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Proteins That Associate With Lamins: Many Faces, Many Functions

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Abstract

Lamin-associated polypeptides (LAPs) comprise inner nuclear membrane proteins tightly associated with the peripheral lamin scaffold as well as proteins forming stable complexes with lamins in the nucleoplasm. The involvement of LAPs in a wide range of human diseases may be linked to an equally bewildering range of their functions, including sterol reduction, histone modification, transcriptional repression, and Smad- and β-catenin signaling. Many LAPs are likely to be at the center of large multi-protein complexes, components of which may dictate their functions, and a few LAPs have defined enzymatic activities. Here we discuss the definition of LAPs, review their many binding partners, elaborate their functions in nuclear architecture, chromatin organization, gene expression and signaling, and describe what is currently known about their links to human disease.

Introduction

The eukaryotic nucleus is a complex organelle with essential functions in genome stability, DNA replication and gene expression. It is structurally and functionally organized into distinct sub-compartments, most prominently the nuclear envelope (NE), which separates nuclear and cytoplasmic activities. The NE is comprised of three major structures (Fig. 1) [1,2]: i) two concentric membrane layers, the outer (ONM) and the inner (INM) nuclear membrane facing the cytoplasm and nucleoplasm, respectively, and separated by the perinuclear lumen; ii) nuclear pore complexes (NPCs) inserted into the double membrane, which mediate nucleo-cytoplasmic exchange of components, and iii) the nuclear lamina protein meshwork that is tightly associated with the INM and provides mechanical stability. The ONM is directly linked to and functionally related to the endoplasmic reticulum (ER) and

thus contains ribosomes and other ER proteins. Yet a subset of ONM proteins is unique and not shared with the ER. The INM (although joined with the ONM at NPCs) contains its own unique group of integral membrane proteins that selectively and efficiently target to the INM [3,4]. Many of these INM proteins are components of the nuclear lamina, the core of which is formed by the nuclear-specific, type V intermediate filament lamin proteins. Details of lamins are covered elsewhere in this issue; however, critical to this review is that there are different lamin subtypes: B-type lamins expressed throughout development, and A-type lamins found predominantly in differentiated cells [5,6]. Lamins are post-translationally modified by farnesylation [7]. B-type lamins are permanently farnesylated and thus tightly associated with the INM, in contrast with A-type lamins that either are not farnesylated at all or have the farnesyl group removed by an additional post-translational proteolytic cleavage of the C-terminal 15 residues. The transient farnesylation of lamin A may facilitate its incorporation into the lamina, but after cleavage it should be less tightly associated with the membrane and accordingly, a subfraction of lamin A (estimated at ~5 to 10%) can also be found in the nuclear interior [8]. Multiple stable interactions of lamins with INM proteins within the nuclear lamina are fundamental for the mechanical integrity of the NE. This review focuses on mammalian lamin-associated proteins, most of which are components of the NE and the lamina. We attempt to resolve confusion between the terms lamin-versus lamina-associated polypeptides, which have been synonymously used in literature, and describe interactions, dynamics and potential functions of the best studied lamin-associated polypeptides in mammals, as well as their potential involvement in human diseases.

Definition of LAPs as lamin-associated polypeptides

The term LAP was originally used to designate “lamina-associated polypeptides”, INM proteins stably associated with the lamina at the nuclear periphery. These proteins bound lamins and cofractionated with lamins upon extraction with buffers containing high concentrations of monovalent salts and non-ionic detergents [9,10]. As the lamina is restricted to the nuclear periphery, LAPs by this definition are located at the NE. However, thereafter the term LAP has also been used for lamin-associated polypeptides, including proteins associated with the ~5% of lamins not located at the nuclear periphery. As the intranuclear lamins are presumably not assembled into a filamentous structure like in the lamina, epitopes may be accessible in nucleoplasmic lamins that are masked in filaments and thus enable interactions with a different set of binding partners. In both cases, however, the term “associated” is meant to indicate a stable or protracted interaction with lamins or lamin complexes biochemically defined by resistance to extraction with salt and detergent. In contrast proteins that interact with lamins transiently upon activation of specific signaling pathways or during the cell cycle or differentiation would be referred to as lamin-interacting polypeptides. In this review we use the more general definition of LAPs defining proteins stably associated with peripheral and internal lamin complexes. One aspect of this biochemical definition of LAPs is that the interaction with lamins can also be indirect. A LAP could bind another LAP that binds to lamins and still resist the harsh extraction conditions.

Clearly defined LAPs

LAP1 and LAP2 (now known as LAP2β) were identified by monoclonal antibodies generated against lamina-enriched fractions of rat liver nuclei [9,10]. Both proteins were determined to be integral membrane proteins by biochemical means and to

reside at the NE and INM by immunofluorescence and immunogold electron microscopy. Moreover, they bound lamins *in vitro*. Thus LAPs 1 and 2 have all the characteristics of the most stringent definition of a LAP, although they showed quite different extractabilities at different salt concentrations [9]. The antibody to LAP1 recognized three proteins designated LAP1A, B and C, and cloning of LAP1C [11] indicated that these forms are derived by alternative splicing and expressed in a developmentally regulated manner. Co-precipitation confirmed binding of LAP1C to lamin B [12], but the other LAP1 variants have not been analyzed further. Analysis of the human genomic sequence indicates that the LAP1 gene may have a plenitude of splice variants but this has not been followed up yet.

Much more has been revealed on the LAP2 proteins since their initial identification. Cloning of the rat, mouse and human cDNAs and genes [13,14,15,16] identified 6 alternatively spliced isoforms LAP2α, β, γ, δ, ε, and ζ, all of which, except for LAP2α and ζ, contain a single C-terminal transmembrane domain and a long nucleoplasmic N-terminus [17]. The first described LAP2 antibodies recognized only LAP2β, the largest transmembrane LAP2 that binds preferentially lamin B [9,18]. The smaller isoforms, which lack specific regions in the nucleoplasmic domain, have yet to be tested for their lamin binding activity. LAP2α is unique, as it shares only the N-terminal third with the other isoforms and contains a distinct large C-terminus without a transmembrane domain. Accordingly LAP2α localizes to the nuclear interior [19], where it forms stable complexes with A-type lamins [20]. Although LAP2α is resistant to detergent/salt extraction [19] and binds lamins it clearly is not a lamina-associated protein as it is not part of the peripheral lamina. It represents an example of a lamin-associated protein that forms stable and protracted complexes with lamins outside the lamina.

Database searches have revealed a 40 amino acid-long motif of two helices [21] termed the LEM domain (for LAP2, Emerin and MAN1) that is shared by LAP2 isoforms, MAN1, emerin [22,23], LEM2/NET25 [24,25], and three uncharacterized proteins LEM3-5 in mammals [26]. The LEM domain binds the chromatin protein BAF (Barrier to autointegration Factor; [27,28,29]. Although LEM-domain proteins have no sequence similarity outside the LEM domain and contain different numbers of predicted transmembrane domains, all tested localize to the NE and bind lamins [24,30,31,32,33]. Moreover, emerin [34,35] and LEM2 [24] require A-type lamins for localization in the INM.

The lamin B-receptor (LBR) is another well-studied INM protein, identified by a biochemical screen for lamin-binding proteins in a lamin-depleted NE fraction [36]. LBR preferentially bound to lamin B over lamin A, was shown to have similar resistance to extraction with 0.1 M NaOH or 8 M urea, and antibodies yielded nuclear rim staining. LBR is solubilized only in highly stringent conditions and can thus also be considered a lamin<u>a</u>-associated polypeptide. Cloning and further analyses have shown that LBR is a polytopic protein with eight potential transmembrane domains [37].

Since these initial studies on LAPs 1 and 2 and LBR, around 80 additional putative NE transmembrane proteins have been identified (*reviewed in* [4]). Almost all of the potential LAPs tested thus far have been shown to target to the NE and those that have passed the criteria for inclusion as LAPs are shown in Table 1.

SUN1 [38,39,40] and SUN2 [41,42] are INM proteins containing a Sad1-UNC homology domain (SUN) that extends into the perinuclear space and interacts with ONM proteins termed nesprins [43,44]. The latter proteins form a huge family of isoforms encoded by three genes, *Nesprin 1, 2,* and *3*, but many isoforms are localized

on different cellular membranes, including mitochondria. Nevertheless, many of them target to the ONM and bind SUN domain proteins and some of them, such as nesprin 2 isoforms even localize to the INM and bind lamins [45] or their localization depends on lamin A [46].

A caveat of the above-mentioned definition of LAPs is that NPCs co-isolate with the lamina fraction and share some biochemical characteristics with lamina components. GP190/POM121, GP210, and NDC1/NET3 were also shown to localize to the NE, were resistant to extraction with Triton X-100, and were retained in membrane vesicles in the presence of chaotropes [47,48,49]. Yet these would not be considered LAPs because they are integral proteins of the NPC.

LAPs function as assembly points for multi-protein complexes

Most LAPs have been characterized in terms of their interactions. An LBR-complex was found in chicken erythrocytes that contains an LBR-specific kinase, p32, and an 18 kDa protein of the INM and ONM [50,51,52]. A second LBR complex has at its core an interaction with heterochromatin protein 1 alpha and gamma (HP1; [53,54]) and also contains histones H3/H4 [55] and epigenetically marked heterochromatin [56]. Interactions within this complex are likely epigentically regulated, as histone deacetylase inhibitors impaired the transient accumulation of microinjected HP1 at the NE [57].

A multitude of interactions have also been found for LEM proteins: first is their interaction with the DNA- and histone-binding protein barrier to autointegration factor (BAF) [28,58] that has essential functions in chromatin organization and gene regulation [59]. Biochemical and structural studies have identified the LEM domain in LAP2β to mediate the interaction with BAF [27,29] and binding of BAF to other

LEM proteins has been experimentally confirmed for MAN1 [32], emerin [31] and LAP2α [60]. Thus, all LEM domain proteins can bind chromatin via BAF. Moreover, some have additional interactions with chromatin. MAN1 has a second BAF-binding region outside its LEM domain [32] and a predicted DNA binding winged Helix C-terminal domain [61]. A second LEM-like motif at the N-terminus of all LAP2 proteins interacts with DNA [27] and LAP2β additionally binds DNA and chromatin protein HA95 via C-terminal isoform-specific regions [62,63].

Furthermore, several LEM proteins have been found to bind to different regulatory proteins, including the transcriptional repressor germ cell-less [32,64], transcriptional repressor Btf [32,65], transcriptional regulator Lmo7 [66], splicing factor YT521-B [67], epigenetic modifier enzymes [68], and signaling molecules (see below), indicating that lamin-LAP complexes can serve as scaffold structures, regulating gene expression and signaling pathways.

There is evidence that different LAP complexes may be segregated at the NE. LBR-lamin B-containing complexes are excluded from LEM protein-lamin A microdomains formed in LEM2 expressing cells [24]. Similarly a lamin mutant that redistributed lamin subtypes into distinct microdomains correspondingly redistributed LAPs known to bind each lamin subtype [69].

The fact that many LAP interaction partners were found in different cell types suggests that LAPs may have a wider range of binding partners that are tissue-specific. Tissue-specific LAP-complex formation may be regulated by the availability of potential binding partners in a specific cell type, the relative abundance of both LAPs and binding partners, and their relative affinities (Fig. 2). Further evidence that some LAPs are at the center of large tissue-specific complexes comes from a recent study on epitope masking at the NE. Six different monoclonal antibodies to different

epitopes of emerin were generated, each of which recognizes the protein by immunofluorescence in some cell types. However, none recognized emerin in spleen, even though it was clearly present by Western blot analysis [70] suggesting that all six epitopes are masked by binding partners. Such an extreme degree of epitope masking is consistent with the large number of identified binding partners that associate with distinct and/or overlapping regions of the emerin protein [71]. A parallel result was obtained for lamin B1. Three different lamin B1 antibodies were used to stain heart and hippocampus sections: one stained just cardiomyoctyes, another stained just hippocampus, and the third stained both [70]. As each antibody recognized a different region, different partners may occupy different binding sites in the different tissues. These data suggest that LAPs and lamins form large tissue-specific multiprotein complexes, the perturbation of which may underlie tissue-specific phenotypes in lamin-linked diseases (see below).

LAP-complexes function in nuclear architecture

Among the original LAPs studied, only one had a clearly determined enzymatic activity. The C-terminus of LBR is homologous to yeast sterol C-14 reductase (Erg24p; [72]) and the human gene can functionally complement a yeast Erg24p mutant [73]. All other functions reported for LAPs are structural ones involved in nuclear architecture and higher order chromatin organization, and in providing scaffolds for gene regulatory and signaling complexes (Fig. 3).

Given the structural roles of cytoplasmic intermediate filament proteins, it is not surprising that their nuclear counterparts, the lamins together with LAPs are to a large extent providing mechanical support for the NE [69,74,75]. The relevance in nuclear architecture has been demonstrated for several LAPs. Lower expression levels

of LBR, as seen in LBR-associated diseases affect nuclear shape and chromatin organization in blood granulocytes [76]. Emerin-deficient cells have normal structural integrity but impaired mechanotransduction [77] and knockdown of LEM2 leads to abnormally shaped nuclei [78]. Functional redundancy of LEM proteins in mammalian cells is most likely the reason for only mild phenotypes typically observed with single LEM-protein deficiencies. In *C.elegans*, only the simultaneous downregulation of the major LEM proteins, Ce-emerin and Ce-lem2 (formerly referred to as Ce-MAN1), caused severe chromatin organization phenotypes [79].

A structural link between the nucleoskeleton and cytoskeleton across the NE is provided by the INM SUN domain proteins, which form dimers and interact with ONM nesprins that in turn associate with the cytoskeletal filament systems (Fig. 3; for review see [80]). In *C. elegans* these proteins were linked to nuclear positioning and migration [43]. Transgenic mice overexpressing a dominant negative nesprin/syne-1 fragment exhibited impaired anchorage of nuclei at neuromuscular junctions [81], indicating that this function is evolutionarily conserved. The SUN-nesprin complex may also be involved in regulating the space between INM and ONM, since downregulation of SUN1 and 2 in HeLa cells caused detachment of the ONM and extension of the perinuclear space [38].

LAP-complexes function in chromatin organization

Given the numerous interactions of LAPs with chromatin proteins and DNA, it is plausible that they are involved in higher order chromatin structure (Fig. 3). LBR interactions with heterochromatin determinants [54,56], and LEM protein interactions with the DNA-crosslinking BAF [59] argue for a role of LAPs in forming heterochromatic regions. In support of this LAP2β binds HDAC3 that mediates H4

deacetylation [68], linked to heterochromatic, transcriptionally inactive genomic regions. Since most LEM proteins have been shown to bind BAF they may be functionally redundant. However, most of the other interaction partners of LEM-domain proteins bind outside the conserved LEM domain, suggesting also considerable specificity in the functions of these proteins.

LAP-chromatin interactions may also be important for nuclear assembly after mitosis. LBR is among the first proteins to assemble around sister chromatids in anaphase [82,83]. Its immunodepletion from cell lysates impaired *in vitro* NE assembly [84], suggesting that LBR is a key component for targeting membranes to chromosomes. LAP2β was suggested to be important for NE enlargement in G1 phase, because LAP2β fragments in *Xenopus* nuclear assembly reactions [85] or microinjected into mitotic mammalian cells [86] blocked nuclear growth in G1-S-phase. More recently, a basic domain in LAPs was suggested to mediate binding of INM proteins to negatively charged DNA during the initial docking of membranes [87]. This domain is a basic ($pI > 8.5$) nucleoplasmic region of more than 100 amino acid residues found in 46% of putative nuclear envelope proteins recently identified [25]. However, more specific targeting and assembly pathways have to be involved as well, since LBR and LAP2 bind different chromatin regions during assembly. LAP2α initially is targeted to telomeres in anaphase and further assembles to chromatin-associated "core structures" in telophase [60]. The chromatin-bound core structures, located adjacent to the mitotic spindle, contain also emerin, LAP2β, and BAF, while LBR complexes bind equatorial regions of anaphase chromatin [60,83]. The complexes at the core regions were determined to be stable by FRAP (fluorescence recovery after photobleaching) [88] and important for NE assembly because emerin, LAP2α, and BAF mutants that disrupted LAP-BAF interactions failed to assemble

NEs *in vivo* and *in vitro* [89,90].

LAP-complexes function in gene expression and signaling

LAPs may affect gene expression by epigenetic mechanisms and by binding to repressor proteins or sequestering transcription factors (Fig. 3). LAP2β binds the repressor germ cell less (gcl), which is known to affect E2F/DP transcription factor heterodimers [64]. Emerin also interacts with gcl [91], and with the repressor Btf [65]. The binding of LEM-domain proteins with BAF could similarly influence gene regulation, as BAF has been found to repress gene expression in retinal cells via binding to the homeodomain transcription factor Crx [92]. Furthermore, pull-down studies identified an emerin-binding transcription factor, Lim domain only (Lmo7), which controls the expression of emerin and many muscle specific genes [66]. Since overexpression of emerin inhibited Lmo7, the Lmo7-emerin interaction is tightly regulated by feedback pathways. Finally, LAP2α-lamin A complexes in the nuclear interior bind the tumor supressor retinoblastoma (Rb) [93], which is a negative regulator of E2F-dependent transcription controlling the balance between proliferation and differentiation [94]. Accordingly LAP2 can associate with E2F/Rb-responsive promoters and impair expression of E2F-target genes upon cell cycle exit and differentiation [95].

Novel data point towards a function of LAPs in signaling. Mammalian MAN1 binds Receptor-Smads 2/3, downstream effectors of the TGFβ and bone morphogenic protein (BMP) signaling cascades [96]. MAN1 overexpression impaired Smad 2/3 phosphorylation, heterodimerization (with Smad 4), and nuclear translocation - events that are linked to an active pathway – and thus antagonizes signaling [97]. Given the ubiquitous expression of MAN1, the physiological relevance of MAN1 in TGFβ and

BMP signaling is unclear: MAN1 could either recruit and de-activate Smads as a mechanism to eliminate weak signals, or alternatively, MAN1 might have a major role in regulating Smad signaling only in a subset of tissues due to the presence of additional tissue-specific MAN1 binding partners.

Emerin may also have signaling functions as it interacts with β-catenin, a dual function protein in cell-cell adhesion and transcriptional activation. Overexpression of emerin causes cytoplasmic accumulation of β-catenin, leading to the inhibition of its transcriptional activity. Conversely, emerin-null cells have increased nuclear, transcriptionally active β-catenin [98].

How do INM LAPs reach their destination?

In theory, for LAPs to access the INM they must either (1) diffuse laterally in the membrane around the outside of the NPCs; (2) bud off of the ONM as vesicles into the lumen of the NE, then fuse again with the INM; or (3) be removed from the ER membrane, maintained in a soluble form during transport through the NPC, and re-inserted into the membrane inside the nucleus (Fig. 1). The latter two mechanisms would be energetically unfavorable and have little or no support in the literature. The first mechanism obtained its support initially from studies, in which fusions of the nucleoplasmic region of LBR to the membrane-spanning segment of a ER/plasma membrane protein resulted in its targeting to the INM [99], while inserting an increasing mass of protein between the LBR nucleoplasmic region and the transmembrane segment resulted in the fusion protein no longer being able to access the INM [100]. It was proposed that the limited space between the membrane and the body of the NPC would set a size limit for the diffusion of the nucleoplasmic region in the membrane. Once in the nucleus, the nucleoplasmic LBR domain could be

retained in the NE by binding to lamin B [101] and become immobile as shown by FRAP analysis [82].

More recent studies have demonstrated that transport is more complex, involving an energy-dependent gated mechanism. Ohba and colleagues developed a system for testing inducible INM accumulation of a protein [102]. They fused the LAP2β membrane-spanning segment to both GFP and FRB, and fused the lamin-binding LAP2β nucleoplasmic region to FKBP. Upon addition of rapamycin, FRB binds to FKBP so that the lamin-binding region of LAP2β fuses to the integral membrane component. In agreement with the lateral diffusion-retention model, the membrane-spanning piece diffused at equilibrium between the ER and INM in the absence of rapamycin, but rapidly accumulated in the INM upon rapamycin addition. Unexpectedly, temperature and ATP-depletion significantly reduced accumulation while inhibitors of vesicle fusion and of soluble transport across the NPC had no deleterious effect. These data strongly argue against vesicle fusion and soluble transport mechanisms, but support an active, energy-dependent transport along the membrane. A recent study in yeast reports the presence of nuclear localization sequences (NLS) in INM protein orthologues, which are essential for their transport into the nucleus via a karyopherin- and Ran-GTPase dependent pathway [103]. It remains to be seen whether karyopherin and Ran-GTPase mediate a gated in-membrane lateral diffusion indicated for mammalian systems, or whether this represents a distinct mechanism.

Impairment of LAP functions gives rise to human diseases

Mutations in lamins, particularly the *LMNA* gene, were linked to a hetergeneous group of diseases, collectively termed laminopathies, that affect tissues ranging from

muscle to fat, bone, and skin [104,105]. The molecular mechanism for how missense mutations in lamins give rise to the diverse phenotypes is poorly understood, but may involve disruption of functional protein complexes associated on LAPs [106]. This is supported by the observation that mutations in some LAP genes also give rise to laminopathy-type diseases.

Emerin mutations cause the X-linked form of Emery-Dreifuss Muscular Dystrophy (EDMD), characterized by progressive muscle wasting, shortening of tendons, and heart and conduction system defects [23,107]. Most disease-causing mutations result in reduced levels of emerin [108]. When expressed, several mutants of emerin (S54F, P183H, P183T, and Del95-99) mislocalized to the cytoplasm [109]. Intriguingly, mutations in lamin A cause the autosomal type of EDMD [110] and similarly, the expression of some of these lamin A mutants (L85R, N195K, E358K, M371K, R386K, R453W, W520S, and R527P) also resulted in mislocalization of emerin [111]. This suggests that functional lamin-emerin complexes are lost by mutations in either protein. The disease-relevant functions of this complex are unknown, but gene-expression profiling on muscle biopsies from patients [112] and on emerin deficient mouse cells [113] suggest a defect in the Rb1-MyoD pathway, involved in muscle differentiation.

LAP2α is linked to a laminopathy-type dilated cardiomyopathy, and loss of LAP2α-lamin A binding was observed for the mutated protein *in vitro* [114]. This finding again argues that disease-causing mutations in lamin A and LAP2α impair the functions of the nucleoplasmic complex. Because LAP2α can regulate Rb function during differentiation [95], it was proposed that mutations in LAP2α may affect the differentiation of adult muscle stem cells, which may impair homeostasis and regeneration of heart muscle tissues in patients [106].

Heterozygous loss-of-function mutations in MAN1 cause osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis, characterized by increased bone density [115]. The pathological phenotype may be caused by impairment of MAN1 function in Smad signaling, which is important for bone development. Accordingly, mutated MAN1 was unable to antagonize Smad signaling like the wild type, and TGFβ/ Smad-responsive genes were upregulated in patient fibroblasts versus controls.

Heterozygous LBR mutations mostly resulting in reduced protein levels cause Pelger-Huet anomaly (PHA), an autosomal dominant syndrome characterized by abnormal nuclear shape and chromatin organization in blood granulocytes [76]. Homozygous individuals lacking LBR have varying degrees of developmental delays, epilepsy and skeletal abnormalities. LBR is also linked to autosomal recessive Greenberg's skeletal dysplasia, characterized by lethal skeletal and visceral anomalies [116]. It is unclear, to what extent the loss of LBR sterol reductase activity or the impairment of LBR chromatin-organizing function contributes to the phenotype.

Nesprin/Syne-1 (*SYNE1*) is associated with autosomal-recessive cerebellar ataxia [117], characterized by impaired walking due to a lack of coordination of gait and limbs. Since a brain-specific Nesprin/Syne-1 splice variant localizes to the postsynaptic endocytotic zone of excitatory synapses, mutations may disrupt glutamate receptor turnover and function. Thus, this disease is likely not caused by defects in nuclear specific functions of Nesprin/Syne-1.

Finally, LAP1 has recently been linked to primary dystonia, a CNS-based autosomal-dominant movement disorder caused by mutations in the AAA+ATPase torsin A. Normally, ATP bound torsin A is recruited to the NE by LAP1, while ATP-free torsin A is in the ER lumen. Disease-causing mutated torsin A is stably bound to LAP1 in the NE [118,119]. As a dilation of the perinuclear space was observed in

patients, torsin accumulation may affect nuclear architecture, but it could also interfere with yet unknown functions of LAP1.

Concluding comments and future directions

Although we have much to discover about the functions of LAPs, their involvement in such a wide range of human diseases indicates that these functions are critical. The plethora of functions already uncovered range from the mechanical integrity of the nucleus to genome organization and regulation of signaling. It will be essential in the future to identify and analyze tissue-specific LAP complexes and their functions.

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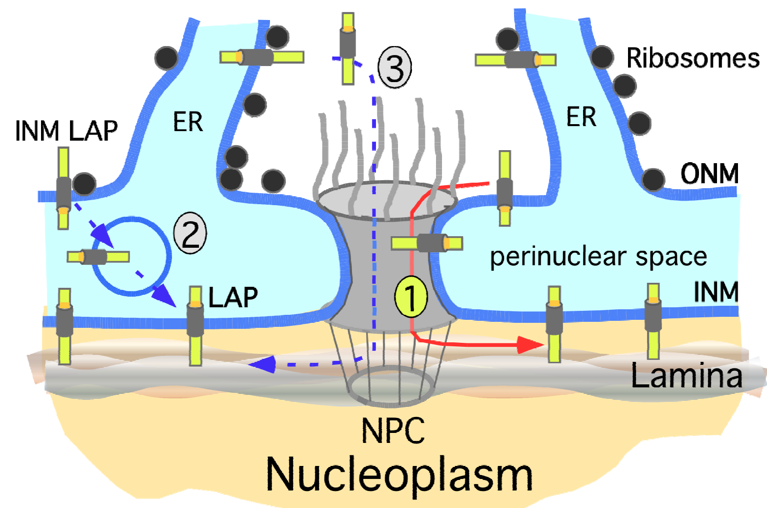
Figure Legends:

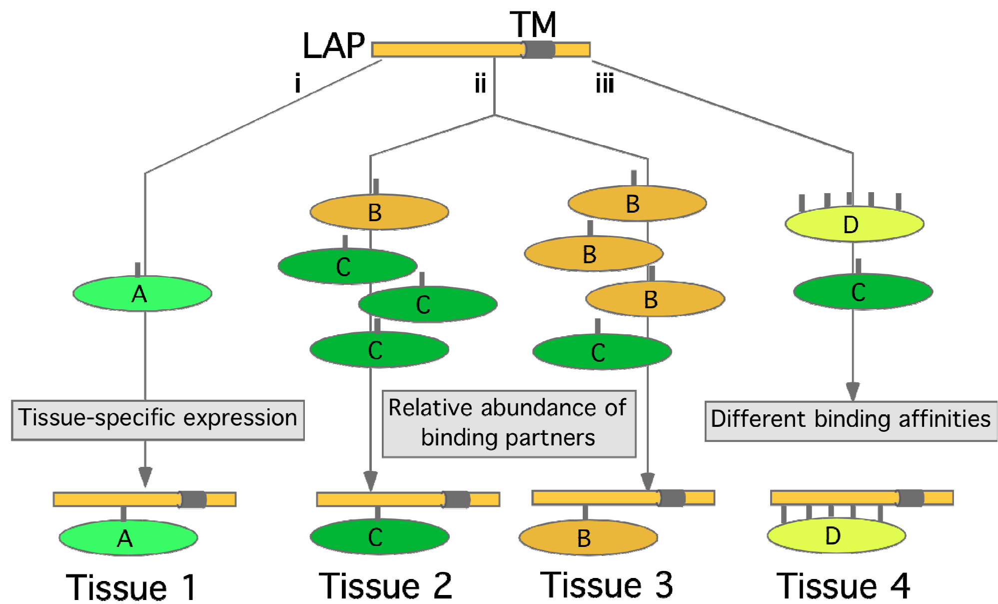
Figure 1.



Schematic representation of the nuclear envelope and potential transport mechanisms of membrane proteins to the inner nuclear membrane: the nuclear envelope (NE) is

comprised of a double membrane system of outer (ONM) and inner (INM) nuclear membranes. The ONM is continuous with the endoplasmic reticulum (ER). Several integral membrane proteins are embedded in both ONM and INM. Many of those in the INM physically interact with the lamin polymer that underlies the INM (LAP, Lamina-associated polypeptides). Nuclear pore complexes (NPC) perforate the nuclear membrane where ONM and INM join and appear to be involved both in transport of soluble factors between the nucleus and cytoplasm through their inner channel and in transport of INM LAPs on their outer face. INM proteins may diffuse laterally along the membrane from the ER to the INM in an energy-dependent manner and are retained by interaction with the lamina (pathway 1). In theory, alternative pathways for INM protein transport into the nucleus include budding off of vesicles with the protein from the ONM and fusion with the INM (pathway 2); or release of the INM protein from ER membranes, transport through NPC by classical transport pathways and insertion into the INM (pathway 3). Pathways 2 and 3, however, are energetically unfavorable and have little or no support in the literature.

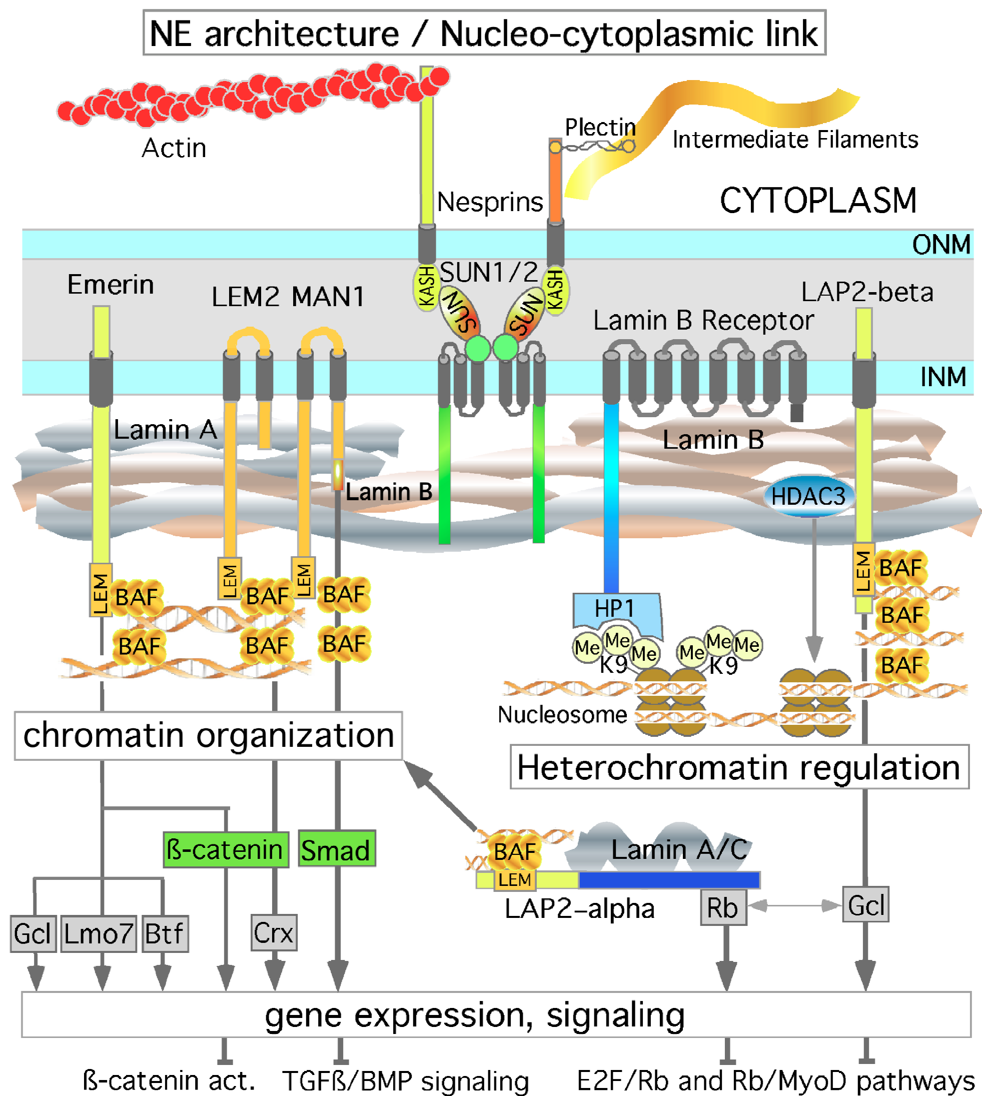
Figure 2.



Abundantly expressed LAPs can form tissue-specific complexes with different functions through (i) the availability of specific binding partners in specific tissues, (ii) the relative abundance of partners in tissues, and (iii) distinct binding affinities of

partners. TM, transmembrane domain; A-D, LAP binding partners; number of connecting lines denote binding strength.

Figure 3.



Functions of LAP complexes: Lamin A/C-associated emerin, MAN1 (may also bind lamin B), and LEM 2 interact with BAF and may be involved in chromatin

organization. Lamin B-associated LAP2β and LBR bind BAF and HP1, respectively and provide anchors for compacted heterochromatin (with H3K9-trimethyl marks). LAP2β also binds histone deacetylase HDAC3, which contributes to heterochromatin formation. Both types of complexes are also involved in nuclear architecture. SUN-dimer/Nesprin complexes, interacting in the perinuclear space via their SUN and KASH domains, respectively, provide the link between the lamina and cytoplasmic actin and intermediate filaments (via plectin). In addition, emerin binds to the transcriptional repressors gcl and Btf and the transcription factor Lmo7, and LAP2β to gcl, and BAF to cone-rod homeobox protein Crx. Thus, these proteins may be involved in the regulation of gene expression. MAN1 antagonizes TGFβ signaling by binding Smads, and emerin binds to and impairs β-catenin transcriptional activity. Finally, nucleoplasmic lamin A/C-LAP2α complexes bind to BAF and Rb and thus affect chromatin organization and E2F/Rb and Rb/MyoD pathways, respectively. LAP2β may also affect the Rb pathway via gcl.

Table 1 — Properties described for LAPs

LAPs	NE targeting	Integral membrane protein	Triton-resistant	Lamin binding	Other LAP binding	Lamins affect targeting
LAPs at the nuclear periphery						
LAP1	+ [10]	+ [10]	+ [10]	A,B [9]		+ [120]
LBR	+ [36]	+ [36]	+ [36]	B [36]		+ [99,101]
LAP2β	+ [9]	+ [9]	+ [9]	B [9]		+ [18]
Emerin	+ [23]	+ [41]	+ [23]	A [30,33,109]	MAN1 [32], Nesprin-1-alpha [121], Nesprin-2 [45]	+ [34,122]
MAN1	+ [123]	+ [22]	+ [123]	A,B [32]	Emerin [32]	+ [124]
Nurim	+ [125]	+ [41]	+ [125]	nt*		nt
UNCL	+ [126]	+ [126]	+ [126]	nt		nt
Syne/Myne/Nesprin-1	+ [127]	+ pred	+ [128]	A [129]	Emerin [121], SUN1 [39]	nt
Syne/Nesprin-2 alpha and beta	+ {130]	+ pred	+ [45]	A [45]	Emerin [45], SUN1 [39, 40]	+ [45,46]
Syne/Nesprin-2G**	+ [38]	+ pred	nt	nt	SUN2 [38]	nt
Nesprin-3/NET53**	+ [131]	+ [25]	nt	nt		nt
Unc84A/SUN1	+ [41]	+ [41]	+ [41]	A [39]***	Nesprin 1/2 KASH domain [39, 40]	- [38,39]
SUN2	+ [42]	+ [42]	+ [42]	A [38]	Nesprin 2G [38]	+ [38]
LUMA	+ [41]	+ [41]	+ [41]	nt		nt
NET8	+ [25]	+ pred	+ [25]	nt		nt
NET39	+ [25]	+ [25]	+ [25]	nt		nt
NET51	+ [25]	+ [25]	+ [25]	nt		nt
NET56	+ [25]	+ pred	+ [25]	nt		nt
LEM2/NET25	+ [24]	+ [24]	+ [24]	A [24]	LAP2-alpha [24]	+ [24]
NET9	+ [132]	+ pred	+ [132]	nt		nt
NET32	+ [132]	+ pred	+ [132]	nt		nt
NET37	+ [132]	+ [25]	+ [132]	nt		nt
Titin	+ [133]****	- [134]	nt	+ [133,135]		nt****
Nup53	+ [136]	- [136]	+ [136]	+ [136]		nt
SREBP-1	+ [137]	- [138]	nt	+ [137,139]		+ [137]
c-Fos	+ [140]	- [141]	nt*****	+ [140]		+ [140]
LAPs in the nucleoplasm						

LAP2α	- [19]	- [16]	+ [19]	A [20]		+ [20]
Lco1	- [142]	- [142]	nt******	+ [142]		+ [142]
MOK2	- [143]	- [144]	+ [145]	+ [145]		+ [145]

nt=not tested; pred=predicted.
* It was suggested that nurim targets to the NE independent of lamin-binding as expressed protein failed to target to the NE in heterologous systems upon expression of lamins. However, only A-type lamins were tested and direct binding has yet to be examined.
** Although not all Nesprin subtypes have been fully characterized yet, we define both proteins as LAPs, based on the properties reported for Nesprin-2 and the similarities between the two proteins.
*** UNC84A was shown to bind lamins. However, NE localization of human UNC84A was recently shown to not depend upon binding to lamins [146].
**** In this study only a slight enrichment was observed at the NE for a fragment of human titin shown to bind lamins in a 2-hybrid screen [135], when fused to GFP [133]. However, this weak result was strengthened because in C. elegans the authors demonstrated a cell cycle-dependent strong targeting of Ce-titin to the NE that was dependent on lamins.
***** Ectopic expression of lamin protected c-Fos from detergent solubilization, but NP-40 instead of Triton X-100 was used.
****** Although resistance to Triton X-100 was not tested, binding affinity was high and reactions were performed in the presence of high salt; so a more stable interaction is indicated.

Table 2 — LAPs linked to human diseases

Mutated LAP	Disease	Phenotype	Comments	Reference
Emerin	Emery-Dreifuss muscular dystrophy	Contractures of elbows, Achilles tendon, neck and spine. Progressive weakness in upper arms and lower limbs. Often associated with dilated cardiomyopathy.	Other variants caused by lamin mutations	[23,107,147]
LAP2α	Familial dilated cardiomyopathy	Ventricular dilation and impaired cystolic function. Cardiac pump failure may occur after conduction abnormalities.	Other variants caused by lamin mutations	[114]
LBR	Pelger-Huet anomaly	Neutrophil nuclei in heterozygotes have fewer segments and course chromatin, with no effects on normal health. Homozygotes have epilepsy and skeletal abnormalities.		[76]
LBR	Greenberg/HEM skeletal dysplasia	Widespread tissue edema in fetus. Disorganized bone structure, short limbs and conversion of cartilage to bone. Early *in utero* lethality.		[116]
MAN1/LEMD3	Buschke-Ollendorff syndrome	Skeletal defects include spots of increased bone density and sclerosis in a flowing pattern. Accompanied by joint contractures, skin lesions and muscle atrophy.		[115]
Torsin A	Torsion dystonia	Prolonged, involuntary muscle contractions induce abnormal posture and twisting or repetitive	TorsinA only accumulates at the NE when mutated, binds to LAP1	[118, 119, 148]

		movements in arms and legs.		
SYNE1	Autosomal recessive cerebellar ataxia	Impaired walking due to a lack of coordination of gait and limbs. Often also pyramidal features, peripheral neuropathy, cognitive loss or retinopathy.	Mutation may affect a Syne variant not localized to the NE.	[117]