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Review

The role of the nuclear envelope in cellular organization

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Abstract. Over the last years it has become evident that the nuclear envelope (NE) is more than a passive membrane barrier that separates the nucleus from the cytoplasm. The NE not only controls the trafficking of macromolecules between the nucleoplasm and the cytosol, but also provides anchoring sites for chromosomes and cytoskeleton to the nuclear periphery. Targeting of chromatin to the NE might actually be part of gene expression regulation in eukaryotes. Mutations in certain NE proteins are associated with a diversity of human diseases, including muscular dystrophy, neuropathy, lipodistrophy, torsion dystonia and the premature aging condition progeria. Despite the importance of the NE for cell division and differentiation, relatively little is known about its biogenesis and its role in human diseases. It is our goal to provide a comprehensive view of the NE and to discuss possible implications of NE-associated changes for gene expression, chromatin organization and signal transduction.

Key words. Nuclear envelope; chromatin; nuclear pore complex.

Introduction

The nuclear envelope (NE) is a highly specialized membrane that delineates the cell nucleus. The NE is composed of the nuclear membranes, the nuclear pore complexes (NPCs) and the lamina (fig. 1). The nuclear membranes can be divided into two morphologically distinct but interconnected domains named the outer nuclear membrane (ONM) and the inner nuclear membrane (INM). The ONM is continuous with the rough endoplasmic reticulum (rER), studded with ribosomes [1–4] and provides attachment sites for structural elements of the cytoplasm [5–11]. The INM contains a unique set of integral and membrane-associated proteins that provide binding sites for the lamina and chromatin [12–15]. The nuclear lamina, which underlines the INM, is a dense network composed of intermediate filaments made of the lamin proteins and lamin-associated proteins [16] and is essential for nuclear integrity.

NPCs span the nuclear envelope at sites where ONM and INM are fused and are the exclusive mediators of transport between the nucleus and cytoplasm [17–22]. NPCs are large protein assemblies with an estimated mass of ~125 MDa in metazoa [23] and ~66 MDa in fungi [24, 25]. The molecular mechanisms of transport across the NE have been analyzed in great detail, and since this subject has been covered recently by major reviews [17–19, 26–30] we will not discuss it here.

Over the last few years it has become clear that the NE participates in a variety of cellular processes that are not necessarily linked to nuclear transport. Proteins that specifically localize to the nuclear periphery interact with the cytoskeleton and are involved in the positioning of the nucleus within the cell and its movement during certain developmental stages. Proteins of the INM interact with chromatin and might regulate gene expression and DNA replication. Some proteins of the NPC seem to be promiscuous and have been shown to interact with kinetochores and the mitotic spindle during mitosis. In this review we want to summarize recent insights into these 'unex-

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pected' behaviors of NE components and discuss some of the exciting new concepts that have emerged in the field of nuclear function and dynamics.

The NPC

Significant progress has been made in the biochemical analysis of the NPC composition and organization of individual nucleoporins within NPC sub-complexes. Immuno-electron microscopy has revealed the relative location of proteins within the overall NPC framework.

The NPC is an eightfold symmetrical structure [31] built from about 30 different proteins [32, 33], called nucleoporins. Accordingly, the nucleoporins are present in copies of eight or multiples of eight, per NPC, and thus this large structure is assembled from a relatively low number of components. Two major classes of nucleoporins were identified, one class with FG repeats that functions directly in nucleo-cytoplasmic transport by binding the soluble transport receptors, and a second class that lacks FG repeats and is thought to represent the structural components of the NPC [19, 27]. In addition to the well-accepted roles of the nucleoporins in the nuclear transport process, it is becoming evident that these proteins have additional functions.

Transport-independent functions of nucleoporins

The yeast Nup84 complex is essential for NPC assembly and is considered to make up the bulk of the NPC [34]. It consists of seven subunits (yNup84p, yNup85p, yNup120p, yNup133p, yNup145C, Sec13p, Seh1p) [35] and has a mass of 375 kDa [34]. The Nup84 complex has been reconstituted from purified recombinant proteins and was shown to be Y-shaped [35]. Taking the eightfold symmetry of the NPC into account, it is possible that eight such Y-shaped subunits aligned around the pore could form part of the central structure of the NPC, perhaps corresponding to the 'star ring' observed by surface scanning electron microscope (EM) [36]. Mutations in either *NUP133* or *NUP120* result in clustering of NPCs into one or a few aggregates in the NE, implying a role in NPC or NE organization [37]. Mutations in several members of the complex also lead to accumulation of polyA+ RNA in the nucleus, suggestive of a function in messenger RNA (mRNA) export [38, 39].

The mammalian homologue of the yNup84 complex is the Nup107–160 complex. Within this substructure, Nup107 is homologous to yNup84p, Nup160 to yNup120p, Nup96 to yNup145C and there are also mammalian equivalents of yNup, Sec13 and Seh1 [40–43]. In addition, the mammalian complex has two subunits, Nup37 and Nup43, that are not found in yeast [44]. Similarly to its yeast counterpart, the Nup107–160 complex has been implicated in mRNA export from the nucleus [42, 45, 46]. The Nup107–160 complex has been shown to associate with kinetochores during cell division, suggesting a mitotic function of this complex [44].

The primary structure of yNup84 and Nup107–160 complex nucleoporins has recently been investigated, and α solenoids, β propellers or a combination of both structural features were predicted in all of them. Interestingly, this

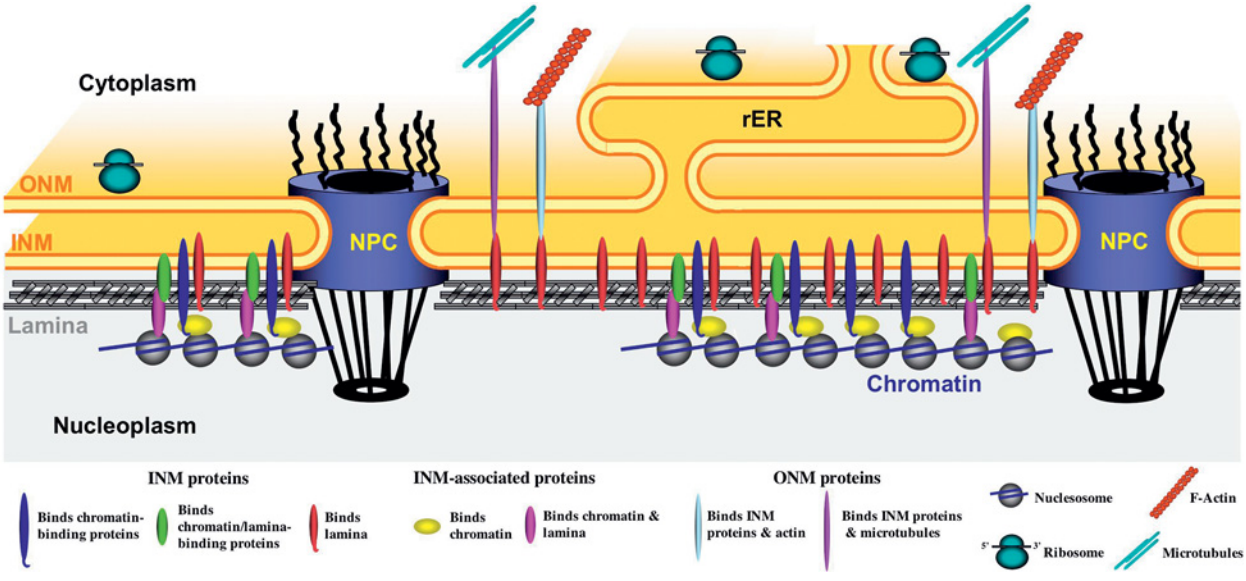

Figure 1. Schematic representation of the NE. ONM, outer nuclear membrane; INM, inner nuclear membrane; NPC, nuclear pore complex; rER, rough endoplasmic reticulum. Ribosomes associated with the ONM as well as proteins of the INM associated with the lamina meshwork (red) and with chromatin (blue and green) are indicated. INM proteins associate with chromatin through chromatin-binding proteins (yellow and pink). ONM proteins (purple and gray) associate with INM proteins and the cytoskeleton through F-actin or microtubules.

Table 1. Nuclear envelope protein-associated diseases.

Protein	Disease	Mechanism	References
INM			
emerin	X-EDMD	mutation	[218–219]
LBR	PHA, HEM, AD	mutation, autoantibodies	[227, 228, 234, 235]
LAP1	AD	autoantibodies	[236]
LAP2	AD	autoantibodies	[236]
MAN1	osteopoikilosis, Buschke-Ollendorf syndrome, melorheostosis, AD	mutation, autoantibodies	[232]
NPC			
Nup62	PBC, picornavirus infection	autoantibodies, virus replication	[217]
Nup88	breast, ovarian and several other malignant cancers	overexpression	[237, 238]
Nup98	AML, CML, MDS, T-all, VSV-infection	chromosomal translocation, virus replication	[195, 202]
Nup153	AD, picornavirus infection	autoantibodies, virus replication	
Nup214	AML, MDS, T-all, adenovirus infection	chromosomal translocation, virus docking	[198, 239–242]
Nup358	IMT	protooncogene activation by translocation	[243]
gp210	PBC	autoantibodies	
ALADIN	AAAS	mutation	[205]
Tpr	osteosarcoma, fibroblastoma, carcinoma, AD adenocarcinoma, papillary thyroid	protooncogene activation by translocation, autoantibodies	[204]
Lamina			
lamin A/C	A-EDMD, LGMD1B, DCM, CMT2, FPLD, MAD, HGPS, WS, AD, tight-skin contracture syndrome	mutations, autoantibodies	[106, 220, 221, 223–225, 244–253]
lamin B	AD	autoantibodies	

X-EDMD, X-linked Emery-Dreifuss muscular dystrophy; PHA, Pelger-Huet anomaly; HEM, Greenberg skeletal dysplasia; AD, autoimmune disesase; PBC, primary biliary cirrhosis; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; T-all, T-cell acute lymphoblastic leukemia; VSV, vesicular stomatitis virus; IMT, inflammatory myofibroblastic tumor; AAAS, triple A syndrome; A-EDMD, autosomal EDMD; LGMD1B, limb girdle muscular dystrophy; DCM, dilated cardiomyopathy with conduction system disease; CMT2, charcot-Marie-Tooth disorder type 2; FPLD, Dunnigan-type familial lipodystrophy; MAD, mandibulo acral dysplasia; HGPS, Hutchinson-Gilford progeria syndrome; WS, Werner's syndrome; SLE, systemic lupus erythematosus; AD: among the reported autoimmune diseases are SLE, PBC, rheumatoid arthritis, autoimmune cytopenias, chronic active hepatitis, rheumatoid arthritis and polymyalgia rheumatica

architecture resembles that of the complexes that form membrane vesicle coats and a common evolutionary origin of these complexes, and even a common function in producing domains of high membrane curvature has been suggested [45, 47]. Consistent with this notion, Sec13p is both a Nup84/Nup107–160 complex component and a subunit of the COPII membrane vesicle coat complex [38, 47]. β propellers are a common domain involved in protein-protein interactions and are a more widespread feature of nucleoporins. In the case of Nup159, a β propeller domain has been shown to interact with the helicase Dbp5, and this interaction is implicated in targeting Dbp5 to the NPC and allowing it to function in mRNA export [48].

Similar to the Nup107–160 complex, Nup358/RanBP2 associates with kinetochores during mitosis [49, 50]. During interphase, Nup358/RanBP2 is the main constituent of the cytoplasmic filaments of the vertebrate NPC. Nup358 is a large protein containing four RanGTP binding domains, similar to that found in cytoplasmic, soluble RanBP1 [51, 52]. Nup358 also contains two zinc-finger domains that are predicted to bind RanGTP [51] and a number of loosely spaced FG repeats. It serves as a binding site for SUMO-modified forms of RanGAP, the GTPase-activating protein for Ran [53], and is an active E3 ligase in the sumoylation reaction [54, 55]. During metaphase, Nup358, together with RanGAP, localizes to kinetochores and the mitotic

spindle. Depletion of Nup358/RanBP2 by small interfering RNA (siRNA) impaired kinetochore assembly and resulted in chromosome segregation defects in mammalian cells. In yeast, kinetochore assembly also seems to require the function of a nucleoporin. Strains lacking Nup170p, the homologue of mammalian Nup155, resulted in kinetochore integrity defects [56].

Nup155 has recently been shown to be essential for nuclear envelope formation in nematodes and vertebrates. Combining RNA interference (RNAi) techniques in *C. elegans* with *in vitro* nuclear assembly reactions using *Xenopus* egg extracts, the authors of this study showed that Nup155 is critical for correct nuclear membrane fusion and NPC assembly in both organisms [57].

Another unexpected function of a nucleoporin was identified also using *Xenopus* egg extracts. Depletion of Nup153 from extracts inhibited nuclear envelope breakdown (NEBD) during mitosis. Nup153 was shown to recruit the COPI complex, which mediates retrograde transport from the Golgi to the ER, to the nuclear envelope [58]. A direct role of the COPI complex in this process was further supported by antibodies against β-COP which inhibited NEBD [58]. What role the COPI complex plays in this process remains to be analyzed.

The nuclear basket emanates from the central framework of the NPC into the nucleoplasm. Several studies have suggested that Tpr and its yeast homologues, Mlp1p and Mlp2p, protrude from the NPC into the nucleoplasm. Careful studies employing several antibodies suggested that Tpr is the main constituent of the nuclear basket, with its long coiled-coil segments forming the fibrils emanating from the nuclear face of the NPC [59, 60]. Mlp1p and Mlp2p had earlier been suggested to form intranuclear fibrils, facilitating delivery of cargoes to the NPC [61]. Although an attractive model, the fact that *mlp1Δ mlp2Δ* mutants are viable and only show a slightly reduced nuclear import rate [61] render this suggestion unlikely.

A genetic screen in yeast has highlighted an unforeseen function of Nup2p [178]. Working in concert with several transport receptors, Nup2p physically tethers chromatin to the NPC, blocking the propagation of heterochromatin in a defined chromatin domain and impeding its silencing. How Nup2p prevents heterochromatin spreading is not known yet; however, since Nup2p is a dynamic nucleoporin that can shuttle to the nucleoplasm and the cytoplasm, a role of this protein when detached from the NPC cannot be ruled out.

A connection between NPCs and spindle pole body (SPB) has been described in yeast. Ndc1p was the first nucleoporin to be linked to SPB. Ndc1p, Pom34p and Pom152p are the three transmembrane-domain nucleoporins present in yeast [62, 63]. Ndc1p is essential for the viability of yeast cells [64, 65], and it is a stable component of the spindle pole body, being involved in the duplication and insertion of SPBs into the nuclear membrane [63, 65, 66]. Since the spindle pole body and nuclear pore complex are topologically similar, it has been suggested that Ndc1p is involved in the biogenesis of both complexes.

The NE and nuclear positioning

In eukaryotes, the nucleus occupies a well-defined, often non-centered position within the cell. Correct nuclear positioning is essential for many cellular processes, including cell division, migration, differentiation, fertilization and polarization. The coordinated action of microtubules and actin filaments has been shown to be required for nuclear migration (for reviews see [67–69]). Anchoring of the nucleus to the cytoskeleton has been attributed to a conserved family of giant nuclear membrane proteins (800 kDa or more) present in mammals, *Caenorhabditis elegans* and *Drosophila*, and commonly known as Nesprins [70]. The proteins of this family (including ANC-1, Nesprin 1 and 2, and Msp-300) are characterized by the presence of an N-terminal actin-binding domain, a large central rod domain containing spectrin-like repeats and a C-terminal transmembrane domain responsible for their NE localization [8, 10, 67]. Nesprins have been suggested to be ONM proteins, although controversial data exist regarding their INM localization [9, 10, 71, 72]. Nesprins connect the nuclear membranes with the cytoskeleton by interacting with INM proteins, lamins and actin [72–74]. A good example of how such an interaction functions in nuclear anchoring is the *C. elegans* UNC-84 that binds ANC-1 proteins. UNC-84 is an INM protein member of the SUN family [75]. UNC-84 requires lamin for its NE localization and is responsible for anchoring ANC-1 to the nuclear membrane. ANC-1, on the other hand, interacts with the actin network [76, 77]. UNC-84 also interacts with UNC-83, a nuclear envelope protein that binds microtubules [78]. Because all three proteins have been linked to nuclear migration and/or to nuclear positioning in *C. elegans,* a bridging model has been proposed in which the INM protein UNC-84 would connect the nucleus to different components of cytoskeleton by interacting with the ONM proteins UNC-83 and ANC-1 [8, 67, 76–78]. In an analogous way, SUN1 (or Matefin), another INM-specific protein, interacts with the ONM protein ZYG-12, an interaction that is necessary for the attachment of *C. elegans* centrosomes to the NE [5, 79]. The SUN family of proteins is conserved in metazoa, and similar complexes are being identified in other organisms [74]. In this way, an INM-ONM bridge connecting the nuclear periphery to the cytoskeleton is established that is responsible for nuclear positioning, nuclear movement and possibly other functions, i.e. the transduction of mechanical signals from the cell surface to the nucleus [80–83]. Changes in nuclear shape, induced by modulations of cell morphology, resulted in alterations of gene expression programs [83].

The INM and nuclear organization

Over the last few years it has become clear that the NE participates in the coordination of intranuclear chromatin organization and in certain aspects of gene expression. Chromosomes occupy the nuclear interior in a non-random fashion [84, 85]. Chromatin organization is mainly achieved by the separation of chromosomes into non-overlapping territories and by specific contacts with the NE. The subject of chromatin territories has been discussed in multiple reviews [86–90]. The idea that the NE might be involved in chromatin organization was inferred from early EM studies which revealed that heterochromatin is tightly associated with the NE in many cell types [91]. This was interpreted as selective attachment of specific loci to the nuclear periphery. Recent fluorescent in situ hybridization experiments suggest the existence of NE-associated regions of chromatin from throughout the genome [85]. Specific DNA sequences that mediate NE association have not been identified, but repetitive sequences might play an important role [92, 93]. We will discuss the assembly of peripheral, NE-associated structures below, and then examine their effects on gene expression and their roles in disease.

The nuclear lamina

The proteins of the NE that provide contact with chromatin, and therefore might be involved in its organization, can be grouped into three classes; lamins, INM proteins and nucleoporins. The lamin proteins, the main components of the nuclear lamina, form a peripheral fibrous meshwork that underlies the INM in metazoa. Certain mechanical properties of the lamina suggest that the network is extensible but limited in compressibility, functioning as a molecular shock absorber [94] and helping confer tensile strength on the nuclear periphery [95–97]. In addition, lamins are required in as yet poorly understood ways for fundamental nuclear processes such as replication and transcription [71].

Nuclear lamins constitute the type-V intermediate filament protein family and have been grouped into B-type lamins and A/C-type lamins on the basis of their amino acid composition [98, 99]. They share overall organization, consisting of globular N- and C-terminal domains separated by a central α-helical segment. The helical regions associate during lamin dimerization, while the globular domains mediate higher-order interactions. In vertebrates, B-type lamins are expressed ubiquitously, including meiotic and early embryonic cells, while A/C-type lamins are found only in differentiated cells [100, 101]. Two B-type lamins, B1 and B2, which are encoded by distinct genes, are present in mammalian somatic cells. In contrast, the various A-type lamins, including lamins A and C, are alternative splicing variants [102]. The lamin C-terminal domains contain several motifs, including an NLS, that are required for its localization. Lamins B1 and B2 contain a C-terminal CaaX motif that constitutes a site for the addition of a farnesyl lipid group [103]. This feature is shared by lamin A [104, 105]. However, proteolytic processing of the C-terminus of newly synthesized lamin A, soon after its incorporation into the nuclear lamina, results in the loss of its farnesyl lipid modification [106]. Thus, while the mammalian B-type lamins are farnesylated proteins that are always membrane-associated, the mature A- and C-type lamins are not farnesylated and are soluble during mitosis. In mammalian cultured cells, RNAi studies demonstrated that B-type lamins are essential for individual cell growth while A-type lamins are not [107].

The plant nucleoskeleton contains coiled-coil proteins apparently unrelated to lamins [108, 109], and nothing equivalent to the lamina is found in yeast [110]. *C. elegans* has only one lamin gene (lmn-1), and its knock-down produces pleotropic phenotypes, including aberrant nuclear shape, clustering of NPCs, mislocalization of some INM proteins and abnormal chromosome segregation during mitosis [111, 112]. *Drosophila* has both a B-type (Dm0) and A/C-type lamin (C). Lamin Dm0 is expressed ubiquitously, whereas lamin C is found in late-embryonic and differentiated cells [100, 113]. Reduced Dm0 expression affects NE assembly and induces the formation of annulate lamellae [114]. A mutant Dm0 allele profoundly affects nuclear morphology and impairs *Drosophila* development [115].

Several INM proteins have been shown to interact directly with lamins. LBR, which was named lamin B receptor on being shown to bind to lamin B [116], is among the best characterized. The nucleoplasmic N-terminal domain of LBR interacts *in vitro* not only with B-type lamins, but also with chromatin via HP1 [117]. Other examples of lamin-binding INM proteins are emerin, which interacts with A- and B-type lamins [118] and the lamin-associated proteins (LAPs) [119]. All transmembrane LAPs contain nucleoplasmic N-termini, a single transmembrane domain and a short C-terminal domain [120]. LAP1 A and B interact with lamins A, C and B1; LAP2β interacts with lamin B1 and chromatin.

INM proteins link the INM to chromatin as well as to the lamina [121–127]. As referred to above, LBR binds HP1. In addition, a shared motif common to the nucleoplasmic domains of the INM proteins LAP2, emerin and MAN1, the so-called LEM domain, binds to a protein named Barrier to Autointegration Factor (BAF) originally discovered because of its effects on retroviral integration into the host genome [128]. Studies using overexpressed or *in vitro* produced protein fragments have since indirectly implicated BAF-LEM domain interaction in NE assembly [125, 126].

In summary, a network of direct and indirect protein-protein interactions forms a highly interconnected sub-NE domain that consists of NPCs, the lamina, INM proteins, specific regions of chromatin and, as detailed in an earlier section of the review, extends to the microtubule and actin cytoskeleton.

In addition to the peripheral lamina, two different types of intranuclear lamin-containing structure have been reported. Tubular invaginations of the NE that penetrate deeply into the nucleus and have an associated lamina have been observed, but their function is unclear [129]. Second, A-type lamins have been suggested to be part of an internal nucleocytoskeleton as well as being associated with specific nuclear bodies [130]. Intranuclear lamins have been implicated in DNA replication, transcription and overall nuclear organization [15, 131–136]. Together with the rest of the NE, the lamina is disassembled and reformed during mitosis. Several phosphorylation sites, including those for cdc2 kinase, protein kinase C and protein kinase A, are important in lamin disassembly [98, 137, 138]. Protein phosphatases, which reverse the mitotic phosphorylation of lamins, have been implicated in lamina reassembly [139, 140].

A-type lamins are present only in a subset of cell types, and the majority of these lamins is imported into the nucleus in G1 [129, 141]. On the other hand, lamin B has been detected in the nuclear periphery in telophase, although its concentration occurs at late telophase/cytokinesis, when the nuclear membrane is likely closed around chromatin [142, 143]. Controversial reports exist on the role of lamin B in nuclear assembly. While some studies show that depletion of B-type lamins inhibited nuclear formation *in vitro* [144, 145], in others the removal of the bulk soluble lamins had no effect [120, 146, 147], although the nuclei formed were fragile and failed to replicate their DNA. Nuclei lacking lamins also mislocalize some INM proteins [97] and exhibit NPC clustering [112]. In cells where assembly of B-type lamins was inhibited, NE formation still occurred, but interestingly, apoptosis rates increased dramatically [140, 148]. The lack of effect on NE assembly observed in these studies is consistent with the finding that B-type lamins accumulate at the nuclear periphery after a closed NE is formed [142, 143]. It is therefore beyond question that lamins are important for nuclear integrity and function, but unlikely that they play a direct role in NE assembly per se.

Targeting proteins to the INM

A heterogeneous group of trans-membrane proteins selectively localizes to the INM. More than 20 integral proteins have been identified, largely by biochemical and proteomic approaches. For several (e.g. nurim, LUMA), almost no information beyond their sequence and localization is available. The list of INM proteins is growing, however, and a recent study identified 67 additional candidate INM proteins [149]. Potential INM proteins have also been identified by genomic analysis [150].

During interphase, new integral proteins are synthesized continuously and inserted into the rER where they can freely diffuse throughout the tubular ER network. To reach the INM the proteins have to pass the highly curved pore membrane domain of the NPC. The localization of only a few INM proteins has been studied in detail. Rapid passive diffusion through the NPC of these proteins requires that their cytoplasmic domains have a diameter of 9 nm or less [151, 152]. Much larger proteins have been proposed to reside in the INM [74], although their location is not definitively established. If they are INM proteins, it is likely that they will have to translocate actively through the NPC rather than diffusing passively (fig. 2).

Recently a ‘nuclear trap’ assay was developed to monitor the movement of integral protein fusions from the ONM to the INM using live cell fluorescence imaging [153]. This assay confirmed earlier findings about the influence of the size of the cytoplasmic domain of INM proteins on their ability to transit from the rER and outer nuclear membrane to the inner nuclear membrane. The assay was also used to demonstrate the ATP dependence of protein accumulation in the INM. Interestingly, the translocation of the reporter protein studied was blocked at temperatures of 20 °C or below, a surprisingly high temperature [153]. As for the ATP requirement, the step that is blocked at this temperature has not yet been elucidated. A link between INM-associated protein targeting and protein modification was found in a yeast genetic screen. Localization of the green fluorescent protein (GFP) tagged reporter Trm1 was dependent on its N-acetylation, which may affect interaction between Trm1 and an integral membrane protein proposed to act as a ‘receptor’ during Trm1 localization [154].

How NE proteins might regulate gene expression

The functional consequences of localization of chromatin to the NE have been analyzed in some detail. Peripheral chromatin located in the proximity of the NE has been shown to have increased nuclease sensitivity, implying that the NE might be a positive regulator of transcriptional activity [155]. This model was not supported by the finding that the majority of actively transcribed genes is not located at the nuclear periphery [156]. In fact, gene-poor chromosomes with lower levels of transcription are more likely to be associated with the NE than gene-rich chromosomes [157–159]. Findings like these have suggested that chromatin-NE interactions might in fact play a negative role in gene expression. In support of this con-

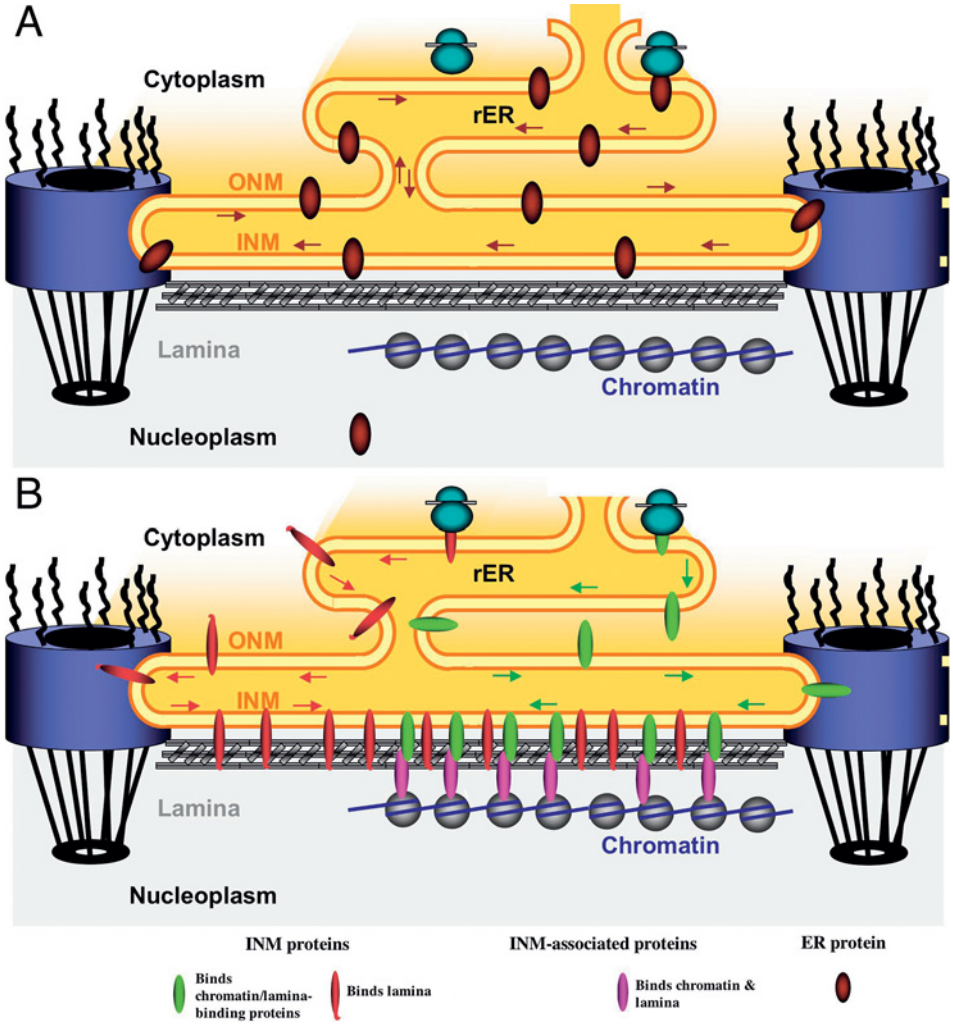

Figure 2. Targeting of proteins to the INM. INM-specific proteins are synthesized in the rER and freely diffuse within the ER and through the pore membrane domain. Proteins with cytoplasmic domains of 9 nm or less can freely diffuse through the pore membrane; larger proteins might be translocated by an energy-dependent mechanism. Subsequently, INM proteins bind to the lamin or chromatin, which leads to their retention and concentration in the INM.

cept, a reporter gene artificially tethered to the yeast NE was silenced [160].

Lamins might regulate transcription through association with transcriptional regulators. Lamin A/LAP2 complexes might, for example, stabilize repressive complexes on DNA, preventing gene activation. The retinoblastoma gene product, Rb, interacts with lamin A *in vivo*, and this interaction seems to require LAP2α [161, 162]. Since Rb binds to E2F, this interaction might influence transcription of E2F-responsive genes. LAP2 α and lamin A are not exclusively located at the nuclear periphery, and the Rb-E2F repression mechanism could therefore function independent of the NE [161, 163].

Germ cell less (GCL), a ubiquitously expressed transcription regulator, has been shown to interact with LEM domain INM proteins and localizes together with E2F-DP3 to the NE [124, 164]. GCL additionally binds this member of the E2F family and thereby represses E2F-dependent gene transcription [165–167]. GCL and LAP2β overexpression efficiently repressed transcription of E2F target genes. Mice lacking GCL show severe defects in sperm development as well as abnormal nuclear structure in their differentiated tissues [164], providing support for the importance of this NE-associated regulation.

The idea that the NE forms a repressive environment is supported by the finding that negative regulators of transcription localize to the nuclear periphery. For instance, the adipocyte differentiation factor, sterol response element binding protein 1 (SREBP1), was identified as a novel lamin A-interacting protein [168]. The cellular aging-associated transcriptional repressor Oct-1 was shown to associate with lamin B at the NE and may form a transcriptional repressive apparatus by anchoring nuclear ma-

trix attachment regions onto the nuclear lamina in the nuclear periphery [169]. Mouse BAF was also shown to be capable of decreasing the transcriptional activity of CRX, which regulates photoreceptor differentiation [170]. Finally, the NE protein MAN1 antagonizes bone morphogenic protein signaling and neural induction by interacting with the coactivator Smad1 during *Xenopus* embryogenesis [171]. These examples support the idea that the NE may generally participate in the establishment and maintenance of transcriptional repression.

How might the NE regulate chromatin structure? Likely candidates for providing a link between the NE and chromatin are LEM domain proteins (LAP2, emerin, MAN1, otefin, Bocksbeutel). These proteins interact, via their LEM domains, with the metazoan DNA- and chromatin-binding protein BAF. Depletion of BAF from *C. elegans* or *Drosophila* resulted in aberrant chromosome segregation and other phenotypes typical for impaired mitosis [172, 173]. Distortions in nuclear structure were also observed, including abnormal lamin distribution, large chromatin aggregates and lobulation of the NE, possibly resulting from impaired chromatin interaction with the NE following mitosis [172]. Overexpression of a mutant form of BAF displaces emerin, LAP2β and lamin A from reforming nuclei [126, 174]. On the basis of these results it has been proposed that BAF might recruit emerin and possibly other INM proteins to chromatin during nuclear assembly, but this is an area where careful depletion or knockout studies are needed to test models based largely on dominant negative approaches.

The NE might also participate in the overall architecture of the cell nucleus. For example, in a positioning known as the Rabl configuration, the three-dimensional localization of both telomeres and centromeres are found in close proximity to the NE at opposite sides of the nucleus. The Rabl configuration is found specifically in embryonic cells and has been analyzed in detail in Drosophila [175, 176]. However, in mammals this configuration does not exist, and the organization of the chromosomes in the nucleus varies as a function of cell type [110].

Telomeres and centromeres are organized into heterochromatin and represent chromatin regions of mainly repressed genes. In *Saccharomyces cerevisiae*, the association of telomeres with the NE is mediated by the Ku heterodimer and Sir4 [177–179]. The NPC-associated proteins Mlp1p and Mlp2p as well as the nucleoporin Nup145 have been reported to be required for telomere anchoring and the telomere position effect in yeast [180], although some of these findings are controversial [181]. The telomere position effect silences genes in the close proximity of yeast telomeres and is mediated by Sir proteins and Esc1, which binds Sir4 [177, 181]. On the basis of Sir protein overexpression and artificial NE tethering studies, it has been postulated that a high concentration of Sir proteins in the nuclear periphery leads to the establishment of a 'repressed domain' [182] analogous to that proposed on the basis of the studies in metazoa described above.

The idea of the nuclear periphery as a repressive domain cannot be generalized. It was recently shown that Sir-dependent repressed domains remain silent even when they are not located at the nuclear periphery [183]. Additionally, chromatin boundary activities, i.e. factors that bind to the so-called boundary elements which prevent the spread of repressive chromatin structures into neighboring, transcriptionally active areas, were identified in *S. cerevisiae* by genetic screening [184]. Identified factors with boundary activity included several that are involved in nucleo-cytoplasmic traffic, such as the exportins Cse1p, Mex67p and Los1p. These all exhibited robust boundary activity when artificially attached to a boundary element. The transport proteins were shown to block spreading of heterochromatin by physical tethering of chromosome sites to Nup2p, a nucleoporin [184]. This led to the proposition that association of genomic loci to the NPC can positively alter their transcriptional state [184].

Another exception is the process of activation of *INO1* (the rate-limiting enzyme of inositol synthesis), which can occur at the nuclear periphery. Artificially tethering *INO1* to the nuclear periphery is sufficient to bypass the requirement for Scs2p, which is otherwise required to relieve INO1 repression [185]. Direct association of the transcription regulation machinery with the NPC and nuclear transport factors has been analyzed in the yeast genome [186]. It was shown that many nucleoporins and karyopherins associated with highly transcribed genes, whereas other components of the nucleo-cytoplasmic transport machinery, such as Ran's nucleotide exchange factor, or RanGEF, preferentially associated with transcriptionally inactive genes [186]. This raises the interesting possibility that the organization of the genome is coupled via transcriptional state to the nuclear transport machinery. One specific case involves the highly transcribed GAL genes, which associate with certain nucleoporins [186]. In spite of these observations, the early 'gene gating' hypothesis [187], which suggested genes might be localized close to specific NPCs and that their transcripts would leave the nucleus via the adjacent NPCs, seems unlikely to be correct. There are now a few examples where the movements of single transcripts through the nucleoplasm have been followed [188, 189]. The studied transcripts behave as if they diffuse randomly away from their sites of transcription to the whole nuclear periphery.

Finally, a protein of the inner nuclear membrane has been implicated as a signaling link between the ER lumen and gene expression in the nucleoplasm. The unfolded protein response (UPR) is a cellular program initiated by the accumulation of unfolded or misfolded proteins in the ER that results in the expression of many genes, including chaperones and foldases. A critical member of this signal-

ing pathway is the inositol-requiring kinase1 (IRE1), a kinase with an endoribonuclease activity that resides in the INM [190, 191]. Ire1p was first identified in yeast, where it cleaves the mRNA of Hac1p, a transcription factor of the bZIP family, by an unconventional splicing reaction [192]. The splicing product gives raise to a potent leucin-zipper-containing transcription factor that induces the expression of genes associated with UPR. Two mammalian homologues of the yeast Ire1p had been identified and, through an analogous splicing mechanism, they regulate the activity of the transcription factor XBP1 [191, 193].

NE in disease

There have been reports of abnormalities in NE morphology in cells from various tumors [194], but the molecular cause of these irregularities remains largely obscure. However, in the last few years a variety of human diseases have been linked to mutations in components of the NE. The list of human disorders that are linked to a misfunctional NE is growing rapidly and can be roughly categorized into three groups: diseases associated with mutations in nucleoporins, proteins of the INM and lamins.

Defects in transport between the nucleus and cytoplasm have been associated with certain types of human cancer, but since this aspect was recently reviewed [30], it will not be considered further here. Translocations involving several nucleoporin genes have also been linked to human cancer. The first such chromosomal rearrangement was identified in patients with acute myelogenous leukemia (AML) and resulted in the fusion of parts of Nup98 and the homeobox transcription factor HOXA9 [195, 196]. Other examples include fusion of Nup214 and the nuclear DNA-binding protein DEK, which is also associated with AML [197, 198]. Interestingly, both Nup98 and Nup214 fusion proteins retain the FG-rich repeats that are proposed to be involved in nucleo-cytoplasmic transport and are thought to function as docking sites for transport receptors and receptor-cargo complexes [199, 200]. The FG-rich repeats in the AML-causing fusions are required for cell transformation and have been shown to bind to the transcriptional modulators CREB binding protein and p300 [201, 202]. This led to the model that the nucleoporin-derived segments of these fusions interact with various transcription factors and in this way disrupt normal transcription. A number of distinct translocation and overexpression events that involve other nucleoporins have been correlated with cancerous states [203, 204], suggesting that the ability of nucleoporins to disrupt normal gene expression, if mislocalized or misexpressed, could be rather general.

Triple A syndrome is a human autosomal recessive disorder characterized by an unusual array of tissue-specific defects. Triple A syndrome arises from mutations in a WD-repeat protein called ALADIN (also termed Adracalin or AAAS) that was shown to localize to NPCs [205]. A variety of disease-associated missense, nonsense and frameshift mutations in ALADIN caused the mutant proteins to fail to localize to NPCs. Instead, these forms of ALADIN were found predominantly in the cytoplasm. Microscopic analysis of cells from a triple A patient revealed no obvious morphological abnormalities of the nuclei, nuclear envelopes or NPCs, suggesting that defects in NPC function, rather than structure, may give rise to triple A syndrome. In any case, these findings provide a foundation for understanding the molecular basis of triple A syndrome [205, 206].

Laminopathies and INM protein-related disorders

Mutations in A-type lamins give rise to a range of rare, dominant, genetic disorders, including Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy with conduction-system disease, Dunnigan-type familial partial lipodistrophy and Hutchinson-Gilford progeria syndrome [95, 207–210]. As described above, lamins are proposed to perform a variety of nuclear functions, making it difficult to provide a simple explanation for disease-causing mutations. Insight might come from a recent study that analyzed homotypic and heterotypic binding interactions of lamins. This study suggested that the distinctive arrangement of heterotypic lamin interactions in a particular cell type might affect the stability of the overall lamin polymer [211], and could at least in part explain the tissue-specific properties of laminopathies. An examination of how lamins A/C, emerin and other integral membrane proteins interact at the INM provides the basis for a second model for how mutations that promote disease phenotypes might influence these interactions and cause cellular pathology through a combination of weakness of the lamina and altered interaction with factors that regulate gene expression [97]. Although these models for disease provide a good starting point, providing detailed explanations of laminopathies is an area where much work remains to be done.

The NE is a major intracellular target of the autoimmune response in patients with autoimmune liver disease, systemic lupus erythematosus and related conditions [212–215]. Human autoantibodies identified to date bind to specific components in three NE compartments. Targets include lamins A, B and C, the nucleoporins gp210, Nup153, Tpr and proteins of the Nup62 complex, LBR, MAN1, LAP1 and LAP2 from the INM, and the chromatin-associated BAF [212, 213, 216, 217]. Now that the proteome of the NE is expanding, other autoantibodies to components of the NE are likely to be defined [213]. These antibodies can be extremely valuable research tools, but whether they are important for pathology or can

provide insight into the etiology of these autoimmune conditions is unclear.

The X-linked form of Emery-Dreifuss muscular dystrophy (X-EDMD) was the first human disease found to result from an NE-specific defect [218, 219]. This disorder is characterized by childhood onset with progressive muscle wasting and weakening [220] and is caused by a significant reduction in, or absence, of the INM protein emerin [219]. Emerin is composed of an N-terminal nucleoplasmic domain, a single transmembrane segment and a short lumenal domain [219]. Emerin binds A- and B-type lamins and, via its LEM domain, the BAF protein [122, 127]. A clinically identical autosomal dominant form of EDMD has been mapped to the lamin A gene (at least 22 distinct disease-causing mutations have now been identified in this gene). AD-EDMD is caused by missense mutations in lamins A and C, two components of the nuclear lamina that interact directly with emerin [221]. Lamin A/C mutations also cause one form of dilated cardiomyopathy (CMD1A) and one form of limb-girdle muscular dystrophy (LGMD1B), both of which have clinical features in common with EDMD, as well as a rare, unrelated form of lipodistrophy (FPLD) [222–225]. Why only heart and skeletal muscle, and possibly connective tissue, are affected in EDMD and why expression of the disease is so extremely variable between individuals remain to be explained [219, 226]. Finally, mutations in the INM protein LBR cause the rare Pelger-Huet anomaly [227, 228].

Based on what we know about lamins, a logical hypothesis for pathology caused by defective lamins proposed that aberrant nuclear structure would lead to increased susceptibility to cellular damage by physical strain [209]. Indeed, purified hepatocyte NEs from Lmna –/– mice do exhibit increased fragility and have an increased tendency to break when compared with wild-type nuclei [97]. The notion of increased fragility is an attractive explanation for cardiac and skeletal muscle pathologies, since forces generated during muscle contraction might potentially lead to preferential breakage of nuclei containing a defective nuclear lamina. The mechanical stress model is less convincing with respect to the etiology of lipodistrophy. It seems unlikely that nuclei of adipocytes are subject to forces comparable to those encountered by nuclei in muscle cells. An alternative proposal is that interactions between the NE and chromatin might regulate tissue-specific gene expression patterns [136, 222, 229]. Transcriptional regulators (repressors and activators) have been shown to interact with lamins, and these interactions are proposed to affect chromatin organization and gene expression (see above). In Lmna –/– mice and EDMD patients changes in chromatin organization have indeed been observed [230, 231].

Mutations in MAN1 result in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis, autosomal dominant disorders characterized by increased bone density [232]. Osteopoikilosis represents skeletal dysplasia in different parts of the skeleton, Buschke-Ollendorf syndrome refers to the association of osteopoikilosis with disseminated connective-tissue nevi (hamartomas in which one or several components of the dermis is altered). Melorheostosis is a rare condition of hyperostosis of the cortical bone which affects both bone and soft tissue [232]. Recently it was shown that the nucleoplasmic, carboxyl-terminal domain of human MAN1 binds to Smad2 and Smad3 and antagonizes signaling by BMP, a transforming growth factor beta family member. BMP-induced cell proliferation arrest was also inhibited in stable cell lines overexpressing MAN1 [233]. These results suggest that MAN1 might regulate a signal transduction pathway and thus cause changes in gene expression via a mechanism that has nothing to do with the architectural roles of the protein in the NE. Here, as in all the cases described in this section, much remains to be learned, but study of these pathological conditions is sure to provide new insight into the normal functions of the NE.

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