Nuclear LIM interactor, a rhombotin and LIM homeodomain interacting protein, is expressed early in neuronal development

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Communicated by Susan S. Taylor, University of California at San Diego, La Jolla, CA, July 25, 1996 (received for review May 29, 1996)

ABSTRACT LIM domain-containing transcription factors, including the LIM-only rhombotins and LIM-homeodomain proteins, are crucial for cell fate determination of erythroid and neuronal lineages. The zinc-binding LIM domains mediate protein–protein interactions, and interactions between nuclear LIM proteins and transcription factors with restricted expression patterns have been demonstrated. We have isolated a novel protein, nuclear LIM interactor (NLI), that specifically associates with a single LIM domain in all nuclear LIM proteins tested. NLI is expressed in the nuclei of diverse neuronal cell types and is coexpressed with a target interactor islet-1 (*Isl1*) during the initial stages of motor neuron differentiation, suggesting the mutual involvement of these proteins in the differentiation process. The broad range of interactions between NLI and LIM-containing transcription factors suggests the utilization of a common mechanism to impart unique cell fate instructions.

LIM domains are zinc-binding motifs that mediate protein–protein interactions (1–4) and are found in a wide variety of cytoplasmic and nuclear proteins. The nuclear LIM domain family members are more closely related to each other than to the cytoplasmic LIM domain-containing proteins (5) and appear to function as transcription factors. They can be classified as LIM-only proteins, which contain little more than two LIM domains, or as LIM homeodomain proteins, containing a DNA binding homeodomain in addition to the two N-terminal LIM domains (for review, see refs. 5 and 6).

Genetic studies have demonstrated an essential role for nuclear LIM domain-containing proteins in embryonic development. Disruption of the rhombotin 2 (*Rbtn2*) gene in mice resulted in embryonic lethality at day 10.5 due to a lack of erythropoiesis (7). Misexpression of *Rbtn2* and the related *Rbtn1* in T cells due to T-cell translocation events leads to T-cell acute lymphoblastic leukemia in children (8–10) or to a leukemia with similar properties in transgenic mice (11–13). The leukemias apparently result from progression of lymphocytes through an inappropriate differentiation pathway, eventually leading to the malignant phenotype (14).

The disruption of several LIM homeodomain genes has demonstrated their importance predominately in the development of neuronal lineages. Mutation of the *Caenorhabditis elegans* *Mec3* gene prevented the generation of touch receptor neurons (15), while deletion of *Lhx1* (*Lim1*) in mice precluded head structure formation (16). These mouse embryos died around E10 and lacked forebrain, midbrain, and some hind-brain elements. *Lhx3* (*m-Lim3*, *P-Lim*) $-/-$ mice lacked specific pituitary cell lineages and both anterior and intermediate lobes of the pituitary (17). Additional evidence indicating the importance of the LIM domain-containing transcription factors in development is the targeted mutation of the murine *islet-1* gene (*Isl1*). Inhibition of *Isl1* expression, the earliest

marker for motor neuron differentiation (18, 19), prevented the development of motor neurons and interneurons (20).

While the biological importance of the LIM domain-containing transcription factors is clear, comparatively little is known about their gene targets and the molecular mechanisms through which they function. Interaction between *Rbtn2* and the erythroid specific basic helix–loop–helix factor *Tal1* and the zinc-finger protein *GATA1* has been observed (3, 21, 22). Further evidence for the assembly of LIM domain proteins into nuclear complexes that regulate gene expression is the association of the LIM homeodomain protein *Lhx3* with the POU-domain protein *Pit-1* (23). In addition to interacting, these proteins synergize in transcriptional activation of the *Pit-1*, prolactin, and thyroid stimulating hormone β subunit promoters. To date, no common binding partners or shared transcriptional mechanisms for the nuclear LIM proteins have been identified.

In search of new binding partners for *Rbtn2*, we identified a nuclear protein that associates with the LIM domains of every rhombotin and LIM homeodomain protein analyzed. Nuclear LIM interactor (NLI) is found in the nuclei of developing embryonic neuronal cells and is coexpressed with *Isl1* early in motor neuron differentiation. The synchronous expression of both proteins in such cells during the initial stages of differentiation suggests a role for NLI in the *Isl1*-dependent development of motor neurons.

MATERIALS AND METHODS

Isolation of *Rbtn2* cDNA. The 474-bp human *Rbtn2* coding sequence was amplified from a λ gt11 SK-N-MC cell cDNA library provided by E. Turner (University of California at San Diego). Each primer (100 pmol) was incubated with 5 μ l library for 5 min at 94°C, and 35 cycles of synthesis were carried out in the presence of 2 μ M tetraethylmethylammonium chloride, 200 μ M of each dNTP, and 2.5 units *Taq* DNA polymerase (Promega). The PCR product was initially cloned into pEG202 (24), sequenced in its entirety, and then subcloned into pGEX-2TK (Pharmacia) for expression library screening.

Protein Purification. For library screening, glutathione *S*-transferase (GST)-TK-*Rbtn2* was expressed in BL21DE3pLys cells in the presence of 1 mM $ZnCl_2$ and purified essentially as described by Frangioni and Neel (25). GST-NLI (amino acids 1–375) used for antibody affinity column generation was expressed from the pGEX-KG vector (Pharmacia) and purified by the procedure used for GST-TK-*Rbtn2*. For *in vitro* GST-LIM binding assays, fusion proteins were expressed from pGEX-KG in the presence of 1 mM $ZnCl_2$ and purified by standard procedures (26). (His) $_6$ -NLI

Abbreviations: Rbtn, rhombotin; *Isl1*, islet-1; GST, glutathione *S*-transferase; NF-M neurofilament M.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. U69270).

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(amino acids 178–375) used for antigen production was expressed from the pRSET-B vector (Invitrogen) and purified using Ni-NTA resin (Qiagen, Chatsworth, CA) under non-denaturing conditions.

Library Screening. For protein–protein interaction screening, 2 μ g GST-TK-Rbtn2 was labeled in 20 mM Tris, pH 7.5/100 mM NaCl/12 mM MgCl₂/1 mM DTT/200 μ Ci (1 Ci = 37 GBq) [γ -³²P]ATP/1.5 μ g protein kinase A catalytic subunit provided by S. Taylor (University of California at San Diego) for 2 h at 30°C. The probe was bound to 12.5 μ l glutathione-agarose (Sigma), washed six times with NTN buffer (20 mM Tris, pH 8/100 mM NaCl/0.5% Nonidet P-40; Promega), and eluted. Typically, at least 30% of the probe was labeled by this procedure.

³²P-labeled GST-TK-Rbtn2 was used to screen a λ ExLox mouse embryonic d16 expression library (Novagen) as follows: 1.1×10^6 phage were plated at a density of 40,000 per 15 cm $2 \times$ YT plate on the BL21DE3pLysS host strain and incubated at 37°C until plaques were visible. Phage were induced overnight at 37°C by overlaying plates with nitrocellulose filters impregnated with 10 mM isopropyl β -D-thiogalactoside (IPTG). After induction, the filters were briefly washed in HBB (25 mM Hepes, pH 7.7/25 mM NaCl/5 mM MgCl₂/1 mM DT/0.05% Nonidet P-40). Filters were blocked in HBB containing 5% skim milk for 1 h, and then in the same buffer with 1% skim milk for 30 min. Hybridization was performed overnight in Hyb buffer (20 mM Hepes, pH 7.7/75 mM KCl/2.5 mM MgCl₂/0.1 mM EDTA/1% skim milk/0.05% Nonidet P-40/1 mM DTT) containing 250,000 cpm/ml GST-TK-Rbtn2. Prewashing, blocking, and hybridization steps were performed at 4°C. After hybridization, filters were washed three times in Hyb buffer without probe for a total of 30 min at 22°C before autoradiography. Positive clones were plaque purified and subcloned from the phage.

For nucleic acid library screening, a *Pvu*II–*Pst*I fragment of the NLI partial cDNA was labeled with [α -³²P]dCTP and the Pharmacia Ready-To-Go DNA labeling kit. Phage ($n = 500,000$) from the same library were hybridized by standard procedures.

Cloning and Sequencing. Expression constructs were generated by PCR using *Pfu* DNA polymerase (Stratagene) followed by cloning into pGEX-KG, pcDNA3M (modified from Invitrogen pcDNA3 to include a 5' luciferase translation initiator and hemagglutinin epitope tag), or pcDNA3L (modified from pcDNA3 to include a 5' luciferase translation initiator). All plasmids were sequenced at the 5' fusion junction to ensure an intact reading frame. Sequencing of constructs and cDNAs was performed using the Sequitherm Cycle Sequencing kit (Epicentre Technologies, Madison, WI) and [α -³⁵S]dATP.

GST-LIM Binding Assay. Full-length NLI protein was generated from the pcDNA3M vector and labeled with ³⁵S-labeled methionine using Promega's TNT Coupled Reticulocyte Lysate system. Each GST-fusion (100 pmol) was bound to 12.5 μ l glutathione-agarose, washed with PBS, suspended in 90 μ l binding buffer (PBS/1% Triton X-100/25 mg/ml BSA/1 mM DTT), and incubated 1 h at 4°C. Ten milliliters of the *in vitro* translation reaction was added and the mixture was incubated for an additional hour at 4°C. Beads were washed once with PBS plus 25 mg/ml BSA, and then three times with PBS. Beads were boiled in SDS sample buffer and separated by SDS/12.5% polyacrylamide gel. The gel was stained with Coomassie blue to ensure equivalent loading, and equilibrated in sodium salicylate prior to drying and autoradiography.

Antibodies. Rabbit anti-NLI 4508 polyclonal antibodies were generated against (His)₆-NLI (amino acids 178–375) (Lampire Laboratories, Ottsville, PA). Antisera was affinity purified using a GST-NLI (amino acids 1–375) affinity column. The 12CA5 anti-hemagglutinin epitope mouse mAb was pur-

a

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MET Leu Asp Arg Asp Val Gly Pro Thr Pro Met Tyr Pro Pro Thr Tyr Leu Glu Pro Gly 20
Ile Gly Arg His Thr Pro Tyr Gly Asn Gln Thr Asp Tyr Arg Ile Phe Glu Leu Asn Lys 40
Arg Leu Gln Asn Trp Thr Glu Glu Cys Asp Asn Leu Trp Trp Asp Ala Phe Thr Thr Glu 60
Phe Phe Glu Asp Asp Ala Met Leu Thr Ile Thr Phe Cys Leu Glu Asp Gly Pro Lys Arg 80
Tyr Thr Ile Gly Arg Thr Leu Ile Pro Arg Tyr Phe Arg Ser Ile Phe Glu Gly Gly Ala 100
Thr Glu Leu Tyr Tyr Val Leu Lys His Pro Lys Glu Ala Phe His Ser Asn Phe Val Ser 120
Leu Asp Cys Asp Gln Gly Ser Met Val Thr Gln His Gly Lys Pro Met Phe Thr Gln Val 140
Cys Val Glu Gly Arg Leu Tyr Leu Glu Phe Met Phe Asp Asp Met Met Arg Ile Lys Thr 160
Trp His Phe Ser Ile Arg Gln His Arg Glu Leu Ile Pro Arg Ser Ile Leu Ala Met His 180
Ala Gln Asp Pro Gln Met Leu Asp Gln Leu Ser Lys Asn Ile Thr Arg Cys Gly Leu Ser 200
Asn Ser Thr Leu Asn Tyr Leu Arg Leu Cys Val Ile Leu Glu Pro Met Gln Glu Leu Met 220
Ser Arg His Lys Thr Tyr Ser Leu Ser Pro Arg Asp Cys Leu Lys Thr Cys Leu Phe Gln 240
Lys Trp Gln Arg Met Val Ala Pro Pro Ala Glu Pro Ala Arg Gln Gln Pro Ser Lys Arg 260
Arg Lys Arg Lys Met Ser Gly Gly Ser Thr Met Ser Ser Gly Gly Gly Asn Thr Asn Asn 280
Ser Asn Ser Lys Lys Lys Ser Pro Ala Ser Thr Phe Ala Leu Ser Ser Gln Val Pro Asp 300
Val Met Val Val Gly Glu Pro Thr Leu Met Gly Gly Glu Phe Gly Asp Glu Asp Glu Arg 320
Leu Ile Thr Arg Leu Glu Asn Thr Gln Phe Asp Ala Ala Asn Gly Ile Asp Asp Glu Asp 340
Ser Phe Asn Asn Ser Pro Ala Leu Gly Ala Asn Ser Pro Trp Asn Ser Lys Pro Pro Ser 360
Ser Gln Glu Ser Lys Ser Glu Asn Pro Thr Ser Gln Ala Ser Gln * 375

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b

c

anti-NLI

FIG. 1. Amino acid sequence and nuclear localization of NLI. (a) Predicted amino acid sequence of NLI. The amino terminal boundary of the original clone isolated by the protein–protein interaction screen is designated by an arrow. The putative nuclear localization sequence is underlined. (b) Immunoblot of untransfected, fractionated human 293 cells using affinity purified rabbit polyclonal antisera generated against residues 178–375 of NLI. Preimmune serum did not recognize the ≈ 46 kDa NLI band. (c) Immunocytochemistry of 293 cells using NLI antisera, visualized by a Texas Red-conjugated goat secondary antibody and fluorescence microscopy. Nuclear staining was not seen when using preimmune serum.

chased from Babco (Emeryville, CA). The 39.4D5 mouse Isl1 mAb developed by Thomas M. Jessell was obtained from the Developmental Studies Hybridoma Bank (Johns Hopkins University School of Medicine, Baltimore). The 39.4D5 cross reacts with the closely related Isl2 that is expressed later than Isl1 during chicken neural tube development (27). The rat anti-intermediate unphosphorylated neurofilament M (NF-M) antibody has been described (28). Texas Red conjugated goat anti-rabbit secondary antibody was purchased from Molecular Probes, and the fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibody was purchased from Antibodies, Inc.

Cotransfection and Coimmunoprecipitation from 293 Cells. Full-length NLI and LIM domain proteins were cloned into pcDNA3L and pcDNA3M, respectively. Each plasmid (10 μ g) was cotransfected into 293 cells by calcium phosphate precipitation. Cells were harvested and immunoprecipitated essentially as described (3) using 5 μ l of NLI 4508 antiserum. Immunoprecipitated complexes or 20% of the volume of nuclear extract used for immunoprecipitation were immunoblotted with the 12CA5 mAb or NLI antiserum.

Immunohistochemistry. Embryos were fixed in 4% paraformaldehyde in PBS for 1–2 h at 4°C, soaked in 15% sucrose/PBS before embedding in OCT (Baxter), and frozen in a dry ice ethanol bath. Frozen embryos were either stored at –20°C or sectioned immediately. 10–14 μ m frozen sections were collected onto superfrost plus glass slides (Fisher) using a Reichert-Jung 2800 cryostat. Sections were washed in PBS and incubated in primary antibody for 3 h to overnight at 22°C. Antibody dilutions were: mouse monoclonal anti-Isl1 39.4D5, 1:200; rabbit anti-NLI 4508, 1:1000; and rat anti-intermediate

unphosphorylated neurofilament (NF-M) 1:300. Fluorescein isothiocyanate-conjugated or Texas Red-conjugated secondary antibodies were used at 1:300 and 1:200, respectively. Images were collected on a Bio-Rad MRC1000 confocal microscope.

RESULTS

Identification of NLI as a Rbtl2 Interacting Protein. To identify interactors with nuclear LIM domain-containing proteins, ³²P-labeled GST-Rbtl2 was used to screen 1.1×10^6 phage from a mouse embryonic day 16 expression library. Three individual clones were isolated that bound to the probe but not to GST alone. These partial cDNAs originated from the same gene and were subsequently used to isolate a 2.3-kb cDNA clone corresponding to a 2.3-kb messenger RNA seen on Northern blots. This cDNA encodes a novel protein of 375 amino acids (NLI) (Fig. 1a). cDNA homology searches identified several identical mouse expressed sequence tags and a >90% identical human expressed sequence tag likely representing the human homolog of NLI; a highly related but less well-conserved human expressed sequence tag may be a family member. The predicted amino acid sequence revealed a nuclear localization sequence and two acidic residue-rich regions. NLI antisera recognized an $\approx$ 46-kDa protein in various cell lines including human 293, monkey CV-1, and mouse NIH 3T3 and erythroleukemia MEL cells (Fig. 1b and data not shown). Fractionation experiments and immunocytochemistry with 293 cells showed that NLI was predominantly localized in nuclei (Fig. 1b and c).

FIG. 2. *In vitro* association of NLI with nuclear LIM domain-containing proteins. (a) Schematic representation of GST fusion constructs tested in b and c. Numbering reflects the amino acid sequence and HD stands for homeodomain. (b) *In vitro* assay testing the binding of GST-LIM fusions of predominantly nuclear (group I) and predominantly cytoplasmic (group II) proteins to [³⁵S]methionine-labeled full-length NLI. (c) GST-binding assay testing separated LIM domains of Rbtl2, Isl1, and Mec3. Numbers indicate residues included.

Binding Specificity to Nuclear LIM Domain-Containing Proteins. To determine if NLI interacted only with Rbtl2 or with LIM domains of other proteins, GST fusion constructs were generated containing the LIM domains from various cytoplasmic and nuclear LIM domain proteins. Assays in which GST-LIM fusions were incubated with [³⁵S]methionine-labeled NLI showed that NLI bound with high affinity to all nuclear LIM domain proteins tested, including Rbtl2, Rbtl1, Lmx1, Isl1, Lhx1, and Mec3 (Fig. 2b, group I). In contrast, the LIM domains of the predominately cytoplasmic LIM kinase, Enigma, Paxillin, Zyxin, and CRP, did not bind to NLI (Fig. 2b, group II). NLI is the first protein demonstrated to interact with a wide variety of LIM domain-containing transcription factors, including both the rhombotins and LIM homeodomain proteins. Binding of NLI to the *C. elegans* protein Mec3, the most distantly related of the nuclear LIM proteins (5), suggests that the binding of NLI to nuclear LIM proteins will extend across all related members.

The LIM domains of Rbtl2, Isl1, and Mec3 were separated and tested for binding to NLI to determine if both LIM domains are required for interaction. Fig. 2c illustrates that the N-terminal LIM domain of Rbtl2 and Isl1 (LIM1) and the C-terminal LIM domain of Mec3 (LIM2) specified high-affinity binding. Since NLI binding can be mediated by a single LIM domain, simultaneous association with additional factors through the remaining LIM domain is likely. Target specificity for individual LIM domains of a protein has been described (1,

2, 29). In support of the high affinity *in vitro* binding data, *in vivo* association between NLI and Rbtl2, Rbtl1, and Lmx1 (Fig. 3, lanes 4–6) but not CRP (Fig. 3, lane 3) was demonstrated by cotransfection and coimmunoprecipitation of NLI and LIM proteins using 293 cells.

NLI Expression Within the Developing Nervous System. We used immunohistochemistry to detect expression of NLI, its interactor Isl1, and NF-M (28, 30) during the onset of neuronal differentiation in mouse embryos. Differentiation of neural tube neurons begins at ≈embryonic day 9.5 when the first neurons leave the cell cycle (31). Chicken, rat, and mouse embryos have a similar pattern of neuronal development within the neuroepithelium. In the developing neural tube, there is a mediolateral gradient of motor neuron production such that the proliferating neuronal precursors predominate in the ventricular zone while postmitotic, differentiated neurons migrate laterally to populate the intermediate zone (31–33). Isl1 positive motor neurons are located in the ventrolateral region of the neural tube in a E9.5 (25 somite) stage embryo (20). The first Isl1+ motor neurons are also NLI+ at the level of the developing limb bud (Fig. 4 a–c). Since all Isl1+ motor neurons are also NLI+, the onset of expression for Isl1 and NLI appears to be synchronous in neural tube motor neurons.

A number of NLI+/Isl1+ neurons also express NF-M at E9.5 (Fig. 4d). NF-M is a neuronal phenotypic marker that is classically considered to be one of the earliest characteristics of nerve cells (34). However, in some cases, NF-M expression was not detected in NLI+ neural tube cells. Whereas some NLI+ neurons located in the peripheral most region of the intermediate zone did not appear to express NF-M in 18 somite staged embryos, NLI+ neurons at this location did show NF-M immunoreactivity by the 24 somite stage (data not shown and Fig. 4d). Therefore, NLI and Isl1 expression seems to precede NF-M expression in at least some motor neurons. The early expression of NLI occurring prior to the expression of NF-M, a protein required for neuronal function, is consistent with a role for NLI in neuronal differentiation.

At E9.5, NLI is predominantly expressed by cells located in the lateral most region of the intermediate zone. The location of NLI+ cells and the subsequent expression of NF-M are characteristic of differentiated neurons. These observations taken together with the coincident expression of NLI and Isl1 suggest that NLI is expressed shortly after neurons leave the cell cycle (18, 19). The simultaneous expression of NLI and Isl1 during the initial events of neural tube motor neuron development is consistent with an interaction between NLI and Isl1 throughout this process is likely to be of functional importance.

Multiple neuronal cell types within the central and peripheral nervous systems express NLI. By E9.5, NLI/NF-M+ expression is seen in ventral cells of the hindbrain epithelium; however, NLI expression is not detectable in dorsal cell types in this region until later stages (data not shown). In the brachial level E10.5 neural tube, NLI+ cells include commissural neurons, interneurons, and motor neurons in addition to some medial cells that are NF-M– (Fig. 4 f–h). In the peripheral nervous system, NLI+/Isl1+/NF-M+ cells are found in the dorsal root ganglia and cranial ganglia (Fig. 4 e–h and data not shown). *In situ* hybridization was used to verify the presence of NLI mRNA in all cell types that showed immunoreactivity (data not shown).

DISCUSSION

We have identified NLI as a nuclear protein that strongly interacts with members of the LIM domain transcription factor family and whose expression in the embryonic nervous system suggests a potential involvement in neurogenesis. While previous gene deletion studies have demonstrated the biological

FIG. 3. *In vivo* association of NLI with nuclear LIM domain-containing proteins. Immunoprecipitation and immunoblotting was of crude nuclear extracts from cotransfected 293 cells. R2, Rbtl2; R1, Rbtl1; V, empty vector.

FIG. 4. Neuronal expression of NLI, Isl1, and NF-M in 9.5 day (24 somite) (*a–d*) and 10.5 day (*e–h*) mouse embryos. Anti-NLI (red) (*b* and *f*) and anti-Isl1 (green) staining (*a* and *e*) of brachial level transverse sections revealing the nuclear coexpression of NLI and Isl1 in neural tube motor neurons (*c* and *g*). Arrows in *a–c* mark a single motor neuron that is double labeled in *c*. Insets of *a*, *b*, and *c* show higher magnification of motor neurons double labeled for NLI and Isl1. Arrows in *e–g* mark a single motor neuron that is double labeled, while arrowheads mark a sensory neuron of the dorsal root ganglion that is double labeled. (*d* and *h*) Neural tube cells express NF-M immunoreactivity shortly after expression of NLI. Arrows in *d* mark neuronal cells that coexpress nuclear NLI (red) and cytoplasmic NF-M (green). Arrow in *h* marks a medial neural tube cell that shows NLI immunoreactivity but not NF-M immunoreactivity. (*a–c* and *e–h*, bar = 40 μ m; *d*, bar = 12 μ m.) (The bar shown in *h* represents 40 μ m in *a–c* and *e–h* and 12 μ m in *d*.)

importance of the rhombotins and LIM homeodomain proteins during development, little is understood about the nature of their molecular mechanisms. The properties of NLI suggest that the rhombotins and LIM homeodomain proteins are likely to use a common mechanism to regulate the expression of specific gene subsets.

Preliminary evidence does not support a role for NLI in direct transcriptional activation or repression. An attractive hypothesis is that the relatively widely expressed NLI may couple diverse LIM domain-containing transcription factors to the basal transcription machinery or to other general transcription factors by binding to a single LIM domain. In support of this hypothesis, synergistic transcriptional relationships between LIM homeodomain and ubiquitously expressed transcription factors such as E47 and CREB have been described (35–37). However, direct interaction between these proteins has yet to be shown. The second LIM domain not involved in NLI binding may interact with more tissue specific DNA binding factors to stabilize promoter specific protein–DNA interactions.

Since many neuronal cell types in the neural tube express NLI at later stages in development (E10.5 and onward), specific association of NLI with multiple nuclear LIM domain-containing proteins is likely to occur in neuronal lineages other than motor neurons. At least seven LIM homeodomain proteins are expressed in the chicken neural tube throughout its development including Isl1, Isl2, Lhx1, Lhx3, Lhx5 (Lim2), Lhx2 (LH-2), and Lmx1 (27). In motor neurons, the combinatorial expression patterns of Isl1, Isl2, Lhx1, and Lhx3 defined the identity of discrete motor neuron columns that innervate specific muscle groups (27). It is likely that other neuronal cell types in the neural tube are defined by the combinatorial expression of LIM homeodomain genes as well. NLI may therefore play a common role in neuronal differen-

tiation involving its association with each of the position specific LIM domain-containing transcription factors.

We thank R. Wu for discussion and plasmid constructs, E. McGuire, W. Shawlot, R. Tjian, M. Montminy, M. Beckerle, D. Edwards, and M. Brown for LIM domain plasmids; E. Turner for a λ gt11 phage library; D. Winge for atomic spectroscopy; and S. Taylor for protein kinase A catalytic subunit. We thank J. Sechrist for informative discussions and recommending the applicability of the NF-M antibody and H. Matthies and J. Lee for technical assistance. These studies were supported by National Institutes of Health Grant DK13149. L.W.J. was supported by the Lucille P. Markey Charitable Trust and a National Cancer Institute Cancer Cell Biology Training Grant (1-T32-CA67754-01).

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