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Reorganization of the Nuclear Envelope during Open Mitosis

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Abstract

The nuclear envelope (NE) provides a selective barrier between the nuclear interior and the cytoplasm and constitutes a central component of intracellular architecture. During mitosis in metazoa, the NE breaks down leading to the complete mixing of the nuclear content with the cytosol. Interestingly, many NE components actively participate in mitotic progression. After chromosome segregation, the NE is reassembled around decondensing chromatin and the nuclear compartment is reestablished in the daughter cells. Here, we summarize recent progress in deciphering the molecular mechanisms underlying NE dynamics during cell division.

1. Introduction

The nuclear envelope (NE) is composed of two adjacent membranes of distinct protein composition. The outer nuclear membrane (ONM) is continuous with the rough endoplasmic reticulum (rER) and both share a similar protein composition. The inner nuclear membrane (INM) contains a different set of membrane proteins, which establish connections to chromatin and, in metazoan cells, to the nuclear lamina. INM and ONM are fused at numerous sites, generating pores that are occupied by nuclear pore complexes (NPCs) – macromolecular channels assembled from proteins called nucleoporins. At the onset of mitosis, changes in NE architecture occur, which range from subtle alterations in lower eukaryotes with a closed mitosis to the complete disassembly of the NE during nuclear envelope breakdown (NEBD) in vertebrate cells. At the end of mitosis, NE components are recruited back to chromatin in a coordinated fashion to reestablish the nuclear boundary. Here, we will summarize recent insights into the topological and molecular reorganization of the nuclear membrane during cell division.

2. The NE at the entry into mitosis

Onset of mitosis is marked by the formation of microtubule (MT) asters from centrosomes and their migration around the NE. Centrosome migration requires dynein, a cytoplasmic minus-end directed MT motor [1], which associates with the NE at the end of G2 [2]. MT interactions with the NE aid later events in mitotic entry such as a dynein-dependent tearing process, which leads to a rupturing of the NE during prophase and facilitates NEBD and the movement of NE components towards centrosomes [3–5]. However, the mechanism by which dynein is tethered to the ONM is not yet clear. A subpopulation of the INM protein emerin, which resides in the

ONM, has recently been proposed to interact with tubulin and facilitate the attachment of centrosomes to the NE in interphase cells [6]. Whether centrosome association with emerin or other NE components are used for centrosome migration remains to be seen.

Notably, NE components have also been implicated in other dynamic nuclear changes occurring early during cell division, like telomere attachment to the INM during prophase I of meiosis. In this case, the INM proteins SUN1 and SUN2, help pairing of homologous chromosomes in the nuclear periphery to facilitate homologous synapsis and recombination [7–9]. SUN1 and SUN2 are members of the evolutionary conserved LINC complexes, which establish interactions between the NE and the cytoskeleton [10].

3. NEBD

One of the most dramatic changes in cell organization during mitosis is the loss of nuclear compartmentalization induced by the breakdown of the NE at the transition from prophase to prometaphase (Figure 1). NEBD is marked by an increase in the permeability of the NE accompanied by the initiation of NPC disassembly [11]. In mammalian somatic cells, NPC dissociation is completed within minutes and is initiated by the loss of the peripheral nucleoporin Nup98, which is followed by a wave of synchronous nucleoporin dissociation [12]. Many nucleoporins are released from the NPC in the form of stable nucleoporin subcomplexes. Subsequent steps of NEBD involve the depolymerization of the nuclear lamina [13], and the detachment and removal of the nuclear membranes from chromatin [3,4]. As a consequence, nuclear membrane proteins are redistributed into the membrane system of the mitotic ER (see below). In *C. elegans*, this process requires the transmembrane nucleoporin GP210 [14], the GTPase Rab5 and reticulons [15]. The latter family of integral membrane proteins, together with a distinct class of membrane-bending proteins including mammalian DP1 and its yeast homolog Yop1p, has also been shown to shape the tubular ER [16]. This suggests that tubulation of disassembling nuclear membrane sheets might assist the dispersal of NE components into the ER. *In vitro* experiments using *Xenopus* egg extracts have also implicated the COPI coatomer complex in NEBD [17], but its contribution to NEBD *in vivo* still needs to be characterized.

Many, if not all, of these events are triggered by the activation of mitotic kinases, which phosphorylate NE proteins, including nucleoporins, lamins, INM proteins and chromatin-associated factors. Clearly, Cdk1/cyclin B plays a major role in nuclear disassembly and it directly causes lamina depolymerization. Early *in vitro* studies also implicated the PKC isoform β II in dissolving lamin interactions [18]. A recent study using light-activatable kinase sensors in living cells supports the involvement of PKC β in NEBD [19].

In addition, other kinases play a role in the nuclear disassembly process in various species, including NIMA [20], Aurora A [21,22] and a cyclin A2/Cdk complex [23]. The role of NIMA in NEBD has been established in the filamentous fungus *A. nidulans*, which uses partial NPC disassembly during its semi-open mitosis to allow for Cdk1/cyclinB and tubulin influx into nuclei and intranuclear spindle formation. NPC disintegration in *A. nidulans* is dependent on CDK1 and NIMA and involves the dispersal of a subset of nucleoporins, including Nup98 which is phosphorylated in a NIMA-dependent manner [20]. If NIMA-related kinases play a role in NPC disassembly in higher eukaryotes has not been addressed so far.

The mechanism by which Aurora A affects NEBD is not yet clear. Aurora A depletion in *C. elegans* delays NEBD relative to the completion of chromatin condensation [21,22]. Future experiments will tell whether Aurora A directly phosphorylates NE components and/or activates factors required for NEBD like Cdk1/cyclin B. Surprisingly, in human cells, there is little effect on the timing of NEBD upon depletion of B-type cyclins, whereas the reduction of cyclin A2 causes a significant delay. A constitutively nuclear cyclin B1 can rescue the loss of

cyclin A2, suggesting that at least a part of the function of Cdk/cyclinA complexes in NEBD lies in controlling nuclear accumulation of Cdk1/cyclinB1 [23].

Although several kinases are promoting nuclear disassembly, little is known about how their action is linked to the disassembly of NPCs and subsequent events. An increasing number of mitotic phosphorylation sites are being identified in nucleoporins and other NE proteins through large-scale approaches and individual analyses [24–29]. NPC disassembly is likely a point of no return in nuclear disintegration and therefore must be tightly linked to signaling events controlling mitotic entry. To understand the nature of this connection will require deciphering the molecular events underlying the disruption of the NE permeability barrier. A valuable tool for this task is provided by a recently developed NEBD *in vitro* system, which allows for dissecting the contribution of factors to individual steps in this process [5].

4. Role of disassembled nucleoporins in mitosis

A number of proteins required for nucleocytoplasmic transport in interphase have important functions during mitosis. For instance, the RanGTPase system in conjunction with the transport receptor importin β plays a pivotal role in controlling NEBD [5], spindle assembly and NE assembly (for review see [30,31]). In addition, certain nucleoporins have been implicated in mitotic events independent of their function in mediating transport through the NPC.

RanBP2/Nup358 is a large, multifunctional component of the cytoplasmic filaments of the NPC, which has SUMO E3 ligase activity and tethers SUMOmodified RanGAP to the NPC during interphase [32,33]. After NPC disassembly, a fraction of RanBP2/SUMO-RanGAP complexes associates with spindle microtubules and, importantly, the MT-bound kinetochores [34,35]. Targeting of RanBP2 to kinetochores is dependent on MT, SUMO-1 and the nuclear export receptor CRM1 [36]. Depletion of RanBP2 by RNAi affects proper chromosome alignment and bipolar spindle formation [34,35]. Recently, an elegant study revealed severe aneuploidy and tumor formation in mice expressing low levels of this essential nucleoporin [37]. The tumorigenicity was linked to defects in the resolution of sister chromatids at the meta-anaphase transition, which requires the recruitment of topoisomerase II α to the inner centromeres. Centromere targeting of Topo II α was shown to be dependent on its sumoylation mediated by RanBP2 and compromised in mice with low RanBP2 expression. These data suggest that RanBP2 functions as tumor suppressor by ensuring faithful chromosome segregation during mitosis.

Mitotic functions have also been attributed to the multimeric Nup107/160 complex and its associated partner ELYS/MEL-28. A fraction of the Nup107/160 complex and ELYS/MEL-28 localize to kinetochores [38–40]. Kinetochores targeting of the Nup107 complex depends primarily on the Ndc80 complex [41], which is part of the outer kinetochores layer and involved in establishing MTkinetochores attachments. In addition, the Nup107/160 complex is also found on spindle poles and proximal microtubules during prometaphase [42]. *In vitro*, depletion of the Nup107/160 complex impairs correct bipolar spindle formation, likely by affecting the maintenance of MT fibers between spindle poles and chromosomes [42]. If recruitment of the Nup107/160 complex to kinetochores is compromised in living cells, a striking prolongation of mitosis is induced [41]. This mitotic delay is caused by an extended prometaphase and accompanied by failures in chromosome congression, originating from defects in stable MT-kinetochores interactions. Strikingly, loss of the Nup107/160 complex at kinetochores also affects the recruitment of RanBP2/RanGAP. Although the defects upon loss of the Nup107/160 complex or RanBP2/RanGAP resemble each other, the Nup107/160 complex may harbor an independent function at kinetochores, as RanBP2/RanGAP fail to target to kinetochores in the *Xenopus in vitro* spindle assembly system [36]. Interestingly, such a function might be linked

to CENP-F, an outer kinetochore protein that associates with the dynein partners Nde1 and Nde11 as well as with Nup133 [41,43].

Rae1 (Gle2; Mnrp41) is a nucleoporin involved in mRNA export during interphase, which symmetrically associates with the NPC through binding to its partner subunit Nup98. One important mitotic role of the Rae1/Nup98 complex is the inhibition of securin degradation during prometaphase/metaphase, until the spindle assembly checkpoint (SAC) has been satisfied [44]. The SAC prevents onset of anaphase until chromosome alignment at the metaphase plate is completed. Securin is an inhibitor of separase that cleaves cohesin complexes to allow for sister chromatid separation [45]. Binding of Rae1/Nup98 to the E3 ubiquitin ligase APC/C^{Cdh1} inhibits securin polyubiquitinylation and degradation [44]. Accordingly, cells of double heterozygous mice (Rae1^{+/-}/Nup98^{+/-}) expressing reduced levels of both Nup98 and Rae1 display severe aneuploidy caused by premature securin degradation and separation of sister chromatids. It is, however, currently unclear by which mechanism Rae1/Nup98 are dissociated from APC/C^{Cdh1} to allow its timely activation once all kinetochores are correctly attached to microtubules at the metaphase plate.

In addition, Rae1 can directly bind to MTs and supports spindle assembly *in vitro* [46]. Surprisingly, the Rae1 activity was linked to a Rae1-containing ribonucleoprotein complex promoting MT nucleation/stabilization. Its activity is impaired by RNase treatment, suggesting that RNA plays a role in mitotic spindle assembly. However, it remains to be seen if Nup98 contributes to the function of Rae1 in spindle assembly and if any of the various RNA-binding proteins associated with Rae1 promotes this effect. The function of Rae1 in spindle formation is supported by the observation that RNAi against Rae1 in HeLa cells results in a twofold increase in the mitotic index, accompanied by failures in chromosome alignment and a high incidence of multipolar spindles [46,47]. Recently, NuMA, a microtubule-associated protein involved in spindle assembly, has also been identified as an interaction partner of Rae1 in mitotic HeLa cell extracts [47]. Codepletion of NuMA partially rescued spindle bipolarity in Rae1 depleted cells, suggesting that the proper balance of NuMA and Rae1 is a critical determinant for proper spindle formation.

Taken together, many nucleoporins affect the progression or control of mitosis and it becomes obvious that there is an important connection between nucleoporins and spindle assembly. The strong link between kinetochores and NPCs suggests that a possible outer pre-kinetochore complex may be a constituent of the NPC during interphase, which is recruited to kinetochores during mitosis. In evolutionary terms, it is interesting to consider whether this is a recent invention or a remnant of an old mechanism. Intriguingly, dinoflagellates, which are unicellular protists derived from typical eukaryotes by early divergence, divide through a closed mitosis using a cytoplasmic spindle [48]. During 'dinomitosis', kinetochore-like chromatin regions are closely associated with the nuclear envelope (perhaps NPCs), which forms channel-like structures that allow for penetration of microtubules.

Mitotic ER

During NEBD, INM proteins are redistributed into the membrane system of the mitotic ER [49,50]. This process not only requires the phosphorylation of INM proteins [51] but recent studies in *C. elegans* also revealed an involvement of two factors that control the formation of ER tubules, namely the GTPase Rab5 and YOP-1/RET-1 [15]. YOP-1/RET-1 belong to a class of evolutionary conserved integral membrane proteins [52], which can be grouped into two distinct families that structurally shape ER tubules [16]. The first group of proteins, the reticulons, includes two genes in yeast (Rtn1, Rtn2) and four in mammals (Rtn1–4). The second group consists of proteins related to DP1 (Yop1p in yeast) [16]. These proteins localize to the tubular ER and are excluded from low-curvature membranes such as the NE and peripheral

ER sheets. Reticulons share with DP1 a conserved domain of 200 aa containing two hydrophobic segments, each thought to form a hairpin in form of a wedge within a lipid bilayer [53]. These wedge-like domains are thought to increase the surface area of the outer leaflet to create asymmetry in the lipid bilayers and reticulons have been shown to be sufficient to form tubules in vitro [54]. The involvement of YOP-1/RET-1 in NE dynamics during *C. elegans* mitosis [15] suggests that the intrinsic propensity of the ER to oscillate between tubules and sheets is utilized during mitosis and affects the fate of the NE. Indeed, consistent with the idea of ER restructuring during mitosis, three-dimensional EM reconstruction demonstrated that the ER is entirely composed of tubules in metaphase and no sheets are observed [55]. Since the NE is also reabsorbed in the ER during mitosis, NE reformation must likely occur from tubular ER [56].

NE formation from tubular ER

Consistent with such a mechanism, *in vitro* analyses suggest that an intact tubular ER is required for NE formation at the end of mitosis [50]. Live imaging revealed that the ER is targeted to chromosomes via tubule-end binding and subsequently immobilized on the chromatin surface. This chromatin-bound network then flattens and seals into a closed NE. *In vitro* data further suggests that targeting of membranes to chromatin is at least partially regulated by NE-specific transmembrane proteins binding to DNA and/or chromatin [50,57].

Recent proteomic studies have revealed a large number of INM proteins [58] and many of them can bind chromatin and even DNA directly [50,57]. This property might help to ensure that they reassociate with decondensing chromatin to aid chromatin enclosure in a concerted fashion – possibly triggered by their dephosphorylation as well as by changes of chromatin.

One chromatin-associated protein implicated in the process of NE assembly is the barrier-to-autointegration factor (BAF) [59]. BAF is known to bind a class of INM proteins sharing a common sequence motif referred to as LEM domain (based on its presence in Lamina-associated polypeptide (LAP2), Emerin and MAN1). In *C. elegans*, BAF depletion leads to defects in post-mitotic NE assembly and mislocalization of lamin, emerin and MAN1 [60]. The chromatin association of BAF during mitosis is controlled through its phosphorylation by vaccinia-related kinase 1 (VRK-1), which promotes the release of BAF from DNA after mitotic entry. Depletion of VRK-1 leads to defects in NE formation, likely caused by a failure to dissociate BAF and its partner INM proteins from chromatin in first place. NE reformation requires chromatin reassociation of BAF, presumably in its dephosphorylated form. These data suggest that the dynamic localization of BAF to chromatin is required for proper NE assembly.

In mammalian cells, oligomerized complexes of BAF and LAP2 α , a soluble LEM protein, have been suggested to serve as a nucleation site for nuclear membrane assembly by recruiting membrane-anchored LEM proteins such as emerin and LAP2 β to specific chromatin regions in late anaphase [61].

While the formation of flat membrane sheets on chromatin could in principal be initiated by the recruitment of DNA-binding INM proteins [57], it is difficult to imagine how they can mediate the final sealing of patches to form a fully closed NE. However, considering the fact that a typical mammalian cell nucleus contains thousands of pores, the formation of a completely closed membrane sphere is not necessary. Instead, membranes might expand across the chromatin surface with the last remaining holes being stabilized and occupied by newly forming NPCs (Figure 2). This step might involve interactions between chromatin-associated nucleoporins [62] and transmembrane nucleoporins like POM121 and NDC1 [63–65], the latter being delivered from the ER network. Such a mechanism would not require a final closing step of the membrane network and therefore could occur without specific NE fusion machinery. Previously observed inhibition of NE formation by non-hydrolyzable GTP analogues [66] and

the dependence on SNARE-mediated membrane fusion [67] could be explained by defects in ER reconstitution due to a block in ER fusion.

NPC assembly: filling gaps and making holes in the NE

New insights have uncovered that chromatin compaction is highest in midanaphase just before NE formation occurs [68]. Interestingly, Aurora B kinase has to be removed from chromatin to allow decondensation to occur. This step is mediated by Cdc48/p97, an AAA-ATPase and ubiquitin-dependent chaperone that promotes NE formation by extracting ubiquitinated Aurora B from chromatin [69]. This novel function of Cdc48/p97 provides the first mechanistic link between chromatin coordination and NE formation.

Recent studies suggest that NPC assembly and NE reformation are coordinated processes initiated from chromatin-bound nucleoporins [12,40,62,70]. The Nup107/160 complex is essential for pore assembly since its depletion results in pore-free NEs [62,71]. It is targeted to chromatin by ELYS/MEL-28, a process that can occur independently of membranes *in vitro* [70,72]. ELYS binding to chromatin occurs via its AT hooks, small DNA binding motifs [73]. Two additional nucleoporins, Nup153 and Nup50, associate with chromatin prior to recruitment of membranes [12]. However, since most of both proteins bind to the intact NE only when nuclear import is reinitiated, they are probably not involved in NE formation.

Known spatial regulators for the deposition of nucleoporins on chromatin are the small GTPase Ran and its regulator, the transport receptor importin β [74]. Ran, when bound to GTP, dissociates importin β from its binding partners and thereby regulates nucleocytoplasmic transport in interphase cells [75], as well as spindle formation and nuclear assembly in mitosis [31]. Several nucleoporins including Nup153, Nup358 and the Nup107/160 complex bind to importin β during mitosis, an interaction that negatively regulates NPC assembly [76,77]. RanGTP is generated on chromatin by its exchange factor RCC1. This produces a local gradient of high RanGTP concentration around chromatin [78], which triggers the local release of importin β from nucleoporins and consequently assembly into NPCs. In addition to Ran, recruitment of nucleoporins is likely controlled by their timely dephosphorylation, which also may control their ability to bind to chromatin and to each other upon anaphase onset, although clear data is lacking. A potential candidate for nucleoporin dephosphorylation is PP1, which is recruited to the NE by AKAP149 and has been shown to remove mitotic phosphorylation marks from lamin B in telophase to allow lamina assembly [79].

After the initial recruitment of some nucleoporins to chromatin, a crucial yet poorly understood step in NPC reassembly is the establishment of contacts to the NE membrane, presumably to the transmembrane nucleoporins POM121 and NDC1 [63,65]. Interestingly, POM121 and the Nup107/160 complex may have a checkpoint function in coordinating the assembly of NPCs and nuclear membranes [65]. How this postulated checkpoint functions in molecular terms is still unknown.

The incorporation of Nup107/160, POM121 and NDC1 most likely mark the formation of a pore spanning the INM and ONM. In order to stabilize the highly curved fusion pore membrane (i.e. connections of INM and ONM) additional nucleoporins have to be attached. Examples are the Nup93 and Nup62 subcomplexes, which combined add almost a third of all known NPC components [12]. The members of the Nup62 complex and several other nucleoporins contain phenylalanine-glycine (FG) repeats, which establish transport capability and the permeability barrier. Import activity of NPCs starts concomitantly with the association of the Nup93 and Nup62 subcomplexes [12]. Nup53, a member of the Nup93 complex, interacts with the transmembrane nucleoporin NDC1, establishing a link between the pore membrane and the soluble nucleoporins [63,80]. Nup155, another subunit of the Nup93 complex, is required for NPC and NE formation in *Xenopus* egg extract and *C. elegans* [81]. In addition, the Nup93

complex contributes significantly to the exclusion limit of the NPC [82] and may play a major structural role in the assembly and formation of NPCs from chromatin-bound NPC intermediates.

The final step of NPC assembly is the addition of peripheral nucleoporins such as Nup214, Nup153, Tpr and Nup50 as well as the membrane nucleoporin gp210 [12].

In summary, NPC assembly at the end of mitosis is a highly complex multi-step process that involves molecular interactions between chromatin, nuclear membrane and soluble nucleoporins. It is important to note that new pores are also formed during interphase [83], when the total number of NPCs doubles. Whether mitotic and interphase assembly of pores exhibit different molecular requirements remains to be determined.

Outlook

The dynamic reorganization of the NE during mitosis is of fundamental importance for chromosome segregation and re-establishment of the nuclear compartment in the daughter cells. Many open questions and technical challenges in studying NEBD and nuclear assembly remain. For instance, membrane reorganization events such as NEBD occur rapidly, making it difficult to dissect and analyze individual steps of this process. In addition, membrane intermediates are difficult to preserve by fixation protocols, requiring detailed studies in living cells. Another obstacle is the complexity of nuclear disassembly and reformation, which involves molecular interactions between chromatin, membranes and soluble components. Importantly, only a small fraction of proteins that associate with the NE have been characterized. Since many of these NE-specific proteins can interact with chromatin or the cytoskeleton, it can be expected that the NE will become of central interest for our understanding of eukaryotic chromatin and cell organization. Recent advances in the structural analysis of nucleoporins, characterization of the NE proteome, advances in fluorescence microscopy and cell-free reconstitution systems hold the promise of exciting new insight into the biology of the NE in the near future.

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Figure 1.

Schematic illustration of NEBD. In G2, the cell nucleus has completed DNA replication and NPC duplication. The NE (dark green), which is continuous with the ER network (green), encloses the chromosomes (blue). NPCs (red) mediate nuclear transport. At this stage, the ER is composed of tubules and sheets. When cells enter mitosis, NPCs disassemble and the NE gets reabsorbed into the ER, which at this stage is composed of tubules. NPC components are dispersed into the cytoplasm, except for three transmembrane nucleoporins, which together with other NE proteins are distributed into the ER (dashed lines). Centrosomes move to the NE (orange dots) and microtubules (purple) participate in the rupturing of the NE. In metaphase, a subset of NPC components has associated with kinetochores and the spindle is established. At this stage, chromosomes at the metaphase plate are devoid of membranes.

Figure 2.

NE reformation around segregated chromosomes in one of the two daughter cells. In anaphase ER tubules associate with chromatin (purple arrows) and a subset of nucleoporins associates with chromatin. This step involves the small GTPase Ran and phosphatases such as PP1 [79, 84,85]. Additional membrane tubules and NE proteins are recruited to the chromatin surface and mediate NE flattening. At this stage most NE proteins are cleared from the ER network. In late anaphase/early telophase a closed NE has formed with pores being assembled in a step-wise manner. Once pores become transport competent, the NE expands and cells move into G1.