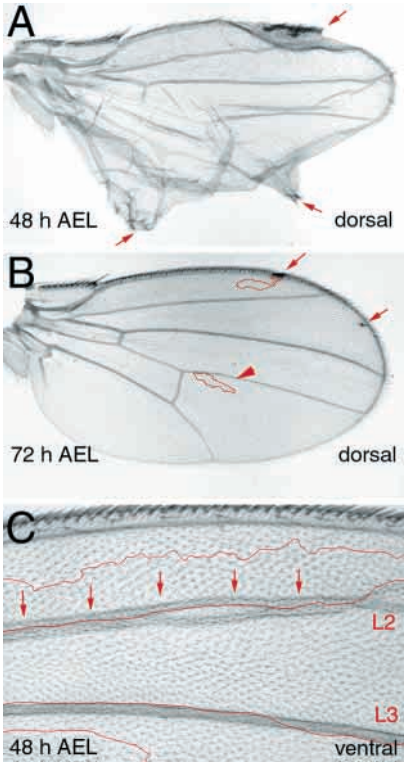

Fig. 4. Wing phenotypes caused by removing *fringe* at different developmental stages. Cuticle preparations of adult wings carrying *fng*^{13} mutant clones. Clones were induced in *hs-FLP*; *mwh fng*^{13} *FRT80/FRT80* larvae by heat-shock treatment. Mutant cells are homozygous for *mwh* (not visible at this magnification). (A) Dorsal clones induced in early second instar (48 h AEL) induce ectopic wing margin tissue and cause outgrowth of the wing (arrows). (B) Dorsal clones induced in early third instar (72 h AEL) at or near the endogenous margin induce ectopic wing margin tissue (arrows). Clones at a distance from the margin do not show any phenotype (arrowheads). (C) Detail of a wing showing portions of two ventral clones induced in early second instar (48 h AEL). The clone located adjacent to the second longitudinal vein (L2) autonomously induces ectopic vein differentiation (arrows). The clone located at the third longitudinal vein (L3) produces no phenotype. The clones are outlined in red.

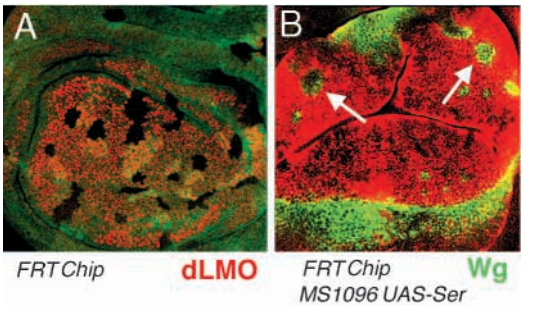

Fig. 5. Spatial differences caused by removing Ap activity are due to Serrate levels. (A) *Chip*^{e55} mutant clones in the wing pouch labeled by the absence of β-galactosidase expression (green). dLMO expression (red) was lost in *Chip*^{e55} mutant cells in D and V compartments. (B) *Chip*^{e55} mutant clones induced in a disc carrying *MS1096* and *UAS-Serrate.* Clones were marked by the absence of β-galactosidase expression (red). Arrows indicate clones in the proximal wing pouch that induced ectopic Wg expression (green) and caused axis bifurcation.

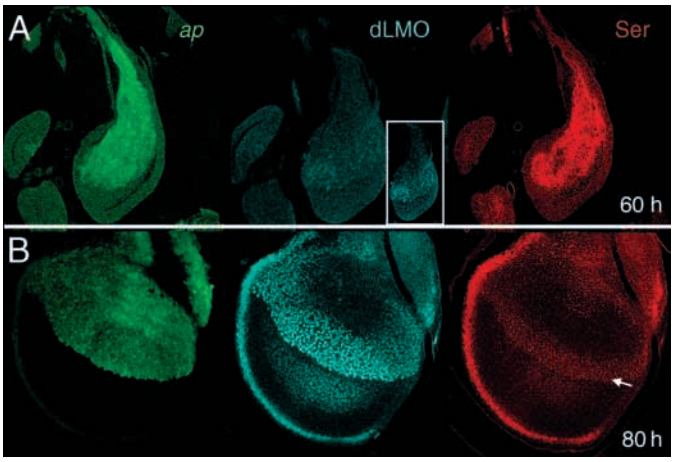

Fig. 6. Comparison of Serrate and dLMO expression in second and early third instar. Wing discs labeled for *ap*^{GAL4} expression using *UAS-GFP* (green). dLMO expression is shown in blue. Serrate expression is shown in red. (A) Second instar wing disc (60 h AEL). (B) Early third instar disc (80 h AEL). The images in A and B were taken under the same conditions to allow comparison of the relative expression levels at the two stages. dLMO expression is barely detectable in second instar. The inset shows the same disc with the dLMO signal intensified to visualize the low level of expression. The level of Serrate decreases considerably in the dorsal wing pouch, but remains higher near the DV boundary than in the more proximal region (arrow).

interactions between mutant cells that are functionally Ap^{-} and adjacent wild-type cells that are functionally Ap^{+}. Under normal circumstances, the early Ap-dependent expression of *Serrate* decays in the dorsal wing pouch, but a low level of Ap-dependent expression of *fringe* persists (Fig. 1D). When Ap or Fringe activity is removed, cells become responsive to induction by Serrate on nearby cells, if the level of Serrate expression is sufficiently high. This suggests that the level of Serrate expression in the dorsal wing pouch should change in a graded manner during the transition from second to early third instar. Serrate is strongly expressed throughout the dorsal compartment in second instar wing discs (red in Fig. 6A; see also Diaz-Benjumea and Cohen, 1995; Bachmann and Knust, 1998). At this stage, dLMO expression begins to be detectable in dorsal cells (blue). Early dLMO expression is Ap dependent and can be induced in ventral cells by ectopic expression of Ap (data not shown; see also Milán et al., 1998). During early third instar Serrate expression is reduced in the proximal part of the wing pouch, but is maintained at an intermediate level in dorsal cells close to the DV boundary (arrow, Fig. 6B). At this stage dLMO begins to be expressed in both D and V compartments. The time at which Serrate expression decreases corresponds to the time at which dLMO levels increase. The increase in dLMO may be due to the superimposition of the early Ap-dependent dLMO expression in D cells in second instar and the subsequent expression in D and V cells. This secondary dorsal and ventral expression of dLMO is not affected by reducing Ap activity, suggesting that this expression is Ap-independent (in *ap*^{GAL4}/*ap*^{UGO35}; *UAS-fringe* larvae; data not shown).

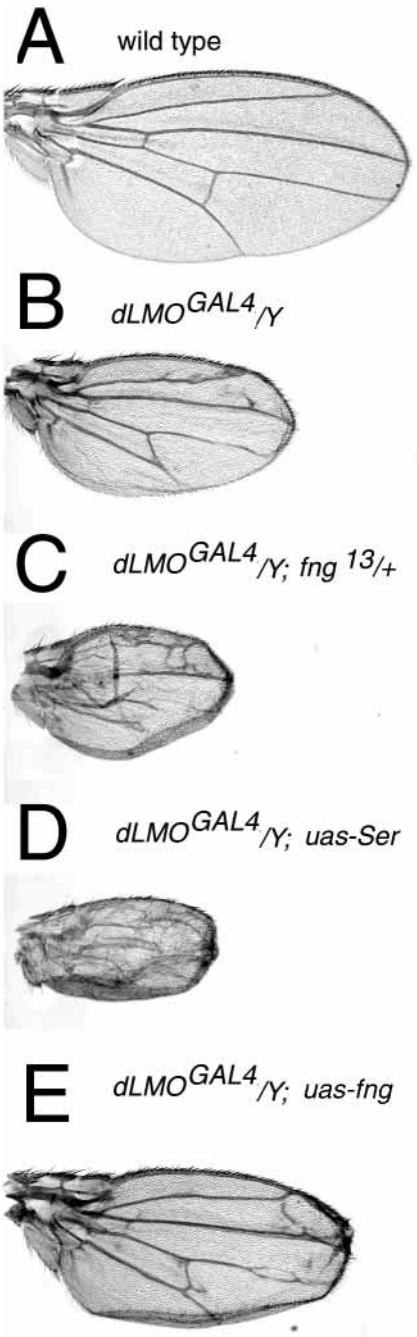

Fig. 7. Serrate and Fringe levels modify the dLMO phenotype. (A) Cuticle preparation of a wild-type male adult wing. (B) Cuticle preparation of a $dLMO^{GAL4}$ male adult wing. (C) Cuticle preparation of a $dLMO^{GAL4}$; $fringe^{13}$/+ male adult wing. (D) Cuticle preparation of a $dLMO^{GAL4}$; *UAS-Ser*/+ male adult wing. (E) Cuticle preparation of a $dLMO^{GAL4}$; *UAS-fng*/+ male adult wing. All wings are shown at the same magnification.

However, dLMO expression was lost in clones of *chip* mutant cells in both compartments, indicating that its expression requires Chip activity. This observation is intriguing in that it opens the possibility that another LIM homeodomain protein may function as a cofactor for Chip in wing development.

Loss of dLMO activity leads to elevated Ap activity in the dorsal compartment. This causes a reduction in the size of the wing and abnormalities in vein formation (Fig. 7A,B; Milán et al., 1998; Milán and Cohen, 1999a). As a consequence of elevated Ap activity, *Serrate* and *fringe* expression fail to undergo normal modulation and persist at elevated levels in the dorsal compartment of the *dLMO* mutant disc (Fig. 1I,K). To evaluate the contribution that overexpression of these two genes makes to the *dLMO* phenotype, we examined the effects of decreasing or further increasing their activities in the *dLMO* mutant background. This is of interest because Serrate and Fringe can be considered to have opposing activities in dorsal cells. Fringe reduces sensitivity of dorsal cells to Serrate (Panin et al., 1997; Fleming et al., 1997). Removing one copy of the *fringe* gene in a *dLMO* mutant background enhanced the severity of the *dLMO* mutant phenotype (Fig. 7B,C). Conversely, increased Fringe activity partially suppressed the *dLMO* phenotype (Fig. 7E). These observations are consistent with the possibility that the defects in the *dLMO* mutant are due to excess Serrate activity. This interpretation gains further support from the observation that overexpression of Serrate further enhanced the severity of the *dLMO* mutant phenotype (Fig. 7D). The finding that overexpression of Fringe only partially suppressed the *dLMO* mutant phenotype suggests the existence of additional targets for Ap that are misregulated in *dLMO* mutant discs (Fig. 7E).

dLMO is required to allow Delta expression in dorsal cells

The pattern of expression of Delta also changes from early to late stages of wing development. In second instar wing discs, Delta is expressed in ventral cells adjacent to the DV boundary (Fig. 8A). By early third instar, Delta begins to be expressed in dorsal cells (Fig. 8B; see also de Celis and Bray, 1997). In mature third instar wing discs, Delta is expressed on both sides of the DV boundary (Fig. 8C). The change from ventrally restricted expression of Delta to symmetric expression at the DV boundary requires dLMO activity. In *dLMO* mutant wing discs, Delta expression is reduced in dorsal cells (Fig. 8D). Dorsal expression of ChAp, a constitutively active form of Ap that is not subject to inhibition by dLMO, also reduces Delta protein level in D cells (Fig. 8E). Delta expression adjacent to the DV boundary is under Wg control (de Celis and Bray, 1997; Micchelli et al., 1997). Expression of other Wg targets, including Vestigial and Distal-less, is not affected by the absence of dLMO activity or by expression of ChAp (data not shown). The fact that overactivation of Ap is capable of repressing Delta in dorsal cells suggests that Ap is responsible for limiting expression of Delta to ventral cells in second instar. Once dLMO levels increase in early third instar discs, Ap activity is reduced and *Delta* can then be induced by Wg on both sides of the DV boundary. To evaluate the contribution that downregulation of *Delta* makes to the *dLMO* phenotype, we examined the effects of decreasing its activity in the *dLMO* mutant background. Removing one copy of the *Delta* gene in a weak *dLMO* mutant background (MS1096) showed an enhanced vein phenotype (Fig. 8F-H). These observations are consistent with the possibility that reduced Delta activity in dorsal cells contributes to the venation defects observed in stronger *dLMO* mutant wings (Milán et al., 1998).

dLMO is required in dorsal cells to allow cell survival

Overactivation of Ap in the *dLMO* mutant leads to reduced wing size and venation defects. To examine the consequences

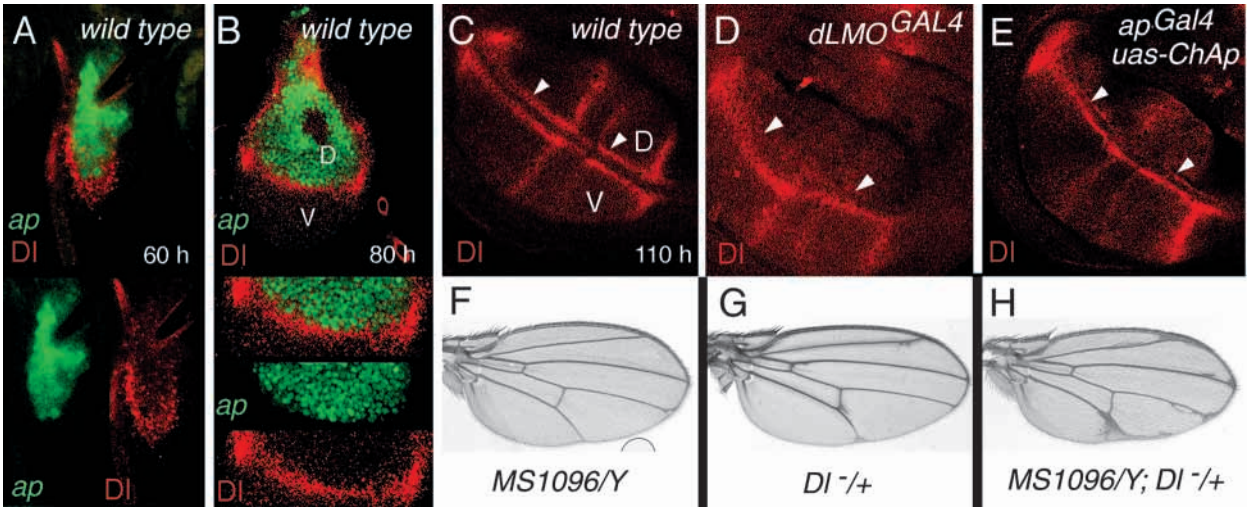

Fig. 8. Delta downregulation is responsible of the dLMO phenotype. Wing discs labeled for ap^{GAL4} expression using *UAS-GFP* (green). Delta (Dl) expression is shown in red. (A) Second instar wing disc (60 h AEL). Lower panels show single channel stainings of the same disc. Delta protein is expressed in ventral cells near the DV boundary at this stage. (B) Early third instar disc (80 h AEL). Lower panels show magnification of single channel stainings of the same disc. Delta protein is expressed in both dorsal and ventral cells although at higher levels and in a broader domain in ventral cells. (C) Late third instar wing disc (110 h AEL). Delta was expressed at similar levels on both sides of the DV boundary (arrowheads) and along the longitudinal veins. (D) Late third instar *dLMO* mutant wing disc double labeled for Ap (not shown) and Delta (red). Delta was expressed along the ventral side of the DV boundary and along the longitudinal veins in the V compartment, but not in dorsal cells (arrowheads). (E) Late third instar ap^{GAL4} *UAS-ChAp* wing disc double labeled for Ap (not shown) and Delta (red). Delta protein expression was reduced in the dorsal wing pouch (arrowheads). (F) Cuticle preparation of a MS1096 male adult wing. (G) Cuticle preparation of a Dl^{6B37}/+ male adult wing. (H) Cuticle preparation of a MS1096; Dl^{6B37}/+ male adult wing. The ectopic vein phenotype due to reduced Dl activity was enhanced by reducing dLMO activity.

of overactivating Ap in genetic mosaics, we produced clones mutant for the $dLMO^{GAL4}$ allele (hdp^{R590}) in larval and adult tissues. When observed in adult tissues, clones of mutant cells did not show any observable abnormality in the notum or in the ventral wing surface (not shown). However, $dLMO^{GAL4}$ clones survived poorly in the dorsal wing surface compared to the ventral surface. Dorsal clones induced in early-mid third instar (84 h AEL) were smaller and fewer in number than clones in the ventral compartment of the same wings (Fig. 9B). Clones induced in mid second instar (60 h AEL) did not survive until the end of third instar in the dorsal compartment, though many wild-type twin spots were recovered (Fig. 9A). The number of clones and twin spots in the ventral compartment was approximately equal suggesting that mutant clones were lost from the dorsal compartment. Using the Minute technique to give the *dLMO* mutant cells, a growth advantage with respect to surrounding cells did not overcome the small size and reduced number of dorsal clones recovered in the adult wing (Fig. 9D) or in the wing disc (Fig. 9C). Thus, dLMO seems to be required in the dorsal compartment of the wing for cell survival. Indeed, the frequency of dying cells was increased in *dLMO* discs with respect to wild-type discs, and the excess cell death was restricted to the dorsal wing pouch (Fig. 9E,F). In the notum and the ventral pouch of *dLMO* wing discs, the number of dying cells was similar to wild-type control discs. Thus the reduced size of the *dLMO* mutant wing appears to be due to cell death in the dorsal compartment. Although it is not evident in the third instar, there is likely to be compensating cell death or reduced cell proliferation in the ventral compartment at later stages to give the overall size reduction in the *dLMO* mutant wing.

If overactivation of Ap is responsible for dorsal cell death in *dLMO* mutant wings, we reasoned that removing Ap activity using *Chip* mutant clones might improve the survival of *dLMO* mutant cells in the dorsal compartment. In dLMO-positive (wild-type) wings, *Chip* mutant clones were comparable in size to their twin spots in dorsal and ventral compartments (Fig. 3). In *dLMO* mutant wing discs, *Chip* mutant clones located in the D compartment were much bigger than their twin clones; whereas those located in the V compartment were comparable in size to their twins (Fig. 9G,H). These observations suggest that overactivation of Ap may indeed be responsible for the growth and cell survival defects in the dorsal compartment of the dLMO mutant wing.

DISCUSSION

Apterous is expressed in the presumptive dorsal compartment in second instar wing discs where it confers dorsal identity and induces expression of *Serrate* and *fringe* (Diaz-Benjumea and Cohen, 1993; Williams et al., 1993; Blair et al., 1994; Irvine and Wieschaus, 1994; Diaz-Benjumea and Cohen, 1995). Ap-dependent interaction between dorsal and ventral cells leads to restricted activation of the Notch pathway along the DV compartment boundary. Notch signaling is required to establish the DV boundary organizer and thereby control growth and patterning of the wing disc (Diaz-Benjumea and Cohen, 1995; Kim et al., 1995; de Celis et al., 1996; Doherty et al., 1996). Early in development Apterous induces the expression of dLMO, which functions as an antagonist of Ap activity (Milán et al., 1998; Shoresch et al., 1998). Thus Apterous negatively regulates

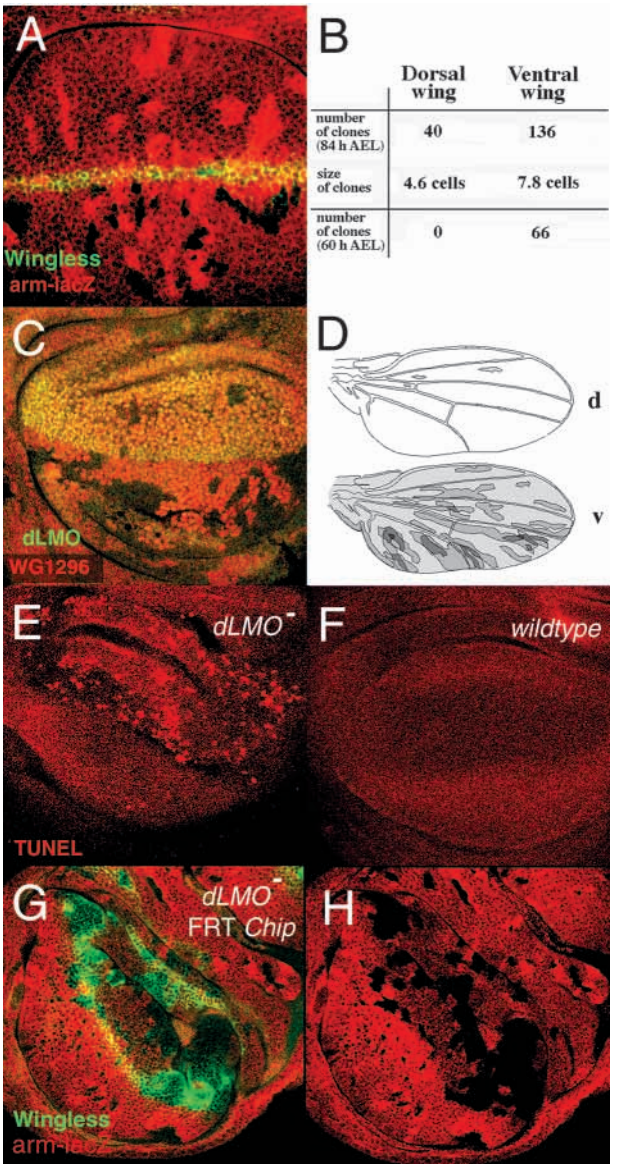

Fig. 9. dLMO is required for cell survival in the dorsal wing pouch. (A) hdp^{R590} mutant clones in the wing pouch labeled by the absence of β-galactosidase expression (red). Clones were induced in *hdp^{R590} FRT18/arm-lacZ FRT18; hs-FLP (II)* larvae at 60 h AEL and visualized 120 h AEL. Wild-type twin-spot clones show stronger levels of β-galactosidase. Wingless protein is shown in green to mark the DV boundary. (B) Table summarizing analysis of f^{36a} hdp^{R590} mutant clones in the adult wing. Clones were induced in f^{36a} hdp^{R590} *FRT18/FRT18; hs-FLP (II)* larvae at 60 or 84 h AEL. Number of clones recovered in D and V compartments of three wings and average clone size are indicated. (C) hdp^{R590} M^{+} mutant clones in the wing pouch labeled by the absence of β-galactosidase (red) and dLMO protein expression (green). Clones were induced in *hdp^{R590} FRT18/WG1296-lacZ M(1)15D FRT18; hs-FLP (III)* larvae at 60 h AEL and visualized 120 h AEL. (D) Diagram of the positions and size of f^{36a} hdp^{R590} *M(+)* mutant clones induced at 84 h AEL and visualized in the adult wing showing those located in the dorsal (d) or ventral (v) wing surface. Overlapping clones show stronger gray color. Clones were induced in f^{36a} hdp^{R590} *FRT18/WG1296-lacZ M(1)15D FRT18; hs-FLP (III)* larvae. Number of wings=4. (E,F) TUNEL staining of hdp^{R590} (E) and wild-type (F) wing discs showing apoptotic cells (in red). (G) $Chip^{e55}$ mutant clones labeled by the absence of β-galactosidase expression (in red) in a dLMO loss-of-function background. Wg expression is shown in green. Note the bigger clonal size in the dorsal wing pouch than in the ventral pouch.

its own activity by inducing expression of an inhibitor. These observations have suggested that Ap activity is highest at early stages and subsequently decreases due to the effects of dLMO.

Removing Apterous activity at different stages of wing development shows that Ap is needed throughout larval stages to confer dorsal cell identity, but its role in Notch activation along the DV boundary is temporally and spatially modulated. This can be explained in terms of changes in *Serrate* and *fringe* expression. Some of the changes in *Serrate* and *fringe* expression are caused by reducing Ap activity, whereas others are Ap independent. In early second instar wing discs, Ap activity is required in the entire dorsal compartment. Removing Ap activity in mitotic recombination clones at this stage induces Notch activation at the interface between wild-type and mutant cells. This response is independent of the position of the clone within the wing pouch. In early third instar wing discs, Ap-dependent expression of *Serrate* and *fringe* is reduced by dLMO. Serrate expression gradually becomes restricted to the region near the DV boundary and, subsequently, by mid-third instar is induced only in cells adjacent to the boundary (Micchelli et al., 1997; de Celis and Bray, 1997). The effects of removing Ap activity in clones reflects the gradual retraction of Serrate expression toward the DV boundary. Clones of cells lacking Ap activity induced in early third instar activate the Notch pathway and induce Wg if they are located close to the DV boundary. Clones located more proximally do not show this response. We have shown that this spatial difference can be overcome by providing Serrate in proximal cells.

By mid-third instar, new Ap-independent patterns of *Serrate* and *fringe* expression are observed. *Serrate* is expressed on both sides of the DV boundary by the activity of Wg and *fringe* is expressed in four quadrants flanking the DV and AP compartment boundaries. Maintenance of Notch activation along the DV boundary is now under control of a feedback loop between Wg and Serrate and Delta (Micchelli et al., 1997; de Celis et al., 1997). Ap is no longer required for Notch activation at the DV boundary and removing Ap activity no longer leads to activation of the Notch pathway.

In the absence of dLMO, Ap activity remains at high early levels as development proceeds. *Serrate* and *fringe* expression remain high throughout the dorsal compartment and fail to undergo normal modulation. In addition, *Delta* is not expressed in dorsal cells. Ap-dependent repression of Delta at early stages is needed to prevent ectopic activation of Notch in dorsal cells, which are inherently Delta-sensitive due to the activity of Fringe (de Celis et al., 1996; Doherty et al., 1996; Panin et al., 1997; Fleming et al., 1997). We have presented evidence that some of the defects observed in dLMO mutant wings are correlated with excess Serrate activity and insufficient Delta activity. In addition, we observed that abnormally high levels of cell death in the dorsal compartment of the *dLMO mutant* wing disc are due to excess Ap activity and that this leads to overall reduction in the size of the wing. These findings indicate the need to downregulate Ap activity to allow normal wing development. However, Ap activity continues to be required for dorsal cell fate specification and for proper

adhesion of D and V wing surfaces. Thus we propose that Ap different target genes may be controlled at different levels of Ap activity. *Serrate*, *fringe* and *Delta* may be regulated by a higher level of Ap activity than the target genes involved in surface apposition or fate specification. Temporal changes in the level of Ap activity may be required to modulate activity of different genes at different times to allow normal wing development.

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REFERENCES

Axelrod, J. D., Matsuno, K., Artavanis-Tsakonas, S. and Perrimon, N. (1996) Interaction between wingless and Notch signaling pathways mediated by Dishevelled. *Science* **271**, 1826-1832.

Bachmann, A. and Knust, E. (1998) Positive and negative control of *Serrate* expression during early development of the *Drosophila* wing. *Mech. Dev.* **76**, 76-78.

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

Blair, S. S. (1995) Compartments and appendage development in Drosophila. *BioEssays* **17**, 299-309.

Blair, S. S., Brower, D. L., Thomas, J. B. and Zavortink, M. (1994) The role of *apterous* in the control of dorsoventral compartmentalization and PS integrin gene expression in the developing wing of Drosophila. *Development* **120**, 1805-1815.

Brook, W. J. and Cohen, S. M. (1996) Antagonistic interactions between Wingless and Decapentaplegic responsible for dorsal-ventral pattern in the Drosophila leg. *Science* **273**, 1373-1377.

Brook, W. J., Diaz-Benjumea, F. J. and Cohen, S. M. (1996) Organizing spatial pattern in limb development. *Ann. Rev. Cell Dev. Biol.* **12**, 161-180.

Calleja, M., Moreno, E., Pelaz, S. and Morata, G. (1996) Visualization of gene expression in living adult Drosophila. *Science* **274**, 252-255.

Cohen, B., McGuffin, M. E., Pfeifle, C., Segal, D. and Cohen, S. M. (1992) *apterous:* a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins. *Genes Dev.* **6**, 715-729.

Dahmann, C. and Basler, K. (1999) Compartment boundaries: at the edge of development. *Trends Genet.* **15**, 320-326.

de Celis, J. F. and Bray, S. (1997) Feed-back mechanisms affecting Notch activation at the dorsoventral boundary in the *Drosophila* wing. *Development* **124**, 3241-3251.

de Celis, J. F., Garcia-Bellido, A. and Bray, S. J. (1996) Activation and function of *Notch* at the dorsal-ventral boundary of the wing imaginal disc. *Development* **122**, 359-369.

Diaz-Benjumea, F. J. and Cohen, S. M. (1993) Interaction between dorsal and ventral cells in the imaginal disc directs wing development in Drosophila. *Cell* **75**, 741-752.

Diaz-Benjumea, F. J. and Cohen, S. M. (1995) Serrate signals through Notch to establish a Wingless-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing. *Development* **121**, 4215-4225.

Doherty, D., Fenger, G., Younger-Shepherd, S., Jan, L.-Y. and Jan, Y.-N. (1996) Dorsal and ventral cells respond differently to the *Notch* ligands *Delta* and *Serrate* during *Drosophila* wing development. *Genes Dev.* **10**, 421-434.

Fernandez-Funez, P., Lu, C. H., Rincon-Limas, D. E., Garcia-Bellido, A. and Botas, J. (1998) The relative expression amounts of apterous and its co-factor dLdb/Chip are critical for dorso-ventral compartmentalization in the Drosophila wing. *EMBO J.* 6846-6853.

Fleming, R. J., Gu, Y. and Hukriede, N. A. (1997) Serrate-mediated activation of Notch is specifically blocked by the product of the gene fringe in the dorsal compartment of the *Drosophila* wing imaginal disc. *Development* **124**, 2973-2981.

Irvine, K. and Wieschaus, E. (1994) *fringe*, a boundary specific signalling molecule, mediates interactions between dorsal and ventral cells during Drosophila wing development. *Cell* **79**, 595-606.

Johnston, S. H., Rauskolb, C., Wilson, R., Prabhakaran, B., Irvine, K. D. and Vogt, T. F. (1997) A family of mammalian Fringe genes implicated in boundary determination and the Notch pathway. *Development* **124**, 2245-2254.

Kim, J., Irvine, K. D. and Carroll, S. B. (1995) Cell recognition, signal induction and symmetrical gene activation at the dorsal/ventral boundary of the developing Drosophila wing. *Cell* **82**, 795-802.

Micchelli, C. A., Rulifson, E. J. and Blair, S. S. (1997) The function and regulation of cut expression on the wing margin of *Drosophila*: Notch, Wingless and a dominant negative role for Delta and Serrate. *Development* **124**, 1485-1495.

Milán, M., Campuzano, S. and Garcia-Bellido, A. (1997) Developmental parameters of cell death in the wing disc of Drosophila. *Proc. Natl. Acad. Sci. USA.* **94**, 5691-5696.

Milán, M., Diaz-Benjumea, F. and Cohen, S. M. (1998) *Beadex* encodes an LMO protein that regulates Apterous LIM-homeodomain activity in *Drosophila* wing development: a model for LMO oncogene function. *Genes Dev.* **12**, 2912-2920.

Milán, M. and Cohen, S. M. (1999a) Regulation of LIM homeodomain activity in vivo: A tetramer of dLDB and Apterous confers activity and capacity for regulation by dLMO. *Molec. Cell* **4**, 267-273.

Milán, M. and Cohen, S. M. (1999b) Notch signaling is not sufficient to define the affinity boundary between dorsal and ventral compartments. *Molec. Cell* **4**, 1073-1078.

Morcillo, P., Rosen, C., Baylies, M. K. and Dorsett, D. (1997) Chip, a widely expressed chromosomal protein required for segmentation and activity of a remote wing margin enhancer in *Drosophila*. *Genes Dev.* **11**, 2729-2740.

Neumann, C. J. and Cohen, S. M. (1996) A hierarchy of cross-regulation involving *Notch*, *wingless*, *vestigial* and *cut* organizes the dorsal/ventral axis of the *Drosophila* wing. *Development* **122**, 3477-3485.

Neumann, C. J. and Cohen, S. M. (1998) Boundary formation in the Drosophila wing: the POU-domain protein Nubbin attenuates Notch activity. *Science* **281**, 409-413.

O'Keefe, D. D., Thor, S. and Thomas, J. B. (1998) Function and specificity of LIM domains in *Drosophila* nervous system and wing development. *Development* **125**, 3915-3923.

Panin, V. M., Papayannopoulos, V., Wilson, R. and Irvine, K. D. (1997) Fringe modulates Notch-ligand interactions. *Nature* **387**, 908-913.

Pignoni, F. and Zipursky, S. L. (1997) Induction of *Drosophila* eye development by decapentaplegic. *Development* **124**, 271-278.

Shoresch, M., Orgad, S., Schmueli, O., Werczberrger, R., Gelbaum, D., Abiri, S. and Segal, D. (1998) Overexpression *Beadex* mutations and loss of function *heldup-a* mutations in Drosophila affect the 3′ regulatory and coding components, respectively, of the *Dlmo* gene. *Genetics* **150**, 283-299.

Speicher, S. A., Thomas, U., Hinz, U. and Knust, E. (1994) The *Serrate* locus of *Drosophila* and its role in morphogenesis of the wing imaginal discs: control of cell proliferation. *Development* **120**, 535-544.

Strigini, M. and Cohen, S. M. (1999) Formation of morphogen gradients in the Drosophila wing. *Semin. Cell Dev. Biol.* **10**, 335-344.

Thomas, U., Speicher, S. A. and Knust, E. (1991) The *Drosophila* gene *Serrate* encodes an EGF-like transmembrane protein with complex expression patterns in embryos and wing discs. *Development* **111**, 749-761.

van Meyel, D. J., O'Keefe, D. D., Jurata, L. W., Thor, S., Gill, G. N. and Thomas, J. B. (1999) Chip and apterous physically interact to form a functional complex during Drosophila development. *Molec. Cell.* **4**, 259-265.

Williams, J. A., Paddock, S. W. and Carroll, S. B. (1993) Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing *Drosophila* wing disc into discrete subregions. *Development* **117**, 571-584.

Williams, J. A., Paddock, S. W., Vorwerk, K. and Carroll, S. B. (1994) Organization of wing formation and induction of a wing-patterning gene at the dorsal/ventral compartment boundary. *Nature* **368**, 299-305.

Yuan, Y. P., Schultz, J., Mlodzik, M. and Bork, P. (1997) Secreted fringe-like signaling molecules may be glycosyltransferases. *Cell* **88**, 9-11.

Zeng, C., Justice, N.J., Abdelilah, S., Chan, Y.M., Jan, L.Y. and Jan, Y.N. (1998) The Drosophila LIM-only gene, dLMO, is mutated in Beadex alleles and might represent an evolutionarily conserved function in appendage development. *Proc. Natl. Acad. Sci. USA* **95**, 10637-10642.