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Topical Review

The Inner Nuclear Membrane

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Introduction

The nuclear envelope surrounds the cell nucleus and is composed of the nuclear lamina, nuclear pore complexes and nuclear membranes (Fig. 1). The nuclear membrane is divided into three distinct but interconnected domains: outer, pore and inner. The outer nuclear membrane is directly continuous with and similar in composition to the endoplasmic reticulum membrane. The pore membranes connect the inner and outer membrane domains at numerous points and are associated with the nuclear pore complexes. The inner nuclear membrane is associated with the nuclear lamina and chromatin. Despite its proximity to the genetic material and the dramatic alterations it undergoes during mitosis and apoptosis, little is known about the inner nuclear membrane and only a few of its proteins have been characterized. Recently, some of these proteins have been associated with human genetic diseases.

Peripheral Proteins of the Inner Nuclear Membrane

The first inner nuclear membrane proteins to be characterized were the nuclear lamins. The lamins are intermediate filament proteins that polymerize to form 10 nm-diameter filaments (Aebi et al., 1986; Fisher, Chaudhary & Blobel, 1986; McKeon, Kirschner & Caput, 1986). The lamins have amino-terminal head and carboxyl-terminal tail domains separated by alpha-helical rod domains that are highly conserved among all intermediate filament proteins (Franke, 1987). The nuclear lamins form the nuclear lamina, a meshwork of intermediate filaments on the inner surface of the inner nuclear membrane.

Nuclear lamins have been identified in many different metazoan species (Erber et al., 1999). In humans, three genetic loci encode lamins (Biamonte et al., 1992; Lin & Worman, 1993, 1995; Wydner et al., 1995; Machiels et al., 1996). The human lamin genes and their encoded proteins are outlined in Table 1. Lamins A, C and A(Δ)10 (A-type lamins) arise by alternative RNA splicing and are encoded by the *LMNA* gene on chromosome 1q21.2–21.3. Lamin B1 is encoded by the *LMNB1* gene on chromosome 5q23.2–31.1 and lamin B2 by *LMNB2* on chromosome 19p13.3. B-type lamins (B1 and B2) appear to be expressed in all somatic cells while A-type lamins are absent from some undifferentiated, hematological and cancer cell types (Guilly et al., 1987, 1990; Stewart & Burke, 1987; Worman, Lazaridis & Georgatos, 1988; Lourim & Lin, 1989; Röber et al., 1990; Cance et al., 1992). Germ cell-specific lamin A and lamin B2 isoforms may also arise by alternative RNA splicing (Furukawa & Hotta, 1993; Furukawa, Inagaki & Hotta, 1994).

Most of the nuclear lamins are prenylated, specifically farnesylated, at their carboxyl-termini (Wolda & Glomset, 1988; Farnsworth et al., 1989; Beck, Hosick & Sinensky, 1998). An exception is lamin C, which is never prenylated. Lamin A is synthesized as a precursor, prelamin A, which is farnesylated and subsequently processed by endoproteolysis to a shorter form (Weber, Plessmann & Traub, 1989; Beck, Hosick & Sinensky, 1990; Sinensky et al., 1994). The prelamin A endopro-

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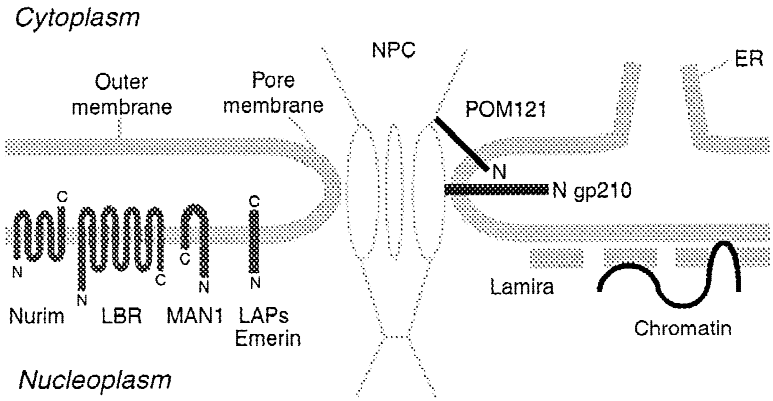

Fig. 1. Diagram showing the three components of the nuclear envelope: the nuclear lamina, nuclear pore complex (NPC), and nuclear membranes. The outer nuclear membrane is in continuity with the endoplasmic reticulum (ER) and the nuclear pore membrane is connected to the nuclear pore complex by integral proteins (gp210 and POM121 shown). The nuclear pore complex is represented with the cytoplasmic and nucleoplasmic. The integral proteins of the inner nuclear membrane are connected to the fenestrated nuclear lamina and to chromatin. Specific integral proteins of the inner nuclear membrane and their topologies are shown at the left. LBR is a polytopic protein with a nucleoplasmic amino-terminal domain followed by a hydrophobic portion, similar to sterol reductases, with 8 putative transmembrane segments. LAP1, LAP2 (transmembrane isoforms) and emerin all have nucleoplasmic amino-terminal domains and one transmembrane segment. MAN1 has a nucleoplasmic amino-terminal domain and two putative transmembrane segments. Nurim lacks a large hydrophilic domain and has 4 putative transmembrane segments. The nucleoplasmic domains of all these proteins are less than 60 kilodaltons, consistent with the being able to diffuse through the lateral channels of the nuclear pore complexes.

Table 1. Human nuclear lamin genes and the encoded proteins

Locus	Chromosome	Proteins	Cell types expressed
LMNA	1q21.2–21.3	Lamin A	Differentiated somatic
		Lamin C	Differentiated somatic
		Lamin A Δ10	Differentiated somatic
		Lamin C2	Germ
LMNB1	5q23.2–31.1	Lamin B1	Apparently all somatic
LMNB2	19p13.3	Lamin B2	All or most somatic
		Lamin B3	Germ

tease activity responsible for this processing appears to be in the nucleus (Kilic et al., 1997). The precise functions of lamin prenylation remain unknown except for the fact that prelamin A must be prenylated to be processed to lamin A (Beck, Hosick & Sinensky, 1990; Kilic et al., 1997). A nuclear protein called Narf has also been identified that specifically associates with prenylated prelamin A, however, its function is not clear (Barton & Worman, 1999).

In addition to lamins, there are likely other peripheral proteins associated with the inner nuclear membrane but few have been identified. Otefin is a peripheral protein of the inner nuclear membrane of *Drosophila* (Padan et al., 1990); Ashery-Padan et al., 1997). So far, an otefin orthologue has not been identified in mammals.

Integral Proteins of the Inner Nuclear Membrane

In interphase cells, several integral membrane proteins are specifically localized to the inner nuclear membrane (Fig. 1). The first to be identified was lamin B receptor or LBR (Worman et al., 1988; Worman, Evans & Blobel, 1990). LBR has a nucleoplasmic, amino-terminal domain of approximately 200 amino acids that binds to B-type lamins and chromatin proteins (Worman et al., 1990; Ye & Worman, 1994, 1996; Ye et al., 1997). This domain is followed by a hydrophobic region with eight putative transmembrane segments. The hydrophobic region of LBR is very similar in sequence to sterol reductases found in yeast, plants and animals (Schuler, Lin & Worman, 1994). These include two human proteins of the endoplasmic reticulum, one of which is a 7-dehydrocholesterol reductase (Holmer, Pezhman & Worman, 1998). LBR has C-14 sterol reductase activity in transformed yeast (Silve et al., 1998), however, the relevance of this enzymatic activity to mammalian cell physiology has not been established.

Three related proteins called lamina associated polypeptide (LAP) 1A, 1B and 1C have been identified by virtue of recognition by a single monoclonal antibody that labels the inner nuclear membrane (Senior & Gerace, 1988). As their name implies, these proteins are associated with the nuclear lamina. LAP1A and LAP1B bind to lamins A, C and B1 (Foisner & Gerace, 1993). LAP1C contains a nucleoplasmic, amino-terminal domain followed by one transmembrane segment (Martin, Crimaudo & Gerace, 1995). The other LAP1 isoforms are of similar overall structure and arise from the same gene by alternative RNA splicing.

LAP2 was identified by another monoclonal antibody and shown to be an integral membrane protein of the inner nuclear membrane that binds to lamin B1 and chromatin (Foisner & Gerace, 1993). Previously, a 49 amino acid protein known as “thymopoietin” was isolated from bovine thymus and observed to affect T-cell differentiation and function (Harris et al., 1994). Complementary DNA cloning and sequencing of three alternatively spliced mRNAs led to the identification of three human protein isoforms that contained the 49 amino acid thymopoietin sequence (Harris et al., 1994). These three thymopoietin isoforms (α, β and γ) contain identical amino-terminal regions. Thymopoietin-α does not contain a transmembrane segment and is present dif-

fusely throughout the nucleus, while the β and γ isoforms have transmembrane domains and are localized to the inner nuclear membrane (Harris et al., 1994). Subsequent cDNA cloning and sequencing of rat LAP2 predicted a protein of 452 amino acids containing a large nucleoplasmic domain and a single membrane-spanning segment (Furukawa et al., 1995). LAP2 had no sequence similarity to LAP1 but was identical to the protein known as thymopoietin-β. A single human gene on chromosome 12q22 was then shown to encode LAP2/thymopoietins α, β and γ (Harris et al., 1995). Characterization of the orthologous mouse gene and reexamination of the human one showed that they each encode at least seven proteins that arise by alternative RNA splicing (Berger et al., 1996). Because the role of these proteins in thymocyte differentiation remains controversial, they are generally referred to as LAP2s. LAP2-β and probably LAP2-γ bind to lamin B1 and chromatin (Foisner & Gerace, 1993). The nucleoplasmic LAP2-α isoform also appears to interact with chromatin (Vlcek et al., 1999). Interactions of LAP2s with chromatin may be mediated by binding to Barrier-to-Autointegration Factor or BAF (Furukawa, 1999).

In 1994, positional cloning resulted in the identification of a gene at chromosome Xq28 responsible for X-linked Emery-Dreifuss muscular dystrophy. Sequencing demonstrated that it encoded a protein, named emerin, that contained a stretch of about 40 amino acids with sequence similarity to a portion LAP2 (Bione et al., 1994). Emerin was soon localized to the inner nuclear membrane and shown to be absent from this location in most patients with X-linked Emery-Dreifuss muscular dystrophy (Manilal et al., 1996; Nagano et al., 1996). Emerin contains a nucleoplasmic, amino-terminal domain followed by a single transmembrane segment and a short carboxyl-terminal tail in the perinuclear space/endoplasmic reticulum lumen. Several lines of evidence suggest that emerin interacts with A-type and possibly B-type nuclear lamins (Fairley, Kendrick-Jones & Ellis, 1999; Sullivan et al., 1999; Clements et al., 2000).

The "MAN antigens" are three polypeptides, recognized by autoantibodies from a patient with a collagen vascular disease, that are localized to the nuclear envelope (Paulin-Levasseur et al., 1996). One of the so-called MAN antigens has been identified as LAP2-β (Lang et al., 1999). Another, MAN1, is a 82.3 kilodalton integral protein of the inner nuclear membrane, encoded by a gene on human chromosome 12q14 (Lin et al., 2000). MAN1 is predicted to have a nucleoplasmic amino-terminal domain followed by two hydrophobic segments and a carboxyl-terminal tail. Protein sequence analysis reveals that MAN1 contains a conserved globular module of approximately 40 amino acids which has been termed the "LEM domain" because it is found in LAP2, emerin and MAN1. The LEM domain is also present in two uncharacterized proteins of *C. elegans*. At the present time, little else is known about MAN1 or the LEM domain. The third "MAN antigen" with a molecular mass of about 40 kilodaltons remains unidentified.

A common feature of LBR, LAP1, LAP2, emerin and MAN1 is that they all contain nucleoplasmic, amino-terminal domains that bind to lamins or chromatin. In contrast to these proteins, a multispanning inner nuclear membrane protein termed nurim does not have a large hydrophilic domain. Nurim was identified as a nuclear envelope component in a visual screen of a green fluorescent protein-fusion library expressed in transfected cells (Rolls et al., 1999). Nurim is present exclusively in the nuclear envelope of cells and is immobilized there, strongly suggesting that it is an inner nuclear membrane protein. The functions of nurim remain unknown at this time.

Protein Targeting to the Inner Nuclear Membrane in Interphase

The inner nuclear membrane can be considered a specialized domain of the endoplasmic reticulum. In principle, integral proteins of the inner nuclear membrane can diffuse freely between the endoplasmic reticulum and the interconnected inner, pore and outer nuclear membranes. Integral membrane proteins localized to the inner nuclear membrane in interphase can freely diffuse laterally in the endoplasmic reticulum membrane, where they are synthesized (Ellenberg et al., 1997; Östlund et al., 1999). Their diffusional mobilities are significantly reduced in the inner nuclear membrane and they cannot readily diffuse back to the endoplasmic reticulum after reaching this location (Ellenberg et al., 1997; Östlund et al., 1999). Retention in the inner nuclear membrane likely occurs as a result of protein-protein interactions, either between the nucleoplasmic domains of these proteins and other nuclear structures or between transmembrane segments in the plane of the membrane. For example, the nucleoplasmic domain of LBR, which confers inner nuclear membrane retention (Soullam & Worman, 1993, 1995; Ellenberg et al., 1997), binds to B-type lamins (Ye & Worman, 1994) and human orthologues of *Drosophila* heterochromatin protein HP1 (Ye & Worman, 1996; Ye et al., 1997). Similarly, the major nuclear envelope targeting domain of LAP2 coincides with its lamin binding region (Furukawa, Fritze & Gerace, 1998). The transmembrane domains of nurim (Rolls et al., 1999) and LBR (Smith & Blobel, 1993; Soullam & Worman, 1995) also contribute to inner nuclear membrane immobilization, likely via interactions with transmembrane segments of other resident proteins.

The results of many studies are consistent with a "diffusion-retention" model for the targeting of integral membrane proteins to the inner nuclear membrane. In

this model, proteins synthesized on the endoplasmic reticulum with cytoplasmic domains less than approximately 60 kilodaltons can laterally diffuse in the plane of the membrane to the inner nuclear membrane, unless this diffusion is inhibited by retention sequences or by binding to cytoplasmic structures. Integral proteins with nucleocytoplasmic domains less than 60 kilodaltons that do not bind to nuclear ligands, cytoplasmic structures or endoplasmic reticulum proteins such as the KDEL receptor ultimately enter the secretory pathway. Lateral diffusion of integral proteins in the interconnected endoplasmic reticulum, outer, pore and inner nuclear membranes is faster than the rate of vesicular transport between the endoplasmic reticulum and Golgi. Therefore, integral proteins that ultimately enter the secretory pathway may transiently spend time in the inner nuclear membrane, diffusing back and forth between it and the endoplasmic reticulum before being transported to the Golgi. Integral proteins with cytoplasmic domains larger than about 60 kilodaltons cannot reach the inner nuclear membrane because of steric constraints imposed by the lateral channels of the nuclear pore complexes (Soullam & Worman, 1995). Resident integral proteins of the inner nuclear membrane are retained there by binding to nuclear ligands or by interacting with the transmembrane segments of other resident proteins.

All of the integral proteins of the inner nuclear membrane characterized so far have nucleoplasmic domains of less than 60 kilodaltons, consistent with the size constraints imposed by the 10 nm lateral channels of the nuclear pore complex (Hinshaw, Carragher & Milligan, 1992). Integral membrane proteins synthesized on the endoplasmic reticulum with larger cytoplasmic domains cannot reach the inner nuclear membrane. Nature has apparently utilized the steric hindrance of the nuclear pore complex lateral channels to evolve regulatory mechanisms that sense conditions in the endoplasmic reticulum and transfer this information to the nucleus. SREBPs are integral proteins of the endoplasmic reticulum membrane that are latent transcription factors which regulate the expression of genes involved in cholesterol biosynthesis (Brown & Goldstein, 1999). When cellular sterol concentrations are low, SREBPs are cleaved by a protease and the freed cytoplasmic domain, which is larger than 60 kilodaltons and contains a nuclear localization signal, is transported into the nucleus. Similarly, the human orthologue of yeast Ire1, which is involved in the unfolded protein response, is a transmembrane protein of the endoplasmic reticulum (Niwa et al., 1999). When unfolded proteins accumulate in the endoplasmic reticulum lumen, Ire1 is cleaved by a protease and the free cytoplasmic domain is then transported into the nucleus where it mediates the spliceosome-independent cleavage of RNA (Niwa et al., 1999).

Inner Nuclear Membrane During Mitosis

The nuclear envelope is a dynamic structure that grows in interphase, breaks down in prophase during mitosis, and reassembles in anaphase, telophase and early G1 (Fig. 2). Two models have emerged from studies on the disassembly and reassembly of the nuclear envelope membranes during cell division. In one model, inner nuclear and pore membranes reversibly fragment during mitosis by vesiculation or/and intraluminal fission, generating vesicles distinct from the endoplasmic reticulum. In a second model, the inner and pore membranes lose their differentiation but remain continuous with the endoplasmic reticulum. In this model, integral proteins of the inner nuclear membrane detach from their nuclear ligands, regain higher diffusion mobility in the lipid bilayer and mix with endoplasmic reticulum integral proteins. These apparently mutually exclusive models originated from the combination of data obtained from experiments on somatic cells and amphibian oocytes.

Early ultrastructural analysis of mitotic cells demonstrated a progressive fragmentation of nuclear membranes, suggesting a disassembly by vesiculation (Zeligs & Wollman, 1979). In the context of available models of membrane traffic by vesiculation, it was proposed that the structure of the nuclear envelope may represent a balance between scission and fusion activity with prevailing scission generating vesicles during mitosis (Warren, 1993). Subsequently, immunofluorescence microscopy and biochemical studies of mitotic cells were performed using antibodies directed against proteins of the inner nuclear and pore membranes (Bailer et al., 1991; Chaudhary & Courvalin, 1993; Foisner & Gerace, 1993; Buendia & Courvalin, 1997). After metaphase, inner nuclear membrane proteins (LBR and LAP2) were shown to be targeted back to chromatin before a pore membrane marker (gp210) and to be enriched in a biochemically separable mitotic membrane fraction, suggesting a domain-specific vesiculation of nuclear membranes (Chaudhary & Courvalin, 1993; Buendia & Courvalin, 1997). However, the sequential targeting to chromatin of inner nuclear and pore membrane proteins does not necessarily infer their presence in isolated vesicles in vivo. As cell homogenization unavoidably provokes membrane vesiculation and fragmentation, isolation of membrane fractions enriched in one protein may also reflect its concentration in microdomains or “rafts” (Jacobson & Dietrich, 1999) of a continuous membrane system.

The terminology of “nuclear membrane vesicle” has emerged from electron microscopic observations and isolation by ultracentrifugation of membrane fractions from amphibian and echinoderm eggs, with distinct abilities to bind chromatin and fuse with one another (Vigers & Lohka, 1991; Poccia & Collas, 1996). Recently, inner nuclear membrane markers, including LBR and LAP2,

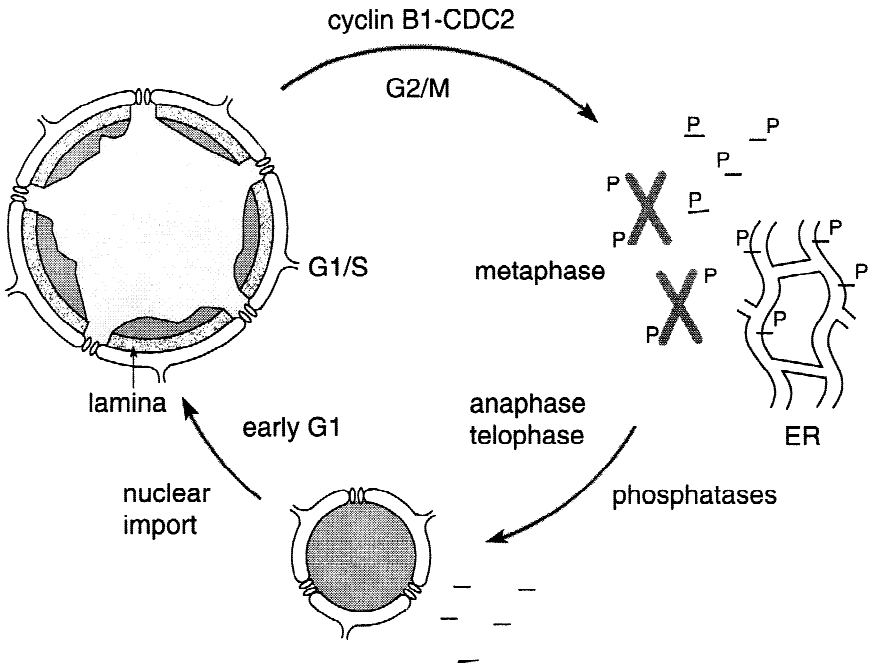

Fig. 2. Cell cycle-dependent modifications of the nuclear envelope. At the end of the G2 phase of the cell cycle, cyclin B1-CDC2 protein kinase is imported into the nucleus, triggering the massive phosphorylation of proteins in chromatin, the lamina, the inner nuclear membrane and the nuclear pore complexes. As a result, in prometaphase, chromosomes condense, the lamina and nuclear pore complex proteins are solubilized and inner nuclear membranes redistribute in the tubular network of mitotic endoplasmic reticulum (ER). Simultaneously with the separation of the two sets of chromosomes in anaphase and telophase, cyclin B1-CDC2 kinase is destroyed and a wave of protein dephosphorylation occurs due to the activation of protein phosphatases. Integral inner nuclear membrane proteins distributed in the endoplasmic reticulum contact chromatin, the membrane surrounds the chromosomes, which remain condensed, and nuclear pore complexes reform. In late telophase and early G1, the lamina and many other nuclear proteins are imported, chromatin partially decondenses and nuclear functions resume. In G1/S, condensed chromatin (dark gray) remains preferentially at the periphery of the nucleus, in contact with the lamina and inner nuclear membrane. During DNA replication in S phase, nuclear membranes and lamina grow in proportion to the increase in volume of the nucleus until late G2 phase.

have been characterized in *Xenopus* and sea urchins using either cross-reacting antibodies or specific antibodies or by cloning. This has enabled the recovery of egg membrane fractions enriched in proteins of the inner nuclear membrane (Collas, Courvalin & Poccia, 1996; Drummond et al., 1999; Gajewski and Krohne, 1999; Gant, Harris & Wilson, 1999; Lang et al., 1999). Using specific antibodies, the sequential targeting of proteins of the inner nuclear membrane and pore membrane to sperm chromatin was demonstrated, suggesting the existence of several populations of nuclear membrane vesicles in egg cytoplasm (Collas, Courvalin & Poccia, 1996; Drummond et al., 1999; Gajewski & Krohne, 1999). These data show that either nuclear vesicles exist in eggs or that they are generated during extract preparation from portions of a membrane continuum enriched in inner nuclear membrane protein (Poccia & Collas, 1996; Jacobson & Dietrich, 1999).

In contrast to the above, recent studies have argued in favor of the disappearance of nuclear membrane domains during mitosis by diffusion of their specific integral proteins throughout a continuous membrane reticulum. Using fluorescence recovery after photobleaching and fluorescence loss in photobleaching, Ellenberg et al. (1997) have demonstrated that LBR-green fluorescent protein diffuses rapidly and freely within endoplasmic reticulum membranes of metaphase cells and become immobile in endoplasmic reticulum regions that contact chromatin in anaphase and early telophase.

Immunofluorescence and confocal imaging studies have also shown that inner nuclear membrane proteins colocalize with endoplasmic reticulum markers at mitosis (Yang, Guan & Gerace, 1997). The reassembly of inner nuclear membrane proteins on decondensing chromatin at the end of mitosis occurs in a non-uniform fashion. While LBR and LAP2 accumulate on chromosomal peripheral regions that are excluded from the spindle (Chaudhary & Courvalin, 1993; Buendia & Courvalin, 1997), emerin first assembles at the central chromosomal core region, located within the spindle (Dabauvalle et al., 1999; Haraguchi et al., 2000). This topological difference in targeting may be secondary to a different localization of the chromatin ligands of these inner nuclear membrane proteins. Video microscopy has further demonstrated that LBR and emerin are targeted to their respective chromosomal locations within 5 minutes after

the onset of anaphase and then become mixed throughout the reforming inner nuclear membrane by 8 minutes (Haraguchi et al., 2000). These data strongly support the presence of a permanently interconnected endoplasmic reticulum-nuclear envelope membrane system during mitosis in which different integral membrane proteins are immobilized both in interphase and anaphase-telophase by specifically binding to a subset of chromatin or lamin components.

Models suggesting a pathway of nuclear membrane reassembly by chromatin-targeting, binding and fusion of nuclear membrane vesicles have generally resulted from studies using cell-free extracts from *Xenopus* or sea urchin eggs and *Drosophila* embryos (reviewed by Poccia & Collas, 1996). Data from egg reconstitution assays and from in vivo somatic cell studies have often been combined to present a unified model of nuclear envelope reassembly. However, as eggs are loaded with stockpiles of molecules and membranes required to support numerous and rapid cell divisions, they may contain nuclear membrane vesicular precursors which are absent from somatic cells. Nuclear reconstitution in egg extracts also generally involves a sperm nucleus as the chromatin binding substrate for membranes, generating the formation of pronuclear envelopes rather than somatic nuclear envelopes.

In summary, studies on the mitotic disassembly and reassembly of the nuclear membrane performed by following markers in living cells strongly favor the hypothesis that integral proteins in nuclear membranes diffuse during mitosis throughout the endoplasmic reticulum, which remains an intact tubular network in many cell types. At the same time, evidence from biochemical studies using oocyte or embryo extracts suggest that inner nuclear membrane vesicles may function in nuclear envelope assembly. As cell homogenization unavoidably provokes membrane fragmentation and vesicularization, and as pronucleus formation in oocytes may differ from nuclear envelope reassembly in dividing cells, the results of these in vivo and in vitro studies are not mutually exclusive.

Role of Inner Nuclear Membrane Proteins in Nuclear Envelope Disassembly During Mitosis

Inner nuclear membrane proteins have specific functions in the disassembly and reassembly of the nuclear envelope during mitosis. Lamins bind to chromatin proteins and DNA and are proposed to play a key role in initiating the mitotic disassembly and reassembly of the nuclear envelope (Burke, 1990; Höger et al., 1991; Glass et al., 1993; Ludérus et al., 1994; Taniura, Glass & Gerace, 1995; Goldberg et al., 1999). However, data obtained by different approaches have argued against a primary role for lamins in nuclear envelope reassembly at the end of mitosis. Immunofluorescence microscopy studies have shown that LBR and LAP2 are targeted to chromosomes early in anaphase, prior to lamins, suggesting a primary role for integral proteins in the reassembly of the nuclear envelope around chromatin (Bailer et al., 1991; Chaudhary & Courvalin, 1993; Foisner & Gerace, 1993). This is also supported by experiments in which antibodies to A-type and B-type lamins were microinjected in mitotic cells (Benavente & Krohne, 1986). These studies have shown that nuclei, surrounded by nuclear membranes containing nuclear pore complexes, could reassemble without a lamina. More recently, a time-lapse study of mitotic cells performed with green fluorescent protein-tagged A-type lamins has shown that the reformation of the lamina does not occur until after cytokinesis is completed (Broers et al., 1999). In addition, the presence of LBR in nuclear envelopes has been correlated with the occurrence of chromatin-membrane contacts but not with the presence of lamins in the membrane in *Xenopus* oocytes and early embryonic cells (Gajewski & Krohne, 1999). The primary argument which has been proposed in favor of a key role for lamins initiating the reassembly of the inner nuclear membrane around chromatin is the association of B-type lamins with membranes, presumably inner nuclear membrane vesicles or remnants, during mitosis (Gerace & Blobel, 1980; Stick et al., 1988). However, B-type lamins have been shown to dissociate from their inner nuclear membrane receptors during mitosis and may merely be nonspecifically associated with various membranes via their farnesyl group (Chaudhary & Courvalin, 1993; Gajewski & Krohne, 1999). In this case, they could not specifically initiate nuclear envelope reassembly around decondensing chromosomes.

Studies in which lamins are depleted from *Xenopus* (Newport, Wilson & Dunphy, 1990) or sea urchin oocyte extracts (Collas, Pinto-Correia & Poccia, 1995; Collas, Courvalin & Poccia, 1996) support a primary role for integral proteins in the reassembly of the inner nuclear membrane around chromatin. Immunodepletion of lamins from these extracts does not prevent the reformation of a nuclear envelope around chromatin. Although these in vitro assembled nuclei were surrounded by a membrane containing nuclear pore complexes, they were unable to undergo nuclear swelling and DNA replication (Newport, Wilson & Dunphy, 1990; Ellis et al., 1997), implicating lamins as essential for further nuclear growth after the nuclear membranes reform. However, similar lamin depletion experiments, performed with extracts from somatic cells (Burke & Gerace, 1986), *Xenopus* eggs (Dabauvalle et al., 1991) or *Drosophila* embryos (Ulitzur et al., 1997), have reached the opposite conclusion, namely that lamins are required for membrane formation around chromatin. To explain these discrepancies, Lourim and Krohne (1993) have proposed that the

apparent lamin-independent reconstitution of nuclear envelopes in vitro is due to the presence of residual lamins or minor lamin isoforms after immunodepletion procedures.

Fewer studies have addressed the breakdown of the inner nuclear membrane at the start of mitosis. Mutations at two serine residues flanking the central rod domain of lamin A, which are phosphorylated at the G2/M transition, block the mitotic disassembly of the nuclear lamina (Heald & McKeon, 1990). However, the effect of this blockage on the disassembly of the inner nuclear membrane has not been studied. Although depolymerization of the lamina in *Xenopus* may occur in the absence of membrane breakdown (Stick & Schwartz, 1983; Miake-Lye & Kirschner, 1985), studies performed in egg extracts have shown that lamina disassembly may be a prerequisite for nuclear envelope breakdown (Newport & Spann, 1987; Collas, 1998).

If lamins play a role in nuclear membrane disassembly or reassembly, it can only be fulfilled by B-type lamins, since A-type lamins are absent from eggs and early developing embryos (Benavente, Krohne & Franke, 1985; Stewart & Burke, 1987), as well as some undifferentiated and cancer cells (Guilly et al., 1987, 1990; Worman, Lazaridis & Georgatos, 1988; Lourim & Lin, 1989; Röber et al., 1990; Cance et al., 1992). A limited role for lamin A in driving mitotic events is further demonstrated by the fact that *LMNA* homozygous knockout embryos develop into mice (Sullivan et al., 1999). Mutant *Drosophila* in which nuclear lamin Dm_0 is poorly expressed results in the development of flies, however, their nuclear envelopes have noticeable structural defects (Lenz-Böhme et al., 1997). One must also keep in mind that nuclear pore complex proteins may also be involved in the disassembly and reassembly of the inner nuclear membrane during mitosis (Sheehan et al., 1988; Bodoor et al., 1999).

The cell cycle-dependent phosphorylation of lamins, integral proteins of the inner nuclear membrane and nuclear pore complex proteins is critical in driving the disassembly and reassembly of the nuclear envelope during mitosis (Fig. 2). The solubilization of lamins during mitosis is due to phosphorylation by cyclin B/$p34^{cdc2}$ and protein kinase C and the phosphorylated amino acid residues have been identified (Heald & McKeon, 1990; Peter et al., 1990; Thompson & Fields, 1996). Several integral proteins of the inner nuclear membrane, including LBR, LAP2 and emerin, are also phosphorylated during mitosis (Courvalin et al., 1992; Foisner & Gerace, 1993; Nikolakaki et al., 1997; Ellis et al., 1998). The specific amino acids phosphorylated by cyclin B/$p34^{cdc2}$ have only been identified for LBR (Courvalin et al., 1992; Nikolakaki et al., 1997). HP1 proteins, chromatin ligands for LBR, and nucleoporins are also phosphorylated in a cell cycle-dependent fashion (Favreau et al., 1996; Minc et al., 1999), suggesting that detachment of the inner nuclear membrane from the chromatin may be due to phosphorylation of a large number of integral and peripheral proteins of the inner nuclear membrane. From in vivo and in vitro studies performed in mammalian somatic cells and in *Xenopus* eggs, it appears that protein kinases promote membrane release from chromatin (Foisner & Gerace, 1993; Pyrpasopoulou et al., 1996), while protein phosphatases stimulate binding (Fernandez et al., 1992; Pfaller, Smythe & Newport, 1991; Vigers & Lohka, 1992).

Inner Nuclear Membrane and Apoptosis

A major structural change in cellular morphology during apoptosis is the condensation of chromatin, which is secondary to the DNA cleavage and the proteolysis of key nuclear proteins such as NuMa and DNA topoisomerase II (Earnshaw, 1995; Thornberry & Lazebnik, 1998). In parallel with chromatin condensation, the inner nuclear membrane detaches from condensing chromatin, nuclear pore complexes cluster in the plane of the membrane but the overall ultrastructure of the nuclear envelope remains well preserved (Buendia, Santa-Maria & Courvalin, 1999). Concurrent with these morphological alterations, A-type and B-type lamins are cleaved early in apoptosis by caspase 6 (Lazebnik et al., 1993) and LAP2 and nup153 by caspare 3 (Buendia, Santa-Maria & Courvalin, 1999). Later in apoptosis, LBR is also cleaved (Duband-Goulet, Courvalin & Buendia, 1998). The apoptotic proteolysis of nuclear envelope proteins is conserved in different cell lines which have been committed to apoptosis by different inducing agents (Buendia, Santa-Maria & Courvalin, 1999). Lamins and inner nuclear membrane proteins are therefore among the small group of nuclear components that cooperate to trigger the dramatic nuclear alterations that occur during apoptosis.

Inner Nuclear Membrane and Human Disease

Recently, inner nuclear membrane proteins have been intriguingly associated with human genetic diseases. Emery-Dreifuss muscular dystrophy (OMIM #310300) can be inherited in an X-linked fashion and is characterized by early contractures of the elbows, Achilles tendons and posterior neck, progressive muscle wasting and cardiomyopathy with atrioventricular conduction block (Emery & Dreifuss, 1966; Rowland et al., 1979; Emery, 1989; Tsuchiya & Arahata, 1997). The spectrum of these symptoms can vary from case to case. In 1994, positional cloning of the gene responsible for X-linked Emery-Dreifuss muscular dystrophy was shown to encode emerin (Bione et al., 1994), an inner nuclear mem-

brane protein and absent from this location in most patients with the disease (Manilal et al., 1996; Nagano et al., 1996). More than 50 different pathogenic mutations in emerin have been described (http://www.path.cam.ac.uk/emd/mutation.html), most resulting in truncated proteins which are not expressed and others causing amino acid substitutions.

A phenotypically similar disorder to X-linked Emery-Dreifuss muscular dystrophy, with cardiomyopathy and conduction abnormalities as frequent prominent features, is inherited in an autosomal dominant manner (OMIM #181350) (Emery, 1989). In 1999, Bonne et al. (1999) described mutations in the *LMNA* gene on chromosome 1 encoding lamins A and C in individuals with this disorder. The mutations ranged from a termination codon at the position encoding amino acid 5, resulting in essentially no protein production from this allele, to point mutations throughout the coding region. Subsequent work by Fatkin et al. (1999) confirmed these findings and described mutations in the lamin A/C gene in individuals with autosomal dominantly inherited cardiomyopathy with conduction block (OMIM #115200).

Patients with Dunnigan-type familial partial lipodystrophy (OMIM #151660) are born with normal fat distribution and normal cardiac and skeletal muscle function (Dunnigan et al., 1974). After puberty, affected individuals experience regional and progressive adipocyte degeneration, often associated with profound insulin resistance and diabetes mellitus. In 1998, the gene for Dunnigan-type lipodystrophy was mapped to chromosome 1q21-22 (Jackson et al., 1998; Peters et al., 1998). Based on this chromosomal localization, Cao and Hegele (2000) hypothesized that the regional muscle wasting in autosomal dominant Emery-Dreifuss muscular dystrophy and the regional adipocyte degeneration in Dunnigan-type lipodystrophy were similar and sequenced the *LMNA* gene in affected individuals. They showed that 5 Canadian probands with Dunnigan-type lipodystrophy had a missense mutation (R482Q) in lamins A and C that was absent from 2,000 normal alleles (Cao & Hegele, 2000). Using a positional cloning approach, Shackleton et al. (2000) also described 5 different mutations at amino acid residues 482 and 486 in lamins A and C in subjects with Dunnigan-type lipodystrophy.

How do mutations in emerin and A-type lamins cause phenotypically similar disorders? Some insights into this question can be obtained from studies of *LMNA* gene knockout mice (Sullivan et al., 1999). In cells from mice homozygous for the *LMNA* knockout, some emerin is found in the endoplasmic reticulum rather than only in the inner nuclear membrane. In normal cells, emerin does not appreciably diffuse out of the inner nuclear membrane back to the endoplasmic reticulum (Östlund et al., 1999). An interaction between emerin and lamins A and C may therefore be responsible in-part for retaining emerin in the inner nuclear membrane. Disruption of the lamin A/C-emerin interaction may underlie the disease phenotype in both X-linked and autosomal dominant forms of Emery-Dreifuss muscular dystrophy. An interaction between emerin and lamin A has also been shown by in vitro binding assays (Fairley, Kendrick-Jones & Ellis, 1999; Clements et al., 2000).

Why do phenotypes as dramatically different as skeletal muscle dystrophy, partial lipodystrophy and cardiomyopathy result from different mutations in the same lamin proteins? One possibility is that different mutations directly result in different types of cellular dysfunction. So far, mutations at only two amino acid residues in lamin A/C have been associated with Dunnigan-type lipodystrophy (Cao & Hegele, 2000; Shackleton et al., 2000), but others may still be discovered. Several different mutations in lamins A and C have been shown to cause Emery-Dreifuss muscular dystrophy with cardiomyopathy (Bonne et al., 1999) while subjects suffering predominantly from cardiomyopathy have mutations in the rod domains of lamins A and C (Fatkin et al., 2000). If particular lamin A/C mutations indeed cause tissue-predominant phenotypes, it is possible that lamins A and C function differently in different cells. Cell type-specific differences in lamin function may arise as a result of interactions with different protein partners in different cells. Another possibility is that the different phenotypes resulting from mutations in lamin A and/or lamin C depend upon the genetic background or environment of the affected individual. This possibility is suggested by studies on the *LMNA* knockout mice. Mice with homozygous mutations in *LMNA* and no detectable lamin A or lamin C expression suffer from skeletal muscular dystrophy, cardiomyopathy and partial lipodystrophy (Sullivan et al., 1999). Heterozygous *LMNA* knockout mice are essentially normal. In contrast, all human subjects described so far with disorders resulting from mutations in lamins A and/or C are heterozygotes. Perhaps different strains of mice heterozygous for *LMNA* mutations, or heterozygous mice raised in different environments, will have different disease phenotypes and this may also be the case for humans with various *LMNA* mutations.

Why do mutations in emerin and lamins A/C, which are expressed in almost all differentiated somatic cells, cause disorders apparently only in muscle and fat, tissues not characterized by high rates of cell division (Table 2)? The implication is that mutations in inner nuclear envelope proteins found in these disorders affect cells during interphase. One hypothesis is that mutations in lamins or emerin make cells more susceptible to apoptosis as lamins and other inner nuclear membrane proteins are known to be key substrates for caspases. Another hypothesis is that interactions between the inner nuclear membrane and chromatin influence tissue-specific gene

Table 2. Hypotheses on how mutations in inner nuclear membrane proteins could cause abnormalities in skeletal muscle, cardiac muscle and lipodystrophy

Increased susceptibility to apoptosis — role of nuclear lamins and inner nuclear membrane proteins in programmed cell death
Regulation of cell type-specific gene expression by chromatin-inner nuclear membrane interactions — example: interaction of LBR with HP1 chromatin proteins
Increased susceptibility to mechanical stress — lamina and inner nuclear membrane connected to cytoskeleton via nuclear pore complexes
Functions in locations other than the nuclear envelope in some cells — controversial localization of emerin to cardiac muscle intercalated discs

expression, perhaps regulating genes that are required for muscle or adipocyte survival. A role for the inner nuclear membrane in regulating gene expression is suggested by the interaction between LBR and human orthologues of *Drosophila* HP1 (Ye & Worman, 1996; Ye et al., 1997). In *Drosophila*, HP1 is known to suppress the expression of genes translocated localized near heterochromatin (Eissenberg et al., 1990). One can also hypothesize that mutations in nuclear envelope proteins may subject cells to injury resulting from recurrent mechanical stress, which is characteristic of skeletal and cardiac muscle. The nuclear lamina likely interacts with the inner aspect of the nuclear pore complex. The outer aspect of the nuclear pore complex has attached filaments that extend into the cytoplasm (Hinshaw, Carragher & Milligan, 1992). Filaments extending from the pore complexes could potentially interact with cytoskeletal components such as desmin, actin or tubulin. Hence, the lamina on the inner aspect of the inner nuclear membrane may be part of an elaborate internal support network of cells. Minor disruptions in this support network could make cells susceptible to injury from mechanical stress. Emerin, via its interaction with lamins, may also be a part of this putative support network. Finally, it is possible that, in some cell types, inner nuclear membrane proteins are targeted to other subcellular locations where they have alternative functions. This hypothesis is suggested by the controversial finding of a protein recognized by some anti-emerin antibodies in intercalated discs of cardiac muscle (Cartegni et al., 1997; Manilal et al., 1999). Which if any of these hypotheses explain how mutations in inner nuclear membrane protein cause human diseases remain to be established.

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