Mutations in the Caenorhabditis elegans unc-84 gene cause defects in nuclear migration and anchoring. We show that endogenous UNC-84 protein colocalizes with Ce-lamin at the nuclear envelope and that the envelope localization of UNC-84 requires Ce-lamin. We also show that during mitosis, UNC-84 remains at the nuclear periphery until late anaphase, similar to known inner nuclear membrane proteins. UNC-84 protein is first detected at the 26-cell stage and thereafter is present in most cells during development and in adults. UNC-84 is properly expressed in unc-83 and anc-1 lines, which have phenotypes similar to unc-84, suggesting that neither the expression nor nuclear envelope localization of UNC-84 depends on UNC-83 or ANC-1 proteins. The envelope localization of Ce-lamin, Ce-emerin, Ce-MAN1, and nucleoporins are unaffected by the loss of UNC-84. UNC-84 is not required for centrosome attachment to the nucleus because centrosomes are localized normally in unc-84 hyp7 cells despite a nuclear migration defect. Models for UNC-84 localization are discussed.

INTRODUCTION

The wholesale movement or “migration” of nuclei is required for the growth and development of all eukaryotes. Genes involved in nuclear migration have been identified and characterized in Saccharomyces cerevisiae, filamentous fungi, Caenorhabditis elegans, Drosophila, and vertebrates (reviewed in Morris, 2000). Most of these genes encode molecular motors (dynein, or dynein-associated proteins) or proteins that mediate microtubule assembly or disassembly.

In C. elegans, the development of several specific cell types depends on nuclear migration events, and these migrations in turn depend on the unc-83 and unc-84 genes. For example, during the formation of the embryonic hypodermal syncytium, 17 of the 23 hyp7 nuclei undergo migration from one side of the cell to the other (Malone et al., 1999). Likewise, in the ventrolateral layer of P cells, nuclei migrate toward the ventral cord, allowing the P cells to form a single row along the ventral cord. In both unc-83 and unc-84 lines, these nuclear migration events fail to occur (Horvitz and Sulston, 1980; Malone et al., 1999). The UNC-84 protein is predicted to be split approximately in half by a single transmembrane domain, and its C-terminal region was found to be homologous to the Schizosaccharomyces pombe Sad1 gene in a region termed the SUN (Sad/UNC) domain, the function of which is unknown. Sad1 is an integral membrane protein associated during meiosis and mitosis with the spindle pole body in S. pombe, and its overexpression results in its accumulation at the nuclear periphery (Hagan and Yanagida, 1995). Expression of an UNC-84:GFP transgene suggested that UNC-84 is present in many cells and is localized to the nuclear periphery. Based on this nuclear peripheral localization and the presence of the SUN domain, it was proposed that UNC-84 is localized to the outer nuclear membrane, where it is associated with centrosomes (Malone et al., 1999; Raff, 1999; Morris, 2000).

The nuclear envelope is the boundary between the nucleus and the cytoplasm. The outer nuclear membrane is continuous with the endoplasmic reticulum. The outer nuclear membrane is separated from the inner nuclear membrane by the perinuclear space. The nuclear lamina is a filamentous meshwork that lies between the inner nuclear membrane and the peripheral chromatin. The lamina also extends throughout the nuclear interior (Broers et al., 1999;
Lamin-dependent Localization of UNC-84

Liu et al., 2000; Moir et al., 2000). An increasing number of integral and peripheral membrane proteins have been identified that associate with lamins. Thus, we define the term “nuclear lamina” broadly to include lamins and lamin-binding proteins (Cohen et al., 2001).

Because the nuclear outer membrane is continuous with ER membranes, all known proteins present in the outer membrane are also distributed throughout the ER. Membrane proteins that localize exclusively to the nuclear envelope are believed to do so by a retention mechanism, in which they diffuse along the “pore membrane domain” to the inner membrane, where they are retained by binding to intranuclear components such as lamins (Soulam and Worman, 1995). There is currently no precedent for a protein localized exclusively at the outer membrane of the nucleus. We therefore decided to further examine the localization of the UNC-84 protein. Our results suggest that UNC-84 is a nuclear lamina protein whose nuclear envelope localization requires lamin.

MATERIALS AND METHODS

Strains and Antibodies

All unc-84, unc-83, and anc-1 strains were described previously (Malone et al., 1999). Antiserum against Ce-lamin was described previously (Liu et al., 2000). Mouse polyclonal antipeptide antibodies against Ce-emerin (serum 3272) and Ce-MAN1 (serum 3266) are described (Lee et al., 2000). To obtain polyclonal antibodies against UNC-84, one mouse (serum 3398) and one rat (serum 3595) were immunized at 3-week intervals with synthetic peptides conjugated to keyhole limpet hemocyanin (KLH; peptide synthesis and conjugation were done by Boston BioMolecules, Woburn, MA). Immunizations and serum production were done by Covance Research Products (Denver, PA). The peptide antigen was CAVWKWIGN-QSQKRW-COOH, which corresponds to the first 14 residues of UNC-84 plus an N-terminal Cys residue. Both antisera against UNC-84 worked well for protein blots and indirect immunofluorescence staining. Antibodies against the mtf-1 gene product (matefin) was obtained by immunizing rats with a synthetic peptide corresponding to the matefin N-terminus.

MAb414, which recognizes several different nucleoparins, was purchased from BAbCO (Richmond, CA). An MAb against tubulin was purchased from Sigma Chemical Co. (catalogue number T-9026; St. Louis, MO). Cy3-conjugated goat anti-mouse and goat anti-rat antibodies and FITC-conjugated goat anti-rabbit antibodies were purchased from Jackson Laboratories (West Grove, PA). G. Hermann and J. Priess (Fred Hutchinson Cancer Research Center, Seattle, WA) kindly provided MAb IFA, which was used to detect embryonic centrosomes (Leung et al., 1999).

Immunostaining

Immunostaining was performed as described (Lee et al., 2000; Liu et al., 2000). Nematodes were fixed for 20 min at −20°C in methanol and 10 min at −20°C in acetone. Antibodies were diluted in PBS (1:200 for UNC-84, Ce-emerin, or Ce-MAN1, 1:400 for lamin and 1:1000 for MAb414). Nematodes were viewed with a Zeiss (Thornwood, NY) Axioskop microscope equipped with epifluorescence illumination with the use of a 63×/numerical aperture 1.4 APOCHROMAT objective lens. Confocal samples were acquired with the Noran Oz confocal laser scanning microscope system with the use of Intervision Software (version 6.3) on a Silicon Graphics Indy R5000 platform (Silicon Graphics Inc., Mountain View, CA). A krypton–argon laser (Omnichrome series 43, Noran Instruments Inc., Middleton, WI) that excites at wavelengths of 488 and 568 nm was used to obtain optical sections. Narrow-band emission filters (525 and 605 nm) were used to eliminate channel cross-talk, and 0.5-mm z-plane sections (as determined by full-width half-maximum intensity values) were collected using a 10-mm fixed slit. Slides were imaged with the use of a 100× oil-immersion planar apochromatic objective lens (numerical aperture = 1.35) through an Olympus (Tokyo, Japan) IX-50 inverted microscope.

Centrosomes were immunostained using MAb IFA as described (Leung et al., 1999). Images were collected using an Axioplan2 microscope (Carl Zeiss Inc.) and a Hamamatsu C4742–95 CCD camera (Hamamatsu Photonics KK, Bridgewater, NJ). A stack of images taken in the z plane at 0.3-mm intervals was deconvolved and analyzed using Openlab 2.0.7 (Improvision, Lexington, MA) software.

Protein Extracts

To prepare protein samples for SDS-PAGE, mixed-stage nematodes were boiled for 5 min in 2× SLB solution (25 mM Tris-HCl, pH 6.8, 20% glycerol, 0.2 M β-mercaptoethanol, 4% SDS, 0.001% bromphenol blue), and the extract was then passed through a 25-gauge, ½-inch syringe. Protein extracts were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with specific antibodies.

Cell Extracts

C. elegans nuclei were prepared essentially as described (Lee et al., 2000). For chemical extraction, 1 volume of nuclei was either used directly or thawed on ice, washed once in PBS-Inh (PBS containing 1 mM PMSF, 1 mg/ml leupeptin, and 1 mg/ml aprotinin), centrifuged at 4000 × g for 1 min at 4°C, and then extracted for 30 min at 4°C in 10 volumes of PBS-Inh plus the extraction reagent (e.g., 1 M NaCl, 1% Triton X-100, or 8 M urea). After extraction, the residual nuclear pellet was separated from the supernatant by centrifugation at 9000 × g for 1 min at 4°C. The nuclear pellet was washed in PBS.

Figure 1. Colocalization of endogenous UNC-84 and Ce-lamin at the nuclear envelope in wild-type embryos. C. elegans embryos were double-stained by indirect immunofluorescence using antibodies against endogenous UNC-84 (red) and endogenous Ce-lamin (green) and viewed by confocal microscopy. Overlap between UNC-84 and Ce-lamin appears yellow in the combined panel. Bar, 10 μm (all panels).
The supernatant was further purified by centrifugation at 14,000 \times g for 5 min at 4°C.

To prepare protein samples for SDS-PAGE, we boiled each sample for 5 min in 2X SLB solution (25 mM Tris-HCl, pH 6.8, 20% glycerol, 0.2 M β-mercaptoethanol, 4% SDS, 0.002% bromphenol blue) and then passed the extract through a 25-gauge, ½-inch syringe. Protein extracts were subjected to SDS-PAGE, transferred to polyvinylidene difluoride membrane, and immunoblotted with specific antibodies.

**RESULTS**

**UNC-84 Protein Colocalizes with the Nuclear Lamina**

Polyclonal antibodies were raised in both rat and mouse against an N-terminal peptide of UNC-84 (see MATERIALS AND METHODS) and used to localize endogenous UNC-84.
in *C. elegans* embryos by indirect immunofluorescence. Both the mouse antibodies (Figure 1, left) and the rat antibodies (Figure 2) localized UNC-84 to the nuclear envelope. This localization was specific for UNC-84, because envelope staining was not detected with preimmune sera or in unc-84(n369) null embryos (see below).

The localization of UNC-84 was similar to that of nuclear lamins (Liu et al., 2000). We therefore used confocal microscopy to analyze embryos double-stained for both UNC-84 and Ce-lamin and found that UNC-84 and Ce-lamin colocalized at the nuclear envelope (Figure 1, right). To determine if UNC-84 behaves as an integral membrane protein, we tested its resistance to extraction by detergents, salt, and chaotrophic agents (Singer, 1974). Isolated *C. elegans* nuclei were extracted with PBS containing 1% Triton X-100, 1 M NaCl, or 8 M urea reagent and analyzed as described (Lee et al., 2000). UNC-84 and Ce-lamin both pelleted after treatment with 1% Triton X-100. As predicted for an integral membrane protein, UNC-84, but not Ce-lamin, pelleted after extraction with 1 M NaCl or 8 M urea, similar to the extraction properties of Ce-MAN1 and Ce-emerin (Lee et al., 2000), suggesting that UNC-84 is an integral membrane protein.

**Staining Endogenous UNC-84 in Wild-type, unc-84, unc-83, and anc-1 Lines**

We used Western blotting to detect endogenous UNC-84 protein on blots containing total protein extracts from mixed-stage wild-type (N2) *C. elegans*. Antibodies against UNC-84 recognized one major band and several minor bands (Figure 3). The major band and one minor band were absent in mixed-stage extracts from unc-84(n369) (arrows in Figure 3), which is a predicted null with a nonsense mutation early in the coding region of UNC-84 (Malone et al., 1999). We therefore concluded that the large and small bands were both UNC-84 proteins. The two UNC-84–specific bands migrated on SDS-PAGE close to the predicted masses (125 and 98 kDa) of the two alternatively spliced products of unc-84. The large (125 kDa) isoform is sufficient for UNC-84 function (Malone et al., 1999).

The UNC-84 antibodies were also used to determine if genetically identified point mutations in the N-terminal or SUN-domains of UNC-84, which disrupt UNC-84 activity (Malone et al., 1999), also interfered with the expression or localization of each mutant protein. Both isoforms of UNC-84 were present in nematodes carrying mutations in the SUN-domain (class 3; unc-84(sa61) and unc-84(n399); Malone et al., 1999), as shown by immunoblotting (Figure 3), and the mutant proteins were properly localized at the nuclear envelope (Figure 2). UNC-84 protein was also present in nematodes carrying mutations in the N-terminal domain (class 4; unc-84(n1411); Malone et al., 1999; Figure 3). The class 2 alleles unc-84(n371) and unc-84(n323) (Malone et al., 1999) also did not disrupt UNC-84 localization to the nuclear envelope. As expected, UNC-84 was not detected in unc-84(n322) embryos, which lack the peptide epitope, nor was UNC-84 protein detected in immunoblots (Figure 3) or by antibody staining (Figure 2) in the unc-84(n369) null line. These results confirmed that unc-84(n369) is devoid of UNC-84 protein and demonstrated that mutant UNC-84 proteins were expressed and properly localized in lines carrying unc-84 mutant alleles from classes 2, 3, and 4.

Unc-84 and unc-83 have significantly overlapping nuclear migration defects and they were proposed to be involved in the same pathway (Horvitz and Sulston, 1980). In addition, a recent study shows that UNC-83 requires the SUN domain of UNC-84 for its nuclear envelope localization (Starr et al., 2001). It was therefore interesting to analyze if UNC-84 localization depends on UNC-83 activity. We found that UNC-84 does not depend on UNC-83 for its expression or envelope localization, because UNC-84 was expressed (Figure 3) and localized (Figure 2) normally in lines carrying unc-83(n1408), a null allele (Malone et al., 1999). Anc-1, mutations in which cause anchoring defects similar to unc-84, was also not required for UNC-84 expression, because normal levels of UNC-84 protein were detected in extracts from the anc-1 null line (Figure 3). Thus, UNC-84 is likely to act upstream of both UNC-83 and ANC-1 or in parallel pathways.

**The Pattern of UNC-84 Expression during *C. elegans* Development**

We used rat and mouse anti–UNC-84 antibodies to independently determine the pattern of UNC-84 expression during *C. elegans* development; both sera gave similar results. UNC-84 was not detected in the nuclear envelope from fertilized oocytes until just before the 26-cell stage (Figure 4). Weak staining was first detected in all nuclear envelopes at the 26-cell stage (Figure 4). This signal became stronger as embryonic development proceeded, with UNC-84 localized in every nuclear envelope. We also double-stained larval
stages L1–L4 for both endogenous UNC-84 and Ce-lamin (Figure 4). We stained for Ce-lamin because it is present in the nuclear envelope of every cell except spermatocytes (Liu et al., 2000), and therefore served as a positive control for antibody penetration. Almost all nuclear envelopes in L1–L4 stage larvae stained positively with UNC-84 antibodies. Likewise, all somatic adult cells, including the distal tip cell (DTC) in the gonad, stained positive for UNC-84 (see Figure 4 for anterior view of C. elegans adult). Germ cells in the mitotic and transition zones of the gonad stained negative for UNC-84 (Figures 4 and 5), whereas Ce-lamin staining was normal (Figure 4; see also Liu et al., 2000). UNC-84 was detectable in nuclear envelopes after the pachytene stage and continued to be positive in oocytes before fertilization (Figure 5). These results showed that endogenous UNC-84 is expressed in the nuclear envelopes of essentially all adult and embryonic cells, except between fertilization and the 26-cell stage. This result confirms the localization of UNC-84::GFP (Malone et al., 1999), but was surprising, given that loss of UNC-84 affects only a small number of migrating nuclei in the developing embryo, when the protein is expressed in most cell types.

**UNC-84 in C. elegans Remains in the Nuclear Envelope until Late Anaphase**

We recently showed that during mitosis in C. elegans embryos, the nuclear membranes and lamina are completely disassembled only during late anaphase (Lee et al., 2000). To determine if UNC-84 fits this same pattern, we used antibodies to follow the fate of endogenous UNC-84 protein during the different stages of mitosis in 64 to 200-cell embryos. UNC-84 maintained a nuclear rim-staining pattern during interphase, prophase, prometaphase, metaphase, and early anaphase. UNC-84 was completely released from chromatin only during late anaphase, and began to reaccumulate around chromatin in early telophase (Figure 6). This unusual pattern was strikingly similar to the mitotic disassembly and reassembly dynamics of the inner nuclear membrane proteins, Ce-emerin and Ce-MAN1, which are integral proteins of the inner nuclear membrane (Lee et al., 2000).

**Ce-lamin, Ce-emerin, Ce-MAN1, and Nucleoporins Do Not Depend on UNC-84 for Their Nuclear Envelope Localization**

The unc-84(n369) line, which has no detectable UNC-84 protein (Figures 2 and 3), was used to determine if UNC-84 is
required for the nuclear envelope localization of other known lamina proteins. unc-84(n369) embryos containing between 50 and a few hundred nuclei were stained pair-wise for UNC-84 and either nucleoporins (using mAb414) or each of three other nuclear lamina proteins: Ce-lamin (Liu et al., 2000), Ce-emerin, and Ce-MAN1 (Lee et al., 2000). The nucleoporins and all three lamina proteins remained properly localized to the nuclear envelope in the absence of UNC-84 (Figure 7).

**UNC-84 Requires Ce-lamin for Its Nuclear Envelope Localization**

In mammals, lamins are essential for the efficient localization of at least one tested nuclear membrane protein, emerin (Sullivan et al., 1999; Olins et al., 2001). We used indirect immunofluorescence to localize endogenous UNC-84 in lamin-deficient lmn-1(RNAi) embryos, created by injecting double-stranded lamin RNA into the syncytial gonad of adult hermaphrodites (Liu et al., 2000). Embryos with more than 50 cells were triple-stained for DNA, endogenous Ce-lamin, and endogenous UNC-84 (Figure 8). UNC-84 staining at the nuclear envelope was not detectable in lamin-deficient embryos, demonstrating that UNC-84 requires Ce-lamin for stable retention at the nuclear envelope (Figure 8). In parallel, lmn-1(RNAi) embryos were stained for nucleoporins (mAb414), Ce-emerin, and matefin, a novel nuclear membrane protein. Nucleoporins were clustered to one side of the nucleus as expected in lamin-deficient cells (Liu et al., 2000). Ce-emerin was displaced from the nuclear envelope, similar to UNC-84. In contrast, matefin remained localized to the nuclear envelope in the lmn-1(RNAi) embryos (K. Lee, J. Liu, K. Wilson, and Y. Gruenbaum, unpublished observations). These results demonstrate the dependence of UNC-84 on Ce-lamin for its nuclear envelope localization.

**Centrosome Attachment to the Nuclear Envelope Is Not Disrupted in unc-84 Mutants**

UNC-84 was previously proposed to reside in the outer nuclear membrane and mediate attachment to centrosomes or microtubules during nuclear migration (Malone et al., 1999; Raff, 1999). This model predicted that in an unc-84 null background, centrosomes would migrate normally across the hyp-7 cell, but the nucleus would fail to follow because of lack of UNC-84. Detachment of centrosomes was found in one-cell stage C. elegans embryos mutated in the heavy chains of the molecular motor, dynein, resulting in migration defects (Gonczy et al., 1999). Likewise, cytoplasmic dynein is required for the nuclear attachment and migration of centrosomes during mitosis in Drosophila embryos (Robinson et al., 1999). To test the hypothesis that loss of UNC-84 might similarly uncouple the nucleus from the centrosome, we localized the centrosomes and nuclei in migrating hyp7 cells in wild-type and unc-84(n369) null embryos. In wild-type hyp7 cells, centrosomes associated closely with migrating nuclei (Figure 9, A and B). These centrosomes did not associate with the leading edge of nuclei, but instead were associated with random sides, suggesting that the force that pulls (or pushes) migrating nuclei may not be exerted through the centrosome. Also surprisingly, centrosome localization in unc-84 null embryonic hyp7 cells was indistinguishable from wild-type (Figure 9C). This result strongly suggested that UNC-84 is not required for centrosome attachment to the nuclear envelope.
DISCUSSION

As the structural scaffold for the nucleus, the nuclear lamina, which includes lamins and lamin-associated proteins, is involved directly or indirectly in many biological functions. The lamina provides structural support for chromosomes, maintains nuclear shape, and spaces nuclear pore complexes. The lamina is also required for DNA replication and is proposed to mediate transcriptional regulation (Cohen et al., 2001). Our findings for UNC-84 add a novel role to the nuclear lamina in regulating nuclear migration and nuclear, the possible mechanisms of which are discussed below.

UNC-84 Is Probably a Nuclear Lamina Protein

The number of integral and peripheral membrane proteins that localize to the nuclear inner membrane is growing steadily (Cohen et al., 2001). Most inner membrane proteins bind directly to nuclear lamins and are therefore defined as part of the lamina (Gruenbaum et al., 2000). Each lamin-binding protein is likely to have a unique function, none of which are currently understood; in some cases lamin-binding activity may only be needed to localize the protein. A previous study suggested that UNC-84 is part of the nuclear envelope (Malone et al., 1999). Our current results suggest in several independent ways that UNC-84 directly or indirectly interacts with the nuclear lamina: UNC-84 colocalizes with the single C. elegans lamin protein, Ce-lamin, during interphase and exhibits the same distinct dynamics as inner membrane proteins Ce-MAN1 and Ce-emerin during mitosis (Lee et al., 2000). UNC-84 also depends on lamins for its nuclear envelope localization in vivo. In addition, a recent study reveals that the mouse homolog of UNC-84 is localized in the nuclear envelope, most probably to the inner nuclear membrane (Dreger et al., 2001). Finally, ectopic expression of C. elegans UNC-84 in mouse NIH-3T3 cells resulted in its association with the nuclear envelope. Thus, UNC-84 probably joins the growing number of lamin-associated proteins and adds new functions to this structure. Because the loss of UNC-84 had no effect on the localization of any other tested envelope proteins (Ce-emerin, Ce-MAN1, nucleoporins), we propose that UNC-84 associates with lamins and organizes factors important for nuclear migration and anchorage, independent of any complexes formed by other known nuclear membrane proteins. One of these proteins is UNC-83, which depends on UNC-84 for its nuclear envelope localization (Starr et al., 2001).

UNC-84 Expression during C. elegans Development

UNC-84 is expressed in most cells during C. elegans development. This result is consistent with a previous report showing UNC-84::GFP expression in most larval and adult cells (Malone et al., 1999). In contrast, loss of UNC-84 affects only a small number of migrating nuclei. We therefore hypothesize that UNC-84 functions may overlap with other protein(s), which is not present in migrating nuclei. Alternatively, UNC-84 might have a binding partner that is expressed uniquely in migrating nuclei, and depends on UNC-84 for its function.

UNC-84 staining was not detected in germ cells before the pachytene stage, or between fertilization and the 26-cell stage. This is a unique expression pattern for a nuclear envelope protein. The lack of staining between fertilization and the 26-cell stage could be due to 1) degradation of UNC-84 after fertilization, 2) release and diffusion in the ER resulting in cytoplasmic staining which is too weak to be detected, or 3) loss of antibody recognition due to posttranslational modification at the UNC-84 N-terminus.

UNC-84 Expression in Different Mutant Lines

Existing unc-84 mutations have been divided into four distinct complementation groups, termed classes 1–4 (Malone et al., 1999). Alleles in class 3 and class 4 completely complement each other. It was therefore suggested that class 3
and class 4 mutations, which cluster in the C- and N-terminal halves of UNC-84, respectively, do not grossly affect protein levels or protein structure. We verified this hypothesis by showing that UNC-84 expression levels in class 3 and 4 mutants were roughly similar to wild-type animals. We also showed that the envelope localization of UNC-84 was normal in these mutants. Because both class 3 and class 4 alleles affect nuclear migration, we suggest that mutations in either domain specifically disrupt interactions between UNC-84 and unknown factors required for nuclear migration. The best such candidate is UNC-83, because the nuclear migration phenotypes in \textit{unc-83} and \textit{unc-84} lines overlap and UNC-83 localization depends on UNC-84 (Malone \textit{et al.}, 1999; Starr \textit{et al.}, 2001). UNC-84 expression and envelope localization were also normal in class 2 (\textit{unc-84 (n371)}) mutants. Class 3 mutations lie in the C-terminal SUN domain and disrupt nuclear anchoring. We therefore suggest that the SUN-domain of UNC-84 interacts with factors involved in nuclear anchoring. One candidate is ANC-1, because mutations in the \textit{anc-1} gene cause nuclear anchoring phenotypes similar to class 3 alleles. Because UNC-84 expression is normal in \textit{unc-83} and \textit{anc-1} mutant lines, we suggest that both UNC-83 and ANC-1 act downstream or in parallel to UNC-84.

### Models for UNC-84 in Gene Expression, Signaling, or Nuclear Envelope "Bridging"

Several possible models for UNC-84 function are consistent with its lamin-dependent localization and nuclear migration function, as diagrammed in Figure 10. Because UNC-84 is expressed in nearly all cells but its loss causes a phenotype in only a few cells, we speculate that UNC-84 may have multiple binding partners, some of which are cell specific in their expression. Putative binding partners are depicted as proteins X and Y in Figure 10. In the gene expression model, we propose that UNC-84 and its binding partners might play an active role in regulating the transcription of proteins required for nuclear migration and anchoring, similar to the manner in which mammalian transcriptional regulators Rb and Oct-1 are proposed to repress transcription when associated with the nuclear lamina (Cohen \textit{et al.}, 2001). In the signaling model, we propose that binding to UNC-84 might serve a signaling role by retaining, sequestering, or activating downstream proteins that mediate nuclear migration and anchoring. Finally, we propose a bridging model in which UNC-84 interacts with the luminal domain of an outer membrane protein, to form a structural "bridge" through the nuclear envelope; this bridge would connect UNC-84 to outer membrane proteins that interact.
with microtubule-based motors or the centrosome. Alternatively, it is also possible that UNC-84 is actually located in the outer membrane and its lamin-dependent localization depends on linking to an inner membrane protein that binds lamins (Figure 10). In this case, UNC-84 would represent a completely novel outer-nuclear-membrane-specific protein, which remains connected to the nuclear lamina until late anaphase during mitosis. To our knowledge, there is no precedent for such a translumenal structural bridge through the nuclear envelope, but this model is attractive because it provides a mechanism to structurally link the pushing or pulling forces of nuclear migration to the nuclear lamina, via UNC-84. In theory, the attachment of cytoskeletal elements to nuclear pore complexes, which are anchored in the lamina, would achieve the same goal, but there is currently no evidence for functional links between pore complexes and the cytoskeleton. Determining the orientation of UNC-84 in the inner membrane and identifying and localizing its binding partners will be essential for distinguishing between these models.

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Lamin-dependent Localization of UNC-84


