Linker of Nucleoskeleton and Cytoskeleton Complex Proteins in Cardiac Structure, Function, and Disease

Matthew J Stroud,* Indroneal Banerjee,* Jennifer Veevers,* Ju Chen

Abstract: The linker of nucleoskeleton and cytoskeleton (LINC) complex, composed of proteins within the inner and the outer nuclear membranes, connects the nuclear lamina to the cytoskeleton. The importance of this complex has been highlighted by the discovery of mutations in genes encoding LINC complex proteins, which cause skeletal or cardiac myopathies. Herein, this review summarizes structure, function, and interactions of major components of the LINC complex, highlights how mutations in these proteins may lead to cardiac disease, and outlines future challenges in the field. (Circ Res. 2014;114:538-548.)

Key Words: cardiomyopathies • cell nucleus • myocytes, cardiac

The division between cytoplasm and nucleus is defined by the nuclear envelope (NE), which consists of 2 lipid bilayers, the inner nuclear membrane (INM) and the outer nuclear membranes (ONM; Figure). The lumen between the 2 layers is known as the perinuclear space (PNS). The INM and ONM are fused periodically at nuclear pore complexes that regulate bidirectional macromolecular trafficking across the NE1–3 (Figure). Immediately underlying the INM is the nuclear lamina, a meshwork of intermediate filaments composed of A-type and B-type Lamins that plays a critical role in providing structural integrity to the NE and provides anchoring sites for chromatin domains and regulatory proteins, including signaling molecules and transcription factors.4 Proteins within the INM and ONM act as a linker of the nucleoskeleton and cytoskeleton (LINC) complex5,6 (Figure).

The LINC complex provides structural support to the nucleus and physically couples the nucleoskeleton with the cytoskeleton.7–11 This NE-spanning supramolecular chain may serve as a mechanosensor, translating mechanical cues, which include physical forces (eg, tension, compression, or shear stress) and alterations in extracellular matrix (ECM) mechanics, into biochemical signals, thus allowing cells to adapt to their physical environment.12,13 By mediating changes in cytoskeletal and nuclear organization/structure/positioning, these mechanical signals may also influence chromatin localization and organization and thereby modulate gene expression by altering interactions with active transcription complexes or by altering intracellular signaling pathways.12–16

A wide range of cardiac and skeletal myopathies have been linked to mutations in LINC complex proteins. These diseases include, but are not limited to, dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy, and Emery–Dreifuss muscular dystrophy (EDMD).17–21 In this review, we discuss the structure, function, and interactions of major components of the LINC complex, including Nesprins, Sun proteins, Emerin, Luma, and associated Lamins, and highlight how mutations in these proteins may lead to cardiac disease.

Nesprins
The ONM components of LINC complexes comprise a 4-member family of spectrin-repeat (SR) transmembrane proteins termed NE spectrin-repeat proteins (Nesprins).22,23 The founding member of this protein family, Nesrin 1, was alternatively named synaptic NE-1 (Syne-1),24 Enaptin,25 or myocyte NE protein-1 (Myne-1)26 because of its simultaneous discovery by several independent groups. Nesrin 1 was first discovered during a search for specific gene markers of contractile differentiated vascular smooth muscle cells (VSMCs). In a differential expression screen of differentiated versus de-differentiated VSMCs, a cDNA clone (IRA1) was identified that was more strongly expressed in differentiated VSMCs.27

Isolation and full-length sequencing of the human ortholog of IRA1 identified a gene encoding a protein (hereafter referred to as Nesrin 1) that localized to the NE in C2C12 myoblasts and human VSMCs.28 Nesrin 1 was also found to be highly expressed in nuclei that lie beneath the postsynaptic membrane at the neuromuscular junction of adult skeletal muscle fibers,24 and at the NE of smooth, skeletal, and cardiac muscle.26 The expression pattern of mammalian Nesrin 1 therefore implies a specific role in muscle function, further suggested by its abundance in the sarcomeric Z-line of both human skeletal and cardiac muscle.22

Circulation Research is available at http://circres.ahajournals.org

© 2014 American Heart Association, Inc.
Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAs</td>
<td>amino acids</td>
</tr>
<tr>
<td>ABD</td>
<td>actin-binding domain</td>
</tr>
<tr>
<td>ARVC</td>
<td>arrhythmogenic right ventricular cardiomyopathy</td>
</tr>
<tr>
<td>CH</td>
<td>calponin-homology</td>
</tr>
<tr>
<td>DCM</td>
<td>dilated cardiomyopathy</td>
</tr>
<tr>
<td>DKD</td>
<td>double knockout</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EDMD</td>
<td>Emery–Dreifuss muscular dystrophy</td>
</tr>
<tr>
<td>INM</td>
<td>inner nuclear membrane</td>
</tr>
<tr>
<td>HP1</td>
<td>heterochromatin protein 1</td>
</tr>
<tr>
<td>KASH</td>
<td>Klarsicht, ANC-1, and Syne homology</td>
</tr>
<tr>
<td>LBR</td>
<td>Lamin B receptor</td>
</tr>
<tr>
<td>LINC</td>
<td>linker of nucleoskeleton and cytoskeleton</td>
</tr>
<tr>
<td>MEF</td>
<td>mouse embryonic fibroblast</td>
</tr>
<tr>
<td>MKL1</td>
<td>megakaryoblastic leukemia 1</td>
</tr>
<tr>
<td>NE</td>
<td>nuclear envelope</td>
</tr>
<tr>
<td>NELS</td>
<td>nuclear envelope localization signal</td>
</tr>
<tr>
<td>Nesprin</td>
<td>nuclear envelope spectrin-repeat protein</td>
</tr>
<tr>
<td>MEF</td>
<td>mouse embryonic fibroblast</td>
</tr>
<tr>
<td>Myne</td>
<td>myocyte nuclear envelope protein</td>
</tr>
<tr>
<td>ONM</td>
<td>outer nuclear membrane</td>
</tr>
<tr>
<td>PNS</td>
<td>perinuclear space</td>
</tr>
<tr>
<td>RACE</td>
<td>rapid amplification cDNA ends</td>
</tr>
<tr>
<td>SR</td>
<td>spectrin-repeat</td>
</tr>
<tr>
<td>SUN</td>
<td>Sad1/UNC-84</td>
</tr>
<tr>
<td>Syne</td>
<td>Synaptic nuclear envelope</td>
</tr>
<tr>
<td>TM</td>
<td>transmembrane</td>
</tr>
<tr>
<td>VSMC</td>
<td>vascular smooth muscle cell</td>
</tr>
</tbody>
</table>

Sharing >60% homology with Nesprin 1, Nesprin 2 was discovered simultaneously in both a differential cDNA screen and a yeast 2-hybrid screen used to identify proteins concentrated in the postnysaptic membrane. Accordingly, Nesprin 2 is otherwise known as Syne-2, or nucleus and actin connecting element (NUANCE), having been identified as a novel protein during a database search using the peptide sequence of the actin-binding domain (ABD) of known α-actinin–related proteins. Nesprin 2 was found to be predominantly expressed at the ONM and in the nucleoplasm of multiple cell types, a rather uncharacteristic feature of actin-binding proteins.

Multiple Nesprin isoforms that vary markedly in size are produced by alternative transcriptional initiation, RNA splicing, and termination of the 2 independent Nesprin 1 and Nesprin 2 genes. Giant isoform Nesprin 2 (Nesprin 2G) was first cloned by combining rapid amplification cDNA ends (RACE)-PCR with the analysis of data available from human EST and genomic databases. Independently, Zhang et al performed bioinformatic examination of the genomic regions of human Nesprin 1 and Nesprin 2 and predicted the existence of giant isoforms, Nesprin 1G and Nesprin 2G, with calculated molecular weights of 1.01 MDa and 796 kDa, respectively. Nesprin 1G cDNA was later cloned with mRNA extracted from mouse brain. Both Nesprin 1G and Nesprin 2G consist of an N-terminal tandem repeat of calponin homology (CH) domains, an SR-containing rod domain, and a C-terminal transmembrane Klarsicht, ANC-1, and Syne homology (KASH)-domain. The 2 giant proteins bind to the actin cytoskeleton via their CH domains, whereas the C-terminal transmembrane KASH domains mediate their localization and stabilization at the NE by interacting directly with Sad1/UNC-84 or SUN domain–containing proteins residing in the INM. The existence of these giant isoforms in striated muscle has yet to be confirmed by Western blot and cDNA data.

The smaller Nesprin 1 and Nesprin 2 isoforms that have been documented include, but are not limited to, Nesprin 1α, Nesprin 1β, Nesprin 2α, Nesprin 2β, and Nesprin 2γ. For a detailed review and schematic representations of the various Nesprin isoforms, we refer readers to recent publications. In comparison with their respective giant isoforms, the short isoforms either vary in the length of their SR-containing rod domains, lack both the N-terminal CH domain and the C-terminal KASH domain, or lack either the CH domain or the KASH domain. For example, the Nesprin 1α isoform contains the KASH domain and SRs but lacks the CH domains. Nesprin 1α localizes to the NE and has been reported to interact with itself, Emerin, and Lamin A/C. Like Nesprin 1α, Nesprin 2α is expressed predominantly in heart and skeletal muscle, as detected by Northern blot and RT-PCR analysis, and colocalizes with Emerin and Lamin A/C in the NE of VSMCs. Although direct interactions between Nesprin 1α, Nesprin 2α, Emerin, and Lamin A/C have been reported, the recently solved crystal structure of SUN domains interacting with KASH peptides of Nesprins implies that KASH-containing Nesprin isoforms reside in the ONM. Because Nesprins are a multi-isoform protein family, many antibodies have been generated against different regions/domains of each protein isoform. However, it should be noted that the specificities of many of the antibodies described to date have not been thoroughly validated in suitable Nesprin knock out animal models. Furthermore, the lack of isoform-specific sequences in many of the Nesprin variants makes it difficult to design antibodies targeting a single isoform.

Recent data suggest that missense mutations in Nesprin 1 and Nesprin 2 may be involved in the pathogenesis of EDMD-like phenotypes, including cardiomyopathy. Screening for DNA variations in genes encoding Nesprin 1 and Nesprin 2 was performed on 190 EDMD or EDMD-like patients lacking Lamin or Emerin mutations. Four heterozygous missense mutations were identified (R257H, V572L, and E646K in Nesprin 1α and T89M in Nesprin 2β), which occurred at positions that are highly conserved evolutionarily and which lie within the Lamin and Emerin binding domains of Nesprin 1 and Nesprin 2. Fibroblasts from these patients exhibited nuclear morphological defects and mislocalization of Emerin and SUN2. These observations could be recapitulated by siRNA knockdown of Nesprin 1 or Nesprin 2 in normal fibroblasts. In addition, diminished NE localization of Nesprins and impaired Nesprin/Emerin/Lamin–binding interactions were common features of all fibroblasts of patients with EDMD. These results suggest that defective LINC complexes and uncoupling of the nucleoskeleton and cytoskeleton may play a significant role in the muscle-specific pathogenesis of EDMD.
In an independent study, a patient with the R374H missense variant in Nesprin 1α, but no mutations in LMNA, was identified in a screen of 46 unrelated patients with nonischemic cardiomyopathy. This individual developed severe DCM and required cardiac transplantation at 26 years of age. Patient-derived fibroblasts displayed increased expression of LINC complex proteins Nesprin 1α and Lamins A and C. Because of the association of Nesprin 1 mutations with cardiac disease, Puckelwartz et al. also characterized the cardiac phenotype of a mutant mouse model in which the KASH domain of Nesprin 1 was specifically replaced by a stretch of 61 unrelated C-terminal amino acids (Nesprin1rKASH). Homozygous mutant mice exhibited lethality, with approximately half dying at or near birth from respiratory failure. Surviving mice displayed progressive muscle weakness, a characteristic of EDMD, and with increasing age they developed cardiomyopathy with associated cardiac conduction defects. Absence of the KASH domain prevented Nesprin 1 from binding to SUN proteins, thereby disrupting the LINC complex. Furthermore, cardiomyocyte nuclei were found to be elongated with reduced heterochromatin in Nesprin1rKASH hearts. These findings mirror what has been described for Lamin A/C mutations and reinforce the importance of an intact LINC complex for normal cardiac function. Of note, however, is the observation that in this model, mutant Nesprin 1α protein was similar in size (120 kDa) because of the replacement of 100 amino acids of the KASH domain with an alternate 61 amino acids and was produced at the same level as wild-type Nesprin 1α in skeletal muscle samples. It is unclear from the data presented as to whether other mutant Nesprin 1 isoforms were present because no proteins with molecular weights >120 kDa were shown by Western blot analysis.

In other mouse models, ablation of the KASH domain of either Nesprin 1 (Nesprin1ΔKASH) or Nesprin 2 (Nesprin2ΔKASH) has been reported to have no effect on either viability or fertility; however, double mutants die of respiratory failure within 20 minutes of birth. Nesprin 1α is highly expressed in synaptic nuclei of syncytial muscle fibers and is upregulated during myotube differentiation. In Nesprin 1ΔKASH mutants, clusters of synaptic nuclei are abolished and mice display abnormal repositioning of nonsynaptic nuclei in skeletal muscle. Heart and muscle functions of these mice have not been reported. In the absence of Western blot analysis, however, it is unclear whether truncated mutant proteins lacking the KASH domain are produced.
domain or native Nesprin isoforms that lack the KASH domain are still present in these mouse models.42 Interestingly, expression of a dominant-negative form of Nesprin 1, which encodes the C-terminal KASH domain, results in mislocalization of neuromuscular junction nuclei.43 Another group has reported that mice lacking the ABD of Nesprin 2G were viable and almost indistinguishable from wild-type mice, except for slight epidermal thickening.44 Interestingly, fibroblasts from these mice exhibited abnormal nuclear morphology and an uneven distribution of Emerin in the NE.

The 2 Nesprin 1 mutant mouse lines discussed were generated by either partially removing42 or completely replacing39 the last exon of Nesprin 1, which encodes the KASH domain. Because KASH-less Nesprin 1 isoforms have been shown to exist, we generated Nesprin 1 mutants by targeting an exon that is shared by all Nesprin 1 isoforms containing the C-terminal SR region with or without the KASH domain (hereafter referred to as Nesprin 1−/− mice).45 Nesprin 1−/− mice have markedly decreased survival rates, growth restriction, increased variability in body weight, and compromised exercise capacity compared with wild-type animals. In agreement with the previous studies,39,42 we found that Nesprin 1 is critical for nuclear positioning and anchorage in skeletal muscle. Of the LINC complex–associated proteins examined, only SUN1 and SUN2 were slightly upregulated in Nesprin 1−/− cardiac and skeletal muscles, respectively.45 No defects in cardiac contractile function were observed in our Nesprin 1−/− mice up to 12 months of age, although function was not assessed in older mice.45 To this end, it would be of great interest to investigate whether Nesprin 1 and Nesprin 2 have distinct and overlapping roles in cardiac muscle nuclear positioning, nuclear membrane integrity, and cardiac muscle function by generating mouse lines in which Nesprin 1 and Nesprin 2 are specifically ablated in developing cardiac muscle and adult cardiomyocytes. It will also be important to determine the roles of different isoforms of Nesprin 1 and Nesprin 2 by generating isoform-specific knockout mice.

**Nesprin 3, Nesprin 4, and KASH5**

Nesprin 3 was first identified in a proteomics screen to identify novel NE proteins and was subsequently found to interact with the ABD of human Plectin 1C using a yeast 2-hybrid screen.46,47 Nesprin 3 has 2 protein isoforms, 3α and 3β, which contain a C-terminal KASH domain but lack N-terminal CH domains.47 The KASH domain interacts with SUN1 and SUN2 to retain Nesprin 3 at the ONM, and the N-terminus of Nesprin 3α interacts with the ABD of Plectin, which in turn interacts with intermediate filaments.47,48 Nesprin 3 is conserved throughout evolution and is ubiquitously expressed in mouse tissues, importantly localizing to the NE in both skeletal and cardiac myocytes.47,49,50 Nesprin 3 knockout zebrafish and mice are viable and do not display any basal phenotype.49,50 However, it would be interesting to investigate whether these mice have an abnormal cardiac response to stress or present an age-related phenotype because Nesprin 3 has been found to regulate cell morphology during flow-mediated mechanical loading and cell migration in a 3D collagen matrix.51,52

Nesprin 4 was discovered by performing a BLASTP search for sequences similar to the KASH domain of Nesprin 2.53 Nesprin 4 localizes to the ONM and contains a single SR domain and a C-terminal KASH domain. Nesprin 4 interacts with Kinesin-1, as shown by coimmunoprecipitation and a yeast 2-hybrid screen, and recruits Kinesin-1 to the NE when expressed in heterologous HeLa cells.53 Nesprin 4 knockout mice seem overtly normal with no obvious loss of viability; however, they do display defects in hearing.45 Whether Nesprin 4 is expressed in the heart remains to be determined.

**KASH5** was identified by performing a yeast 2-hybrid screen using a testes cDNA library and the mouse cohesin protector protein shugoshin-2 as bait.51 KASH5 contains a C-terminal KASH domain that interacts with SUN1 and SUN2 and a central coiled-coil region. Its expression seems to be limited to the testes and has yet to be described in the heart.

**SUN Proteins**

Sad1/UNC-84 or SUN proteins were originally described in fission yeast (Sad1)50 and Caenorhabditis elegans (UNC-84).57 BLAST searches using the conserved C-termius (now termed the Sad1/UNC-84 or SUN domain) revealed the mammalian homologs, SUN1 and SUN2, which were subsequently cloned using a cDNA library from human brain.57 Since the identification of SUN proteins in mammals, 5 mammalian family members (SUN1-5) have been identified.39 Whereas SUN1 and SUN2 are ubiquitously expressed, SUN3, SUN4 (SPAG4), and SUN5 seem to be specifically expressed in the testes.58–60 For the purposes of this review, we focus on the roles of SUN1 and SUN2 as they have been identified in the mouse heart and skeletal muscle.5,39,45 For a more extensive overview of SUN proteins in other tissues and cell types, we refer readers elsewhere.61

SUN1 and SUN2 are type II membrane proteins.5,62,63 Human SUN1 is the largest member of the SUN domain family containing 812AAs and having a molecular mass of ≈90 kDa. SUN2 contains 717AAs and a predicted molecular mass of ≈80 kDa. Overall, SUN1 and SUN2 proteins show high degrees of similarity and share 64% homology.5,7 SUN1 and SUN2 both have an N-terminal region localized at the nucleoplasm,5,7 which is abutted to a single transmembrane domain that spans the INM.6,61 The bulk of SUN1 and of SUN2 comprise the stalk region, which spans the PNS and comprises coiled-coil repeats that are thought to be essential for trimerization.5,64,65 The most highly conserved region between family members is the C-terminal SUN-domain, which comprises ≈175AAs and interacts with Nesprins.

To our knowledge, only the longest isoforms of SUN1 and SUN2 have been shown to be expressed in the heart at the protein level.45 Interestingly, a recent study found 6 potential splice isoforms of SUN1 expressed in the heart using RT-PCR.46 All predicted isoforms contain the canonical SUN domain, stalk region, and transmembrane domain but have varying lengths that protrude into the nucleoplasm. At present, there is no experimental evidence to suggest differential splicing of SUN2.62 It remains to be seen whether these isoforms are translated into protein and, if so, what distinct roles they may have.

SUN1 and SUN2 have been shown to interact directly with nuclear Lamins.5,7 Specifically, overexpressed HA-tagged SUN1 coimmunoprecipitates with GFP-conjugated Lamin A in U2OS cells. Furthermore, an in vitro transcribed/translated
N-terminus of SUN1 was found to interact with in vitro transcribed/translated Lamin A, but not Lamin C, Lamin B1, or Lamin B2. In another study, a GST-conjugated N-terminus of SUN1 was found to preferentially interact with in vitro transcribed/translated pre-Lamin A over mature Lamin A, and weakly interacted with Lamin C and Lamin B1. In HeLa cells, overexpression of Myc-tagged pre-Lamin A, but not Lamin B1, relocalized an HA-tagged N-terminus of SUN1 from the nucleoplasm to the NE. This interaction seems to be evolutionarily conserved, as the localization of one of the two SUN protein homologs expressed in C. elegans, UNC-84, was found to be dependent on Lamins. Given the foregoing in vitro interaction and immunofluorescence data, it was somewhat surprising that in Lamin A/C-null mouse embryonic fibroblasts (MEFs), SUN1 was still able to localize to the NE. In support of this, others have shown that SUN1 was able to localize to the NE when Lamin A/C and Lamin B1/B2 were knocked down in HeLa cells.

Similarly to SUN1, the HA-tagged N-terminus of SUN2 could be recruited from the nucleoplasm to the NE by overexpressing Myc-tagged pre-Lamin A, but not Lamin B1 in HeLa cells. In addition, in vitro pull-down assays revealed that the N-terminus of SUN2 interacts weakly with Lamin A, Lamin C, and Lamin B1. In contrast to SUN1, the localization of SUN2 showed some dependence on Lamin A/C because SUN2 was unable to localize to the NE in the majority of MEFs devoid of Lamin A/C.

These data clearly imply some role of nuclear Lamins in the localization of SUN proteins. However, in light of recent evidence in which lmnα−/− mice were shown to produce truncated Lamin A that may be capable of interacting with SUN proteins, data using the Lamin A/C-null MEFs must be interpreted with caution. The region of Lamin A that interacts with SUN1 maps to residues 389–664 and the truncated Lamin A produced by the Lamin A/C-null MEFs retains 78 of 275 AAAs. It is therefore possible that the remaining 78 AAs could mediate the interaction between SUN1 and truncated Lamin A. In contrast, for SUN2, the 197 AA of the 275 AA interaction domains that are lost in the Lamin A/C-null MEFs may be critical for retention of SUN2 at the INM.

Whether Lamin-independent mechanisms exist to localize SUN1 and SUN2 to the INM remains to be seen. However, it is clear that other mechanisms exist to recruit SUN proteins to the INM. For example, in C. elegans, UNC-84 requires a combination of 2 nuclear localization signals, an INM-sorting motif and an NE-localization signal (NELS) that is evolutionarily conserved with mammalian SUN1. In support of the hypothesis that other localization mechanisms exist, Turgay et al demonstrated that, in addition to Lamin A/C, SUN2 requires a combination of its nuclear localization signal, Golgi retrieval sequence, and the SUN domain for localization to the INM.

Various mechanisms have been speculated to retain SUN1 and SUN2 at the INM, including binding to the Lamins and heterochromatin. Intriguingly, one study found that the N-termini of SUN1 and SUN2 were able to interact with Emerin and short isoforms of Nesprin 2 that localize to the INM. Clearly, further work is required to investigate whether these interactions have functional consequences in vivo.

Whereas the localization and connection between SUN1 and SUN2 to the nucleoskeleton seem to require a combination of factors, it is clear that the connection between the cytoskeleton and SUN1 and SUN2 is directly through members of the Nesprin family. The crystal structure of SUN2 revealed that it forms a hexameric complex with 3 KASH peptides of the Nesprins and coordinate them using a combination of hydrogen bonds, a KASH lid and disulfide bridges. The extensive covalent and noncovalent attachments as well as the binding avidity between 3 KASH domains with 3 SUN domains are thought to enable the LINC complex to transmit force between the cytoskeleton and the nucleoskeleton. This is of critical importance in cardiomyocytes that are constantly undergoing mechanical stress for maintenance of the NE architecture as well as sensing and responding to changes in force.

Given the interaction partners and localizations of SUN1 and SUN2, they are likely candidates for playing a role in the pathogenesis of cardiomyopathy. For example, mutations in Lamin A/C that result in progeria show upregulated levels of SUN1 in patient-derived fibroblasts. Interestingly, downregulation of SUN1 in mouse models for progeric (lmnaΔ9) and dystrophic (lmna−/−) laminopathies ameliorated the phenotypes observed and significantly extended the lifespan of the mice. Specifically, histological sections from lmna−/− hearts revealed an increase in sarcoplasmic vacuoles and an increase of inflammatory cells in the myocardium. These features were ameliorated in the lmna−/− SUN1−/− double knockout (DKO) mice. In addition, the cardiac function as measured by ejection fraction was restored to near wild-type levels of ≈70% in the lmna−/− SUN1−/− DKO compared with ≈50% in lmna−/− mice. Intriguingly, SUN1 accumulated in the Golgi of lmna−/− and LmnaA9 MEFs, which resulted in cytotoxicity. However, the underlying molecular mechanisms behind the pathogenesis remain unknown.

Interestingly, one study in mice using a global knockout approach to ablate SUN1 and SUN2 expression in all tissues resulted in perinatal lethality. Although the causes of death were not ascertained in great detail, it seems that the lungs were not fully inflated in the DKO. Interestingly, the DKO was rescued by expressing SUN1 using a transgenic approach under a neuron-specific promoter. Also, it is intriguing that this phenotype is similar to that of Nesprin 1 and Nesprin 2 double global knockout mice.

Another potential association between SUN1 and SUN2 and disease is through a mutation in Emerin that results in X-linked EDMD. It has been shown that the disease-causing mutation in Emerin reduces the strength of binding between Emerin and SUN1 or SUN2. Despite these data, the precise role of SUN1 and SUN2 in the heart remains elusive. Studies of cardiac-specific knockout mouse models will further our understanding of these key factors.

**Emerin**

The gene coding for Emerin was first identified in 1994 by genetic mapping of X-linked recessive EDMD. Emerin is a type II integral membrane protein, which contains a nucleoplasmic...
N-terminal domain, followed by a single transmembrane region that spans the INM, and a short luminal tail that resides in the PNS.75,76 Emerin is ubiquitously expressed in tissues and predominantly localizes to the NE in skeletal and cardiac muscle, where it is thought to be retained at the INM via its interaction with A-type Lamins.75–79 Emerin has multiple binding partners, including but not limited to SUN1, SUN2, Nesprin 1τ, and the chromatin-interacting protein, barrier to autointegration factor, which provides a link between Emerin and chromatin.55,71,80 For a comprehensive list of binding partners and their interaction regions, we refer readers to a recent review.81

Mutations in Emerin cause X-linked EDMD and lead to cardiac conduction defects and DCM, as well as to defects in skeletal muscle.74,75,82–85 The number of disease-causing mutations in Emerin is vast, and we refer readers elsewhere for a comprehensive overview.86,87 Many of the Emerin disease-causing mutations lead to its depletion in cells.75–76 It is therefore surprising that Emerin knockout mice have been reported to display either a mild age-related atrioventricular conduction defect or no overt skeletal or cardiac phenotype.86,89

Interestingly, Emerin-deficient MEFs have abnormal nuclear shape,88 altered NE elasticity,90 and display an impaired response to mechanical stimulation, as measured by expression levels of mechanosensitive genes ies-1 and egr-1.88 After sustained mechanical strain, the number of apoptotic cells is higher in Emerin-null MEFs compared with wild-type cells.88

It was therefore postulated that Emerin might play a role in mechanosensing and regulate expression of genes to enable the cell to adapt to mechanical load. In support of this, a recent report suggests that Emerin indirectly regulates the localization and therefore signaling of the mechanosensitive transcription factor megakaryoblastic leukemia 1 (MKL1)90 by modulating actin dynamics. MKL1 is a coactivator of serum response factor, which is a master regulator of genes encoding numerous cytoskeletal proteins, including both Vinculin and Actin.

Although it is clear that mutations in Emerin are the unequivocal cause of X-linked EDMD, studies with cardiomyocytes derived from human EDMD patient-derived induced pluripotent stem cells may reveal the molecular basis behind the disease.

Luma

Luma (TMEM43) was first identified in a proteomics screen for new INM proteins in neuroblastoma cells and was subsequently cloned and shown to localize to the NE in COS-7 cells.86,82 Luma is widely expressed in human tissues and importantly contains 4 transmembrane (TM) domains that are thought to play a role in self-oligomerization.83,85 The majority of the protein resides in the PNS and comprises a large hydrophilic domain that is located between TM1 and TM2. Luma has been shown to interact with Lamins A/C, B1, Emerin, and SUN2.83,93,96 and may play a role in regulating Emerin localization.

Luma has been identified as the unequivocal cause of arrhythmogenic right ventricular cardiomyopathy type 5 (ARVC5).84,96,97 The mutation in Luma causing ARVC5 changes residue Serine 358 to Leucine residue (S358L) in the third TM domain.96 The ARVC5 locus was mapped in an extended 8-generation family from the genetically isolated population of the Canadian island of Newfoundland. ARVC5 is a lethal, fully penetrant, sex-influenced, autosomal-dominant disorder. Typically, it affects males more than females because affected men have a median lifespan of 41 years compared with 83 years in the control group, whereas affected women have a median lifespan of 71 years. The disorder is fully penetrant by age 63 and age 76 in men and women, respectively. The most prominent clinical features of ARVC5 were premature ventricular contractions and left ventricular dilatation, resulting in heart failure and sudden cardiac death. Interestingly, another study identified 2 patients with EDMD with 2 novel mutations in Luma, E85K and I91V.95 Unfortunately, the patient with the E85K mutation died after a muscle biopsy was performed, and the patient’s son who reportedly had the same phenotype was lost to follow-up. Therefore, no mutation analysis or segregation studies could be performed. For the patient with I97V, her parents died with no mention of any specific causes, and she did not have any children. Therefore, unlike the S358L mutation that is genetically traceable, the E85K and I91V mutations can only be associated with EDMD, and it remains to be seen whether they are causative factors in EDMD.

The underlying molecular mechanism(s) behind the pathogenesis of ARVC5 caused by the S358L mutation in Luma remains to be elucidated. However, Luma was identified as a potential target gene of the adipogenic transcription factor, PPARγ,98 indicating a potential link between the fibrofatty replacement of cardiomyocytes in the hearts of patients with ARVC5. Further work using mouse models and patient-derived induced pluripotent stem cells are needed to reveal the molecular function of Luma in cardiac function and disease.

Nuclear Lamins

Nuclear Lamins were first described in 1978.99 Nuclear Lamins are type V intermediate filaments that readily self-associate to form parallel coiled-coil homodimers. These homodimers are then able to form higher-order filamentous structures to form the nuclear lamina, situated beneath the INM. The main components of the nuclear lamina are the A-type and B-type nuclear Lamins, which share a common overall structure, comprising a central α-helical rod, which is flanked by nonhelical globular domains at either terminus.100

The A-type Lamins are encoded by a single gene, LMNA, that is alternatively spliced to generate the major Lamins, A and C, as well as the minor Lamins, Aα10 and C2.101,102 The expression of A-type Lamins is ubiquitous and is developmentally regulated because their expression is only detected in differentiated cells.103 In contrast, B-type Lamins are constitutively expressed and are found in many tissues.104 The B-type Lamins, Lamin B1 and B2, are encoded by LMNB1 and LMNB2, respectively.105,106 For a comprehensive overview of the discovery and description of nuclear Lamins, we refer the readers to another review.107

Mutations in LMNA cause a broad range of human diseases, collectively known as laminopathies.108,109 Laminopathies comprised more than a dozen previously defined clinical disorders and include several forms of muscular dystrophies,
such as autosomal-dominant EDMD, limb-girdle muscular dystrophy, DCM, heart-hand syndrome, and a newly discovered LMNA-related congenital muscular dystrophy or L-CMD. The myriad of diseases caused by mutations in the gene coding for Lamin A/C surpasses any other known gene and is beyond the scope of this review. For an overview of the laminopathies that result in cardiac diseases, we refer readers elsewhere.

The many roles of Lamins are mediated by interactions with numerous Lamin-binding proteins both at the nuclear periphery and in the nucleoplasm. In addition to providing structural integrity to the NE, playing a role in cytoskeletal organization and nuclear positioning, there is also growing evidence that Lamins regulate chromatin organization and gene expression and influence cell signaling. Lamins can interact with chromatin either directly or through histones and other INM and nonintegral membrane proteins, such as Lamin B receptor (LBR), heterochromatin protein 1 (HP1), Emerin, LBR, and barrier to autointegration factor (Figure).

Because substantial data support a role for Lamin A/C in its interaction with chromatin and gene regulation, Mewborn et al. examined gene expression changes in hearts and fibroblasts resulting from a dominant LMNA mutation, E161K, associated with inherited cardiomyopathy, and correlated this with changes in chromosome positioning. In addition to having a high percentage of misexpressed genes, chromosome 13 was found to be less tightly associated with the nuclear membrane in LMNA E161K mutant cells, and the entire chromosome territory was displaced to a more intranuclear position compared with control cells, thereby linking abnormal gene expression and intranuclear position. Gross mislocalization of chromosome 13 was also observed in another fibroblast line with an LMNA gene mutation, D596N, associated with both cardiomyopathy and muscle disease. However, the chromosome 13 territory was found completely abutted to the nuclear periphery.

The authors hypothesized that changes in nuclear positioning and therefore chromatin organization can modulate the epigenetic regulation of gene expression. This may be via altering interactions with active transcription complexes and subsequent accessibility to transcription factors (Figure). Interestingly, cells lacking A-type Lamins have defective nuclear mechanics and impaired expression of mechanosensitive genes, such as iex-1 and egr-1. Further evidence that Lamin A/C may play a mechanosensing role is from studies using Lmna−/− mice. These mice display a significantly attenuated response to pressure overload, evidenced by reduced ventricular mass and myocyte size. In addition, analysis of pressure-overload–induced transcriptional changes also revealed an impaired activation of egr-1. The most recent data suggesting Lamin A/C plays a role as a mechanosensor were derived from analysis of Lamin A/C-null mice or Lamin (N195K/N195K) mutant mice. MEFs derived from both mouse lines showed impaired nuclear translocation of the mechanosensitive transcription factor MKL1. Furthermore, cardiac sections from both mouse lines had significantly reduced fractions of cardiomyocytes with nuclear MKL1. MKL1 is a coactivator of serum response factor, which in turn is a master regulator of genes encoding many cytoskeletal proteins.

Many mouse models have been generated to mimic mutations found in humans. Interestingly, it seems that some autosomal-dominant mutations in humans, for example, the H222P mutation that causes autosomal-dominant EDMD, only causes a phenotype in mice when both alleles are mutated. Mutations in other factors or greater dosage sensitivity may exacerbate the phenotype in humans.

One of the better-characterized mouse models used to mimic EDMD in humans is the Lmna−/− mouse. These mice develop severe cardiac and skeletal myopathy, bearing a striking resemblance to human EDMD, which results in premature death between 6 to 8 weeks of postnatal development. Lmna−/− MEFs and tissues exhibit aberrant nuclear morphology, partial loss of peripheral heterochromatin, and mislocalization of Emerin. In another study using the same Lmna−/− model, mutant mice develop rapidly progressive DCM by 4 to 6 weeks of age. Isolated lmm−/− cardiomyocytes exhibit altered nuclear morphology and architecture with central displacement and fragmentation of heterochromatin. These studies lend further credence to the notion that Lamins and their associated proteins play an important role in nuclear mechanics, chromatin organization, and modulation of gene expression, which if impaired causes striated muscle damage in subjects with certain Lamin A/C mutations.

A recent report demonstrated that the original Lmna−/− mouse is not a null allele and actually expresses a truncated form of Lamin A that arises as a result of an unforeseen splicing event. The resulting protein is 54 kDa in size and contains the N-terminal globular domain and rod domains, whereas a large proportion of the C-terminal globular domain is missing. The C-terminus is where most of the interaction partners are thought to bind to Lamin A; hence, many of the conclusions from the Lmna−/− mice are still likely to be valid.

In another Lamin mutant mouse line generated using gene-trap technology, Lmna−/− mice have a more severe phenotype than the Lmna−/− mice and die before weaning at 2 to 3 weeks postpartum, providing evidence that the truncated Lamin A protein in Lmna−/− mutants retains some function. The phenotype of Lmna−/− mice is more consistent with what is observed in humans, because a patient who lacked Lamin A/C died at birth. Clearly, some re-evaluation of the data collected from the Lmna−/− mouse is required despite the value it has added to the field in terms of understanding laminopathies.

Unlike A-type Lamins, there is a paucity of diseases linked to mutations in B-type Lamins. This may be because mutations result in early lethality, as observed for Lamin B1 knockdown mice and Lamin B2 knockout mice. To date, no B-type Lamin mutations have been reported as causing either skeletal or cardiac myopathies.

Concluding Remarks and Future Directions

The importance of the LINC complex in numerous fundamental cellular functions has been established by the discovery of cardiac and skeletal muscle disease-causing mutations in genes encoding LINC complex–associated proteins. The LINC complex provides structural support to the nucleus and physically couples the nucleoskeleton and is hypothesized to serve as a mechanosensor, translating mechanical cues, which include...
physical forces and alterations in ECM mechanics, into bio-chemical signals.12,13 By mediating changes in cytoskeletal and nuclear organization, structure, and positioning, these mechanical signals may also influence chromatin localization and regulate gene expression.12–16 These mechanisms are not mutually exclusive, and alterations in one of these cellular features could influence the other. Consequently, any disruption in the LINC-associated protein complex could result in defects in cellular structure and function and contribute to the development of cardiac and skeletal myopathies, as described throughout this review.

The use of integrated approaches that combine animal models and cell-based assays, where LINC complex–associated proteins have been targeted, has been instrumental in beginning to uncover the molecular mechanism(s) by which NE proteins act as crucial regulators in diverse cellular processes. These include cytoskeletal organization, nuclear architecture, chromatin dynamics, and gene expression. The next step is to determine molecular mechanisms by which cardiac and skeletal muscle-specific complexes coordinate mechanical and signaling pathways throughout the cell, and to determine how malfunction of this process contributes to disease.

As described in this review, several NE-associated proteins, including Nesprins, SUNs, Emerin, Luma, and Lamins, have been studied in detail. To further investigate their involvement in cardiac and skeletal muscle function, future work should involve more in-depth studies characterizing each of the individual proteins and individual isoforms by generating global and cardiac-specific knockout mice for each of the proteins and specific isoforms. Another key future challenge is to further determine LINC complex interaction networks with other NE-associated and proximal proteins, as well as yet-to-be-identified proteins, in cardiomyocytes.

It is also important to generate mouse models that mimic disease mutations identified in humans to elucidate molecular mechanisms underlying cardiac and skeletal myopathies in vivo. Furthermore, complementary studies are essential to investigate mechanisms by which mutations in LINC complex–associated proteins impact human cardiomyocyte function using human patient–derived induced pluripotent stem cells. In addition to uncovering fundamental biology, insights gained from these studies can potentially lead to novel therapeutic approaches for treating the devastating diseases caused by LINC complex–associated mutations, including cardiac and skeletal myopathies.

Acknowledgments

We apologize to all authors whose work could not be cited because of space constraints.

Sources of Funding

J. Chen is funded by grants from the National Heart, Lung, and Blood Institute. J. Chen is the American Heart Association (AHA) Endowed Chair in Cardiovascular Research. M.J. Stroud and I. Banerjee are supported by AHA postdoctoral fellowships (13POST17060120 and 12POST12030256). J. Lowe is supported by a California Institute for Regenerative Medicine (CIRM) postdoctoral fellowship (TG2-01154).

Disclosures

None.


Linker of Nucleoskeleton and Cytoskeleton Complex Proteins in Cardiac Structure, Function, and Disease
Matthew J Stroud, Indroneal Banerjee, Jennifer Veevers and Ju Chen

Circ Res. 2014;114:538-548
doi: 10.1161/CIRCRESAHA.114.301236

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/114/3/538

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/