Proteins that bind A-type lamins: integrating isolated clues

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Summary
What do such diverse molecules as DNA, actin, retinoblastoma protein and protein kinase Cα all have in common? They and additional partners bind ‘A-type’ lamins, which form stable filaments in animal cell nuclei. Mutations in A-type lamins cause a bewildering range of tissue-specific diseases, termed ‘laminopathies’, including Emery-Dreifuss muscular dystrophy and the devastating Hutchinson-Gilford progeria syndrome, which mimics premature aging. Considered individually and collectively, partners for A-type lamins form four loose groups: architectural partners, chromatin partners, gene-regulatory partners and signaling partners. We describe 16 partners in detail, summarize their binding sites in A-type lamins, and sketch portraits of ternary complexes and functional pathways that might depend on lamins in vivo. On the basis of our limited current knowledge, we propose lamin-associated complexes with multiple components relevant to nuclear structure (e.g. emerin, nesprin 1α, actin) or signaling and gene regulation (e.g. LAP2α, retinoblastoma, E2F-DP heterodimers, genes) as ‘food for thought’. Testing these ideas will deepen our understanding of nuclear function and human disease.

Key words: Laminopathy, Emerin, Emery-Dreifuss muscular dystrophy, Hutchinson-Gilford progeria syndrome, Nuclear envelope

Introduction

Lamins are intermediate filament proteins found only in the nuclei of multicellular eukaryotes. They form stable filaments at the nuclear inner membrane and stable structures in the nucleoplasm that might or might not be filamentous. Lamins are important for nuclear architecture; they provide mechanical strength, determine nuclear shape, and anchor and space the nuclear pore complexes. Perhaps more surprisingly, lamins are important for nuclear architecture; they provide mechanical strength, determine nuclear shape, and anchor and space the nuclear pore complexes. Lamins continue to be expressed in most differentiated adult cells, whereas A-type lamins (encoded by LMNA) are still unknown. A-type lamins, although dispensable during development, and cell-type-appropriate isoforms of A-type lamins begin to be expressed in a tissue-specific manner during development, and cell-type-appropriate isoforms of A-type lamins continue to be expressed in most differentiated adult cells (Machiels et al., 1996; Rober et al., 1989). A-type lamins appeared late in metazoan evolution; they are not found in the nematode Caenorhabditis elegans, which has differentiated cells and tissues, but are found in more complicated eukaryotes, including Drosophila, birds and vertebrates (Cohen et al., 2001; Zimek et al., 2003). Despite their clearly different roles, the conserved similarities between A- and B-type lamins suggest similar mechanisms of function in the nucleus.

Like other intermediate filament proteins, lamins consist of an N-terminal head domain, a central coiled-coil (rod) domain responsible for dimerization, and a large globular C-terminal tail. The tail includes an immunoglobulin (Ig)-fold domain, the backbone organization of which is unique to lamins, formed by residues 430-545 (Dhe-Paganon et al., 2002; Krimm et al., 2002). All lamins except lamin C and lamin C2 also contain a CAAX (Cys-aliphatic-aliphatic-any residue) motif at the C-terminus, which is farnesylated on Cys in vivo prior to proteolytic removal of the AAX sequence. Prenlim A is then carboxymethylated and re-cleaved to remove the modified Cys plus 14 additional residues (647-661), yielding mature lamin A (Fig. 1) (Moir et al., 1995). A site-specific protease named Zmpste24 is proposed to perform both cleavage events (Pendas et al., 2002). Lamin A is (so far) the only known substrate for Zmpste24 in mammals. Consistent with lamin A being a major substrate is the observation that loss of Zmpste24 causes muscular dystrophy and bone degeneration phenotypes in mice (Bergo et al., 2002; Pendas et al., 2002; see below) and mandibuloacral dysplasia in humans (Agarwal et al., 2003), disorders associated with compromised A-type lamin function.

The assembly mechanisms and structures of lamin filaments are reviewed elsewhere (Goldman et al., 2002; Stuurman et al., 1998). However, several properties are noteworthy. First, the abundance and organization of lamin filaments vary between...
Fig. 1. Regions in lamins A and C to which partners bind. The structural domains (head, rod, tail) of prelamin A are shown. Exons 1-12 encoding residues 1-664 are numbered; the last residue encoded by each exon (except exon 11) is given above. The Ig-fold domain, which includes exons 8 and 9, is shaded gray. Residues 1-566 of lamin A and lamin C are identical. Unique lamin C tail residues 567-572 are produced by alternative mRNA splicing; thus the extreme C-terminal regions of lamins A and C may have distinct binding properties. The zigzag represents the farnesyl moiety on prelamin A; the farnesylated C-terminal peptide is normally removed by proteolytic cleavage after residue 646 (bold; dotted line) to generate mature lamin A. Colored bars indicate the region(s) in lamins A and C required for each named partner to bind, as detailed in Table 1 (e.g. actin can bind two different regions in the tail). For partners with question marks, the binding region in lamin A/C is unmapped. Based on current incomplete knowledge, interactions were loosely color-coded (top to bottom) as blue (architectural), orange (chromatin), yellow (gene regulation), pink (signaling) and green (unknown). Some partners (e.g. BAF) will continue to defy categorization until more is known about their functions.

species and cell types. In *Xenopus* oocyte nuclei, lamins form square networks of 10 nm filaments, all of which are tightly associated with the inner membrane (Stuurman et al., 1998). By contrast, in mammalian somatic nuclei, a significant number of lamins are found in the nuclear interior (Moir et al., 2000). The ultrastructure of interior lamins is unknown, except that they are unlikely to form 10 nm filaments. Second, although A-B heterodimers can form in vitro (Georgatos et al., 1988; Schirmer et al., 2001; Ye and Worman, 1995), there is growing evidence that A-type and B-type lamins form independent networks in vivo (Izumi et al., 2000; Moir et al., 2000; Haraguchi et al., 2001). Finally, and most interestingly from our current viewpoint, lamin polymers comprise a three-dimensional network that displays at least a million copies of each binding site in the exposed rod and tail domains of each lamin, per nucleus. (HeLa cell nuclei contain ~10⁶ copies each of lamins A, B1 and C) (L. Gerace, personal communication). Most biochemical studies have measured partner binding to lamin dimers, not polymers; thus the corresponding interaction in vivo could have increased avidity owing to the large number of available sites on lamin polymers. It is also possible that lamins form a variety of distinct oligomers or polymers that have distinct ‘ docking’ properties. The variety of human diseases linked to A-type lamins suggests that these sites, and the partners that bind to them, are important for nuclear function.

**Laminopathies**

At least seven diseases are caused by mutations in *LMNA* (Burke and Stewart, 2002; Östlund and Worman, 2003). Each disease affects specific combinations of tissues such as the heart, skeletal muscle and tendons (Emery-Dreifuss muscular dystrophy; EDMD), skeletal muscle and heart (limb-girdle muscular dystrophy 1B), the heart only (dilated cardiomyopathy type 1A), neurons and muscle (Charcot-Marie-Tooth disorder type 2B1), adipocytes (Dunnigan-type familial partial lipodystrophy), or adipocytes and bone (mandibuloacral dysplasia). The most devastating laminopathy is Hutchinson-Gilford progeria syndrome (HGPS), which affects multiple tissues and resembles premature aging (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003; Chen et al., 2003). Patients who have this disease usually die in their teens. Whereas most laminopathies are caused by missense mutations spread throughout the molecule, the most frequent known cause of HGPS is a point mutation that creates a new splice donor site, resulting in the deletion of 50 residues (608-657), including the proteolytic cleavage site at residue 646.
Thus, HGPS patients are predicted to produce an internally deleted form of the farnesylated and carboxymethylated lamin A precursor. Zmpste24-null mice lack the processing protease and also have bone- and muscle-disorder phenotypes (Bergo et al., 2002). This suggests that severe laminopathy, if not progeria, might correlate with the absence of unprocessed lamin A precursors. Interestingly, a protein named nuclear prelamin A recognition factor (Narf), which is expressed at its highest levels in heart, skeletal muscle and brain, localizes to the nuclear envelope and intranuclear foci and binds specifically to farnesylated lamin A precursors (Barton and Worman, 1999). Thus, these forms of lamin A have the potential to saturate Narf, the function of which is unknown. When expressed exogenously, disease-associated laminas can disturb the organization of endogenous lamins (e.g. Raharjo et al., 2001; Capanni et al., 2003). In the few cases studied, laminopathy mutations can disrupt binding of lamins to specific partners (see below).

The first genetic model for laminopathy was the LMNA-knockout mouse; these mice appear normal at birth but develop severe muscular dystrophy and die within eight weeks (Sullivan et al., 1999). In the first reported human LMNA-null case, a baby possessing a homozygous nonsense mutation in LMNA, with no detectable A-type lamin proteins, died after premature birth, exhibiting severe joint contractures, muscular dystrophy, fibrosis and absence of muscle fibers in the diaphragm (Muchir et al., 2003). This premature birth and death cannot be attributed exclusively to loss of A-type lamins, because of parental consanguinity and the consequent potential for homozygous defects in other genes. Nonetheless, the resemblance to laminopathy suggests that A-type lamins, although not essential for cell viability, are essential for human life.

Mice have been constructed to express only the L530P-mutated forms of lamins A and C, which causes EDMD in humans (Mounkes et al., 2003). A side-effect of the targeting strategy was that the ‘knocked-in’ L530P allele also changed the mRNA splicing pattern to yield two predicted protein products: mature A-type lamins that lack exon 9, and a C-terminally-truncated lamin A that has 19 extra (intron-encoded) internal residues (see supplementary data in Mounkes et al., 2003). Neither mutation is expected to disrupt C-terminal processing of lamins, but this possibility has not been ruled out. These mice have ‘progeria-like’ pathologies of bone and skin, and die prematurely (Mounkes et al., 2003). Loss of human Zmpste24 gene activity causes severe mandibuloacral dysplasia with progeroid appearance and general lipodystrophy (Agarwal et al., 2003), which is altogether less severe than HGPS. Thus, large deletions in the lamin A/C tail (i.e. loss of 50 residues in HGPS, or loss of exon 9 residues in L530P-knock-in mice) might correlate with progeria, and must certainly disrupt lamin organization and interactions. The effects of ‘progeric’ mutations on the assembly and partner-binding activities of A-type lamins will be important to test.

Many plausible disease mechanisms have been proposed. Some models emphasize possible disruption of the mechanical properties of lamin filaments, which control the shape, stiffness and structural integrity of nuclei. Other models view lamins as sites (‘scaffolds’) for the assembly of other proteins that regulate transcription, cell fate or apoptosis (Burke and Stewart, 2002; Goldman et al., 2002). Both models are probably correct. We have taken a fresh look at the published binding partners for A-type lamins (Table 1, Fig. 1). Some bind A-type lamins in two-hybrid assays and in vitro, but their in vivo significance remains untested. In other cases, the interactions have also been studied in vivo. Many partners were familiar to us, but others were not. They form four loose groups: architectural partners, chromatin partners, gene-regulatory partners and signaling partners. Clearly, some partners fit multiple categories. What emerges, however, is that a rich variety of protein complexes and biochemical pathways could depend on lamins in vivo.

### Table 1. Current knowledge of binding regions in A-type lamins and each partner

| Partner   | Binding region in partner | Binding region in lamin A/C |
|-----------|---------------------------|----------------------------|
| Actin     | ND*                       | Lamins A and C residues 461-536 (Zastrow et al., unpublished) |
|           |                           | Lamin A residues 563-646 (Sasseville and Langelier, 1998) |
| BAF       | ND                        | ND                         |
| DNA       | Minor groove              | Lamins A and C residues 411-553 (Sisterle et al., 2003) |
| EIB 19K   | Residues 1-146            | Lamins A and C residues 252-390 (Rao et al., 1997) |
| Emerin    | Residues 70-170           | Lamins A and C residues 384-566 (Lee et al., 2001; Sakaki et al., 2001) |
| Histones  | ND                        | Lamin C residues 396-430 (Taniura et al., 1995) |
| Lamin B   | ND                        | ND                         |
| LAP1      | ND                        | ND                         |
| LAP2α     | Residues 616-693          | Lamins A and C residues 319-566 (Dechat et al., 2000) |
| 12(S)-LOX | ND                        | Lamin A residues 463-664 (Tang et al., 2000) |
| MOK2      | Residues 1-173            | Lamins A and C residues 243-387 (Dreuilhet et al., 2002) |
| Narf      | ND                        | Farnesylated prelamin A residues 389-664 (Barton and Worman, 1999) |
| Nesprin 1α| Residues 476-1109         | ND (Mislow et al., 2002a) |
| PKCα      | Residues 200-217          | Lamin A residues 500-664 (Martelli et al., 2002) |
| Rb        | Residues 660-672          | ND for lamin C (Markiewicz et al., 2002) |
| SREBP     | Residues 227-487          | Lamin A residues 389-664 (Lloyd et al., 2002) |

*ND, not determined.
stable complexes during interphase (on the basis of their resistance to chemical extraction or their immobility in FRAP experiments), (2) bind tightly to A-type lamins in vitro, or (3) have known structural roles in other contexts (e.g. actin). Such partners might connect lamins to the nuclear envelope, chromatin or other subnuclear structures (Burke and Stewart, 2002; Cohen et al., 2001). All integral membrane proteins that bind to lamins fit this definition, including lamina-associated polypeptide 1 (LAP1) and emerin (Foisner and Gerace, 1993; Östlund et al., 1999). LAP1 has an unusually large luminal domain (Foisner and Gerace, 1993; Martin et al., 1995) and is expressed as multiple isoforms but has not been further characterized. More information is available for emerin, which is relatively immobile in the nuclear inner membrane (Östlund et al., 1999) and binds lamin A with an affinity of 40 nM in vitro (Holaska et al., 2003) (reviewed by Bengtsson and Wilson, 2004). Interestingly, emerin also binds directly to barrier-to-autointegration factor (BAF), a DNA-binding chromatin protein, and might thereby anchor lamins and chromatin to the nuclear envelope during interphase (Lee et al., 2001). Other nuclear membrane proteins (e.g. LAP2β) bind to B-type lamins, BAF, DNA and chromatin, extending this architectural theme (Cai et al., 2001; Foisner and Gerace, 1993; Furukawa, 1999; Shumaker et al., 2001). Emerin and LAP2β both belong to the LEM-domain family of nuclear proteins (Lin et al., 2000). The defining feature of LEM-domain proteins is their LEM domain: a folded motif of at least 40 residues that binds directly to BAF (Zheng et al., 2000; Cai et al., 2001). The LEM-domain family in vertebrates also includes at least six splice forms of LAP2 (Berger et al., 1996), MAN1 (which binds Smad transcription factors and regulates development) (Osada et al., 2003; Raju et al., 2003) and a smaller MAN1-related protein provisionally named Lem2 (Lee and Wilson, 2004), which is conserved in metazoans and essential for viability in C. elegans embryos (Lee et al., 2000; Liu et al., 2003). Lem2 (also known as NET-25) was identified in a proteomic study that also revealed more than 60 additional potential nuclear membrane proteins (Schirmer et al., 2003). We assume that many of these novel proteins bind A-type lamins. Further work will undoubtedly increase our appreciation of the complexity of the nuclear lamina network and possibly also extend its range of known functions.

The best-studied architectural partner for A-type lamins in the nuclear interior is LAP2α, which has separate binding sites for BAF, A-type lamins and chromatin (Dechat et al., 1998; Dechat et al., 2000; Vlcek et al., 1999). LAP2α binds tightly to A-type lamins during interphase (Dechat et al., 2000), but has dynamic architectural roles during nuclear envelope assembly (Vlcek et al., 2002). It is proposed to tether A-type lamins to intranuclear sites and to cooperate with lamins in organizing chromatin (Foisner, 2003). LAP2α (and other LEM-domain proteins) also binds transcriptional regulators (see below), establishing a theme in which ‘architectural’ partners for lamins also influence gene regulation, directly or indirectly.

We do not yet know whether B-type lamins are direct architectural partners for A-type lamins in vivo. A- and B-type lamins have distinct assembly pathways in vivo (Moir et al., 2000), which could mean they form distinct networks. However, B-type lamins might ‘set the stage’ for the assembly of A-type lamins. A-type lamins are known to be architectural partners for each other, since lamin C depends on lamin A for its localization and assembly in the nucleus (Vaughan et al., 2001).

Actin, actin-related proteins and numerous actin-binding proteins (including a nuclear-specific isoform of myosin I) are present in the nucleus, where their functions are now slowly emerging (Pederson and Aebi, 2002). There is currently no evidence for long actin filaments (F-actin) in the nucleus. However, actin can also form a multitude of special dimers, short protofilaments and tubular, flat or branched oligomers that would suit the chromatin-dominated nuclear space (Pederson and Aebi, 2002). Interestingly, nuclear actin polymers adopt a unique conformation that is recognized by specific antibodies (Milankov and De Boni, 1993). Actin binds directly to two regions in the lamin A/C tail: residues 461-536 (in the Ig-fold domain) (M.S.Z. and K.L.W., unpublished) and residues 563-646 (Sasseville and Langelier, 1998). A-type lamin filaments might thus bind to actin polymers in the nucleus. The possibility that actin polymers are architectural partners for lamin filaments deserves serious attention.

Nesprin 1α, a 131 kDa nuclear membrane protein, binds directly to A-type lamins and emerin (Mislowl et al., 2002a; Mislowl et al., 2002b). All nesprin-family proteins (also known as Syne, Myne and NUANCE) have multiple spectrin repeat (SR) domains, and many also have an actin-binding domain (Zhang et al., 2002; Zhang et al., 2001; Starr and Han, 2003). Human nesprins are encoded by two genes, which yield multiple protein isoforms through alternative mRNA splicing and use of internal transcription initiation sites. These isoforms range in mass from huge (~1 MDa for nesprin 1α) to modest (e.g. 131 kDa for nesprin 1β) (Mislowl et al., 2002a; Zhang et al., 2002; Zhang et al., 2001). Nesprins are anchored to specific membranes, such as the Golgi complex or the outer or inner membrane of the nuclear envelope (Gough et al., 2003; Mislowl et al., 2002b; Zhang et al., 2001). Nesprin 1α was proposed to anchor lamin A and emerin at the inner nuclear membrane. However, this model was overthrown by the finding that nesprin 1α and emerin both mislocalize to the endoplasmic reticulum (ER) in human fibroblasts that lack A-type lamins (Muchir et al., 2003). Transient expression of either lamin A or lamin C is sufficient to relocalse both membrane proteins to the nuclear envelope. These results suggest an architectural hierarchy based on the integrity of A-type lamins.

Chromatin partners

Lamins bind DNA directly but nonspecifically in vitro, by contacting the minor groove (Luders et al., 1994). The DNA-binding region is identical in lamin A and lamin C, and includes both the Ig-fold domain and the nuclear localization signal in the tail (Stierle et al., 2003). The affinity of lamin A for DNA is 420 nM (Stierle et al., 2003). The R482W missense mutation, which causes lipodystrophy, reduces this affinity for DNA by seven-fold (to 2.95 nM), and also weakens binding of lamin A to the adipocyte differentiation factor sterol response element-binding protein (SREBP; see below).

DNA is wound around core histone octamers to form nucleosomes, the fundamental unit of chromatin structure. The human lamin A/C tail binds to mixtures of core histones with an apparent affinity of 300 nM (Taniura et al., 1995), which is similar to its affinity for naked DNA. Since histones and DNA
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Gene-regulatory partners
Lamin A/C filaments are thought to provide scaffolds for protein complexes that regulate gene expression (Cohen et al., 2001). Hypophosphorylated retinoblastoma protein (Rb) binds specifically to A-type lamins in vitro (Mancini et al., 1994; Markiewicz et al., 2002; Ozaki et al., 1994). Rb is a transcriptional regulator that has central roles in cell-cycle control. Hypo-phosphorylated Rb binds to E2F-DP heterodimers and blocks E2F-dependent gene expression through a variety of mechanisms, including the recruitment of histone deacetylase complexes (HDACs) (Chau and Wang, 2003). Rb also regulates expression of apoptosis protease-activating factor 1 (Apaf1) and thus apoptosis, which is proposed to contribute to muscle loss in EDMD patients (Bonne et al., 2003). During G1 phase, hypophosphorylated (repressive) Rb is anchored to the nucleoskeleton by its ‘pocket-C’ domain, which binds directly to A-type lamins and LAP2α (Mancini et al., 1994; Markiewicz et al., 2002). Overexpression of LAP2α interferes with cell-cycle progression and leads to apoptosis (Vleek et al., 2002), which supports ‘scaffolding’ models in which Rb activity depends on its attachment to lamins and LAP2α (Fig. 2). Such complexes might be weakened by disease-causing mutations, because Rb and LAP2α interact with the rod and rod/tail domains, respectively, of lamins A and C (Fig. 1). Biochemical studies using a different repressor of E2F activity, germ-cell-less (GCL), independently support scaffolding models, showing that GCL, emerin and lamin A form stable tertiary complexes in vitro (Holaska et al., 2003). Indeed, stable, lamin-based scaffolds might be useful to tether the enormous (1-2 MDa) chromatin-remodeling machines recruited by Rb and other regulators (Neely and Workman, 2002) (Fig. 2).

SREBP1a and SREBP1c are additional lamin-binding proteins. Encoded by alternatively spliced mRNAs, they are both basic helix-loop-helix leucine zipper transcription factors that, when activated, are released from the ER by proteolytic cleavage and move into the nucleus. Once in the nucleus, they activate genes required for cholesterol biosynthesis and lipogenesis (reviewed by Horton, 2002) and promote adipocyte differentiation (Kim and Spiegelman, 1996). SREBP proteins bind to the Ig-fold domain of the lamin A/C tail, as do many other partners (Fig. 1). Interestingly, lipodystrophy-causing mutations in A-type lamins reduce SREBP binding by 25-40% (Lloyd et al., 2002). It will be important to determine which other partners (in addition to SREBP and DNA) are affected by lipodystrophy-causing mutations.

MOK2 is a DNA-binding transcriptional repressor that interacts with lamins A and C. It represses genes activated by cone-rod homeobox protein (Crx) transcription factors by competing with them for binding sites (Dreuillet et al., 2002). MOK2 also binds to RNA in vitro, and might thus influence RNA processing (Arranz et al., 1997). Like Rb, MOK2 binds to the coil 2 region of A-type lamins (Dreuillet et al., 2002) (Fig. 1). It therefore seems that A-type lamins have binding site(s) for transcriptional repressors not only in their tail domain, but also in the rod domain. Optimal access to rod domain sites might require a specific oligomeric (dimer, tetramer or octamer) or assembly (filamentous) state.

Another gene-regulatory partner is BAF (see above). BAF binds directly to several different homeodomain transcription activators, including Crx, and represses Crx-dependent gene expression in retinal cells (Wang et al., 2002) (reviewed by Segura-Totten and Wilson, 2004). Thus, BAF and MOK2 both repress CRX-activated genes, but by different mechanisms (Fig. 2). It is curious that two out of four identified lamin-binding repressors influence Crx-regulated genes, but this might simply reflect the low number of proteins studied so far.

The BAF-binding partner emerin is proposed to be a fifth gene-regulatory partner for A-type lamins. Other LEM-domain proteins (e.g. LAP2β and MAN1) that bind lamin B inhibit gene expression in vivo (Liu et al., 2003; Nili et al., 2001; Osada et al., 2003). Emerin has not yet been tested for such activity in vivo. However, it does form a complex with lamin A and the transcriptional repressor GCL in vitro (Holaska et al., 2003). Emerin is very special among known binding partners for lamin A/C, because loss of emerin causes the same disease – EDMD – as many dominant mutations in A-type lamins (Bione et al., 1994). Perhaps in future other laminopathies will be analogously ‘paired’ to loss of a specific lamin-binding protein(s). Meanwhile the wide expression of emerin in human tissues, and functional overlap between emerin and other LEM-domain proteins make it difficult to pinpoint which function(s) of emerin are critical to the EDMD disease mechanism (Bengtsson and Wilson, 2004). Further molecular and in vivo dissection of emerin functions is needed to clarify its role in lamin A/C complexes, which might combine roles in nuclear architecture (Fig. 2) and gene regulation.

Various gene regulators thus bind A-type lamins and/or emerin, and this supports the idea that dysregulation of gene expression is an important contributing factor in laminopathies.
Signaling partners

Signal transduction pathways regulate many activities in the nucleus. Three signaling proteins are known to bind A-type lamins in vivo: lamin filaments might therefore provide a nucleus. Three signaling proteins are known to bind A-type lamins.

Emerin has many direct binding partners including nesprin-1α, lamins, actin (not depicted), BAF and several transcription regulators (not shown) (Bengtsson and Wilson, 2004). The number of distinct oligomeric complexes that include emerin is not known. (Top left) Emerin, representing all LEM-domain proteins, binds BAF. BAF and MOK bind lamins in vitro and inhibit Crx-dependent gene activation in vivo, implicating both proteins in lamin-dependent gene-regulation events. Not depicted are potential gene-regulatory complexes involving nuclear membrane proteins LAP2β (Nili et al., 2001; Foisner, 2003) or lamin B receptor (Östlund and Worman, 2003).

Furthermore, studies with LEM-domain proteins (emerin, LAP2α and LAP2β) and transcriptional regulators (GCL, BAF and Rb) also provide the best current evidence that lamins scaffold multiprotein complexes (Holaska et al., 2003; Markiewicz et al., 2002; Nili et al., 2001). Interestingly, the gene regulators known to bind A-type lamins are all repressors. Gene silencing is certainly key to cell differentiation, but it is premature to assume that all gene regulators act repressively when complexed with A-type lamins.

**Signal transduction pathways regulate many activities in the nucleus. Three signaling proteins are known to bind A-type lamins in vivo: lamin filaments might therefore provide a structural basis for signaling. Protein kinase Cα (PKCα) – a serine-threonine kinase activated by many signals, including diacylglycerol (DAG) and 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE) – binds to the C-terminal half of the lamin A/C tail (Martelli et al., 2002) (Fig. 1). The lamin-binding and substrate-binding regions of PKCα are distinct. In response to elevated DAG levels, PKCα translocates to the nucleus and interacts with A-type lamins, which suggests that signal transduction by PKCα might involve its attachment to a lamin scaffold (Martelli et al., 2000; Ron and Kazanietz, 1999) (Fig. 2).

12(S)-lipoxygenase [12(S)-LOX] also binds to the lamin A/C tail, perhaps overlapping the binding site for PKCα (Tang et al., 2000) (Fig. 1). Interestingly, 12(S)-LOX is part of a lipid-signaling pathway that converts arachidonic acid to 12(S)-HETE, which activates PKCα in prostate tumor cells (Liu et al., 1994). The 12(S)-LOX protein is detected in both the cytoplasm and nucleus in western blots of cell fractions (Tang et al., 2000). We speculate that PKCα might translocate to the nucleus and co-dock with 12(S)-LOX on lamin A/C filaments (Fig. 2). Alternatively, nuclear-localized PKCα might be activated in a 12(S)-LOX-dependent manner. The knowledge that PKCα and 12(S)-LOX are both direct partners for lamin A can be used to test the hypothesis that lamin scaffolds are important for PKCα-mediated signal transduction.

The third signaling partner, E1B 19K, is an adenovirus early protein (Cuconati and White, 2002). E1B 19K binds directly to lamin A in two-hybrid assays, and cofractionates with
lamin A in vivo. In adenovirus-infected cells, E1B 19K, which shares a low level of sequence similarity with Bcl-2, localizes to the ER and nuclear membranes, and blocks apoptosis in a lamin-dependent manner (Rao et al., 1997). E1B 19K also interacts with (and potentially inactivates) a death-promoting repressor named Btf (Kasof et al., 1999), which binds to emerin in vitro (Haraguchi et al., 2004). Does E1B 19K bind lamin A in order to co-assemble with Btf on emerin-lamin complexes, or might it displace Btf? By considering each strand in this web of interactions, we may be able to understand how each partner ‘uses’ lamin A.

Perspectives

There are at least a million copies of each binding site on A-type lamins per nucleus. This repetition should ensure that all binding partners have access to lamin structures. To understand the functions of A-type lamins, we must now search for new binding partners, particularly those expressed in human tissues affected by laminopathy. However, it would be a mistake to neglect known partners, which provide tools to take this field to the next level. Old-fashioned (but still powerful) biochemical analysis, specific functional assays both in vitro and in vivo, and real-time analysis in living cells will all be needed if we are to understand how and why these complexes interact with lamins and what goes wrong in disease. Known partners for A-type lamins can also be mined for structural information to facilitate discovery of new partners. For example, partners whose binding sites on A-type lamins overlap (e.g. MOK2, Rb and E1B 19K in Fig. 1) might share a lamin-binding motif. Such motifs might allow investigators to identify potentially disease-relevant lamin-binding proteins rapidly in databases. We are hopeful that further work on lamin A/C partners, both known and novel, will lead to a better understanding of nuclear lamins and clinical treatments for diseases in which they are implicated.

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