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Inner nuclear membrane proteins: functions and targeting

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Abstract. We summarize the properties of integral membrane proteins that reside in the inner nuclear membrane, including lamin B receptor (LBR), lamina-associated polypeptide (LAP) 1, LAP2, emerin, MAN1 and nurim. Most of these proteins interact with lamins and chromatin. Some data also suggest more speculative functions such as gene regulation and possibly sterol metabolism. Mutations in emerin and nuclear lamins have been associated with muscular dystrophies and lipodystrophy, raising new questions about the functions of inner nuclear membrane proteins. Integral proteins of the inner nuclear membrane are synthesized on the rough endoplasmic reticulum (ER) and reach the inner nuclear membrane by lateral diffusion in the connected ER and nuclear envelope membranes. Associations with nuclear ligands retain them in the inner nuclear membrane. Further investigation of the functions and targeting of inner nuclear membrane proteins are needed to determine how they are involved in human disease.

Key words. Nuclear envelope; nuclear lamina; Emery-Dreifuss muscular dystrophy; limb-girdle muscular dystrophy; Dunnigan-type partial lipodystrophy; intracellular protein trafficking; lamin B receptor; lamina-associated polypeptide; nurim.

Introduction

The nuclear envelope is a complex structure that separates the nucleoplasm from the cytoplasm. It is composed of the nuclear membranes, the nuclear pore complexes and the nuclear lamina [1]. The nuclear lamina is located on the inner aspect of the inner nuclear membrane and is a filamentous meshwork of proteins called lamins. Lamins are members of the intermediate filament protein family and are thought to provide a structural framework for the nuclear envelope. Two classes of lamins, A-type and B-type, which are differentially expressed during development and differentiation, can be distinguished in mammals. The nuclear pore complexes are responsible for protein and nucleic acid import and export to and from the nucleus. The outer nuclear membrane is continuous with the ER. The perinuclear space is located between the outer and inner nuclear membranes and is continuous with the ER lumen.

The inner nuclear membrane is home to a number of resident integral membrane proteins: lamin B receptor (LBR), lamina associated polypeptide (LAP) 1, LAP2, MAN1, emerin and nurim (fig. 1). Whereas some of these proteins bind to lamins and chromatin (LBR, LAP1, LAP2 and emerin), contributing to the architecture of the nuclear envelope, the functions of MAN1 and nurim are completely unknown and remain to be explored. New excitement and puzzling questions have been brought to this field by the relatively recent discoveries linking inner nuclear membrane protein and lamin mutations to Emery-Dreifuss muscular dystrophy, Dunnigan-type partial lipodystrophy and limb girdle muscular dystrophy type 1b.

LBR, LAP1 and LAP2: inner nuclear membrane proteins with lamin- and chromatin-binding properties

The lamina-associated proteins LBR, LAP1 and LAP2 all have amino-terminal domains of at least 200 residues, followed by variable numbers of transmembrane segments. These proteins, are type II integral membrane proteins and their amino-terminal domains, which face the

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nucleoplasm, are potential binding partners for nuclear ligands.

LBR was the first integral protein of the inner nuclear membrane to be identified and have its complementary DNA (cDNA) sequenced [2, 3]. LBR was identified by experiments in which radioactive lamins were incubated with turkey erythrocyte nuclear envelopes. B-type (but not A-type) lamins bound to an abundant protein of ~60 kDa, the 'lamin B receptor' or LBR [2]. LBR is an integral membrane protein, extractable only by a mixture of high salt plus Triton X-100 or ionic detergents. LBR has an amino-terminal domain that faces the nucleoplasm followed by a hydrophobic C-terminal domain [3, 4]. The C-terminal domain has eight predicted membrane-spanning domains and is highly homologous to yeast and plant sterol reductases [5, 6]. The human LBR gene is localized to chromosome 1q42.1 [7].

The first 60 amino acids of LBR seem to be critical for binding to B-type lamins, as this is the minimal epitope recognized by anti-LBR antibodies from rare patients with primary biliary cirrhosis that are anti-idiotypic to some anti-lamin B1 antibodies [8]. The N-terminal domain of LBR also binds to DNA and residues 70–100 appear to be critical for this interaction [4]. LBR's binding to nucleosomal DNA is similar to that of histone H1 and non-histone proteins HMG1/2, which both bind preferentially to linker DNA, and whose binding is enhanced by DNA curvature and supercoiling [9]. LBR also binds to human chromodomain proteins $HP1^{Hs\alpha}$ and $HP1^{Hs\gamma}$ [10]; residues 97–174 appear to mediate this interaction by binding the chromoshadow domain of HP1 proteins [11]. These human proteins are orthologs of *Drosophila* HP1, a heterochromatin protein involved in position-effect variegation [12, 13]. Because a portion of the transcriptionally inactive heterochromatin is adjacent to the inner nuclear membrane in higher eukaryotic cells [14] and because the nuclear lamina is a discontinuous structure [15], direct interactions between heterochromatin and the inner nuclear membrane can be envisioned. Furthermore, we speculate that interactions between LBR and heterochromatin proteins may promote heterochromatin binding to the inner nuclear membrane. Chromatin binding by LBR might be important for chromatin attachment to the nuclear envelope, since LBR is cleaved late during apoptosis [16, 17], and both LBR [18] and HP1 proteins [19] are phosphorylated during mitosis, presumably to allow dynamic changes of nuclear structure and chromatin organization during mitosis.

More speculatively, LBR might be indirectly involved in gene regulation by nuclear receptors. Residues 208–615 of human LBR share high homology with human, plant and yeast sterol reductases [6]. In two complementation studies, different LBR constructs were able to rescue strains of *Saccharomyces cerevisiae* deficient in sterol-14 reductase [20] and *Neurospora crassa* strains deficient in sterol δ 14–15 reductase [21]. It is not known whether LBR functions as a sterol reductase in humans. However, if it does, LBR could modulate the effects of oxysterols, via oxysterol nuclear receptors, on transcription.

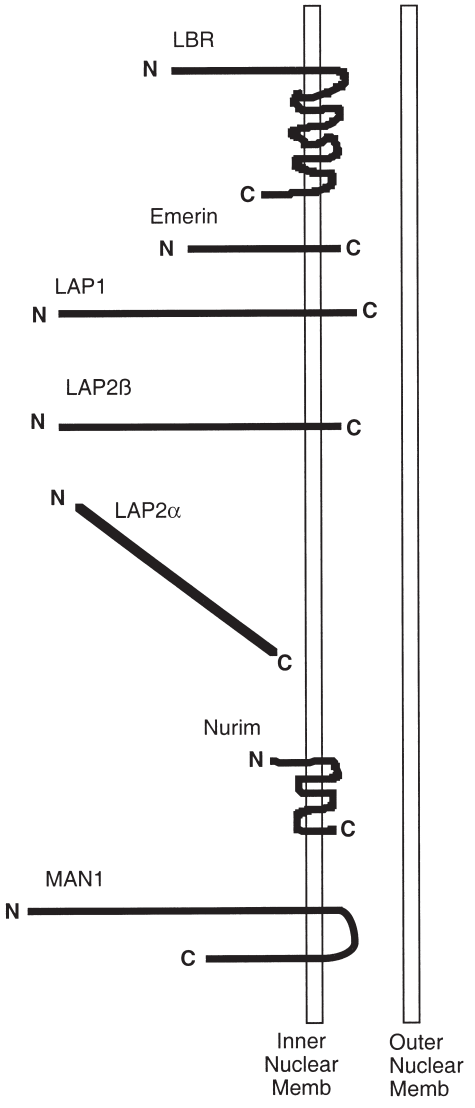

Figure 1. Integral proteins of the inner nuclear membrane. The inner and outer nuclear membranes are indicated. Amino-termini are indicated by N, and carboxyl-termini are indicated by C.

LAP1 and LAP2

LAP1 and LAP2 proteins were identified using monoclonal antibodies against isolated nuclear envelopes [22, 23]. One monoclonal antibody recognized three inner membrane proteins of approximately 75 kDa (LAP1A), 68 kDa (LAP1B) and 55 kDa (LAP1C) [22]. Only the cDNA for LAP1C has been completely sequenced in rat, but partial characterization of other cDNAs suggested that the three LAP1 proteins arise from the same gene by

alternative RNA splicing [24]. The human LAP1 gene has been localized to human chromosome 1p36 [L. Holmer and H. J. Worman, unpublished data]. Each LAP1 isoform binds to lamins with different affinities [22, 23]. In cell fusion experiments where rat and mouse, or rat and hamster cells were fused to form heterokaryons, rat LAP1C accumulated in the nuclei of mouse or hamster cells only when lamins A and C were expressed [25].

The human gene encoding LAP2 on chromosome 12q22 encodes several different proteins by alternative RNA splicing [26]. Three LAP2 isoforms, named α, β and γ (also known as thymopoietins), were originally characterized [26, 27]. LAP2β and LAP2γ are integral membrane proteins of the inner nuclear membrane, whereas LAP2α does not have a transmembrane segment and resides in the nuclear interior [26–28]. All LAP2 isoforms share a common N-terminal region of 187 amino acids, which may be involved in chromatin binding [29]. Additional messenger RNAs (mRNAs) highly related to human LAP2 were subsequently isolated from mice and it now appears that seven different mammalian LAP2 isoforms arise from a single gene [30].

The best-studied LAP2 isoform, LAP2β, is a 53-kDa protein with a hydrophilic N-terminal domain of 409 residues, followed by a single transmembrane segment [26, 27]. A region for lamin binding is located between residues 298 and 409 and binds to part of the α-helical rod domain of lamin B1 [27, 31]. The chromatin-binding domain of LAP2β is localized to the N-terminus [32] and this domain binds barrier to autointegration factor (BAF), a DNA-associated protein of unknown function (see below). LAP2β binding to lamins and chromatin is inhibited by mitotic phosphorylation [23]. In in vitro assembly assays, various fragments of the LAP2β amino-terminal domain disrupt nuclear envelope assembly and chromatin expansion [33]. Microinjection of a recombinant polypeptide comprising the nucleoplasmic domain of rat LAP2β from residues 1–398 into metaphase HeLa cells does not affect the reassembly of the nuclear envelope but strongly inhibits nuclear volume increase [34]. The interactions of LAP2β with lamins and chromatin, their regulation by mitosis-specific phosphorylation and the effects of LAP2β fragments on nuclear envelope assembly and volume increase in in vitro assays strongly suggest a role for the protein in nuclear envelope assembly and disassembly.

Emerin

Emerin is a nuclear lamin-binding protein of the inner nuclear membrane. In 1994, positional cloning of a gene at chromosome Xq28, which was responsible for X-linked Emery-Dreifuss muscular dystrophy, led to the discovery of emerin [35]. Emerin localizes to the inner nuclear membrane and contains a domain of approximately 40 amino acid residues at its N-terminus that is homologous to a domain near the N-terminus of LAP2β [35–37]. Several lines of evidence suggest that emerin interacts with lamins. When emerin was immunoprecipitated from cultured myoblasts or purified hepatocyte nuclei, both A- and B-type lamins copurified with emerin [38]. In lamin A/C 'knockout' mice, emerin becomes partially mislocalized to the ER, suggesting that emerin depends on A-type lamins for retention in the inner nuclear membrane [39]. Lamin A was also shown by biomolecular interaction analysis to bind directly to emerin [40].

During early reassembly of nuclear envelopes, emerin is concentrated in areas of the mitotic spindle and in the midbody of mitotic cells [41, 42]. About 5 min after the onset of anaphase, emerin accumulates on chromosomes at a location that is distinct and separate from LBR [42]. Later in telophase, their localizations become uniform [42]. As emerin targeting to decondensing chromosomes in mitosis occurs prior to that of lamins, these findings suggest that emerin may interact directly with chromatin components different from those that bind to LBR.

In summary, emerin, as well as LBR, LAP1 and LAP2, appears to have important functions in anchoring the nuclear lamina to the inner nuclear membrane during interphase and might have roles in the mitotic disassembly and reassembly of the nuclear envelope. LBR, LAP2 isoforms and possibly emerin also interact with chromatin, leading to the speculation that some inner nuclear membrane proteins might also function in gene regulation by direct interactions with heterochromatin. As mentioned above, LBR might influence gene expression in the oxysterol signaling pathway by hypothetically reducing the oxysterols that bind to nuclear receptors that regulate various genes [43].

Nuclear envelope proteins, muscular dystrophy and lipodystrophy

The discovery that human diseases are caused by mutations in emerin and lamins has raised new questions about the functions of inner nuclear membrane proteins. Emery-Dreifuss muscular dystrophy (EDMD) is characterized by early contractions of elbows and Achilles tendons and a cardiomyopathy with atrioventricular conduction block that is life threatening [44]. Two modes of inheritance have been described: X-linked and autosomal dominant. Mapping the gene, responsible for X-linked EDMD led to the discovery of emerin (see above) [35]. Mutations that cause EDMD are scattered throughout the emerin gene and most result in truncated, unstable proteins that are not expressed to any significant degree in muscle cells [35–37, 45, 46]. Subsequently, Bonne et al.

[47] showed that mutations in *LMNA*, the gene encoding lamins A and C, cause the autosomal dominant form of EDMD. Furthermore, mutations in lamins A and C are also linked to limb girdle muscular dystrophy type 1b [48] and familial cardiomyopathy with conduction defect and variable skeletal muscle involvement [49, 50], indicating that these muscle disorders, are allelic variants of autosomal dominant EDMD. A wide range of mutations in the *LNMA* gene causes these disorders and there is significant intra- and interfamilial variability in phenotype [51]. Intriguingly, mutations clustered in exon 8 of the *LMNA* gene, which encodes part of the C-terminal tail common to lamins A and C, cause Dunnigan-type partial lipodystrophy, which is characterized by regional adipose tissue degeneration, insulin resistance and diabetes mellitus [52–54].

As mutations in emerin and lamins both cause EDMD, abnormalities in these proteins may cause a common structural defect in the nuclear envelope that leads to muscle disease. In *Drosophila*, reduced expression of lamin Dm0 causes impaired locomotion and neuromuscular defects with defective nuclear envelopes, clustered nuclear pore complexes and accumulation of annulate lamellae [55]. Mice lacking lamin A/C are indistinguishable at birth from normal mice but develop severe muscle wasting, contractures, cardiomyopathy and decreased fatty tissue within weeks after birth [39]. Their nuclei are misshapen, and whereas lamin B2, LAP2β and nuclear pore complexes seem unaffected, emerin is mislocalized and accumulates in the ER [39]. Complete knockout of both lamin A/C genes in mice cannot be compared directly to humans with EDMD who have at least one normal copy of the gene. Nonetheless, the findings in 'knockout' mice suggest that nuclear structure may be disrupted to some extent in humans with the disease. Indeed, ultrastructural abnormalities in muscle nuclei have been observed in patients with X-linked EDMD [56]. Although these data are suggestive, a cause-and-effect relationship between altered nuclear structure and muscle cell dysfunction has not been established.

Several hypotheses have been proposed to explain how mutations in emerin and lamins, or changes in nuclear structure, might cause muscle or adipose tissue disease. One hypothesis is that nuclear envelope defects make cells more susceptible to apoptosis, since lamins and other inner nuclear membrane proteins are key substrates for caspases [16, 17, 57]. Another hypothesis, based on interactions between LBR and heterochromatin proteins [10, 11], is that interactions between the inner membrane and chromatin can influence tissue-specific gene expression, perhaps by regulating genes required for muscle or adipocyte survival. Some investigators suggest that abnormal nuclear structure may cause cell injury from recurrent mechanical stress, which is characteristic of skeletal and cardiac muscle [1]. Finally, some investigators hypothesize that the mislocalization of inner membrane proteins to other subcellular locations may cause problems. Clearly, more experiments are needed to test these hypotheses.

Recently identified nuclear envelope proteins – new or common functions?

In 1999, Rolls et al. [58] identified nurim in a visual screen for green fluorescent protein-fusion proteins that localized to the nuclear envelope. They identified nurim, which is present exclusively in the nuclear envelope of cells and is immobilized there, strongly suggesting that it is an inner nuclear membrane protein. Nurim has 262 amino acids and a molecular mass of approximately 29 kDa, but lacks any significantly hydrophilic amino-terminal domain. Nurim consists of five transmembrane segments with short intervening loops, a structure unique among inner nuclear membrane proteins. Experiments using fluorescence recovery after photobleaching suggest that nurim interacts very strongly with other nuclear components, but nothing is known about its functions or potential binding partners.

In 2000, Lin et al. [59] reported the molecular characterization of a new inner nuclear membrane protein, MAN1. Three proteins ('MAN antigens') recognized by autoantibodies from a patient with a collagen vascular disease were previously localized to the nuclear envelope by immunoelectron and immunofluorescence microscopy [60]. One 'MAN antigen' was identified as LAP2β [61]. Lin et al. [59] screened expression libraries with these autoantibodies to obtain a partial cDNA clone for a mouse MAN protein, which led to the human MAN1 cDNA. Hydropathy analysis predicts that MAN1 has an amino-terminal domain of approximately 470 amino acid residues facing the nucleoplasm, two transmembrane segments and a C-terminal tail that also faces the nucleoplasm. Northern blots reveal a single MAN1 transcript in all tissues examined, with the highest expression in placenta. By radiation hybrid mapping, the MAN1 gene was localized to chromosome 12q14 [59]. In cell fractionation experiments, MAN1 behaves as an integral membrane protein, and confocal immunofluorescence microscopy of cells transfected to express epitope-tagged MAN1 is exclusively localized at the nuclear envelope.

MAN1 shares a common domain with both LAP2 and emerin, and also with a novel *Caenorhabditis elegans* protein [59], which has been called LEM-3 [62]. This domain, called LEM (LAP2, emerin and MAN1), is a highly conserved stretch of approximately 40 residues located at or near the N-termini of the mammalian proteins (fig. 2). Secondary structure predictions suggest that the LEM domain consists of two α helices separated by a loop. A second globular domain of similar size and pre-

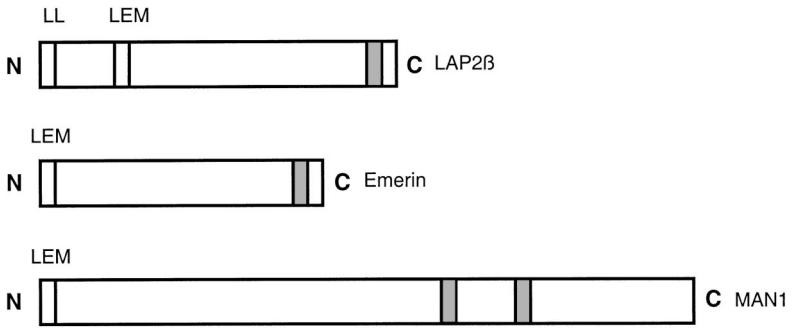

Figure 2. Schematic diagrams of LAP2β, emerin and MAN1 showing the positions of the LEM domain (LEM) and 'LEM-like' domain (LL). Transmembrane segments are indicated by shaded boxes. Amino-termini are indicated by N, and carboxyl-termini are indicated by C.

dicted secondary structure to the LEM domain, called the 'LEM-like' domain, is also present at the N-terminus of LAP2. Furukawa [63] showed that the LEM domain of LAP2β binds to the rat ortholog of human BAF: the minimal domain of LAP2β necessary for binding to BAF (LAP2 residues 68–195) overlap the LEM domain, and the deletion of residues 67–137 abolishes the interaction. Thus, the LEM domain of LAP2β, and possibly other LEM domain-containing proteins, may mediate binding to BAF and, in turn, chromatin.

Targeting of proteins to the inner nuclear membrane

Integral membrane proteins are synthesized cotranslationally on ER-bound ribosomes, and their topologies are determined by signal- and stop-transfer sequences [64]. Membrane proteins that are destined to remain in the ER or nuclear envelope membranes contain specific targeting and retention signals. Integral proteins destined for the inner nuclear membrane must pass along the 'pore membrane domain' through the lateral channels of nuclear pore complexes. The diameters of these channels are approximately 10 nm [65], large enough to allow the diffusion of globular proteins with masses under approximately 60 kDa. A so-called 'diffusion-retention' model of protein targeting to the inner nuclear membrane is supported by several studies of inner nuclear membrane proteins [27, 66–71]. In this model, membrane proteins diffuse freely in the interconnected membranes of the rough ER and nuclear envelope after synthesis, move along the lateral channels of the nuclear pore complex and arrive at the inner nuclear membrane. Binding to nuclear ligands such as lamins or chromatin then prevents the inner membrane proteins from escaping.

Evidence for the diffusion-retention model comes from studies of LBR. LBR contains two non-overlapping targeting signals, one at the N-terminus and one in its first transmembrane segment [66–69]. These two domains can each mediate targeting of a nonnuclear type II integral membrane protein to the inner nuclear membrane. Furthermore, movement through the lateral channels of the nuclear pore complex is limited by protein size. The amino-terminal targeting domain of LBR, with a mass of 22.5 kDa, can diffuse and concentrate in the nucleoplasm by binding to nuclear ligands. A chimeric membrane protein with two copies of LBR's amino-terminal domain fused to a cytosolic transmembrane protein is able to localize to the inner nuclear membrane, but adding a third copy prevents the chimeric protein from reaching the inner membrane, resulting in a distribution throughout the ER [67]. This result indicates that nucleoplasmic domains larger than ~67 kDa cannot diffuse through the lateral channels of the nuclear pore complexes. Notably, all known inner nuclear membrane proteins have nucleoplasmic domains with masses less than 60 kDa. Experiments testing the mobility of an inner-membrane-localized LBR-green fluorescent protein (GFP) fusion protein by fluorescence loss in photobleaching suggest that the fusion protein cannot return to the ER to any appreciable degree [68]. Similar results are obtained for emerin-GFP [70], where two regions in the N-terminal domain are required for efficient retention at the inner nuclear membrane [70, 71].

Perspectives

A small but growing number of integral proteins have been localized to the inner nuclear membrane. Several associate with lamins and/or chromatin, providing potential connections between the inner nuclear membrane and the genetic material. The relatively recent discoveries that mutations in emerin and lamins A/C cause muscular dystrophies and partial lipodystrophy offer challenges to basic cell biologists who study the nuclear envelope to learn the functions of these proteins and distinguish between different models for their role in human disease.

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