

Lamins in disease: why do ubiquitously expressed nuclear envelope proteins give rise to tissue-specific disease phenotypes?

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Summary

The nuclear lamina is a filamentous structure composed of lamins that supports the inner nuclear membrane. Several integral membrane proteins including emerin, LBR, LAP1 and LAP2 bind to nuclear lamins in vitro and can influence lamin function and dynamics in vivo. Results from various studies suggest that lamins function in DNA replication and nuclear envelope assembly and determine the size and shape of the nuclear envelope. In addition, lamins also bind chromatin and certain DNA sequences, and might influence chromosome position. Recent evidence has revealed that mutations in A-type lamins give rise to a range of rare, but dominant, genetic disorders, including Emery-Dreifuss

muscular dystrophy, dilated cardiomyopathy with conduction-system disease and Dunnigan-type familial partial lipodystrophy. An examination of how lamins A/C, emerin and other integral membrane proteins interact at the INM provides the basis for a novel model for how mutations that promote disease phenotypes are likely to influence these interactions and therefore cause cellular pathology through a combination of weakness of the lamina or altered gene expression.

Key words: Nuclear lamina, Lamin, LAP, Emery-Dreifuss muscular dystrophy, Congenital cardiomyopathy, Partial lipodystrophy

Introduction

The nuclear envelope (NE) creates a compartment within the interphase cell in which DNA replication, transcription and RNA processing can be regulated independently of translation. Components of the NE include the inner nuclear membrane (INM) and outer nuclear membrane (ONM), nuclear pore complexes (NPCs) and the nuclear lamina. The lamina is a fibrous nucleoskeletal structure associated with the INM through interactions with integral membrane proteins. The lamina also interlinks adjacent NPCs (reviewed by Vaughan et al., 2000).

Nuclear lamins are the major components of the lamina. Analysis of cDNA sequences that encode nuclear lamins has confirmed that they are closely related to the cytoplasmic intermediate filament (IF) protein family (Fisher et al., 1986). As such, the lamins have been classified as type V IFs, and share a common primary sequence consisting of globular N- and C-terminal domains and a central α -helical rod domain. The rod domain can be divided into four α -helical segments, coil 1a, coil 1b, coil 2a, and coil 2b, which are separated by non- α -helical linker sequences (Fig. 1). Unlike the rod domains of cytoplasmic IFs, the rod domains of lamins contain an additional six heptad repeats in coil 1b. Lamins are classified as either A-type or B-type, depending on their primary sequence, behaviour at mitosis and tissue-specific expression patterns. B-type lamins are constitutively expressed in all embryonic and somatic tissues studied to date. In vertebrates, lamins B1 and B2 are closely related in sequence but are the products of separate genes. In amphibian and avian species, these lamins are expressed from the blastula stage of development onwards (Stick and Hausen, 1985; Lehner et al.,

1987). B-type lamins, which are specialised for meiosis and early embryogenesis are expressed in at least some organisms. A sperm-specific B-type lamin (lamin LIV) has been identified in *Xenopus* (Benavente et al., 1985). In addition, lamin B3 is expressed in *Xenopus* oocytes and early embryos (Stick and Hausen, 1985). Lamins LIV and B3 are both the products of individual genes. A spermatocyte-specific lamin also termed lamin B3, which is a splicing variant of lamin B2, has also been identified in mouse (Furukawa and Hotta, 1993), but note that mouse lamin B3 and *Xenopus* lamin B3 are not homologous. The A-type lamins, lamin A and lamin C, are also alternatively spliced products of the same gene, as is the more recently described lamin A Δ 10, but are expressed exclusively in differentiated cells and tissues (Stick and Hausen, 1985; Lehner et al., 1987; Rober et al., 1989). Developmental control of A-type lamin expression has led to the suggestion that they stabilise differential gene expression (Nigg, 1989).

Lamin modifications

The C-terminal tail domains of lamins contain several motifs that are essential for their correct distribution. A nuclear-localisation signal (NLS) is located close to the α -helical rod domain, although the exact position within the tail domain does vary between different lamins. The NLS is required for nuclear import and is homologous to the prototype NLS of the SV40 large T antigen. Furthermore, the C-terminal ends of all the B-type lamins and lamin A contain a sequence motif CaaX (C, cysteine; a, any aliphatic amino acid; X, any amino acid). The motif is modified by farnesylation of the C-terminal cysteine residue, followed by cleavage of the three N-terminal residues and carboxy-methylation of the cysteine. The added

hydrophobic prenyl moiety appears to target (and anchor) lamins to the nuclear membrane (Vorburger et al., 1989). B-type lamins remain farnesylated throughout their lifetime, whereas lamin A is processed further. The C-terminal 15 residues of lamin A (including the prenyl tail) are removed by proteolytic cleavage to yield mature lamin A (Sasseville and Raymond, 1995) (Fig. 1). The different processing pathways for B-type lamins and lamin A probably explain their different behaviours at mitosis (see below).

Along with the rest of the NE, the lamina is disassembled and reassembled during mitosis. Both *in vivo* and *in vitro* assays suggest that the mitotic CDC2 kinase, protein kinase C (PKC), and cyclic-AMP-dependent kinase (PKA) phosphorylation sites are important in lamin assembly as well as disassembly (Moir et al., 1995). In addition, protein phosphatases have been implicated in lamina assembly (Murphy et al., 1995). Phosphorylation/dephosphorylation of lamins during interphase can modulate lamina filament assembly in a number of ways. Phosphorylation at sites adjacent to the NLS by PKC can influence the rate at which lamins enter the nucleus, thus limiting the availability of filament subunits (Hennekes et al., 1993). Phosphorylation by PKA facilitates the incorporation of new lamin subunits into the assembled lamina structure as the nucleus grows (Peter et al., 1990). Finally, the rate of dephosphorylation by PP1a at CDK1 sites can influence the initial rate of lamin filament assembly at telophase (Thompson et al., 1997). All these regulatory events probably ensure the assembly of 10–13-nm filaments *in vivo*. Following disassembly, lamins A and C are 'soluble' during mitosis (they are probably present as dimers and/or tetramers), whereas the rest of the time, B-type lamins generally remain attached to nuclear membrane vesicles (Gerace and Blobel, 1980).

Lamin associations and binding proteins

Several INM proteins have been shown to interact directly with nuclear lamins *in vitro* and to remain closely associated with the nuclear lamina after extraction of nuclei with non-ionic detergents, nucleases and/or high-salt buffers (Foisner and Gerace, 1993; Stuurman et al., 1998; Fairley et al., 1999). The lamin B receptor (LBR), also termed p58, was the first of these to be identified (see Moir et al., 1995) and is probably the best characterised. LBR has an N-terminal nucleoplasmic domain and a C-terminal region that includes eight predicted transmembrane domains. The nucleoplasmic domain interacts with B-type lamins, a human chromatin-

associated protein (HP1) and chromatin *in vitro* (see Stuurman et al., 1998). This domain also contains phosphorylation sites for PKA, CDC2 and an arginine-serine (RS)-specific kinase (Simos et al., 1996; Nikolakaki et al., 1996; Nikolakaki et al., 1997). The RS kinase co-isolates with LBR and other NE components as a multimeric complex from avian erythrocyte NEs. The complex includes nuclear lamins, p34/32 (which might be involved in RNA splicing) and the integral membrane protein p18 (which binds to B-type lamins *in vitro*: Simos et al., 1996). Using chicken hepatoma cells, Meier and Georgatos have also shown that LBR and B-type lamins co-segregate in the same mitotic vesicles (Meier and Georgatos, 1994). Similarly, in *Xenopus* eggs, xLBR and lamin B₃ also co-segregate in the same vesicle population (Drummond et al., 1999).

Lamin-associated proteins (LAPs), LAP1A, LAP1B, LAP1C, and LAP2, were initially identified in rat liver NE fractions (Foisner and Gerace, 1993). All LAPs contain predicted long N-terminal nucleoplasmic domains, a single transmembrane segment and a short C-terminal luminal tail. Three of these proteins, LAP1A, LAP1B and LAP1C, are closely related and are probably alternatively spliced products of the same gene. LAP2 proteins are also expressed as a variety of isoforms (α , β , γ , δ , ϵ and ξ), all being alternatively spliced products of a single gene. Of these isoforms all except LAP2 α possess a conserved membrane-spanning domain and are therefore type II integral membrane proteins. LAP2 α possesses an extended and unique C-terminal domain and forms part of the nuclear matrix (Foisner and Gerace, 1993; Dechat et al., 1998; Harris et al., 1995). Analysis of extracted NE fractions,

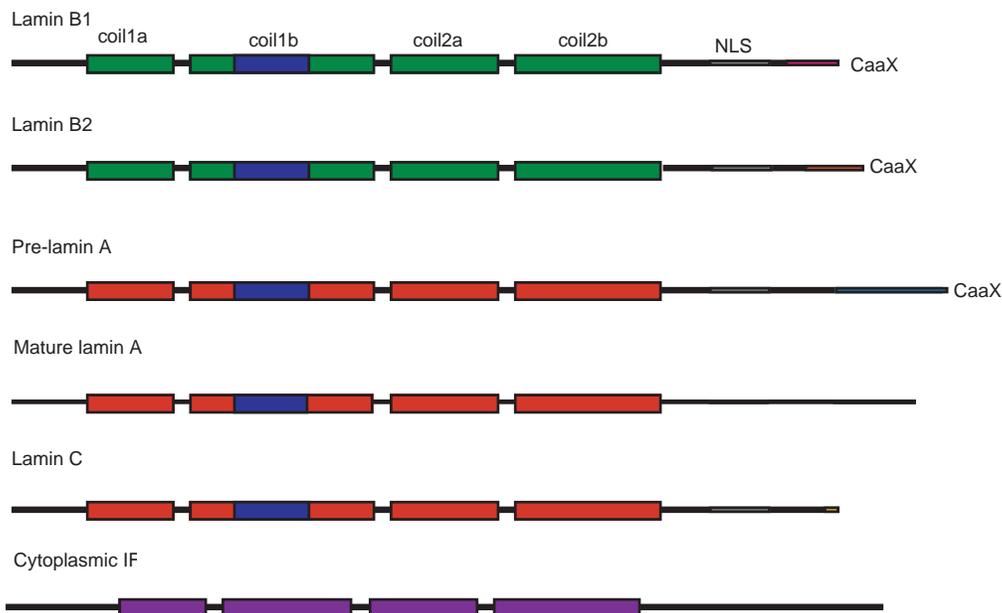


Fig. 1. Schematic representation of somatic cell lamins. Coloured rectangles represent α -helical coiled-coil domains; B-type lamins are shaded in green, and A-type lamins are shaded in red. The blue shaded area within coil 1b shows the position of the heptad repeat extension, which is unique to the lamins. NLS=nuclear localisation signal sequence; CaaX is the site for carboxy methylation, prenylation and proteolytic cleavage. Coloured regions in the C-terminal tail domains show the major sites of amino acid residue variation among the lamin subtypes. The stylised cytoplasmic IF illustrates differences in organisation for comparison. The central rod domain is shorter (owing to the absence of the heptad repeat extension in coil 1b). The head domain is generally longer and varies between IF types. The tail domain lacks an NLS and CaaX.

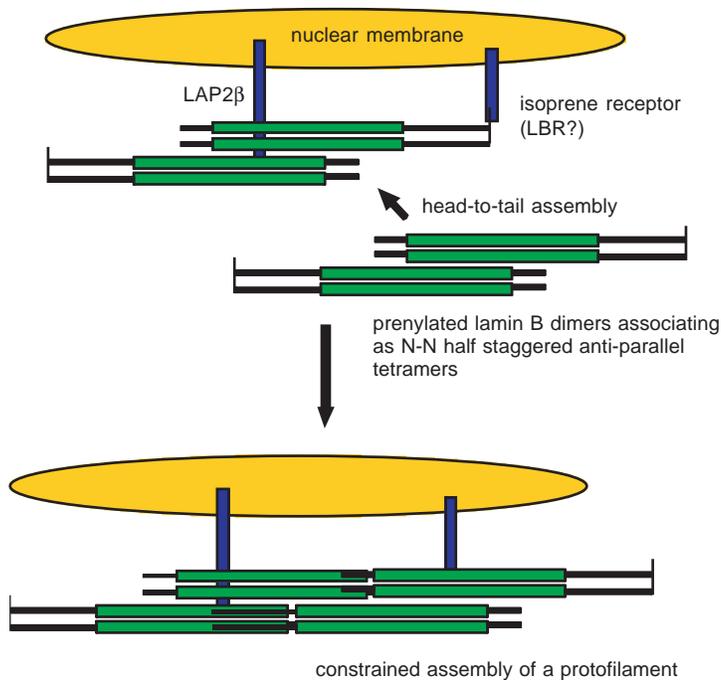


Fig. 2. Cartoon showing the constrained polymerisation of B-type lamin filaments at the INM. Tethering of B-type lamins to the INM in this model is through association with LAP2 β through coil 1b and through an isoprene receptor (possibly LBR) (after the work of Gant et al., 1999 and Mical and Montiero, 1998). Lamin B dimers form N-N half-staggered anti-parallel associations to make tetramers and head-to-tail associations to form higher-order structures such as proto-filaments.

combined with *in vitro* binding studies, has indicated that LAP1A and LAP1B (but not LAP1C) interact with lamin A, lamin C and lamin B1, whereas LAP2 β associates specifically with lamin B1 and chromatin. The affinities of the integral membrane LAP2 isoforms for lamins have not as yet been determined, although the affinity of LAP2 α for lamins is described below. The affinity of LAP2 β for lamin B1 and chromatin is reduced in the presence of CDC2 activity (Foisner and Gerace, 1993).

Although LAP1C has not been shown to interact with nuclear lamins *in vitro*, its localisation within the nuclear envelope appears to be lamin dependent. Powell and Burke reported that LAP1C translocates between the INM and ONM in undifferentiated P19 cells (Powell and Burke, 1990). When P19 cells are transfected with lamin A, LAP1C becomes immobilised at the INM. In contrast to these findings, other investigations suggest that LAP1C is anchored at the NE as part of a multimeric complex that includes LAP1A, B-type lamins (but not A-type lamins) and a protein kinase activity distinct from that associated with LBR (Maison et al., 1997). LAP2 β has been characterised more fully and does not appear to have a role in the initial phase of NE assembly (Pyrpasopoulou et al., 1996). Yang et al. have mapped distinct lamin-binding and chromatin-binding domains to the N-terminal nucleoplasmic domain of LAP2 β (Yang et al., 1997a). Moreover, LAP2 β also binds to specifically to lamin B1 within coil 1b. Yang and co-workers (1997) were able to inhibit lamina assembly, NE growth and entry into S phase by

microinjecting peptides corresponding to the lamin-binding domain of LAP2 β (but not the chromatin-binding domain) into mitotic and early G₁ phase cells. Similarly, Gant and co-workers demonstrated that GST-LAP2 fusion peptides containing regions of the LAP2 N-terminal nucleoplasmic domain also inhibited some aspects of NE assembly and, in particular, lamina assembly in cell-free extracts of *Xenopus* eggs (Gant et al., 1999). Thus, there is strong evidence to suggest that LAP2 β plays an important role in lamina assembly.

Recently, Dechat et al. have identified a splice variant of LAP2, LAP 2 α (Dechat et al., 1998). LAP2 α does not possess a C-terminal transmembrane-spanning domain but instead has a long N-terminal extension that includes an NLS. Thus, LAP2 α is distributed in the nucleoplasm (rather than in the NE), where it associates with chromatin (Vlcek et al., 1999). Recently, Dechat et al. have also shown that LAP2 α associates specifically with A-type lamins *in vivo* and *in vitro* and that its nuclear distribution is influenced by lamin A (Dechat et al., 2000).

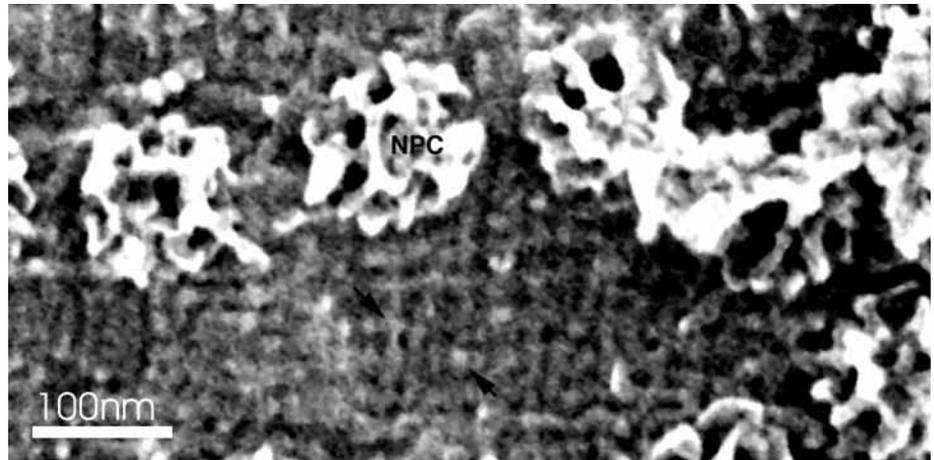
The INM protein emerin is related to both the LAPs in that it is also a type II integral membrane protein and its N-terminal nucleoplasmic domain possesses the LEM (LAP, Emerin, MAN) motif common to several INM proteins. Emerin binds to A-type and B-type lamins in nuclear extracts and *in vitro*. In addition, the distribution of emerin at the INM is dependent upon the presence of A-type lamins and specifically lamin A (Fairley et al., 1999; Sullivan et al., 1999; Vaughan et al., 2000). Thus it seems likely that emerin specifically associates with lamin A or lamin C *in vivo*.

Lamina filament assembly and organization

Most cytoplasmic IF proteins can self-assemble into 10–13-nm filaments *in vitro*. In contrast lamin polypeptides form paracrystals *in vitro* (Quinlan et al., 1995). Paracrystals are assemblies of intermediate filaments that overextend laterally to give rise to thickened structures (>13 nm diameter) with a repeated (every 23 nm) striated appearance through their length. This makes an investigation of lamin filament assembly intrinsically more difficult. However, several methods have been used to investigate the mechanism(s) of lamin filament assembly, including the EM analysis of the self-assembly properties of purified recombinant wild-type and mutant lamins, and chemical cross-linking within native laminae (reviewed within Stuurman et al., 1998). These studies suggest that the initial events in lamin assembly *in vitro* involve lateral interaction between two lamin chains to form parallel unstaggered homodimers, through coiled-coil association of the α -helical rod domains.

The lamin dimers can then associate longitudinally to form head-to-tail polymers. *In vitro* binding studies of mutant *Drosophila* lamins have shown that amino acid residues at the N-terminal end of coil 1a and at the C-terminal end of coil 2 are essential for head-to-tail polymerisation of lamin dimers. Lamin filaments of 10 nm diameter can form *in vivo* by lateral anti-parallel association of head-to-tail polymers. This type of association certainly does occur *in vitro* but results in formation of large paracrystals (Moir et al., 1991; Heitlinger et al., 1991). Therefore, do lamina filaments only assemble at the NE? Early studies revealed that post-translational modification

Fig. 3. Field emission in-lens scanning electron micrograph showing the lamina of a manually isolated oocyte germinal vesicle envelope from the amphibian *Xenopus laevis*. The lamina appears as a square lattice flattened to the membrane and interacting with the nuclear pore ring. Arrows illustrate 'press-stud-like' structures positioned where lamin filaments cross. NPC=nuclear pore complexes. Picture courtesy of Martin W. Goldberg, Patterson Institute for Cancer Research.



of the C-terminal CaaX motif is essential for targeting of lamins to the NE (Holtz et al., 1989; Krohne et al., 1989; Kitten and Nigg, 1991; Hennekes and Nigg, 1994). Indeed, mutant lamins that lack this motif preferentially assemble into crystalline structures in the nucleoplasm (Mical and Montiero, 1998; Izumi et al., 2000). Moreover, although the CaaX motif is necessary for NE localisation of the lamins, it is not in itself sufficient to anchor lamins to nuclear membranes; this finding has given rise to the notion that an isoprenyl receptor resides at the NE (Firmbach-Kraft and Stick, 1995). Since a lamin B1 mutant lacking the CaaX motif segregates independently of LBR at mitosis (Mical and Montiero, 1998), it is possible that LBR is the isoprene receptor. In more recent studies, LAP2 β , which binds specifically to a region within coil 1b of B-type lamins, has been shown to play an essential role in the initial assembly of the lamina (Yang et al., 1997a; Yang et al., 1997b; Gant et al., 1999).

Thus, we can develop a model for how lamina filaments polymerise as a 2D array at the INM (Fig. 2). Lamin dimers are localised at the INM through interactions with an isoprene receptor and LAP2 β . Because of the nature of these interactions (i.e. the first occurs through the tail of the lamin, whereas the second involves the N terminus of the rod domain) and because lamin dimers are intrinsically stiff molecules, these initial associations force the lamin dimer to lie parallel to the INM. Since in vitro evidence suggests that the preferred mode of assembly is head-to-tail, and because lamin dimers are forced into parallel alignment with the INM, presumably 10–13-nm filaments assemble as a flattened array subjacent to the INM. Take away this anchorage to the INM, and the proteins form paracrystals in the nucleoplasm. We wish to make two important points here. First, the basic lamina structure must be based around B-type lamins (see below). Second, we propose a constrained mechanism for filament assembly that is directly analogous to the constraints that influence the formation of cytoplasmic IF networks. In the nucleus, the constraining elements are probably LAP2 β and the putative isoprene receptor (possibly LBR), whereas in the cytoplasm the constraining elements are small heat-shock proteins and molecular chaperones such as $\alpha\beta$ crystallins (Quinlan and Van Den Ijssel, 1999).

An important factor is of course missing: why does the lamina form an orthogonal meshwork? The evidence for such a meshwork is from spread nuclear envelopes manually

dissected from amphibian oocyte germinal vesicles (GVs; see Fig. 3). Note that ultrastructural examinations of this type have so far only been possible in oocyte GV, and the precise arrangement of lamina filaments might differ in somatic cell nuclei. Two structural elements are self evident in the micrograph: first, the filaments extend between NPCs and physically associate with the nucleoplasmic ring of the NPCs; second, where filaments cross, there is a 'press-stud-like' structure. The meshwork might therefore be generated because lamin filaments polymerise between adjacent NPCs, and proteins within the press stud act analogously to cytoplasmic IFAPs to control the 2D organisation of the network. There is now considerable evidence, both from in vitro studies and from mutant strains of *Drosophila* that do not express house-keeping lamins, for a structural relationship between lamina assembly and NPC assembly and stability (Smythe et al., 2000; Lenz-Böhme et al., 1997). There is no data to suggest how the press stud is formed and what proteins reside within the structure.

How do A-type lamins fit into this model? Several lines of evidence suggest that A-type lamins are organised into the lamina in a way that is distinct from that in which B-type lamins are organised. Since A-type lamins are not expressed in embryonic cells (Stick and Hausen, 1985; Lehner et al., 1987; Rober et al., 1989) and are dispensable for development (Sullivan et al., 1999), they are clearly not required for assembly of the basic lamina. Moreover, although lamin A is readily incorporated into the lamina of in vitro assembled sperm pro-nuclei, this occurs through the formation of hetero-oligomeric associations with lamin B3, and, in the absence of lamin B3, lamin A forms small aggregates in the nucleoplasm (Dyer et al., 1999). During NE reassembly after mitosis in somatic cells, B-type lamins appear at the nuclear periphery early in telophase, whereas A-type lamins first appear in the nucleoplasm and arrive at the NE only during G₁ phase (Bridger et al., 1993; Broers et al., 1999; Dechat et al., 2000). A significant proportion of A-type lamins is readily extracted from the lamina of a range of cell lines, whereas B-type lamins are completely insoluble. This finding is consistent with the fact that several dominant negative A-type and B-type lamin mutants that form nucleoplasmic aggregates cause the relocation of A-type lamins from the NE to the aggregates but do not affect the distribution of B-type lamins (Vaughan et al., 2000; Izumi et al., 2000). These reports highlight two properties of lamina filaments: (1) the basic structure is highly

insoluble; and (2) the stability of this structure is based around strong molecular interactions presumably between adjacent B-type lamin dimers and between B-type lamin dimers and their INM-protein binding partners. A-type lamins appear to be incorporated into the lamina through hetero-oligomeric associations with B-type lamins, although this association may well be stabilised by additional interactions between A-type lamins and the INM proteins LAP1C and emerin.

We propose the following model to explain the association of lamin A with the lamina. Lamin A dimers form tetramers within the nucleoplasm through half-staggered anti-parallel associations. The tetramers then associate with lamin B dimers through head-to-tail and anti-parallel associations at the NE. If associations between A-type and B-type lamins occur at the NE, there may be a ligand that recruits lamin A tetramers to the NE. For pre-lamin A, the ligand is probably an isoprene receptor. However, for mature lamin A, the ligand must be another protein, and obvious candidates are emerin and LAP1C. Thus, we envisage that emerin recruits mature lamin A to a position where it can integrate into the lamina filament (Fig. 4). LAP1C might further stabilise the association of lamin A with the NE, since the amount of LAP1C in the NE is proportional to the amount of lamin A and/or C (Powell and Burke, 1990).

How does lamin C fit with this model? Several lines of investigation now suggest that lamin C associates with the lamina by interacting with lamin A. In several cancer cell lines in which lamin A is not expressed, lamin C is located in the nucleolus rather than at the NE (Vaughan et al., 2000; Venables et al., 2000). Moreover, when fluorescently labelled lamin C is injected into the nuclei of Swiss 3T3 cells, it forms aggregates in the nucleoplasm that persist for several hours. In contrast, when the same fluorescently labelled lamin C is co-injected into Swiss 3T3 cells with lamin A, it is incorporated into the NE rapidly (Pugh et al., 1997). The fact that lamin C expressed transiently in murine embryonal carcinoma cells that are arrested in S phase accumulates in the nucleoplasm is consistent with these data (Horton et al., 1992). Finally, in Sw13 cells that express very low levels of lamin A, lamin C is located in the nucleolus. When GFP-lamin-A is overexpressed in these cells, endogenous lamin C relocates to the NE (Vaughan et al., 2000). If lamin C localisation at the NE depends on lamin A, then the model is modified as follows: lamin A and lamin C form anti-parallel tetramers in the nucleoplasm. These tetramers are then incorporated into the lamina through the formation of head-to-tail associations with lamin B oligomers at the NE (Fig. 4).

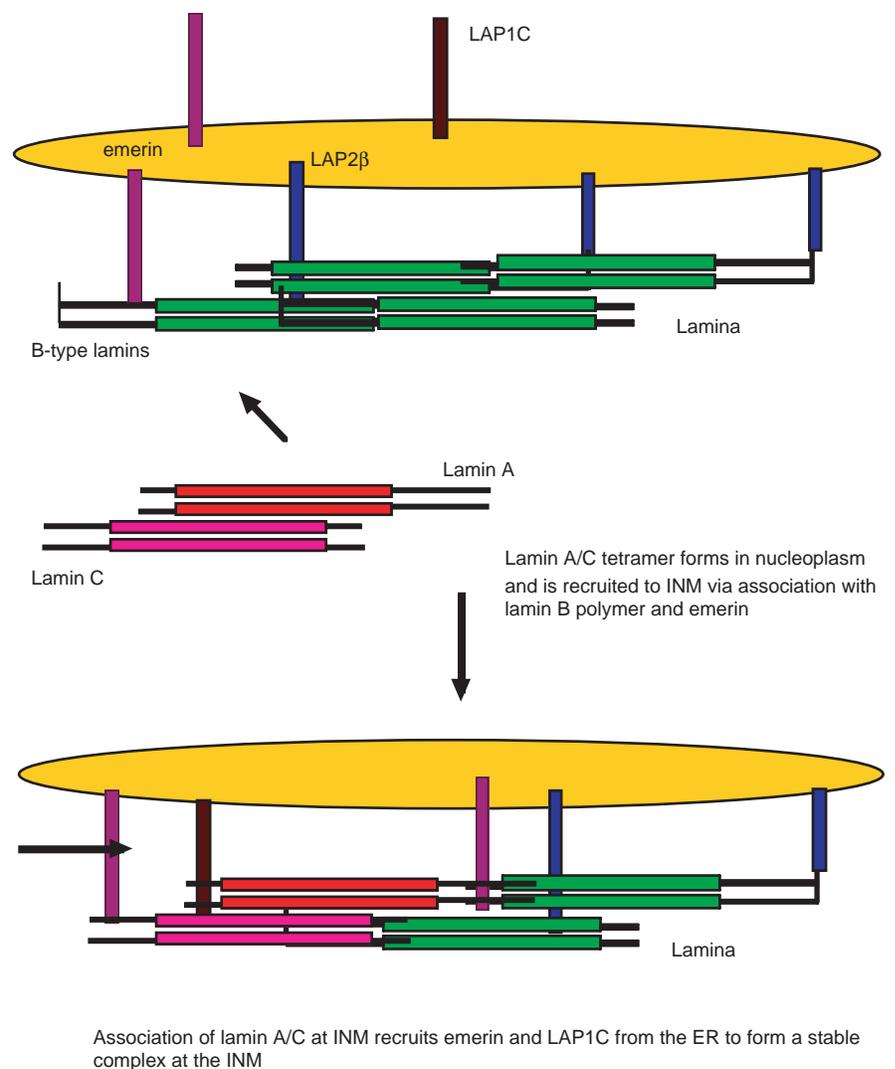


Fig. 4. Incorporation and stabilisation of A-type lamins at the INM. A-type lamin tetramers are incorporated into the INM by head-to-tail and anti-parallel associations with B-type lamin filaments. Recruitment of these lamins to the INM leads to further recruitment of emerin and LAP1C from the ONM/ER to the INM, stabilising the A-type lamins. 'Lamina' indicates where lamin filaments are assembling.

A number of points about this model should be made explicit. The amounts of both LAP1C and emerin at the INM are probably proportional to the amount of lamin A and lamin C (Powell and Burke, 1990; Sullivan et al., 1999; Vaughan et al., 2000). Therefore, how can A-type lamins be recruited to the NE by either protein, if both proteins are recruited to the INM by lamins A and C? One possibility is that pre-lamin A initially recruits a seeding population of emerin and LAP1C to the INM, and this population then recruits mature lamin A. Alternatively, since LAP1 proteins and emerin bind to lamin B₁ *in vitro*, perhaps the assembly of B-type lamins at the INM during telophase recruits sufficient emerin or LAP1C to initiate recruitment of lamin A. Differences in the behaviour of A-type and B-type lamins at telophase support this model. Recruitment of B-type lamins to the NE at telophase is both rapid and occurs over the entire surface of chromosomes. In contrast, recruitment of lamin A and lamin C is much slower and occurs at focal points in which emerin is also present

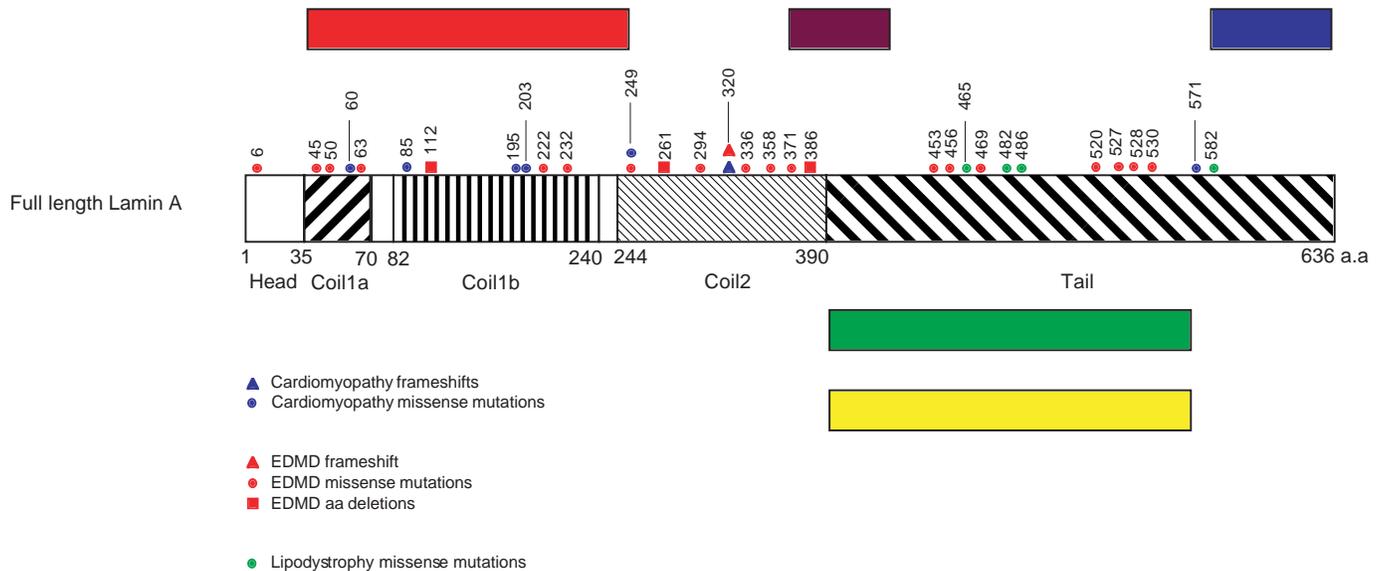


Fig. 5. Distribution of lamin A and lamin C mutations causing dilated cardiomyopathy with conduction block disease, EDMD and Dunnigen-type partial lipodystrophy in relation to known lamin interaction domains. In the diagram these domains include the LAP2 α -binding domain, emerin-binding domain, lamin B-binding domain, chromatin-binding domain and chromosome-binding domain. The positions of the various binding domains are indicated by coloured bars above or below the cartoon as follows: red bar—a site of lamin-B–lamin-A/C interaction; purple bar—chromosome-binding domain; green bar=LAP2 α -binding domain; yellow bar=emerin-binding domain; blue bar=chromatin-binding domain.

(Dabauville et al., 1999). These dynamics fit the constraints of the model, since the model predicts that emerin and lamin A limit the rate of each other's incorporation at the INM.

Internal lamins

The concept of internal lamin structures was first proposed some years ago (Goldman et al., 1992; Bridger et al., 1993). Since that time a number of investigations have supported the notion that A-type lamins not only associate with the NE but also form some sort of internal nucleoskeleton (Hozak et al., 1995). Two distinct types of structure appear to exist. First, tubular invaginations of the NE project into the nucleus, often making contact with the nucleolus; these structures are dynamic and continually reorganise (Fricker et al., 1997; Broers et al., 1999). Second, A-type lamins and specifically lamin A have been reported to be components of an internal nucleoskeleton (Hozak et al., 1995) and are associated with nuclear bodies such as nuclear speckles (Jagatheesan et al., 1999). FRAP studies in CHO cells that stably express GFP–lamin-A also reveal the presence of an internal lamin A population that has distinct solubility properties compared with the population of lamin A present at the NE (Broers et al., 1999). Moreover, lamin A and lamin C associate specifically with chromatin-binding proteins such as LAP2 α and p110^{RB} in vitro and appear to influence the cellular distributions of each protein (Ozaki et al., 1994; Dechat et al., 2000; Markiewicz et al., 2000; unpublished data). Thus, internal populations of lamin A and lamin C might anchor or organise components of a nuclear matrix.

Dominant mutations in A-type lamins and their relationship to nuclear structure

Recent reports have identified several mutations in the gene encoding lamin A and lamin C that give rise to a variety of

dominant congenital diseases. These include Emery-Dreifuss muscular dystrophy (Bonne et al., 1999; Raffaele di Barletta et al., 2000), a dilated cardiomyopathy (Fatkin et al., 1999) and most recently a congenital lipodystrophy (Cao and Hegele, 2000; Shackleton et al., 2000). Mutations giving rise to the cardiomyopathy occur mainly within the rod domain and affect coil 1a and coil 1b, although a single missense mutation is located at the C terminus (Brodsky et al., 2000; Fatkin et al., 1999). One mutation giving rise to EDMD leads to insertion of a stop codon after residue six and is effectively null. More commonly, EDMD is caused by missense mutations in two separate clusters of highly conserved residues within regions of the tail domain common to lamin A and lamin C or throughout the rod domain (Bonne et al., 1999; Raffaele di Barletta et al., 2000). Most interestingly, the mutations that cause the lipodystrophy are missense mutations but on this occasion in residues lying between the two EDMD clusters (Cao and Hegele, 2000) (Fig. 5).

Why do these mutations give rise to such a diverse group of diseases, and why do mutations within essentially the same general region of the lamin A and lamin C tail cause two distinct phenotypes? At a molecular level, a number of simple explanations might be put forward.

Lamin-A/C–emerin interactions

EDMD occurs in two distinct forms: an autosomal dominant form caused by mutations in the lamin A/C gene (Bonne et al., 1999; Raffaele di Barletta et al., 2000), and an X-linked form caused by mutations in emerin (Maninal et al., 1996). Several lines of evidence suggest that emerin binds to lamins in vitro (Fairley et al., 1999; Clements et al., 2000). In addition, emerin co-localises with A-type lamins but not B-type lamins during NE reassembly (Dabauville et al., 1999). Our own data as well as evidence from the lamin-A- and lamin-C-knockout mouse

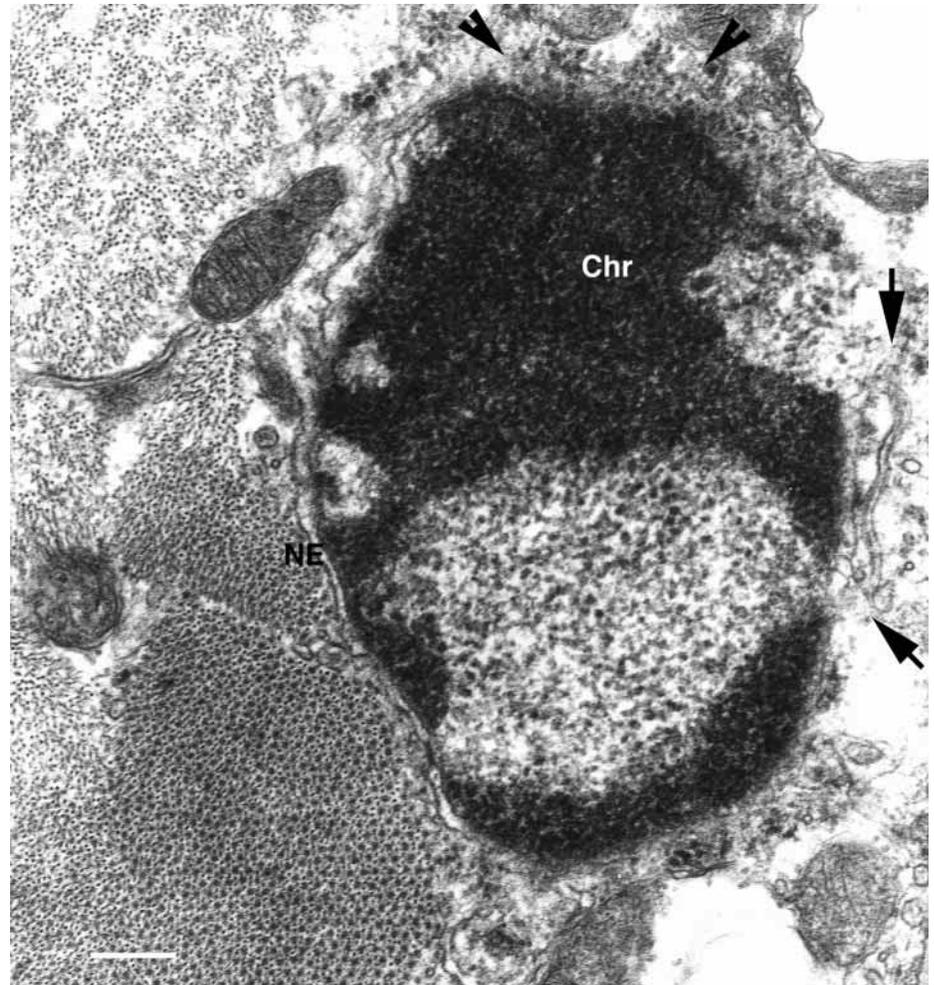


Fig. 6. Electron micrograph illustrating fragile NEs in skeletal muscle from a patient suffering from X-EDMD. NE=nuclear envelope; Chr=chromatin; the arrow shows areas in which the NE is disrupted; arrowhead shows areas in which chromatin is extruded into the cytoplasm. Bar, 200 nm. Micrograph courtesy of Prof. I. Hausmanowa-Petriciewicz and Prof. A. Fidzianska, Warsaw, Poland.

suggest that emerin is anchored at the INM through a specific association with lamin A (Vaughan et al., 2000; Sullivan et al., 1999). In yeast two-hybrid screens, the site of emerin-lamin interactions has been mapped to the tail domain. Moreover, in competition reactions, emerin interacts preferentially with lamin C. Thus we anticipate that emerin-lamin interactions are mediated by residues in the lamin A and lamin C tail that are mutated in EDMD.

Lamin-A/C–lamin-B interactions

B-type lamins are also lamin-A-binding proteins. In extracts of *Xenopus* eggs, human lamin A forms stable oligomeric associations with lamin B3, which appear to mediate the association of lamin A with the lamina in sperm pronuclei (Dyer et al., 1999). In addition, dominant negative mutants of lamin B1 recruit lamin A and lamin C but not lamin B1 or lamin B2 from the nuclear envelope to nucleoplasmic aggregates, when expressed in a range of tissue culture cell lines (Izumi et al., 2000; Vaughan et al., 2000). These dominant negative mutants are created by deletion of the CaaX motif. Further deletion of the first 33 residues of the N terminus and of the C terminus up to the NLS does not affect the ability of the mutant protein to form nucleoplasmic aggregates or to recruit lamin A and lamin C to those aggregates. Mutant proteins in which coil 1a and part of coil 1b (including the helix initiation sequence) are deleted also form nuclear aggregates.

However, lamin A and lamin C are not recruited to these aggregates, which suggests that coil 1a and coil 1b of lamin B1 are essential for lamin A and lamin C associations (Izumi et al., 2000). If lamin A and lamin C and lamin B1 tetramers form, as predicted, through anti-parallel associations (Quinlan et al., 1995), the lamin A and lamin C residues that are mutated in congenital cardiomyopathies fall within a region that would be required for the formation of tetramers with lamin B1 (Fig. 5). Finally, mutations in the rod domain that cause EDMD might influence anti-parallel associations either between A-type lamins and B-type lamins or between lamin A and lamin C, or might prevent helix initiation and thus destabilise dimers.

Lamin-A/C–LAP2 α interactions

How might one explain the fact that mutations lying between the EDMD clusters in the lamin A and lamin C tail cause lipodystrophy? Is there another lamin-A- and lamin-C-binding partner that associates with this region of each protein? We have recently shown that the non-nuclear-membrane LAP family member LAP2 α specifically associates with lamin A and lamin C in vivo and in vitro. The region within the lamin A and lamin C tail that binds to LAP2 α spans that containing the lipodystrophy mutations (and indeed the EDMD mutations). Therefore, is LAP2 α involved in EDMD or lipodystrophy or both? As yet there is no direct evidence for

any of these possibilities; however, consider one factor: LAP2 α is a chromatin-associated protein that forms part of the structure referred to as the nuclear matrix. As such, this protein does not associate with the NE and indeed does not co-localise with NE lamins (Dechat et al., 1998; Dechat et al., 2000). However, A-type lamins within the nucleoplasm do co-localise with LAP2 α and, importantly, LAP2 α distribution is influenced by the distribution of A-type lamins *in vivo* (Dechat et al., 2000). Therefore, we can assume that LAP2 α and emerin associate with completely different subpopulations of A-type lamins, even though they might associate through similar regions of the tail domain. Whether mutations in NE-associated lamins are involved mainly in EDMD, whereas mutations in nucleoplasmic lamins are involved in lipodystrophies, remains to be seen. Remember, in the lamin-A- and lamin-C-knockout mouse, complete absence of lamin expression leads to EDMD-like phenotypes and an absence of white fat. Clearly, under these circumstances, neither NE-associated lamins nor nucleoplasmic lamins are present.

Nuclear defects associated with EDMD

A number of nuclear architectural defects associated with EDMD have now been described. These are mainly associated with the X-linked form of the disease and include aberrant distribution of heterochromatin, fragility within the NE and leakage of lamins into the cytoplasm (Fidzianska et al., 1998). In the lamin-A- and lamin-C-knockout mouse, similar nuclear defects are also observed (Sullivan et al., 1999). These defects appear to be similar to those evident in IF-fragility syndromes. Most IF diseases result in tissue or cell fragility. For example, skin-blistering disorders arise through mutations in several keratins (Chan et al., 1993; Chipev et al., 1994; Rugg et al., 1993) or plectins. These mutations compromise the IF cytoskeleton, weakening associations with desmosomes or bundling of filaments. At the cellular level the result is fragile associations between cells such that fractures occur between specific epithelial layers within the skin and at sites that are vulnerable to wear and tear (Irvine and McLean 1999). Preliminary immunofluorescence investigations of lamin and emerin organisation in EDMD tissues have been carried out, but no gross differences in organisation of either protein was reported (Bonne et al., 1999). There has as yet been no thorough ultrastructural examination of lamina filament organisation in EDMD tissues. Indeed, lamina filament organisation in nuclei other than large amphibian germinal vesicles has been achieved only in one study, which depended upon FESEM microscopy (Zhang et al., 1986). Therefore, it is unclear whether or not filament organisation is compromised in affected tissues. However, fragility of the NE is an obvious feature of absence either of emerin or of lamin A and lamin C (Fidzianska et al., 1998; Sullivan et al., 1999 – see Fig. 6). If we consider this disease, and indeed the cardiomyopathy, to be an IF-fragility syndrome, then we can propose the following model to explain the disease at both a molecular and a cellular level.

Lamin A and lamin C are incorporated into the lamina through a minimum of two molecular interactions. The basic and necessary interaction is through anti-parallel associations between A-type lamin dimers and B-type lamin dimers (Fig. 4). The B-type lamin dimers form stable associations with the INM (as explained above), but in this configuration A-type

lamins are not stably associated with the INM. Therefore a second stabilising interaction occurs through associations between the lamin A and lamin C tail, and emerin. Other associations, such as interactions between A-type lamins and LAP1C (Powers and Burke, 1990), might make the association of this lamin with the NE even more stable. If this model is correct, one should be able to predict the results of a number of experiments. For example, in FRAP studies involving GFP-lamin-A, the recovery time for bleached regions of NEs is 2-4 hours (Broers et al., 1999). If emerin stabilises the association of lamin A with the NE, then mutations in emerin or an absence of emerin should increase the rate of dissociation and re-association of lamin A with the lamina and therefore decrease the recovery time after photobleaching. The same outcome should be true of the reverse experiment. Emerin localisation at the INM is dependent upon its interaction with lamin A (Vaughan et al., 2000). Therefore EDMD mutations in the lamin A/C tail (but not mutations associated with dilated cardiomyopathy or partial lipodystrophy) ought to lead to increased mobility of emerin and again an increase in the rate of recovery after photobleaching. With respect to dilated cardiomyopathy, the model suggests that association of lamin A and lamin C with lamin B is principally through anti-parallel associations. This could be tested in assays that measure protein-protein interactions *in vitro*, such as BIACORE or the yeast-two hybrid system (Clements et al., 2000; Vaughan et al., 2000). Moreover, one might also predict that identical mutations in the rod domain of lamin B1 or B2 affect the stability of lamin A within the lamina, and again this could be investigated by FRAP. Alternatively, some EDMD mutations within the rod domain might disrupt the helix, and one would predict that such mutations give rise to unstable proteins. Finally, mutations that affect lamin-A/C-LAP1C interactions might also result in a muscular dystrophy or a cardiomyopathy.

The cellular question is clearly this: if lamin A and lamin C are ubiquitously expressed, why do mutations in these proteins principally affect cardiac and skeletal muscle? At one level this question might be fairly simple to address. Both the dilated cardiomyopathy and EDMD are notable for very variable penetrance (Brodsky et al., 2000). In EDMD, skeletal muscle wastage progresses very slowly, and patients are mainly at risk of sudden heart failure (Emery, 1989). Therefore, the differences between EDMD and dilated cardiomyopathy are more apparent than real. Both diseases can emerge over some thirty to forty years. Presumably, the NE is vulnerable to damage in contractile tissues such as skeletal and cardiac muscle. This vulnerability may be increased because, in muscle, B-type lamins are apparently expressed at reduced levels (Manilal et al., 1999). If the absence of emerin from the NE or mutations in lamins A and C destabilises the association of these lamins with the NE, the lamina as a whole would become less effective as a load-bearing structure, particularly in muscle. Eventually, this general fragility translates into physical damage leading to cell death and tissue damage. Physical damage manifests itself as fractures at the NE and the leakage of lamins and chromatin into the cytoplasm (Fidzianska et al., 1998 and Fig. 6). In skeletal muscle, the damage might be limited because muscle fibres are a syncytium, and not all nuclei will be damaged within a single muscle fibre. If sufficient numbers of nuclei become damaged, and the fibre is compromised, individual muscle fibres can be

replaced. In contrast, in cardiac muscle, loss of individual cardiomyocytes in adults will be cumulative and will eventually lead to conduction blocks. Therefore, we propose that the aetiology of both diseases can be explained by an accumulation of damaged nuclei as a result of a reduction in the load-bearing properties of the lamina. Note that a novel A-kinase-associated protein termed mAKAP is expressed exclusively in skeletal and cardiac muscle, localises to the INM and maps to a region of chromosome 14 that is linked to Arrhythmogenic right ventricular dysplasia (ARVD) (Kapiloff et al., 1999). The presence of mAKAP at the INM of these tissues localises PKA to the lamina. Perhaps the function of PKA at this site is to detect and respond to damaged lamina filaments.

Although fragility may explain the cellular pathology associated with EDMD and cardiomyopathy it is more difficult to use this model to explain the lipodystrophy. An alternative hypothesis is that this disease (and possibly the other diseases) are promoted because lamin complexes (either lamin A/C and emerin or lamin A/C and LAP2 α) are involved in tissue-specific gene regulation. Some evidence for this hypothesis has been reported in that lamins bind to the transcriptional regulators p110^{RB} (Ozaki et al., 1994) and BAF1 (Furukawa, 1999). Moreover, ectopic expression of lamin A in myoblasts has been reported to induce the synthesis of muscle-specific genes (Lourim and Lin, 1990). Therefore an alternative hypothesis termed the 'gene expression' hypothesis has been proposed recently, and on the basis of this hypothesis one might assume that the diseases arise through altered gene expression in either cardiac muscle, skeletal muscle or in adipocytes. Since both skeletal and cardiac muscle develop normally in the lamin-A- and lamin-C-knockout mouse (Sullivan et al., 1999), and penetrance in the diseases is very variable, one would expect any change in gene expression resulting from mutations in lamin A/C or emerin to be subtle. Detecting and cataloguing of these changes is likely to require DNA chip technology (Cohen et al., 2001).

Conclusions

Mutations in A-type lamins and their associated proteins are likely to give rise to an increasing range of inheritable diseases. An understanding of the aetiology of each disease will arise from an understanding of lamina structure and function. The models proposed here relate mainly to the likely organisation of A-type lamins at the INM and how this organisation influences some INM proteins. In the future, similar models for the organisation of internal lamin structures will become necessary if we are to achieve a complete understanding of molecular mechanisms underlying the various lamin-related diseases.

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