Synthesis, transport and incorporation into the nuclear envelope of A-type lamins and inner nuclear membrane proteins

Jose M. González, Vicente Andrés

Department of Epidemiology, Atherothrombosis and Imaging, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro 3, 28029 Madrid, Spain

Corresponding authors:

Vicente Andrés

e-mail: vandres@cnic.es

Jose M. Gonzalez

e-mail: jmagonzalez@cnic.es

Phone: +34-914531200

Fax: +34-914531265

Abstract

The mammalian nuclear envelope (NE), which separates the nucleus from the cytoplasm, is a complex structure composed of nuclear pore complexes, the outer and inner nuclear membranes, the perinuclear space and the nuclear lamina (A- and B-type lamins). The NE is completely disassembled and reassembled at each cell division. Here, we review recent advances in the understanding of the mechanisms implicated in the transport of inner nuclear membrane and nuclear lamina proteins from the endoplasmic reticulum to the nucleus in interphase cells and mitosis, with special attention on A-type lamins.

The nuclear envelope. The nuclear envelope contains the nuclear pore complexes (NPCs), large multiprotein channels through which the nucleus and cytoplasm communicate, and which control the active transport of ions and small molecules. The NPCs are embedded in the outer and inner nuclear membranes, which are separated by a luminal space. The outer nuclear membrane (ONM) is continuous with the endoplasmic reticulum (ER) and is fused to the inner nuclear membrane (INM) at the sites where NPCs are inserted. Within the nucleus of metazoan cells, the INM is lined by the nuclear lamina, a meshwork of intermediate-filament proteins that is tightly associated with several INM-associated proteins and interacts with portions of the chromatin (Figure 1). The nuclear lamina provides mechanical stability to the INM and is implicated in nuclear positioning, chromatin structure, NPC organization, NE breakdown and reassembly during mitosis, DNA replication, DNA damage responses, cell cycle progression, cell differentiation, cell polarization during cell migration, and transcriptional control [1-5]

In mammals, the nuclear lamina is mainly composed of type-V intermediate filaments called lamins, which are classified as A-type or B-type based on their primary sequence and their biological properties. The *LMNA* gene, through alternative splicing, generates at least four isoforms: the two major isoforms lamin A and C (A-type lamins) and the two minor isoforms C2 and AΔ10. A-type lamins are expressed in a developmentally regulated manner, with lamin A and C being mainly expressed in differentiated cells and not in highly proliferating tissues. Of the two *LMNB* genes, *LMNB1* encodes lamin B1 and *LMNB2,* through alternative splicing, encodes lamin B2 and B3. B-type lamins are constitutively expressed in all somatic cells, with lamins B1 and B2 being expressed in most cells and lamin B3 only in spermatocytes. Lamins form coiled-coil dimers that associate longitudinally to form head-to-tail polymers [3].

Lamins A, B1, and B2 are expressed as prelamins, and are processed to mature lamins through a series of posttranslational modifications to their C-terminal end [3, 6]. The C-terminal CAAX motif is first isoprenylated by a farnesyltransferase (FTase), promoting attachment to the INM. This modification is required for the subsequent cleavage of the 3 terminal amino acids, catalyzed by the endoproteases Rce1 (Ras-converting enzyme 1) and Zmpste24 (Zinc metallopeptidase STE24 homolog, *S. cerevisiae*; also known as FACE-1 in humans: farnesylated-proteins converting enzyme). Next, isoprenylcysteine carboxyl methyltransferase (ICMT) methylates the newly accessible cysteine residue. Unlike lamin B, lamin A undergoes a

second proteolytic cleavage by Zmpste24, which removes the 15 C-terminal residues, including the farnesylated and carboxylmethylated C-terminal cysteine. Therefore mature lamin A, like lamin C (which lacks the CAAX motif), is not an isoprenylated protein. The enzymes for lamin maturation have been detected both in the ER [7] and in the INM [8], and there are conflicting views as to whether maturation takes place in the cytoplasm or the nucleus [9]. The current view is that isoprenylation facilitates the initial assembly of lamin A in the nuclear lamina, while subsequent removal of the isoprenoid moiety allows it to enter into solution, for instance during mitosis [6]. However, lamin C is found in the NE despite the fact that it lacks the CAAX motif, which would suggest that isoprenylation is not essential for NE localization [10]. Other posttranslational modifications to lamins that might regulate their import into the nucleus and their assembly and disassembly include phosphorylation, sumorylation, ADP-ribosylation and glycosylation [11].

Protein incorporation into the INM and the nuclear lamina in interphase. Four non-exclusive models have been proposed for the transport of proteins to the INM: diffusion-retention, vesicle fusion, targeting with classical nuclear localization signals (NLSs), and targeting with specific INM-sorting motifs [12] (Figure 2). The diffusion-retention model [13] suggests that integral membrane proteins synthesized in the ER diffuse throughout the ER membranes and reach the ONM. Subsequent transfer from the ONM to the INM is not fully understood, but has been proposed to operate by passive lateral diffusion at the sites of NPC insertion into the membrane [14]. In this model, INM proteins move freely between the ER and INM and become immobilized on the inner face of the NE through interactions with chromatin or the nuclear lamina [15]. Consistent with this view, fluorescence recovery after photobleaching (FRAP) experiments with fluorescent-tagged proteins demonstrated that the mobility of some LINC (linker of nucleoskeleton and cytoskeleton) proteins is increased in embryonic fibroblasts from mice lacking A-type lamins, and these experiments also suggest that A-type lamins and the INM protein Sun2 anchor nesprin-2 in the ONM [16]. Doubts about the diffusion-retention model stem from the fact that it envisages transport of proteins across four continuous membrane domains (ER, ONM, ONM–INM contacts at NPCs, and INM) by diffusion alone, which seems unlikely [12]. Moreover, the model does not explain the transport of INM proteins

with large nucleoplasmic or lumenal domains, which cannot diffuse across ONM–INM contacts, or why the presence of a lamin-binding domain in a membrane-bound protein is not sufficient for INM localization [17]. Additionally, there is evidence that protein trafficking to the INM is an energy-consuming process [12].

The fusion of vesicles to membranes is a temperature-, energy- and calcium-dependent phenomenon that occurs in ER, Golgi apparatus and plasma membrane [18]. The vesicle fusion model for protein transport to the NE is supported by studies showing that depletion of regulators of vesicle fusion, such as p47 in Xenopus oocytes, impairs NE formation [19]. However, further experiments are needed to corroborate the involvement of vesicle fusion in the incorporation of INM proteins to the NE.

A third model proposes that INM proteins are transported to their final destination by an active process regulated by the same mechanisms than regulate nuclear import of soluble proteins [20]. INM proteins contain a classical NLS that is recognized by importins and karyopherins. According to this model, importins interact with phenylalanine-glycine repeats in the NPCs and then transport INM proteins to the nuclear interior along gradients of soluble Ran-GTP/Ran-GDP created by Ran-GTPases [21-22]. This model does not explain the transport of INM proteins lacking an NLS [23] or whose NLS is dispensable for their INM localization [21]. However, recent papers suggest a role of importin-β through direct interaction with the NLS of INM proteins [24-25]

The forth model proposes active transport by signal-sequence-mediated events initiated in the ER [26-27]. Importin-α-16, a truncated form of importin-α, recognizes INM-sorting motifs in INM proteins and facilitates their transport into the nucleus [26]. These INM-sorting motifs have an extremely hydrophobic sequence of 18 aminoacids with a sequence rich in positively charged aminoacids at its C-terminus [27]. INM-sorting motifs that interact with importin-α have been detected in several INM proteins, such as lamin B receptor and nurim in mammals [26-27] and Heh2 in yeast [24]. An important role for this mechanism in the transport of INM proteins to the NE is attested by the existence of truncated importin-α isoforms in insects, yeast and mammals, and the identification of putative INM-sorting motifs in many INM proteins [12] Supporting this notion, Tapley et al. recently demonstrated that INM-sorting motifs are among multiple signals that contribute to the efficient transport of INM proteins to the NE; the authors found that the

SUN domain protein UNC-84 is transported to the NE during early embryogenesis in *Caenorhabditis elegans* by processes mediated by classical NLSs and INM-sorting motifs [12]. Additionally, recent studies combining FRAP and photoactivation in both the ER and the NE suggest that the binding of many NE transmembrane proteins to the INM depends more on exchange of proteins between the ER and INM than on mobility within the INM [28]. These studies also corroborate the existence of several mechanisms in NE protein dynamics, including unregulated lateral diffusion, mechanisms requiring ATP or Ran GTPase function or facilitation of protein transport by the phenylalanine-glycine repeats in NPCs [28].

Incorporation of A-type lamins into the nucleus. Unlike other intermediate filaments, lamins accumulate exclusively in the nucleus during interphase. All lamins have classicall NLSs and are predicted to be transported to the nucleus by importin α/β followed by internalization across the NPCs. Within the nucleus, lamins associate with the INM, where they form the nuclear lamina [10, 29]. The import of lamins into the nucleus seems to be regulated by the phosphorylation of serine and threonine residues close to the NLS [30-31]. In interphase cells, lamins are distributed in the perinuclear rim and nucleoplasmic areas [3]. Analysis by FRAP and fluorescence loss in photobleaching (FLIP) using GFP-tagged proteins indicates low turnover of of A- and B-type lamins in the nuclear periphery, with recovery within hours for lamin A and 45 minutes for lamin B1 [3]. In contrast, lamin C is more mobile [32]. Although these experiments corroborate earlier biochemical studies [33], it is unknown exactly how A-type lamins incorporate into the nuclear lamina and how this process is influenced by other INM and trafficking members. A- and B-type lamins form separate networks in the nuclear lamina [34-35]. Interaction between these two structures has been confirmed by fluorescence resonance energy transfer (FRET) and live cell imaging studies [34, 36] and in experiments with *LMNA*-null cells and mice with an insertional mutation in *LMNB1* that results in the synthesis of a lamin B1 protein lacking several key functional domains [37-38]. Mouse embryonic fibroblasts lacking lamin A/C exhibit reduced expression of B-type lamins in the nuclear lamina, and the expression of the mutant *LMNB1* altered lamin A/C distribution [37-38]. Accordingly, lamin A mobility in the nuclear lamina is increased when the amount of lamin B1 is reduced [39]. Lamins are also organized into intranuclear tubules and aggregates that contain lipids and NPC proteins, and

which could serve as transport channels from cytoplasmic regions [3]. These channels are stable and lamins in these regions have a low turnover similar to that of lamins in the perinuclear rim. Finally, polymerized lamins are dispersed throughout the nucleus from the nucleolar periphery to the NE, frequently connecting with NPCs [3]. Polymerized lamins may provide a scaffold for processes such as transcription and DNA replication [3].

The nuclear envelope in mitosis. The NE is completely disassembled and reassembled during cell division. Higher eukaryotes form a cytoplasmic spindle, which requires NE breakdown to allow access of spindle microtubules to kinetochores. Restructuring of the NE during breakdown involves the disassembly of all its components (including membranes, NPCs and nuclear lamina), chromatin condensation, formation of the microtubule aster from centrosomes, and centrosome separation. All the nuclear components are then coordinately reassembled after DNA segregation into daughter cells [40-41] (Figure 3).

Activation of mitotic kinases at the end of G2 triggers entry into prophase through the phosphorylation of nucleoporins, lamins, INM proteins and chromatin-associated factors. The phosphorylation of nucleoporins by cyclin-dependent kinase 1 (CDK1, also called CDC2) controls NPC disassembly. Nucleoporins are dispersed in the cytoplasm within minutes, and transport between the cytoplasm and nucleus through NPCs is inhibited [42-43]. Microtubules then exert pulling forces that cause invaginations of the NE around centrosomes [44], and the nuclear lamina undergoes depolymerization [45]; firstly A-type lamins are released to the nucleoplasm [46], followed by B-type lamins [44]. CDK1 contributes to lamin depolymerisation in vitro [47], and mutagenesis of the CDK1 phosophorylating sites in lamin A/C blocks nuclear lamina disassembly at the onset of mitosis [48]. Simultaneous with nuclear lamina depolymerization, CDK1 phosphorylation of INM proteins and other lamin-associated proteins, including lamina-associated polypeptide 2α and 2β (LAP2α, LAP2β) and lamin B receptor LBR), causes their detachment from lamins and chromatin [40, 44]. NE breakdown also involves contributions from protein kinase C (PKC), aurora A, cyclin A2/CDK complex polo-like kinase 1 (PlK1) and never-in-mitosis A (NIMA) [40-41].

After NE breakdown, NE membrane proteins retract into the membrane system of the ER [49]. A-type lamins become dispersed throughout the cytoplasm, while B-type lamins initially remain associated with the nuclear membranes before being dispersed into the ER [45]. The ER is also reorganized during mitosis, and this reorganization seems to be related to NE disassembly, since NE breakdown is impaired by disruption of key ER proteins such as reticulons and the DP1 family members YOP1 and RET1 [50-51]. Chromatin is condensed into chromosomes before NE breakdown. During spindle assembly microtubules are reorganized and interact with chromosomes, aligning them on the metaphase plate [40-41].

From late anaphase to telophase, the NE starts to reassemble around chromatin, a process controlled spatially and temporally by the production of RanGTP on the surface of the chromatin. The production of RanGTP together with the inactivation of kinases and activation of phosphatases (e.g. protein phosphatase 1-a (PP1a)) release NPC components and importins [52] and recruit some nucleoporins to form the prepore [53]. Thereafter membranes and other soluble nucleoporins associate with the prepore to form a closed NE that exhibits nuclear import activity.

NE membrane reformation starts with the binding of the tips of the ER tubules to chromatin [54], a process mediated by NDC1, POM121 and Sun1 and the complex Nup107-160. Removal of the reticulon proteins leads to additional recruitment of ER tubules to form flattened NE patches [55]. Some NE proteins, including LBR, LAP2β, and B- and A- type lamins, then bind to chromatin [56], although the majority of lamins are not reassembled into the nuclear lamina until after the nucleus has recovered competence for nuclear import [11].

The disassembly of A-type and B-type lamins at the transition from prophase to prometaphase requires their CDK1- and PKC-dependent phosphorylation on specific serine residues [40, 57], whereas their reassembly during the telophase/early G1 transition is controlled by PP1a [45, 48]. Mitotic phosphorylation and disassembly of A-type lamins precedes B-type lamin phosphorylation and disassembly [46, 57]. Nuclear lamina reassembly begins with the association with the chromosome ends of LAP2α and BAF, followed by LBR and a small fraction of emerin, and then by LAP2β [11]. Accumulation of A-type lamins within the nucleus mostly occurs after the assembly of other major NE components, including NPCs, in daughter cells in late telophase [56, 58]. Remaining A-type lamins are transported into the nucleus after

enclosure of the chromatin and formation of an intact NE. Newly-synthesized lamins are transported through NPCs and continue to be incorporated into the NE of the interphase nucleus [59]. Other elements begin to assemble in daughter cells after the formation of an intact NE [56, 58]. It is clear that the majority of A-type lamin molecules only reassemble during and after cytokinesis, and that lamins also regulate nuclear assembly [11]. Further experiments are needed to increase knowledge about the mechanisms regulating the assembly of A-type lamins in cells.

Conclusions. During the cell cycle, the NE is partially or completely remodeled in perfect coordination with the changes occurring simultaneously in chromatin and the ER. These processes are regulated by phosphorylation events that are still incompletely understood. Further efforts are needed to identify additional proteins that participate in NE dynamics (such as kinases, phosphatases, and other regulatory or trafficking proteins) and to determine how they interact to control NE formation and turnover. Given that *LMNA* mutations or defective posttranslational processing of pre-lamin A cause the human diseases termed laminopathies, further work is also needed to assess if the etiology of these diseases is related to alterations to NE reorganization as a consequence of the expression of mutant lamins or lamin-associated proteins.

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Abbreviations.

CDK1: cyclin-dependent kinase 1

ER: endoplasmic reticulum

FRAP: fluorescence recovery after photobleaching

FRET: fluorescence resonance energy transfer

INM: inner nuclear membrane

NE: nuclear envelope.

NPC: nuclear pore complex

NLS: nuclear localization signal

LBR: Lamin B receptor.

LAP2: Lamina-associated polypeptide.

ONM: outer nuclear membrane

PKC: protein kinase C

PP1a: protein phosphatase 1-a

Key words

A-type lamins, nuclear envelope, inner nuclear membrane proteins, nuclear lamina, nuclear envelope assembly and disassembly, nuclear envelope turnover.

Figure legends

Figure 1. Schematic representation of the structure of the mammalian nuclear envelope and endoplasmic reticulum (ER).

Figure 2. Incorporation of transmembrane proteins into the inner nuclear membrane in interphase. Four non-exclusive models have been proposed for the transport of proteins to the INM: (A) diffusion-retention, (B) vesicle fusion, (C) targeting with classical nuclear localization signals (NLSs), and (D) targeting with specific INM-sorting motifs. Green rectangles: INM proteins. RCC1: Regulator of Chromosome condensation 1; RanBP1 and 2: Ran-binding protein 1 and 2; RanGAP: Ran GTPase-activating protein; α-16: importin α-16; α: importin α; β: importin β. INM-SM: Inner Nuclear Membrane Sorting Motif. See text for details.

Figure 3. Scheme of the events associated with nuclear envelope disassembly and assembly during mitosis. ER: Endoplasmic Reticulum; INM: Inner Nuclear Membrane; NE: Nuclear envelope; NPC: Nucleopore complexes; ONM: Outer Nuclear Membrane. See text for details.

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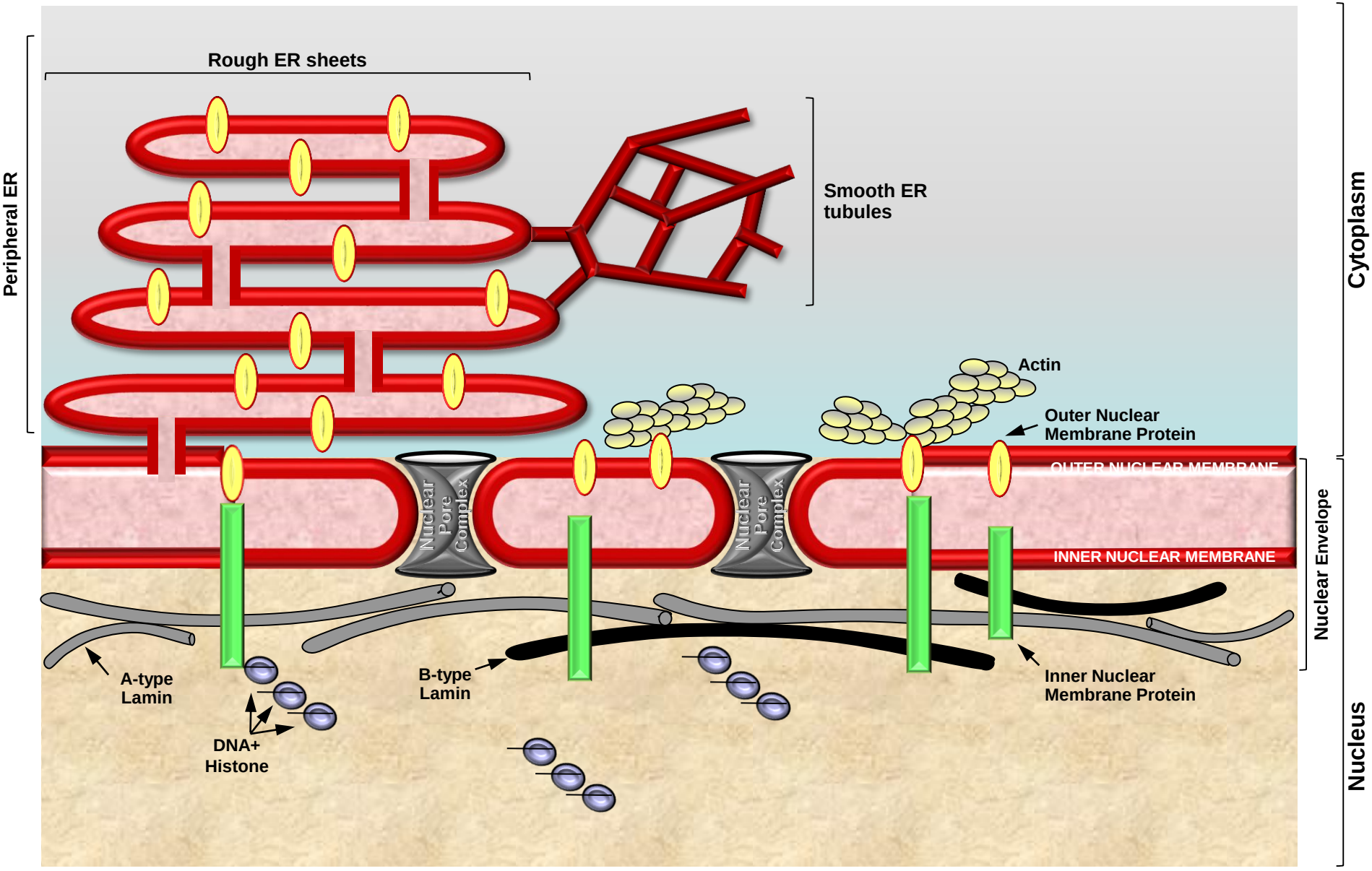
Figure 1


Rough ER sheets
Peripheral ER
Smooth ER tubules
Cytoplasm
Actin
Outer Nuclear Membrane Protein
OUTER NUCLEAR MEMBRANE
Nuclear Pore Complex
Nuclear Pore Complex
INNER NUCLEAR MEMBRANE
Nuclear Envelope
A-type Lamin
B-type Lamin
Inner Nuclear Membrane Protein
DNA+ Histone
Nucleus

Figure

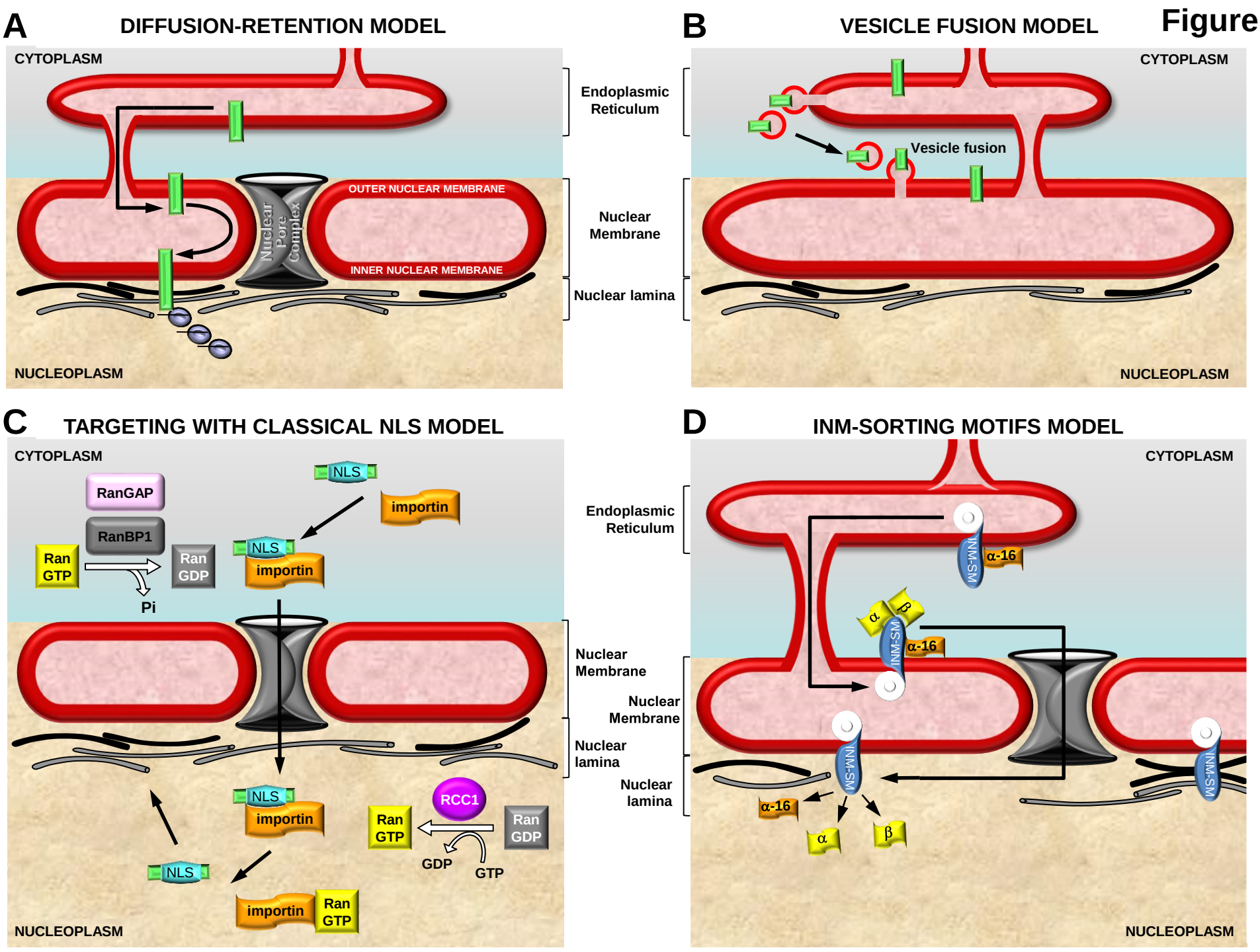

A
DIFFUSION-RETENTION MODEL
CYTOPLASM
OUTER NUCLEAR MEMBRANE
Nuclear Pore Complex
INNER NUCLEAR MEMBRANE
NUCLEOPLASM
Endoplasmic Reticulum
Nuclear Membrane
Nuclear lamina
B
VESICLE FUSION MODEL
CYTOPLASM
Vesicle fusion
NUCLEOPLASM
C
TARGETING WITH CLASSICAL NLS MODEL
CYTOPLASM
RanGAP
RanBP1
Ran GTP
Ran GDP
Pi
NLS
importin
NUCLEOPLASM
RCC1
GDP
GTP
D
INM-SORTING MOTIFS MODEL
CYTOPLASM
INM-SM
α-16
α
β
NUCLEOPLASM
Endoplasmic Reticulum
Nuclear Membrane
Nuclear Membrane
Nuclear lamina
Nuclear lamina

Figure 3

- NPC disassembly
- NPC dispersion in the cytoplasm
- NE Breakdown
- INM/ONM dispersal
- Nuclear lamina depolymerization
- INM proteins and lamin-associated protein detachment from chromatin and lamins
- ER reorganization

- Mitotic spindle is established
- Nups at the kinetochores
- Lamin B dispersed into ER
- Lamin A dispersed in the cytoplasm

- Formation of prepores
- Recruitment to the chromatin surface of RE membrane tubules and NE proteins

- NE flattening
- Membrane closure
- Completed NPC assembly and nuclear transport reestablishment
- Lamin import
- Lamina assembly