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Breaking and Making of the Nuclear Envelope

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Abstract During mitosis, a single nucleus gives rise to two nuclei that are identical to the parent nucleus. Mitosis consists of a continuous sequence of events that must be carried out once and only once. Two such important events are the disassembly of the nuclear envelope (NE) during the first stages of mitosis, and its accurate reassembly during the last stages of mitosis. NE breakdown (NEBD) is initiated when maturation-promoting factor (MPF) enters the nucleus and starts phosphorylating nuclear pore complexes (NPCs) and nuclear lamina proteins, followed by NPC and lamina breakdown. Nuclear reassembly starts when nuclear membranes assemble onto the chromatin. This article focuses on the different models of NEBD and reassembly with emphasis on recent data. J. Cell. Biochem. 95: 454–465, 2005.

Key words: cell cycle; nuclear assembly; nuclear lamina; mitosis; nuclear membrane; nuclear pore complexes

NUCLEAR ENVELOPE (NE)

In eukaryotic cells, nucleoplasmic and cytoplasmic activities are separated by the NE. The NE is composed of two lipid bilayer membranes. The outer nuclear membrane (ONM) is continuous with the endoplasmic reticulum (ER), studded with ribosomes and is a site for protein translation and modifications. The ONM and the inner nuclear membrane (INM) are separated by a 25–45-nm thick lumen and are fused at sites where the nuclear pore complexes (NPCs) are embedded. Underlying the INM and abutting the peripheral chromatin, there is a protein meshwork termed the nuclear lamina [Gruenbaum et al., 2005].

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PROTEIN COMPOSITION OF THE NE

The NPC is composed of ~30 different proteins, collectively termed nucleoporins. It has an eightfold symmetry that forms a selective aqueous channel for macromolecule transport between the nucleoplasm and the cytoplasm. The structure of the NPC consists of eight spokes located at the core of the NPC, a cytoplasmic ring, cytoplasmic filaments, a nucleoplasmic ring, and a nuclear basket [Fahrenkrog and Koser, 2004]. In vertebrates, two integral membrane proteins connect the NPC to the nuclear membranes: POM121 is unique to vertebrates, while gp210 is evolutionarily conserved in metazoan and in plants [Cohen et al., 2003].

The nuclear lamina is composed of lamins and lamin-associated proteins, most of which are integral proteins of the INM. Lamins, which are type V intermediate filament proteins, are also present in the nuclear interior [Gruenbaum et al., 2005]. They are grouped into A- and B-types. B-type lamins have an acidic isoelectric point, undergo isoprenylation, and remain attached to membranes throughout the cell cycle, while A-type lamins have a neutral isoelectric point and remain soluble in mitosis (see below). Lamin A also undergoes isoprenylation but loses the farnesyl moiety in a second cleavage event that removes its last 15 residues. Every metazoan cell expresses B-type lamins, while A-type lamins are expressed only in

differentiated cells [Stuurman et al., 1998]. The genome of single cell organisms including *S. cerevisiae* and *S. pombe*, as well as the genomes of plants contain no lamin genes, and also lack other genes encoding INM proteins known in metazoans [Cohen et al., 2001].

The number of integral proteins at the mammalian INM is 78 and still increasing [Gruenbaum et al., 2005]. Most of these proteins interact directly or indirectly with lamins and many of them share common protein domains including the LEM-domain and the SUN-domain. In addition, many nucleoplasmic proteins interact with lamins and/or integral proteins of the INM. These include proteins of the DNA replication, RNA Polymerase II transcription, and splicing complexes, as well as specific transcriptional regulators and chromatin modifiers. Lamins and their associated proteins, emerin, SUN-domain proteins, and nesprins also interact with actin [Gruenbaum et al., 2005].

LEM-DOMAIN

The LEM-domain is a ~40 residue motif found in a group of proteins that interact directly or indirectly with A-, B-type lamins, or both. All tested LEM-domain proteins, including the INM proteins LAP2β, emerin and MAN1, and the nucleoplasmic protein LAP2α, a binding partner of nucleoplasmic A-type lamins, also bind via the LEM-domain to barrier-to-autointegration factor (BAF), a ~10-kDa protein that binds dsDNA, chromatin, and transcription activators [Foisner, 2003; Segura-Totten and Wilson, 2004]. In *C. elegans*, BAF enrichment at the nuclear periphery requires lamins and the two integral LEM-domain proteins: Ce-emerin and Ce-MAN1, which in turn require lamin for their NE localization [Liu et al., 2003a].

INTERACTIONS OF LAMINA PROTEINS WITH CHROMATIN

The nuclear lamina is closely associated with the peripheral chromatin, and lamina proteins have many chromatin targets. Lamins themselves can bind DNA and core histones via their rod and tail domains, respectively [Gruenbaum et al., 2003]. The LEM domain is involved in the association of the LEM-domain proteins and their complexes with chromatin via BAF. Additional domains in LEM-domain proteins also mediate chromatin interactions [Gruenbaum et al., 2003]. For example, LAP2β contains binding domains for DNA and for the chromosomal protein HA95 in its C-terminus. The C-terminus of LAP2α is both essential and sufficient for its chromosome association during nuclear assembly. In addition, it binds pRb [Johnson et al., 2004]. LBR forms complexes with DNA, heterochromatin HP1-type chromodomain proteins, HA95, and histones H3/H4 in a histone-acetylation-dependent manner [Gruenbaum et al., 2005].

NE BREAKDOWN (NEBD) DURING MITOSIS

In higher eukaryotes, the NE completely disassembles during mitosis in a process called "open mitosis". Nuclear membranes, nuclear lamina, and NPCs disassemble between prophase and prometaphase stages of the cell cycle allowing engagement of the chromosomes with the cytoplasmic mitotic spindle. In contrast, single-cell eukaryotes, such as *S. cerevisiae*, have "closed mitosis" where the different components of the NE (membranes and NPCs) probably remain intact [Cohen et al., 2001]. In these cells, tubulin proteins are imported into the nucleus and the spindle assembles within the nucleus.

The process of mitotic disassembly of the NE has probably evolved gradually and appears to have co-evolved with the increased complexity of the nuclear scaffold and lamina. For example, in *Aspergillus nidulans* during mitosis NPCs partially disassemble; Nup98, Nup159, Nup42, Nsp1, and Gle2 homologs are dispersed in the cytoplasm, while Nup96, Nup133, and POM152 homologs remain associated with the NE throughout mitosis [De Souza et al., 2004]. The partial disassembly of the NPCs makes the nucleus permeable to cytoplasmic proteins and the cytoplasm permeable to nuclear proteins [De Souza et al., 2004]. Other intermediate types of NE disassembly are found in *C. elegans* and syncytial *Drosophila* embryos. In *C. elegans*, lamin, integral INM proteins, and nuclear membranes completely disassemble only at mid-late anaphase, leaving a spindle envelope during most stages of mitosis. NPCs disassemble just before metaphase and spindle microtubules penetrate the nucleus only late in prometaphase [Lee et al., 2000]. In *Drosophila* early embryos, the nuclear lamina starts to disassemble at metaphase, completing its disassembly by late anaphase, while fenestrated membranes remain in a spindle envelope throughout the cell cycle. Following prometaphase, an

additional layer of membranes begins to form around the spindle envelope [Gruenbaum et al., 2003].

NEBD IS TRIGGERED BY PHOSPHORYLATION

According to the phosphorylation model, the rapid activation of cyclin B1-Cdk1 (MPF) is the event that commits the cell to undergo mitosis. In vertebrate cells, during the G2 phase of the cell cycle, MPF shuttles between the nucleus and the cytoplasm in a process that requires import (NLS) and export (NES) signals. When G2 ends, about 10 min before NEBD, Polo-like kinase 1 (Plk1) phosphorylates cyclin B at its amino terminal cytoplasmic retention sequence, causing its accumulation in an active form in the nucleus [Pines and Rieder, 2001; Jackman et al., 2003]. In support of the pivot role of cyclin dependent kinase 1 (Cdk1) in triggering NEBD, human HT2-19 cells deficient of Cdk1 skip mitosis stopping all cell divisions, while DNA replication alternates with both G1 and G2, and the centrosomes duplicate. However, centrosome migration, a typical prophase event, occurs and the timing of these cell cycle events is unaffected by Cdk1 inactivation as compared to normally dividing cells [Laronne et al., 2003].

Upon nuclear accumulation, Cdk1 phosphorylates nucleoporins, lamins, and integral proteins of the INM, as well as many other nuclear proteins. Cdk1 phosphorylation triggers the disassembly of the lamin filaments, which in vertebrates are phosphorylated on serines S22 and S392 symmetrically flanking the lamin rod domain. Mutations in these residues in the mammalian A-type lamins block its Cdk1 phosphorylation, inhibit lamina disassembly and NEBD, and induce cell cycle arrest [Heald and McKeon, 1990]. Moreover, Cdk1 phosphorylation of lamins is sufficient to depolymerize lamin filaments in vitro [Stuurman et al., 1998]. Cdk1 phosphorylation of lamin-binding proteins is also required for their mitotic disassembly. In LAP2α, up to seven mitotic-phosphorylated serines are clustered in its chromatin-binding domain. When all these serines are mutated, the mutant LAP2α fails to dissociate from chromatin during mitosis, but does not prevent NEBD [Gajewski et al., 2004]. Cdk1 phosphorylation also affects LAP2β binding to lamins and/or chromatin [Foisner and Gerace, 1993]. In line with these observations, nuclear lamina, nuclear membranes, and NPCs do not disassemble in cells deficient of Cdk1, and nuclear import remains normal [Laronne et al., 2003; and Y.G., unpublished observations]. The role of Cdk1 in NEBD is also apparent in *Aspergillus nidulans*, where nucleoporins phosphorylation by Cdk1 and NIMA kinases is required for the mitotic disassembly of NPCs.

Lamins, integral proteins of the INM, and nucleoporins also serve as substrates for many other protein kinases including protein kinase C, protein kinase A, MAP kinase, and casein kinase II [Gruenbaum et al., 2003]. It has yet to be determined whether these protein kinases are active during mitosis and what roles they play in NEBD.

Do microtubules have a role in NEBD? Jan Ellenberg and colleagues recently proposed a model in which the beginning of NEBD starts with spindle microtubules causing NE folds and invaginations, up to 1 h before NEBD. The mechanical tension produced by the spindle microtubules and associated dynein motors tear holes in the nuclear lamina and nuclear membranes. The first hole forms at the site of maximal tension, making the NE permeable to cytoplasmic macromolecules. The holes in the NE then expand, the NE becomes fragmented and the fragments are pulled toward the centrosomes [Beaudouin et al., 2002; Salina et al., 2002]. In support of this model, just before NEBD, the microtubule minus end motor, dynein, and the dynactin component p62 are translocated to the outer face of the NE. Overexpression of p62 dissociates dynein from the NE, reduces NE invaginations, and delays NEBD [Salina et al., 2002].

The above model seems to contradict the phosphorylation model, in which NEBD is triggered by phosphorylation of its components through MPF, which is actively transported to the nucleus up to 10 min before detectable NEBD (see above). Formation of holes in the NE by tearing forces of microtubules up to 1 h before NEBD, as suggested in the microtubule-dependent model, would predict that MPF passively enters the nucleus much earlier without causing lamina disassembly.

The question here is what came first, the chicken (lamins and NPC phosphorylation) or the egg (tension created by microtubules)? MPF phosphorylation probably plays a key role in NEBD, while microtubule tearing may play a minor, if any, role in this process. In cells lacking Cdk1, duplication and migration of centrosomes occur normally, suggesting that both

microtubules and dynein are normally distributed and active in these cells, yet NEBD does not occur. This suggests that microtubules and dynein are not sufficient to tear holes in the NE without phosphorylation of NE components [Laronne et al., 2003; and Y.G., unpublished observations]. A recent study assessing the mechanical resilience of lamin B1 network to microtubules tension support this notion [Panorchan et al., 2004]. This study demonstrated that lamin B1 networks possess an elastic stiffness, which even increases under tension and shows an exceptional resilience against shear deformations [Panorchan et al., 2004], concluding that lamin B1 polymers resist a much higher tension than that created by microtubules pulling the NE surface. Therefore, NE tearing by microtubules can only occur when lamin B1 is in a state of (phosphorylation-dependent) depolymerization and weakening. Indeed, loss of lamins and/or changes in lamina composition weaken lamina structure and affect nuclear shape also in interphase. Nuclei of *lmna*-null mouse embryo fibroblasts become fragile, stretched, and misshapen in response to mechanical stress [Lammerding et al., 2004]. Dramatic changes in nuclear shape are also seen in human cells expressing mutant A-type lamins and in *C. elegans* cells lacking lamin [Gruenbaum et al., 2005]. Microtubule tearing is thus apparently not sufficient to cause NEBD and is also not essential for NEBD, as both nocodazole-induced depolymerization of microtubules or laser-ablation of centrosomes cannot inhibit NEBD [Georgatos et al., 1997; Hinchcliffe et al., 2001].

The microtubule-based tearing model, which is mostly based on live imaging of mammalian cells overexpressing marker proteins fused to fluorescence proteins, is also dispensable for triggering NEBD in other experimental systems. Observations in starfish show that NPCs disassembly is the first step in NEBD leading to a fenestration of the NE [Terasaki et al., 2001]. Likewise, during mitosis in *C. elegans*, NPCs disassemble earlier than nuclear lamina and nuclear membranes [Lee et al., 2000]. It is, therefore, tempting to speculate that NPCs are also the first to disassemble in higher eukaryotes, causing leakiness of the NE.

Altogether, the current knowledge suggests a model in which the first step in NEBD is the accumulation of active MPF in the nucleus, where it phosphorylates lamins, nucleoporins, and INM proteins (Fig. 1). The phosphorylated nucleoporins leave the NPCs forming holes in the NE and causing its permeabilization. In parallel, lamin phosphorylation weakens lamin filaments and induces lamin filament depolymerization. Lamin molecules are then rapidly transported from the filaments on spindle microtubules towards the centrosomes by dynein motors. The first hole in the NE occurs probably where lamin filaments disassembly and lamins removal is most efficient. The phosphorylation of INM and chromatin proteins helps dissociating the NE from chromatin and causes the complete disassembly of the NE.

FATE OF NE COMPONENTS DURING MITOTIC NEBD

Disassembly of Membranes

There are currently two models, which are not necessarily mutual exclusive, to describe the fate of nuclear membranes during mitosis. According to the first model, nuclear membranes form vesicles. These vesicles are used at the end of mitosis for reassembly of the nuclear membranes around segregated chromatin (see below). In support of this model, in *Xenopus* oocytes nuclear membranes are found in a population of vesicles that is different from the bulk of the ER-derived membranes. These maternal pools are composed of two distinct vesicle populations termed non-fusogenic and fusogenic vesicles, each containing specific protein markers [Vigers and Lohka, 1992; Drummond et al., 1999]. Membrane vesicles are also found in other invertebrate and vertebrate oocytes and in *Drosophila* early embryos [Ulitzur and Gruenbaum, 1989]. This model is also supported by a recent study on the role of COPI in *Xenopus* NEBD [Liu et al., 2003b]. The COPI coatomer complex is required for vesicle budding during trafficking between the Golgi and ER and within the Golgi. COPI is recruited to the NE during early prophase by the nucleoporin Nup153, which requires ARF, a small GTPase protein. Depletion of COPI from mitotic extracts or inhibiting ARF recruitment to the COPI complex prevented NEBD.

The second model suggests that after NEBD, NE membranes are fused to the ER network [Ellenberg et al., 1997; Yang et al., 1997]. The main support for this model comes from studies showing that following NEBD in mammalian cells, integral proteins of the INM are localized

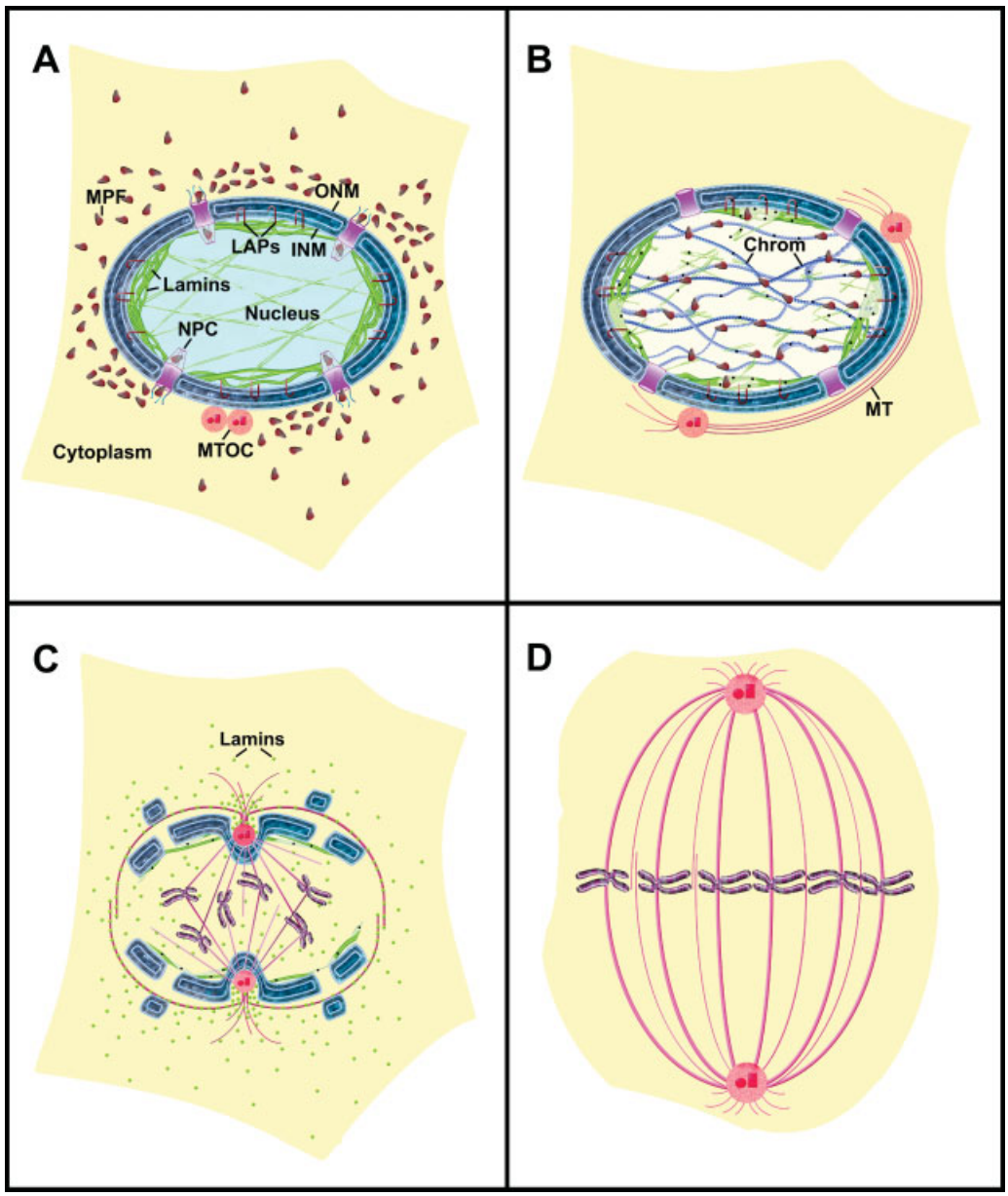

Fig. 1. A model describing the different sequential events of nuclear disassembly during mitosis in vertebrate cells (not to scale). **A**: During late G2, MPF enters the nucleus by transport through NPCs and starts phosphorylating its target proteins. **B**: During early prophase, phosphorylation by MPF (black dots) causes chromosome (Chrom) condensation, weakening of the nuclear lamina by partial disassembly of lamin filaments (light green), loss of interaction between integral proteins of the INM (LAPs) and chromatin, and NPC disassembly. In addition, centrosomes (MTOC) migrate along the ONM. **C**: At late prophase/prometaphase, chromosomes further condense and the nuclear membranes fold and invaginate next to the centrosomes, which have migrated to opposite positions. At that stage of mitosis, NPCs disassembly is probably completed and lamin dimers are probably rapidly removed by the microtubules and dynein (not shown) and transiently accumulate next to the centrosomes. While most of the nuclear membranes probably become fused to the ER network, membrane vesicles attached to microtubules may also occur. The disassembled membranes contain integral proteins of the INM and type B lamins (not shown). **D**: At metaphase, the chromosomes are aligned in the mid-plane and the nuclear envelope completed its disassembly. For simplicity, the different components of the nuclear envelope are not shown in (D).

in the ER membrane network, which cause the nuclear membranes to loose their unique identity [Ellenberg et al., 1997; Yang et al., 1997]. In addition, thin-section electron microscope analyses of mammalian cells do not show a massive budding of vesicles from the NE. Opponents of the first model argue that the vesicles result from shearing of the NE-ER membranes

during sample preparation. However, this does not provide a sufficient explanation for the existence of two distinct populations of vesicles. The different fates of NE membranes upon disassembly could also be explained by cell-type specific differences (oocytes vs. somatic cells). It cannot be excluded that in mammalian cells both mechanisms of membrane disassembly occur during NEBD. While a fraction of nuclear membranes may vesiculate (e.g., nuclear membranes next to the NPCs), another fraction may merge with the bulk of the ER. The latter fraction would then include many of the integral proteins of the INM.

Disassembly of Lamins and INM Proteins

The sequence of disassembly of nuclear lamina proteins is currently poorly understood. A- and B-type lamins seem to disassociate at different times of NEBD. In mammalian cells, A-type lamins start dissociating from the nuclear lamina at early prophase, whereas B-type lamins disassociate only later [Georgatos et al., 1997]. Since the NE localization of many integral proteins of the INM, as well as nucleoplasmic lamin-associated proteins depends on A- or B-type lamins [Gruenbaum et al., 2005], it will be extremely difficult to accurately follow the kinetics of disassembly of each of these proteins. Furthermore, the process is very fast. Thus, live imaging of fluorescently fused and overexpressed proteins is not very reliable to determine the sequence of disassembly of various lamina and NE proteins. Moreover, immunofluorescence analyses with specific antibodies face the problem of possible differences in the accessibility of the recognized epitopes following the phosphorylation of the protein, and the fluorescence signal is hard to quantitate.

Disassembly of NPCs

Several nucleoporins leave the NPC early in mitosis and become associated with kinetochores including Gle2, Nup133, Nup107, Nup37, Nup43, Seh1, Sec13, and Nup358 [Joseph et al., 2004], suggesting additional roles for these proteins in mitotic progression. Depletion of Nup358 causes mitotic defects and inhibition of kinetochore assembly [Salina et al., 2003], indicating that Nup358 has an essential role in kinetochore maturation and function. Likewise, RanGAP1-RanBP2 complex is essential for microtubule-kinetochore interactions in vivo, since depletion of this complex causes mislocalization of the kinetochore proteins RanGAP1, Mad1, Mad2, CENP-E, and CENP-F and loss of cold-stable interactions between the kinetochore and the microtubules. Experiments in *D. melanogaster* and *C. elegans* also indicate a requirement for NPC components including Ran, Nup358, RanGAP, importin α, and importin β in spindle assembly, suggesting that these proteins must dissociate from the NPC early in mitosis [Harel and Forbes, 2004].

NUCLEAR REASSEMBLY DURING MITOSIS

During anaphase and telophase, the NE and its associated structures reassemble around the segregated chromosomes to form functional daughter nuclei. Nuclear reassembly is based on a highly regulated sequence of molecular interactions, which include in a temporal order: (i) targeting of individual nucleoskeletal proteins to the chromosomal surface, (ii) membrane recruitment and fusion, (iii) assembly of NPCs, (iv) transport of the bulk of lamins into the nucleus through newly formed NPCs, and (v) formation of the nuclear lamina. At early G1, the chromatin becomes fully enclosed by an intact NE. The nucleus then enlarges by import of nuclear proteins through NPCs, the NE expands and the chromatin becomes fully decondensed.

Our knowledge about the underlying mechanisms of NE assembly mostly derive from in vitro nuclear assembly studies in a variety of systems, particularly *Xenopus laevis* egg extracts, and from immunofluorescence microscopic analyses in fixed and living cells [Foisner, 2003].

Initial Phases of Lamina Protein Assembly

The speed with which the nucleus reforms after sister chromatid separation and the different cell systems used to study nuclear assembly make it difficult to determine a detailed general order and sequence of the molecular interactions of NE proteins with chromatin during early nuclear assembly stages [Foisner, 2003]. Time-lapse microscopy of GFP-tagged integral INM proteins in HeLa cells showed that they are targeted to chromosomes ~5 min after the metaphase-anaphase transition, while a uniform distribution of INM proteins around chromatin can be detected ~3 min later [Haraguchi et al., 2000]. LBR and a small fraction of emerin can be identified at the chromosomal surface before LAP2β [Haraguchi et al., 2000; Dechat et al.,

2004]. LAP2α is also detected at the chromosomal surface before LAP2β (Fig. 2), reflecting different chromosome binding properties of LAP2 isoforms during nuclear assembly despite the presence of the common LEM and LEM-like domains [Vlcek et al., 2002]. The initial association of LAP2α with chromosomes involves its unique C-terminal chromosome binding region, while the N-terminus including the LEM domain, common to all LAP2 isoforms, is dispensable at these stages [Vlcek et al., 2002].

Kinetics of Lamins During Nuclear Assembly

The assembly of A- and B-type lamins follows different pathways. B-type lamins are detected at chromosomes at early stages of NE reformation, shortly after LAP2β is visible around the chromatin. As B-type lamins bind membranes, they could be targeted to chromosomes with membrane structures initially, but their assembly into a stable lamina occurs in subsequent stages at the inner surface of the newly formed membrane, as shown by fluorescence recovery after photobleaching (FRAP) analyses of GFP-tagged lamin B1. In contrast, the bulk of A-type lamins translocates through the newly formed NPCs and redistributes to the nuclear interior in telophase/G1, although one study reported detection of a minor fraction of lamin C at chromosomes in late anaphase in LAP2α-containing structures [Dechat et al., 2004]. Consistent with the idea of independent lamin assembly pathways of B- and A-type lamins, inhibition of B-type lamin polymerization at the end of mitosis does not interfere with the assembly of A-type lamins [Steen and Collas, 2001].

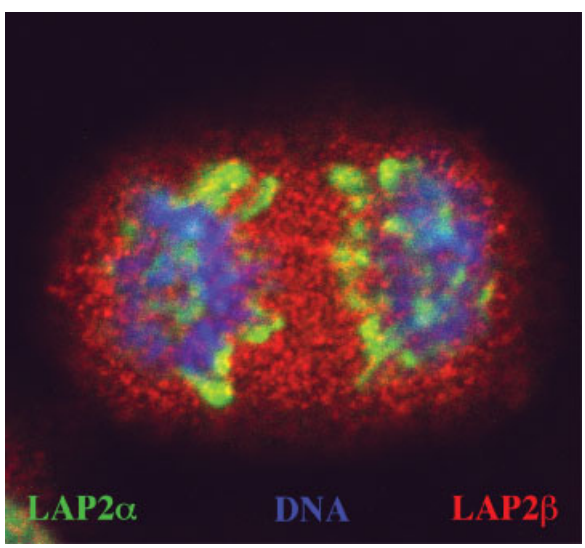

Fig. 2. Confocal immunofluorescence image of a dividing HeLa cell in anaphase, showing the localization of LAP2β (red) and LAP2α (green) on chromosomes (blue). Note that LAP2α associates with the ends of chromosomes, while LAP2β is still mostly cytoplasmic.

LAP2α/BAF Complex and Nuclear Assembly

In mammalian cells, different groups of lamin-binding proteins are initially recruited to discrete regions at the chromosomal surface, indicating the presence of distinct nuclear substructures. LAP2α initially accumulates on telomeric regions of chromosomes during anaphase and subsequently forms so-called 'core' structures on anaphase chromatin adjacent to the spindle pole and mid-spindle area [Dechat et al., 2004] (Fig. 3). A large fraction of BAF colocalizes with LAP2α at telomeres (Fig. 3A) and core structures [Haraguchi et al., 2000]. Both proteins show low exchange rates at core

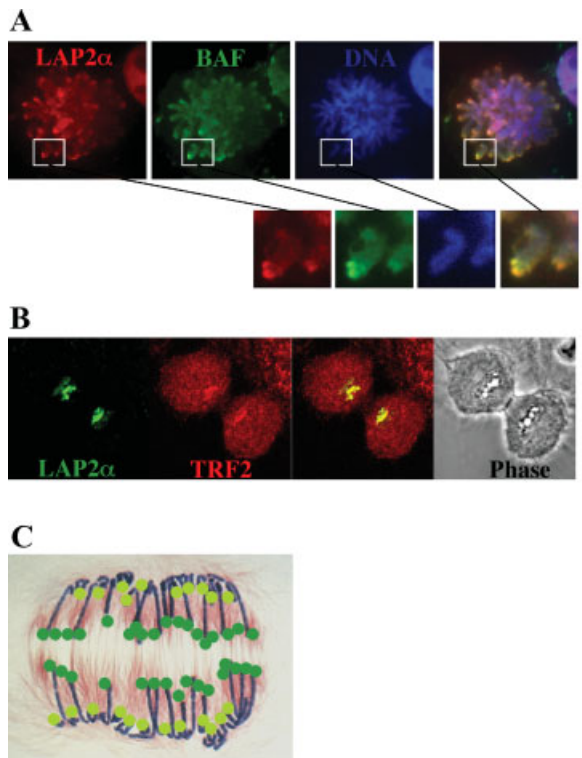

Fig. 3. Confocal immunofluorescence image of (**A**) a mitotic chromosome spread showing the localization of LAP2α (red) and BAF (green) on telomers (blue). Overlap between LAP2α and BAF (yellow). **B**: A dividing HeLa cell in telophase showing LAP2α (green), telomere-binding TRF2 (red), and merge image (yellow). Phase contrast image of the cells is shown on the right. **C**: Model depicting the formation of inner and outer core regions by binding and assembly of LAP2α to telomeres of chromosomal long (dark green circles) and short (light green circles) arms, respectively.

regions measured by FRAP [Dechat et al., 2004; Shimi et al., 2004], suggesting the formation of stable LAP2α/BAF complexes at early stages of nuclear reassembly. As the telomeric protein TRF2 colocalized with LAP2α in core structures (Fig. 3B), it was suggested that LAP2α transiently helps to anchor telomeres to defined stable regions in the reforming nuclei during chromatin decondensation [Dechat et al., 2004]. Assembly of inner and outer core regions located adjacent to spindle poles and mid-spindle microtubules, respectively, could both be initiated by LAP2α binding and assembly at telomeres (Fig. 3C). These LAP2α/BAF core structures may also trigger changes in chromatin structure, providing docking sites for other NE proteins and may define sub-areas of chromatin in the fully assembled interphase nucleus.

Other NE proteins, such as LBR, LAP2β, emerin, and lamins, do not bind telomeres at early stages of NE assembly. However, emerin, to some extent LAP2β, and a sub-fraction of lamin C are targeted to core regions slightly after LAP2α/BAF. We hypothesize that the association of these proteins with core regions is mediated by the direct interaction between LAP2α and lamin C, and by the binding of the LEM-domain proteins emerin and LAP2β to BAF. In contrast, LBR is initially detectable at more peripheral chromatin regions that do not overlap with the core structures, indicating different types of interactions between LBR and chromatin [Ellenberg et al., 1997].

Roles of Protein Phosphatase 1 (PP1) in Nuclear Assembly

Phosphorylation/dephosphorylation-dependent mechanisms ensure the temporally and spatially coordinated accumulation of lamina and NE proteins at chromosomes. Nuclear reassembly requires phosphatase-activity, and at least for B-type lamins, involves PP1 [Steen and Collas, 2001]. AKAP149 recruits PP1 to the reforming NE, which in turn removes phosphates from mitosis-specific sites on lamin B. The identification of other PP1 target proteins, other phosphatases, or regulatory mechanisms during the complex processes of post-mitotic nuclear reassembly awaits further investigation.

Assembly of Nuclear Pore Complexes

Functional pore complexes are assembled after the formation of a continuous double membrane around chromosomes and require specific nucleoporins [Fahrenkrog and Koser, 2004]. The first defined event in NPC formation is the association of the Nup107-160 complex with chromatin [Walther et al., 2003a; Harel et al., 2003b]. This highly conserved sub-complex, which is localized at both sites of the central core of NPCs and consists of up to nine nucleoporins, is predicted to form stable NPC scaffold structures during assembly [Rabut et al., 2004]. Other nucleoporins that assemble early include the nucleoplasmic basket protein Nup153 and the vertebrate-specific pore membrane protein, POM121, followed by the soluble p62 complex proteins and CAN/Nup214 [Bodoor et al., 1999; Haraguchi et al., 2000]. One potential implication is that POM121 is required for NPC assembly within the nuclear membrane. The evolutionarily conserved pore membrane protein gp210, and the basket protein Tpr concentrate at the nuclear periphery at detectable levels only at late telophase/G1. Interestingly, in vitro nuclear assembly studies in *Xenopus* egg extracts using gp210 fragments or antibodies, and RNAi experiments in *C. elegans* and HeLa cells indicated an essential function of gp210 in nuclear pore formation, including the close apposition of INM and ONM before fusion to form pore membranes [Drummond and Wilson, 2002; Cohen et al., 2003]. Gp210 may not be required for all vertebrate nuclear membrane assembly events, since its expression varies in different cell types [Olsson et al., 2004].

While the sequential association of NPC proteins with chromosomes during NE reformation is regulated by phosphorylation/dephosphorylation-dependent mechanisms, as suggested for nucleoporins Nup153, Nup214, or gp210 [Favreau et al., 1996], the positional signal for NPC to reassemble on chromatin is mediated by Ran, a small GTPase, and involves importin β [Wozniak and Clarke, 2003]. RanGTP is highly compartmentalized and accumulates around chromatin during mitosis, as Ran and its nucleotide exchange factor Rcc1, which mediates conversion of RanGDP to RanGTP, bind histones H3, H4 and H2A, H2B, respectively [Nemergut et al., 2001; Bilbao-Cortes et al., 2002]. The specific production and accumulation of RanGTP at mitotic chromosomes are required to dissociate Nup107, Nup153, and Nup358 from importin β, making them available for binding chromatin and initiating NPC assembly [Harel et al., 2003a; Walther et al., 2003b].

Assembly of Membranes

Nuclear membrane assembly involves two clearly distinguishable events: (i) Targeting of membranes to chromatin, and (ii) membrane fusion. The segregation of NE-specific membranes and ER membranes is a key event in NE assembly and has to be seen in the context of the different models describing the fate of membranes during mitotic diassembly (see above). In the case of the existence of different vesicle populations, as described in *Xenopus* egg extracts, assembly requires only subpopulations of vesicles. Indeed, at least two different vesicle populations, one with a capacity to bind chromatin and a different population required for fusion have been described [Vigers and Lohka, 1992; Drummond et al., 1999]. In the case of a uniform mitotic membrane system, in which nuclear membranes loose their identity within the ER, a segregation of NE membranes during NE assembly can be envisaged by formation of membrane sub-compartments within the ER, probably by forming protein complexes involving INM components, which diffuse laterally in the membrane and bind to chromatin. In both cases, however, membrane fusion of chromatin-bound membranes occurs subsequently to form a continuous double membrane around the chromatin. In *Xenopus* oocytes, this includes the formation of a chromatin associated ER-like membrane network, followed by fusion of the membranes into the closed NE.

Functions of Ran and Importin β in Membrane Fusion

Membrane fusion involves importin β and Ran that are also involved in NPC assembly (see above). Importin β and Ran interact with yet unknown target proteins [Harel and Forbes, 2004]. Addition of an excess of importin β to *Xenopus* in vitro nuclear assembly reactions inhibited nuclear membrane fusion, whereas initial targeting of membranes to chromatin was not affected [Harel et al., 2003a]. Furthermore, depletion of either Ran or Rcc1 in *Xenopus* egg extracts [Hetzer et al., 2000] or their RNAi-mediated downregulation in *C. elegans* [Askjaer et al., 2002] inhibited nuclear membrane fusion. Thus, the relative abundance of importin β and RanGTP seems to be critical for two distinct major mechanisms of nuclear reassembly: membrane fusion and NPC assembly, but specific targets of these proteins remain to be identified.

Experiments in the *Xenopus* in vitro nuclear assembly system have also implicated p97, a member of the AAA-ATPase family, in two separate steps of membrane fusion using different adapter molecules [Hetzer et al., 2001]. Early lateral fusion events to seal the nuclear double membrane involve the trimeric p97-Ufd1-Np14 complex. A different, p97-p47 complex has been suggested to be required for expansion of a sealed nuclear membrane during nuclear growth at late stages of assembly and during interphase, which is likely mediated by point fusions of membranes.

Specific Roles of Individual NE and Chromatin Proteins in Nuclear Reassembly

Various lamina and nucleoskeletal components have been directly implicated in nuclear assembly. The role of nuclear lamins in assembly has been a matter of debate for many years. Immunodepletion of lamins in various in vitro nuclear assembly assays from both invertebrate and vertebrate organisms has led to conflicting results showing that lamins are either essential or dispensable for nuclear membrane formation. These conflicting results probably arose due to experimental difficulties in completely removing lamins from both the soluble and the membrane fractions of these extracts [Gruenbaum et al., 2003]. In a different approach, addition of a dominant negative C-terminal *Xenopus* lamin B3 fragment, to in vitro nuclear assembly extracts, inhibited nuclear membrane assembly [Lopez-Soler et al., 2001], suggesting essential roles for lamins in nuclear membrane assembly. However, lamin polymerization is probably not required for this process, since preventing lamin polymerization by inhibiting PP1 did not interfere with nuclear membrane assembly [Steen and Collas, 2001]. This hypothesis is consistent with the observation that lamin polymerization probably occurs only after nuclear membranes and NPCs are formed (see above).

Lamin-binding proteins in the INM, including LBR and LAP2β, are also involved in NE assembly [Foisner, 2003]. Immunodepletion studies in avian cells and sea urchin egg extracts suggested a role of LBR in targeting nuclear membranes to chromosomes and in post-mitotic nuclear reformation [Collas et al., 1996]. The role of LAP2β in nuclear assembly is still controversial. While addition of an N-terminal LAP2 fragment to *Xenopus* assembly reactions

inhibited NE assembly [Gant et al., 1999], expression of a similar fragment in HeLa cells or its addition to mammalian in vitro nuclear assembly extracts had no effect on NE assembly [Vlcek et al., 2002]. The nucleoplasmic LAP2 isoform, LAP2α, probably has a key role in early post-mitotic assembly of nuclear structures in mammalian cells, as the addition of dominant-negative C-terminal LAP2α fragments to in vitro assembly extracts of mammalian cells prevented the assembly of the NE [Vlcek et al., 2002].

BAF is also involved in NE reformation in all systems tested: *Xenopus* extracts, mammalian cells, and *C. elegans*. In *Xenopus*, BAF interactions with both DNA and LEM-domain proteins are critical for membrane recruitment and chromatin decondensation during nuclear assembly [Segura-Totten and Wilson, 2004]. In HeLa cells that express a mutant BAF, emerin, LAP2β, and lamin A fail to localize at the NE during interphase. Interestingly, the same mutation had no effect on the assembly of B-type lamins [Haraguchi et al., 2001]. In *C. elegans*, downregulation of BAF causes gross defects in chromosome segregation, chromatin decondensation, and mitotic progression. Nuclear pores reassembled, whereas lamin and LEM-domain proteins bound chromatin but remained patchy and disorganized. Thus, the assembly of BAF, lamins, and LEM-domain proteins is mutually dependent, and is required to capture segregated chromosomes within the nascent NE (A.M. and Y.G. unpublished observations).

Recent reports show that actin and its binding proteins play many roles in the nucleus [Bettinger et al., 2004]. Not surprisingly, actin and its binding protein 4.1 are also implicated in NE assembly. Addition of the actin inhibitor, latrunculin A, strongly perturbed nuclear assembly and produced distorted nuclear structures containing neither actin nor protein 4.1 [Krauss et al., 2003]. Since actin can interact with both lamin and emerin [Holaska et al., 2004], the formation of emerin/lamin/actin-complexes at the chromosomal surface probably is important for nuclear reassembly.

Taken together, an increasing number of structural components of the NE have an essential role in NE assembly and cooperate to form functional daughter nuclei after mitosis. The molecular details, which involve a complex temporal and spatial coordination of the individual steps, are just beginning to emerge.

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