INTRODUCTION

The LIM-homeodomain (LIM-HD) gene family encodes a large group of transcriptional regulators, whose members have been implicated in a variety of developmental processes. Early embryonic patterning events (Shawlot and Behringer, 1995; Taira et al., 1997), limb and eye formation (Vogel et al., 1995; Porter et al., 1997), and the development of imaginal discs in Drosophila (Cohen et al., 1992; Curtiss and Heilig, 1997) all depend on the function of LIM-HD family members. LIM-HD proteins are also expressed in discrete subpopulations of developing neurons (Way and Chalfie, 1989; Ericson et al., 1992; Tsuchida et al., 1994; Appel et al., 1995; Matsumoto et al., 1996; Varela-Echavarria et al., 1996) and play key roles in their differentiation (Way and Chalfie, 1988; Lundgren et al., 1995; Pfaff et al., 1996; Sheng et al., 1996; Hobert et al., 1997; Porter et al., 1997; Sheng et al., 1997; Thor and Thomas, 1997; Hobert et al., 1998).

Although many of the developmental processes that the LIM-HD transcription factors control have been elucidated, the mechanisms by which they regulate target genes remains unknown. It is generally thought that these factors become localized to the regulatory regions of appropriate target genes through recognition of specific genomic sequences by their homeodomains. However, many homeodomains, including those of the LIM-HD class, appear to bind similar target sequences in vitro (Hoey and Levine, 1988; Kalionis and O’Farrell, 1993). Therefore, specificity of target gene selection is not likely to be controlled entirely by the DNA-binding properties of the homeodomain.

In addition to the DNA-binding homeodomain, members of the LIM-HD family of transcription factors have two highly conserved domains in the N-terminal portion of the protein, called the LIM domains (Freyd et al., 1990; Karlsson et al., 1990). LIM domains received their name from the first three founding members of the family: C. elegans Lin-11 and Mec-3, plus mammalian Islet-1. LIM domains are cysteine-rich, zinc-finger like domains (Michelsen et al., 1993; Archer et al., 1994) which do not bind DNA, but instead have been shown to mediate protein-protein interactions (Schmeichel and Beckerle, 1994; Arber and Caroni, 1996, for recent review see Jurata and Gill, 1998).

Interactions between LIM domains and a number of different proteins have been well characterized. One family of interacting proteins binds the LIM domains of LIM-HD and nuclear LIM-only proteins, with high affinity. This family includes mouse NLI/Ldb1/Clim-2, Xenopus Xlb1, Zebrafish Ldb1-4, and Drosophila Chip (Agulnick et al., 1996; Jurata et al., 1996; Bach et al., 1997; Morcillo et al., 1997; Toyama et al., 1998). NLI binds LIM domains via a specific LIM interacting domain, and is also capable of self-dimerization (Jurata and Gill, 1997). In this manner, NLI forms a bridge between two LIM-HD proteins, building homo- or heterodimeric complexes of LIM-
reaction (PCR), ligated into pKS by TA cloning (Marchuk et al., 1990),
encoding aa 1-338 were amplified from pKS16-4 by polymerase chain
DNA constructs
3916
(Stratagene).
cDNA clone (Bourgouin et al., 1992) in pKS Bluescript vector
MATERIALS AND METHODS
these two developmental processes.
phenotypes. Analysis of chimeric LIM-HD proteins establishes
negative fashion to disrupt Ap function, causing
expression of LIM domains alone can act in a dominant-
cells, we have found that the LIM domains, as well as the
domains of the LIM-HD member Apterous (Ap).
Drosophila
Here we use
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interactions between LIM domains and other transcription
factors are highly specific. For example, E47 binds Lmx-1, but
does not interact with Islet-1 (Johnson et al., 1997).
Collectively
1991). Adults mutant for
al., 1995) and juvenile hormone production (Altartz et al.,
While the nature of protein interactions mediated by LIM
domains are beginning to be elucidated in vitro, the functional
contribution of the LIM domains in vivo is virtually unknown.
Here we use Drosophila to investigate the role of the LIM
domains of the LIM-HD member Apterous (Ap). ap plays a
key role in a number of developmental processes, including
wing and haltere development (Butterworth and King, 1965;
Cohen et al., 1992; Diaz-Benjumea and Cohen, 1993; Blair et
1994), muscle differentiation (Bourgouin et al., 1992),
axon guidance within the embryonic nervous system (Lundgren et
al., 1995) and juvenile hormone production (Altartz et al.,
1991). Adults mutant for ap eclose at a low frequency and are
wingless, highly uncoordinated, sterile and short-lived, dying
within 1-2 days after eclosion. We have chosen to examine two
aspects of the ap phenotype in detail: wing development and
embryonic axon pathfinding.
Using the GAL4 system (Brand and Perrimon, 1993) to
direct expression of transgenes specifically in ap-expressing
cells, we have found that the LIM domains, as well as the
homeodomain, are essential for Ap function. Furthermore,
expression of LIM domains alone can act in a dominant-
negative fashion to disrupt Ap function, causing ap mutant
phenotypes. Analysis of chimeric LIM-HD proteins establishes
that LIM domains are interchangeable between family
members in the generation of wing structure. In contrast, for
axon pathfinding of the Ap neurons, the LIM domains confer
functional specificity and cannot be replaced by those of
another family member. This suggests that the Ap LIM
domains mediate qualitatively different protein interactions
in these two developmental processes.

MATERIALS AND METHODS
DNA constructs
All manipulations of the ap CDNA are based on the published full length
cDNA clone (Bourgouin et al., 1992) in pKS Bluescript vector
(Stratagene). UAS-ap was generated by Xbal excision of the entire ap
open-reading frame from pKS16-5 and insertion into the pUAS vector
(Brand and Perrimon, 1993). To generate UAS-apHD, sequences
encoding aa 1-226 were PCR amplified and inserted into the pUAS
above.

RESULTS
The ap mutant phenotype
During larval development ap is expressed in the dorsal
compartment of the wing disc, the region giving rise to the
notum, scutellum, wing hinge and dorsal surface of the wing
blade (Cohen et al., 1992). ap functions to restrict these cells to
a dorsal identity, and is necessary for normal wing margin
formation (Diaz-Benjumea and Cohen, 1993; Blair et al.,
1994). In flies homozygous for ap^P44, a null allele of ap (Bourgouin et
al., 1992), the wing is completely eliminated. Within the
embryonic ventral nerve cord (VNC), ap is expressed by three
of the approximately 200 neurons in each abdominal

HD transcription factors (Jurata et al., 1998). In addition to
binding the LIM domain of the LIM-HD family member
Apterous (Ap), the Drosophila NLI homolog, Chip, genetically
interacts with ap in the developing wing, evidence that the Ap-
Chip interaction is functional (Moricollo et al., 1997). LIM
domains can also bind directly to other transcription factors,
resulting in synergistic activation of transcription in cultured
cells. For example E47, a HLH transcription factor, binds the
LIM domains of Lmx-1 (Johnson et al., 1997), and the POU
domain of Pit-1 binds the LIM domains of Lhx-3 (Bach et al.,
1995). In contrast to NLI-LIM interactions, which appear to be
global since NLI binds all nuclear LIM domains tested,
interactions between LIM domains and other transcription
factors are highly specific. For example, E47 binds Lmx-1, but
does not interact with Islet-1 (Johnson et al., 1997).
Collectively

hemisegment (Bourgouin et al., 1992). Using promoter fusions to the axon-targeting tau-lacZ reporter (Callahan and Thomas, 1994), we previously showed that the ap-expressing neurons are interneurons that extend axons ipsilaterally and anteriorly along a single pathway within each longitudinal connective (Lundgren et al., 1995). Upon reaching the adjacent anterior segment the Ap neurons tightly fasciculate with their homologues, forming a discrete axon bundle running the length of the VNC (Fig. 1F).

In apP44 mutant embryos the ap neurons fail to recognize their appropriate pathway and instead wander within the connective, failing to fasciculate with one another.

**apGAL4 directs transgene expression specifically in ap cells**

To express the Ap variants in this study we made use of the GAL4-UAS system (Brand and Perrimon, 1993). We used a P[GAL4] enhancer trap insertion in the ap locus, isolated by Calleja et al. (1996). We found that this P[GAL4] line, which we have named apGAL4, is capable of driving reproducibly high levels of UAS transgene expression in the ap cells within the wing disc and the central nervous system (CNS). In apGAL4/+ individuals carrying a UAS-lacZ transgene, β-galactosidase (β-gal) activity is specifically localized to the cells of the dorsal compartment of the wing disc where ap is normally expressed (Fig. 1A). We determined the identity and behavior of the GAL4-expressing cells in the CNS using a UAS-tau-lacZ transgene to label axon processes. Double labeling of apGAL4/+; UAS-tau-lacZ/+ embryos for Ap and β-gal revealed that apGAL4 drives high levels of Tau-β-gal specifically in ap neurons, and that the axon processes of these neurons project normally within the connectives (Fig. 1B).

**apGAL4 is a strong mutant allele of ap**

By several criteria, apGAL4 acts as a strong hypomorphic allele of ap. apGAL4/apP44 individuals show only a slightly more extreme wing phenotype than apGAL4 homozygotes. Like apP44 homozygotes, apGAL4/apP44 individuals have no wings, but often have a ribbon-like outgrowth in the wing region that lacks any recognizable structures (Fig. 1G). This ribbon-like outgrowth is similar to that observed when apP44 is placed in trans to the temperature-sensitive allele apP789 (Wilson, 1981) and the flies are raised at non-permissive temperatures (data not shown). Such an outgrowth is rarely seen in apP44 homozygotes, suggesting that apGAL4 retains a low level of ap function. Like null mutants, apGAL4 mutant individuals are sterile, uncoordinated and exhibit precocious adult death.

Within the CNS of apGAL4/apP44 embryos, Ap protein levels are undetectable and axon pathfinding errors, as assayed with a UAS-tau-lacZ reporter transgene, are indistinguishable from those of apP44 homozygotes (Fig. 1C,H). As in apP44 homozygotes, the Ap neurons still project anteriorly but fail to choose a common pathway, wandering within the connectives and remaining highly defasciculated.

A full-length ap transgene rescues the ap mutant phenotype

We first tested whether rescue of the ap mutant phenotype was possible by re-supplying wild-type function using the apGAL4 allele to drive expression of an ap cDNA (Fig. 2). A single copy of a UAS-ap transgene almost completely rescues the wing defects of apGAL4/apP44 individuals (Fig. 3A). The wing blade is of appropriate size with a normal margin and vein pattern,
but is held at right angles away from the body, resembling the phenotype of mild ap hypomorphic mutations in which only the hinge region is affected (Wilson, 1981; Bourgouin et al., 1992). This mild wing defect may result from failure of apGAL4 to drive completely wild-type expression in the developing wing disc, or from the inherent delay in the timing of expression using GAL4/UAS-mediated transactivation (Brand and Perrimon, 1993; Lin et al., 1995). A single copy of UAS-ap is also capable of rescuing both the sterility and uncoordinated behavior of apDapLIM individuals.

To examine the ap neurons in these rescued individuals, we recombined the UAS-tau-lacZ and UAS-ap transgenes onto the same chromosome. Within the VNC of apGAL4/apP44 embryos carrying one copy each of UAS-ap and UAS-tau-lacZ, Ap immunoreactivity is clearly restored in the Ap neurons (Fig. 1D) and the behavior of these neurons is indistinguishable from wild-type (Figs 3B, 6). The axons of the Ap neurons in these rescued individuals fasciculate tightly with one another and choose a single pathway as they project anteriorly within the connectives. Thus, re-supplying ap function with UAS-ap fully rescues the nervous system phenotype.

Both the LIM and homeodomain of Ap are necessary for function

We capitalized on the apGAL4/UAS-ap-mediated phenotypic rescue to ask whether the Ap LIM domains are required for function. To generate ApLIM, we specifically eliminated the LIM domains, and left the rest of the protein intact (Fig. 2). This Ap derivative is unable to rescue any element of the ap phenotype when expressed using apGAL4 in ap mutant cells, although ApLIM protein is present at high levels and is properly localized to the nucleus as assayed with the anti-Ap antibody (data not shown). In apGAL4/apP44; UAS-apLIM+/+ adults, the wings remain ribbon-like outgrowths, devoid of any identifiable structures (Fig. 3C). Using the UAS-tau-lacZ transgene we found that the ap neurons remain defasciculated, indistinguishable from those of apGAL4/apP44 mutant individuals (Figs 3D, 6). Therefore, the LIM domains are essential for ap function.

We also tested whether the Ap homeodomain is necessary for function. To generate ApHD, we truncated Apterous between the LIM domains and the homeodomain, thereby eliminating the homeodomain and the C-terminal end of the protein (Fig. 2). Like ApLIM, this construct is unable to rescue either the ap wing or CNS phenotypes when expressed in ap mutant cells under the control of apGAL4, indicating that the homeodomain is also required for ap function (Figs 3E, 6).
We found that ApΔHD, but not ApLIM, is capable of modifying the wing phenotype of apGAL4/apP44 flies. In apGAL4/apP44; UAS-apΔHD/+ flies the ribbon-like outgrowth commonly observed in apGAL4/apP44 individuals is entirely eliminated, suggesting that the ApΔHD protein acts in a dominant-negative fashion to disrupt residual ap function (Fig. 3E).

To further examine the potential dominant-negative activity of ApΔHD, we analyzed its effects in apGAL4/+ heterozygous individuals, which have normal wings. The wings of apGAL4/+ flies carrying a single copy of UAS-apΔHD are blistered and exhibit numerous margin defects (Fig. 4A). The dorsal and ventral wing surfaces often fail to fuse, resulting in a fluid-filled balloon-like structure. Supplying additional wild-type ap function with a copy of UAS-ap (genotype = apGAL4/+; UAS-apΔHD/UAS-ap) fully restores wild-type wing structure, indicating that ApΔHD interferes with ap function (Fig. 4B).

We further tested whether ApΔHD could modify the phenotypes of an intermediate ap hypomorphic allelic combination. The apts78j temperature-sensitive allele has nearly normal wing morphology when raised at 15°C, but completely lacks all wing structures at 29°C (Wilson, 1981). When raised at 18°C, apGAL4/appts78j flies have defective wing margins and unfused wing surfaces (Fig. 4C), a phenotype similar to the ApΔHD dominant effect in an apGAL4/+ heterozygous background. Introduction of the UAS-apΔHD transgene into this hypomorphic mutant background at 18°C (genotype = apGAL4/appts78j; UAS-apΔHD/UAS-ap+) eliminates the wings altogether (arrow), mimicking the ap null mutant phenotype. (E) A single UAS-lim3ΔHD transgene also disrupts wing formation in apGAL4/+ individuals, causing blistering and loss of margins (arrow). (F) The UAS-lim3ΔHD dominant phenotype is suppressed completely with a UAS-ap transgene (genotype = apGAL4/+; UAS-lim3ΔHD/UAS-ap).

**Fig. 4.** Expression of LIM domains disrupts ap function in the developing wing. (A) Expression of ApΔHD in apGAL4/+; UAS-apΔHD/+ flies produces a dominant wing phenotype consisting of blisters (arrow) and margin defects (arrowhead). (B) The dominant phenotype of UAS-apHD is suppressed completely with a single UAS-ap transgene (genotype = apGAL4/+; UAS-apΔHD/UAS-ap). (C) apGAL4/ap78j flies have wings with blisters (arrow) and margin defects (arrowhead) when raised at 25°C. (D) Introduction of a single UAS-apΔHD transgene into apGAL4/ap78j individuals raised at 25°C (genotype = apGAL4/ap78j; UAS-apΔHD/UAS-ap) eliminates the wings altogether (arrow), mimicking the ap null mutant phenotype. (E) A single UAS-lim3ΔHD transgene also disrupts wing formation in apGAL4/+ individuals, causing blistering and loss of margins (arrow). (F) The UAS-lim3ΔHD dominant phenotype is suppressed completely with a UAS-ap transgene (genotype = apGAL4/+; UAS-lim3ΔHD/UAS-ap).

**apΔHD disrupts ap function**

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The same dominant effect on wing development is also observed using a HD-deleted version of Islet (Isl), a different LIM-HD protein normally not expressed in the wing disc (Thor and Thomas, 1997). In apGAL4/+ individuals carrying a UAS-islΔHD transgene, wing structure is severely disrupted (Fig. 4E). As found for ApΔHD, the IslΔHD dominant effect is suppressed by the addition of full-length Ap with a UAS-ap transgene (Fig. 4F). Thus, the ability to act as a dominant-negative inhibitor of Ap function in the wing is not restricted to the Ap LIM domains.

In contrast to the wing, neither ApΔHD nor IslΔHD has any dominant effects on the development of the ap neurons within the CNS. This suggests that for these two developmental processes, generation of wing structures and axon pathfinding, there are differences in the protein interactions involving the Ap LIM domains.
Ap and Lim3 are not interchangeable

The above results establish that the LIM domains are essential for Ap function. We next tested whether the LIM domains of a different LIM-HD family member might be interchangeable with those of Ap. For these studies we chose Drosophila Lim3, which is normally expressed in subsets of post-mitotic neurons, none of which co-express Ap (S. T., S. G. E. Andersson, A. Tomlinson and J.B.T., unpublished). Conservation of LIM domain sequence within the LIM-HD family ranges from 25% to 86% aa identity. Ap and Lim3 are relatively divergent, sharing only 37% identity within the LIM domains.

Expression of Lim3 in Ap cells results in lethality during larval or early pupal stages. To circumvent these lethal effects we raised individuals carrying both apGAL4 and UAS-lim3 at 18°C to reduce levels of GAL4-mediated transactivation (Staehling-Hampton et al., 1994). At this temperature a small number of apGAL4/apP44; UAS-lim3/+ individuals emerge and these flies have small outgrowths of tissue in the hinge region of the thorax (Fig. 5A). Although unstructured, the presence of a row of bristles on this tissue suggests the development of a rudimentary dorsal/ventral margin, and indicates that Lim3 can partially rescue the ap wing phenotype.

Although non-viable at 25°C, apGAL4/apP44; UAS-lim3/+ embryos survive through embryonic stages, allowing us to examine the behavior of the ap mutant interneurons ectopically expressing Lim3. Using the UAS-tau-lacZ transgene, we found that Lim3 partially rescues the ap neuronal pathfinding defects (Figs 5B, 6). The axons are more highly fasciculated than those in ap mutants, but 74% of the segments still display clear pathfinding errors. Thus, although Lim3 promotes some degree of axon fasciculation and the formation of a rudimentary wing margin in ap mutants, it is not interchangeable with Ap.

### LIM domains are interchangeable for wing formation but not axon pathfinding

To determine whether LIM domains are interchangeable between Lim3 and Ap, we created a fusion between the N-terminal half of Lim3, including the LIM domains, to the C-terminal half of Ap, containing the homeodomain (Fig. 2). This Lim3:Ap chimera, rescues the ap wing phenotype to the same extent as full-length Ap, likely because the Lim3 LIM domains also bind Chip. Thus, interchanging the LIM domains has no effect on formation of the Ap-Chip complex in the generation of wing structure. (B) During pathfinding of the Ap neurons, the Ap LIM domains are involved in additional protein interactions independent of Chip. These interactions are specific to the Ap LIM domains and cannot be mediated by the Lim3 LIM domains. Taken together, this data suggests that LIM domains mediate different types of protein interactions in different developmental processes.

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**Fig. 6.** Quantitation of axon pathfinding data. Each embryonic segment was scored either as fasciculated or defasciculated. Fasciculated segments contained only two bundles of axons with no axons crossing the midline or choosing a separate pathway. For each embryo, all eight abdominal segments were scored and the percentage of fasciculated segments was calculated. An average of 12 embryos were examined for each genotype (minimum = 9). Asterisks denote a statistically significant improvement in axon fasciculation compared to the apGAL4/apP44 genotype (P≤0.05, Student’s t-test). Error bars indicate standard error of the mean.

**Fig. 7.** Model of protein interactions mediated by the Ap LIM domains. (A) Our data suggests that during wing formation the primary function of the Ap LIM domains is to interact with Chip. This interaction is required since ApALIM fails to rescue the ap wing phenotype and it can be disrupted by the expression of LIM domains alone. The Lim3:Ap chimera rescues the wing phenotype to the same extent as full length Ap, likely because the Lim3 LIM domains also bind Chip. Thus, interchanging the LIM domains has no effect on formation of the Ap-Chip complex in the generation of wing margin. (B) During pathfinding of the Ap neurons, the Ap LIM domains are involved in additional protein interactions independent of Chip. These interactions are specific to the Ap LIM domains and cannot be mediated by the Lim3 LIM domains. Taken together, this data suggests that LIM domains mediate different types of protein interactions in different developmental processes.
and uncoordinated behavior of \( ap^{GAL4}/ap^{P44} \) individuals also can be rescued with \( UAS-lim3:ap \).

In contrast to its ability to rescue the wing phenotype, the Lim3:Ap chimera only partially rescues the axon pathfinding phenotype. The level of rescue seen with the Lim3:Ap chimera is not significantly different from Lim3 itself (Figs 5D, 6). Thus, despite their sequence divergence, the Ap and Lim3 LIM domains are interchangeable in the generation of normal wing structure, but not in the control of pathfinding of the Ap neurons, revealing a functional difference between the Ap and Lim3 LIM domains.

DISCUSSION

The Ap LIM domains are essential for function

Although the ability of LIM domains to interact with other proteins has been clearly established in vitro and in cultured cells, their in vivo role is unknown. Within the LIM-HD family, it has been suggested that LIM domains function to negatively regulate LIM-HD activity by interfering with the ability of the homeodomain to bind DNA. This model is based primarily on two observations. First, LIM-less versions of Islet-1 and Meca-3 bind target DNA sequences more effectively in vitro than the full length proteins (Sanchez-Garcia et al., 1993; Xue et al., 1993). Second, site-directed mutagenesis of the LIM domains of Xlim-1 potentiates its ability to form secondary axes when misexpressed in Xenopus embryos (Taira et al., 1994). However, our genetic data indicate that negative regulation of LIM-HD function is not the primary role for the LIM domains. LIM-less versions of Ap clearly do not act as activated forms of the protein, as the Xlim-1 data might predict, but instead are incapable of mediating any discernible \( ap \) function within the wing disc or CNS.

LIM domains act as dominant negative factors in the wing

Not only does expression of \( ap^{\Delta HD} \) fail to rescue the \( ap \) mutant phenotype, but it disrupts normal \( ap \) function in the wing disc. This result supports previous studies in which overexpression of Islet-3 LIM domains was found to disrupt eye and tectal development in the zebrafish embryo, presumably due to a dominant-negative effect of the LIM domains on Islet-3 function (Kikuchi et al., 1997). Our finding that Isl\( ^{\Delta HD} \) was just as effective in disrupting \( ap \) function as Ap\( ^{\Delta HD} \) suggests that in some circumstances LIM domains show little or no specificity when interfering with LIM-HD function. The most likely explanation for this lack of specificity is that the Ap LIM domains normally mediate a functional complex with Chip (Morcillo et al., 1997). Extrapolating from studies of NLI binding (Jurata et al., 1996), Chip is likely to be capable of binding the LIM domains of most, if not all, LIM-HD proteins. Thus we would predict that in addition to Isl\( ^{\Delta HD} \), a \( ^{\Delta HD} \) variant of any family member would compete with full length Ap for binding to Chip and prevent formation of a functional complex.

Why do we not see a dominant effect of Ap\( ^{\Delta HD} \) and Isl\( ^{\Delta HD} \) in the CNS? One possibility is that Chip or Ap (or both) are expressed at sufficiently high levels in the Ap neurons that they are refractory to the levels of Ap\( ^{\Delta HD} \) driven by \( ap^{GAL4} \). Alternatively, Ap function in axon pathfinding may require other, non-Chip-dependent interactions that are unaltered by Ap\( ^{\Delta HD} \). The latter possibility is supported by the finding that the LIM domain-dependent synergism exhibited by Lmx-1 and E47 in transcriptional activation cannot be abolished by overexpressing LIM domains (German et al., 1992).

Ap and Lim3 are not interchangeable

While there is a clear difference in the ability of Ap and Lim3 to rescue the \( ap \) mutant phenotype, Lim3 is capable of partial rescue. In \( ap \) mutants, Lim3 can induce a wing-like structure with an irregular margin and unfused dorsal and ventral surfaces. In the CNS, Lim3 enables Ap mutant interneurons to fasciculate more tightly. This result is surprising given the divergence between the two family members (only 37% aa identity within the LIM domains and 46% within the homeodomain). Although the molecular basis for this partial rescue remains unknown, there are two extremes among the various possibilities. First, when expressed in Ap cells, Lim3 may regulate those genes normally regulated by Ap, albeit not as effectively. However, it seems unlikely that Lim3 regulates exactly the same set of genes as Ap since individuals that misexpress high levels of Lim3 using \( ap^{GAL4} \) (at 25°C) are completely non-viable, while individuals expressing high levels of full-length Ap under identical conditions are viable. The other extreme predicts that Lim3 regulates an entirely different set of effector target genes, but that these effectors serve similar functions and are sufficient to give some degree of phenotypic rescue. For example, within the CNS, Ap and Lim3 control specific pathfinding behaviors of neurons that normally express them, presumably by regulating the expression of distinct sets of cell recognition molecules (Lundgren et al., 1995; S. T., S. G. E. Andersson, A. Tomlinson and J. B. T., unpublished). Thus it is conceivable that both LIM-HD members regulate similar types of target genes involved in controlling pathway recognition and axon fasciculation.

Specificity of LIM domains

Our results with the Lim3:Ap chimera demonstrate that in the developing wing the LIM domains of Ap and Lim3 are completely interchangeable. The simplest explanation for this interchangeability is that the primary function of the Ap LIM domains is to bind Chip. Thus, any nuclear LIM domain would be able to carry out this function. Based on studies of Chip and NLI (Morcillo et al., 1997; Jurata et al., 1998), we predict that in the developing wing Ap forms Chip-mediated homodimers or possibly heterodimers with another, as yet unidentified LIM-HD family member (Fig. 7A).

In contrast, our analysis of the Lim3:Ap chimera in the CNS indicates that the Ap and Lim3 LIM domains are not interchangeable. While full-length Ap fully rescues the \( ap \) pathfinding phenotype, Lim3:Ap shows only partial rescue, to an extent no greater than Lim3 itself. This result suggests that there are functional differences between LIM domains, a notion supported by the finding that LIM domains of different LIM-HD family members show specificity in their ability to bind other proteins (e.g., Jurata et al., 1998). Whereas Ap-Chip complexes may be sufficient to regulate target genes in the wing disc, in the Ap neurons it is likely that additional interactions with other factors are required for full Ap function, and that these interactions are specific to the Ap LIM domains (Fig. 7B).
Our findings support the notion that interactions among transcription factors play critical roles in the specificity of target gene regulation (Xue et al., 1993; Copeland et al., 1996; Guichet et al., 1997; Yu et al., 1997; Zelzer et al., 1997). For example, within the bHLH/PAS family of transcription factors, it is the protein-interacting PAS domain and not the DNA binding domain which dictates target specificity (Zelzer et al., 1997). It has also been shown that a homeodomain-deleted version of the Fushi tarazu (Ftz) protein is capable of regulating ftz-dependent segmentation, presumably through interactions with other transcription factors such as the pair-rule protein Paired (Copeland et al., 1996) or the nuclear hormone receptor Ftz-F1 (Guichet et al., 1997; Yu et al., 1997). Our analysis of the LIM-HD protein Apterous further underscores the importance of protein-protein interactions in transcription factor function, and suggests that LIM domains are involved in different types of protein-protein interactions in different developmental processes.

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