LIM domain-containing transcription factors are required for embryonic survival and for the determination of many cell types. The combinatorial expression of the LIM homeodomain proteins Isl1, Isl2, Lhx1, and Lhx3 in subsets of developing motor neurons correlates with the future organization of these neurons into motor columns with distinct innervation targets, implying a functional role for LIM homeodomain protein combinations in the specification of neuronal identity. NLI is a widely expressed, dimeric protein that has been shown to specifically interact with the LIM domains of LIM domain-containing transcription factors. The present studies demonstrate that NLI mediates homo- and heteromeric complex formation between LIM domain transcription factors, requiring both the N-terminal dimerization and C-terminal LIM interaction domains of NLI. Although the interaction between most LIM homeodomain proteins is dependent on NLI, a direct interaction between the LIM domains of Lhx3 and the homeodomains of Isl1 and Isl2 was also observed. This interaction was disrupted by NLI, demonstrating that the conformational state of Lhx3-Isl1/Isl2 complexes is modified by NLI. Evidence indicating that NLI facilitates long range enhancer-promoter interactions suggests that NLI-dependent LIM domain transcription factor complexes are involved in communication between transcriptional control elements.

The LIM domain transcription factor family includes LIM homeodomain proteins, which consist of two N-terminal LIM domains and a C-terminal DNA-binding homeodomain, and LIM-only (LMO) proteins, which contain little more than two LIM domains (for review see Refs. 1 and 2). Gene deletion studies in mice have revealed that both LIM homeodomain and LMO proteins are essential for embryonic viability, as well as for the development of motor neurons and interneurons, the pancreas, the pituitary, the head, and erythrocytes (3–7). Most LIM domain transcription factors have limited patterns of expression, but all have been shown to be expressed within subregions of the developing nervous system (8–13). Studies investigating the role of LIM homeodomain proteins in neuronal development have largely focused on motor neuron differentiation, for which expression of the LIM homeobox gene Is1/I is required (3). Along with Isl1, the LIM homeodomain proteins Isl2, Lhx1, and Lhx3 show overlapping patterns of expression in developing motor neurons, which are located in the ventral, postmitotic region of the spinal cord (8). Furthermore, the combinatorial expression patterns of these four proteins in subsets of developing motor neurons correlates with the future organization of these neurons into specific motor columns that innervate distinct targets. Because the expression of these factors precedes the organization of motor columns and axonal migration, the combinatorial pattern of LIM homeodomain gene expression appears to play a functional role in the specification of motor neuron identity. The combinatorial expression of other LIM domain transcription factors may similarly dictate neuronal identity and axonal pathway selection in the development of interneurons and commissural neurons, which are located dorsal to the motor neurons in the neural tube, and of sensory neurons located in the dorsal root ganglia. Indeed, the Drosophila LIM homeodomain protein apterous has been demonstrated to mediate axonal pathway selection of embryonic interneurons (10). It has been proposed that LIM domain transcription factors regulate the expression of cell surface molecules involved in axon fascilitation and pathfinding (10, 14). However, neuron-specific gene targets for the LIM homeodomain proteins have not yet been identified, and little is understood about the biochemical basis for the proposed LIM homeodomain combinatorial code for neuronal identity.

NLI (Ldb1), a recently identified nuclear protein, binds with high affinity to the LIM domains of all LIM homeodomain and nuclear LMO proteins but not to cytoplasmic LIM domain-containing proteins (15–18). Although widely expressed, highest levels of NLI protein are found in postmitotic cells of the embryonic neural tube and in other regions of the developing central nervous system, significantly overlapping the expression patterns of many LIM domain transcription factors (15). The functional domains of NLI include an N-terminal homodimerization domain and a C-terminal LIM interaction domain (16). It has also been demonstrated that NLI forms a tetrameric complex with the LIM homeodomain protein Lmx1.1, in which each NLI molecule of an NLI homodimer interacts independently with an Lmx1.1 molecule (16). Based on these observations, we sought to determine if NLI is capable of mediating interactions between heterologous LIM domain transcription factors in an effort to understand the biochemical basis for the motor neuron combinatorial code.
EXPERIMENTAL PROCEDURES

Materials

The Lmx1.1, Lhx1, and Lhx3 cDNA clones were generous gifts from M. German, W. Shawlot, and H. Westphal. The LMO2 and NLI cDNAs were isolated previously (15). For in vitro binding assays, hemagglutinin epitope (HA)-tagged proteins were transcribed and translated from the T7 promoter of pcDNA3M (19), FLAG epitope-tagged proteins were translated from pcDNA3-FLAG (16), and untagged proteins were translated from pcDNA3L (15). The same vectors were used for transfection and protein expression from the cytomegalovirus promoter in Epstein-Barr nuclear antigen human kidney 293 cells (Invitrogen). The TNT reticulocyte lysate system (Promega) and [35S]methionine (Amer sham Corp.) were used to produce labeled proteins for the in vitro assays and [35S]translabel (NEN Life Science Products) was used for metabolic labeling of transfected cells. The anti-FLAG M2 monoclonal antibody was purchased from Eastman Kodak, the anti-HA monoclonal antibodies 12CA5 and HA.11 were purchased from Berkeley Antibody Co., and protein A-Sepharose was obtained from Sigma.

Methods

Con structs—The mutant and full-length NLI constructions in pcDNA3L were generated by subcloning restriction fragments from pcDNA3M (16). Full-length LIM domain proteins and the mutants Isl1 ΔN (amino acids 134–349), Lhx3 ΔN (amino acids 151–402), Lhx3 N' (amino acids 1–153), and Lhx3 LIMs (amino acids 33–153) were generated by ligating Pfu polymerase (Stratagene)-generated polymerase chain reaction products in frame into pcDNA3M or pcDNA3-FLAG. Intact reading frames for all constructs were verified by sequencing.

In Vitro Double Immunoprecipitation (IP) Assay—0.75 μg of DNA in equivalent amounts of pcDNA3M, pcDNA3-FLAG, or pcDNA3L constructs was transcribed and translated for 2 h at 30 °C in 25-μl TNT reactions containing [35S]methionine and 1 mM ZnCl2 to ensure proper LIM domain folding. Samples were then diluted with 100 μl of FLAG lysis buffer (25 mM Tris, pH 7.5, 300 mM NaCl, 1% Triton X-100, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 200 μg/ml ethidium bromide) to inhibit DNA-dependent protein degradation (20). The clarified supernatant was cleared with 10 μl of protein A-Sepharose prior to immunoprecipitation with 1 μl of anti-FLAG monoclonal antibody for 1 h at 4 °C. Immune complexes were collected with protein A-Sepharose, washed twice with FLAG lysis buffer and twice with FLAG final wash buffer (25 mM Tris, pH 7.5, 140 mM NaCl), and then boiled for 5 min in 25 μl of SDS lysis buffer (20 mM Tris, pH 7.5, 50 mM NaCl, 1% SDS, and 1 mM dithiothreitol). The supernatant was diluted with 225 μl of RIPA buffer (10 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS) and then immunoprecipitated with 2.5 μl of anti-HA (12CA5) antibody and protein A-Sepharose. After four washes with RIPA buffer, proteins were analyzed by 10% SDS-polyacrylamide gel electrophoresis (PAGE) (see Figs. 1 and 2) or 12.5% SDS-PAGE (see Fig. 3) and fluorography. The translation panels for Figs. 1 and 2 were exposed for 12 h at −70 °C, the double IP panels for Figs. 1 and 2 were exposed for 4 days at −70 °C, the double IP panel for Fig. 2B was exposed for 2 days at −70 °C, and both panels of Fig. 3 were exposed for 8 h at −70 °C. In Vivo Double Immunoprecipitation—293 cells in 100-mm dishes were transfected by standard procedures (21) with 20 μg of DNA containing equivalent amounts of pcDNA3M, pcDNA3-FLAG, and pcDNA3L constructs. 24 h after transfection, cells were metabolically labeled overnight with 1 μCi [35S]translabel/dish in medium containing 5% dialyzed calf serum. Nuclei were prepared as described previously (16) and then lysed in 100 μl of RIPA buffer containing 200 μg/ml ethidium bromide and diluted to 1 ml with FLAG lysis buffer. After shearing, clarified extracts were subjected to the double immunoprecipitation procedure described above. Binding was visualized by 10% SDS-PAGE and fluorography, with 24 h of exposure at −70 °C. For Western blotting, nuclear extracts from unlabelled, parallel transfections were separated by 10% SDS-PAGE and immunoblotted with anti-FLAG/anti-HA (HA.11) and anti-NLI (4508b) antibodies (15).

RESULTS

To determine if NLI is capable of mediating homodimeric and heterodimeric association between LIM homeodomain proteins, a double immunoprecipitation procedure that allows visualization of protein-protein interactions between similarly sized species was used. This method has been utilized in the past to illustrate the ability of NLI to form homodimers (16). In the assay, FLAG epitope-tagged, HA epitope-tagged, and untagged proteins were cotranslated in vitro, after which complexes were immunoprecipitated with anti-FLAG antibodies. Following denaturation of the complexes, coprecipitating HA-tagged proteins were isolated with anti-HA antibodies and visualized by SDS-PAGE and autoradiography. Thus, this assay allows evaluation of direct interactions occurring between FLAG and HA-tagged proteins as well as indirect interactions occurring through an untagged protein. The cell free system also provides an efficient means for producing and labeling LIM domain proteins in a eukaryotic environment, avoiding the insolubility and misfolding problems that occur when LIM domain transcription factors are produced in Escherichia coli. In addition, because all cell lines thus far tested have been shown to express NLI (15, 16) and many cell lines express LIM domain proteins in vitro, with untagged derivatives of NLI. Complexes were first immunoprecipitated with an anti-FLAG monoclonal antibody and denatured, after which coprecipitating HA-tagged proteins were captured by an anti-HA-specific monoclonal antibody and visualized by SDS-PAGE and fluorography. The upper panel shows the autoradiogram of 2.5% of the translation reaction used for the immunoprecipitation experiment, and the lower panel shows the results of the double immunoprecipitation assay. The comigrating NLI and Lmx1.1 run slightly larger than Isl1 on SDS-PAGE. — represents the empty vector pcDNA3L, ΔID refers to an NLI construct in which the LIM interaction domain (amino acids 300–338) has been deleted, and ΔDD refers to an NLI construct lacking the homodimerization domain (amino acids 1–200).

Because Isl1 is expressed in all pancreatic Islet cell types (22, 25) and Lmx1.1 has similarly been shown to be expressed in pancreatic Islet cells (26), the ability of these LIM homeodomain proteins to form NLI-mediated homo- and heterodimers was examined (Fig. 1). Although FLAG-Isl1 and HA-Isl1 were incapable of interacting directly with one another (Fig. 1, lane 1), the addition of untagged NLI to the binding reaction allowed coprecipitation of HA-Isl1 with FLAG-Isl1 (Fig. 1, lane 2). Likewise, FLAG-Lmx1.1 and HA-Lmx1.1 were only capable of interacting with each other in the presence of NLI (Fig. 1, lanes 5 and 6). Similarly, heterodimeric association between FLAG-Isl1 and HA-Lmx1.1 depended on the presence of NLI (Fig. 1, lanes 9 and 10). NLI has been shown to be coexpressed and associated with Isl1 in the Tu6 pancreatic tumor cell line (16), suggesting that the NLI-dependent interaction observed between Isl1 and Lmx1.1 is likely to represent a physiologically

FIG. 1. NLI-mediated homo- and heterodimerization of the LIM homeodomain proteins Isl1 and Lmx1.1. Double immunoprecipitation assays in which [35S]methionine-labeled FLAG and HA epitope-tagged proteins were cotranslated in vitro with untagged derivatives of NLI. Complexes were first immunoprecipitated with an anti-FLAG monoclonal antibody and denatured, after which coprecipitating HA-tagged proteins were captured by an anti-HA-specific monoclonal antibody and visualized by SDS-PAGE and fluorography. The upper panel shows the autoradiogram of 2.5% of the translation reaction used for the immunoprecipitation experiment, and the lower panel shows the results of the double immunoprecipitation assay. The comigrating NLI and Lmx1.1 run slightly larger than Isl1 on SDS-PAGE. — represents the empty vector pcDNA3L, ΔID refers to an NLI construct in which the LIM interaction domain (amino acids 300–338) has been deleted, and ΔDD refers to an NLI construct lacking the homodimerization domain (amino acids 1–200).
significant complex found in the endocrine cells of the pancreas.

If the NLI-dependent association between two LIM domain transcription factors results from the simultaneous binding of an NLI homodimer to two LIM domain proteins, both the homodimerization domain (amino acids 1–200) and LIM interaction domain (LID) (amino acids 300–338) of NLI (16) should be required for this effect. Indeed, deletion of either the dimerization domain or the LID was sufficient to prevent the NLI-dependent association between LIM domain proteins (Fig. 1, lanes 3, 4, 7, 8, 11, and 12). The dimerization domain deletion mutant binds LIM domains as efficiently as full-length NLI, and the LID deletion mutant is capable of homodimerization (16). Reduced signal intensity of the dimerization domain deletion in the translation reaction results from less efficient labeling of this protein compared with full-length NLI due to fewer methionine residues rather than decreased protein expression. Thus, through its ability to simultaneously homodimerize and bind monomerically to LIM domains in the formation of a tetrameric complex, NLI mediates the indirect association between LIM domain transcription factors.

Because motor neuron subtypes express combinations of the LIM homeodomain proteins Isl1, Isl2, Lhx1, and Lhx3 (8), we investigated whether NLI was also capable of mediating interactions between these factors. NLI-dependent association between FLAG-Lhx1 and HA-Lhx1 was demonstrated (Fig. 2A, lanes 1 and 2). Likewise, NLI-dependent heterodimerization was clearly evident between Isl1 and Lhx1, Isl2 and Lhx1, and Isl2 and Isl1 (Fig. 2A, lanes 3–8). A minor amount of NLI protein, which migrates at approximately 50 kDa, can also be seen in the double immunoprecipitation panel and results from the coprecipitation of partially renatured NLI with HA-tagged LIM proteins. We have previously shown that the highest levels of NLI protein in the developing embryo are found in the postmitotic cells of the neural tube, including the motor neurons, and that NLI is coexpressed with Isl1 early in motor neuron development (15). Thus it is likely that the NLI-dependent complexes of LIM homeodomain proteins demonstrated in vitro also occur in vivo.

The LIM-only protein LMO2 was also capable of interacting with Isl1 in the presence of NLI (Fig. 2A, lanes 9 and 10). LMO2 is widely expressed in the embryo including the central nervous system (13), and expression of the closely related LMO1 is restricted to the ventral postmitotic cells of the brain and spinal cord during embryonic development (11, 13, 27). Intriguingly, Isl1 is also expressed in the thymus (23), where overexpression of LMO1 and LMO2 results in leukemogenesis (28, 29), suggesting that an NLI-dependent complex between Isl1 and LMO1 proteins play a role in the development of cancer by misdirecting T cell development. Thus, although LIM-only proteins lack a DNA binding homeodomain, they are able to interact with other LIM homeodomain proteins through NLI dimers. This result explains a previous observation that the LIM domains of Isl1 were capable of redirecting the subcellular localization of LMO1 when these proteins were coexpressed in cells (30). Because direct LIM-LIM interactions were not observed in our study, the apparent LIM-LIM interactions observed in situ were most likely a consequence of NLI-mediated complex formation.

Next we evaluated the ability of the LIM homeodomain protein Lhx3 to interact with other LIM homeodomain proteins expressed in the developing nervous system (Fig. 2B). Although NLI was required for the formation of Lhx3-Lhx3 homodimers, Lhx1-Lhx3 heterodimers, and Lmx1.1-Lhx3 heterodimers (Fig. 2B, lanes 1–6), a direct NLI-independent interaction between Lhx3 and the related proteins Isl1 and Isl2 was observed (Fig. 2B, lanes 7 and 9). Significantly, Lhx3 and Isl1 are coexpressed early in the development of most motor neurons,3 and Lhx1, Isl1, and Isl2 are later coexpressed specifically in the developing neurons of the medial subdivision of the median motor column (MMCₚ) (8) that eventually innervate the axial muscles (14). In addition, coexpression of the homologues of the Lhx3 and the Islet genes in motor neuron subsets is conserved in Drosophila and zebrafish, suggesting that the direct interaction between Lhx3 and Isl1/Isl2 may be evolutionarily conserved (31).4 Aside from motor neurons, Lhx3 and Isl1 are coexpressed in the anterior pituitary, retina, and pineal gland (22, 24, 25, 32–34), indicating that the interaction between these proteins may occur in additional physiological environments.

To investigate the determinants of the direct interaction between Lhx3 and Isl1, deletion mutants of both proteins were tested using the double immunoprecipitation assay (Fig. 3). The direct interaction between Lhx3 and Isl1 was of compara-

3 S. L. Pfaff, unpublished observations.

4 J. Thomas and S. Thor, personal communication.
NLI Mediates LIM Domain Transcription Factor Dimerization

Fig. 3. Inhibition of the direct interaction between the LIM domains of Lhx3 and the Isl1 homeodomain by NLI. Similar double immunoprecipitation experiments to those described for the previous figures were performed except that only two proteins (a FLAG and an HA-epitope tagged) were cotranslated for lanes 1–6. In lane 7, untagged NLI was additionally cotranslated from pcDNA3L, and the reaction volume was correspondingly increased. The upper panel shows 2.5% of the translation reaction used for the binding assay, and the lower panel shows the results of the double immunoprecipitation assay. ΔN (amino acids 134–349) represents an N-terminal deletion of Isl1 retaining all of the protein C-terminal to the LIM domains, which includes a linker region, homeodomain, and Islet-specific domain. Lhx3 ΔN likewise represents an N-terminal truncation retaining essentially all but the LIM domains of Lhx3. The Lhx3 N’ construct includes the alternatively spliced N-terminal 32-amino acid peptide and the LIM domains, whereas the Lhx3 LIMs construct includes only the LIM domains.

Fig. 4. In vivo interactions between LIM domain transcription factors. In vivo interactions were evaluated by double immunoprecipitations from nuclear extracts of cotransfected 293 cells. – refers to the empty vector pcDNA3L. The top and middle panels show the immunoblot results from the transfection reactions from nuclear extracts of cotransfected 293 cells. Of interest, overexpression of NLI diminished Lmx1.1-Isl1 complex formation (Fig. 4, lane 7). Indeed, the transition from a direct interaction between Isl2 and Lhx3 to an indirect NLI-dependent interaction is also evident in lanes 7 and 8 of Fig. 2B and less clearly in the interactions between Isl1 and Lhx3 in lanes 9 and 10 of Fig. 2B. Thus, Lhx3 can either interact directly with Isl1 or Isl2 or interact indirectly with these proteins through NLI, which complex is formed is likely to be dictated by the relative amount of NLI expressed in the cell.

Interactions between LIM transcription factors were also evaluated in vivo. Cotransfected FLAG-Lmx1.1 and HA-Isl1 were capable of interacting even in the absence of transfected NLI (Fig. 4, lane 1), suggesting that these proteins utilize endogenously expressed NLI to form a complex in 293 cells. Of interest, overexpression of NLI diminished Lmx1.1-Isl1 complex formation (Fig. 4, lane 2), presumably by diluting out the LIM proteins with excess NLI, thereby preventing tetrameric complex formation. The exact stoichiometry of NLI-LIM proteins is thus critical for formation of the tetrameric complex, and precise regulation of the expression levels of each protein throughout developmental processes is likely to dictate whether or not tetrameric complexes are formed and which LIM proteins are incorporated into such complexes. As expected, deletion of the LIM domains of Isl1 prevented interaction between Lmx1.1 and Isl1 (Fig. 4, lane 3). Although incapable of interacting with Lmx1.1, the Isl1 C terminus interacted strongly with full-length Lhx3. In this experiment, a significant amount of FLAG-Lhx3 renatured and coprecipitated with the HA-Isl1 C terminus. As demonstrated in vitro, the Lhx3-Isl1 C terminus interaction was dependent on the LIM domains of Lhx3 (Fig. 4, lanes 4 versus 5). Curiously, the phosphorylation state of the Isl1 C terminus appeared to be altered by the coexpression of full-length Lhx3 (Fig. 4, top panel).
intrinsic transcriptional activity (16), it is probable that such chromatin structure in the promoter region, allowing access to proteins, such as thyroid hormone receptors or enhancer-associated histone acetylases may destabilize bound LIM domain transcription factor (Fig. 5B). By recruiting a promoter-bound LIM domain transcription factor and a promoter-acting long range interactions, potentially by bridging an enhancer and the Islet proteins occurs at a local DNA level and results in transcriptional synergy. The regulation of NLI levels, either through transcriptional or translational control or subcellular relocalization, might therefore be involved in a molecular switch between short range and long range interactions (Fig. 5B).

The Drosophila homologue of NLI (Chip) has recently been identified as a gene capable of facilitating enhancer-promoter interactions over very long distances (85 kilobases), mediating expression of both the cut and Ultrabithorax genes (36). Although the role of LIM domain transcription factors in this function has not been established, Chip interacts both physically and genetically with the Drosophila LIM homeodomain protein apterous. The very high level of conservation between Lmx1.1 and E47 (26). The presence of NLI and perhaps the phosphorylation of the Islet proteins, however, prevent the direct Lhx3-Isl1/2 interaction and promote tetrameric complex formation, potentially supporting long range DNA interactions at the expense of local DNA interactions.

**DISCUSSION**

The Drosophila homologue of NLI (Chip) has recently been identified as a gene capable of facilitating enhancer-promoter interactions over very long distances (85 kilobases), mediating expression of both the cut and Ultrabithorax genes (36). Although the role of LIM domain transcription factors in this function has not been established, Chip interacts both physically and genetically with the Drosophila LIM homeodomain protein apterous. The very high level of conservation between NLI and Chip suggests that NLI may also be capable of mediating long range interactions, potentially by bridging an enhancer-bound LIM domain transcription factor and a promoter-bound LIM domain transcription factor (Fig. 5A). By recruiting the enhancer to within close proximity of the promoter, additional enhancer-bound factors may help to activate transcription, or enhancer-associated histone acetylasmes may destabilize chromatin structure in the promoter region, allowing access to other positive regulatory factors (37). Because LIM domain transcription factors show little transcriptional activity in the absence of other proteins (26, 34) and because NLI shows no intrinsic transcriptional activity (16), it is probable that such additional factors are necessary to activate gene transcription.

One apparent exception to the NLI dependence of LIM transcription factor association is the direct interaction observed between Lhx3 and Isl1/Isl2. This interaction appears to be a special case, although our experiments did not exhaustively test every possible combination of LIM domain transcription factors. The LIM homeodomain protein Lhx4 (Gsh-4) is highly related to Lhx3 (38, 39), and is coexpressed with Lhx3 throughout motor neuron development. It is, however, evaluation of whether the LIM domains of Lhx4 are also capable of interacting directly with Isl1 and Isl2 awaits the isolation of a full-length Lhx4 cDNA.

In the presence of NLI, NLI-containing complexes appear to be preferential to the direct Lhx3-Isl1/Isl2 complex. We have previously described a similar effect of NLI in which NLI disrupted the transcriptional synergy between Lmx1.1 and E47 from the rat insulin I mini-enhancer by competing with E47 for direct interaction with the LIM domains of Lmx1.1 (16, 40). In addition, it is possible that phosphorylation of the Isl1 C terminus blocks the interaction between Isl1 and Lhx3, further promoting the formation of NLI-dependent complexes. Alternatively, the Lhx3-Isl1 interaction may prevent Isl1 phosphorylation, thereby affecting Isl1 activity in some way.

Due to the superficial resemblance of the Lhx3-Isl1/2 interaction to the Lhx3-Pit-1 interaction that results in transcriptional synergy from pituitary-specific promoters (34), it is tempting to speculate that the direct interaction between Lhx3 and the Islet proteins occurs at a local DNA level and results in transcriptional synergy. The role of NLI, levels, either through transcriptional or translational control or subcellular relocalization, might therefore be involved in a molecular switch between short range and long range interactions (Fig. 5B). Such a switch might occur at a particular time in development and significantly affect which genes are transcribed in cells coexpressing Lhx3 and Isl1/Isl2. Indeed, there is evidence that Isl1, Lhx3, and Lhx4 may have both early and late functions in motor neuron development. Lhx3 and Lhx4 are expressed in motor neuron precursor cells prior to the onset of Isl1 expression and then coexpressed with Isl1 after the final cell division, implying a general role for these factors in motor neuron development. Later, Lhx3 and Lhx4 expression is down-regulated in all but the MMC4 neurons, which maintain Isl1 expression and up-regulate Isl2 expression, at which time these factors are likely to be involved in determining neuronal subtype identity and directing axon guidance (8). It will be important, therefore, to investigate the exact timing of the onset of NLI expression in developing motor neurons to determine when NLI-dependent and NLI-independent complexes between Lhx3 and Isl1/Isl2 are likely to exist.

The current studies suggest that NLI-dependent and NLI-independent complexes between LIM domain transcription factors are involved in the proposed LIM homeodomain combinatorial code for the specification of motor neuron identity. Because nearly all postmitotic neurons of the developing neural tube express NLI (15) and various combinations of LIM domain transcription factors (8, 9), it is likely that these factors and the complexes they form are involved in the specification of neuronal subtype identity for sensory, commissural, and interneurons, in addition to motor neurons. However, to evaluate the molecular significance of NLI-LIM domain transcription factor complexes, neuron-specific gene targets for LIM domain transcription factors must be identified.

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The Nuclear LIM Domain Interactor NLI Mediates Homo- and Heterodimerization of LIM Domain Transcription Factors
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