Interactions between LIM Domains and the LIM Domain-binding Protein Ldb1*  

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*LIM domains mediate protein-protein interactions and, within LIM-homeodomain proteins, act as negative regulators of the transcriptional activation function of the protein. The recently described protein Ldb1 (also known as NLI; LIM domain-binding protein) binds LIM domains in vitro and synergizes with the LIM-homeodomain protein Xlim-1 in frog embryo microinjection experiments. In this study we localized the transcriptional activation domain of Xlim-1 to its carboxyl-terminal region, and characterized the interactions of the amino-terminally located LIM domains with Ldb1. Ldb1 binds LIM domains through its carboxyl-terminal region, and can form homodimers via its amino-terminal region. Optimal binding to Ldb1 required tandem LIM domains, while single domains could bind at lower but clearly measurable efficiency. In animal explant experiments, synergism of Ldb1 with Xlim-1 in the activation of downstream genes required both the region containing the dimerization domain of Ldb1 and the region containing the LIM-binding domain. The role of Ldb1 may be to recruit other transcriptional activators depending on the promoter context and LIM-homeodomain partner involved.

LIM domains, cysteine-rich motifs which bind zinc and mediate protein-protein interactions, are found in diverse proteins which may also contain homeodomains, kinase domains, cytoskeletal components, or other conserved domains (reviewed in Refs. 1 and 2). LIM domain proteins have been classified into three groups (3). The first group contains paired LIM domains, classified as type A and B, near the amino terminus, and often but not always also contain a homeodomain (LIM-homeodomain proteins). This group contains LMO (LIM-only) proteins which contain little sequence not within the paired LIM domains. The second group contains one or two copies of a single sequence type of LIM domain (type C). The third group includes a heterogeneous collection of LIM domains that are generally localized near the carboxyl terminus and are often associated with additional domains, some of which bind to cytoskeletal components.

The biological consequences of disruption of LIM-homeodomain genes has been studied in several cases, demonstrating the importance of these genes in development, especially in neuronal cells. For example, mutation of the Caenorhabditis elegans mec-3 gene prevented the generation of touch receptor neurons (4). Disruption of the murine lim-1 gene led to lack of head formation (5), affecting forebrain, midbrain, and some hindbrain elements. Lhx3 null mice miss the anterior and intermediate lobes of the pituitary gland (6), while mice that lack Isl-1 function are deficient in motorneuron and interneuron formation (7). Thus, the importance of the LIM-homeodomain proteins is clear, but the molecular mechanism of their action is not fully understood.

The LIM-homeodomain protein Xlim-1 is specifically expressed in the Spemann organizer during the gastrula stage in Xenopus (8). Overexpression experiments indicate that the LIM domains of Xlim-1 exert a negative regulatory function, since mutant forms in which the LIM domains are disrupted can initiate neural and muscle induction in animal explants (9). Other types of experiments have also provided evidence that LIM domains play a negative regulatory role in the activity of LIM-homeodomain proteins (10, 11). These studies implied that LIM-homeodomain proteins may be activated in vivo by interaction with other proteins that would abrogate the inhibitory role of the LIM domains and, possibly, exert an additional activating or modulating function.

Recently, we have isolated a LIM-domain-binding protein, named Ldb1,1 from mouse and Xenopus (12). These proteins are 98% identical over their entire length. Ldb1 binds to LIM domains from all LIM-homeodomain proteins tested and to LMO1 and LMO2, but not to LIM domains of group II or group III proteins (1). In explant experiments, Ldb1 synergizes with Xlim-1 in the activation of downstream target genes like goosecoid and chordin. Since in embryo microinjection experiments, the activity of Xlim-1 plus Ldb1 is similar to that of Xlim-1 with mutated or deleted LIM domains we concluded that Ldb1 relieves the inhibitory effect of the LIM domains (12). The same protein was isolated independently by Jurata et al. (13) and named NLI, and more recently by Bach et al. (14).

In our previous work (12) Ldb1 was isolated because of its ability to bind LIM domains in an overlay assay using a mouse expression library, while the Xenopus ortholog emerged from a two-hybrid screen using LIM domains as bait. In the initial characterization of the interaction with the aid of the overlay assay we found that tandem LIM domains bound Ldb1 effectively, whereas proteins that carried a point mutation in either LIM domain or in both LIM domains bound poorly (12). These results differ to some extent from those of Jurata et al. (13) who found that NLI (identical to Ldb1) was able to bind the individual LIM A domain of Isl-1 and LIM B domain of Mec-3. Since the experiments of Agulnick et al. (12) and Jurata et al. (13) used different methods they may not be directly comparable, yet it is clear that the precise requirements for LIM-domain binding to Ldb1/NLI are not fully understood.

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¶ The abbreviations used are: Ldb, LIM domain-binding protein; NLI, nuclear LIM interactor; DBD, DNA-binding domain; AD, activation domain; PCR, polymerase chain reaction.
at present.

To better understand the molecular mechanism of activation of Xlim-1 by Ldb1 we wished to better define the binding of Ldb1 to Xlim-1 LIM domains. We report here the result of quantitative two-hybrid assays which show that single LIM domains of Xlim-1 do bind Ldb1 but tandem AB LIM domains show the highest affinity. Using a combination of in vitro and in vivo methods we show that the amino-terminal region of Ldb1 is able to form homodimers, and that the LIM-binding portion of Ldb1 is localized to the carboxyl terminus. Furthermore, using microinjection experiments we show that both the region containing the dimerization as well as the region containing the LIM-binding domain of Ldb1 are required for synergy with Xlim-1 in activation of downstream genes. These studies begin to define the molecular details of the synergistic interaction between Ldb1 and LIM domains in LIM-homoeo-

Experimental Procedures

Two-hybrid System—Plasmids pAS2-1, pLAM, pACT2, and yeast strains Y187 and CG-1945 were from CLONTECH. ABL60, ABL60-1m, -2m, and LIM A, and LIM B (Fig. 1) were generated by PCR using Xlim-1 (9) as template, and fragments were cloned into pAS2-1. pXdb1-ACT2 was generated by subcloning Xdb1 from pXdb1SP64RI (12) to pACT2. pLdb1-ACT2 and subsequent deletions were generated by PCR for ligation into pACT2 and/or pAS2-1. DBD-Xlim-1 constructs were made by subcloning full-length Xlim-1, followed by generating deletions using PCR for cloning into pAS2-1. All constructs were verified by DNA sequencing.

The two-hybrid screen using Ldb1 as probe was performed using pLdb1(1–310)-AS2–1 in yeast CG-1945. This screen was done using a Xenus gastrula pGAD10 library essentially as described (12). Approximately 2 × 10^5 transformants were plated and there were 2 His+ lacZ clones.

The quantitative interaction assay (Figs. 1 and 4) was performed as described by Moehle and Hinnebusch (15). Fusion genes were introduced into Y187 by the lithium acetate transfection procedure (CLONTECH, Ref. 16). Transformants were grown to stationary phase at 30 °C with agitation. Precultures were diluted 1:50 into fresh media and grown to OD_600 of approximately 0.6–0.8, at which point cells were washed and frozen at −70 °C. Cells were lyzed by sonication in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 2 mM β-mercaptoethanol and protease inhibitors, and lysates were centrifuged at 27,000 × _g_ for 30 min at 4 °C. The supernatant was applied to a FLAG M2 antibody-agarose column (Kodak/IBI) as recommended by the manufacturer and washed with 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM β-mercaptoethanol. Protein A was eluted with FLAG peptide (Kodak/IBI) in the same buffer. Glycerol was added to a final concentration of 10%, and aliquots were frozen at −80 °C.

Overaly Assay—Purified Lhx1 protein was 32P-labeled with the catalytic subunit of PKA (New England Biolabs) as described (19). Labeled protein was purified on a G-50 column in overlay buffer (modified from Ref. 17) with 20 mM HEPES (pH 7.7), 10 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 1% Nonidet P-40, 1% Carnation non-fat dry milk. Supernatant containing extracts were subjected to SDS-polyacrylamide gel electrophoresis (8–16% acrylamide gels, Novex), and proteins were transferred to nitrocellulose filters. For Western blots, the filters were probed with the anti-FLAG M2 monoclonal antibody (Kodak/IBI) according to the manufacturers instructions. For Far Western analysis, filters were blocked for at least 1 h in overlay buffer, probed with labeled Lhx1 (approximately 1 × 10⁶ dpm/μg) in the same buffer overnight at 4 °C, or for 4 h at room temperature, washed in the same buffer 3 times for 20 min total, and autoradiographed on Kodak X-Omat AR film. Quantitation was accomplished using NIH image software on scanned autoradiographs.

Animal Cap Assay—Animal cap experiments were carried out essentially as described (12). Briefly, Ldb1 (full-length), Ldb1(1–310), and Ldb1(210–375) were cloned into pCS2+ using PCR and pSP64RI-Xdb1 (12) as template. Constructs were verified by double-stranded DNA sequencing. RNA was generated using MEGAscript (Ambion) according to the manufacturers specifications and injected into the animal hemisphere of both blastomeres at the 2-cell stage. During blastula, animal caps were dissected and cultured in cap media (67% L15 (Leibovitz medium), 1 mg/ml bovine serum albumin, 50 μg/ml gentamycin, 7.5 mM Tris, pH 7.5) until mid-gastrula (stage 11) or tailbud (stage 25) and flash frozen in dry ice. Collected animal caps were extracted for RNA and subjected to Northern blot analysis as described (12). Plasmids used for probes were: otx2 (21), chordin (22), goosecoid (23), and nrp-1 (24). Images were collected using a Molecular Dynamics Storm 860 PhosphorImager.

Results

LIM Domain Binding to Ldb1—To characterize the sequence requirements for this interaction we used a quantitative yeast two-hybrid system to measure in vitro binding efficiency between Ldb1 and several LIM domain constructs (Fig. 1). A Gal4-DBD fusion containing the tandem LIM domains A and B and the adjacent 60-residue linker region (ABL60) bound strongly to Ldb1 (LDB). This binding was reduced substantially by a single point mutation in either LIM domain in which a critical cysteine residue, involved in zinc binding, was changed to glycine (9). Mutation of either LIM A (1m) or LIM B (2m) had about the same effect, decreasing β-galactosidase activity approximately 7-fold; this level of interaction, resulting in 250–300 units of β-galactosidase activity, was quite sufficient to give a positive reaction in the qualitative two-hybrid assay (blue colonies). Oxytalan mutation in both LIM domains (3m) decreased binding dramatically to background levels (3m, 3 units/μg; reporter, 7 units/μg), resulting in negative (white) colonies. These data are in general agreement with in vitro binding data shown previously, which showed a reduction of binding by each mutation (2m, 1m) but was not sufficiently sensitive to distinguish between them (12). We tested whether isolated LIM domains are sufficient for binding Ldb1, and found that LIM A alone bound at a similar level as mutant 2m, while LIM B bound very weakly at an average of 19 units/μg or 1.2% of ABL60. Nevertheless, the interaction between Ldb1 and Ldb1 is still sufficient to yield blue colonies in the qualitative assay, attesting to its high sensitivity. Clearly, LIM B alone bound less efficiently that mutant 1m in which the LIM A domain structure is disrupted. This observation suggested that the 60-residue linker region may assist Ldb binding by LIM B in the 1m construct. We
conclude from these results that both LIM domains of Xlim-1 are required for optimal binding of Ldb1, and furthermore, that isolated LIM A but not LIM B can interact with Ldb1 at a substantial if suboptimal level. These conclusions are in general agreement with those of Jurata et al. (13) for Isl-1 LIM domains, given the increased sensitivity of the yeast assay.

Binding of LIM Domains Requires the Carboxyl Terminus of Ldb1—Ldb1 has no sequence similarity to other proteins in the database, and no domain structure could be inferred from its sequence. Therefore, truncation experiments were used to test for residues required for LIM domain binding. The protein overlay assay shown in Fig. 2A used truncated forms of Ldb1 transferred to a filter in equal amounts, as seen in the top panel. The lower panel shows the binding of labeled Lhx1 (mouse Lim-1) to the various Ldb1 truncations. Full-length Ldb1 (1–375) binds strongly to Lhx1, and so do the truncated forms missing 25 or 45 residues from the carboxyl terminus. However, truncating an additional 20 amino acids (construct 1–310) dramatically decreased binding. Whether the apparent 7% binding relative to full-length Ldb1 is significant could not be determined by this assay, but two-hybrid data shown below suggest that it is not. This result indicates that the carboxy-terminal 45 amino acids of Ldb1 are not required for high efficiency binding to Lhx1, but the adjacent region between amino acids 310 and 330 is required for binding.

Ldb1 Can Form Homodimers via Its Amino-terminal Region—Ldb1 was originally found using LIM domains as bait in both an overlay-type assay and a two-hybrid screen. A Gal4-DBD fusion of the ABL60 region of Xlim-1 binds effectively to the Gal4-AD-Ldb1 fusion, as shown in Fig. 1. In agreement with the in vitro results from panel A, the 65-amino acid carboxyl-terminal deletion of Ldb1 (1–310) does not bind in this assay. Since the qualitative two-hybrid assay is very sensitive (see “Experimental Procedures”), Western blotting confirmed proper size of each fusion protein. Qualitative results are shown to the right as W (white) or B (blue) colonies.
DBD-Ldb1 fusion was positive for β-galactosidase without partner, i.e. in the one-hybrid assay, implying the presence of an activation domain in Ldb1; both the carboxyl-terminal truncation (1–310) and amino-terminal truncation (200–375) are inactive. Thus, the DBD-Ldb1(1–310) fusion could be used as a partner in two-hybrid assays with Gal4-AD-Ldb1 fusions. The results show that the amino-terminal 200 residues of Ldb1 can support dimerization with the Ldb1 (1–310) tester constructs, but constructs containing residues 1–150 or 50–375 cannot. All fusion proteins were subjected to Western analysis to confirm size and relative amount as described under “Experimental Procedures.”

The interaction between Ldb1 molecules was characterized further in the two-hybrid assay (Fig. 3). Dimerization requires the amino-terminal region, as constructs from which 50 or more residues have been deleted were unable to interact with the tester construct, Ldb1 (1–310). Turning to truncations from the carboxyl terminus we found that an Ldb1 fusion containing the amino-terminal 201 residues was capable of interacting with the Ldb1 (1–310) tester, but construct 1–150 could not. Thus, the amino-terminal half of Ldb1 is responsible for homodimer formation. Since residues in intervals 1–49 and 150–201 are required, it appears likely that the entire region from the amino terminus to about residue 200 forms a dimerization domain in Ldb1.

**Xlim-1 Contains a Transactivation Domain in Its Carboxyl Terminus**—While functional studies in *Xenopus* embryos have shown the importance of the LIM domains in Xlim-1, the domain(s) of Xlim-1 which are responsible for transcriptional activity have not been well studied. The carboxyl-terminal region of Xlim-1 has been assumed to be involved in transcriptional activation, by comparison to studies of the related LIM-homeodomain proteins Isl-1 and Mec3 (25, 26). Furthermore, a Gal4-DBD fusion with the amino-terminal portion of Xlim-1 containing both LIM domains, the linker, and the homeodomain, is inactive in the yeast one-hybrid assay (Fig. 2). To analyze the activation function of Xlim-1 further we measured the potential transactivation activity of different Xlim-1 constructs by quantitative one-hybrid analyses in yeast (Fig. 4).

![FIG. 3. Ldb1 dimerizes via its amino-terminal region. Results of qualitative one-hybrid and two-hybrid assays are shown for different combinations of constructs. A DBD-Ldb1 fusion is positive in the one-hybrid assay, implying the presence of an activation domain in Ldb1; both the carboxyl-terminal truncation (1–310) and amino-terminal truncation (200–375) are inactive. Thus, the DBD-Ldb1(1–310) fusion could be used as a partner in two-hybrid assays with Gal4-AD-Ldb1 fusions. The results show that the amino-terminal 200 residues of Ldb1 can support dimerization with the Ldb1 (1–310) tester constructs, but constructs containing residues 1–150 or 50–375 cannot. All fusion proteins were subjected to Western analysis to confirm size and relative amount as described under “Experimental Procedures.”](http://www.jbc.org/)

![FIG. 4. Xlim-1 contains a transactivation domain in its carboxyl terminus. A quantitative one-hybrid assay was carried out in yeast Y187. Plasmids encoding Gal4-DBD fusion proteins as shown at the top, were transformed and quantitated as described under “Experimental Procedures.” The assay was performed with three individual transformants with the average and standard deviation from the mean shown. Western blotting verified the presence of intact fusion proteins in Y187.](http://www.jbc.org/)

The yeast reporter strain Y187, with or without a DBD-lamin C control plasmid, has very low transcriptional activity. As mentioned already (see Fig. 3), Ldb1 is capable of expressing a measurable activation function although its activity is quite low and its biological function unclear (Fig. 4). In contrast, full-length Xlim-1 mediates high level transactivation of the reporter construct in the yeast cells. The isolated carboxyl terminus of Xlim-1 (see DBD-CT239) shows a decrease in activity to only approximately 3% of full-length Xlim-1. However,
removal of 27 amino acids from the amino terminus in the fusion protein (DBD-CT266) leads to recovery of activity to a level approximately 31% of full-length Xlim-1. Further deletion of 39 amino acids results in low activity that does not appear to be further affected by the deletion of an additional 56 amino acids. The diminished activity of all of these constructs containing carboxyl-terminal regions of Xlim-1 are above background as they are positive in the qualitative assay (blue colonies), which requires just a low level of activity (see Fig. 1). From these results we conclude that the transcriptional activation domain of Xlim-1 resides in the carboxyl terminus. The region 266–403 is sufficient for strong, albeit not maximal, activation and the region 266–305 is an indispensable part of the activation domain. The low activity of the fusion containing the entire carboxyl-terminal domain (DBD-CT239) suggest the possibility of a transcriptional repressor function in the region between residues 239 and 266, but possible effects of protein folding may also account for this apparent inhibition.

Full-length Ldb1 Is Required for Synergy with Xlim-1 in Vivo—While results presented here show Ldb1 to contain a dimerization domain as well as a LIM-binding domain, it is not clear how these domains are involved in the activation of Xlim-1. *Xenopus* microinjection experiments were performed to test the ability of these molecules to activate expression of various molecular markers in naive ectodermal explants. In animal cap assays, coinjection of Xlim-1 and Ldb1 synergized to activate the Spemann organizer markers goosecoid and chordin, as reported previously, and the neural markers nrp-1 and otx2 (Fig. 5, Ref. 12). However, coinjections of Xlim-1 and a fragment of Ldb1 containing its LIM domain-binding portion did not synergize to activate these gastrula or neurula specific genes. The fragment of Ldb1 which contains the dimerization domain, when coinjected with Xlim-1, was likewise unable to induce goosecoid, chordin, or otx2, while very weakly inducing nrp-1. As an additional comparison, Fig. 5 shows the level of gene activation achieved by the activated mutant of Xlim-1, 3m (9), and also includes RNA from whole embryos at the equivalent stage and from uninjectected animal caps as positive and negative controls, respectively. These results indicate that both halves of Ldb1 are required to synergize with Xlim-1 to activate downstream genes and that the interaction of truncated Ldb1 with LIM domains is not sufficient for this activity in vivo.

**DISCUSSION**

We have analyzed the interaction of Xlim-1/Lhx1 with Ldb1 to better understand the molecular action of both proteins. Xlim-1 appears to have latent properties and can be activated in *Xenopus* animal explant experiments by mutation or deletion of both LIM domains (9). More recently, we have shown that wild-type Xlim-1 also can be activated by coexpression of Ldb1 in animal explants (12). In the present report we show that tandem LIM domains of Xlim-1 are required for optimal binding of Ldb1, confirming and expanding our previously published *in vitro* results (12) with the use of the two-hybrid technology. While tandem LIM domains are required for optimal binding, individual domains can bind Ldb1. This was concluded from studying the Cys to Gly mutations 1m (LIM A mutation) and 2m (LIM B mutation) which interact with Ldb1 at a lower but still clearly measurable level as compared with the intact wild-type tandem LIM domains. However, the double LIM domain point mutation 3m showed no binding to XLdb1. In testing deletion constructs containing single LIM domains, we found that the LIM A domain of Xlim-1 bound to Ldb1 with an affinity approximately equal to the 1m and 2m mutants, while the LIM B bound very weakly but still gave a positive interaction signal in the qualitative assay (blue colonies). Of the various LIM domain constructs tested, only the double LIM domain mutation 3m showed no binding, i.e. yielded white colonies. These results agree in general with those of Jurata et al. (13), who found that the Isl-1 LIM A domain binds to Ldb1/NLI but the LIM B does not. The fact that we were able to detect low level binding of the Xlim-1 LIM B domain to Ldb1 may be due to the increased sensitivity of the two-hybrid assay, or possibly to subtle differences in affinity of Ldb1/NLI for LIM domains from Xlim-1 and Isl-1. The major conclusion we draw from this portion of these studies is that tandem LIM domains bind much better to Ldb1 than single A or B LIM domains.

While our results suggest that optimal binding to Ldb1 requires tandem AB LIM domains they do not address the question of possible affinity changes in response to other interacting proteins. Such additional interactions clearly occur, as, for example, Wadman et al. (27) recently demonstrated the existence of a complex containing Ldb1 together with GATA-1, E47, Tal1, and LMO2. This study implicated Ldb1 as a component of a bridging complex for transcriptional activation in hematopoiesis. In addition, it is clear that LIM domains from groups II and III, i.e. other than LIM-homeodomain or LMO proteins, interact individually with a variety of ligands (Refs. 28–30 and reviewed in Refs. 1 and 2), and furthermore, LIM domains may have more than one binding interface (28).
Since Ldb1 is a novel LIM-binding protein we investigated which portion of Ldb bound to LIM domains, and found that the carboxyl-terminal portion of Ldb1 is sufficient and necessary for binding in vivo and in vitro. This conclusion is in agreement with the fact that we isolated a carboxyl-terminal portion of Ldb1 (amino acids 210–375) in our original two-hybrid screen for LIM domain-binding proteins (12). In addition, Jurata et al. (13) reported in their identification of NLI/Ldb1 that they isolated one example of the carboxyl-terminal portion of NLI/Ldb1 which bound MLO2 LIM domains.

To better understand the role of Ldb1 in vivo we performed a two-hybrid screen with a portion of Ldb1, using the first 310 amino acids which are incapable of binding LIM domains. This portion of Ldb1 did not autoactivate the lacZ reporter in yeast, i.e. did not contain a transactivation domain. The screen yielded two clones, both of which were full-length Xenopus Ldb1. Further experiments confirmed the ability of Ldb1 to form homodimers and showed that Ldb1-Ldb1 interaction was mediated by the first 201 amino acids of the protein. This result is particularly relevant as at least one other member of the Ldb family exists in both mice and humans (12), and it is possible and perhaps likely that Ldb1 could form a heterodimer with Ldb2. Given the relatively nonspecific binding of LIM-homeodomain and LMO proteins to Ldb1, this potential for cross-talk between members of the Ldb family may be important for establishing specificity through recruitment and/or maintenance of other factors which are presumably present in the LIM-homeodomain-Ldb transcriptional complex.

Xlim-1 contains multiple domains with apparent distinct roles in the overall function of this protein as a transcription factor. The LIM domains are thought to be negative regulators of Xlim-1 function, and mediate interactions with other proteins, amongst which Ldb1 is a major example. The homeodomain is well known as a DNA-binding domain and mediates additional protein-protein interactions (31), but the carboxyl terminus of Xlim-1, which is slightly proline rich (17%, Ref. 8), is largely uncharacterized. We show here that the carboxyl terminus of Xlim-1 contains a transactivation domain as defined in the yeast one-hybrid system. This is in agreement with studies showing transcriptional activity in the carboxyl terminus of the LIM-homeodomain proteins Isl-1 (25) and Mec-3 (26). Using a quantitative assay we found that the full-length protein gave the highest level of activity even though we have shown that the portion of the protein up to and including the homeodomain is transcriptionally silent (Ref. 12, Fig. 2), as also shown for the Isl-1 homeodomain (32). It is notable that wild-type Xlim-1 is a strong transcriptional activator in yeast when fused to the Gal4-DBD, whereas in Xenopus animal explants the LIM domains appear able to inhibit the transcriptional activity of Xlim-1. We conclude from studies presented here that the transcriptional activation domain of Xlim-1 resides in the carboxyl terminus, and that this domain is maximally active when associated with its neighboring domains in the native protein.

This work shows that both the region of Ldb1 containing the dimerization domain and the region containing the LIM domain-binding domain are required for activation of Xlim-1 in vivo. In support of this conclusion, preliminary experiments showed that Ldb1 (1–310) and Ldb1 (210–375) were not capable of inducing a partial secondary axis when coinjected with Xlim-1; we have shown previously that full-length Ldb1 is able to perform this activity (12). If the entire role of LIM domains in LIM-homeodomain proteins were a negative one it could be predicted that LIM binding alone should activate Xlim-1. The fact that the carboxyl-terminal domain, which binds LIM domains efficiently, cannot synergize with Xlim-1 in vivo implies that Ldb1 may recruit other transcriptional activators to the Ldb1-Xlim-1 complex. The work in this report is in general agreement with a recent paper by Jurata and Gill and coworkers (33) that appeared as this work was being revised. These authors, using different methods from those reported here, show that NLI/Ldb1 dimerizes via its amino terminus, binds LIM domains via amino acids 300–338, and interacts with the LIM-homeodomain protein Lmx1 with highest affinity when both LIM domains are present. This portion of the work is fully consistent with our results. However, when Jurata et al. (13) performed transfections in cell culture to test activity of Ldb1, they found that Ldb1 inhibited the synergy that was previously demonstrated for Lmx1 and the transcription factor E47 on an insulin mini-enhancer response element. This contrasts with our finding of stimulation of gene activation in vivo by Xlim-1 plus Ldb1, emphasizing the role of context in LIM domain-Ldb interactions. The particular sequence of the enhancer-promoter element involved and the nature of additional protein factors that participate in the regulation of the gene under study may determine not only the strength of the functional interactions between LIM-homeodomain proteins and Ldb1 but also whether such interactions lead to the enhancement or repression of transcriptional activity.

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