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Regulation and coordination of nuclear envelope and nuclear pore complex assembly

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Abbreviations: AKAP, a-kinase anchoring protein; BAF, barrier-to-autointegration factor; CDK, cyclin dependent kinase; CPC, chromosomal passenger complex; ER, endoplasmic reticulum; FG-repeats, phenylalanine-glycine repeats; HP1, heterochromatin binding protein; HEM, hydrops-ectopic calcification-"moth-eaten"/Greenberg skeletal dysplasia; GAP, GTPase-activating protein; GEF, GTP-exchange factor; GTP/GDP, guanosine triphosphate/diphosphate; INCENP, inner centromere protein; INM, inner nuclear envelope; KASH domain, named after proteins Klarsicht, Anc-1, Syne homology; Lap, lamin associated polypeptide; LBR, lamin B binding protein; LEM domain, named after INM proteins Lap2, emerin, Man1; LINC, linker of nucleoskeleton and cytoskeleton complex; MKLP1, motor-kinesin like protein 1; NE, nuclear envelope; NLS, nuclear localization signal; NPC, nuclear pore complex; Nup, nucleoporin; ONM, outer nuclear envelope; PHA, Pelger-Huët anomaly; Pom, pore membrane protein; PP2A, PP1, protein phosphatase 2A, protein phosphatase 1; SR-specific kinase, serine/arginine-rich specific kinase; SUN domain, named after proteins Sad1, UNC-84; TM7SF2/DHCR1, transmembrane 7 superfamily member 2/dehydro-cholesterol reductase1; VRK-1, vaccinia-related kinase-1

In metazoans with "open" mitosis, cells undergo structural changes involving the complete disassembly of the nuclear envelope (NE). In post-mitosis, the dividing cell faces the difficulty to reassemble NE structures in a highly regulated fashion around separated chromosomes. The de novo formation of nuclear pore complexes (NPCs), which are gateways between the cytoplasm and nucleoplasm across the nuclear membrane, is an archetype of macromolecular assembly and is therefore of special interest. The reformation of a functional NE further involves the reassembly and organization of other NE components, the nuclear membrane and NE proteins, around chromosomes in late mitosis.

Here, we discuss the function of NE components, such as lamins and INM proteins, in NE reformation and highlight recent results on coordination of NPC and NE assembly.

Introduction

An overview of the nuclear envelope (NE). The nuclear envelope (NE) is the physical barrier between the cytoplasm and nucleoplasm. The NE is composed of two lipid bilayer membranes: the outer nuclear membrane (ONM), which is continuous with the endoplasmic reticulum (ER), and the inner nuclear membrane (INM), which contains a specific subset of more than 60 INM or NE transmembrane proteins.[1] The ONM and the INM, which are ~20–40 nm apart,[2] fuse at places where the nuclear pore complex (NPC) is embedded. NPCs provide an aqueous channel for molecular exchange between the cytoplasm and nucleoplasm. The NPC is a large structure (60–125 MDa) with octahedral symmetry assembled by multicopies of ~30 different proteins called nucleoporins or Nups.[3,4]

Small molecules below ~30 kDa freely diffuse through the NPC, while larger molecules cannot pass the permeability barrier of the NPC, established by hydrophobic phenylalanine-glycine (FG) repeats of Nups (FG-Nups).[5] For passage of larger molecules through the NPC, they must bind to nuclear transport carriers, such as importin β family members, p10/NTF2, TAP/NFX or Hikeshi that interact with FG-Nups and possess ability to translocate through NPCs (for reviews, see refs. 6–8).

The small GTPase Ran plays key roles in determining the directionality of nuclear transport, mediated by importin β family members, by regulating cargo binding and release.[6,7] The GDP form of Ran is converted into the GTP form within the nucleus by chromatin bound Ran GTP-exchange factor (GEF) RCC1. The GTP form of Ran is converted into the GDP form by the Ran GTPase-activating protein (GAP) RanGAP1 in the cytoplasm, thereby creating a steep RanGTP concentration gradient at the boundary of the NE. For the nuclear import, importins bind to their cargoes in the cytoplasm where RanGTP concentration is low, but dissociate from the bound cargoes within the nucleus when RanGTP bind to importins. The nuclear export occurs in the opposite manner: exportins bind cargoes in the presence of RanGTP and forms a trimeric complex, but release them when RanGTP is converted into RanGDP.

In multicellular organisms, the nuclear lamina, a meshwork of type V intermediate filaments, A-type and B-type lamins, lies

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beneath the INM and maintains the nuclear shape and structure.[9,10] Moreover, the nuclear lamina stays in contact with the cytoskeleton through its interaction with the highly conserved linker of nucleoskeleton and cytoskeleton (LINC) complex (see refs. 11–13). The LINC complex consists of KASH (Klarsicht, Anc-1 and Syne homology) and Sad1 and UNC-84 (SUN) proteins, which are membrane spanning proteins that localize at the ONM and INM, respectively. Sun proteins form dimers through their coiled-coiled domain and bind KASH proteins in the perinuclear lumen via the SUN domain at their C-terminus.

At the ONM, KASH proteins interact either with actin filaments,[13] microtubules,[14] intermediate filaments[15] or centrosomes[16] via their cytoplasmic tail.

Besides the structural role, the nuclear lamina functions in gene stability and chromatin organization, which is associated with various human diseases, collectively called laminopathies.[17-19]

The fate of the nuclear envelope in mitosis. In the course of "open" mitosis, the NE disassembles, also referred to as nuclear envelope breakdown, and follows the fate described below.

In prophase, the permeability of the NE initially increases as NPCs disassemble in a stepwise manner upon phosphorylation by mitotic kinases such as CDK1 and Neks.[20] The nuclear membrane is then partially ruptured in a process assisted by microtubule-motor proteins, dynein and its regulator dynactin[21] and finally becomes completely retracted into the mitotic ER.[22]

Upon disassembly of the NE, the nuclear barrier dissolves and nuclear transport is annihilated. However, RanGTP gradient continues to exist around mitotic chromosomes through the action of chromatin-bound RCC1. By this way, nuclear localization signal (NLS)-containing cargoes can be recruited and released at the vicinity of mitotic chromosomes, where the concentration of RanGTP is high.[23-26] Moreover, mitotic chromosomes establish a spatial environment for phosphorylation gradients, e.g., by scaffolding Aurora B kinase,[27] which is the main activity of the chromosomal passenger complex (CPC) and is important for spindle assembly checkpoint and cytokinesis.[28]

During metaphase, most NE components are either dissolved or membrane-bound until reformation of the NE in anaphase. It was recently shown that lamin B and Nups also have important, mitotic functions.[29,30]

In late anaphase, NE reformation takes place on mitotic chromosomes, which serve as assembly platform for the highly controlled de novo assembly of NPCs in space and time and the reconstruction of the nuclear lamina as well as of the nuclear membrane, originating from the mitotic ER. Further, the compaction and structure of chromatin (condensed/decondensed) regulates binding of NE proteins and supports reformation of a functional NE.[31]

Nups, lamins and some INM proteins localize on sister chromosomes in a mutually related manner, indicating that NPC assembly and reconstruction of other NE structures involving INM proteins and lamins are coordinated. However, the molecular basis for such coordination is still very poorly understood.

Recruitment of Nuclear Membrane Proteins and Lamins at the End of Mitosis

The role of lamins in NE reformation. In mammals, one class of lamins, the A-type lamins, is derived from LMNA gene locus, which expresses the two splicing variants lamin A and lamin C. The other class of lamins is called B-type lamins, including three different proteins. Lamin B1 is encoded by the gene locus of LMNB1, and lamin B2 and B3 (B3 is only expressed in testis) are encoded by LMNB2 (reviewed by refs. 9, 10, 32 and 33). At least one form of B-type lamin is present in each cell, while expression of A-type lamins is tissue specific and regulated during development. A- and B-type lamins share the same structural elements such as a α-helical rod domain and several IgG-folds but are processed differently. Mature B-type lamins are carboxymethylated and farnesylated at the C-terminus, which favors attachment of B-type lamins to lipid membranes. Precursors of A-type lamins are modified in the same way but the last 15 amino acids are cleaved by the metalloprotease Zmpste24/FACE1, which renders mature A-type lamins membrane-unattached.[10] Various human diseases like Emery-Dreifuss muscular dystrophy are related to A-type lamins and its interaction partners, but only a few diseases are known, which derive from B-type lamins.[18,19]

Early experiments tested the function of lamins during NE reformation in vitro with *Xenopus laevis* and *Drosophila* embryo extracts. *X. laevis* has three different B-type lamins (B1–3),[34] whereby lamin B3 is the most abundant one. *Drosophila melanogaster* expresses only one B-type lamin (lamin Dm0). Immunodepletion of B-type lamins from *Drosophila* embryo extracts inhibited vesicle attachment to chromatin[35] and chromatin decondensation in nuclear assembly assays in *Xenopus* egg extracts.[36] Further immunodepletion of *Xenopus* lamin B3 gave rise to fragile and small nuclei yet with a sealed nuclear membrane,[37] which were devoid of a lamina and lost their ability for DNA replication.[38] In an alternative approach, Lopez-Soler and colleagues used a C-terminal peptide of *Xenopus* lamin B3 that bound and inhibited the function of endogenous lamin B3 and disturbed nuclear membrane targeting to chromatin, NPC formation and lamin polymerization in vitro.[39] Apparent contradictions of lamin B depletion in *Xenopus* or *Drosophila* extracts could be a result of cross-reactivity of the lamin antibodies, unspecific interactions of the lamin peptide or the inefficient removal of lamin B3 from extracts. In total, these results demonstrated a role of B-type lamin in establishment of a functional nucleus. In mammalian cells, recruitment of B-type lamin to mitotic chromosomes is critical for cell survival.[40,41] However, it was reported, that B-type lamin is essential for organogenesis but not for differentiation of embryonic stem cells.[42] Accumulating evidences indicate that lamins have a significant, physiological role during development in various organisms,[43-45] as well as in senescence,[46] which might be due to their function in the maintenance of the nuclear morphology and the interaction network with other proteins at the NE that involves signaling pathways.

During NE reformation, recruitment of lamin B to mitotic chromosomes requires dephosphorylation by PP1. Steen et al.[41] identified the PP1-substrate specifier AKAP (A-kinase anchoring protein) 149 as an integral membrane protein of the NE and ER

membrane. In HeLa cells, when association of AKAP 149 with PP1 was inhibited, localization of B-type lamin was abolished at the end of mitosis, while it had no effect on NE localization of A-type lamin or INM proteins such as emerin and LBR. The different effect of AKAP149 on localization of B-type lamin and A-type lamin suggests that their way of assembly at end of mitosis is not interdependent and is regulated differently. Live imaging and immunofluorescent staining demonstrated that reformation of the nuclear lamina in mammalian cells occurs later than recruitment of other INM proteins like emerin, Lap2α, LBR or transmembrane Nups such as Pom121,[37,47-49,59] demonstrating chromosome recruitment of some INM proteins does not depend on the assembled lamina.

INM proteins in reformation of a functional nuclear membrane. Many INM proteins can bind either or both of A- or B-type lamins.[50] In addition, recruitment of INM proteins is involved in the attachment of the ER to mitotic chromosomes.[22,51-53] INM proteins, which have been studied in relation to their function in NE formation, are described below.

LBR. One of the best-studied INM proteins is the lamin B receptor (p58/LBR), which was initially identified from avian erythrocytes as lamin-binding protein.[54] The N-terminus of human LBR extends into the nucleoplasm, whereas the C-terminal region of LBR contains eight predicted transmembrane segments with homology to the sterol reductase ERG24 (human TM7SF2/DHCR1).[55] The N-terminus interacts with chromatin,[56,57] chromatin-binding proteins like heterochromatin binding protein (HP1), HA95, histone H3/4, the nuclear transport receptor importin β[57] and the nucleoporins Pom121[58] and ELYS/Mel28.[59]

There are two rare human diseases related with LBR: hydrops-ectopic calcification-"moth-eaten" (HEM)/Greenberg skeletal dysplasia and Pelger-Huët anomaly (PHA). The PHA syndrome, a hematological condition, induces hypolobulation of nuclei in granulocytes and detachment of heterochromatin from the NE.[60] HEM leads to abnormalities in skeletal growth and displays parallels to a deficiency of 3β-hydroxysterol Δ(C-14) reductase, which implies that LBR has a role in cholesterol metabolism.[61] Overexpression of *Xenopus* LBR in HeLa cells caused membrane overproduction and stack formation, suggesting a role of LBR in membrane growth.[62] Whether LBR functions as sterolreductase is unclear and awaits in-depth investigation.

Multiple studies support the relevance of LBR for NE formation. LBR is required in vitro for nuclear membrane assembly in sea urchin (sea urchin homolog p56),[63] rat hepatocyte or turkey erythrocyte extracts,[64] and in *Xenopus* egg extract, depending on importin β and RanGTP.[62] Depletion of hLBR in HeLa cells increased apoptotic marker caspase-3 in G1-phase[65] and delayed NE formation in vivo.[52] It was suggested that LBR plays a redundant function with other INM proteins (see next section).

LBR is phosphorylated by several kinases such as a SR-specific kinase and cdc2.[66] Tseng et al.[67] demonstrated that the mitotic phosphorylation of hLBR on residue S71 and S86 by CDK1 hindered the premature attachment of the LBR-associated nuclear membrane to chromatin, from which they concluded that mitotic phosphorylation of hLBR is important for temporal control of NE assembly.

LEM domain-containing proteins. Another group of INM proteins, which interacts with lamins and is potentially involved in NE formation, are LEM (named after INM proteins Lap2, emerin, Man1) domain containing proteins. The LEM domain, formed by 40 aa of two parallel α-helices,[68,69] is shared by non-related INM proteins, including emerin, isoforms of lamina-associated polypeptide (Lap)2 (α, β, δ, ε, γ, ζ), Man1, Lem2, 3 and 4 and yet uncharacterized Lem5.[70] The LEM domain is specifically recognized by the protein Barrier-to-Autointegration Factor (BAF). BAF, an A-type Lamin binding protein, forms a dimer and displays DNA-looping activity, necessary for chromatin organization.[71-73] The role of BAF in chromatin decondensation supports proper NE assembly.[74-76] The chromatin-binding affinity of BAF is decreased after phosphorylation by VRK-1 kinase upon mitotic entry. Recently, it was reported that *C. elegans* protein LEM4-L and the human ortholog Lem4 inhibited the phosphorylation activity of VRK-1 in vitro and interacted with PP2A[77] indicating that Lem4 regulates the BAF kinase and the phosphatase that controls the function of BAF in chromatin binding and NE reformation.

In U2OS cells, RNAi-induced depletion of the LBR, Lap2β, MAN1, BAF and the transmembrane Nups Ndc1 and Pom121 delayed reformation of a transport competent NE. Double depletion of the LBR and Lap2β enhanced the delay of NE formation, whereas exogenously expressed LBR or BAF rescued NE formation in Lap2β-depleted cells. Further, overexpression of each INM protein caused accelerated NE formation in contrast to overexpression of the ONM protein nesprin-3a,[52] indicating that INM proteins and their interacting partners such as BAF have a redundant role in nuclear membrane recruitment.

Contribution of Nucleoporins to NE Formation

NPC structure. The basic molecular architecture of the NPC is well conserved among eukaryotes.[78-80] Several Nups behave as subcomplexes throughout the cell cycle. Nups are divided into four groups based on their structural roles in the NPC structure (**Fig. 1**): transmembrane Nups, scaffold Nups, central Nups and peripheral Nups. In vertebrates, three transmembrane Nups, Pom121, NDC1 and gp210, exist, which are considered to link the scaffold structure with the pore membrane. Scaffold Nups, consisting Nup107–160 and Nup93–205 subcomplexes, build up the three ring-shaped structure for the basic body of the NPC. The Nup107–160 subcomplex forms the nucleoplasmic and cytoplasmic rings, which sandwich the central ring (also known as spoke ring), composed of the Nup93–205 subcomplex. The central transport channel, enclosed by the spoke ring, is assembled by the central Nups (Nup62, Nup58 and Nup54).[81] About one third of nucleoporins, including the central Nups, possess unstructured (or disordered) phenylalanine-glycine-repeats, reaching into the central transport channel of the NPC to form the permeability barrier.[82,83] Peripheral Nups organize into cytoplasmic filaments and the nuclear basket, but their exact composition is unclear and several associated factors exist at the cytoplasmic and

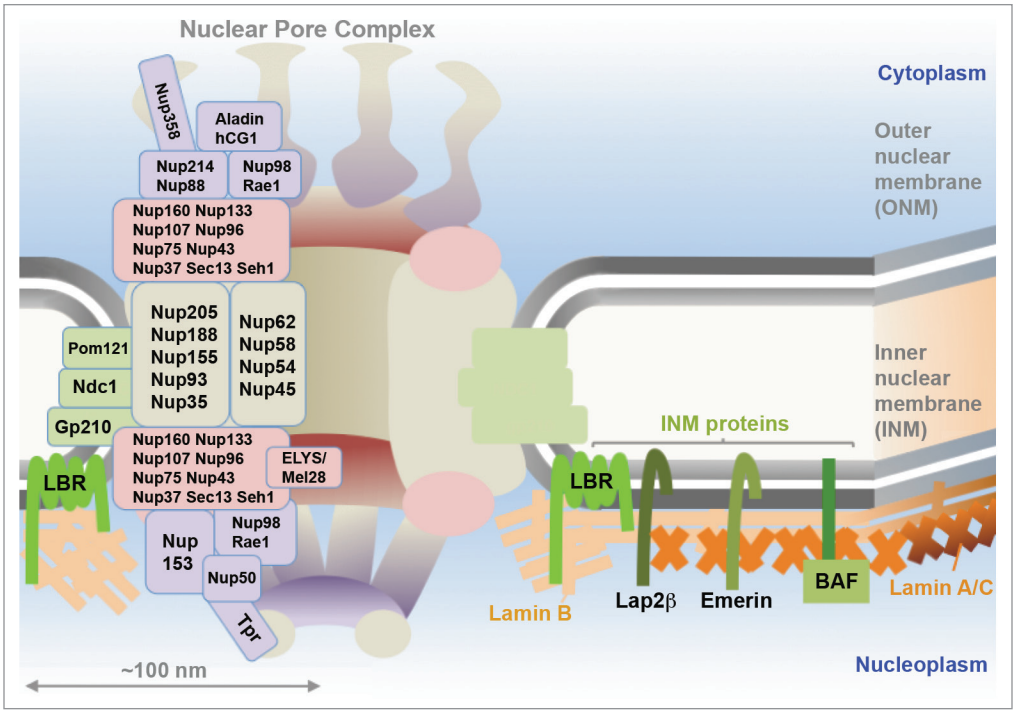

Figure 1. Structure of the metazoan nuclear envelope (NE). Scheme of the NE. A nuclear pore complex (NPC) is inserted into the NE at places where inner nuclear membrane (INM) and outer nuclear membrane (ONM) fuse. The body of NPC consists of scaffold Nups forming three ring-like structures; cytoplasmic ring (red, cytoplasmic side: Nup107–160 complex), central ring (beige, Nup93–205 complex) and nuclear ring (red, nucleoplasmic side: Nup107–160 complex). The permeability barrier is established by central Nups (Nup62 complex). Peripheral Nups (purple) constitute the cytoplasmic fibrils and the nuclear basket. In vertebrates, there are three integral membrane pore membrane Nups (green), which are thought to anchor the NPC scaffold to the NE. Each block indicates a subcomplex of Nups. The ONM is continuous with the endoplasmic reticulum (ER), whereas the INM contains a distinct subset of INM proteins (green) that interact with the filamentous meshwork of A-type (beige) and B-type lamins (orange) beneath the INM.

nucleoplasmic side.[84,85] The permeability barrier and peripheral Nups are important for the function of the NPC in exchanging macromolecules.[86]

Two different models of NPC assembly in interphase and in mitosis. In metazoans, NPCs are assembled twice during cell cycle: during interphase and in postmitosis.[87-90] It is proposed that interphase NPC formation has different preconditions from postmitotic NPC formation: it requires insertion of nucleoporins into sealed double nuclear membranes from both sides.[91] For this, ONM and INM must fuse[92] and the underlying nuclear lamina must locally restructure. Furthermore, the CDK activity is necessary for NPCs assembly during interphase, but not in post-mitosis.[93] Recently, it was reported that Pom121 plays a critical role for early steps of interphase NPC assembly.[58,94,95]

Postmitotic NPC assembly occurs in a highly temporally and spatially ordered manner.[96,97] There are two favored models. The pre-NPC model suggests that formation of a pre-pore structure takes place on chromatin prior to membrane attachment and fusion.[97-99] In contrast, the NPC insertion model expects that NPCs are assembled into pre-existing double membranes during postmitosis,[100] which is comparable to interphase NPC formation.

In line with the pre-NPC model, it was proposed that ELYS/Mel28 is the very first Nup, which targets mitotic chromosomes via its chromatin-binding, AT-hook domain.[101] ELYS/Mel28 recruits the subcomplex Nup107–160, followed by the transmembrane nucleoporin Pom121[101] that interacts with the Nup93–205 complex and Nup107–160 complex.[102] Some members of the Nup107–160 complex such as Nup133 contain an ALPS motif, which senses and assists binding to membrane curvature.[103]

The vertebrate nucleoporin ELYS/Mel28, was identified as putative transcription factor during embryogenesis in mouse.[104-106] Evidences accumulated, pointing out that the major function of ELYS/Mel28 in metazoans lies at the NPC. Depletion of ELYS/Mel28 blocks postmitotic NPC formation.[107-110] ELYS/Mel28 directly interacts with the Nup107–160 complex, which forms the body of NPCs and is essential for NPC formation.[99] It was recently demonstrated that ELYS/Mel28 and the Nup107–160 complex are involved in regulation of the CPC,[111] composed of Survivin, Borealin, INCENP and Aurora B kinase. Depletion of Seh1, a component of the Nup107–160 complex, which is responsible for targeting the Nup107–160 complex to kinetochores, caused mislocalization of Survivin and Aurora B in mitosis and induced reduction of the active kinesin MKLP1, which is an Aurora B substrate and necessary for cytokinesis.[111] Similarly, depletion of ELYS/Mel28 increased the number of cells with unresolved midbody structures.[108] However, mechanisms of regulation of the CPC through ELYS/Mel28 and the Nup107–160 complex are unknown. Interestingly, depletion of the nuclear basket components Nup153 and Nup50 induced a delay in the Aurora B-mediated abscission checkpoint.[112] Together, these

evidences indicate a connection between postmitotic NPC formation and cytokinesis through Aurora B regulation.

NE reformation through ELYS/Mel28 and other Nups. Beside the primary role of ELYS/Mel28 in postmitotic NPC formation, a function in NE formation was reported for *C. elegans* embryos, where reduction of MEL-28 inhibited formation of a functional NE, which had neither NPCs nor a lamina and was only partially sealed.[107,113] In comparison, in vitro assembled nuclei from *Xenopus* egg extracts depleted of MEL-28 were smaller and devoid of NPCs but enclosed by a thin nuclear membrane.[109] Interestingly, in HeLa cells, reduction of ELYS/Mel28 disturbed the recruitment and distribution of INM proteins.[59] While LBR was dispersed upon ELYS/Mel28 depletion, core binding INM proteins such as emerin, BAF and Lap2α still targeted to chromosomes but were affected in their accumulation at the core region, implying a role for ELYS/Mel28 in the overall regulation of the NE architecture during postmitotic assembly.[59]

Other nucleoporins, which have an essential role in NE formation, are components of the Nup93–205 complex, Nup155[114,115] and Nup53[116-118] as well as the transmembrane pore proteins Ndc1 and Pom121.[102,119,120] In Nup53-depleted *Xenopus* extracts, NE formation was completely inhibited.[118] A truncated Nup53, which binds Nup155, in *X. laevis*, can restore NE formation in Nup53-depleted extracts.[117] Comparably, in *C. elegans*, a mutated Nup53, where the mutation affects the Nup155-interacting region, demonstrated a similar defect in NE formation.[116] These results indicated that the interaction of Nup53 and Nup155 is necessary for successful NE formation in *C. elegans* and *X. laevis*.[116,118] Moreover, Nup155 knockout is lethal in mouse and dysfunctional Nup155 is linked to a cardiovascular disease, on the cellular level basing on decreased nuclear permeability and impaired nuclear transport,[121] pointing out the significance of Nup155 for a functional NPC. Nup155 interacts with other members of the Nup93–205 complex such as Nup53 and Nup93 and also binds to Pom121 and Ndc1,[102,115] suggesting that the interaction of Nup93–205 with transmembrane pore proteins is essential for NE formation. Comparably to blocking the Nup93–205 complex, reduction of Pom121 inhibited NE formation in *X. laevis* egg extracts as observed by TEM analysis[119] and induced aberrant nuclear morphology in HeLa cells.[122] Depletion of Pom121 did not only disturb the NPC assembly but decreased the protein level of Nup155, Nup53, Nup93 and Nup107,[102] implying an important role of Pom121 in regulation of other Nups. The similar phenotypes observed upon depletion of Pom121, Ndc1, Nup53 or Nup155, led to the conclusion that they have a comparable function in NE assembly in invertebrates. As these Nups are essential for postmitotic NPC formation, the regulation of both processes, NPC and NE formation, appear to be closely entwined,[114,119] whereby Nups play a crucial role. Evidences from higher eukaryotes confirmed the close relation between NPC and NE assembly, however, transmembrane pore proteins, Pom121 and Ndc1, displayed redundancy with other INM proteins in NE formation.[52]

Formation of NE subdomains. In early interphase, NPCs and lamins are unevenly distributed in cultured mammalian cells, e.g., HeLa, U2OS and IMR90 cells,[123,124] human prostate cancer cell lines[125] and in vivo in *D. melanogaster*.[126] The NE region, at which the density of NPCs is high, is termed pore-rich region, in contrast to the NE region, which is devoid of NPCs, designated as pore-free island.[123] Generally in human cells, the pore-rich region contains B-type lamin, Sun1, LBR and NPCs, whereas A-type lamin and binding partners such as emerin, Sun2 and BAF are accumulated at pore-free islands.[123,127] These structural differences disappear during cell-cycle progression from G1 to S, and all NE components are uniformly spread at the NE in G2. Large pore-free islands were maintained in cells in which their interphase NPC formation and cell cycle progression was suppressed by a CDK inhibitor.[93] This result indicated that CDK activity and consequently de novo NPC formation are necessary for the reduction of pore-free islands. Furthermore, CDK activity was required for the uniform distribution of NPCs as well as of LBR and B-type lamin, which are part of the pore-rich region. On the other hand, the de-accumulation of A-type lamin and emerin, however, was not dependent on CDK activity.[93] These evidences strongly suggest that there is a relationship between NPC assembly and other NE components, especially INM proteins and lamins at the pore-rich region, during interphase. A relation between the NPC and NE components is further supported by the observation that depletion of the LINC complex protein Sun1, which can be found at the pore-rich region, induced defects in interphase NPC assembly and led to NPC clustering.[94,127]

In HeLa cells, both NE subdomains, the pore-rich region and the pore-free island, are established postmitotically in telophase (**Fig. 2A and B**), where they correspond to the chromosomal noncore region and to the chromosomal core region, respectively.[123,128] The core region is the region on chromosomes next to the spindle pole and central spindle areas, whereas the noncore regions are the peripheral regions on chromosomes surrounding the core region. The uneven distribution of NE constituents can be traced back to asymmetric targeting of A- and B-type lamins and their interacting INM proteins to telophase chromosomes. Thereby, BAF recruits LEM domain containing proteins such as emerin and Lap2α to the core region.[128-130] The majority of LEM domain containing proteins specifically binds A-type lamin and A-type lamin itself requires BAF for localization at the core region.[129] In contrast, B-type lamins and interacting INM proteins as well as Nups bind to the noncore regions. It was proposed that the mitotic spindle facilitates recruitment of INM proteins to the core region as microtubule (MT) disturbing chemicals diminished accumulation of proteins at the core region.[129]

Conclusion

In metazoans, the overall NE architecture is established during postmitotic formation of the NE. The NE reformation requires the reassembly of the NPCs embedded into a sealed nuclear membrane, which is formed from the mitotic ER and reorganized into an ONM and INM, as well as the assembly of the nuclear meshwork of A- and B-type lamins. The coordinated sequence of reassembly during the end of mitosis poses an organizational challenge, which must be overcome to ensure the successful

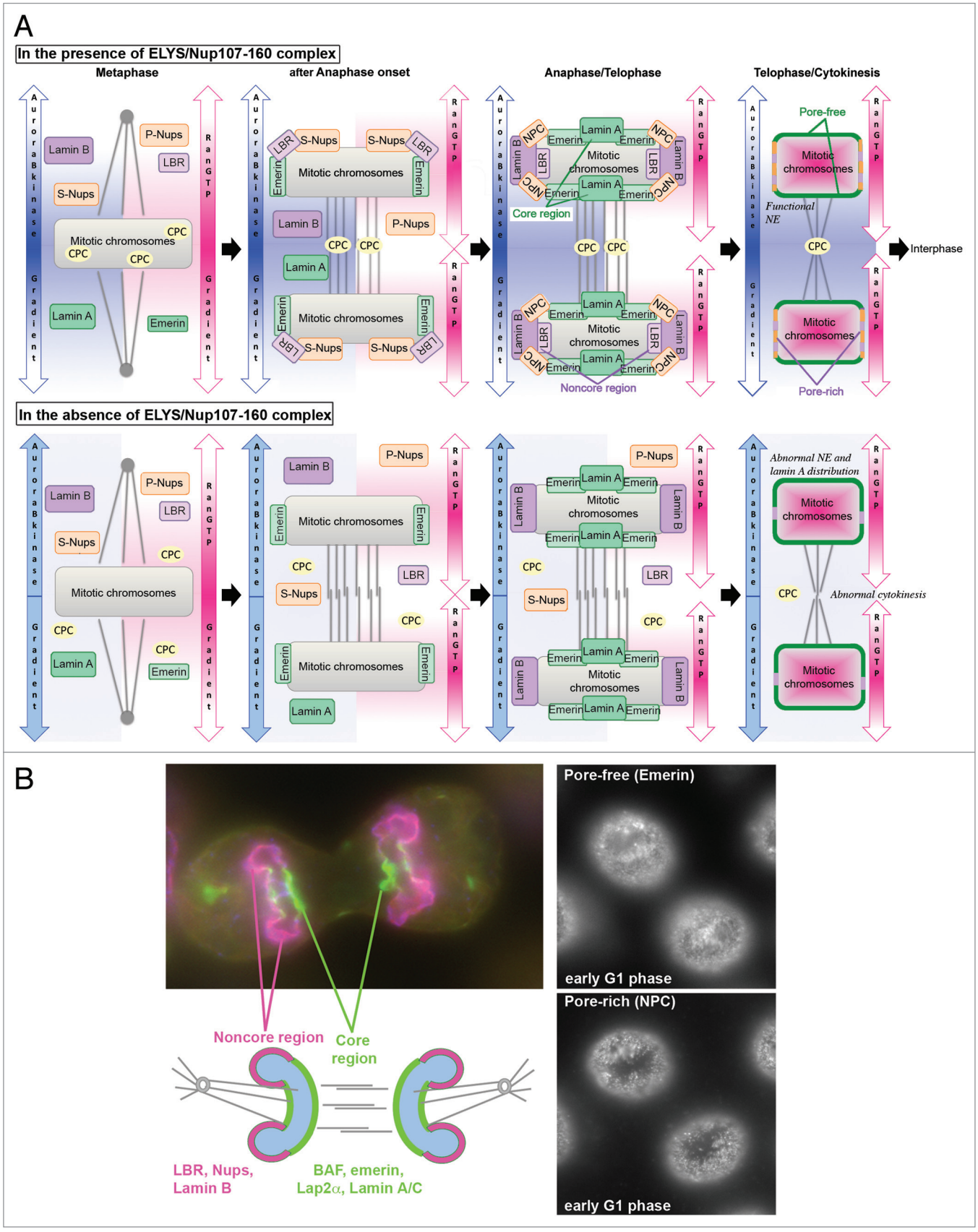

Figure 2. For figure legend, see page 111.

Figure 2 (See opposite page). Coordination of NPC and NE assembly at post-mitosis. (**A**) Scheme showing effects of ELYS/Nup107–160 complex on postmitotic NE/NPC assembly. In the presence of ELYS/Nup107–160 complex, scaffold Nups (Nup107–160 complex, Nup93–205 complex, also Pom121 and Ndc1), LBR and emerin, target to the noncore region of mitotic chromosomes soon after anaphase onset. During anaphase/telophase, A-type lamin binding proteins (represented by emerin) and A-type lamins accumulate at the core region of mitotic chromosomes. The core regions become the pore-free islands whereas noncore regions become the pore-rich region on the assembled, functional NE at early G1. In the absence of ELYS/Nup107–160 complex, neither scaffold Nups nor LBR can target mitotic chromosomes, although B-type and A-type lamins can localize to chromosomes in ananphase/telophase. Because absence of ELYS/Nup107–160 complex affects proper localization of the chromosomal passenger complex (CPC) that contains Aurora B kinase, the phosphorylation gradient is disrupted, which causes an aberrant distribution of core region proteins, including A-type lamins and their binding partners and also disturbs cytokinesis. RanGTP–gradient is established throughout cell cycle by chromosome bound RCC1. S-Nups, scaffold Nups; P-Nups, peripheral Nups; NPC, nuclear pore complex. (**B**) Left, photograph showing core region and noncore region in telophase (HeLa cell): red, LBR; green, emerin; blue, ELYS/Mel28. Right, photographs of the nuclear surface of early G1 HeLa cells shows IF staining of emerin (upper picture), which accumulates at pore-free islands and IF staining of NPCs (lower picture), representing the pore-rich region. Pore-free islands originate from the mitotic core regions, whereas pore-rich regions derive from mitotic noncore regions as depicted in the scheme.

generation of two daughter cells with a functional nucleus. On the one hand, coordination of the NE assembly on mitotic chromosomes is dependent on the condensation/decondensation state of chromatin, which is only fragmentally understood so far. On the other hand, mitotic chromosomes itself are a center of phosphorylation and RanGTP gradients, which spatially and temporally control recruitment of NE proteins. If local chromatin changes facilitate NE subdomain formation is still an open question. Vice versa, NE subdomains could change the chromatin structure or serve as scaffold for specialized chromatin in interphase, which needs to be addressed.

Besides roles in NE reformation, Nups play important roles throughout mitosis and regulate mitotic progression. Therefore Nups are required earlier than in anaphase, although their mitotic roles must be examined in more detail.

The interplay of NE proteins during mitosis provides a basis for steady and dynamic interactions at the interphase NE. Tackling open questions about postmitotic NE reformation will give deeper insights into the control and organization of the concerted, molecular assembly of a stable NE structure in interphase, which is capable of molecular transport, signaling and chromatin regulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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