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# The structure and function of nuclear lamins: implications for disease

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**Abstract.** The nuclear lamins polymerize to form the nuclear lamina, a fibrous structure found on the inner face of the nuclear membrane. The lamins also appear to form structures within the nucleoplasm. These various lamin structures help to establish and maintain the shape and strength of the interphase nucleus, but recent work also suggests that the lamins have a role in nuclear processes such as DNA replication. Furthermore, mutations in the human lamin A/C gene have recently been linked to several diseases, including Emery-Dreifuss muscular dystrophy. This review discusses the nature of these mutations and the possible effects of lamin mutations on nuclear function.

**Key words.** Nuclear lamin; Emery-Dreifuss muscular dystrophy; DNA replication.

## Introduction

The nuclear lamina is often considered to be a static exoskeleton that serves primarily to give the nucleus shape and strength. However, the nuclear lamins, the major proteins that form the lamina, also play a role in a number of nuclear processes including DNA replication. Recent work using gene disruption and RNA interference demonstrate that the lamins are essential for life. The impulse for elucidating the functions of lamins has increased with the surprising discovery that mutations in the human lamin A/C gene are found in patients with several inherited diseases. These include Emery-Dreifuss muscular dystrophy, EDMD [1], partial lipodystrophy [2] limb girdle muscular dystrophy and dilated cardiomyopathy/conduction system diseases [3]. In this review we discuss the role(s) of the lamins in nuclear processes. We also summarize the human mutations that correlate with disease and speculate on the effect of these mutations on nuclear functions.

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## The lamin gene family

Nuclear lamins have been found in all metazoans examined although they have not been identified in any unicellular organisms. The genome of *Saccharomyces cerevisiae* does not contain a lamin gene. There is some immunological evidence for nuclear lamins in plants although the genes have not been cloned [4].

The nuclear lamins are closely related to the cytoplasmic intermediate filaments (IFs) and have the typical domain structure of IFs [5, 6]. In fact, analyses of lamin and cytoplasmic IF sequences from invertebrates suggest that the nuclear lamins were the progenitor IF and cytoplasmic sequences arose through gene duplication of the lamin sequence [7]. The central rod domain of the lamins (approximately 360 amino acids) consists of a heptad repeat that is characteristic of proteins forming an $\alpha$ helix. In vertebrates, the lamin rod contains six heptads that are absent from the cytoplasmic IF [8]. As in cytoplasmic IF, the lamin rod domain contains the short sequences within the rod (linker regions) that do not have heptad repeats. However, unlike the cytoplasmic sequences, the lamin linker regions do not contain proline residues that introduce a stutter in the $\alpha$ helix and, therefore, it is likely that the lamin rod domain is a continuous helix [8, 9]. The rod

domain drives the dimerization of lamin protein chains. It is also essential in higher order interaction between dimers [10]. The rod domain is flanked by a non-helical N-terminal (30–40 amino acids) and C-terminal domains (170–265 amino acids). These domains are not well conserved when compared to other IFs. The C-terminal domain contains a nuclear localization sequence (NLS) for nuclear transport. A chromatin binding site has also been mapped to the C-terminal domain [11]. In most lamin isotypes, a C-terminal cysteine that undergoes isoprenylation is present in a CaaX motif at the very C-terminus of the molecule [11].

Most invertebrates appear to have a single lamin gene that is transcribed to produce a single messenger RNA (mRNA)/lamin protein. The exception is *Drosophila melanogaster* which contains two genes, each of which is transcribed to produce a single mRNA [12, 13]. In vertebrate species, there are three or four lamin genomic sequences. In *Drosophila* and in vertebrates, the lamin multigene family is divided into A and B types [11]. The B-type lamins are expressed constitutively while the A-type lamins are expressed later in development. For example, lamin A is not expressed in the mouse brain until several days after birth [14]. Alternative splicing of the lamin genes increases the number of lamin gene products and recent work suggests that some of the newly-identified lamins have tissue-specific functions (see fig. 1). In humans, there are at least three different mRNAs transcribed from the single lamin A type gene on chromosome 1 [11]. Lamins A and C are identical except lamin A has a unique 90 amino acid region at its C-terminus, whereas lamin C has a unique 6 amino acid sequence [6]. Lamin C lacks the C-terminal cysteine that is isoprenylated in lamin A. These isotypes are usually expressed in approximately equal amounts in differentiated, somatic cells but their relative expression levels can differ in some tumors [15]. The variant lamin A$\Delta$10 has a 30-amino acid deletion in the lamin A-specific part of the carboxy-terminal domain and is found in normal and tumor cells [16]. There are two human somatic B-type lamins, B1 and B2, encoded by separate genes on chromosome 5 and 19 respectively. These two isotypes are expressed in various ratios in different tissues [14].

In rodents, two sperm-specific lamins have been identified. Both are products of alternative splicing events that result in a shorter rod domains. Lamin C2 is a male germ cell variant where the N-terminal 86 amino acids of lamin C are replaced by 6 unique amino acids [17]. Alternative splicing of RNA encoded by the B2 gene gives rise to B3, a male germ cell specific variant [18]. In lamin B3, the N-terminal 208 amino acids of lamin B2 are replaced by a unique 85 amino acid insertion (see fig. 1). It is very likely that these isotypes are also present in humans. Interestingly, these sperm-specific lamins may have a role in the distinctive nuclear shapes seen in sperm cells [18]. In particular, lamin C2 seems concentrated at sites where sperm chromatin contacts the nuclear envelope, suggesting it may have a role in organizing sperm chromatin [19].

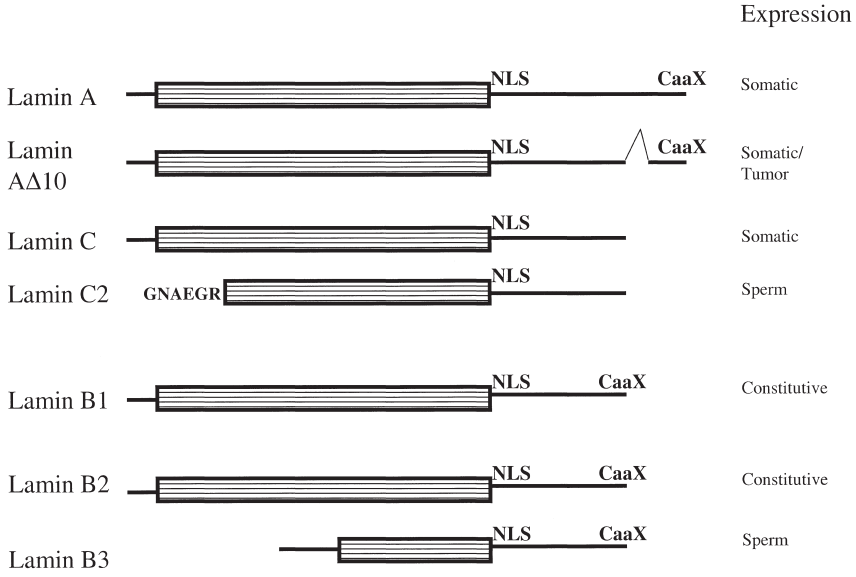


Figure 1. Schematic drawing of the different lamin isotypes found in mammalian tissues. In mammals, seven different lamin proteins have been identified. Lamins A, C, A$\Delta$10 and C2 result from differential splicing of the lamin A gene. Only one transcript has been identified for the lamin B1 gene. Lamins B2 and B3 result from transcription of the B2 gene. The rod domain ($\alpha$ helical region) is indicated by a rectangle filled with horizontal lines. The N-terminal and C-terminal nonhelical domains are presented as thick lines. The unique six amino acids that form the N-terminal domain of lamin C2 are shown using the single-letter amino acid code. The nuclear localization signal (NLS) and isoprenylation motif (CaaX) are shown in the C-terminus. Lamins C and C2 lack the CaaX motif.

In chickens, a single mRNA transcript has been identified for each of the lamin B1, B2 and lamin A genes. Similarly, a single transcript has been identified for each of three B-type genes and one lamin A-type gene in *Xenopus laevis***.** A sperm-specific lamin has been identified in Xenopus but it has not been assigned to a particular gene [56]. It is likely that the identification of splicing variants will increase the number of lamin proteins expressed in these species. For example, a splicing variant of the *Xenopus* lamin B3 (not equivalent to mammalian B3) has been recently described where the last 12 amino acids are replaced with a different 12 amino acid sequence [20]. The B3 originally described by cDNA sequencing has a single cysteine which is farnesylated. The recently described variant has a second cysteine residue in this region which is palmitoylated. This modification appears to allow a stronger association with membrane vesicles during nuclear breakdown.

## The functions of the nuclear lamins

### Lamins and nuclear shape during interphase

During interphase, the nuclear lamina supports the nuclear membrane and is involved in determining the shape and the mechanical strength of the nucleus. This is strongly supported by observations of cells carrying lamin gene knockouts. For instance, a knockout of the lamin A gene in mouse, results in cells with elongated nuclei and an apparent nuclear fragility in adult tissues [21]. A *Drosophila* mutant that dramatically reduces the level of lamin $Dm_0$ (a B-type lamin) expression results in defective nuclear envelopes in some tissues [22]. In *Caenorhabditis elegans*, a reduction in lamin expression by RNA interference (RNAi) techniques causes changes in nuclear shape [23]. Finally, nuclei assembled in the *Xenopus* extract after the depletion of the majority of the lamins (a biochemical knockout) are smaller and more fragile than nuclei assembled in normal extracts [24, 25].

The addition of variant or mutant lamins also results in changes in nuclear shape. The transfection of the mouse sperm-specific lamin (B3) into somatic cells results in a elongated nuclear shape like that seen in the sperm nucleus [18]. Similarly, lamin mutants can disrupt lamin organization both when added to nuclei assembled in vitro and when injected into mammalian cells [26]. This treatment alters nuclear shape and appears to decrease the overall strength/ robustness of the nuclei. Together, these observations support the view that nuclear lamina supports the nuclear envelope, influencing both the shape of the nucleus and its mechanical strength. This function for the lamina provides an obvious potential mechanism for the development of muscular dystrophy in people carrying mutations in lamin genes. The mutations may compromise lamina assembly and resulting in reduced nuclear stability (see below).

### Establishment of nuclear shape and structure

Although the nuclear lamins appear to be essential for maintaining the stability of the interphase nucleus, the role of the lamins in reestablishing the nuclear/cytoplasmic boundary following mitosis is controversial. In one view, the lamins have an essential role in the initiation of nuclear envelope assembly through their own self-assembly and through interactions with other molecules [27]. In an alternative view, the lamins are not required for envelope assembly and are imported into the nucleus only after a continuous membrane complete with pore complexes has assembled [24]. Particularly compelling evidence for lamin involvement in envelope assembly comes from the *Drosophila* lamin gene mutation that results in a reduction in the amount of lamin present in the flies [22]. This reduced lamin expression is accompanied by the formation of cytoplasmic clusters of nuclear pores (annulate lamellae) as well as defective nuclear envelopes. In *C. elegans*, the reduction of the amount of lamin by RNAi has a dramatic effect on ability of cells to exit mitosis properly and reassemble nuclei [23].

Other evidence both for and against a prominent role for lamins in nuclear membrane formation comes from immunoadsorbtion experiments. In extracts of mammalian cells or *Drosophila* embryos, nuclear assembly is prevented when lamin antibodies are added to immunodeplete lamins or block lamin function, suggesting a critical role for lamins in envelope assembly [28–33]. In extracts prepared from *Xenopus* eggs, lamin antibodies have blocked membrane assembly in some cases but not in others [24, 25, 33]. These contradictory results have been proposed to be due to variations in the efficiency of immunodepletion of the lamin protein [11].

The results are also contradictory when the timing of lamin targeting to chromatin after mitosis is examined. In some immunofluorescence experiments, lamin A appears to associate with chromatin in late anaphase or early telophase, simultaneously with other envelope markers, suggesting an involvement in envelope assembly [34]. Similarly, in living cells GFP-laminB1 accumulates early in telophase, as chromatin is decondensing [35]. In other immunofluorescence experiments, lamins appear to accumulate on chromatin well after other markers such as the lamin B receptor [36]. In addition, when nuclear assembly in *Xenopus* extracts is followed by scanning electron microscopy and immunofluorescence, lamina assembly appears to occur after membrane enclosure and pore assembly [37]. Finally, when lamin A is followed in living cells using a fusion with the green fluorescent protein (GFP), the GFP-lamin A appears to begin accumu-

lating during in cytokinesis after nuclei have reassembled [38]. These latter experiments suggest that the lamins do not have a role in the initial steps of nuclear envelope enclosure.

It has proposed that these contradictory results can be reconciled if only a fraction of the total lamin complement is required in the initial stages of envelope assembly [40]. This fraction may be difficult to detect by microscopic methods and may not be depleted by some immunoadsorbtion protocols. In fact, nuclei assembled in *Xenopus* extracts following lamin immunodepletion do contain lamin protein (as assayed by Western blotting) that is difficult to detect by immunofluorescence [39]. In this model, assembly of a nuclear lamina is not required for nuclear membrane formation. Instead, lamin interactions with chromatin and with other proteins of the nuclear envelope may be essential in establishing the structure of the nuclear envelope.

This model for lamin function during nuclear assembly is supported by the sequence of events that occur in the in the *Xenopus* extract. In this system, approximately 98% of the lamins are soluble rather than membrane-bound [40]. A small fraction of the soluble lamins have been reported to associate with condensed chromatin before the reassembly of the nuclear envelope [37]. Inhibitors that block nuclear membrane fusion do not prevent this association. The remaining 2% of lamins are associated primarily with a population of membrane vesicles termed the fusogenic fraction. During nuclear assembly, the fusogenic fraction binds to chromatin after the non-fusogenic fraction [41]. Subsequently, nuclear membrane fusion occurs between the two vesicle populations and membrane closure results.

The presence of chromatin-bound and vesicle-bound populations of lamins suggests a model for lamin involvement in nuclear envelope closure. Interactions of the fusogenic population with chromatin and/or with the non-fusogenic population could be facilitated by the interactions of the two populations of lamin molecules (see fig. 2). Furthermore, the interaction of the vesicles and chromatin certainly involves other proteins. The lamins could also act to bridge vesicles and chromatin by interacting with these molecules. There is now large family of lamin-associated proteins (LAPS) some of which are involved in this process. For example, fragments of LAP2$\beta$, a membrane-bound form of the protein, inhibit nuclear assembly when added to the *Xenopus* extract possibly by disrupting normal lamin-LAP2 interaction during nuclear assembly [42]. The lamins also interact with NUP153, a nucleoporin, and disruption of the lamina with mutants prevents NUP153 incorporation into the pore, suggesting lamins are also required for proper pore assembly [43]. A detailed description of the interactions between lamins and other molecules will be required to define the role of lamins during nuclear assembly.

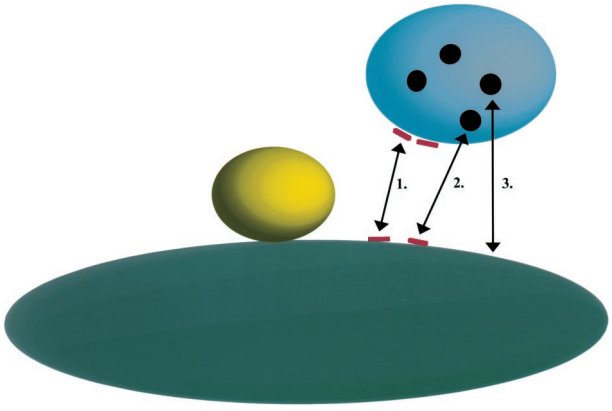


Figure 2. Schematic drawing representing possible interactions that mediate membrane targeting during nuclear assembly. The model is drawn largely from data obtained in vitro with *Xenopus* egg extracts (see text). Chromatin is presented as a green ellipse. The non-fusogenic vesicle population (yellow) is shown bound to chromatin. The binding of the non-fusogenic vesicles to chromatin may be facilitated by three types of interactions: (i) Chromatin-bound lamin (red) may interact/assemble with vesicle-associated lamin (red) to promote vesicle association. (ii) Chromatin-bound lamin (red) may interact with lamin-associated proteins (LAPs; black circles). (iii) Lamin-associated proteins (black circles) may interact directly with chromatin.

## Nuclear lamins and DNA replication

Nuclei assembled in a *Xenopus* extract from which the majority of the lamins have been immunodepleted have an unexpected phenotype. Although these nuclei are competent for nuclear transport and have intact nuclear envelopes, they fail to replicate DNA [24, 25]. These results suggest that proper lamin organization is required for DNA replication to occur. Furthermore, DNA replication is also prevented when lamin mutants lacking the amino terminal domain ($\Delta$N) are added to normal, non-depleted extracts during nuclear assembly [26]. These fragments have altered in vitro assembly properties and appear to act as a dominant negative mutants to disrupt the endogenous lamin organization, either when added to the *Xenopus* extract or microinjected into cells [26]. The block in DNA synthesis is not due to incomplete nuclear assembly as replication is also blocked when lamin organization is disrupted by $\Delta$N fragments after nuclei have assembled [48]. The involvement of lamins in DNA synthesis is also suggested by the findings that different fragments of LAP2 (a lamin associated protein) can block or enhance DNA replication. For example, LAP2 fragments that lead to an accumulation of lamin in the nucleus also lead to increased replication efficiency [42].

Two other intriguing examples suggest that lamin dynamics correlate with DNA replication. In early *Xenopus* development, the cell cycle is only 30 min in length and DNA replication begins on individual chromosomes before mitosis is complete [44]. In this case nuclear lamins

(and probably other envelope components) begin to assemble around each chromosome in anaphase before replication begins in early telophase. In the second example, lamin assembly around double minute chromosomes is correlated with the replication of these structures [45, 46]. The double minute chromosomes are present in the cytoplasm and during S phase the minichromosomes become surrounded by lamins and are replicated. The findings that these special cases of DNA synthesis are accompanied by unusual timing or subcellular localization of lamin assembly further supports the involvement of lamins in DNA replication.

In *C. elegans*, the ablation of lamin expression by RNA interference techniques results in numerous phenotypes, including the improper segregation of chromosomes. This could be the result of defects in DNA replication [23]. The genetic tools available in this system may allow the identification of molecules that interact with lamins during replication.

Although proper lamin organization appears to be required for replication to proceed, the exact role of the lamins is not clear. One explanation is based on the involvement of lamins in the maintenance of nuclear envelope integrity [47]. Selective transport across the envelope allows a high concentration of replication factors to be established and maintained in the nucleus. In this model, the loss of normal lamin structure would lead to breaches in the nuclear envelope, which would result in a drop in the nuclear concentration of replication factors. Indeed, nuclei assembled in lamin-depleted extracts are fragile and subject to rupture under mechanical strain. The report of the development of a cell-free system that executes semi-conservative replication in the absence of a nuclear membrane or detectable lamin structures is also consistent with this model [47]. In this system, replication factors are very concentrated, for example cyclin E is 25-fold more concentrated in than standard egg extract. This high concentration of replication factors apparently overcomes the requirement for lamins. These results also appear to support the suggestion that the primary role for lamins in replication is to help maintain the integrity of the nuclear membrane. The principal caveat to this model is the finding that disruption of lamin organization with lamin mutants does not appear to inhibit nuclear transport or compromise the nuclear envelope [27]. These later results suggest that lamins serve another role in DNA synthesis.

Other experiments suggest that lamins are more directly involved in DNA synthesis [26, 48]. Nuclei assemble normally in extracts containing the nucleotide analog AraC, although DNA replication is blocked at the switch from initiation to elongation. This inhibition can be reversed by the addition of dCTP. However, if $\Delta$N lamin mutants are added to the extracts before the dCTP addition, replication does not resume [26, 48]. The lamin mutant disrupts the endogenous lamin network, resulting in lamin aggregates in the nucleoplasm. These aggregates also contain proliferating cell nuclear antigen (PCNA) and replication factor (RFC), two cofactors of DNA polymerase $\delta$, which is responsible for the elongation phase of replication. An effect on PCNA is also seen in lamin-depleted nuclei such that the PCNA becomes less resistant to extraction [39]. These experiments suggest that lamins are involved in the elongation phase of replication where they may facilitate the organization of DNA replication factors.

It is not clear how the nuclear lamina, a structure found at the nuclear periphery, can organize and influence replication which occurs throughout the nucleus. One possibility is that the lamina serves as a scaffold for chromatin and influences chromatin organization, thereby affecting nuclear processes. There is substantial evidence that the lamins/lamina interacts with chromatin. However, it remains to be established how this interaction translates into a functional role in replication. A second, compatible, possibility is that the lamins can exist in structures within the nucleus that interact with replication factors. Numerous examples of nucleoplasmic structures have been described [49–52]. In tissue culture, nucleoplasmic lamin B foci colocalize with sites of DNA replication and with PCNA by immunofluorescence microscopy [52]. In addition, nucleoplasmic lamin A foci colocalize with the retinoblastoma protein and with sites of DNA replication in early S phase in primary human fibroblasts [53].

In addition to reports of nucleoplasmic foci, lamin structures that occupy a small fraction of the nucleoplasm, there have also been descriptions of 10-nm filaments throughout the nucleoplasm in electron microscopic preparations. These filaments stain with lamin antibodies [51, 54, 55]. Furthermore, FRAP experiments with GFP-tagged lamins also suggest that lamins can assemble within the nucleoplasm [35]. Because photobleaching recovery of this network is relatively slow (>5 h), these structures do not represent diffusible pools of lamins but instead may be a polymer. The presence of nucleoplasmic lamin structures raises the possibility that nuclear lamins form an internal scaffold upon which nuclear processes are organized. Interestingly, the biochemical properties of nucleoplasmic lamin structures appear to differ from those of the peripheral lamina [35]. This may reflect different structures and polymeric states for nucleoplasmic lamin structures. Further investigation of these differences may provide important clues to function of these structures in DNA replication and other nuclear processes.

## Nuclear lamins and transcription

Early suggestions that nuclear lamins might have a role in transcription were based on the correlations between changes in lamin expression and altered gene expression. For example, the expression patterns of lamin isotypes change when zygotic transcription begins in the *Xenopus*

embryo [57–59]. In addition, the induction of lamin A expression during development is correlated with differentiation [14, 59].

Additional evidence suggesting that lamins may be involved in transcription centers around the observations that nuclear lamins are a major constituent of the nuclear matrix/scaffold. Transcriptionally active DNA is enriched in nuclear matrix preparations, and transcription continues in nuclear scaffold preparations after the removal of the majority of nuclear proteins and DNA [60, 61]. Furthermore, components of the transcription machinery often bind to the nuclear matrix. For example, the active phosphorylated form of RNA polymerase II associates with a nuclear matrix protein [62]. Based on these findings, some have suggested DNA becomes transcriptionally active by associating with the nuclear matrix [60]. However the matrix/scaffold preparations contain many proteins, and the wide variety of methods used in these experiments yield many different protein profiles, therefore these experiments have not conclusively demonstrated a role lamins in transcription. A few direct tests of the involvement of lamins on transcription have been attempted by inducing lamin A expression in cell lines that normally lack lamin A. Unfortunately, observations of subsequent alterations in gene expression have yielded ambiguous results [63, 64].

Recent studies of insulator or boundary elements provide additional evidence that the regulation of gene activity may involve nuclear lamins [65]. Insulators appear to regulate the effects of enhancers on promoters and have been found in a variety of eukaryotic organisms ranging from *Drosophila* to vertebrates. In addition, when a gene is placed in a new chromosomal location insulators act as barriers to the transcriptional state of the surrounding chromatin and allow independent regulation of the transgene [65]. In *Drosophila*, the protein su(Hw) is required for the function of the insulator element gypsy [66]. Recently, su(Hw) was shown to be involved in the localization of gypsy close to the lamina at the periphery of the nucleus. In contrast following heat shock when transcription of most genes is repressed, su(Hw) and gypsy containing DNA sequences are dispersed throughout the nucleus. These observations led to the proposal that gypsy insulator function may require an attachment to a substrate, possibly the nuclear lamins [67]. The reported interaction of Rb (retinoblastoma protein) with nuclear lamins also supports a role for lamins in transcription as Rb binds to the transcription factor, E2F, to repress the transcription of a number of cell cycle genes [68]. These reports and the identification of lamin mutants that result in disease indicate that a re-examination of the role of lamins in transcription is merited. These lamin mutants together with the remarkable sensitivity of gene chip technology may provide the means to directly test if normal lamin organization is a requirement for transcription.

## Mutations in the human lamin a gene lead to disease

More than 50 mutations have been identified in the lamin A genes of individuals having the autosomal dominant form of Emery-Dreifuss muscular dystrophy, EDMD [1, 69–72]. EDMD is characterized by progressive muscle wasting, contractures of elbows and achilles tendons and cardiomyopathy with conduction system disease. Although this is an inherited disease, there can be substantial variation in the symptoms of affected individuals from the same family [69]. Mutations in emerin, a protein of the inner nuclear membrane, have been identified as causative for the X chromosome-linked form of EDMD [72]. Significantly, emerin has been proposed to interact with nuclear lamins [72–75]. Moreover, lamin A gene mutations have since been identified in patients with limb girdle muscular dystrophy, dilated cardiomyopathy and lipodystrophy [2, 76–86]. There has not yet been a report of mutations in the lamin B genes leading to disease.

As lamins form a subtype of the intermediate filament (IF) family of proteins, the lamin A/disease connection has an obvious analogy with a set of diseases that are caused by mutations in the keratins, a cytoplasmic family of IFs [87, 88]. The common feature of the keratin-associated diseases is fragility of epithelial cells as a consequence of mutant keratin protein expression. This was first observed in the K5/K14 keratin gene pair where point mutations lead to one of several epidermylosis bullosa simplex (EBS) disease subtypes. The mutations result in aberrant assembly of the keratin IF network and the disruption of cell/cell and cell/substrate interactions at desmosomes and hemidesmosomes respectively [87, 88]. As a consequence, the cell's resistance to mechanical stress is reduced and skin blistering results. The mutations lie almost exclusively in the rod domain that is required for keratin assembly, and there is a strong correlation between the severity of the disease and the effect on keratin assembly in vitro [87, 88]. In the most severe form of the disease, mutations are present in residues that are highly conserved amongst all IFs and which have been shown to be essential for assembly.

As with the EBS diseases, the symptoms of Emery-Dreifuss muscular dystrophy suggest the degeneration of a tissue under great stress. In addition, mutations in other cytoskeletal proteins, including desmin and actin, have been found in types of muscular dystrophy, supporting the idea that a loss of cytoskeletal integrity ultimately leads to tissue degeneration [89, 90]. By analogy with the keratin example, the mutant lamins may act as disruptors of lamin organization.

However, unlike the keratin example, the mutations of lamin A linked to human diseases are not concentrated in regions that are thought to be essential for IF assembly, but instead are spread throughout the molecule. In the case of EDMD, mutations of the lamin A/C gene occur in the C-

terminal nonhelical domain, a region dispensable for in vitro assembly, as well as in the rod domain a region essential for assembly [1, 69–72]. The mutations are almost all single base changes that lead to point mutations, or truncation of the protein resulting from introduction of a stop codon. In some cases, there is the deletion of a single codon that retains the reading frame. There does not appear to be a correlation between the type of mutation and the clinical presentation of the disease. The mutations in limb-girdle muscular dystrophy and dilated cardiomyopathy are also found both in the rod and C-terminal domain [76]. The point mutations identified in the lipodystrophy cases are clustered in the region of the C-terminal domain common to both lamins A and C with the exception of two mutations in the C-terminal region unique to lamin A [2, 77, 82–86]. Interestingly, individuals with two mutations have a more severe case of lipodystrophy [77].

The interpretation of the consequences of lamin A mutations is further complicated by the relative lack of data on the mechanism of lamin assembly. Nuclear lamin assembly appears to differ from cytoplasmic IF polymerization. Purified lamins isolated from cells or made by expression of cDNAs in *Escherichia coli* do not assemble in vitro into 10-nm filaments as do all other IF proteins. Instead, lamins form paracrystals that appear to result from stacking of filaments [91, 92]. It is unlikely that these paracrystals exist in vivo except in unusual circumstances [94]. Instead, the lamina appears as a network of filaments underlying the nuclear envelope in the few cases where the lamina has been visualized [94].

The differences between the structures that assemble in vitro and those observed in the nucleus suggest that lamin assembly in vivo may be regulated through interactions with other molecules. In vitro studies using lamin mutants suggests that, unlike cytoplasmic IF, the C-terminal domain influences the pathway of lamin assembly [95]. This raises the possibility that the binding of LAPs to the C-terminal domain in vivo may influence lamin assembly. The consequence of mutations in the lamin C-terminal domain may be altered lamin-LAP interactions and less stable lamin assembly. This might explain the cases of lipodystrophy, EDMD, limb girdle muscular dystrophy and dilated cardiomyopathy where the mutation occurs in the C-terminus.

Changes in lamin-LAP interactions could have other consequences besides those affecting lamin assembly. It has been proposed that there are a unique set of lamin-LAP interactions in each tissue and the mutations related with each disease affect particular lamin–LAP interactions. This model assumes that disrupting lamin–LAP interactions would result in the specific alterations in gene regulation in the affected tissues [72].

## Conclusion

In summary, there appear to be two possible mechanisms to explain how mutations in the lamin A gene result in disease. In one model, the mutations cause incorrect lamin-lamin or lamin-LAP assembly, resulting in unsupported nuclear envelopes and nuclear fragility. This nuclear fragility under stress would result in the generalized loss of nuclear functions with the consequence being cell death and tissue degeneration. The specificity of mutations for a particular disease could result from tissue-specific lamin-LAP interactions that affect lamin assembly. A second model suggests that lamin-LAP interactions have functions in nuclear processes distinct from the role of the lamina in nuclear stability. For example, it has been suggested that tissue-specific LAP expression would affect gene transcription [72]. However, the lamins also have roles in DNA replication, chromatin organization and nuclear assembly and altered LAP-lamin interactions might just as likely to affect these processes.

The symptoms of the human diseases linked to mutations in the lamin A gene typically arise well after adulthood. Furthermore, the lamin A knock out mouse survives to birth before obvious symptoms are detected. Finally, cell lines that do not express A-type lamins have been successfully propagated. These observations suggest that the mutation of A-type lamins in EDMD (and other lamin-related diseases) appears to be subtly altering instead of completely blocking the nuclear functions required for cell proliferation such as DNA replication, transcription and nuclear assembly.

A number of in vitro and in vivo systems can be exploited to study the functions of the lamins and the consequences of lamin mutations. For instance, the effects of mutant lamin proteins that disrupt lamin organization on a number of nuclear functions can now be analyzed in vitro using cell free systems derived from *Xenopus laevis* eggs, mammalian cells and *Drosophila* embryos [28, 32, 96]. These effects can then be compared to those observed in cells through the use of single cell injection of protein or mRNA encoding lamin mutants. Transient transfection studies are problematic when analyzing the affects of lamin mutants as cells expressing mutant proteins that inhibit nuclear assembly, replication or transcription will be difficult to propagate. However, the emergence of more reliable inducible promoter systems should alleviate this problem. The identifications of lamin mutants that block a particular aspect of lamin function without disrupting the overall organization of the lamins would be an especially welcome aid in determining the precise roles of lamins in nuclear processes. More subtle mutations should also help identify other proteins that interact with lamins to carry out these processes.

The rapid development of genetic systems to study protein function will also allow the effects of lamin muta-

tions to be examined in vivo. The insertional inactivation of the mouse lamin A/C gene has provided a great deal of new information on lamin function [21]. The symptoms of mice carrying a targeted disruption of the lamin A/C gene are very similar to those seen in human patients [21]. Mice that completely lack lamin A /C expression (homozygotes) develop apparently normally until birth. However, after birth, these mice grow much more slowly than the normal or heterozygotes and rapidly developed gait and posture difficulties. The homozygotes die within eight weeks of birth, and analysis of tissues suggests that the mice had developed symptoms reminiscent of muscular dystrophy. Other symptoms were consistent with those found in patients suffering from other diseases linked to mutations in the lamin A/C gene such as cardiomyopathy (degeneration of cardiac muscle) and lipodystrophy (lack of white fat). The ability to culture cells from these animals provides a unique opportunity to analyze the effects of lamin A knockouts using cell biological and biochemical methods. For example, an examination of the cells from the mice lacking A-type lamins revealed changes in nuclear shape and an apparent fragility of the nucleus. Furthermore, the distribution of emerin is altered in the lamin A knockout mouse. Recently, gene knock-in technology has made it feasible to substitute a mutated gene for the wildtype version. This procedure will allow the introduction of the lamin A/C mutations involved in different diseases. Therefore it may be possible to recapitulate the tissue-specific effects of the diseases and determine the molecular interactions of lamins that altered in each disease.

Other genetic systems that have recently emerged are also promising for the dissection of the roles of lamins in the nucleus. For instance standard *Drosophila* mutagenesis techniques and the RNAi mutagenesis technique in C elegans should facilitate the identification of the factors that associate with lamins to regulate nuclear function [97].

In closing, we believe that continued analysis of roles of lamins will provide valuable insight into the causes of a dehabilitating disease. These studies should also increase our understanding of the complicated seemingly interlocking regulation of nuclear processes.

*Note added in proof:* A recent publication provides additional evidence that lamins are involved in nuclear membrane formation (Lopez-Soler et al. [2001] J. Cell Biol 154: 61–70). The C-terminal domain of Xenopus lamin B3 appeared to both inhibit lamin polymerization in vitro and block membrane formation around chromatin in situ.


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