Introduction
Spectrin repeats are units of approximately 106 residues, with a characteristic structure that includes three $\alpha$-helices separated by two loop regions, forming a triple-helical bundle (Pascual et al., 1996; Pascual et al., 1997; Yan et al., 1993). Spectrin repeat (SR)-containing proteins provide critical structural roles, and mutations in SR-protein genes have been implicated in human disease. For example, mutations in the $\beta$-spectrin gene are associated with hereditary elliptocytosis (Delaunay, 1995). $\beta$-spectrin contains 17 SRs within its rod region and is a major contributor to the stability of the erythrocyte membrane, where it interacts with an array of proteins including ankyrin and actin (Bennett and Gilligan, 1993). Dystrophin, the protein product of the Duchenne muscular dystrophy locus, contains 24 SRs, and mutations that disrupt dystrophin lead to muscle membrane instability and muscular dystrophy (Anderson and Kunkel, 1992). The SRs of these and other cytoskeletal proteins serve as sites for protein-protein interaction, actin and microtubule crosslinking, and molecular scaffolding and stabilization. Additionally, SRs mediate dimerization in $\alpha$- and $\beta$-spectrin, and $\alpha$-actinin (Djinovic-Carugo et al., 1999).

In contrast to the plasma membrane, the stability of the nuclear membrane derives, in part, from an assembly of intermediate filaments underlying the inner nuclear membrane. Lamins are intermediate filament proteins that provide significant structure to the inner nuclear membrane, forming a meshwork that interacts with nuclear membrane receptors and associated proteins (Gruenbaum et al., 2000; Stuurman et al., 1998). Nuclear lamins display a typical intermediate filament domain profile, including a globular N-terminal head, a central rod domain and a C-terminal globular tail. Lamins are classified as type A or type B, depending on sequence, expression pattern and mitotic behavior. Lamins A and C, alternatively spliced products of the same precursor gene, are expressed in differentiated cells and tissues, whereas type B lamins are constitutively expressed within all embryonic and somatic tissues (Broers et al., 1997). In addition, during the mitotic disassembly of the nuclear membrane, A-type lamins solubilize and are dispersed throughout the cell, whereas B-type lamins remain firmly bound to nuclear membrane vesicles (Moir et al., 2000a; Moir et al., 2000b). A growing number of inner nuclear membrane proteins have been found to interact with nuclear lamins in vivo and in vitro and to regulate nuclear membrane function and assembly (Clements et al., 2000; Dechat et al., 2000; Gant and Wilson, 1997; Martins et al., 2000). For example, the lamin B receptor (LBR) contains a predicted eight-transmembrane segment in its C-terminus and a nucleoplasmic N-terminal domain that can be phosphorylated by protein kinase A and cdc2 kinase (Courvalin et al., 1992; Worman et al., 1990). Additionally, members of the LAP2 (Lamin-associated protein 2) family and LBR bind to chromatin (Chu et al., 1998; Foisner and Gerace, 1993; Furukawa et al., 1998; Ye and Worman, 1994). Such
Northern blot analysis was carried out by amplifying a 432 bp 32P-labeled cDNA probe amplified from the myne-1 3’UTR region with the following primers: 5’TACAGGAATGCCCCCTCCTCC3’ and 5’ACA TGGTGTGTGGAGGGCTC3’. The probe was hybridized to a human multi-tissue mRNA blot (Clontech) and visualized on a Molecular Dynamics Phosphorimager (Amersham Pharmacia Biotech) and Kodak MS film. Using an EST-derived sequence, a forward primer 5’CTCTCTTCTCTCGGCGAGCACGAGTGGCGC3’ was designed. A full-length clone of myne-1 was generated by using this forward primer and the reverse primer 5’CATGTAATCTGGAGGGCTAAG GCTG3’ to amplify a 3644 bp product from human skeletal muscle cDNA (GenBank accession number AF444779). The product was then cloned into pCRII-TOPO vector (Invitrogen) and fully sequenced to confirm the sequence of myne-1. Predicted amino-acid sequence alignments were performed using the MacVector (v. 6.0) program (Oxford Molecular Group). Prediction of orientation of transmembrane helices was performed on the TMHMM server (v. 2.0) at http://www.cbs.dtu.dk/services/TMHMM/. Prediction of amino-acid domain structure was performed on the Simple Modular Architecture Research Tool (SMART) at http://smart.embl-heidelberg.de/ and the ISREC ProfileScan protein domain analysis server at http://www.isrec.isb-sib.ch/

**Materials and Methods**

Identification of myne-1 cDNA, northern blot and sequence analysis

A partial cDNA for myne-1 was obtained from the Kazusa DNA Research Institute, Kisarazu, Japan (http://www.kazusa.or.jp/en/). Northern blot analysis was carried out by amplifying a 432 bp 32P-labeled cDNA probe amplified from the myne-1 3’UTR region with the following primers: 5’TACAGGAATGCCCCCTCCTCC3’ and 5’ACA TGGTGTGTGGAGGGCTC3’. The probe was hybridized to a human multi-tissue mRNA blot (Clontech) and visualized on a Molecular Dynamics Phosphorimager (Amersham Pharmacia Biotech) and Kodak MS film. Using an EST-derived sequence, a forward primer 5’CTCTCTTCTCTCGGCGAGCACGAGTGGCGC3’ was designed. A full-length clone of myne-1 was generated by using this forward primer and the reverse primer 5’CATGTAATCTGGAGGGCTAAG GCTG3’ to amplify a 3644 bp product from human skeletal muscle cDNA (GenBank accession number AF444779). The product was then cloned into pCRII-TOPO vector (Invitrogen) and fully sequenced to confirm the sequence of myne-1. Predicted amino-acid sequence alignments were performed using the MacVector (v. 6.0) program (Oxford Molecular Group). Prediction of orientation of transmembrane helices was performed on the TMHMM server (v. 2.0) at http://www.cbs.dtu.dk/services/TMHMM/. Prediction of amino-acid domain structure was performed on the Simple Modular Architecture Research Tool (SMART) at http://smart.embl-heidelberg.de/ and the ISREC ProfileScan protein domain analysis server at http://www.isrec.isb-sib.ch/

**Generation of AM1**

The peptide, TSGRSTPNRQKTPRGK, representing residues 970–985 of GenBank accession number AB018339 (KIAA0796) was synthesized and injected into rabbits to raise a polyclonal antisera (Zymed Laboratories, South San Francisco, CA). This sequence shows no significant homology to the related sequence KIAA1011/DKFZ 434G173 and no significant homology to any other sequence in the available electronic databases. A glutathione s-transferase (GST) fusion protein expressing a fragment of myne-1 (aa 979 to 1105) was expressed in *E. coli* using pGEX4T-1 (Amersham Pharmacia Biotech) and was used to affinity purify AM1 as described (McNally et al., 1996).

**Immunocytochemistry and immunoblotting**

Tissues from a C57/BL6 mouse were harvested and frozen in liquid-nitrogen-cooled isopentane. Frozen 7 μm sections were fixed in –20°C methanol for two minutes, washed twice in PBS and blocked with 5% FBS in PBS. Primary antibodies were used at the following dilutions: lamin A/C 1:200; emerin 1:250; anti-smooth muscle actin 1:100; and AM1 1:50. Each antibody was diluted in blocking solution and incubated overnight at 4°C. CY3-conjugated goat anti-rabbit and FITC-conjugated goat anti-mouse secondary antibodies (Jackson Immunochemicals) were used at 1:2000 in blocking solution. Results were visualized using a Zeiss Axioscam digital camera mounted on a Zeiss Axiosphot 50 microscope (Carl Zeiss Inc.). Images were recorded and stored using the Zeiss Axiovision digital imaging software. Immunoblotting was performed as described in (Davis et al., 2000). AM1 was used at 1:200, and HRP-conjugated goat anti-rabbit antibody (Jackson Immunochemicals) was used at 1:5000. ECL plus (Amersham Pharmacia Biotech) and Kodak MS film were used for detection. Anti-smooth-muscle actin monoclonal antibody, 1A4, was purchased from Sigma (catalogue number A2547). Anti-lamin A/C monoclonal antibody, XB10, was obtained from Covance/BABCo (catalogue number MMS-107P). Anti-emerin monoclonal antibody was from Novoceastra (catalogue code NCL-EMERIN). DAPI mounting medium was from Vector Laboratories. Anti-LAP2β monoclonal antibody was from BD Transduction Laboratories (catalogue number L74520).

**Nuclear preparation and fractionation**

Nuclear membrane preparations were prepared essentially as described in (Davis et al., 2000). Briefly, mouse skeletal muscle tissue was homogenized in PBS then washed in 1×PBS twice at 4°C and
pelleted. Cells were lysed in hypotonic buffer (10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT plus protease inhibitor cocktail (Boehringer Mannheim GmbH., catalogue number 1873580)), incubated on ice for 15 minutes, vortexed for 30 seconds, then nuclei were collected at 14,000 g for 10 seconds at 4°C. The light microsomal fraction was collected from the supernatant by centrifugation (30,000 g for 30 minutes). The heavy microsomes were subjected to 105,000 g for 30 minutes. The nucleus membrane and the nucleoplasm were separated by resuspending the initial nuclear pellets in high salt buffer (20 mM Hepes, pH=7.9, 25% glycerol, 0.42 M KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM DTT plus protease inhibitor cocktail). Nuclear membranes were pelleted at 14,000 g for five minutes at 4°C. Equal volumes of each fraction (15 µl) were loaded. Immunoblotting was performed as described in (Davis et al., 2000). AM1 was used at 1:200, and HRP-conjugated goat anti-rabbit antibody (Jackson Immunnochemicals) was used at 1:5000. ECL plus (Amersham Pharmacia Biotech) and Kodak MS film were used for detection.

In vitro muscle differentiation and immunofluorescence
Murine muscle C2C12 cells were obtained from American Type Culture Collection and maintained at below 70% confluence to avoid differentiation. Cells were grown on glass coverslips and differentiated by allowing cells to grow to 70% confluence followed by serum starvation. Cells were harvested at sequential stages of differentiation, fixed, and stained and visualized as described above. The antibodies used are listed above.

Immunoprecipitation of lamin A/C and myne-1
For immunoprecipitations, differentiated C2C12 cells were harvested, sonicated on ice in immunoprecipitation buffer (10 mM Hepes, pH 7.4, 10 mM KCl, 5 mM EDTA, 1% Triton X-100 and protease inhibitor cocktail) and then centrifuged at 16,000 g for 10 minutes at 4°C. Lysate was then precleared with Protein A/G sepharose. Immunoprecipitations with relevant antibodies (diluted 1:75) were performed at room temperature for three hours, followed by incubation with Protein A/G sepharose for two hours and centrifugation at 4,000 g for 10 minutes. Immune complexes were washed four times and proteins eluted in SDS sample buffer and run on duplicate 4-12% gradient SDS-PAGE. One gel was stained with Coomassie Blue stain and destained to visualize proteins and the second gel was transferred to a PVDF membrane. Membranes were blocked in 3% BSA/TBS-T for one hour before overnight incubation with lamin A/C, 1:500. Secondary antibodies were applied and visualized as described above.

Results
Domain structure, mRNA expression and chromosomal localization of myne-1
A Basic local alignment search tool (BLAST) search (Altschul et al., 1997) using the dystrophin amino-acid sequence yielded a SR-rich Drosophila sequence, kakapo. Kakapo contains 37 SRs and an α-actinin-type actin-binding domain at its N terminus and is important for attaching muscle to epidermis (Gregory and Brown, 1998; Prokop et al., 1998; Strumpf and Volk, 1998) and for dendritic sprouting of motor neurons (Prokop et al., 1998). BLAST searches with kakapo SRs identified a novel SR protein encoded by the EST KIAA0796. A 5′ EST (GenBank AW952703) was aligned with the existing KIAA0796 to encode the 5′ end. This consensus sequence contains a Kozak sequence and predicted start methionine, completing a 4163 bp cDNA sequence with a 3507 bp open reading frame. This sequence was recently reported as encoding syn-e-1 (synaptic nuclear envelope protein-1) (Apet et al., 2000). We propose the name myne-1 (myocyte nuclear envelope protein-1) as the protein is preferentially expressed in striated and smooth muscle (see below).

Using the Simple modular architecture research tool (SMART) algorithm (Ponting et al., 1999; Schultz et al., 2000; Schultz et al., 1998) and TMHMM transmembrane helix server (Krogh et al., 2001), we found that the primary structure of myne-1 predicts a 131 kDa protein containing seven SRs, a central coiled-coil domain and a type II transmembrane domain followed by a short intraluminal C-terminus (Fig. 1A).

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**Fig. 1.** Domain structure of myne-1. (A) The predicted domain structure of myne-1 is shown. It includes seven spectrin repeats (large gray bars) and a transmembrane domain (black bar) at the C-terminus. Between the fifth and sixth spectrin repeat is a region (small gray bars) with homology to the LEM domain found in LAP2, emerin and MAN1. The LEM domain of myne-1 is interrupted in its midportion by an α-helical domain (white bar). Just before the transmembrane domain is a serine-rich domain (hexagon). (B) This region is not present in one splice form of myne. (C) The amino-acid sequence of the interrupted LEM domain of myne-1 is shown. The alignment of the LEM domains of human MAN1, emerin, LAP2, and two C. elegans homologs (F42H11.2/CAEEL; W01G7.5/CAEEL) is shown. Light gray represents homology; dark gray represents identity.
Analysis of cDNAs encoding myne-1 reveals that alternative splicing in this region results in the loss of the serine-rich region (Fig. 1B). A BLAST alignment indicates that the first 1020 amino acids of the 1169 amino acid myne-1 share 40% overall homology with the central SR-containing domain of dystrophin. The second and third SRs of myne-1 share 50% overall homology with the C-terminal two SRs found in mAKAP, an A-kinase anchoring protein preferentially expressed in muscle (Kapiloff et al., 1999). Myne-1 residues 595-670 share homology with the LEM domain, a 43 residue region of homology found in LAP2, emerin, and MAN1 (Lin et al., 2000). The LEM domain has been demonstrated to bind to BAF (Shumaker et al., 2001), which in turn binds to chromatin (Zheng et al., 2000). A predicted 20 residue coiled-coil domain (Fig. 1C) disrupts the myne-1 LEM domain. Two bipartite nuclear localization signals are found in myne-1 (amino acids 348-365 and 563-580), located in the last α-helix of the third and fifth SRs, respectively. All SRs of myne-1 except for the fourth SR are predicted to be acidic (pI 4.6-6.2). The fourth SR is predicted to be basic (pI 9.7).

Using electronic database searches, most of the intron-exon borders of myne-1 were established (data not shown). The
myne-1 gene maps to human chromosome 6q25.2-6q25.3 and is located close to the marker D6S420. A probe specific to myne-1 was hybridized to multiple mRNAs from human tissues and showed that the prominent myne-1 mRNA was approximately 4.2 kb and was broadly expressed in many tissues, although the highest levels were observed in human heart and skeletal muscle (Fig. 2A). The 4.2 kb transcript represents the major transcript from the gene, but may not be the only transcript since a faint ~9.5 kb mRNA signal was observed throughout all tissues on a human multi-tissue mRNA blot.

Myne-1 is found at the nuclear membrane of skeletal and cardiac muscle
To evaluate the protein expression of myne-1, residues 1057-1072 were used as an epitope to generate rabbit antiserum. Affinity-purified AM1 was used to assess protein expression in multiple mouse tissues (Fig. 2B) and showed that myne-1 is a 131 kDa protein that is most abundantly expressed in cardiac, skeletal and smooth muscle. Studies using AM1 on transverse sections of mouse quadriceps and heart, including both atrium and ventricle, showed staining exclusively at the nuclear membrane, identified by the rim surrounding all DAPI-stained nuclei (Fig. 2C). To confirm the nuclear membrane localization of myne-1, we separated the membrane fractions of mouse muscle into heavy microsomes, light microsomes and nuclear membranes. Myne-1 was enriched in the nuclear membrane fraction (Fig. 3A, lane 3). Additionally, muscle nuclei preparations were separated into nuclear membranes and nucleoplasm using high salt extraction. In this preparation, myne-1 was greatly enriched in the nuclear membrane fraction (Fig. 3B, lane 3). In order to examine the tissue localization of myne-1 in more detail, we examined the immunolocalization of myne-1 in smooth-muscle-containing organs including the stomach (Fig. 4A-D) and bladder (Fig. 4E-H), where staining at the nuclear rim was also seen (red staining). Counterstaining with monoclonal anti-smooth muscle actin (green) identified smooth muscle. Only very faint staining against myne-1 was observed in glandular, epithelial and connective tissues.

Immunolocalization of myne-1, lamin A/C, and emerin
The nuclear distribution of the myne-1 in muscle is similar to that of emerin and lamin A/C, nuclear envelope proteins that organize nuclear architecture and provide structural support to the nuclear envelope (Gruenbaum et al., 2000). To compare immunolocalization of myne-1, emerin and lamin A/C, antibodies to each of these proteins were used to stain transverse sections of mouse quadriceps. We analyzed sections double-labeled with AM1 and antibodies to emerin or lamin A/C, respectively. In all sections observed, myne-1 and lamin A/C displayed an identical coimmunolocalization pattern (Fig. 5A-C). Higher magnification showed consistent coimmunolocalization of myne-1 and lamin A/C (Fig. 5D).
Double-labeling with AM1 and emerin antibodies demonstrated that myne-1 and emerin displayed only a partial overlap (Fig. 5E-G). This can be seen under higher magnification (Fig. 5H) that shows exclusion of emerin in the arterial wall nuclei (arrow) where myne-1 is expressed (Fig. 5H). The lower magnification view (Fig. 5G) shows myocyte nuclei that stain for myne-1 but not emerin. Note the scattered red nuclei throughout.

Myne-1 expression and localization during in vitro muscle differentiation

The C2C12 cell line, a myoblast cell line capable of in vitro differentiation into myotubes, was used to study the expression of myne-1 during muscle differentiation (Fig. 6A-J). Sparsely plated, undifferentiated cells displayed no detectable levels of myne-1 (Fig. 6B). In contrast, emerin has been shown to be present in undifferentiated C2C12 cells (Fairley et al., 1999). Once cells were placed in differentiation medium, myne-1 expression levels increased (Fig. 6D,F,H,J). Early in differentiation as myoblasts fused to myotubes, myne-1 was located at many distinct multiple foci within nuclei. Later in differentiation, myne-1 became localized to the nuclear membrane of the C2C12 cells (Fig. 6J).

We studied colocalization of myne-1 with other known nuclear membrane associated proteins during muscle differentiation. Both myne-1 and lamin A/C display an identical pattern during in vitro differentiation including the localization to intranuclear foci progressing to nuclear membrane localization (Fig. 7A-C). We also studied the localization of myne-1 and LAP2β by double-labeling differentiating C2C12 cells. We similarly found that both myne-1 and LAP2β could be seen in intranuclear foci during in vitro muscle differentiation (Fig. 7D-F).

Myne-1 interaction with A-type lamin

Immunolocalization studies demonstrated that myne-1 displays a nuclear membrane localization identical to lamin A/C. To test the potential interaction between myne-1 and lamin A/C, myne-1 was immunoprecipitated using the AM1 antibody from fully differentiated C2C12 cells. The precipitates were separated by electrophoresis and immunoblotted with a monoclonal antibody against lamin A/C (XB10). Lamin A/C was found associated with the immunoprecipitate using AM1 (Fig. 8, right panel, lane 4), but was not seen with protein A/G beads alone or in the absence of muscle extracts (Fig. 8, right panel, lanes 2 and 3).

Discussion

Myne-1 is a type II transmembrane protein with seven SRs and an interrupted LEM domain found at the nuclear membrane of cardiac, skeletal and smooth muscle. Myne-1 colocalized with lamin A/C, an intermediate filament protein found at the inner nuclear membrane. Protein levels of myne-1 increased during myocyte differentiation, and the intracellular localization of myne-1 progressed from discrete intranuclear foci to nuclear rim staining during myocyte differentiation. Interestingly, we found this same developmental pattern for lamin A/C, a nuclear-membrane-associated protein, and for LAP2β, a
Myne-1 associates with lamin-A/C

Myne-1 associates with lamin-A/C.

Intronuclear foci colocalized with myne-1. In both cases, the intranuclear foci colocalized with myne-1. Intronuclear foci during myocyte development have previously been described for lamin A/C and nuclear-membrane-associated protein mAKAP (Kapiloff et al., 1999; Pugh et al., 1997). Intranuclear foci within developing myocytes may represent either punctate invaginations or membranous structures within the nucleus. The role of these intranuclear foci during myocyte development is not known, but given the potential role of nuclear membrane proteins in chromatin interaction, it may involve regulation of the gene expression that accompanies differentiation.

Previously, this protein was identified as syne-1 (synaptic nuclear expressed protein-1) (Apel et al., 2000). Because of our findings, we propose renaming this as myne-1 (myocyte nuclear envelope protein-1) to account for its expression pattern and potential interactions outside of postsynaptic nuclei. Apel et al. identified the transmembrane domain as a klaristh-like domain. klaristh is a protein critical for migration of nuclei to the cell periphery in Drosophila. This is consistent with the peripheral localization of nuclei in skeletal myocytes but is inconsistent with the centrally located nuclei of cardiac and smooth muscle. Using the SMART and TMHMM server algorithms, we found that this region is highly likely to encode a type II transmembrane domain. This prediction was tested experimentally by a nuclear membrane extraction performed on mouse muscle where myne-1 was greatly enriched in the nuclear membrane fractions.

In addition to SRs and the transmembrane domain, we found that myne-1 contains an interrupted or ‘broken’ LEM domain. The LEM domain is a region of 43 amino acids and is so named for its presence in LAP2, emerin and MAN-1 protein of the inner nuclear membrane (Lin et al., 2000). The function of the LEM domain is not fully understood, but recent data (Shumaker et al., 2001) suggest that this residue is critical for binding BAF, a small molecular weight protein that binds to double stranded DNA (Zheng et al., 2000). Therefore, the LEM domain may be important for crosslinking chromatin to the inner nuclear membrane. The disrupted LEM sequences in myne-1 may function similarly or may have additional functions given to the tissue-specific expression of myne-1.

We identified myne-1 by its homology to the SRs of kakapo. SRs are normally associated with a number of cytoskeletal proteins, such as spectrin, dystrophin, utrophin, α-actinin, plectin and ACF7, and participate in protein-protein interactions with other cytoskeletal proteins such as actin and zyxin (Amann et al., 1998; Crawford et al., 1992; Rybakova et al., 1996; Rybakova and Ervasti, 1997). Because SR proteins are generally associated with the cytoskeleton and the plasma membrane, the nuclear membrane localization of myne-1 is unusual. mAKAP, a protein kinase A anchoring protein targeted to the nuclear membrane of differentiated myocytes, possesses three SRs, two of which are critical for targeting mAKAP to the nuclear membrane (Kapiloff et al., 1999). Downstream regulation of cAMP-dependent proteins such as protein kinase A (PKA) is mediated by anchoring proteins (AKAPs) that sequester PKA to discrete subcellular locations. This compartmentalization is critical for cellular function, as specificity of cAMP-mediated signaling and function is based in a large part on distinct spatial positioning. In the case of mAKAP, it is the SRs of the protein that are critical for the compartmentalization of the protein, targeting mAKAP to the nuclear membrane (Kapiloff et al., 1999). Like mAKAP, myne-1 may serve as a scaffolding protein for kinases. Using the yeast two-hybrid system, Apel et al., demonstrated that syne-1 binds to MuSK, a tyrosine kinase expressed in postsynaptic myocytes (Apel et al., 2000). The in vitro interaction between syne-1 and MuSK occurs in the cytoplasmic domain of MuSK. This cytoplasmic domain contains the tyrosine kinase domain of MuSK (Valenzuela et al., 1995) and demonstrates high

Fig. 6. Immunolocalization of myne-1 in differentiating C2C12 cells. A,C,E,G and I represent nuclei stained with DAPI. AM1 labeling is shown in B,D,F,H and J. UN indicates undifferentiated C2C12 cells. D1, D3, D5 and D9 indicate 1 day, 3 days, 5 days and 9 days of differentiation, respectively. During differentiation, myne-1 is localized to discrete intranuclear foci, whereas late in differentiation, it becomes localized to the nuclear membrane. The bar represents 30 μm.
homology to many tyrosine kinases when subjected to a BLAST search. Thus, syne-1/myne-1 may act as a scaffold for any number of tyrosine kinases, or serine/threonine kinases, as its serine-rich C-terminal domain implies.

We observed complete colocalization between myne-1 and lamin A/C, but only partial overlap between myne-1 and emerin. Although this observation does not exclude the possibility of a myne-1–emerin interaction, it indicates that an interaction is not necessary for myne-1 and emerin to localize to the nuclear membrane of cells. Myne-1 and lamin A/C coimmunoprecipitate from muscle extracts, indicating an interaction between the two proteins. At this point, it is not known whether the lamin-A/C–myne-1 interaction is a direct or indirect interaction. It is possible that other nuclear membrane or nuclear-membrane-associated proteins participate in or in some way mediate the lamin-A/C-myne-1 interaction.

Mutations in the genes encoding emerin and lamin A/C specifically alter the phenotype of skeletal muscle, cardiac muscle and, in particular, the atrio-ventricular node of the cardiac conduction system (Becane et al., 2000; Bonne et al., 2000; Fatkin et al., 1999; Funakoshi et al., 1999). The phenotypic spectrum of lamin A/C and emerin mutations overlaps strikingly and is distinct from a number of other muscular dystrophies that alter genes encoding plasma membrane proteins (Hack et al., 2000). Lamin A/C and emerin are broadly expressed, and the mode by which tissue-specific effects develop from mutations in these genes is not known. We hypothesize that mutations in these genes may disrupt tissue-specific protein interactions. For example, mutations within exon 8 of lamin A/C are associated with a unique adipocyte-wasting disorder, DLPD (Speckman et al., 2000). It is possible that particular lamin A/C mutations disrupt the localization or function of myne-1. Further dissection of the myne-1–lamin-A/C interaction will determine whether there is direct binding between these proteins and what other proteins may participate in the subcortical network of the inner nuclear membrane. A better understanding of the cell biology of these interactions may shed light on the tissue-specific mechanisms of pathogenesis in these disorders.

References


