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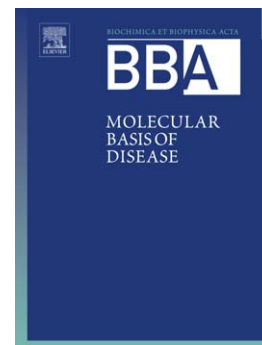
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## Nuclear Envelope Defects in Muscular Dystrophy

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**Abstract**

Muscular dystrophies are a heterogeneous group of disorders linked to defects in 20-30 different genes. Mutations in the genes encoding a pair of nuclear envelope proteins, emerin and lamin A/C, have been shown to cause the X-linked and autosomal forms respectively of Emery-Dreifuss muscular dystrophy. A third form of muscular dystrophy, limb girdle muscular dystrophy 1b, has also been linked to mutations in the lamin A/C gene. Given that these two genes are ubiquitously expressed, a major goal is to determine how they can be associated with tissue specific diseases. Recent results suggest that lamin A/C and emerin contribute to the maintenance of nuclear envelope structure and at the same time may modulate the expression patterns of certain mechanosensitive and stress induced genes. Both emerin and lamin A/C may play an important role in the response of cells to mechanical stress and in this way may help to maintain muscle cell integrity.

## Introduction

Muscular dystrophies (MDs) represent a diverse group of several dozen inherited disorders [1]. While their common feature is always progressive weakness and degeneration of skeletal muscle, these various disorders may differ, quite considerably, with respect to location of affected tissues, disease progression and severity. Disparity in affected muscles can easily be appreciated when comparing facioscapulohumeral MD 1A (FSHMD1A OMIM#158900) to limb girdle MD 1A (LGMD1A OMIM#159000). In the former, the muscle groups affected are in the face, shoulder girdle and lower legs. In the latter, proximal weakness of the hip girdle is observed which only later progresses to the shoulder girdle. Other forms of muscular dystrophy, for example Emery-Dreifuss MD (EDMD OMIM#310300), may feature degeneration of cardiac muscle in addition to skeletal muscle. Finally, certain forms of muscular dystrophy such as EDMD may appear early in life whereas others such as LGMD1A display an adult onset. Mutations in at least 20-30 genes [2, 3] have been associated with MD. Proteins encoded by these genes can be grouped according to their subcellular localization. While this review will focus primarily on MD-linked nuclear proteins, functional parallels between protein groups will be explored.

## Cytoskeletal and extracellular matrix related Muscular Dystrophies

The most common form of MD is Duchenne MD (DMD OMIM#310200) [1]. This is an X-linked disorder with an early onset of about 3-5 years of age. The affected gene in DMD encodes dystrophin, an extremely large (~400kD) protein related to alpha actinin and spectrin. In muscle cells, dystrophin functions to link the actin cytoskeleton to the plasma membrane and extracellular matrix (ECM)[2, 3]. The N-terminus of dystrophin interacts directly with cytoskeletal actin filaments, but not actin filaments of the contractile apparatus. Distal regions of the molecule bind a complex of plasma membrane proteins containing, among others, members of the dystroglycan and sarcoglycan families of glycoproteins. Alpha-dystroglycan in turn binds to alpha2-laminin on the extracellular face of the plasma membrane providing a link to the ECM. Perhaps not surprisingly, mutations in the genes encoding dystroglycans, sarcoglycans and laminin have all been linked to various forms of muscular dystrophy [2, 3]. In addition, forms of MD such as Fukuyama congenital muscular

dystrophy appear to involve proteins that are implicated in the intracellular processing of newly synthesized dystroglycans and sarcoglycans [4, 5]. What all of these proteins have in common is their contribution to the integrity of a structural network, with signaling properties, that connects the muscle cell cytoskeleton to the ECM through the plasma membrane. Other MD-associated genes encode cytosolic proteins like calpain-3 and sarcomeric proteins such as titin. The latter functions both as a molecular ruler in sarcomere assembly as well as an elastic component of the contractile apparatus. In this way titin makes a direct contribution to muscle cell functionality.

### **Nuclear envelope related Muscular Dystrophies**

In recent years an additional group of MDs have been linked to defects in nuclear envelope proteins [1]. The prototype of these is Emery-Dreifuss MD. EDMD displays two inheritance patterns, X-linked (EDMD OMIM#310300) and autosomal (EDMD2 OMIM#181350). Both forms of the disease display similar physical symptoms featuring degeneration of muscles of the upper arms, shoulder girdle and lower legs, and contractures of the Achilles tendons as well as of tendons of the elbows and neck. These contractures have a childhood onset and are one of the early signs of the disease. EDMD also features a very significant cardiac involvement with both cardiac muscle degeneration and associated conduction system block. The latter frequently requires the implantation of a pacemaker in early adulthood and may ultimately necessitate a heart transplant.

In 1994, the X-linked form of EDMD was mapped to a gene encoding emerin, a 29kD membrane protein (named after Professor Alan Emery, who originally described the disease [6]) [7]. Emerin immediately provided two surprises. First, it turned out to be a nuclear envelope membrane protein and second it was not specific to muscle [8, 9]. Instead it is expressed in virtually all human-cell types. Subsequent discussion will delve further into the etiology of both X-linked and autosomal EDMD, as well as other associated disorders, in an attempt to elucidate how defects in ubiquitously expressed proteins might give rise to tissue specific diseases. Finally, recent findings will be examined which might functionally connect nuclear envelope components with dystrophin and dystrophin associated proteins

(dyoglycans and sarcoglycans etc.) that are linked to Duchenne, Becker and related forms of MD.

### **The Nuclear Envelope**

The nuclear envelope (NE) is a selective barrier that forms the interface between the nucleus and the cytoplasm, and as such plays a central role in defining the biochemical identities of each compartment [10, 11]. In addition to its barrier function, it is becoming increasingly clear that the NE is a key determinant of nuclear architecture and may strongly influence cytoplasmic organization. The NE contains several discrete structural elements, the most prominent of which are the inner and outer nuclear membranes (Figure 1). In mammalian somatic cells these two membranes are separated by a uniform gap of about 30-50nm referred to as the perinuclear space (PNS). The INM and ONM are connected at annular junctions which create aqueous channels between the nucleoplasm and cytoplasm. These channels are occupied by nuclear pore complexes (NPCs), massive multi-protein assemblies that regulate the trafficking of macromolecules across the NE. A mammalian somatic cell nucleus typically contains several thousand NPCs .

In addition to its continuities with the INM at the periphery of each NPC, the ONM also displays multiple connections to the peripheral endoplasmic reticulum (ER) to which it is functionally related. Evidently the INM, ONM and ER form a single continuous membrane system. Similarly, the PNS constitutes a perinuclear extension of the ER lumen, and contains both secretory proteins and soluble ER resident proteins, including ER chaperones.

The final major structural feature of the NE is the nuclear lamina [12]. This is a relatively thin (20-50nm) protein meshwork that is closely associated with both the nuclear face of the INM and the underlying chromatin. The key components of the nuclear lamina are a group of proteins known as A- and B-type lamins. The lamin proteins are members of the more extensive cytoplasmic intermediate filament (IF) family and like all IF proteins contain a central coiled-coil domain flanked by non-helical head and tail domains . In contrast to their cytoplasmic counterparts, each of the lamins contains a nuclear localization sequence

(NLS) within the C-terminal non-helical domain required for efficient nuclear import of newly synthesized lamin proteins. Both A- and B-type lamins are known to interact with membrane proteins of the INM [12] as well as with chromatin [13] [14]. In this way, the nuclear lamina may provide anchoring sites at the nuclear periphery for higher order chromatin domains in addition to stabilizing and organizing the NE. While the bulk of the lamins appear to reside at the nuclear periphery, nucleoplasmic lamins have also been observed [15-17] with proposed roles in several aspects of nuclear metabolism, including DNA replication [18-21].

In mammalian cells there are two major A-type lamins, A and C, encoded by a single gene, *LMNA* [22]. These two proteins are identical for the first 566 amino acid residues. Both proteins possess unique C-terminal extensions. In the case of lamin C this consists of a sequence of six amino acids. The unique region of lamin A is considerably larger at 98 amino acids. Two other A-type lamins have been described. The first of these, lamin A $\Delta$ 10 [23], lacks a 30 amino acid sequence within the unique lamin A specific region that is encoded by exon 10 (*LMNA* contains 12 exons). While it is found in somatic cells, its abundance and distribution has yet to be well defined. Lamin C2[24] is male germ cell-specific and essentially consists of a truncated form of lamin C that contains an alternative N-terminus modified by myristoylation.

Mammalian somatic cells also contain two B-type lamins, lamins B1 and B2 [25], encoded by separate genes (*LMNB1* and *LMNB2*) [26, 27]. A third B-type lamin, lamin B3, derived from the *LMNB2* primary transcript by alternative splicing is male germ cell-specific [28]. While B-type lamins as a class are expressed in all nucleated cell types, the expression of A-type lamins is developmentally regulated [29, 30]. As a general rule, A-type lamins are found in most adult differentiated cell types but are absent from both early embryonic cells and adult stem cell populations [31, 32]. In the mouse, A-type lamin expression commences only midway through gestation at embryonic day 8 or day 9, initially in cells of the trophoblast and visceral endoderm [29].



The ubiquitous expression of B-type lamins led to the conclusion early on that these were essential proteins. This has certainly turned out to be true of lamin B1, at least at the organismal level. Gene targeting experiments in mice have revealed that lamin B1 is required for the development of viable embryos [33]. However, mouse embryonic fibroblasts derived from *Lmnb1*-null embryos can nevertheless be maintained in culture. Evidently this protein is dispensable in certain cell types. This observation is supported by findings that HeLa cells depleted of lamin B1 [34] and/or lamin B2 using RNA interference continue to proliferate in culture, at least in the short term (Kyle Roux, Melissa Crisp and Brian Burke, unpublished observations).

Lamin A and lamins B1 and B2, feature C-terminal CaaX motifs (where **C** is cysteine, **a** is an aliphatic amino acid and **X** is usually a hydrophobic residue). The CaaX motif was originally described in small Ras-related GTPases and represents a site of farnesylation [35]. This modification is mediated by a protein farnesyl transferase and occurs on the CaaX cysteine residue soon after completion of lamin synthesis [36-39]. Farnesylation is followed by C-terminal proteolysis to remove the aaX residues [40]. Processing of the CaaX motif is then completed by carboxy methylation of the newly exposed C-terminal cysteine residue [40]. Farnesylation of the CaaX cysteine residue is a prerequisite for the efficient assembly of newly synthesized lamins into the interphase nuclear lamina [41-43]. While the B-type lamins remain permanently farnesylated, lamin A is unique in that this modification is lost following proteolytic cleavage 14 residues upstream from the farnesylated cysteine [44]. This cleavage event is catalyzed by ZmpSte24, a membrane associated proteinase [45, 46], and occurs soon after incorporation into the nuclear lamina, typically within 30-60min of synthesis [47]. In this way, full length, or pre-lamin A exists only transiently in normal cells.

While individual lamin monomers are known to assemble to form coiled-coil homodimers, the higher order organization of the lamina is still a topic of considerable debate. As members of the IF family, the lamins are thought to be organized in the form of filaments. Certainly this has been borne out in ultrastructural studies of *Xenopus* oocyte nuclear envelopes where the lamina appears as an oftentimes orthogonal meshwork of 10nm

filaments [48]. However, the oocyte lamina is composed primarily of a single lamin isoform (lamin L3) [49]. The organization of the more complex mammalian somatic cell lamina containing lamins A, C, B1, B2 and perhaps A $\Delta$ 10, has yet to be satisfactorily addressed.

### **Nuclear membrane proteins**

Despite their numerous connections at the periphery of each NPC, the INM and ONM are biochemically quite distinct. This could be surmised even from early ultrastructural studies since the ONM, but not the INM, contains numerous bound ribosomes. Recent proteomic studies have revealed the existence of as many as 67 integral membrane proteins that are enriched in the NE. The bulk of these appear to reside within the INM [50]. The mechanism by which proteins become localized to the INM has been a topic of some debate. The consensus that has emerged is that it involves, at least in part, a process of selective retention [51-53]. In this model, proteins that are synthesized on the peripheral ER or ONM gain access to the INM by lateral diffusion via the membrane continuities surrounding each NPC. Only proteins that can interact with nuclear, other INM or lamina components will be retained and concentrated. However, recent findings that movement of membrane proteins between the ONM and INM involves an energy dependent mechanism [54] and which appears to operate at the level of the NPC, suggest that we have not yet heard the final word on INM protein sorting.

The recent identification of a number of ONM-specific integral membrane proteins has raised additional questions [55-57]. In particular, what prevents ONM proteins from simply drifting away in to the peripheral ER? The issue of ONM protein localization was originally addressed in *C. elegans*. Starr and Han [58] demonstrated that the appropriate localization of Anc-1, a very large type II ONM protein involved in actin-dependent nuclear positioning, was dependent upon Unc-84, an INM protein [58]. Localization of Unc-84 itself was found to be dependent upon the single *C. elegans* lamin [59]. Based upon these and similar findings, both Lee et al. and Starr and Han [59, 60], proposed a model in which Unc-84 and Anc-1 would interact across the PNS via their respective luminal domains. In this way, Unc-84 would act as a trans-luminal tether for Anc-1 in the ONM.

In mammalian cells two giant (800-1000kDa!) actin binding proteins have been identified (variously termed NUANCE, nesprin 2 Giant, nesprin 1, enaptin, Syne 1, syne 2, myne 1) as integral proteins of the ONM [55-57, 61, 62]. Due to a complex array of alternatively spliced isoforms a very large family of proteins are encoded by the *nesprin 1* and *nesprin 2* genes[56]. Nesprins are related to Anc-1, as well as to a *Drosophila* ONM protein known as *Klarsicht* [63-66] All three proteins contain an ~60 amino acid C-terminal KASH domain (Klarsicht, Anc-1, Syne Homology) that is comprised of a single transmembrane anchor and a short segment of about 40 residues that resides within the PNS.

A third nesprin family member, nesprin 3, has also been described [67]. Like nesprins 1 and 2, nesprin 3 possesses a C-terminal KASH domain. However, its distinct N-terminal cytoplasmic domain features a binding site for plectin, a very large (466kDa) intermediate filament-associated protein. Thus, whereas nesprins 1 and 2 connect the NE to microfilaments, nesprin 3 may function as a link between the NE and the cytoplasmic intermediate filament network.

One of the defining features of the *C. elegans* Unc-84 protein is a 200 amino acid region of homology with Sad1p, an *S. pombe* polypeptide that is associated with the spindle pole body [68]. This region of homology is known as the SUN domain (for Sad1p, Unc-84) and extends into the PNS. Mammalian cells contain several SUN domain proteins. Indeed there are five that are encoded within the human genome. Two of these, Sun1 and Sun2, are INM proteins and have a topology similar to that of Unc-84 with a nucleoplasmic N-terminal domain and a C-terminal SUN domain in the PNS [69-71]. At least in the case of Sun1, its nucleoplasmic domain interacts with farnesylated pre-lamin A raising the possibility that this protein could function in lamin A targeting and/or assembly [69].

Recent reports indicate that both Sun1 and Sun2 cooperate in tethering nesprin 2 Giant (nesp2G) within the ONM [69, 72]. This tethering involves the establishment of molecular interactions that span the PNS [73] similar to that suggested for Anc-1 and Unc84 in *C. elegans* (Figure 2). Circumstantial evidence based upon competition between nesprin 1 and nesprin 2 KASH domains indicates that nesprin 1 Giant (nesp1G, enaptin) is similarly

tethered by Sun1 and Sun2. It follows, therefore, that Sun1 and Sun2 function as links in a molecular chain that connects the actin cytoskeleton, via giant nesprin proteins, to nuclear lamins and other components of the nuclear interior. We now refer to this assembly as the LINC complex (for LInker of Nucleoskeleton and Cytoskeleton) [69]. The recent discovery of nesprin 3 as a link to the IF system [67], suggests that there maybe multiple functionally distinct isoforms of the LINC complex that are responsible for integrating the nucleus with different components of the cytoskeleton. The implication of the existence of these linkages across the NE is that the nucleus and cytoplasm may display interdependent mechanical properties. As will be discussed below, this has recently been shown to be the case.

### **The nuclear envelope and muscular dystrophy**

X-linked EDMD was the first human disorder to be linked to defects in a component (emerin) of the NE. Emerin is a type II transmembrane protein that localizes exclusively to the INM [7-9]. The bulk of its mass resides within its 220 amino acid N-terminal nucleoplasmic domain. The majority of emerin mutations, either point or nonsense mutations, that are associated with EDMD lead to complete loss of the emerin protein or to its mislocalization [74-77]. It would appear that EDMD must be caused by loss of some essential emerin function. The nature of this function, however, is still a matter of debate. Although emerin is expressed in the majority of adult cell types, only skeletal and cardiac muscle seem to be adversely affected by its loss.

Detailed analyses of the emerin protein both *in vivo* and *in vitro* have revealed that the nucleoplasmic domain of emerin interacts with multiple nuclear proteins [78-82]. The N-terminus of emerin shares a sequence of about 40 amino acids with several other proteins including the INM proteins lamina associated polypeptide 2 (LAP2) and MAN1. This region of homology, known as the LEM domain (for LAP2, emerin and MAN1) functions as a binding site for BAF (barrier to autointegration factor) a small DNA binding protein [83, 84]. In this way BAF functions as a link between emerin and chromatin. Emerin also binds to several transcriptional regulators, germ cell less (GCL) [80] and Btf [78]. Binding of GCL and BAF to emerin are mutually exclusive [80]. Btf when overexpressed induces apoptosis. Thus sequestration of Btf by emerin could potentially have an anti-apoptotic function. In

terms of regulatory activities, emerin might also modulate pre-mRNA processing through interactions with YT521-B, a factor involved in splice site selection [82].

In addition to these regulatory molecules, emerin interacts with a number of structural proteins including actin and A-type lamins. Emerin has been shown to promote the polymerization of actin and to cap the pointed end of actin filaments *in vitro*. It likely binds nuclear actin *in vivo*. As will be described further below, the interaction with A-type lamins contributes to the appropriate localization of emerin to the INM.

Studies on the role of emerin *in vivo* have so far shed only a little light on the etiology of X-linked EDMD. In *C. elegans*, depletion of the emerin orthologue by RNA interference yields no detectable phenotype [85]. Emerin depletion is however, synthetic lethal with depletion of MAN1, which like its mammalian orthologue, is also a LEM domain protein [85]. Mice harboring a deletion of the emerin gene have no overt symptoms of muscular dystrophy and display no obvious skeletal or cardiac muscle pathology [86]. However, in common with human EDMD patients, fibroblasts derived from emerin deficient mice often have irregularly shaped nuclei featuring blebbing of the nuclear membranes [87]. Furthermore, emerin deficient fibroblasts exhibit impaired signaling responses to mechanical stress. This may be observed in terms of reduced induction of *iox-1* and *egr-1*, a pair of mechanosensitive genes [87]. The overall effect is that emerin-null cells, when compared with wild type cells, display increased rates of apoptosis when subjected to mechanical strain [87].

Several years after the identification of the emerin as the site of mutations causing X-linked EDMD, the autosomal dominant form of the disease was mapped to the *LMNA* gene [88]. Soon thereafter, limb girdle MD 1B (LGMD1B) and dilated cardiomyopathy (DCM) were also linked to mutations in *LMNA* [89, 90]. In total more than 70 distinct mutations within *LMNA* have now been linked to skeletal and cardiac muscle diseases (Figure 3). A few rare mutations which cause autosomal recessive EDMD have also been mapped to *LMNA*.

Muscular dystrophy (EDMD and LGMD1B) and cardiomyopathy are only three of at least 11 (depending upon definition) other disorders linked to mutations in the *LMNA* gene [91]. These diseases, which are commonly referred to as “laminopathies”, include Dunnigan type familial partial lipodystrophy (FPLD) [92, 93], Charcot-Marie-Tooth disease (CMT2) [94], mandibuloacral dysplasia (MAD) [95], restrictive dermopathy (RD) [96] and two premature aging syndromes, Hutchinson-Gilford progeria (HGPS) [97-99] and atypical Werner’s syndrome (aWRN) [100, 101]. A major goal now is to determine how mutations in the widely expressed *LMNA* gene can give rise to such a bewildering array of tissue specific disorders. What is becoming increasingly clear is that multiple disease mechanisms must be at work [12, 102, 103]. A complete discussion of the laminopathies is beyond the scope of this review. therefore the remainder of the article will focus primarily on the molecular causes of EDMD, LGMD1B and DCM.

Dilated cardiomyopathy is a feature of both EDMD and LGMD1B. These two disorders differ only in terms of the affected muscle groups (e.g. distal versus proximal leg muscles). Evidently, certain *LMNA* mutations may cause heart disease (DCM) but spare skeletal muscle. However, either additional genetic or environmental factors may tip the balance towards skeletal muscle involvement [104]. This view is supported by observations of Brodski et al who have identified a family carrying a single lamin A mutation (a frameshift caused by a single nucleotide deletion at position 959) where different members have been diagnosed with EDMD, LGMD or with DCM [105]. The bulk of *LMNA* mutations associated with muscle disease involve single amino acid changes, although deletions, frameshifts (above) and nonsense mutations are well represented (Figure 3). Indeed the earliest report linking *LMNA* to autosomal dominant EDMD highlighted a nonsense mutation at codon number six [88]. This would in effect represent a functional gene deletion, thus *LMNA* haploinsufficiency is sufficient to cause muscle disease.

Mutations associated with muscle disease are found throughout the *LMNA* gene, including many in exons encoding the coiled-coil domain. These may prevent dimer formation or alter lateral interactions between A-type lamins thereby interfering with the assembly of higher order lamin structures. It is certainly true that some (but by no means all) muscle disease-

linked lamin mutations cause abnormalities in A-type lamin assembly at the nuclear periphery [106-108]. The non-helical lamin A tail is known to be comprised in part by an immunoglobulin type fold consisting of apposed beta sheets [109, 110]. Those EDMD-associated mutations found in this region of the lamin A molecule are predicted to significantly disrupt the 3D organization of the Ig domain. Taken together, these observations are consistent with the notion that muscle pathologies are linked to loss of structurally functional lamin proteins.

Homologous recombination has been used to eliminate the *Lmna* gene in mice [111]. Animals homozygous for the deletion are born without any striking abnormalities, indicating that A-type lamin expression is not a prerequisite for normal embryonic development. However, newborn *Lmna*-null mice fail to thrive and display clear evidence of muscle weakness. Histological analyses reveal extensive muscular dystrophy and cardiomyopathy. These animals, which never survive beyond eight weeks of age, display a syndrome that is very similar to human EDMD. There are, however, two important differences. The first is that *Lmna*-null mice display a peripheral axonal neuropathy similar to CMT2 (in humans this is caused by an R298C mutation) [112]. There is no evidence of any other laminopathy-related pathology in these knock out animals. Secondly, heterozygous animals (*Lmna*<sup>+/-</sup>) are asymptomatic [111]. As described above, loss of just one *LMNA* allele in humans is associated with EDMD. Regardless of these differences, however, the mouse studies lend considerable support for the view that it is loss of A-type lamin function that underlies the skeletal and cardiac muscle pathologies.

At the cellular level, loss of A-type lamin expression is associated with prominent changes in nuclear morphology [111]. In both fibroblasts from *Lmna*-null mice as well as cells from laminopathy patients [113], nuclei are frequently observed to have highly irregular shapes. This is usually associated with the appearance of NE “herniations” in which B-type lamins, INM proteins, NPCs and chromatin are withdrawn from one pole of the nucleus. This is accompanied by localized dilation of the ONM in the NPC free regions. Changes in heterochromatin organization, particularly loss of peripheral heterochromatin, are also

observed [114]. Given that heterochromatin is generally transcriptionally silent, loss of A-type lamins could be associated with changes in gene expression patterns.

A final conspicuous feature of fibroblasts derived from *Lmna*-null mice is the frequent mislocalization of emerin from the INM to the peripheral ER [111]. Introduction of human lamin A into these cells by transfection will restore the normal localization of emerin to the INM. Similar mislocalization of emerin can be observed in HeLa cells following depletion of A-type lamins by RNA interference [34]. These observations clearly demonstrate that A-type lamins contribute to the normal localization of emerin and provide a molecular relationship between X-linked and autosomal EDMD.

Mutations of titin are associated with multiple MD disorders where conventional thought suggests its role as a structural protein of the muscle sarcomere is presumably disrupted [115]. However, a recent report by Zastrow et al. identified nuclear titin as a binding partner for A- and B-type lamins [116], making titin the third NE protein mutated in MD. It remains to be seen if perturbation of titin function at the NE might underlie some aspects of disease pathology within muscle cells.

At present there are two views of how defects in NE proteins might give rise to skeletal and cardiac muscle disease, and involve either mechanical stress or gene expression based models [102] [117]. As will be seen, however, these views are not mutually exclusive. The mechanical stress model proposes that muscle cell nuclei lacking functional A-type lamins or emerin may be excessively prone to mechanical damage caused by repeated cycles of muscle contraction. This notion has some merit. Firstly, we know that nuclei containing either defective A-type lamins or depleted of A-type lamins exhibit structural abnormalities. Furthermore, nuclei isolated from *Lmna*-null mouse livers are far more prone to fragmentation than their wild-type derived counterparts [111]. Recently Lammerding et al. [118] and Broers et al. [119] have demonstrated exactly such nuclear fragility in *Lmna*-null fibroblasts *in vivo*. Basically these investigators used direct mechanical methods to deform nuclei in cells in culture. They were able to show that the nuclei in *Lmna*-null fibroblasts were more deformable and ruptured under lower applied forces than nuclei in wild-type



fibroblasts. Lammerding et al also demonstrated that the cytoplasm of *Lmna*-null cells was mechanically less resilient than that of wild-type cells [87]. This begins to make some sense given that we now know that nuclei are coupled to both the actin and IF cytoskeleton via the LINC complex of SUN proteins and nesprin proteins [56, 57, 67, 69, 72]. In muscle cells actin filaments and desmin IF filaments are linked to dystroglycans and sarcoglycans at the cell surface via contacts with dystrophin and other dystrophin interacting proteins. This raises the possibility that in EDMD and related myopathies, mechanical stress might have detrimental effects on both nuclear and cytoplasmic (and perhaps plasma membrane) organization. In this respect, the etiology of EDMD could have more in common with that of Duchenne and related MDs than immediately meets the eye.

The alternative view of EDMD, LGMD1B and DCM pathology proposes that changes in NE organization due to emerin or lamin mutations might lead to changes in muscle cell gene expression patterns. Both emerin and A-type lamins are known to interact with a number of transcriptional regulators including GCL and Btf (in the case of emerin) [78, 80] and SREBP [120], Rb [121] and MOK2 (in the case of lamin A) [122]. Furthermore, the well documented rearrangements in heterochromatin organization would be entirely consistent with this idea. Compelling evidence in favor of the gene expression model comes from several quarters. As described above, emerin deficient mice exhibit no overt pathology. However, muscle regeneration in these mice show clear abnormalities. In particular, myogenic differentiation is delayed, a phenomenon that is associated with perturbations in transcriptional pathways that are regulated by Rb and MyoD [86]. Similarly, lamin A mutants have been found to interfere with the differentiation program of C2C12 myoblasts [123, 124].

Both mechanical stress and gene expression defects may be integrated in other observations. *Lmna*-null fibroblasts exhibit grossly impaired mechanotransduction and decreased viability under mechanical strain [118, 119]. Induction of the mechanosensitive genes, *iex-1* and *egr-1* is strongly attenuated [118]. Similar but milder effects are observed in *emerin*-null cells [87]. *Lmna*-null cells (but not *emerin*-null cells) also exhibit reduced NF- $\kappa$ B-regulated transcription in response to either cytokine or mechanical stimulation [118]. Taken together, all of these observations suggest that *emerin*- and *Lmna*-null cells exhibit

reduced viability when subjected to mechanical stress. This reduced viability may be due to direct mechanical effects (i.e. physical damage), inability to induce mechanosensitive genes or indeed to both. In this way both the mechanical stress and gene expression models may accurately describe different aspects of the molecular basis of EDMD and related myopathies.

Clearly we have come a considerable way in improving our understanding of NE biology since the original realization that X-linked EDMD was caused by defects in an INM protein. However, the recognition that at least nine or ten more human diseases are linked to defects in A-type lamins has raised puzzling questions. How for instance can defects in a near ubiquitously expressed gene give rise to such an array of tissue specific phenotypes? The answer may well lie with other proteins that are themselves expressed in a tissue specific fashion but which interact with A-type lamins. Some such proteins may be represented among the sixty or more nuclear membrane proteins identified using proteomics approaches. Given the role of the LINC complex in integrating the nucleus with the cytoskeleton a better understanding of some laminopathies may even arise through studies of NE-associated cytoplasmic proteins. What is certain is that this area of research at the interface of medicine and cell biology will continue to present us with surprises that redefine our understanding of cellular structure and function while hopefully revealing novel avenues for disease therapy.

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## Figure Legends

### Figure 1

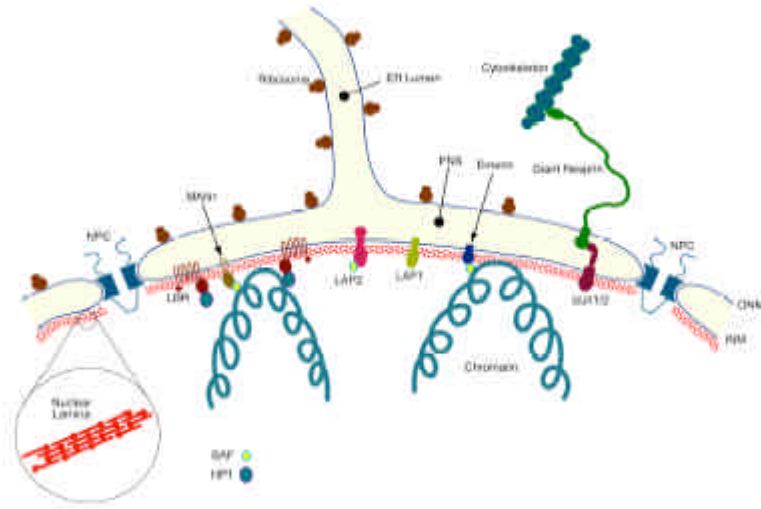
Overview of the organization of the nuclear envelope. Several selected protein components are shown. These include the nuclear lamina composed of lamin oligomers, and both inner and outer nuclear membrane (INM and ONM) proteins. INM proteins include lamin B receptor (LBR), lamina associated proteins 1 and 2 (LAP1 and LAP2), MAN1, emerin and Sun1. The latter acts as a tether for Nesprin 2 in the ONM through interactions which span the perinuclear space (PNS). The latter is continuous with the ER lumen. Soluble proteins, heterochromatin protein 1 (HP1) and barrier to autointegration factor (BAF) interact with the nucleoplasmic domains of certain INM proteins and provide a link for chromatin domains at the NE. Chromatin domains are also anchored to the nuclear lamina.

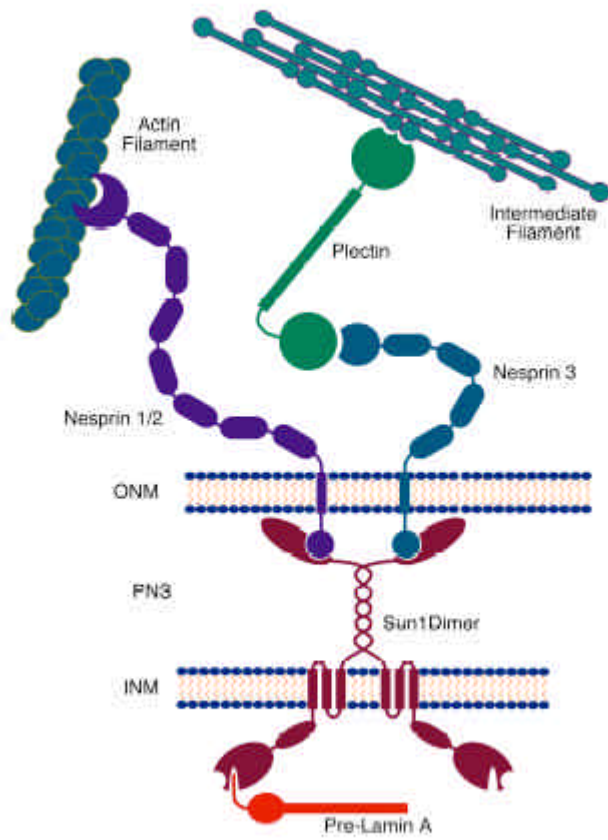
### Figure 2

Sun1 protein dimers in the inner nuclear membrane (INM) function as tethers for nesprin proteins in the outer nuclear membrane (ONM). Dimerization of Sun1 is likely mediated by an extended coiled-coil domain within the perinuclear space (PNS). The nucleoplasmic domain of Sun1 binds farnesylated pre-lamin A. The cytoplasmic domain of nesprins 1 and 2 interacts with actin filaments whereas that of nesprin 3 binds plectin. The latter is likely to provide a link to the cytoplasmic intermediate filament (IF) network.

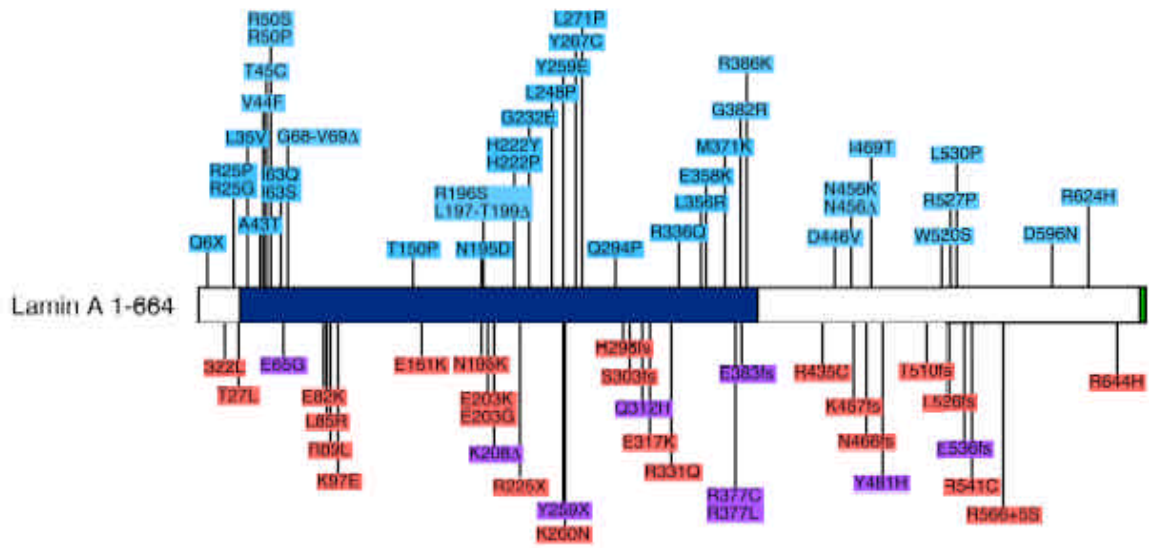
### Figure 3

Myopathy related mutations within human lamin A. The position and nature of EDMD mutations are indicated by blue boxes. Dilated cardiomyopathy (DCM) mutations are indicated in orange. LGMD1B mutations are indicated in purple. Substitutions are in single letter code. X represents a nonsense mutation, fs refers to a frameshift and a deletion is indicated by  $\Delta$ . The lamin A central coiled-coil domain is shaded in dark blue, while the C-terminal CaaX box is in green. This figure was compiled from the Leiden University muscular dystrophy pages and lamin A/C sequence variation database ([http://www.dmd.nl/lmna\\_seqvar.html](http://www.dmd.nl/lmna_seqvar.html)).





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