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The relative expression amounts of *apterous* and its co-factor *dLdb/Chip* are critical for dorso-ventral compartmentalization in the *Drosophila* wing

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Dorso-ventral axis formation in the *Drosophila* wing requires the localized accumulation of the Apterous LIM/homeodomain protein (Ap) in dorsal cells. Here we report that *dLdb/Chip* encodes a LIM-binding co-factor that controls Ap activity. Both lack and excess of *dLdb/Chip* function cause the same phenotype as *apterous* (*ap*) lack of function; i.e. dorsal to ventral transformations, generation of new wing margins, and wing outgrowths. These results indicate that the normal function of Ap in dorso-ventral compartmentalization requires the correct amount of the DLDB/CHIP co-factor, and suggest that the Ap and DLDB/CHIP proteins form a multimeric functional complex. In support of this model, we show that the *dLdb/Chip* excess-of-function phenotypes can be rescued by *ap* overexpression.

Keywords: *apterous*/co-factor/compartment/LDB/LIM domain

Introduction

Growth and patterning of the *Drosophila* appendages require the formation of compartments that are defined by the expression of selector genes (Diaz-Benjumea and Cohen, 1993; Blair, 1995; Tabata *et al*., 1995; reviewed by Lawrence and Struhl, 1996). The restricted expression of the selector gene *apterous* (*ap*) in the dorsal compartment of the wing is required for dorsal cell identity and for maintaining cells of the dorsal and ventral compartment in different lineages. The border between cells differing in *ap* expression defines the dorso-ventral compartment boundary that functions as an organizing center (Diaz-Benjumea and Cohen, 1993; Blair *et al*., 1994). At the dorso-ventral boundary, the interface between dorsal and ventral cells triggers a series of cell interactions mediated by the genes *fringe*, *Serrate*, *Notch* and *Delta* (reviewed in Irvine and Vogt, 1997). These interactions lead to the activation of *wingless* and *vestigial* whose activities mediate the patterning and growth of the wing along the dorso-ventral axis (Couso *et al*., 1995; Kim *et al*., 1995, 1996; Neumann and Cohen, 1997).

ap encodes a putative transcription factor containing a homeodomain, and two LIM domains (Bourgouin *et al*., 1992; Cohen *et al*., 1992). Each LIM domain is composed of two cysteine-rich zinc fingers joined by a two amino acid spacer. Besides homeodomain transcription factors, LIM domains are found in many other proteins including kinases and a variety of LIM-only proteins. These can be nuclear or cytoplasmic, and the latter are often associated with the cytoskeleton. In contrast to other related zinc fingers, LIM domains do not appear to mediate protein–DNA interactions, instead they have been shown to mediate interactions among proteins. Thus, LIM domains are thought to function as adaptor modules bringing different proteins together in a complex (reviewed in Sanchez-Garcia and Rabbitts, 1994; Gill, 1995; Curtiss and Heilig, 1998; Dawid *et al*., 1998). In the context of homeodomain proteins, it was proposed that the LIM domains negatively regulate the activity of the homeodomain, and that a LIM domain-binding co-factor would liberate the homeodomain from the inhibitory action of the LIM domains (Taira *et al*., 1994).

We have isolated a protein factor on the basis of its ability to bind Ap LIM domains. Sequence analysis reveals that it is related to a family of vertebrate proteins known as LDB, NLI or CLIM. These vertebrate homologs interact with a variety of proteins including certain (but not all) LIM domain proteins (Agulnick *et al*., 1996; Jurata *et al*., 1996; Bach *et al*., 1997; Wadman *et al*., 1997). We generated mutations in the *Drosophila Ldb* (*dLdb*) gene. These mutations are allelic to mutations in *Chip*, a previously described gene. *Chip* is known for its functions during segmentation (Morcillo *et al*., 1997), a process in which no LIM homeodomain protein is known to play a role. The DLDB/CHIP protein accumulates in the nuclei of all dorsal and ventral cells of the wing imaginal disc. Surprisingly, however, alterations in *dLdb/Chip* activity produce the specific phenotypes expected for a dorsal selector gene such as *ap*. Both lack and excess of *dLdb/Chip* function mimic *ap* lack-of-function phenotypes without affecting normal Ap protein accumulation. Thus the function of *ap* as a selector gene in cells of the dorsal compartment requires the correct amount of expression of a ubiquitously expressed co-factor. We show that the phenotype produced by excess *dLdb/Chip* can be rescued when *ap* is overexpressed. We conclude that the stoichiometry of Ap and DLDB/CHIP is critical for dorso-ventral compartmentalization.

Results

Identification of a protein binding to Apterous LIM domains

To identify co-factors involved in *ap* function, we used the yeast two-hybrid interactor approach. A third instar

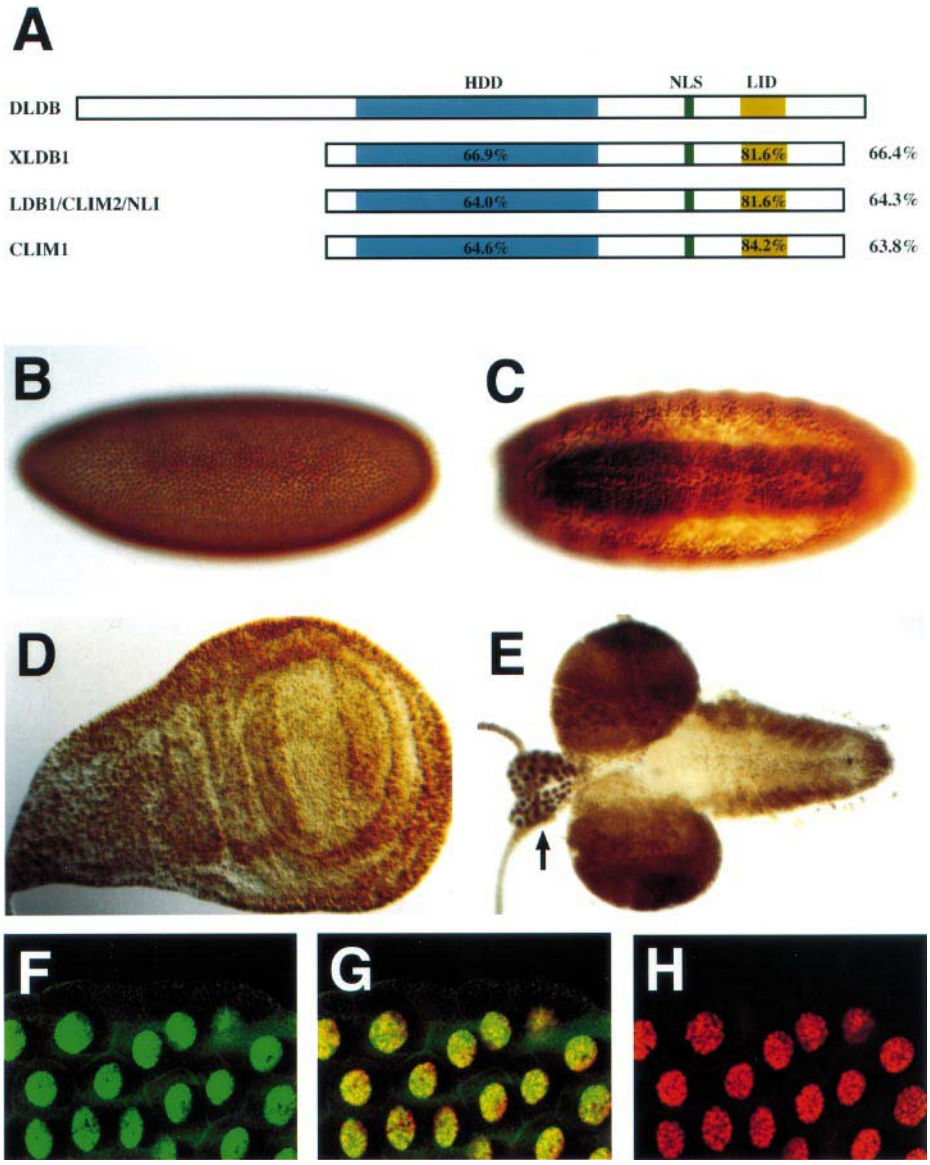

Fig. 1. The *Drosophila* LDB protein: comparison with vertebrate orthologs and pattern of accumulation. (**A**) Comparison of *Drosophila* and vertebrate LDB proteins. XLDB1 is a *Xenopus* protein, LDB1 (also known as CLIM2 or NLI) and CLIM1 are murine proteins (Agulnick *et al*., 1996; Jurata *et al*., 1996; Bach *et al*., 1997). Numbers refer to amino acid percentage identity in the homodimerization domain (HDD), LIM interaction domain (LID) or overall identity (numbers on the right). NLS: nuclear localization signal. (B–H) DLDB accumulates in the nuclei of most if not all cells. (**B**) Lateral view of a stage 5 embryo. (**C**) Ventral view of a stage 16 embryo. (**D**) Third instar wing–notum imaginal disc. (**E**) Third instar central nervous system and ring gland (arrow). (**F**) Nuclear immunodetection of DLDB (green) in salivary glands. (**G**) Merged image from green and red channels shown in (F) and (H), respectively. (**H**) Specimen from (F) showing nuclear staining with propidium iodide (red).

Drosophila cDNA library was screened using the Ap LIM domains as bait. Five positive clones were obtained from ~2×10^6 colonies screened, and two of these identified the fly homolog of Ldb/NLI/CLIM (Agulnick *et al*., 1996; Jurata *et al*., 1996; Bach *et al*., 1997). Recent studies on Ldb/NLI/CLIM have defined an N-terminal homodimerization domain, and a C-terminal domain that mediates interactions with LIM domains (Jurata and Gill, 1997; Breen *et al*., 1998; Jurata *et al*., 1998). The overall amino acid identity of the fly and vertebrate proteins is ~64%, but they are up to 84% identical in the LIM-interacting domain (Figure 1A). Sequence comparison analysis also suggested that *dLdb* may be the same gene as *Chip*, a gene previously characterized for its functions during embryonic segmentation (Morcillo *et al*., 1997). We generated antibodies raised against the DLDB protein in rats. All the antisera revealed the same distribution of DLDB protein. Figure 1B–E shows that DLDB accumulates in the nuclei of cells in all tissues examined, and throughout development. The specificity of the antisera was demonstrated by staining *dLdb* mutants (see below, and Figure 3A–A″). The nuclear localization of DLDB was confirmed further by double staining with DLDB antibodies and propidium iodide (Figure 1F–H).

Generation of dLdb (Chip) mutations

We isolated *dLdb* mutants in a screen for genes involved in wing margin development. One complementation group defined by four lethal ethyl methanesulfonate (EMS) alleles shows a characteristic dominant haploinsufficient

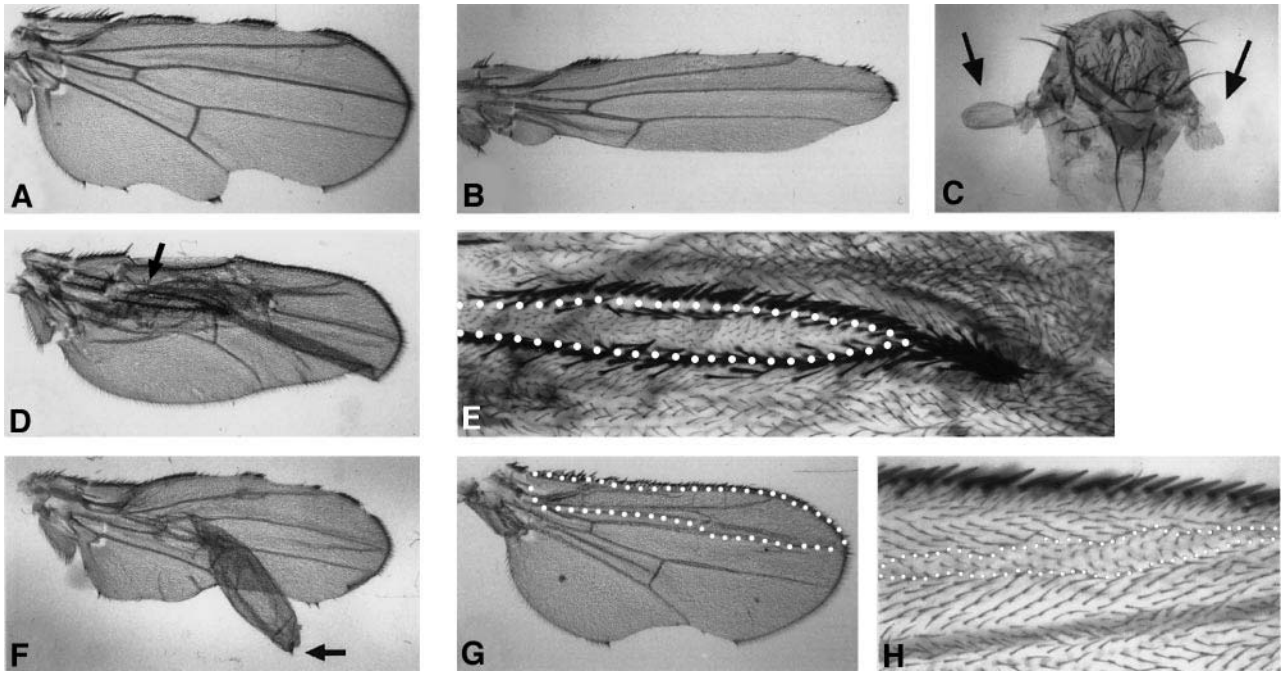

Fig. 2. *dLdb/Chip* lack-of-function phenotypes in the wing. (**A**) $dLdb^{M4}$/+ wing showing nicks in the margin, a characteristic haploinsufficient phenotype of *dLdb/Chip* mutations. (**B**) ap^{78j}/ap^{54} wing showing the margin and size reductions characteristic of weak *ap* mutations. (**C**) Notum and wing rudiments (arrows) of fly with the same genotype as in (B), but also heterozygous for $dLdb^{M4}$. Note that reduction of the wings is much more severe than in (A) or (B), indicating a strong genetic interaction between *ap* and *dLdb* mutations. (**D**) Homozygous mutant $dLdb^{M4}$ clone in the dorsal compartment of the wing inducing an ectopic wing margin (arrow). (**E**) Magnification of (D). The mutant cells are labeled with the cell markers *forked* and *pawn*. The border of the clone (marked by a dotted line) differentiates sensory elements of the ventral wing margin, thus the cells of the clone have changed their identity. Wild-type cells bordering the clone are induced to differentiate as sensory elements of the dorsal surface. (**F**) The small $dLdb^{M4}$ mutant clone (arrow) far from the wing margin induces the outgrowth of the surrounding non-mutant cells. (**G**) The $dLdb^{M4}$ mutant clone in the ventral compartment, indicated with the dotted line, has wild-type phenotype (the somewhat abnormal shape of the wing is caused by the slightly small size of *pawn* cells). (**H**) The phenotype of the $dLdb^{M4}$ mutant clone in the dorsal compartment is rescued by expressing *dLdb* from a transgene at a relatively low level (*C5 GAL4; UAS: dLdb/Chip*). The border of the clone, marked with *pawn*, is outlined.

phenotype consisting of nicks in the wing margin (Figure 2A). This complementation group was mapped to cytological position 60A, the same position as the *dLdb* gene as determined by *in situ* hybridization to polytene chromosomes (not shown). The four alleles of this complementation group were shown to be mutations in *dLdb* using the following approaches: (i) sequencing of the *dLdb* coding region in the mutant $dLdb^{M3}$ revealed a deletion of seven nucleotides resulting in a stop codon that causes the deletion of 122 amino acids (not shown); (ii) rescue of the *dLdb* haploinsufficient phenotype by expressing the *dLdb* cDNA from C5-GAL4 (Yeh *et al*., 1995), a driver directing expression in the wing pouch at relatively low levels (not shown); (iii) lack of anti-DLDB immunoreactivity in somatic recombination clones (Figure 3A–A″); and (iv) rescue of the *dLdb* null phenotype in clones by expression of the *dLdb* cDNA using the C5-GAL4 driver (Figure 2H).

After the work described here was completed, complementation tests between *dLdb* and *Chip* mutations revealed that they are alleles of the same gene.

dLdb/Chip and apterous mutations show genetic interactions and cause identical phenotypes in wing mosaics

As shown in Figure 2A–C, *dLdb/Chip* mutations behave as strong enhancers of wing phenotypes produced by hypomorphic *ap* mutations. This synergistic interaction suggests that *dLdb/Chip* and *ap* have related functions. To investigate further the function of *dLdb/Chip* during wing development, we generated genetic mosaics by induced mitotic recombination using the Minute technique (Morata and Ripoll, 1975).

Clones of *dLdb/Chip* mutant cells in the wing ventral compartment ($n = 145$) show a wild-type phenotype and appear with normal frequencies, indicating that *dLdb/Chip* is not required in this compartment (Figure 2G). In contrast, *dLdb/Chip* clones in the dorsal compartment ($n = 35$) are associated with wing outgrowths and ectopic wing margins (Figure 2D–F). Cells within these clones have a ventral identity revealed by the morphology of the wing margin bristles they differentiate. Normal cells abutting the mutant clone are induced to form the dorsal structures characteristic of the wing margin (Figure 2E). The ectopic margins can be visualized in undifferentiated imaginal discs with the use of molecular markers that label the wing margin (Figure 3B–B″ and C–C″). The largest wing outgrowths correspond to clones far from the normal wing margins. These clones cause the outgrowth of wild-type tissue, with the mutant clone located at the tip of the outgrowth (Figures 2F and 3D').

Thus, although *dLdb/Chip* is expressed in all wing cells, *dLdb/Chip* mutations produce specific phenotypes that are indistinguishable from *ap* phenotypes in clones (Diaz-Benjumea and Cohen, 1993; Blair *et al*., 1994). One possibility is that normal *dLdb/Chip* function is required

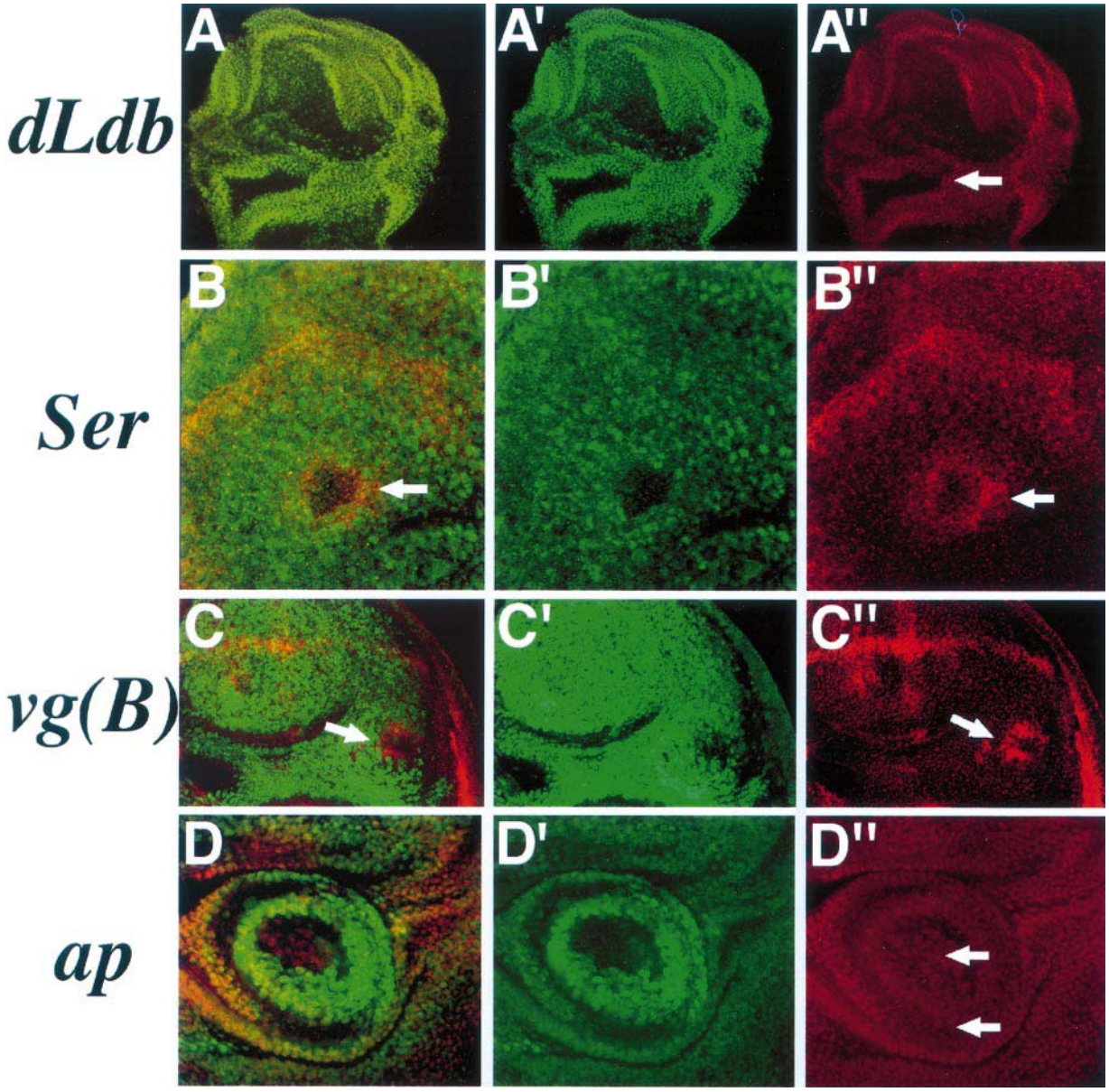

Fig. 3. *dLdb/Chip* regulates the expression of the wing margin markers *Serrate* and *vestigial*, but not of *apterous*. Green panels in the middle rows (′) show cells expressing the c-*myc* marker, and mutant $dLdb^{M4}$ clones appear as unlabeled cells. Red panels on the right (″) show expression of the markers specified to the left of each row. Left panels show the merging of green and red channels. (**A–A″**) DLDB protein is not detectable in *dLdb* mutant clones. The clone (arrow) causes an outgrowth of surrounding tissue deforming the disc. (**B–B″**) *Ser* is expressed ectopically around $dLdb^{M4}$ dorsal clones. Only non-mutant cells close to the clone, that maintain their dorsal identity, accumulate SER (arrows in B and B″). (**C–C″**) *lacZ* expression from the *vestigial-B* dorso-ventral boundary enhancer. Note the *lacZ* ectopic expression in a $dLdb^{M4}$ dorsal clone (arrow). Cells in the border of the clone (mutant and non- mutant) express *lacZ*. (**D–D″**) A mutant $dLdb^{M4}$ clone in a wing disc stained with anti-Ap antibody. Note the presence of Ap label inside the clone (top arrow in D″), and the abnormal growth of wild-type tissue surrounding the clone (bottom arrow). In all discs, anterior is left and dorsal is down.

for *ap* expression. To test this possibility, we monitored *ap* expression in *dLdb/Chip* mutant clones induced in wing imaginal discs. As shown in Figure 3D–D″, Ap protein accumulates normally in *dLdb/Chip* mutant cells. Thus *dLdb/Chip* does not regulate *ap* expression but it shows genetic interactions with *ap*, and it also produces the same mutant phenotypes in genetic mosaics. Taken together, these results are consistent with the hypothesis that *dLdb/Chip* encodes a co-factor required for *ap* function as a dorsal selector gene.

Overexpression of dLdb/Chip causes the same phenotype as apterous lack of function

If Ap and DLDB/CHIP physically interact forming a functional complex, their stoichiometry may be important for the formation of the complex and for dorso-ventral patterning.

To test whether the levels of *dLdb/Chip* expression are important for dorso-ventral patterning, we overexpressed *dLdb/Chip* in various patterns using the GAL4/UAS system (Brand and Perrimon, 1993). Overexpression of *dLdb/*

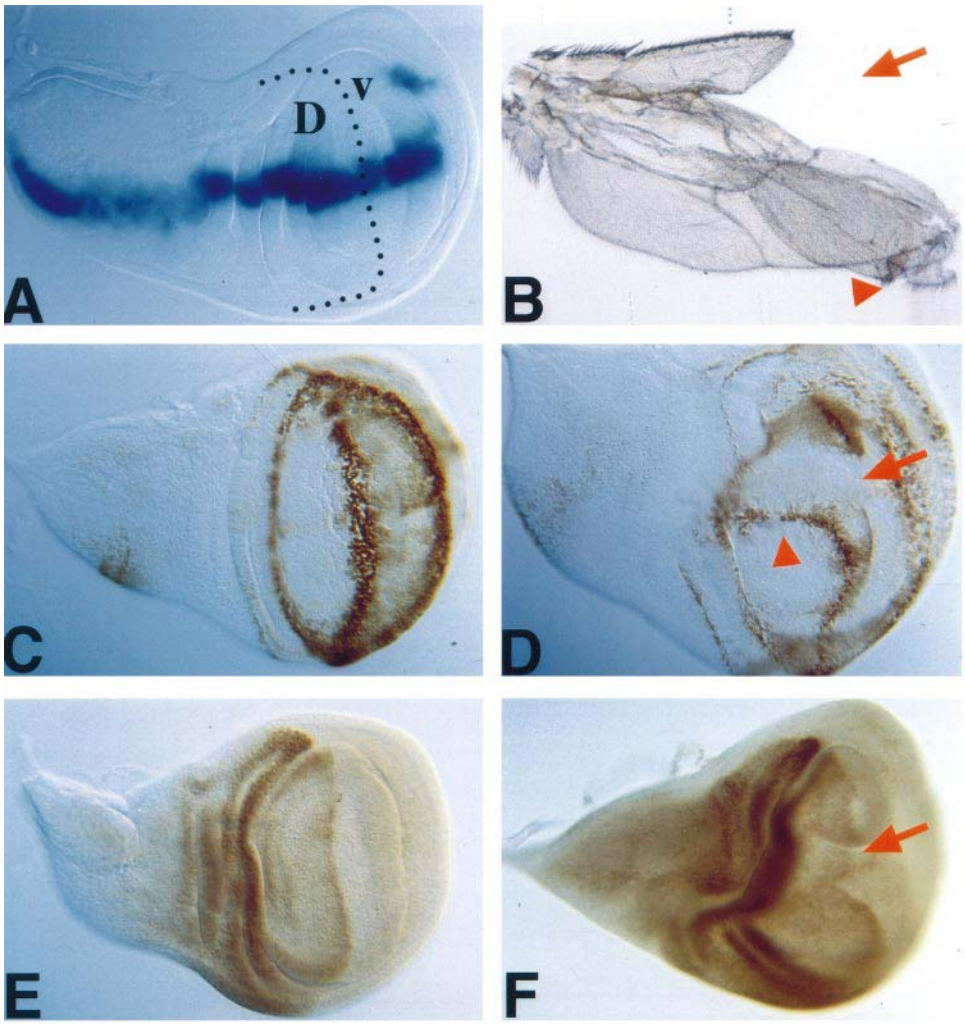

Fig. 4. *dLdb/Chip* overexpression causes *apterous* lack-of-function phenotypes. (**A**) *lacZ* reporter gene expression in *dppGAL4; UAS: lacZ* wing disc. The dorso-ventral compartment boundary is indicated by the dotted line. (**B**) Results of overexpressing *dLdb/Chip* in the *dpp* pattern shown in (A). Note the ectopic wing margin in the distal antero-posterior boundary (arrowhead), and the outgrowth of this region in the dorsal surface. Wings of this genotype also show a cut of the anterior wing margin (arrow), probably the result of the lack of a dorso-ventral compartment boundary where the *dpp* expression domain meets the normal dorso-ventral boundary. (**C**) Wild-type *wglacZ* expression pattern in third instar imaginal disc. Note the *wglacZ* expression along the dorso-ventral boundary. (**D**) *wglacZ* expression on the dorso-ventral boundary is altered when *dLdb* is overexpressed as in (B). Ectopic *wglacZ* expression is observed in the dorsal side of the wing antero-posterior compartment boundary (arrowhead). The arrow points to a gap in the expression of *wglacZ* that corresponds to the cut in the wing margin shown in (B). (**E**) Wild-type distribution of Ap protein. (**F**) Ap protein distribution is unaffected after *dLdb* overexpression as in (B) and (D). Note the normal Ap label where *dLdb* is overexpressed (arrow). Outgrowths in the wing disc can be seen as new disc foldings associated with the formation of ectopic margins shown in (B) and (D).

Chip using a *decapentaplegic* (*dpp*) GAL4 driver (Figure 4A) results in wing outgrowths, the creation of an ectopic wing margin on the dorsal compartment and a cut in the wing (Figure 4B). These phenotypes are also evident in imaginal discs using *wingless-lacZ* (*wglacZ*) expression as a marker of the wing margin (Figure 4C and D). Because lack of *ap* function in clones also causes wing outgrowths and ectopic wing margins (Diaz-Benjumea and Cohen, 1993; Blair *et al*., 1994), we investigated the distribution of Ap protein in these wing imaginal discs. Figure 4E and F shows that *dLdb/Chip* overexpression does not alter the distribution of Ap protein; these results indicate that the phenotypes produced by *dLdb/Chip* overexpression are not caused by *dLdb/Chip* repressing *ap*.

Interestingly, overexpression of *ap* using the drivers *dppGAL4* or *apGAL4* described above does not result in wing abnormalities in the dorsal compartment (not shown).

The results described above show that overexpression of *dLdb/Chip* results in the same phenotype as its lack of function, i.e. transformation of dorsal into ventral cells, and as a consequence wing margin formation and outgrowth. These observations suggest that the relative amounts of Ap and DLDB are important for dorso-ventral patterning.

Overexpression of apterous rescues the phenotypes produced by dLdb/Chip overexpression

If the relative amounts of Ap and DLDB are critical for dorso-ventral patterning, then it should be possible to rescue the excess of *dLdb/Chip* phenotype by overexpressing *ap*. The proposed domain structure of the LDB/NLI family of proteins provides a conceptual framework to understand the phenotypes produced by altering the dosage

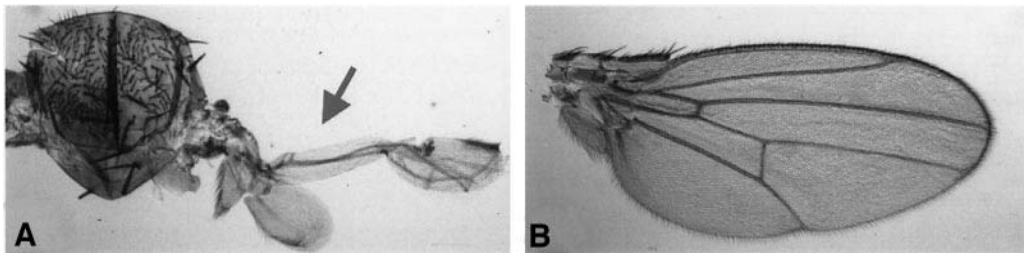

Fig. 5. *ap* overexpression rescues the phenotypes caused by excess *dLdb/Chip*. (**A**) Notum and wing (arrow) of a *apGAL4/+; UAS: dLdb/+* fly. Note that *dLdb/Chip* overexpression in the *ap* domain causes severe reductions in the margin and size of the wing. (**B**) Wing of a *apGAL4/+; UAS: dLdb +/+ UAS: ap* fly. Note the complete rescue of the phenotype shown in (A) by *ap* overexpression.

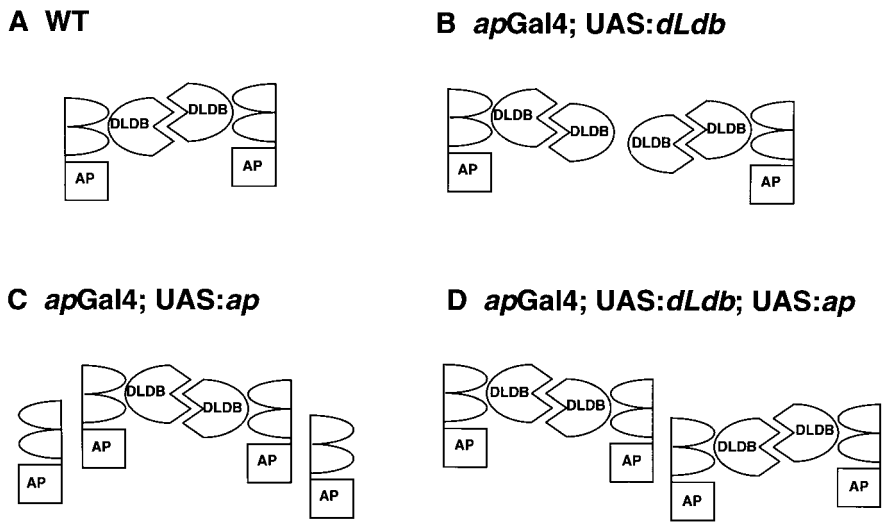

Fig. 6. A model for Ap/DLDB function. (**A**) An DLDB–Ap tetramer may be the functional complex carrying out dorso-ventral patterning functions. For simplicity, the Ap and DLDB molecules are depicted in the absence of DNA. However, one possibility is that DLDB may be instrumental in bringing together otherwise distant Ap-binding sites; DLDB may also facilitate contacts with the target promoter required for activation or repression of transcription. (**B**) Overexpression of DLDB would lead to the titration of Ap, and the formation of non-functional trimers. (**C**) Ap overexpression would not lead to the formation of non-functional complexes because overabundance of Ap should not be able to prevent interactions between DLDB molecules bound to Ap. (**D**) Ap overexpression would reconstitute functional complexes after DLDB overexpression.

of *dLdb/Chip* and *ap*. The presence of homodimerization and LIM-interacting domains in LDB/NLI proteins suggests that LDB and LIM domain proteins may form tetrameric complexes. These complexes would be formed by two LDB molecules interacting through the N-terminal homodimerization domain; in addition, each LDB molecule would interact with a LIM domain through the C-terminal LIM-interacting domain. The occurrence of these complexes has been demonstrated between murine LDB and the hamster LIM/homeodomain protein LMX1 (Jurata and Gill, 1997). In the case of DLDB–Ap, this tetrameric complex (Figure 6A) may be the functional complex carrying out the dorso-ventral patterning functions. This model predicts that *dLdb/Chip* overexpression would lead to the formation of non-functional complexes, and it also predicts that functional DLDB–Ap complexes would be reconstituted by overexpressing *ap* in addition to *dLdb/Chip* (see Figure 6 and Discussion).

To test this model, we used a GAL4 insertion in *ap* (Calleja *et al*., 1996). Expression from this GAL4 driver faithfully reproduces *ap* expression in the wing imaginal disc (not shown). When *dLdb/Chip* is expressed from the *ap* promoter, the wing is reduced or eliminated depending on the UAS:*dLdb/Chip* transgenic line used. Most lines reduce the wings (Figure 5A), whereas the strongest line completely eliminates them (not shown). As shown in Figure 5B, the reduced wing phenotype can be completely rescued by *ap* overexpression. These results provide further evidence for the idea that the stoichiometry of Ap and DLDB is critical for dorso-ventral patterning.

Discussion

Using the yeast two-hybrid system, we cloned *dLdb/Chip*, a gene whose protein product interacts with Ap LIM domains. DLDB/CHIP is the *Drosophila* homolog of a family of vertebrate proteins known for binding certain LIM domain proteins, and at least one non-LIM protein (Agulnick *et al*., 1996; Jurata *et al*., 1996; Bach *et al*., 1997; Wadman *et al*., 1997). As reported recently, DLDB/CHIP is required for normal embryonic segmentation (Morcillo *et al*., 1997). However, no known LIM protein (including Ap) is known to play a role during *Drosophila* segmentation. Thus, this early function of DLDB/CHIP is likely to be independent of LIM proteins. The analysis of DLDB/CHIP function in genetic mosaics described here reveals that it has very specific functions during wing development. We found no requirement for DLDB in cells of the ventral compartment, and an exclusive role for DLDB in dorsal cells specifying dorsal identity in cooperation with Ap.

Mutations in *dLdb/Chip* are haploinsufficient (Figure

2A; see also Figure 3A in Morcillo *et al*., 1997); in addition, *dLdb/Chip* and *ap* show strong genetic interactions (Figure 2A–C). These results suggested that dorsoventral patterning is very sensitive to *dLdb/Chip* dosage. This sensitivity was confirmed in overexpression experiments; we found that excess *dLdb/Chip* expression mimics *ap* lack-of-function phenotypes. How can these results be explained in terms of a DLDB–Ap functional complex? In principle, Ap and DLDB/CHIP may form a dimer. However proteins of the LDB family contain a homodimerization domain, suggesting that they may form tetramers with LIM proteins. The formation of these tetrameric complexes has been demonstrated between the LIM/homeodomain protein LMX1 and LDB (Jurata and Gill, 1997). The LDB homodimerization domain maps to its N-terminus, and this region is ~64% identical between the fly and the vertebrate proteins. Thus, Ap and DLDB may form a tetramer as shown in Figure 6A, and this may be the functional complex carrying out the activity as a dorsal selector protein. We made three observations that support the hypothesis of the DLDB–Ap tetramer as a functional complex. (i) Excess DLDB causes *ap* lack-of-function phenotypes. According to the tetramer model, excess DLDB would lead to the titration of Ap and the formation on non-functional trimers (Figure 6B). This result is more difficult to explain in terms of a DLDB–Ap dimer because Ap would not be titrated. (ii) *ap* overexpression does not produce a mutant phenotype. The tetramer model predicts that excess Ap should not prevent two DLDB molecules interacting with one another; thus it should not interfere with formation of the tetramer (Figure 6C). (iii) The excess DLDB is rescued by overexpressing *ap*. The addition of Ap to an excess of DLDB should result in the transformation of non-functional trimers into functional tetramers (Figure 6D).

It will be interesting to learn if LDB/NLI/CLIM proteins in vertebrates show dosage-sensitive interactions with their protein partners, and how many and what classes of proteins they interact with. DLDB/CHIP was proposed to be a general facilitator of enhancer–promoter interactions (Morcillo *et al*., 1997); accordingly, DLDB/CHIP may help Ap make contacts with the basal transcription machinery at the promoters of genes under Ap control. The hypothesis of DLDB/CHIP as a general enhancer–promoter facilitator implies that it has many partners. However, our mosaic analysis in the wing suggests that the functions of DLDB/CHIP are very specific and that the number of its protein partners may be very limited.

Materials and methods

Library screening

The DNA sequence encoding the *ap* LIM domains was amplified with specific primers and cloned into the yeast bait vector pAS1. The resulting construct was sequenced and transformed into the yeast strain HF7C (Clontech). A λ_{ACT} library from *Drosophila* third instar larvae (a gift from S.J.Elledge) was used for the yeast two-hybrid screening as described (Durfee *et al*., 1993). Two 1.9 kb clones were obtained from the screening (pACT-23-22 and pACT-24-1). Both clones contain the full open reading frame (ORF) but differ by 47 nucleotides in length at the 3′ end. A longer 2.2 kb *dLdb* cDNA was later isolated from a *Drosophila* early pupae cDNA library (Poole *et al*., 1985) using the pACT-23-22 cDNA as probe.

Sequence analysis

All DNA sequence analyses were performed on both strands using the ABI PRISM dye terminator kit (Perkin Elmer). To identify the mutations in *dLdb*, four sets of primers were used to amplify sequences covering all the *dLdb* coding region using genomic DNA from *dLdb* heterozygous flies as templates. The resulting PCR products were cloned into the pGEM T-Vector (Promega) and six independent clones were analyzed for each specific cloning.

Antibody production and immunostaining

The full ORFs of *dLdb* and *ap* cDNAs were cloned into pRSET C (Invitrogen) and pET15b (Novagen), respectively. The resulting constructs were used for protein production in *Escherichia coli* strain BL21DE3 (Novagen). The His-tagged proteins were purified through His-Bind Resin according to the manufacturer's instructions (Novagen). The purified fusion proteins were isolated by SDS–PAGE and used to immunize rats and guinea pigs by Cocolico Co. (Reamstown, PA).

Immunostaining was performed as described (Vachon *et al*., 1992). The following primary antisera were used: mouse anti-β-GAL (1:10 000, Promega), guinea pig anti-Ap (1:2000, see above), rat anti-DLDB (1:3000, see above), mouse anti-C-MYC (1:10, American Type Culture Collection) and rabbit anti-SER (Speicher *et al*., 1994). For horseradish peroxidase staining, subsequent steps were conducted according with the manufacturer of the ABC kit (Vector Laboratories). For confocal image, the secondary antisera conjugated with fluorescein isothiocyanate or Cy5 were used at 1:200 according to the manufacturer's instructions (Jackson ImmunoResearch laboratories). Propidium iodide staining was conducted following standard techniques.

Drosophila stocks and crosses

To generate the *UAS: dLdb* transgenic lines, the *dLdb* cDNA was swapped from pACT-23-22 to the *Xho*I site of pUAST (Brand and Perrimon, 1993). *UAS: ap* was made with a 1740 bp *Xba*I–*Sma*I fragment from the *ap* cDNA that contains the ORF, 5′ and 3′ sequences cloned into the *Kpn*I site of pUAST. The resulting constructs were used to inject *yw* embryos by standard procedures (Rubin and Spradling, 1982). Three independent lines for both *dLdb* and *ap* were tested.

All fly strains were obtained from the Bloomington *Drosophila* Stock Center and crosses were grown at 25°C unless otherwise indicated in the text. The genetic interaction between *ap* and *dLdb* was tested by crossing $ap^{54}/SM5$ flies to ap^{78j}/CyO and to ap^{78j} $dLdb^{M4}/CyO$ flies at 18°C.

Generation of mosaic clones

pwn $dLdb^{M4}$ flies were crossed to f^{36a}; f^{+44} f^{+52} *M(2)58F* and the progeny were irradiated (X-rays, 1000 rad) at 48–72 h after egg-laying. To generate clones on wing imaginal discs, $dLdb^{M4}$*/T(2;3)SM6a-TM6b* and $dLdb^{M4}$*; vg(B)/T(2;3)SM6a-TM6b* flies were mated with heat-shock-inducible 42πM myc flies (Xu and Rubin, 1993). Mitotic recombination was induced in 48–60 h AEL staged larvae using 1000 rad X-rays. After 60 h, larvae were heat-shocked for 2 h at 37°C to induce myc expression. Wing imaginal discs were immunostained after a 90 min recovery as described (Kim *et al*., 1995).

Mutagenesis

dLdb mutations were induced by EMS (0.2 M) in adult males using standard protocols (Lewis and Bacher, 1968) on a Vallecas wild-type strain.

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