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The life cycle of the metazoan nuclear envelope

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The nuclear envelope is a double-layered membrane that encloses the nuclear genome and transcriptional machinery. In dividing cells of metazoa, the nucleus completely disassembles during mitosis creating the need to re-establish the nuclear compartment at the end of each cell division. Given the crucial role of the nuclear envelope in gene regulation and cellular organization, it is not surprising that its biogenesis and organization have become active research areas. We will review recent insights into nuclear membrane dynamics during the cell cycle.

Introduction

A physical membrane barrier around the nucleus was first suspected almost 100 years ago based on micromanipulation studies [1]. Later, electron microscopy (EM) images revealed that these ‘nuclear envelopes’ consist of two parallel membranes, the inner (INM) and outer nuclear membrane (ONM) [2]. Both membranes are penetrated by nuclear pore complexes (NPCs), large protein assemblies that mediate bidirectional exchange of molecules between the nucleoplasm and the cytoplasm [3]. The ONM is continuous with the endoplasmic reticulum (ER) and is studded with ribosomes. Although lipids can diffuse freely between the NE and the ER (Figure 1, Interphase), the protein composition of the NE differs dramatically from ER tubules and sheets. This indicates specific mechanisms of protein targeting and unique roles of the NE in regulating nuclear functions. Research of the last few years has revealed active roles of the NE in the organization of chromatin and the cytoskeleton as well as in cell cycle progression [4]. For instance, recent studies have demonstrated that heterochromatin tightly associates with the INM and the targeting of certain chromatin regions to the NE directly controls gene expression [5-7].

NE proteins, which mediate the multiple NE functions, can be categorized in essentially four classes: components of the nuclear pore, INM- and ONM-proteins and lamins. The first group is composed of ~30 proteins, nucleoporins, which constitute the NPCs, the exclusive sites of nucleocytoplasmic transport [3]. NPCs form hollow cylinders with nucleoplasmic and cytoplasmic filamentous attachments that together form a transport channel across the lipid bilayer [8]. Although NPCs are membrane embedded, only three transmembrane nucleoporins have been identified, leaving us with the puzzle of how this ~90 MDa complex assembles. A detailed model of the molecular architecture of the yeast NPC was recently proposed based on biophysical and proteomic parameters [9], and is likely to provide important insights into the pore structure in metazoa since the overall shape and protein folds seem to have been conserved throughout evolution. The second group of proteins, which specifically localizes to the INM [10], links the NE to chromatin organization. For instance, several integral membrane proteins such as emerin, lamin B receptor, Lap 2 β and MAN1 have been shown to interact with regulators of chromatin organization (e.g. HP1 and BAF) and transcription factors [11,12]. Only recently, INM proteins have also been shown to ‘communicate’ with the third class of

ONM-specific proteins, which interact with the cytoskeleton [13]. A general principle emerging from these studies is that protein ‘bridges’ are established across the perinuclear space, e.g. lamin-interacting SUN proteins and actin-binding proteins such as nesprins [14, 15]. The fourth class of NE proteins constitutes the lamina, a meshwork of intermediate filaments, which is composed of A- and B- type lamins. Strikingly, mutations in lamins and INM proteins are linked to a large number of different human diseases [16,17] and aging [18], highlighting the crucial role of the NE protein network for normal cell function.

The dynamic life cycle of the NE in proliferating cells

Yeast, filamentous fungi and some protists undergo closed mitosis where either the spindle forms inside the nucleus or their microtubules are able to penetrate an intact nuclear envelope [19-21]. In contrast, the NE of metazoan cells completely disintegrates during cell division in order to allow the mitotic spindle to access chromosomes [22]. As a consequence of this open mitosis every dividing cell has to reform the NE and reestablish the identity of the nuclear compartment [23]. In the following paragraphs we will describe new findings regarding the dynamic changes of the vertebrate NE during the cell cycle and describe molecular mechanisms that regulate the life cycle of the nuclear membrane.

NE breakdown

Nuclear envelope breakdown (NEBD) marks the entry into mitosis and precedes the formation of the mitotic spindle apparatus (Figure 1, Prophase). In somatic mammalian cells, interactions of microtubules with the NE generate mechanical forces that contribute to rupture the lamina in a dynein-mediated process [24-26]. Interestingly, the small GTPase Ran has been suggested to regulate microtubule dynamics during NEBD, adding to many functions of Ran throughout the cell cycle [24]. However, nuclear disassembly can also occur in the absence of microtubules [27] indicating that NEBD is regulated at several levels. In *Drosophila* embryos and starfish oocytes, NPC disassembly appears to be an early event in NEBD [27,28]. In the latter system, NEBD proceeds in two defined steps, sequential release of peripheral nucleoporins followed by fenestration of the NE by large gaps [27].

Currently, it is unclear what triggers NEBD, but the phosphorylation of NE components by several kinases such as Cdk1, PKC, NIMA and Aurora A seems to be crucial [29-32]. Hyperphosphorylation of NE proteins is thought to result in disrupting protein complexes and/or in the activation of factors involved in NEBD. Recently, results from *C. elegans* and *Xenopus* egg extracts point to a critical, yet non-essential role of the trans-membrane nucleoporin gp210, which is enriched in its phosphorylated form at the NE just before NEBD occurs [33]. The mechanism by which gp210 triggers disassembly of NPCs the lamina remains to be analyzed.

Depletion of Nup153 from extracts inhibits nuclear envelope breakdown (NEBD) during mitosis. Nup153 was shown to recruit the COPI complex, which mediates retrograde transport from the Golgi to the ER, to the nuclear envelope [34]. A direct role of the COPI complex in this process was further supported by antibodies against β -COP which inhibited NEBD [34]. What roles Nup153 and the COPI complex play in this process has yet to be elucidated.

The Mitotic NE

Between prophase and early anaphase, during which time chromosomes align in the metaphase plate and segregate, chromatin is essentially free of membranes (Figure 1, Metaphase) [35, 36]. Also, the majority of soluble nucleoporin subcomplexes is thought to be distributed throughout the cytoplasm, whereas all transmembrane NE proteins, including the three nucleoporins gp210, Ndc1 and POM121, have been shown to reside in the mitotic ER

[35-37]. The localization of these membrane proteins suggests that the precursor membrane of the NE is the mitotic ER. This notion is further supported by recent findings using 3D modeling of electron tomography, which demonstrates that the ER remains an intact network in mitosis. Interestingly, in contrast to interphase, the ER in metaphase and anaphase is essentially free of sheets and forms a dense yet highly dynamic network of tubules [37].

NE Reformation

In late anaphase, membranes start to associate with chromatin by a poorly understood mechanism (Figure 1, anaphase). Recent studies of intact cells and cell-free nuclear assembly systems show that ER membrane tubules are targeted to chromatin via tubule ends and reorganized into flat nuclear membrane sheets by DNA-binding-NE-specific membrane proteins [38]. In contrast to previous models, which proposed vesicle fusion to be the principal mechanism of NE formation [39], these new studies suggest that the nuclear membrane forms by the chromatin-mediated reshaping of the endoplasmic reticulum [36,37]. This notion is further supported by recent high-resolution EM data of mitotic ER [37] and *in vitro* studies, which show that the formation of ER tubules are required for NE assembly [36].

How are the ER tubules targeted to chromatin? The simplest idea is that integral ER proteins with chromatin-binding capacity are recruited to chromatin. This idea is consistent with recent findings that DNA-binding activity of some INM proteins is required for NE formation *in vitro* [38] and that membrane sheets formed efficiently on protein-free immobilized DNA *in vitro* [36]. Additionally, binding of several INM proteins to chromatin constituents has been implicated in NE assembly. For example, two studies have demonstrated that the integral INM protein LBR, which binds to heterochromatin-binding protein 1 (HP1), is required for targeting and anchoring NE membranes to chromatin *in vitro* [40,41]. Similarly, the barrier-to-autointegration factor (BAF), a chromatin-binding protein [42] and its kinase Vrk have recently been shown to play a direct role in NE formation by recruiting LEM domain proteins to chromatin [43]. Vrk seems to be a critical regulator in this process since BAF phosphorylation reduces chromatin binding and interactions with LEM domain proteins such as emerin [12, 44]. In summary, NE formation is likely to involve a complex interplay of trans-membrane NE proteins distributed into mitotic ER with the reorganizing chromatin.

Chromatin undergoes a number of conformational changes during cell division, mainly massive condensation in prophase, segregation in anaphase, and decondensation in telophase (Figure 1). Interestingly, maximal chromatin compaction is not reached in metaphase but in late anaphase, after sister chromatid segregation [45]. The chromokinesin kinesin-like DNA binding protein (Kid) has recently been shown to be required for the formation of a compact chromosome cluster during anaphase and the proper enclosure of the segregated chromosomes into a single nucleus [46]. Kid targets to the space between anaphase chromosomes and is involved in chromatin condensation. The depletion of Kid results in fenestrated nuclei and multi-nucleation in early embryonic cells. Interestingly, Kid targeting to anaphase chromosomes is an Importin- β -dependent process [47], adding to the growing number of mitotic processes regulated by this transport receptor.

Recent findings support the idea that NE formation and chromatin decondensation are mechanistically linked. It was shown that Cdc48/p97, a hexameric ATPase previously implicated in membrane fusion [48] and ubiquitin-dependent processes [49], inactivates Aurora B by extracting it from chromatin allowing chromatin decondensation. Inhibition of Cdc48/p97 blocked NE formation suggesting that chromatin decondensation is required for NE formation [50]. Therefore, Aurora B removal might be a crucial step in opening chromatin structure. Since NE membranes associate with chromosomes in late anaphase, it will be

interesting to determine how these complex remodeling process regulate recruitment of ER membranes to initiated NE formation (Figure 1, Telophase).

While the above studies are consistent with the idea that NE formation occurs by chromatin-mediated reshaping of the ER, as mentioned above, there are experimental data that suggest that the NE forms by the fusion of vesicles. Results from *Xenopus* have shown that in vitro nuclear assembly initiated from fragmented ER vesicles is blocked by GTP γ S [51,52]. However, these findings do not discriminate between fusion events that are involved in ER reconstitution or NE assembly. When the ER is allowed to preform, NE formation occurs in the presence of GTP γ S and ATP γ S, suggesting that membrane fusion is not required to form flat sheets [36]. Similarly, a recently observed role of SNARE proteins in NE formation could be an indirect effect of blocked ER reconstitution [53].

Simultaneously with membrane coating of chromatin, NPCs are reassembled from disassembled precursors in late anaphase/telophase. This assembly process is coordinated by the stepwise recruitment of NPC proteins to chromatin [54]. Several nucleoporins have been shown to be essential for pore assembly [23]. Most notably, depletion of the Nup107/160 complex, results in NPC-free nuclear membranes [55,56]. In vertebrates only two out of the three transmembrane nucleoporins seem to be involved in NE formation, Ndc1 and POM121 [57,58]. Their exact role is unknown, but an interesting link between POM121 and the Nup107/160 complex has been made. Nuclear membrane formation might actually be linked to pore assembly by a poorly understood checkpoint by which the Nup107/160 complex ‘senses’ nuclear membranes [59]. Interestingly, Nup133, a member of the Nup107/160 complex, has been identified as containing an ALPS-like motif, which contains an amphipathic alpha-helical domain that have been shown to act as a membrane curvature sensor in vitro [60]. It is possible that this domain is involved in targeting the Nup107/160 complex to membranes during NE formation.

While the targeting of soluble nucleoporins to membranes and chromatin during NE formation is still not fully understood, some progress has been made in determining how the Nup107/160 complex is recruited to chromatin. Mel-28/ELYS was identified in a screen in *C. elegans* for factors involved in pronuclear formation and in its absence the Nup107/160 complex is no longer recruited to chromatin [61,62]. Mel-28/ELYS contains an AT-hook domain [63] and the simplest model is that it binds directly to DNA and recruits the Nup107/160 complex. RanGTP stimulates Mel-28/ELYS recruitment [62], revealing yet another Ran-mediated step in NE formation [23]. Other nucleoporins have been implicated in pore assembly, but their exact role remains to be determined. For instance, a complex of Nup53 and Nup155 has recently been shown to be essential for NE formation in nematodes and vertebrates [64,65], however, how Nup53 coordinates interactions between chromatin, membranes and soluble Nup155 remains unclear.

The interphase nucleus

The NE is reformed as a closed membrane barrier at the end of cell division, reestablishing the nuclear compartment by selective nuclear transport, i.e. coordinated redistribution of mitotically dispersed cytosolic and nucleoplasmic components. Relatively little is known about this transition and questions such as when pores become active for transport remain to be analyzed. However, it is clear that even after its formation, the NE undergoes a series of changes necessary for cell cycle progression and transcription. These steps include the assembly of a lamina [66], insertion of new pores, and NE expansion [23]. A recently uncovered interaction between the nucleoporin ELYS and Mcm2–7 replication licensing proteins suggest a direct link between pore assembly and DNA metabolism [67]. The determinants of nuclear size and pore number remain to be determined.

As cells grow and prepare to divide, the number of NPCs doubles and the NE surface increases substantially [68]. New pore insertion into an intact NE occurs by a *de novo* mechanism and requires RanGTP-mediated release of Importin β from a subset of nucleoporins [69]. One of the key questions in this poorly understood process is how the INM and ONM fuse in order to generate a membrane hole. Since membrane pore formation and NPC assembly are likely to be mechanistically coordinated, it is possible that nucleoporins are directly involved in both events. In analogy to vesicle or viral fusion with target membranes, transmembrane proteins residing on either one or both sides of the nuclear envelope could be involved in the fusion of ONM and INM, making transmembrane nucleoporins ideal candidates to fulfill this function. However, none of the three metazoan transmembrane nucleoporins are found in all species, suggesting that they can act redundantly or that a yet unidentified membrane component mediates INM/ONM fusion.

Nuclear expansion requires the supply of additional nuclear membrane and proteins. In vitro nuclear expansion is blocked by disrupting the connection of nuclei with the peripheral ER [70], suggesting that membranes feed into the ONM via connections with ER tubules [36]. In growing *Xenopus* oocyte nuclei the presence of vesicles on the ONM has been interpreted as evidence for vesicle fusion. However, an alternative explanation is that these vesicles were generated during EM sample preparation by rupturing of ER tubules [71].

Growth of the INM requires passage of membrane components through the fusion sites with the ONM at the NPCs. The current view is that INM proteins are retained once they reach the INM by interactions with either the lamina or chromatin. How INM proteins are targeted in metazoa is less clear. One model suggested that ATP-driven changes in nucleoporin interactions might allow membrane proteins to travel across the NPC [72]. In yeast, integral INM proteins have been shown to directly interact with specific nucleoporins and transport receptors to promote their movement past the NPC [73]. By the end of interphase, the NE has undergone major changes in protein composition and is ready to break down at the onset of mitosis to start a new life cycle in the two daughter cells.

Conclusions

The NE has emerged as a critical interface between chromatin and the cytoskeleton. Many questions remain in regard to the regulation of NPC assembly and membrane targeting and flattening during NE formation. A related developing topic of study is addressing how the interaction of chromatin and the nuclear envelope regulates gene expression. It is possible that the early connection points made between NE-membrane proteins and chromatin during NE formation establish a level of chromatin regulation that has lasting effects on gene expression. This idea is supported by the recent observation that repositioning of genes to the nuclear periphery, which resulted in gene silencing, required NE break down and reformation. It will also be interesting to study how the major classes of NE proteins are regulated during the cell cycle and differentiation. Cell-type and development-specific expression of INM proteins [74] certainly suggests that there is still a lot to be learned about the crucial role of the NE in eukaryotic cell function.

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Figure 1.

NE dynamics during the cell cycle. Changes in the organization of the NE throughout open mitosis are shown in representative live-cell images and schematic illustrations. In live-cell images, ER and NE membranes are labeled with Sec61 β (aa1–65)GFP (green), microtubules are labeled with α -tubulin-mcherry (red) and chromatin is stained with Hoechst 33342 (blue). **Interphase:** During interphase the NE completely surrounds the nucleus and is punctuated by nuclear pore complexes (NPC), which are transport channels present where the inner and outer nuclear membrane (INM, ONM) fuse. Distinct transmembrane proteins are present in the INM that bind to the nuclear lamina, a protein meshwork found within the nucleus, and chromatin. The ONM forms a continuous lipid bilayer with the ER, sharing many of the ER-associated proteins. **Prophase:** NE breakdown is initiated during prophase, when many of the constituents of the NE and lamina are phosphorylated to disrupt the protein-protein interactions. This, along with microtubule tearing of the NE (arrows) rapidly exposes the chromatin to the forming spindle. **Metaphase:** By metaphase the membranes of the NE have completely redistributed into the ER, clearing all membranes from the chromosomes. Soluble proteins of the NE and

lamina are distributed mainly through the cytoplasm. **Anaphase:** Late in anaphase, NPC components and ER tubule tips are targeted to the segregated chromosomes, likely by dephosphorylation of the proteins involved in these targeting processes. **Telophase:** In telophase membranes expand around the chromosomes and NPC continues to assemble.