LAMINS: BUILDING BLOCKS OR REGULATORS OF GENE EXPRESSION?

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Intermediate filament (IF) proteins are the building blocks of cytoskeletal filaments, the main function of which is to maintain cell shape and integrity. The lamins are thought to be the evolutionary progenitors of IF proteins and they have profound influences on both nuclear structure and function. These influences require the lamins to have dynamic properties and dual identities — as building blocks and transcriptional regulators. Which one of these identities underlies a myriad of genetic diseases is a topic of intense debate.

METAZOA
A major division of the animal kingdom. It includes all animals except the Protozoa and Parazoa.

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The lamins are the main architectural components of a cage-like structure termed the lamina, which is found underneath the inner nuclear membrane (INM). In the oocyte germinal vesicles of *Xenopus laevis*, the lamina is a two-dimensional lattice of interwoven filaments that interconnect nuclear pore complexes (NPCs)[1,2] (FIG. 1), although in some somatic cells it seems to be a three-dimensional structure[3]. The lamina provides strength and support for the INM[4] and differs considerably as an architectural device from cytoskeletal networks, which are typically three-dimensional and highly branched[5].

Lamins are also found at sites of DNA replication and RNA processing, and in association with replication proteins and RNA polymerases. This has led to the suggestion that lamins influence gene duplication and expression[6]. If this hypothesis is correct, it raises important questions about the way in which 'nucleoskeletal' proteins — which are expressed only in METAZOAN lineages[7] — affect nuclear metabolism (BOX 1). In this article I review what is known about the evolution of lamins and their roles in nuclear structure and function, before going on to discuss why plants and fungi do not have lamins, and how particular lamin mutations promote tissue-specific diseases.

Lamin genes and evolution

Lamins belong to the intermediate filament (IF) gene superfamily. IF proteins are expressed in almost all metazoan cells, where they form part of the cytoskeleton, but they are absent from all plants and fungi studied so far[8,9]. In mammals, the IF superfamily has about 60 members; most fall into five groups[10], four of which are cytoplasmic (I–IV) (TABLE 1). Lamins make up the type V IF family, the members of which are believed to be the evolutionary progenitors of the IF superfamily[11]. In mammals, the type V family consists of three genes that encode seven proteins (TABLE 2). These proteins are classified as A-type and B-type; A-type lamins are expressed mainly in differentiated tissues, whereas the expression of one or more B-type lamins is essential for cell viability[12–15]. Mammals, amphibians and fish all express germline-specific lamins[16,17] (TABLE 2). Invertebrates typically have fewer lamins. For example, *Drosophila melanogaster* expresses two lamins, termed Lamin Dm0 (a B-type lamin) and Lamin C (an A-type lamin) (REF. 18), whereas *Caenorhabditis elegans* expresses a single, B-type lamin, LMN-1 (also known as Ce-lamin) (REF.19) (TABLE 2).

In vertebrates, the primary sequence of the lamins differs from that of the cytoplasmic IF proteins. All IF proteins are organized around a central rod domain — also known as the α-helical coiled-coil dimerization domain — that comprises four coiled-coil domains, which are separated by flexible linker regions, and globular head and tail domains (FIG. 2). The coiled-coil domains are organized around heptad repeats, and the lamins contain an extra 42 residues (six heptads) in coil 1B when compared with vertebrate cytoplasmic IF

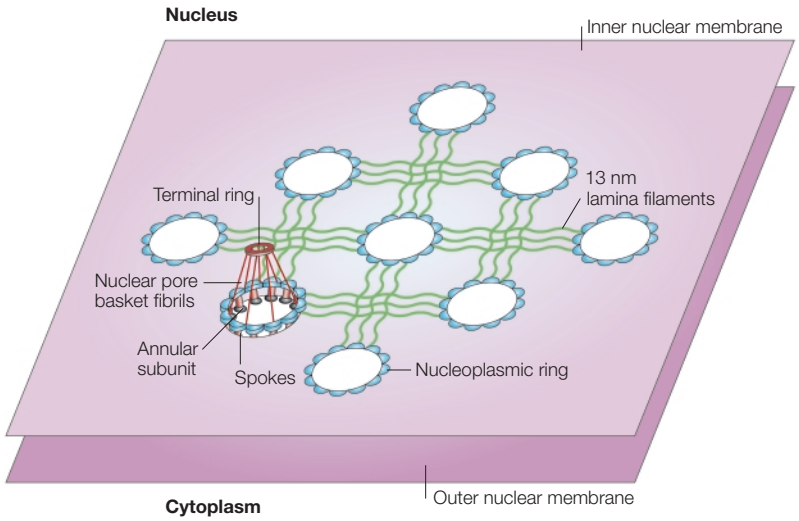


Figure 1 | **Organization of lamina filaments at the inner nuclear envelope.** Ultrastructural studies indicate that lamina filaments might have a two-dimensional orthogonal arrangement in which filaments are interwoven as a square lattice. The filaments also interact with subunits of the nucleoplasmic ring structures of adjacent nuclear pore complexes[1,2].

proteins[20,21]. In addition, the tail domain of the lamins harbours a nuclear-localization signal (NLS) sequence[22] and, in most cases, a carboxy-terminal CaaX box, which is a target for isoprenylation and carboxyl methylation[23–25]. Both of these motifs are absent from the vertebrate cytoplasmic IF proteins; however, they are present in molluscan IF proteins[26], supporting the idea that cytoplasmic IFs evolved from lamins (BOX 2).

Lamins determine nuclear shape and size

Nuclear shape. Six important studies[19,27–31] have highlighted the role of the lamina in determining the shape of the nucleus. In mouse spermatocytes, nuclei are hook shaped rather than spherical, and a spermatocyte-specific lamin — lamin B3 — is expressed in these cells. Indeed, exogenous expression of lamin B3 in somatic cells resulted in their nuclei adopting a hook-shaped morphology[27]. Recently, a dominant-negative mutant of lamin B1 was constructed that lacked four-fifths of the rod domain[28] (B1ΔRod). This mutant was still able to self-assemble into filaments *in vitro* and, when transfected into cultured cells, was incorporated into the lamina. However, its incorporation into the lamina caused massive deformation of the nuclear envelope (NE).

More subtle alterations in nuclear morphology have been reported in fibroblasts from a *lamin A* (*Lmna*) knockout mouse[29] and in fibroblasts from patients carrying rare *lamin A/C* (*LMNA*) mutations[30]. When RNA INTERFERENCE (RNAi) was used to 'knock down' *lmn-1* expression in *C. elegans*, the resulting phenotype was altered nuclear morphology[19]. Finally, the human immunodeficiency virus (HIV) VPR protein induces local disassembly of the nuclear lamina, which leads to regionalized blebbing of the NE[31].

Nuclear size. Several investigations that used cell-free nuclear assembly extracts of *Xenopus* eggs[32] have shown that the lamina also controls the size of the nucleus. When lamins are immunodepleted from the extracts, NE assembly still occurs, but the resulting nuclei are very small[33,34]. Complementary experiments have used dominant-negative lamin mutants to prevent lamina assembly in the same extracts, again resulting in the formation of small nuclei[35,36]. Finally, when certain mutants of the INM protein lamina-associated protein 2-β (LAP2β; first identified in humans as TPβ) — which binds to B-type lamins *in vivo* and *in vitro* — are added to egg extracts, lamina assembly and nuclear growth are both inhibited[37].

Resisting deformation. Recently, green fluorescent protein (GFP)–lamin chimaeras have been used to investigate the lamina assembly properties of living cells[38,39]. Time-lapse movies from both of these studies showed that the NE surface underwent constant deformation. However, resistance to this deformation was evident; in any area where deformation occurred, the original shape was quickly restored. The idea that the lamina is responsible for resisting this type of deformation is supported by time-lapse studies carried out on nuclei in *lmn-1* knockdown worms. In these animals, there seems to be little resistance to deformation, and any deformity of the NE that does occur is maintained for the rest of the cell cycle[19]. The fact that lamin composition, or the presence of mutant lamins, in the lamina affects the shape and size of the nucleus and the strength of the NE indicates that the lamina might act as a TENSEGRITY ELEMENT for the nucleus (BOX 3).

Organization of the nuclear envelope

The lamina has important functions in anchoring the elements of the NE to their correct positions, and the lamins are crucial in this process. Anchorage functions of the lamins include the correct positioning of NPCs and the recruitment of proteins to the INM. As described below, important molecular details of these functions have recently emerged.

Positioning of nuclear pore complexes. In ultrastructural studies, lamina filaments are seen to interact with the nuclear rings (FIG. 3) of the NPCs and to interconnect adjacent NPCs[1,2]. In *Drosophila* mutants that are null for *lamin Dm0*, the NPCs do not seem to have a fixed position in the NE — they seem to float around before eventually clustering together[18]. Similarly, when RNAi is used to knock down the expression of *lmn-1* in *C. elegans*, the NPCs also float around and cluster together[19]. So, lamina filaments seem to position the NPCs correctly by holding them apart.

Recently, the molecular interactions that are involved in positioning NPCs have been clarified. It has been reported that the nuclear-pore protein nucleoporin 153 (Nup153) interacts, through its carboxy-terminal domain, with B-type lamins. Moreover, when lamina assembly is prevented in the *Xenopus* egg extracts with dominant-negative lamin mutants, Nup153 is not

RNA INTEREFERENCE
The process by which an introduced double-stranded RNA specifically silences the expression of genes through degradation of their cognate mRNAs.

TENSEGRITY ELEMENT
A lightweight load-bearing structure that can re-adopt its initial shape after deformation.

Box 1 | **Why are lamins not found in plants and fungi?**

B-type lamins are essential genes in metazoans, and their deletion in *Drosophila melanogaster*, *Caenorhabditis elegans* and cultured human cells leads to death[18,19,66]. This is not true of the cytoplasmic intermediate filament (IF) proteins, as there are none in arthropods[96], and many cytoplasmic IF knockout mice are viable[97]. Although it has been reported[98] that plants and fungi both express lamins, these findings have not been substantiated. Recent analysis of the genomes of *Saccharomyces cerevisiae* and *Arabidopsis thaliana* indicate that direct orthologues of the lamins are not present[99,100].

The lack of lamin orthologues indicates that lamins might have functions that are not required in plants and fungi. But this is hard to believe, as lamins are involved in processes as fundamental as DNA replication. Moreover, chicken lamins assemble as a nuclear rim-like structure when expressed in *Schizosaccharomyces pombe*[101], which indicates that proteins needed for the targeting of lamins to the nuclear envelope and the assembly of lamina filaments must be present in this organism. So, proteins with homologous functions to the lamins will probably be found in plants and yeast.

incorporated into NPCs[40]. At first, this observation was difficult to explain because Nup153 had been thought to be located exclusively at the terminal ring of the nuclear-pore basket[41] (FIG. 3). However, new data have shown that Nup153 is also localized to the nucleoplasmic ring of NPCs, a position in which it could interact directly with lamina filaments. Moreover, when Nup153 is depleted from *Xenopus* egg extracts, the NPCs show increased mobility in the NE and seem to cluster together[42].

The model shown in FIG. 3 can now be used to explain how lamina filaments position NPCs in the NE. In the model, Nup153 is a component of the nucleoplasmic ring of the NPCs. Its presence in that structure allows a direct interaction with lamina filaments through specific associations with B-type lamins. This interaction might support polymerization of lamina filaments between adjacent NPCs, with the result that the filaments anchor the NPCs to relatively fixed positions and hold adjacent NPCs apart. In the absence of Nup153, lamina filaments cannot interact with the nucleoplasmic ring, and the NPCs float in the NE. In the absence of lamina filaments, Nup153 does not associate with the nucleoplasmic ring, and again, the NPCs float. This model implies that rigid lamina filaments are needed to separate NPCs. However, on a closed surface such as the NE, both rigid and flexible filaments could, in principle, maintain NPC separation.

Recruitment of proteins to the inner nuclear membrane. Members of the LAP2 family of INM proteins[43] are believed to be anchored at the INM through interactions with the lamin B rod domain. However, LAP1 (REF. 44) and emerin[45,46] are thought to be anchored at the INM through interactions with lamins A/C. Dramatic new evidence has highlighted the importance of the lamins in recruiting and anchoring proteins to the INM. Incorporation of the dominant-negative mutant B1ΔRod into the lamina results in altered localization of many NE proteins and NPCs[28]. The altered localization of NE proteins includes partial exclusion of lamins A/C from regions that contain B1ΔRod, and partial relocation of the INM proteins LAP2 and LAP1 to the endoplasmic reticulum (ER). Schirmer *et al.*[28] have proposed that the lamin B1 rod has a dominant role in organizing other NE proteins at the INM. It is known that the rod domain of lamin B1 interacts with LAP2β (REF. 27). Therefore, an alternative interpretation is that relocation of LAP2β to the ER might arise because B1ΔRod does not have a LAP2β-interaction domain. In addition, elimination of A-type lamins from the NE causes emerin and LAP1 to relocate from the INM to the ER in many cell types[29,47,48]. So, relocation of LAP1 to the ER in the presence of B1ΔRod might occur because this mutant protein disturbs the normal distribution of lamins A/C (REF. 28). In *C. elegans*, recruitment of the integral membrane proteins UNC-84 and emerin to the INM also occurs through association with LMN-1[49,50]. Also, anchorage of other membrane proteins to the INM, including MAN1 (REF. 19), the lamin B receptor[51] (LBR) and the recently characterized Nesprin family[52], probably occurs through interactions with different lamins.

Recruitment of lamins to the lamina

Several studies indicate that there might be a hierarchy of lamin–lamin associations at the INM. In this hierarchy, the B-type lamins are assembled into lamina filaments first, followed by lamin A and then by lamin C[4]. In *Xenopus* egg extracts, recombinant lamin A is assembled at the NE of *in-vitro*-assembled sperm pronuclei only in the presence of the endogenous lamin B3, indicating that lamin A might be incorporated into existing B-type lamina filaments[53]. In tissue culture cells, lamin A remains in the nucleoplasm until B-type lamin filaments are assembled in the telophase nuclei[39,54]. Finally, dominant-negative lamin mutants that have altered lamin-assembly properties have distinctly different effects on A-type and B-type lamins[28,48,55], indicating that the two types of lamin might be incorporated into the lamina in different ways.

Table 1 | **The cytoplasmic intermediate filament proteins**

Type	Proteins	Tissue expression	Mode of association	References
I	Type I keratins	Epithelia	Obligate heterodimers	107–109
II	Type II keratins	Epithelia	Obligate heterodimers	107–109
III	Desmin, vimentin, GFAP, peripherin	Mesenchymal tissues	Homodimers	4
IV	NF-L, NF-M, NF-H, α-internexin, syncoilin, nestin, synemin	Neurons	Heteromeric associations	109–111

GFAP, glial fibrillary acid protein; NF, neurofilament.

Table 2 | **Lamin polypeptides in different phylogenies**

Organism	Lamin	Mode of synthesis	Expression patterns	References
Type A lamins				
Mammalian	A, AD10*, C C2	Products of alternatively spliced *LMNA* Product of alternatively spliced *LMNA*	Differentiated cells Sperm-specific	20,21,112 113
Avian	A	Product of unique gene	Differentiated cells	14
Amphibian	A, C	Products of the same alternatively spliced gene	Differentiated cells	68
Arthropod	C	Product of unique gene	Most cells from blastocyst	114
Type B lamins				
Mammalian	B1 B2 B3	Product of the *LMNB1* gene Product of alternatively spliced *LMNB2* Product of alternatively spliced *LMNB2*	Most cells Most cells Spermatocytes only	115 27 27
Avian	B1, B2	Product of unique gene	Most cells	14
Amphibian	Li Lii Liii Liv	Products of unique genes	Most cells from blastula stage Most cells from blastula stage Oocyte and eggs Spermatocytes	13,68
Arthropod	Dm0	Product of unique gene	Most cells from egg onwards	18
Nematode	LMN-1	Product of unique gene	Most cells from egg onwards	19

*An alternatively spliced form that is found in some tumour cell lines.

Lamin A and lamin C also show different assembly properties. Expression, or injection, of tagged lamins in mouse cells shows that lamin A is incorporated into the lamina more rapidly than lamin C. These studies also indicate that lamin A directs lamin C to the lamina[56,57]. In many tumour cell lines that do not express lamin A, lamin C is localized to the nucleoplasm or the nucleolus. However, when GFP–lamin A was expressed in a representative cell line, it was incorporated into the lamina with endogenous lamin C (REF. 48), again indicating that lamin A might guide lamin C to the NE.

An investigation of the assembly properties of A-type lamins that carry point mutations in either the rod domain or the tail domain has also been carried out[58]. This study showed that these mutations most often affect the assembly of lamin C more severely than they do the assembly of lamin A. All of these studies indicate that a greater number of protein–protein interactions might be required for incorporation of lamin C into the NE than might be required for lamin A. A summary of these interactions is shown in FIG. 4.

Association of lamins with nuclear bodies

Until the early 1990s, lamins were considered to be exclusive components of the INM. However, the observation that nucleoskeleton filaments have the dimensions of intermediate-type filaments[59], together with the identification of internal lamin structures in human and mouse cells[60,61], led to an acceptance that lamins could occupy sites in the nucleoplasm. More recent evidence has shown that lamins not only move between the NE and the nucleoplasm, but that they also associate with discrete sites of DNA synthesis or RNA processing termed NUCLEAR BODIES[62]. There is considerable interest as to whether these associations are functional or occur by default.

NUCLEAR BODY
A structure of defined size in the nucleus that is identifiable by immunofluorescence or electron microscopy, and contains all of the enzymes required for a specific metabolic function (for example, RNA splicing).

Association with centres of DNA replication

It is widely accepted that lamins have a role in DNA replication, but the nature of this role is controversial. When B-type lamins are depleted, either functionally[33] or physically[34], from *Xenopus* egg extracts, small nuclei that fail to initiate DNA replication are assembled. When purified lamin B (but not lamin A) is re-added to depleted extracts, the nuclei grow and DNA replication is initiated, indicating that lamins might have a direct role in DNA synthesis[53,63]. This finding is supported by the observation that, in somatic cells, a fraction of lamin B1 re-distributes from the NE to centres of DNA replication during S phase[35,64].

To investigate at what stage in the process of DNA replication lamins are required, further experiments using dominant-negative lamin mutants were carried out[35,36,55,65]. All the experiments were done using lamin A or lamin B1 mutants that lacked amino- and/or carboxy-terminal sequences and formed intra-nuclear aggregates in either *in-vitro*-assembled nuclei[35,36,65] or tissue culture cells[55]. In some instances, the mutants recruited endogenous B-type lamins to the aggregates and also recruited proteins that are involved in the elongation phase of DNA replication[35,65]. When B-type lamins were present in the aggregates, stalled replication forks accumulated, which indicates that lamins might be involved in the elongation phase of DNA replication.

In other instances, similar mutants still formed intra-nuclear aggregates. However, whereas A-type lamins were recruited to the aggregates, B-type lamins were not. In these cases, replication proteins were not recruited to the aggregates[36], or, if they were recruited, this resulted from non-specific associations[55]. In both instances, stalled replication forks could be made to function properly, even in the presence of the lamin aggregates, which indicates that lamins might be needed to initiate

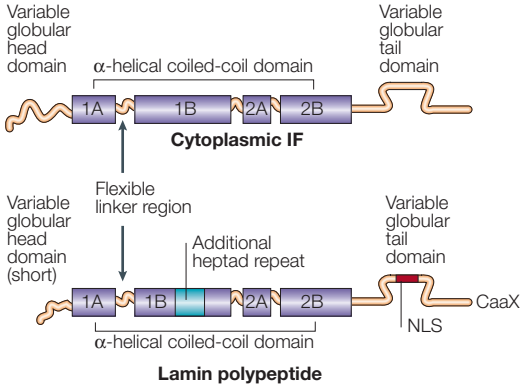


Figure 2 | **Generalized structure of cytoplasmic intermediate filament proteins compared with lamins.** All intermediate filament (IF) proteins have a conserved domain structure that consists of a variable globular head domain, a central α-helical coiled-coil dimerization domain — four coiled-coil domains (organized around heptad repeats) that are interrupted by flexible linker domains — and a variable globular tail domain. The four coiled-coil domains are termed 1A, 1B, 2A and 2B. The linker domains are non-helical. The main differences between the lamins and vertebrate cytoplasmic IFs are that, in lamins: the head domains are very short (~33 amino acids); there is a six-heptad extension of coil 1B; the globular tail domain is usually characterized by having a nuclear-localization signal sequence (NLS) and a site for carboxyl methylation, farnesylation and proteolytic cleavage (CaaX).

DNA replication but are possibly not required for its elongation phase[36,55].

Although it is clear that B-type lamins associate with sites of DNA replication and replication proteins, further studies are needed to clarify the role of the lamins in DNA replication. The prospect of using RNAi to knock down the expression of specific lamins in cultured cells[66] probably provides the best opportunity to address this important issue.

Involvement in RNA synthesis and processing

There is now intriguing evidence that lamins are involved in RNA transcription and processing. However, A-type lamins and B-type lamins seem to influence gene expression in different ways. B-type lamins have relatively fixed locations at the NE, where they associate with PERIPHERAL HETEROCHROMATIN and are probably involved in gene silencing. By contrast, A-type lamins can move between the NE and the nuclear bodies, and can have positive and negative influences on gene expression.

The different patterns of lamin expression during embryogenesis led to the suggestion that lamins have a direct role in gene regulation[67]. B-type lamins are expressed throughout development, and one or more B-type lamins is present in all cell types[13,14,68] — they are, therefore, thought to be essential. RNAi knockdown of either lamin B1 or lamin B2 in cultured cells inhibits cell growth and promotes apoptosis, which shows that both genes are essential[66]. In both somatic cells and genetically active *Xenopus* embryonic nuclei, disruption of the lamina by dominant-negative lamin mutants inhibits RNA POLYMERASE II activity[69], and provides direct evidence for the involvement of lamins in transcription. As *Xenopus* embryonic nuclei only express B-type lamins[13], it seems probable that this effect on RNA polymerase II activity is mediated by B-type lamins. Furthermore, the POU DOMAIN octamer-binding transcription factor 1 (Oct1), which is involved in repression of collagenase genes, associates at the NE with lamin B[70]. The INM protein Lap2β, which associates with the mouse Germ cell-less (Gcl) and E2f-associated protein (Dp) proteins in a complex with lamin B1 at the NE, inhibits E2f activity[71], again indicating that B-type lamins might form part of a repressor complex.

Because A-type lamins are developmentally regulated and their expression patterns in different tissues are correlated with organogenesis[12], they were proposed to have roles in differentiation. However, an *Lmna*$^{-/-}$ mouse develops[29] normally and RNAi knockdown of *Lmna* in cultured cells has no obvious effect[66], which indicates that A-type lamins are not crucial in gene regulation. However, other studies support more subtle roles for A-type lamins in gene regulation. Lamin A has been reported to be an *in vitro* binding partner for the transcriptional regulator retinoblastoma protein (RB)[72]. In addition, lamin A has been reported to associate with RNA SPLICING-FACTOR SPECKLES in interphase cells[62]. The association of lamin A with speckles is clearly a dynamic one, as in skeletal-muscle cultures lamin A associates with speckles in dividing cells but not in cells that are undergoing differentiation[73]. This finding is intriguing, as ectopic expression of lamin A in myoblasts promotes the expression of muscle-specific genes[74]. Perhaps the association of lamin A with splice sites in undifferentiated myoblasts suppresses expression of certain skeletal-muscle genes at a post-transcriptional

PERIPHERAL HETEROCHROMATIN
Heterochromatin that is physically linked to the nuclear envelope.

RNA POLYMERASE II
A eukaryotic enzyme that synthesizes mRNA precursors.

POU DOMAIN PROTEINS
A conserved family of transcription regulators that contain the sequence motif Pit–Oct–Unc.

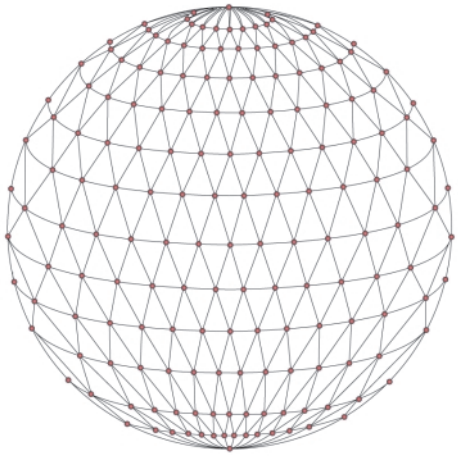
RNA SPLICING-FACTOR SPECKLE
A nuclear body that acts as a reservoir for RNA splicing factors.

Box 2 | **Did cytoplasmic intermediate filament proteins evolve from lamins?**

Cytoplasmic intermediate filament (IF) gene and protein sequences from molluscs have a distinct evolutionary relationship to the vertebrate lamins. The molluscan sequences contain the extra 42 amino-acid residues in coiled-coil 1B, and a 120-amino-acid-residue lamin homology domain in the carboxyl terminus[102–105]. These observations indicate that cytoplasmic IF proteins might have evolved from lamins through a combination of exon shuffling and mutation[11,106]. A wider analysis of the molecular phyologeny of metazoan IF proteins indicates that divergence of vertebrate cytoplasmic IF proteins from the lamins might have occurred in two steps. Deletion of the lamin homology domain in the carboxy-terminal tail occurred before the emergence of the chordates. However, only the chordate cytoplasmic IF proteins lack the lamin homology domain in coil 1B, which argues strongly that the diversification of the vertebrate cytoplasmic family into four groups occurred after the emergence of the chordates[7,99].

Box 3 | **The lamina as a tensegrity element for the nucleus?**

The organization of the lamina at the nuclear envelope is reminiscent of the organization of struts supporting the shell of a GEODESIC DOME. This type of tensegrity element has several functions. By altering the spacing and arrangement of the struts, the shape of the shell can change markedly. In addition, the precise numbers and dimensions of the struts determine the size of the space it surrounds. Finally, the struts are load-bearing elements that effectively resist deformation of the shell. Several investigations have shown that the lamina has all of these properties, providing strong support for the idea of the lamina as a tensegrity element. In the model pictured, which represents the lamina as geodesic dome, lamin filaments (bars) interact with nuclear pore complexes (red spots) to form a cage-like structure that surrounds the 'inner nuclear space', which provides a protective environment for chromatin.



level? Alternatively, the fact that lamin A is a RB binding partner also supports some role for this protein in the activity of RB — perhaps, again, in aspects of specific gene regulation associated with mesenchymal cells that are known to be influenced by RB. A-type lamins were shown to associate with speckles as well as with the nucleoskeleton protein LAP2α (first identified as TPα in humans), which resides in the nucleoplasm[54]. So, in contrast to B-type lamins, A-type lamins might influence transcription at sites that are distant from the NE.

In conclusion, A-type lamins are associated with splicing-factor speckles[62,73] and can reside in the nucleoplasm[54]; so, they might influence gene expression at a transcriptional or post-transcriptional level. A-type lamins might also be involved in silencing at the NE, as peripheral heterochromatin is disrupted in skeletal-muscle cells that harbour mutations in *LMNA*[75]. By contrast, B-type lamins and B-type-lamin binding proteins seem to form repressor complexes at the NE[70,71]. How lamins positively influence RNA polymerase II activity[69] is as yet unclear.

Involvement in disease

During the past three years, A-type lamins, or their binding partners, have been associated with a range of genetic disorders (TABLE 3). There has been considerable debate as to how mutations in the lamin genes promote these particular disease phenotypes, and why certain mutations can give rise to tissue-specific effects. Two hypotheses have been proposed. The 'structural hypothesis' proposes that mutations that give rise to weakness of the lamina lead to fragility of the NE and its breakage in affected people[5,29,76]. The 'gene-expression hypothesis' proposes that the tissue-specific changes in gene expression that are associated with some mutations promote disease[6,77].

EDMD and dilated cardiomyopathy. Emery–Dreifuss muscular dystrophy (EDMD) is caused by mutations in either the *LMNA* gene[78] or the gene that encodes emerin[79]. Mutations in *LMNA* are distributed throughout the coding region and can give rise to dominant point mutations or null phenotypes[80]. *Lmna*$^{-/-}$ mice (as opposed to *Lmna*$^{+/-}$ mice) show features of EDMD[29], but not of other lamin diseases[81]. Mutations in the emerin gene also lead to EDMD and generally give rise to a null phenotype, although some mutations lead to the aberrant targeting of emerin to the ER[82–84]. The absence of lamin A from the NE also leads to mis-localization of emerin to the ER[29,48] and of lamin C to the nucleolus[48]. Because EDMD can result from a null phenotype for emerin or for lamins A/C, EDMD might occur because of a lack of lamin A, lamin C or emerin from the NE. Systematic expression of epitope-tagged lamins, containing EDMD- or dilated cardiomyopathy-causing mutations, showed that lamin C was consistently mis-localized to the nucleoplasm. By contrast, some mutated lamin A remained at the NE[58,85]. Therefore, the absence of lamin C from the NE might be the consistent feature of both EDMD and dilated cardiomyopathy. This defect could result in fragility, as described by Fidzianska and co-workers[76]. Alternatively, the same defect could result in abnormal organization of peripheral heterochromatin, as observed by Sewry and co-workers[75], leading to a loss of silencing.

Familial partial lipodystrophy (FPLD). Mutations that cause familial partial lipodystrophy (FPLD) occur as recurrent missense mutations in exon 10 (REFS 86–88) or, very rarely, in exon 11 (REF. 87) of the *LMNA* gene. These mutations do not overlap with the mutations that cause the other lamin diseases.

The structure of the lamin A globular tail domain has recently been solved[89,90], and has been described as a new member of the IMMUNOGLOBULIN DOMAIN FAMILY. It is composed entirely of β strands, with two large β-sheets (comprising five and four β-sheets, respectively) forming a β-sandwich. Superimposing disease-causing *LMNA* mutations over the predicted structure of the lamin A tail indicates that the mutations that cause EDMD and dilated cardiomyopathy might be distributed throughout the protein core, and would probably destabilize its structure. By contrast, the mutations that give rise to FPLD are clustered at a

GEODESIC DOME
An architectural device, invented by Buckminster Fuller, in which icosahedral structures are used to enclose spaces in a dome.

IMMUNOGLOBULIN DOMAIN FAMILY
A family with structural similarity to immunoglobulin, as defined by the presence and organization of β-sheets and β-strands.

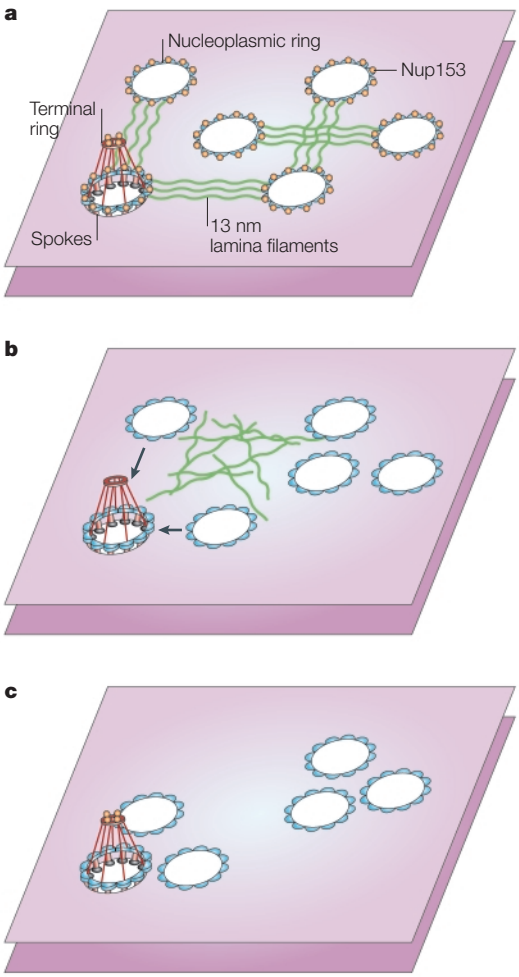


Figure 3 | **Model for a possible interaction between Nup153 and the lamins that anchor nuclear pore complexes in the nuclear membrane. a** | The normal organization of the inner nuclear membrane, in which orthogonally arranged lamina filaments interact with the nucleoplasmic ring of nuclear pore complexes (NPCs) through Nup153. In the model shown, lamina filaments are shown as green lines, the nucleoplasmic rings of the NPCs are shown in blue and the terminal ring and nuclear pore basket fibrils are shown in red. Nup153 (orange spots) is distributed on the terminal ring of the nuclear pore basket and also on the nucleoplasmic ring of NPCs[41,42]. **b** | Organization of NPCs and lamina filaments in the absence of Nup153. In the absence of Nup153, lamina filaments still assemble; however, NPCs are mobile, rather than fixed in the nuclear membrane, and they tend to migrate towards each other and cluster together[42]. This model proposes that, under these circumstances, lamina filaments are not organized as an orthogonal array as this arrangement depends on an interaction with the ring structure of NPCs. The arrows indicate the movement of adjacent NPCs towards each other. **c** | Organization of NPCs in the absence of lamins. In the absence of lamina filaments, Nup153 is still present on the terminal ring of the nuclear pore basket; however, it is no longer present on the nucleoplasmic ring of NPCs. Again, under these circumstances, NPCs are mobile, rather than fixed in the nuclear membrane, and they tend to migrate towards each other and cluster together[18,19,40].

small area of the surface and are unlikely to disrupt the structure severely[89,90]. These findings indicate that the FPLD-causing mutations are unlikely to give rise to fragility. Three independent investigations report that the mutations that cause FPLD do not cause aberrant targeting or incorporation of affected lamins into the NE[23,58,85]. These findings also indicate that the domain in the tail of lamins A/C that is covered by the FPLD-causing mutations is not important for targeting or assembly of the lamina. Perhaps the domain is necessary for the interaction of lamins A or C with an adipocyte-specific component of the INM? However, one study involving skin fibroblasts indicates that FPLD-causing mutations might give rise to severe structural defects at the NE[29], and is consistent with a hypothesis that these mutations influence the structure of the lamina.

Charcot–Marie–Tooth disorders. The Charcot–Marie–Tooth (CMT) disorders are a group of hereditary motor and sensory neuropathies that are characterized by muscle wasting, foot deformities and axonal degeneration[91]. A rare autosomal-recessive form of the disease has been described, which is caused by a specific mutation in the *LMNA* gene that leads to the amino-acid substitution R298C. This substitution has not yet been described in other A-type lamin diseases, leading to the suggestion that the mutation partially defines a distinct functional domain of lamins A/C that is important in axonal survival[91]. A prediction of this study is that lamins A/C are divided into distinct functional domains, each of which has a different relative importance in the maintenance of distinct tissues.

This prediction is supported by a new mouse model[92], which shows the features of several lamin diseases. The mouse is a knockout of the *Face1*/*Ste24* gene product, which is involved in proteolytic processing of isoprenylated proteins. In *Face1*/*Ste24*$^{-/-}$ mice, unprocessed pre-lamin A accumulates at the NE — the mice show late-onset characteristics typical of EDMD and FPLD. The mice also show other phenotypes, including hair-follicle atrophy, thymic hypoplasia and a loss of cortico-medullary demarcation in young mice[92].

These new data indicate that abnormal accumulation of lamin A promotes a range of diseases and supports the view that A-type lamins contain several functional domains. This view is also supported by evidence that lamina mutations also promote mandibuloacral dysplasia (MAD)[93]. Whether these domains can be divided neatly into those involved in transcription, those involved in anchorage and those involved in maintaining nuclear structure has yet to be determined. The emergence of multisystem dystrophy syndrome, in which a range of disease phenotypes are observed, indicates that the picture might be very complicated[94].

Lamins in development

Generation of germ cells. The use of RNAi to knock down expression of *lmn-1* in *C. elegans* occasionally generates animals that are haploinsufficient, rather than null, for this lamin. These animals develop into adults

whose only phenotype is female sterility. There is no indication of abnormality in other tissues and, in particular, in heart or skeletal muscle[19]. In all vertebrates tested, expression of germline-specific lamins is observed, indicating the importance of lamins to the development of an organism[13,17,27]. So, low amounts of lamins could be fundamental in germline development, but not in the development of other tissue types. Indeed, the evidence from knockout mice indicates that A-type lamins might be required to maintain certain tissues in adult animals, but that they are not required in development[29,92]. So, vertebrate lamins can potentially be classified into three groups on the basis of their function. Lamins B1 and B2 have essential functions and are required for the division of all cells[50]. Mouse lamin B3 and frog and fish lamin Liii are potentially involved in germline development — this hypothesis could be tested easily in zebrafish or in *Xenopus tropicalis*. A-type lamins help maintain mesenchymal tissues (and possibly others) in adult organisms[29,92], and perhaps support longevity.

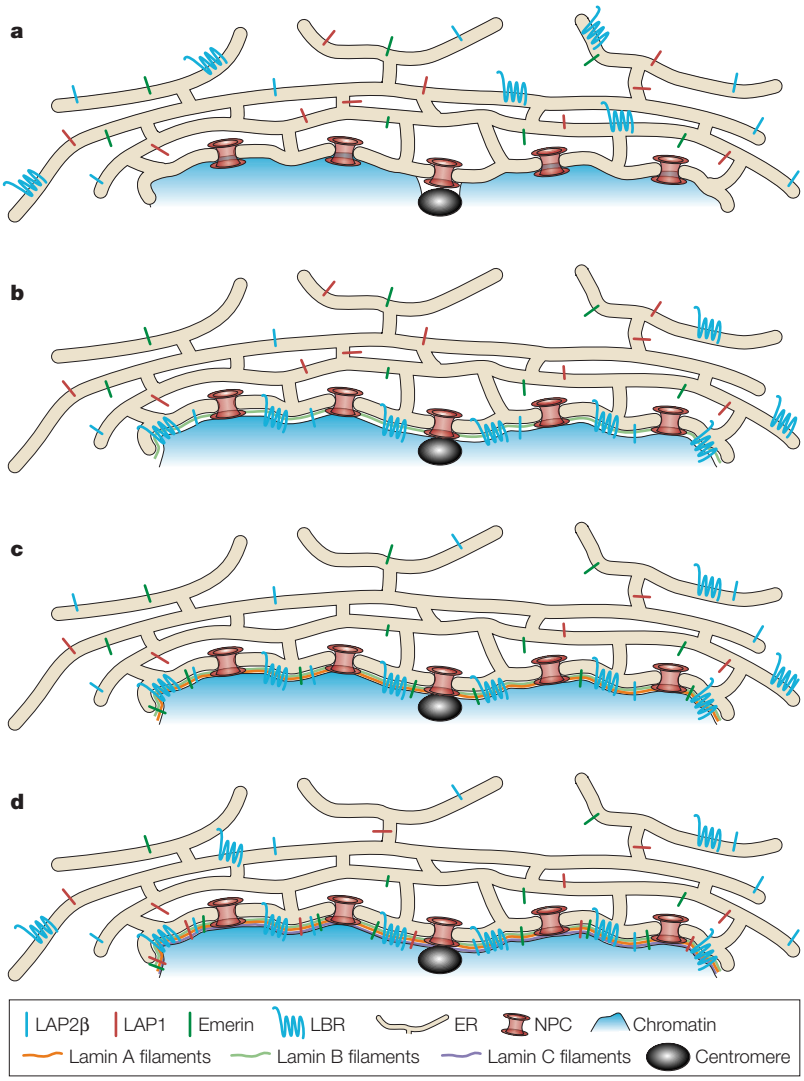


Figure 4 | **Lamin interactions at the inner nuclear membrane. a** | A model proposing a sequence of interactions at the inner nuclear membrane (INM) that support its assembly. The INM is shown as a subcompartment of the endoplasmic reticulum (ER). INM integral membrane proteins are distributed throughout the ER and can migrate between the ER and INM. Four proteins are represented, namely, lamin B receptor (LBR), LAP2β, LAP1 and emerin. **b** | Initially, lamin B assembles into filaments at the INM. Formation of lamin B filaments is dependent on interactions with LAP2β and recruits most of this protein and LBR from the ER to the INM[37,84]. **c** | Formation of lamin B filaments allows the incorporation of A-type lamins into the lamina and allows recruitment of LAP1 to the INM[47,53]. **d** | Incorporation of lamin A into the lamina allows recruitment of lamin C into the lamina, and this in turn allows recruitment of emerin from the ER to the INM[48,56,57]. NPC, nuclear pore complex.

Conclusions

There is now good evidence that lamins are involved in important cellular functions. These include key architectural roles, such as determination of the dimensions, shape and physical strength of the nucleus, and in anchoring proteins, NPCs and chromatin at, or in, the NE. Other functions are metabolic and include roles in DNA replication and transcription. The emerging picture is that lamins have a sophisticated domain structure that allows multifunctionality in different contexts. This is implied by conservation of ancient domains in vertebrate lamins that are dispensable in vertebrate cytoplasmic IFs. In the lamin diseases, the domain in which a mutation occurs to some extent defines the tissue specificity of the disease. This again supports the idea of a sophisticated domain structure that allows lamins to interact with different binding partners in different nuclear locations and in different cell types. A systematic investigation of lamin binding partners using a combination of proteomic technologies and genetic methods, such as the mammalian two-hybrid system, is now required to identify these binding partners, and is a prerequisite to fully understanding domain organization.

In lower metazoan organisms, all of the basic functions of the lamins are carried out by a single lamin polypeptide, whereas in vertebrates these functions are shared between seven lamins. In *C. elegans*, the only tissue defect that results from haploinsufficiency of *LMN-1* is in the testes. By contrast, in mammals, several mesenchymal tissues are affected to various degrees by the failure to express A-type lamins, or through domain-specific mutations in these proteins. All A-type lamin phenotypes are late onset and show variable penetrance. Therefore, the increased complexity of the lamin family in vertebrates is perhaps required to separate germline development from more basic B-type lamin functions, and to help maintain tissues in long-lived animals.

Although lamins are essential genes in metazoans, they are absent from plants and fungi. This finding implies that plants and fungi express distinct proteins with homologous functions. For example, silencing in yeast is carried out by 'silent information regulatory' (SIR) proteins, which have a structural organization that is homologous to the rod and tail domains of the lamins, but lack the head sequences and do not form filaments[95]. Presumably, other proteins, with structural similarity to the lamins, have architectural functions, such as holding NPCs in

Table 3 | **The laminopathies**

Disease	Type of mutation	Principle phenotypes	References
LMNA gene* (chromosome 1q21.3)			
Autosomal-dominant Emery–Dreifuss muscular dystrophy	Throughout the coding sequence. Includes amino-acid substitutions, frameshift and premature stop codons	Early contractures of tendons, slowly progressing muscle weakness and wasting, conduction defects in heart	77,79,116
Autosomal-recessive Emery–Dreifuss muscular dystrophy	A single homozygous mutation at C664T, giving rise to amino-acid substitution H222Y	Early contractures of tendons, slowly progressing muscle weakness and wasting, conduction defects in heart	117
Limb-girdle muscular dystrophy	Amino-acid substitution, codon deletion and splice donor mutations reported	Progessive muscle weakness of hip girdle and proximal arm and leg muscle	118–120
Dilated cardiomyopathy with conduction system defect	Throughout the coding sequence. Includes amino-acid substitutions, frameshift and premature stop codons	Impaired systolic function and dilation of the left or both ventricles. Variable skeletal muscle involvement	121
Charcot–Marie–Tooth disorder	Single mutation in Algerian families giving rise to R298C substitution	Motor and sensory neuropathies characterized by muscle weakness and wasting, foot deformities and axonal degeneration	91
Dunnigan-type familial partial lipodystrophy	Missense mutations clustered in exons 8 and 11	Loss of subcutaneous adipocyte tissue from extremities and trunk with excessive fat deposition in neck. Profound insulin resistance and diabetes	86–88
STA gene‡ (chromosome Xq28)			
X-linked Emery–Dreifuss muscular dystrophy	Throughout the coding sequence. Includes frameshift and missense mutations and premature stop codons	Early contractures of tendons, slowly progressing muscle weakness and wasting, conduction defects in heart	45,46,78,81

**LMNA* encodes lamins A and C. ‡*STA* encodes emerin.

position. In this context, it would be of interest to discover the main binding partners of yeast Nup153. With a better understanding of the domain structures of the lamins, which support their various functions, it will become easier to search yeast and plant genome databases for genes that will probably have corresponding functions. The results of such a search might provide exciting insights into the divergent evolutionary pathways of these three eukaryotic kingdoms.

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