The Nuclear Envelope

Martin W. Hetzer

Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, La Jolla, California 92037

Correspondence: hetzer@salk.edu

The nuclear envelope (NE) is a highly regulated membrane barrier that separates the nucleus from the cytoplasm in eukaryotic cells. It contains a large number of different proteins that have been implicated in chromatin organization and gene regulation. Although the nuclear membrane enables complex levels of gene expression, it also poses a challenge when it comes to cell division. To allow access of the mitotic spindle to chromatin, the nucleus of metazoans must completely disassemble during mitosis, generating the need to re-establish the nuclear compartment at the end of each cell division. Here, I summarize our current understanding of the dynamic remodeling of the NE during the cell cycle.

The NE, a hallmark of eukaryotic cells, is a highly organized double membrane that encloses the nuclear genome (Kite 1913). Early electron microscopy (EM) images revealed that the inner (INM) and outer nuclear membranes (ONM) are continuous with the endoplasmic reticulum (ER) (Watson 1955). Despite the lipid continuity between the NE and the ER, both ONM and INM are comprised of diverse groups of proteins that are typically not enriched in the ER (Hetzer et al. 2005) (Table 1). The *first* group consists of ~30 different polypeptides, called nucleoporins or Nups, which form the ~40–70 MD nuclear pore complexes (NPCs) (Tran and Wentz 2006; D’Angelo and Hetzer 2008). NPCs are aqueous channels that show eight-fold rotational symmetry with an outer diameter of ~100 nm and a central transport channel measuring 40 nm in diameter, through which bidirectional exchange of proteins, RNA, and

ribonucleoprotein complexes between the nucleoplasm and cytoplasm occurs (Beck et al. 2004; Beck et al. 2007; Terry et al. 2007). A subset of Nups is stably embedded in the NE, forming a scaffold structure or NPC core (Rabut et al. 2004; D’Angelo et al. 2009), which is thought to stabilize the highly curved and energetically unfavorable pore membrane (Alber et al. 2007; Boehmer et al. 2008). This core includes the Nup107/160 complex (Nup84 complex in yeast) and the Nup205 complex (yeast Nup170), which together constitute ~50% of the entire NPC (Fig. 1) (Brohawn et al. 2009). Attached to this scaffold are peripheral Nups, many of which contain phenylalanine-glycine (FG) rich repeats that establish a permeability barrier and also mediate active, receptor-dependent transport across the NE (Peters 2009). A *second* group of NE proteins, specifically localizes to the INM (Fig. 1) (Schirmer and Gerace 2005).

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Table 1. List of NE proteins.

NPC	INM	ONM	Lamina
Nup35	LBR	Nesprin-3/ Net35	Lamin A
Nup37	Lap1	Syne/Myne/ Nesprin 1	Lamin B1
Nup43	Lap2b	Nesprin-2a and b	Lamin B2
Nup50	Emerin	Syne/ Nesprin-2G	Lamin C
Nup54	MAN1	Samp1	
Nup58/45	Nurim		
Nup62	NET8		
Nup75	NET38		
Nup88	NET56		
Nup96*	LEM2/ NET25		
Nup98*	NET9		
Nup107	NET32		
Nup133	NET37		
Nup153	Sun1		
Nup155	Sun2		
Nup160	LUMA		
Nup188	~ 60 NETs		
Nup205			
Nup214			
Nup358/ RanBP2			
Sec13R			
Seh1			
Pom121			
Ndc1			
Gp210			
Tpr			
Rae1			
Aladin			
Nlp1/hCG1			

The nuclear pore complex (NPC) contains ~30 nucleoporins (Nups). *Nup98 and Nup96 are synthesized as a single polypeptide that becomes autoproteolytically cleaved to give rise to Nup98 and Nup96. Inner nuclear membrane (INM) proteins: lamin B receptor (LBR), lamin-associated protein (LAP), Nuclear Envelope Transmembrane protein (NET). **More than 60 NETs have no assigned function. Outer nuclear membrane (ONM): spectrin repeat containing nuclear envelope protein (syne), spindle-associated membrane protein 1 (Samp1). Lamina: lamin A and C are products of alternative splicing.

Although most of these >60 integral membrane proteins (also referred to as NE transmembrane proteins or NETs [Schirmer et al. 2003]) remain largely uncharacterized, interaction with lamins (see later) and chromatin have been shown for some of them, such as lamin B receptor (LBR), lamina-associated polypeptide (LAP) 1, LAP2, emerin, and MAN1 (Akhtar and Gasser 2007; Dorner et al. 2007; Schirmer and Foisner 2007). It is becoming increasingly clear that INM proteins play vital and diverse roles in nuclear function such as chromatin organization, gene expression, and DNA metabolism (Mattout et al. 2006; Heessen and Fornerod 2007; Reddy et al. 2008). Importantly, improper localization and function of INM proteins have been linked to numerous human diseases, which has sparked considerable interest in NE biology over the last decade (Vlcek and Foisner 2007; Worman and Bonne 2007; Neilan 2009).

A *third* class of NE proteins specifically resides in the ONM (Fig. 1). This diverse group of integral membrane proteins shares a small KASH (Klarsicht, ANC-1, Syne Homology) domain, which has been shown to interact with Sad1p/UNC-84 (SUN)-domain proteins of the inner nuclear membrane within the periplasmic space of the NE (Starr and Han 2003; Wilhelmsen et al. 2006). Two other related ONM proteins, nuclear envelope spectrin repeat (nesprin)-1 and -2, have been shown to directly interact with the actin cytoskeleton through their amino-terminal actin-binding domain (ABD) (Wilhelmsen et al. 2005). These ONM proteins are implicated in nuclear positioning that is essential for processes such as cell polarization, pronuclear migration, and the organization of syncytia (Fridkin et al. 2009). In addition, ONM and INM proteins form “bridges” across the perinuclear space that might be involved in separating the two NE membrane leaflets at an even distance of ~50 nm (Voeltz and Prinz 2007). These luminal proteinaceous bridges could establish physical connections between the cytoskeleton and chromatin,

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Figure 1. Topology of the NE. Inner and outer nuclear membranes (INM and ONM, respectively) are separated by the ER lumen or perinuclear space (PNS). The nuclear lamina interacts with NE proteins and chromatin. INM proteins link the NE to chromatin and the lamina. ONM proteins provide a connection from the nucleus to the cytoskeleton. The lamin B receptor (LBR) interacts both with B-type lamins and chromatin-associated heterochromatin protein 1 (HP1) in conjunction with core histones. Members of the LEM (lamina-associated protein 2 [LAP2], emerin, MAN1)-domain family (pink) bind to lamins and interact with chromatin through barrier-to-autointegration factor (BAF). SUN proteins (SUN 1 and 2) interact with nesprins in the ONM, thereby forming so-called LINC complexes that establish connections to actin and intermediate filaments in the cytoplasm. Nurim is a multi-pass membrane protein with unknown function. Proteomic approaches have identified ~60 putative transmembrane proteins (NETs), most of which remain uncharacterized.

which might be relevant for transcription, replication, and DNA repair mechanisms (Tzur et al. 2006; Stewart et al. 2007). The *final* group of NE proteins constitutes the lamina, a meshwork of intermediate filaments that is composed of A- and B-type lamins (Gruenbaum et al. 2000). Although the lamina has been shown to be critical for nuclear stability, particularly in tissues that are exposed to mechanical forces such as muscle fibers (Cohen et al. 2008), it has become clear that lamins also play major roles in chromatin function and gene expression (Gruenbaum et al. 2005; Dechat et al. 2008; Reddy et al. 2008). Similar to INM proteins, mutations in lamins are linked to a large number of diverse human diseases (Mounkes et al. 2003; Muchir and Worman 2004; Mattout et al. 2006) and to aging (Gruenbaum et al.

2005; Scaffidi and Misteli 2006), highlighting the crucial role of the NE protein network for normal cell function.

In summary, the NE fulfills a critical role in shielding the genome from cytoplasmic components, but also represents a highly specialized membrane that provides anchoring sites for chromatin and the cytoskeleton (D'Angelo and Hetzer 2006).

NE REMODELING IN DIVIDING CELLS

Although a membrane-enclosed nuclear genome can be found in all eukaryotes, there is a critical difference in the cell-cycle dependent dynamics of the NE between “lower” eukaryotes (e.g., yeast and filamentous fungi) and metazoa (i.e., “higher” eukaryotes). The former undergo

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closed mitosis, where spindle microtubules can either form inside the nucleus or are able to penetrate an intact nuclear membrane (Heywood 1978; Byers 1981; Ribeiro et al. 2002). In contrast, the NE of metazoan cells completely disintegrates during cell division to allow the mitotic spindle to access chromosomes (Kutay and Hetzer 2008). As a consequence, every dividing cell has to reform the NE and re-establish the identity of the nuclear compartment (Hetzer et al. 2005; Anderson and Hetzer 2008b; Guttinger et al. 2009).

NE remodeling in proliferating cells is a highly dynamic process that involves a vast number of molecular players (Figs. 2,3). By the end of interphase in G₂, the nuclei have duplicated their genome, doubled the number of NPCs, and increased the surface area of the NE. The surrounding ER network is continuous with the NE, but not enriched in NE proteins. The entry of mitosis, i.e., prophase, is marked by NE breakdown (NEBD) and the loss of the nucleo-cytoplasmic compartmentalization (Burke and Ellenberg 2002). Between NEBD

and early anaphase, when chromosomes align in the metaphase plate and subsequently segregate, chromatin is essentially free of membranes (Puhka et al. 2007; Anderson and Hetzer 2008a). During these cell-cycle stages, the majority of soluble NE proteins are distributed throughout the cytoplasm and transmembrane NE proteins reside in the mitotic ER (Ellenberg et al. 1997; Anderson and Hetzer 2007; Puhka et al. 2007) (Fig 2). In anaphase, ER membranes begin to reassociate with and rapidly enclose the chromatin mass (Anderson and Hetzer 2008a). Chromatin association of a subset of Nups, decondensation of chromatin, and the assembly of new NPCs occurs concomitantly with NE formation (Anderson and Hetzer 2008c). At the end of cell division, the NE has reformed as a closed membrane barrier and re-establishes the nuclear compartment by enabling selective nucleo-cytoplasmic transport (Dultz et al. 2008). After its formation, the NE expands and undergoes additional structural changes necessary for cell-cycle progression and transcription, including the assembly of new

Figure 2. Schematic illustration of NE breakdown. 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/blue) mediate nuclear transport. 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 and NE proteins are partitioned into the ER (dashed green lines). Centrosomes (orange dots) move to the NE 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.

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Figure 3. Schematic illustration of NE reformation around segregated chromosomes in one of the two daughter cells. In anaphase, ER membranes associate with chromatin (red arrows) and a subset of nucleoporins associates with chromatin. 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.

NPCs (Winey et al. 1997; D'Angelo et al. 2006) (Fig. 3).

BREAKING DOWN THE NUCLEUS

Live cell imaging of mammalian cells has revealed that NEBD, which occurs rapidly within a few minutes in prophase (Lenart et al. 2003), occurs in a step-wise fashion. One of the initial events is the selective loss of Nups from the NPCs (Terasaki et al. 2001; Dultz et al. 2008; Katsani et al. 2008). Nup98, a dynamic NPC component (Griffis et al. 2002), is released before the rest of the NPC components, which disassemble synchronously (Dultz et al. 2008). Collective dispersal of Nups can also be observed in *Drosophila*, suggesting that pore disassembly is evolutionarily conserved (Katsani et al. 2008). Biochemical evidence suggests that the NPC disassembles into stable subcomplexes and not necessarily into individual polypeptides (Miller and Forbes 2000; Harel et al. 2003b; Walther et al. 2003a), which might stabilize the nucleoporins and allow more rapid reformation of NPCs at the end of mitosis.

Unfortunately, we do not have a clear picture of the biochemical nature of the disassembled NPC components. Recent evidence suggests that some nuclear pore components are degraded in a proteasome-dependent manner during mitosis, a process that might regulate total pore numbers. Furthermore, in the same study, Nup43 has been shown to be absent from the mitotic Nup107/160 complex, which therefore seems to differ from its counterpart in interphase (Chakraborty et al. 2008).

THE ROLE OF KINASES IN NE BREAKDOWN

NPC disassembly is critical for early stages of NEBD, such as disrupting the permeability barrier and allowing the influx of molecules, which are thought to be critical for further disassembly steps. For example, protein kinases like cyclin-dependent kinase 1 (Cdk1/CycB1) may require access to proteins of the INM and lamins for proper mitotic regulation (Wu et al. 1998). That is consistent with the idea that the bulk of lamin disassembly occurs after the NPCs are permeabilized (Lee et al. 2000; Beaudouin

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et al. 2002). As the lamina is disrupted, many INM proteins lose their anchor and are released into the ER (Ellenberg et al. 1997; Yang et al. 1997; Puhka et al. 2007; Anderson et al. 2009a; Lu et al. 2009).

One of the critical events during NEBD is the hyperphosphorylation of NE proteins that is thought to disrupt protein complexes and/or play a role in the activation of factors involved in this process. Several kinases, such as Cdk1/CycB1, protein kinase C (PKC), Nima, and Aurora A, have been implicated in NEBD (Collas 1999; Burke and Ellenberg 2002; Gong et al. 2007; Portier et al. 2007). The key kinase seems to be Cdk1, which phosphorylates lamins and Nups, including the Nup107/160 complex, Nup93 subcomplex, Nup53, Nup98, Ndc1, and gp210 (Guttinger et al. 2009). The disassembly of INM proteins, such as Lap2 α and β and LBR, seem to depend on Cdk1 (Courvalin et al. 1992; Dechat et al. 1998; Dreger et al. 1999). Several other kinases have been shown to participate in NEBD including PKC (Goss et al. 1994), Aurora A (Portier et al. 2007), and polo-like kinase (PLK1) (Chase et al. 2000); however, their targets remain to be determined. Lamin B is the only known target of PKC during NEBD (Goss et al. 1994).

In this context, it is worth mentioning that NPCs in organisms with closed mitosis are also partially disassembled. For instance, in *Aspergillus nidulans*, 14 Nups are released, leaving a partial NPC that allows the entry of the Cdk1/cyclin B complex (Osmani et al. 2006). This partial disassembly requires the kinases Nima and Cdk1 (De Souza et al. 2004). Thus, structural reorganization of the NPC during mitosis might be an evolutionarily conserved feature shared by all eukaryotes.

MEMBRANE DYNAMICS DURING NEBD

An interesting aspect of NEBD was recently revealed by the finding that the nuclear membranes are actively participating in the process. Work in the nematode *Caenorhabditis elegans* has shown that depletion of reticulons and the GTPase Rab5 inhibits NEBD (Audhya et al. 2007). The reticulons are part of a family

of membrane-associated proteins that together with a distinct class of membrane-bending proteins like DP1 have been shown to tubulate the ER (Voeltz et al. 2006). These results suggest that active reshaping of the ER might be a critical step in NEBD. Two nucleoporins have also been implicated in this process; however, their roles remain unclear. The transmembrane nucleoporin gp210 is enriched in its phosphorylated form at the NE just before NEBD occurs (Galy et al. 2008). The mechanism by which gp210 contributes to the disassembly of NPCs and the lamina remains to be characterized. Depletion of Nup153, a dynamic component of the nuclear basket, inhibits NEBD and was shown to recruit the COPI complex, which mediates retrograde transport from the Golgi to the ER, to the NE (Liu et al. 2003). What roles Nup153 and the COPI complex play in this process has yet to be elucidated.

New data suggests that lipid synthesis itself might play a role in NE dynamics. The knock down of lipin, a conserved phosphatidic acid (PA) phosphatase that catalyzes the dephosphorylation of PA to yield diacylglycerol (DAG) (Siniosoglou 2009), causes defects in NEBD, abnormal chromosome segregation, and irregular nuclear morphology in *C. elegans*. Interestingly, down-regulation of nematode Lpin-1 was required for disassembly of the nuclear lamina during late NEBD, suggesting that the Lpin-1 requirement appears to be separable from the effect of Lpin-1 on the peripheral ER (Golden et al. 2009; Gorjanacz and Mattaj 2009).

Although phosphorylation is clearly a key event in NEBD, interactions of the NE with microtubules are thought to generate mechanical forces that assist in rupturing the nuclear membrane and presumably the lamina in a dynein-mediated process (Beaudouin et al. 2002; Salina et al. 2002; Muhlhauser and Kutay 2007). This process might also involve the small GTPase Ran, which has been shown to regulate microtubule dynamics during NEBD, potentially uncovering yet another potential mitotic function of Ran (Muhlhauser and Kutay 2007). However, it should be kept in mind that nuclear disassembly can also occur in the absence of microtubules in vitro (Lenart

et al. 2003), indicating that dynein-dependent rupturing of the NE may not be essential.

STRUCTURE AND FUNCTION OF NE COMPONENTS DURING MITOSIS

As mentioned above, chromosomes are essentially membrane-free between metaphase and anaphase. EM and fluorescence microscopy has provided compelling evidence that the NE is reabsorbed into the ER (Ellenberg et al. 1997; Anderson and Hetzer 2007; Anderson and Hetzer 2008a; Anderson et al. 2009a; Lu et al. 2009). For instance, fluorescence time-lapse microscopy in living cells has shown that NE proteins such as the nucleoporins gp210, Ndc1, and Pom121 are partitioned into the mitotic ER (Ellenberg et al. 1997; Puhka et al. 2007). Likewise, INM proteins can be found in the ER during mitosis (Puhka et al. 2007). The topology of the mitotic ER, however, remains controversial. Three-dimensional modeling of the ER by electron tomography suggested that the mitotic ER remains an intact network of membrane tubules that is essentially free of sheets (Puhka et al. 2007). This is supported by recent data suggesting that the intrinsic propensity of the ER to oscillate between tubules and sheets is used during mitosis and affects the fate of the NE during the mitosis of *C. elegans* (Audhya et al. 2007). However, compelling data from nematodes and mammalian cells challenges the view of an entirely tubular ER and instead suggest that the ER is largely composed of membrane sheets. For instance, it was shown that the rough ER of metaphase HeLa cells appears to be exclusively cisternal and concentrates at the cell cortex, often following the contours of the plasma membrane (McCullough and Lucocq 2005). In a study using three-dimensional (3D) reconstructions of living cells, the ER appeared entirely formed of cisternae and very few tubules were observed (Lu et al. 2009). Similar results were obtained in *C. elegans* (Poteryaev et al. 2005). Determining the exact nature of the ER and the disassembled NE components remain critical future goals, because this has important implications for NE formation.

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NE PROTEINS HAVE MITOTIC FUNCTIONS

Although the dynamic organization of the ER membranes remains elusive, it has become clear that disassembled NE components fulfill critical functions in mitosis. For instance, the multimeric Nup107-160 complex, which in interphase is essential for pore assembly and function (D'Angelo et al. 2006), and Nup358, which shows SUMO E3 ligase activity that modifies RanGAP, Ran's GTPase activating protein (Joseph et al. 2002; Pichler et al. 2002), can both be detected in association with kinetochores in mitosis. In addition, ELYS/MEL-28 and Nup107-160 are also found with spindle poles and proximal microtubules (Loiodice et al. 2004; Galy et al. 2006; Rasala et al. 2006). This association is not critical in *Drosophila*, suggesting that the role of Nups in kinetochore function is a relatively late evolutionary event (Katsani et al. 2008). However, in mammalian cells, the absence of the Nup107-160 complex perturbs bipolar spindle formation (Orjalo et al. 2006), and inhibits Nup358 recruitment to microtubule-bound kinetochores (Salina et al. 2003; Joseph et al. 2004). Recently it was shown that the kinesin-binding domain (KBD) of Nup358 associates with kinesin-1, KIF5B/KIF5C. Interestingly, the KBD stimulates the ATPase activity of KIF5B in the presence of microtubules, thereby increasing its activity (Cho et al. 2009).

The Nup107-160 complex might have an independent role at kinetochores because in a *Xenopus* in vitro spindle assembly assay, the Nup358/SUMO-RanGAP complex does not associate with kinetochores (Arnaoutov and Dasso 2005). Because mNup133 (a mNup107-160 member) and CENP-F (a kinetochore protein) interact with each other, this function might be linked to the dynein partners Nde1 and Nde11, which also bind CENP-F (Vergnolle and Taylor 2007; Zuccolo et al. 2007).

Additional roles for other NE proteins in cell-cycle and mitotic progression have also been reported. Depletion of lamin B, a type V intermediate-filament protein and a component of the nuclear lamina, also results in mitotic spindle defects (Zheng and Tsai 2006).

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Interestingly, the spindle-associated lamin B appears to be present in a membranous, matrix-like network and seems to facilitate spindle microtubule organization in a dynein-dependent manner (Tsai et al. 2006; Ma et al. 2009). Although the mechanistic details of spindle-matrix function with respect to lamin B remain to be determined, these results contribute to the emerging paradigm for structural components of the NE having roles in mitosis. This idea is further supported by the recent finding of spindle-associated membrane protein 1 (Samp1), which during interphase is localized to the inner nuclear membrane, specifically localized to the polar regions of the mitotic spindle (Buch et al. 2009). Depletion of Samp1 expression resulted in separation of centrosomes from the NE, indicating that it is functionally connected to the cytoskeleton. This provides evidence for the existence of interactions between mitotic ER membranes with the spindle.

RE-ESTABLISHING ORDER: REFORMATION OF THE NE

NE formation has been studied in various cell-free systems, cell types, and organisms (Burke and Ellenberg 2002; Hetzer et al. 2005; Anderson and Hetzer 2008c). Because membranes are typically delicate structures that are easily disrupted during cell fractionation and fixation, it is easy to see how data obtained from different systems lead to the postulation of sometimes opposing models. There are essentially two different ideas about how the NE is formed: (1) by vesicle fusion and (2) by reshaping of ER into NE sheets.

Based on biochemical data and EM observations, it was initially proposed that the NE fragments into NE vesicles (Collas and Courvalin 2000). This idea is derived from cell-free systems mainly of *Xenopus*, starfish, and *Drosophila* (Lohka and Masui 1983; Newport 1987). Frog egg extracts have the advantage of containing large stockpiles of disassembled NE components (Newport and Spann 1987) and nuclei can be assembled within ~60 minutes in a test tube by mixing a chromatin source, cytosol, and membranes. These artificial nuclei are

capable of nucleocytoplasmic transport, DNA replication, as well as NEBD (Sheehan et al. 1988; Gant and Wilson 1997; Hetzer et al. 2002). The idea of vesicle fusion as the principal mechanism of NE formation was further supported by findings that GTP γ S, a nonhydrolyzable GTP analog, blocks NE formation and results in assembly intermediates covered with chromatin-bound vesicles (Boman et al. 1992; Hetzer et al. 2001). Interestingly, depletion of the small GTPase Ran mimicked the effect of GTP γ S addition (Hetzer et al. 2000; Zhang and Clarke 2000). Because the Ran exchange factor RCC1 (Ohtsubo et al. 1989) is stably associated with chromatin, it was proposed that high levels of RanGTP are generated around chromatin that provide a spatial signal for chromatin-associated processes such as NE formation and NPC assembly (Bilbao-Cortes et al. 2002; Hetzer et al. 2002; Hutchins et al. 2004). It was subsequently shown that Ran releases the nuclear transport receptor Importin β (Nachury et al. 2001; Harel et al. 2003a; Harel and Forbes 2004) from nucleoporins and thereby triggers NPC assembly (Harel et al. 2003a; Walther et al. 2003b). A similar mechanism might be required for the formation of a closed NE, but the targets of Importin β remain unknown. Corroborating the *in vitro* data, Ran has been shown to be required for NE formation in *C. elegans* (Askjaer et al. 2002) and yeast (Ryan et al. 2003).

Although egg extracts provide a unique experimental system to study ER and NE reconstitution, it is important to realize that the assembly reactions are initiated with isolated membranes, which are in a highly fragmented state that does not represent the intact *in vivo* organization of the mitotic ER. Recent evidence shows that an intact NE can also form from preformed ER *in vitro* (Anderson and Hetzer 2007). Strikingly, nuclear assembly from a preformed ER is insensitive to fusion inhibitors such as GTP γ S, ATP γ S, or antibodies that inhibit the function of the AAA-ATPase p97. In addition, factors that are essential for ER tubule formation, such as ATP (Dreier and Rapoport 2000) and reticulons (Voeltz et al. 2006), only block nuclear assembly if added

before the ER network is organized (Anderson and Hetzer 2007). The results strongly support the notion that the ER is the source of NE membranes. According to this idea, the observed inhibition of nuclear assembly by fusion inhibitors and the role of SNARE proteins in NE formation are likely to be an indirect effect of blocked ER reconstitution (Baur et al. 2007).

Although these experiments show that the NE can form from an ER network in vitro, they do not address the question of how NE formation occurs in intact cells. High resolution imaging in intact cells has revealed ER tubules contacting chromatin at early stages of NE formation. Shortly after contact, the rapid coating of chromatin by ER membranes can be observed (Anderson and Hetzer 2007). Recent data suggest that endogenous concentrations of NE-promoting transmembrane proteins are rate-limiting for nuclear assembly. Because NE formation is also affected by endogenous levels of the ER-shaping reticulon proteins that slow NE formation, these findings suggest a tug of war between reticulons and their membrane-curving activity and NE proteins, which promote membrane attachment and spreading around chromatin (Anderson and Hetzer 2008a). The massive membrane-restructuring event that results in the formation of the sheet-like NE involves functionally diverse groups of NE proteins that collaborate during mitosis to tether membranes to the chromatin surface and thereby drive NE formation (Anderson et al. 2009b). Recent findings that DNA-binding activity of some INM proteins is required for NE formation in vitro (Ulbert et al. 2006) and that membrane sheets formed efficiently on protein-free immobilized DNA in vitro support this idea. In addition, NE formation also seems to involve the ability of several INM proteins to bind to chromatin factors. For example, it has been shown 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 (Collas et al. 1996; Pyrpasopoulou et al. 1996). In a similar fashion, the barrier-to-autointegration factor (BAF), a chromatin-binding protein (Segura-Totten et al. 2002) and its kinase Vrk have recently been shown to play a direct role in

NE formation by recruiting LEM domain proteins to chromatin (Gorjanacz et al. 2007). Vrk seems to be a key regulator in this process because BAF phosphorylation reduces chromatin binding and interactions with LEM domain proteins such as emerin (Bengtsson and Wilson 2004; Hirano et al. 2005). Thus, NE formation is likely to involve a complex interplay of transmembrane NE proteins distributed into mitotic ER with the reorganizing chromatin.

ROLE OF CHROMATIN IN NE FORMATION

Recent studies of cell-free nuclear assembly systems suggest that initial ER/chromatin contacts are mediated via tubule ends. In a second step, these tethered ER tubules are reorganized into flat nuclear membrane sheets by DNA-binding-NE-specific membrane proteins (Ulbert et al. 2006; Anderson and Hetzer 2007). In cells, the ER appears to be largely cisternal (Lu et al. 2009) and thus it is likely that pre-existing membrane sheets are directly tethered to the chromatin surface in vivo. This is consistent with the finding that overexpression of reticulons delays NE formation, whereas their knock down accelerates NE formation (Anderson and Hetzer 2008a).

During NE formation, chromatin undergoes a series of conformational changes, from a state of maximal chromatin compaction in late anaphase, right before NE formation (Mora-Bermudez et al. 2007), to transcription- and replication-competent decondensation in interphase. Anaphase compaction requires the kinesin-like DNA binding protein (Kid), which ensures the formation of a compact chromosome cluster during anaphase and the proper enclosure of the segregated chromatin mass into a single nucleus (Ohsugi et al. 2008). Interestingly, Kid loading onto anaphase chromosomes is dependent on Importin β (Tahara et al. 2008), adding to the growing number of mitotic processes regulated by this transport receptor.

Recently, a compelling case has been made that mechanistically links NE formation and chromatin decondensation. The hexameric ATPase Cdc48/p97, which was implicated in membrane fusion (Kondo et al. 1997) and

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ubiquitin-dependent processes (Ye et al. 2003), extracts Aurora B from chromatin, which results in its inactivation and subsequent chromatin decondensation. Interestingly, the inhibition of Cdc48/p97 blocked NE formation, suggesting that chromatin decondensation is required for NE formation (Ramadan et al. 2007), possibly by opening chromatin structure. It is tempting to speculate that this open chromatin structure provides binding sites for INM proteins and therefore drives NE formation.

Concomitantly, with the coating of chromatin by the NE, NPCs assemble in the reforming nuclei. This fascinating example of protein self-assembly is coordinated by the stepwise recruitment of a subset of NPC proteins to chromatin (Dultz et al. 2008) and some progress has been made in determining how this process occurs. A protein called Mel-28/ELYS, which was identified in a screen in *C. elegans* for factors involved in pronuclear formation, is critical for the association of the Nup107/160 complex to chromatin (Rasala et al. 2006; Franz et al. 2007). Because Mel-28/ELYS contains an AT-hook domain (Kimura et al. 2002), a likely scenario is that this step occurs by direct binding to DNA. Interestingly, RanGTP stimulates Mel-28/ELYS recruitment (Franz et al. 2007), presumably by releasing Importin β from one of the Nup107/160/ELYS components (Hetzer et al. 2005). Although other nucleoporins have been implicated in pore assembly, 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 (Franz et al. 2005; Hawryluk-Gara et al. 2008); however, how the membrane-associated Nup53 coordinates interactions between chromatin, membranes, and soluble Nup155 remains unclear.

A group of nucleoporins have been shown to be essential for pore assembly (Hetzer et al. 2005), the largest subcomplex being the Nup107/160 complex, whose depletion results in NPC-free nuclear membranes (Harel et al. 2003b; Walther et al. 2003a). In vertebrates, two transmembrane nucleoporins have also been shown to participate in NE formation, Ndc1 and POM121 (Mansfeld et al. 2006; Stavru

et al. 2006; Anderson et al. 2009b). The third known transmembrane Nup, gp210, is not expressed in all cell types and thus is unlikely to be essential for pore assembly (Eriksson et al. 2004). Whereas the molecular role of these scaffold Nups is unclear, an interesting link between POM121 and the Nup107/160 complex has been made and nuclear membrane formation might actually be linked to pore assembly by a poorly understood checkpoint by which the Nup107/160 complex “senses” nuclear membranes (Antonin et al. 2005).

REMODELING THE INTERPHASE NE

In order for cells to properly progress through multiple cell divisions, the number of NPCs doubles in interphase (Maul et al. 1972). It is unclear if the increase simply reflects the necessity to double pores for daughter cells of the next division cycle, or whether an increase in NPC number is important for interphase cell-cycle progression, e.g., efficient replication or transcription. Interphase nuclear pore assembly is particularly interesting because NPC formation occurs from both sides of the NE (D’Angelo et al. 2006). Thus, the question arises, what is the mechanism of communication between the double membranes? Interestingly, Nup133, a member of the Nup107/160 complex, contains an ALPS-like motif including an amphipathic α -helical domain that has been shown to act as a membrane curvature sensor in vitro (Drin et al. 2007). It is possible that this domain is involved in targeting the Nup107/160 complex to membranes during NE formation. A prediction from this idea is that the membrane hole is formed before Nup107/160 is recruited. Whereas the fusion of INM and ONM remains elusive in mammalian cells, significant progress has been made in deciphering this process in yeast.

Genetic studies in *S. cerevisiae* revealed a network of protein–protein interactions that appears to initiate NPC formation. The nucleoporins Nup59/53 and the integral pore membrane nucleoporins Pom152 and Pom34 tether Nup170 and a third integral membrane nucleoporin Ndc1 to the NE at new assembly sites (Onischenko et al. 2009). Furthermore, depletion

of Nup170 and its homolog Nup157 causes the accumulation of NPC-like structures in the INM and at cytoplasmic foci rather than properly localized to nuclear pores spanning the NE. It is interesting to note that the yeast reticulons and Yop1 not only display genetic interactions with the Poms, but also seem to play an essential role in the formation of new NPCs (Dawson et al. 2009). Similar to the depletion of Nup170, in the absence of reticulons, NPC-like intermediates also accumulate in an aberrant manner in the INM and ONM of the NE. The current idea is that a complex consisting of the three membrane-spanning nucleoporins Pom152, Pom34, and Ndc1 marks a new assembly site by joining or juxtaposing ONM and INM, which is consistent with the observation that NPC assembly proceeds from both sides of the NE (D'Angelo et al. 2006). According to this idea, Nup53/59 and the reticulons Yop1/Dbp1 are recruited to the nuclear membrane and inserted into the outer leaflet of the membrane using monotopic membrane insertion domains, inducing or stabilizing local curvature to the membrane. However, this idea was recently challenged on theoretical grounds, arguing that reticulons induce positive membrane curvature (e.g., giving rise to the convex surface of a tubule), but not negative curvature, which is likely to occur during NPC assembly. Instead, it was proposed that reticulons might participate in the local enrichment of negative curvature-inducing proteins involved in NE formation (Antonin 2009). Whether transmembrane nucleoporins, reticulons, or an unidentified machinery induces the fusion of INM and ONM during NPC assembly remains an open question.

During interphase, the NE expands considerably and thus 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 (D'Angelo et al. 2006), suggesting that membranes feed into the ONM via connections with ER tubules. 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 (Ohba et al. 2004). In yeast, integral INM proteins have been shown to directly interact with specific nucleoporins and transport receptors to promote their movement past the NPC (King et al. 2006). 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.

During interphase, NE connections with the cytoskeleton have to withstand external forces. Recent work has suggested that SUN and KASH domain proteins play key roles in this process (Adam 2001). KASH domain proteins interact with centrosomes/SPBs or cytoskeletal elements in the cytoplasm and bind to SUN domain proteins in the INM to connect the NE to structures on the outside of the nucleus. The nuclear lamina could serve in metazoa to distribute the forces across a broader area (Stewart et al. 2007) (Roux and Burke 2007).

CONCLUDING REMARKS

The NE is well known for its role in shielding the nuclear genome from cytoplasmic components and mediating nucleocytoplasmic transport. Less well understood is its dynamic behavior in dividing cells and its role in the distribution of the genetic material. Furthermore, it has become clear that the NE might actively control the organization of the genome and directly regulate gene expression. This opens some interesting opportunities for future research activities. For instance, the nature of most NE protein-chromatin interactions remain uncharacterized and many of the INM proteins are expressed in a tissue-specific manner. This raises the important question of whether NE composition is a critical component of cell fate determination. If NE proteins control chromatin organization, then is there a link between NE formation and nuclear organization? In other words, are the NE-chromatin contact points that are established

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in anaphase/telophase maintained in interphase? Because many NE proteins are linked to a large number of diseases, answers to these questions are likely to have a direct impact on human health.

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