Orchestrating nuclear envelope disassembly and reassembly during mitosis

Stephan Güttinger, Eva Laurell and Ulrike Kutay

Abstract | Cell division in eukaryotes requires extensive architectural changes of the nuclear envelope (NE) to ensure that segregated DNA is finally enclosed in a single cell nucleus in each daughter cell. Higher eukaryotic cells have evolved 'open' mitosis, the most extreme mechanism to solve the problem of nuclear division, in which the NE is initially completely disassembled and then reassembled in coordination with DNA segregation. Recent progress in the field has now started to uncover mechanistic and molecular details that underlie the changes in NE reorganization during open mitosis. These studies reveal a tight interplay between NE components and the mitotic machinery.

Nuclear pore complex
(NPC). A multisubunit protein complex in the nuclear envelope that serves as a transport channel between the nucleus and the cytoplasm. NPCs are permeable for small molecules but restrict the passive diffusion of macromolecules larger than 30 kDa.

Nuclear lamina
A fibrous intermediate filament network that consists of lamins and underlies the inner nuclear membrane.

Kinetochore
A multimeric protein complex that is formed on centromeric DNA and mediates the attachment of spindle microtubules to chromosomes during mitosis.

Institute of Biochemistry, Eidgenössische Technische Hochschule Zurich, HPM F11.1, Schafmattstrasse18, 8093 Zurich, Switzerland. Correspondence to U.K.
e-mail: ulrike.kutay@bc.biol.ethz.ch
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More than 100 years ago, Schneider and Flemming provided the first comprehensive descriptions of the key structural changes in nuclear organization during mitosis[1,2]. Since then, the model of how mitosis proceeds has been tremendously refined, both on the phenomenological and molecular level.

Of all of the different parts of the nucleus that are reorganized during mitosis, the nuclear envelope (NE) is of special importance as it constitutes its defining boundary. The NE can be seen as an extended sheet of the endoplasmic reticulum (ER) that covers chromatin. It is formed by two closely juxtaposed membranes, termed outer and inner nuclear membrane (ONM and INM, respectively). Although the NE completely encapsulates chromatin, it is not a uniformly closed membrane barrier. Rather, it is perforated by holes that are formed at sites of fusion between the INM and ONM (FIG. 1a). These holes are occupied by nuclear pore complexes (NPCs), which serve as both molecular sieves and transport hubs (FIG. 1b). The ONM is continuous with the rough ER, which it also resembles in protein composition. The INM contains a unique set of membrane proteins that establish contact with the chromatin and the nuclear lamina. In addition to its barrier function, the NE is important for various cellular processes, such as chromatin organization, regulation of gene expression, nuclear positioning and migration (for reviews, see REFS 3–7).

The correct capturing of mitotic chromosomes by spindle microtubules is essential for faithful cell division. Higher eukaryotes form a cytoplasmic spindle, which requires NE breakdown (NEBD) to allow access of spindle microtubules to kinetochores. NEBD not only involves the removal of NE membranes, but also the disassembly of large macromolecular assemblies, such as NPCs and the lamina. After DNA segregation, a NE is reformed around each mass of chromatin in the future daughter cells to re-establish a nuclear boundary[3,8].

The temporal coordination of nuclear disassembly and assembly is subject to the general control of mitotic entry and exit. An important aspect of spatial control relies on the ability of cells to identify chromatin. Chromatin identity is defined by the Ran system throughout the cell cycle[3,9–11]. Ran is a small, Ras-like GTPase that is loaded with GTP by its nuclear, chromatin-associated Ran guanine nucleotide-exchange factor (RanGEF) RCC1, leading to a high concentration of nuclear RanGTP. In the cytoplasm, Ran converts to the GDP-bound form, stimulated by cytoplasmic Ran GTPase-activating protein 1 (RanGAP1). This creates a RanGTP gradient across the NE that is used to control the directionality of nuclear transport in interphase. The generation of RanGTP by chromatin-associated RCC1 continues during mitosis and gives rise to a surrounding cloud of RanGTP[9], which provides spatial information for various processes occuring on chromatin, such as spindle and NE assembly[3,9–11].

Here, we review how structural changes of the NE during 'open' mitosis are mediated and controlled. We cover the processes that lead to NEBD, follow the function of single NE components through metaphase and anaphase, and describe key mechanisms that control the reformation of the NE.

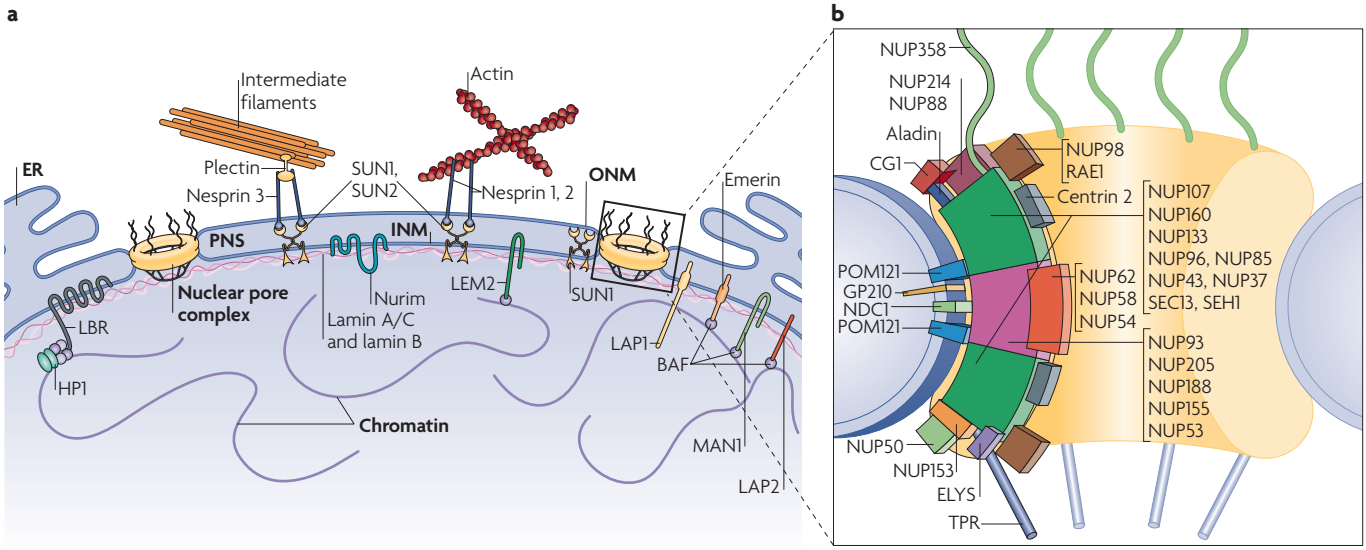

Figure 1 | **The main structural features of the nuclear envelope. a** | The inner and outer nuclear membranes (INM and ONM, respectively) are separated by the perinuclear space (PNS). The nuclear lamina underlies the nucleoplasmic face of the INM. INM proteins link the nuclear envelope (NE) membrane to chromatin and the lamina, whereas 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, and supports postmitotic NE formation[125,138,159]. Members of the LEM (lamina-associated protein 2 (LAP2), emerin, MAN1)-domain family bind to lamins and interact with chromatin through barrier-to-autointegration factor (BAF)[66]. LEM proteins have been implicated in DNA replication, NE assembly and gene regulation[160]. SUN proteins in the INM interact with nesprins in the ONM, thereby forming so-called LINC complexes that establish connections to actin and intermediate filaments in the cytoplasm[6]. Nurim is a multispanning INM protein with no assigned function[161]. Proteomic approaches have identified more than 60 novel putative transmembrane proteins in the NE[162,163] (not shown). **b** | Nuclear pore complexes (NPCs) are large macromolecular assemblies of 60–120 MDa that display eight-fold rotational symmetry[164,165]. They are built from ~30 different proteins, termed nucleoporins (Nups). Three ring-like substructures constitute the major backbone of the NPC and surround the central transport channel. Other major architectural features of the NPC are cytoplasmic filaments and the nuclear basket. Many Nups are part of NPC subcomplexes, which are used as building blocks[166]. One of the most prominent subcomplexes is the NUP107–160 complex (dark green), which localizes to both sides of the NPC and is a major constituent of the NPC scaffold. The central channel of the NPC is filled with FG-repeat-containing Nups, which provide interaction sites for transport receptors. ELYS is known as MEL-28 in *Caenorhabditis elegans*. POM121, pore membrane protein of 121 kDa; RAE1, RNA export 1; SEC13, secretory pathway 13; SEH1, SEC13 homologue 1; TPR, translocated promoter region.

Guanine nucleotide-exchange factor
A protein that catalyses the exchange of GDP for GTP on G proteins.

GTPase-activating protein
A factor that stimulates the intrinsic GTPase activity of small GTPases, thereby inducing the hydrolysis of bound GTP to GDP.

Nucleoporin
A protein constituent of the nuclear pore complex (NPC). Each NPC is composed of ~30 different nucleoporins, which are present in multiples of 8, reflecting the eight-fold rotational symmetry of the NPC. Approximately one-third of nucleoporins contain Phe-Gly repeats that are binding sites for transport receptors.

Centrosome
The microtubule-organizing centre in animal cells. It consists of a pair of centrioles surrounded by the pericentriolar matrix. During mitosis, centrosomes serve as spindle poles.

Taking apart the NE during mitosis

Microscopy studies have contributed to the detailed knowledge of the course of nuclear division in different species (FIG. 2). NEBD during open mitosis comprises several distinct steps, including NPC disassembly, depolymerization of the nuclear lamina and the clearance of NE membranes from chromatin (FIG. 3).

The process of NEBD. An initial event of NEBD is the dispersal of soluble nucleoporins[12–14], many of which are released from the NE as mitotically stable nucleoporin subcomplexes. Upon NPC disassembly, nuclear pores no longer restrain the passive diffusion of nuclear and cytoplasmic components. In mammalian cells, NPC disassembly starts in late prophase and is completed within minutes, as revealed by kinetic analysis[13]. At the temporal resolution of this analysis, it seems that most nucleoporins disassemble synchronously, with the exception of the peripheral FG-repeat nucleoporin NUP98, which starts to disperse slightly ahead of the bulk of nucleoporins[13]. Removal of NUP98 could thus be the initial trigger for the subsequent wave of NPC disassembly. Also, in *Drosophila melanogaster* embryos and starfish oocytes, NPC disassembly is fast, and certain peripheral nucleoporins leave the NE slightly earlier than central NPC subcomplexes[12,14–16].

NEBD is supported by a microtubule-dependent tearing mechanism in mammalian somatic cells[17,18]. Microtubules that are attached to the outer face of the nucleus exert pulling forces that cause NE invaginations around the centrosomes. This leads to the formation of a few holes on the distal side of the nucleus. These holes are apparent as discontinuities in the nuclear lamina and have been interpreted as the result of mechanical stretching of the lamina. It is also conceivable that they represent NE fenestrae initially caused by NPC removal and expanded by microtubule-dependent pulling. Microtubules also assist in clearing chromosomes from NE membranes, which might facilitate spindle assembly[17–19].

Nuclear disassembly continues with lamina depolymerization[20]. The first signs of lamina disassembly are already detectable in early prophase, when A-type

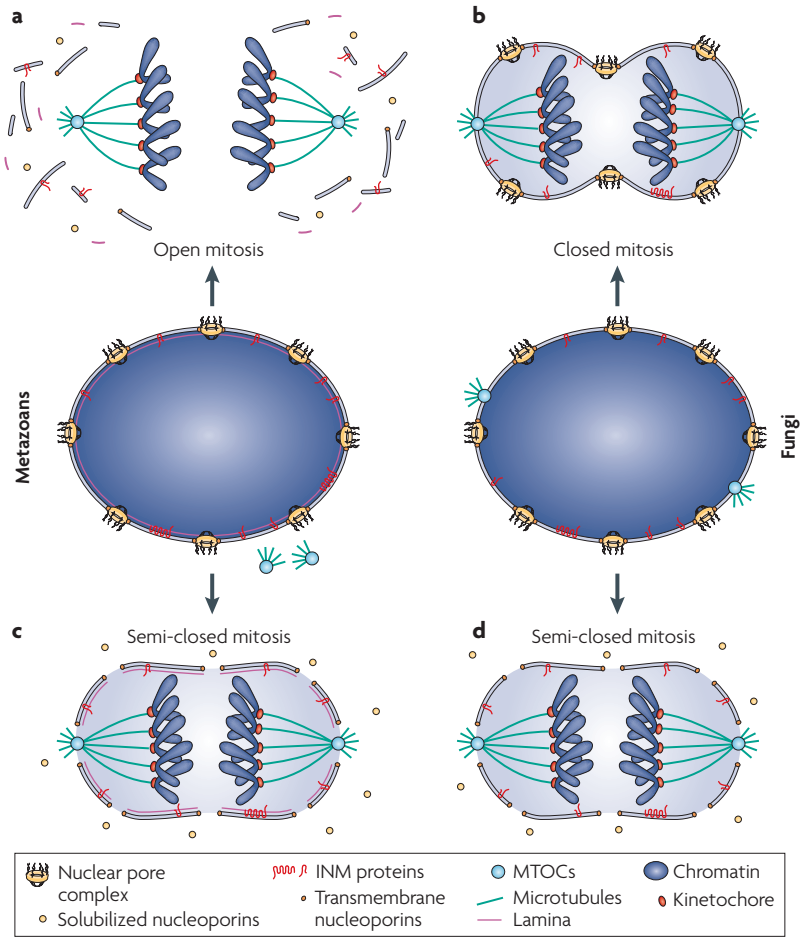

Figure 2 | **From 'open' to 'closed' mitosis.** The terms 'open' and 'closed' mitosis refer to the extremes of a range of possible fates of the nuclear envelope (NE) during mitosis[167]. **a** | In open mitosis, which is used in somatic cells of higher eukaryotes, the NE is completely disassembled and removed from chromatin and a cytoplasmic spindle is formed by microtubules that emanate from cytoplasmic centrosomes. **b** | In closed mitosis, the NE stays intact. Here, microtubule-organizing centres (MTOCs) are either constantly part of the NE (for example, in *Saccharomyces cerevisiae*) or are inserted into the NE during mitotic entry (for example, in *Schizosaccharomyces pombe*), and in both cases MTOCs direct the formation of a nuclear spindle. The establishment of a nuclear spindle requires nuclear uptake of tubulin. Closed mitosis is the most common mechanism in lower eukaryotes. A prevalent intermediate between open and closed mitosis is the partial disassembly of the NE. **c** | In higher eukaryotes, semi-closed mitosis is accomplished by certain cell types, such as in *Caenorhabditis elegans* early embryos or during syncytial embryonic divisions in *Drosophila melanogaster*. Here, the NE only partially opens up near to centrosomes to allow cytoplasmic spindle microtubules to reach the nuclear interior without the need for major rearrangements of NE components. In syncytial cells, this ensures that spindle microtubules capture the correct chromosomes in the common cytoplasm. The NE finally breaks down during anaphase. **d** | Some lower eukaryotes, such as the filamentous fungus *Aspergillus nidulans*, also undergo semi-closed mitosis and partially disassemble their nuclear pore complexes to achieve the rapid influx of tubulin. INM, inner nuclear membrane.

Cyclin-dependent kinase (CDK). A family of protein kinases, the activity of which depends on the formation of a complex with cyclin subunits. Different CDK–cyclin complexes orchestrate distinct steps in the cell cycle.

lamins start to be released into the nucleoplasm[21]. Visible changes in the organization of B-type lamins occur only after the NE permeability barrier has been disrupted by NPC disassembly[18,21,22]. At the same time, INM proteins detach from lamins and chromatin[18], and NE membrane proteins retract into the membrane system of the ER[23–26].

Triggering NEBD: kinases and their targets. NPC disassembly, lamina depolymerization and the dissociation of INM proteins from their nuclear binding sites are controlled by the activation of mitotic kinases, which directly contribute to the phosphorylation of NE proteins (Supplementary information S1 (table)). It is assumed that these phosphorylation events disrupt interactions among NE components during mitosis. However, the functional consequences of phosphorylation have only been assessed for a few kinase targets.

Of all of the kinases that are implicated in NEBD, the role of cyclin-dependent kinase 1 (CDK1) is the best understood. CDK1 directly contributes to the disassembly of the nuclear lamina. The phosphorylation of lamins by CDK1 results in lamina depolymerization *in vitro*[27], and the mutation of CDK1 phosphorylation sites in lamin A/C blocks lamina disassembly at the onset of mitosis[28]. Furthermore, CDK1 might also be directly involved in NPC disassembly. Many nucleoporins are phosphorylated on CDK1 sites during mitosis, including members of the NUP107–160 and NUP53–93 subcomplexes, NUP98, NDC1, GP210 and others[29–33] (Supplementary information S1 (table)). Moreover, the inhibition of CDK1 blocks NE permeabilization during NEBD *in vitro*[19], and recombinant CDK1–cyclin B causes the dissociation of a subset of nucleoporins from isolated *D. melanogaster* embryo nuclei[34]. Also, the release of INM proteins from lamins and chromatin might depend on CDK1 phosphorylation[35]. Known CDK1 targets at the INM are lamina-associated protein 2α (LAP2α)[36], LAP2β[37] and lamin B receptor (LBR)[38]. It is noteworthy that not only B-type cyclins are important for nuclear disassembly, as depletion of cyclin A2 from HeLa cells has been shown to significantly delay NEBD[39].

A couple of additional kinases are known to contribute to NEBD in various species, including protein kinase C (PKC), Aurora A and polo-like kinase 1 (PLK1). In vertebrate cells, high levels of PKCβ activity have been observed immediately before NEBD[40], and the βII isoform of PKC translocates to the nucleus at the G2–M transition[41,42]. This coincides with a rise in the levels of nuclear diacylglycerol[42], which is required for PKC activation. PKC inhibition results in G2 arrest *in vivo*[43] and blocks NEBD *in vitro*[19,44]. So far, lamin B is the only known target of PKCβII in NEBD[41,43], but additional targets are likely to exist. In *Caenorhabditis elegans*, Aurora A has been implicated in NEBD[45,46], but it is not known which of its targets affects nuclear disassembly. Similarly, a lack of PLK1 activity delays NEBD in *C. elegans*[47] and human somatic cells[48].

Notably, also during the semi-closed mitosis of *Aspergillus nidulans,* NPCs are partially disassembled by the release of 14 nucleoporins[49] to allow for nuclear entry of Cdk1–cyclin B[50]. This partial NPC disassembly depends on the activity of two kinases, NimA (never in mitosis A) and Cdk1. The activity of NimA correlates with the phosphorylation of SonBn^{Nup98} and its dispersal from the NPC[51]. Whether NimA-related kinases support nuclear disassembly in organisms undergoing open mitosis remains to be investigated. Notably, however, the expression of NIMA in mammalian cells induces chromatin condensation[52,53] and NEBD[53].

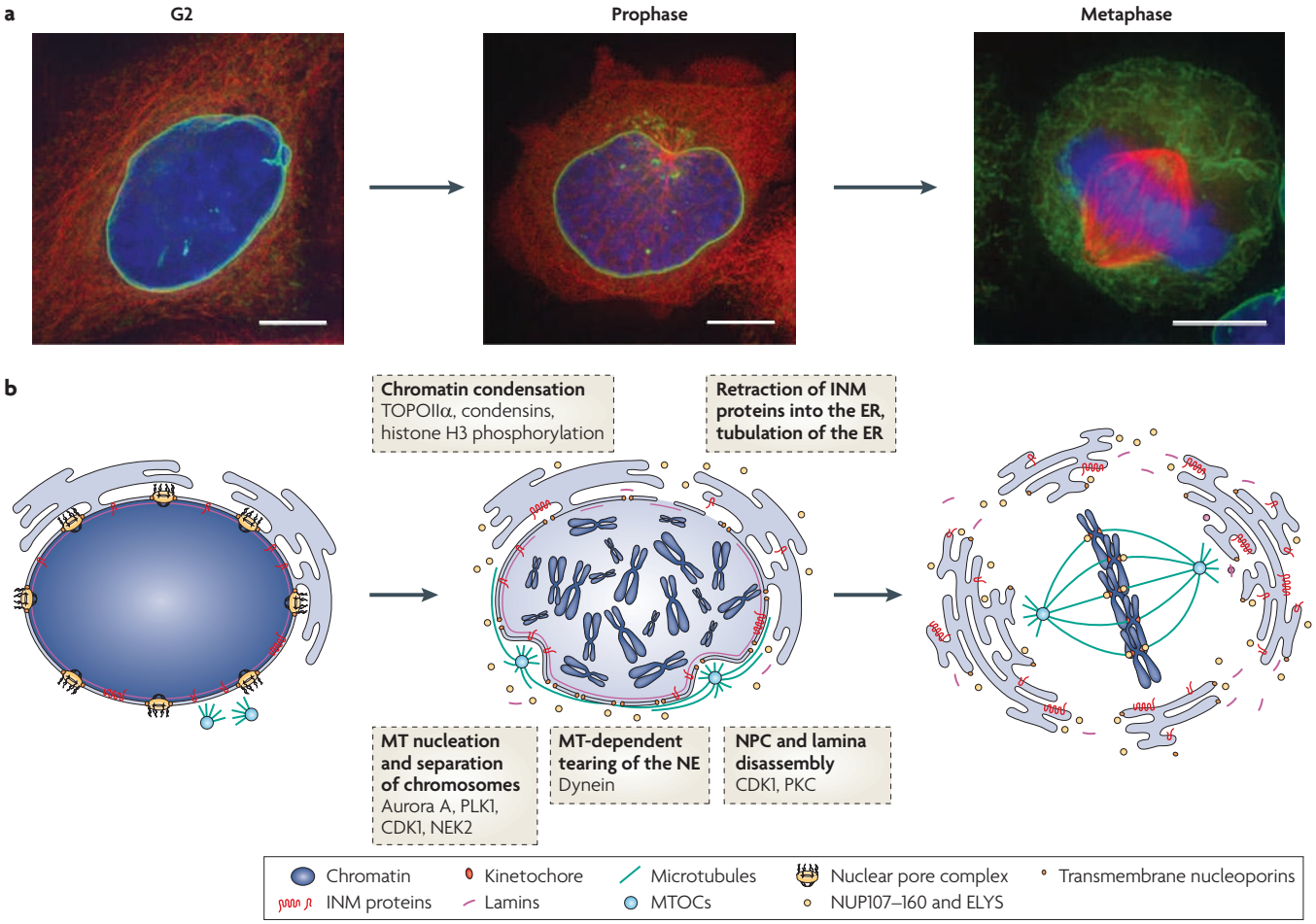

Figure 3 | **Nuclear envelope breakdown during 'open' mitosis. a** | The images show HeLa cells in which the inner nuclear membrane (INM, green; stained by green fluorescent protein fused to lamina-associated protein 2β), DNA (blue; stained with Hoechst) and microtubules (red; stained by red fluorescent protein–α-tubulin) are visualized in G2, prophase and metaphase. Scale bars, 10 μm. **b** | At the end of G2 phase, the activation of mitotic kinases, including the master mitotic regulator cyclin-dependent kinase 1 (CDK1), triggers entry into prophase, which is associated with a series of events that include the start of chromatin condensation, formation of microtubule asters around centrosomes and centrosome separation. Microtubules that are attached to the nuclear envelope (NE) in conjunction with the minus-end-directed motor dynein lead to NE invaginations around centrosomes and to the formation of holes on the opposing site of the NE. At the same time, nuclear pore complex (NPC) disassembly commences and is probably caused by the phosphorylation of nucleoporins. The transition into prometaphase is marked by the loss of the NE permeability barrier. Phosphorylation of nuclear lamins and INM proteins by CDK1, protein kinase C (PKC) and probably other kinases results in lamina disassembly and allows for the retraction of NE membranes into the endoplasmic reticulum (ER). In metaphase, most soluble components of the NE are dispersed throughout the cytoplasm, whereas INM proteins reside in the tubular mitotic ER. ELYS is known as MEL-28 in *Caenorhabditis elegans*. MTOC, microtubule-organizing centre; NEK2, NimA-related kinase 2; PLK1, polo-like kinase 1; TOPOIIα, topoisomerase IIα.

Taken together, the concerted action of multiple kinases might be required to accomplish nuclear disassembly. In the future, it will be important to distinguish between the indirect and direct effects of some of the aforementioned kinases on NEBD.

Centrosomes and microtubules in NEBD. At the onset of mitosis, microtubule asters form at centrosomes and then move apart along the NE[54]. Furthermore, in mammalian somatic cells, microtubule-dependent tearing of the NE during prophase triggers the formation of holes, and supports the removal of membranes from chromatin during prometaphase[17–19]. *In vitro*, importin-β and RanGTP regulate this microtubule-driven process through the Ran gradient by an unknown mechanism[19].

Both centrosome migration and NE tearing require dynein, a cytoplasmic minus-end-directed microtubule motor that associates with the NE at the end of G2 (REFS 17,55,56). The dynein-binding proteins LIS1 and NDEL1 colocalize with dynein to the NE in prophase, and their absence delays the transition into prometaphase in neural stem cells, probably by affecting NEBD[57]. Notably, microtubule-dependent tearing is not essential for the disruption of the NE, as NEBD is delayed rather than blocked in nocodazole-treated cells[17–19].

Nocodazole
A drug that inhibits the polymerization of microtubules. Nocodazole-treated cells enter mitosis but cannot form a mitotic spindle and consequently arrest in prometaphase.

It is still unclear which microtubule attachment sites at the NE are used for centrosome tethering and microtubule-dependent NE remodelling in mammalian cells. In *C. elegans*, the Hook-family member zygote defective protein 12 (ZYG-12) mediates attachment between centrosomes and the ONM by providing a link to the dynein subunit DLI-1 (REF. 58). However, no NE-associated orthologues of ZYG-12 have been described in mammals so far. Rather, the INM protein emerin, which can bind to β-tubulin and partly localizes to the ONM, has been suggested to connect centrosomes to the NE[59]. Whether this potential function of emerin is relevant for mitotic onset is unknown.

Changes in chromatin. Entry into mitosis is accompanied by the structural reorganization of chromatin into condensed chromosomes. Chromatin condensation involves several factors, including topoisomerase IIα (TOPOIIα) as well as condensin I and II complexes. Moreover, histones and changes in their post-translational modifications are important for mitotic chromatin compaction. Specifically, phosphorylation of residue S10 in histone H3 is tightly linked to chromosome condensation (for reviews, see REFS 60–63).

Chromatin starts to condense before NEBD. Once the NE is permeabilized, the rate of condensation in HeLa cells increases more than threefold and is then completed in a few minutes[18]. The increase in condensation rate upon NEBD coincides with nuclear entry of condensin I, which is cytoplasmic in interphase. By contrast, condensin II is nuclear throughout interphase and supports chromosome condensation during early prophase[64,65]. Condensed chromosomes are initially localized at the nuclear periphery[64], probably because chromatin–NE contacts are not yet completely broken. In addition, chromosomes might be captured by NPC–kinetochore interactions, as some nucleoporins are known to localize to kinetochores in mitosis (see below).

In addition to global changes in chromatin organization, the phosphorylation of specific chromatin-associated proteins affects their interaction with INM proteins, which in turn might support NEBD. One example is the mitotic phosphorylation of the barrier-to-autointegration factor (BAF), an essential DNA-binding protein enriched at the NE[66]. BAF phosphorylation *in vitro* reduces its binding to chromatin and to INM proteins of the LEM (LAP2, emerin, MAN1) protein family[67,68], and might promote the dissociation of LEM proteins from chromatin during mitosis.

The NE and the mitotic ER. The disassembly of the nuclear lamina and the phosphorylation of INM and chromatin proteins allow for the complete absorption of NE membranes and their resident proteins into the mitotic ER[23,24]. Not only the NE, but also the ER is dramatically reorganized during mitosis, such that it forms an exclusively tubular network that is devoid of membrane sheets[25]. Recent data obtained using *C. elegans* embryos suggest that mitotic NE and ER remodelling share common requirements, namely for the GTPase RAB-5 as well as for YOP-1 and RET-1 (REF. 69). RET-1 and YOP-1 are membrane-bending proteins of the reticulon and DP1 protein families, respectively, that shape ER tubules[70]. Depletion of RAB-5 or YOP-1 and RET-1 not only affects the structure of the mitotic ER but also inhibits NEBD[69]. Interestingly, lack of RAB-5 or the transmembrane nucleoporin GP-210 lead to similar NEBD phenotypes: delays in NPC and lamina disassembly[69,71]. Although further work is needed to shed light on the underlying mechanism, these data suggest that the sheet-to-tubule transition of membranes might facilitate NEBD. Whether this involves sheet-to-tubule transition of NE membranes, or whether the reorganization of the ER affects NEBD more indirectly, are open questions. Because depletion of the nucleoporin GP-210 phenocopies the effect of RAB-5 or YOP-1 and RET-1 depletion, GP-210 might be a good candidate to support NE-specific membrane remodelling.

Another link between membrane remodelling factors and NEBD stems from studies on the disassembly of *in vitro* assembled nuclei using *Xenopus laevis* extracts[72,73]. Nuclear disassembly in this system was reported to depend on components that are involved in the formation of coatomer protein complex I (COPI)-type vesicles, although evidence for an *in vivo* function of the COPI complex in NEBD is still missing.

NPC components and the mitotic spindle

Although the NE does not exist as a functional unit during mitosis, some of its components have key roles in the regulation of different mitotic processes after NEBD. Interestingly, different nucleoporin subcomplexes influence spindle formation and anaphase onset.

Nucleoporins in spindle assembly and kinetochore function. During spindle assembly, microtubules are reorganized such that minus ends are focused into two poles at centrosomes, whereas plus ends interact with chromosomes through kinetochores and align them on the metaphase plate. Spindle assembly is not only aided by centrosomes and chromatin, but also by microtubule-binding proteins, such as molecular motors and regulators of microtubule dynamics[74]. One nucleoporin that has been implicated in spindle assembly is RNA export 1 (RAE1). RAE1 forms a complex with NUP98, and both are involved in RNA export during interphase[75,76]. In mitosis, RAE1 binds to microtubules and is required for spindle formation *in vitro* and *in vivo*[77] (FIG. 4). Although RAE1 itself has no microtubule-stabilizing or microtubule-bundling activity, it is part of a spindle-associated ribonucleoprotein (RNP) complex, the integrity of which is essential for spindle assembly by an unknown mechanism[77]. In mitotic cells, RAE1 interacts and colocalizes with NUMA (nuclear mitotic apparatus protein), a microtubule-associated protein that promotes microtubule bundling at spindle poles. Both depletion and overexpression of RAE1 lead to increased formation of multipolar spindles, an effect that can be counteracted by NUMA depletion or co-overexpression, respectively[78]. Thus, the exact balance of RAE1 and NUMA levels seems to be crucial for correct bipolar spindle formation *in vivo*.

Topoisomerase
An enzyme that binds to DNA and catalyses its unknotting by transiently breaking phosphodiester bonds.

Condensin
A five-subunit protein complex that is associated with mitotic chromosomes and is implicated in chromosome condensation. Two types of condensin complexes exist in vertebrate cells — condensin I and II — which share the ATPase subunits structural maintenance of chromosomes 2 (SMC2) and SMC4.

Reticulon
A member of the reticulon family of membrane proteins that are associated with the tubular endoplasmic reticulum (ER). Reticulons as well as members of the DP1/YOP1 protein family contain two long hydrophobic domains that are each proposed to insert as wedge-like hairpins into lipid bilayers and to promote the formation of ER tubules.

COPI
(Coatomer protein complex I). A cytosolic protein complex that is composed of seven polypeptides that coats membrane transport vesicles. The COPI complex is required for the formation of Golgi-derived vesicles for retrograde transport to the ER.

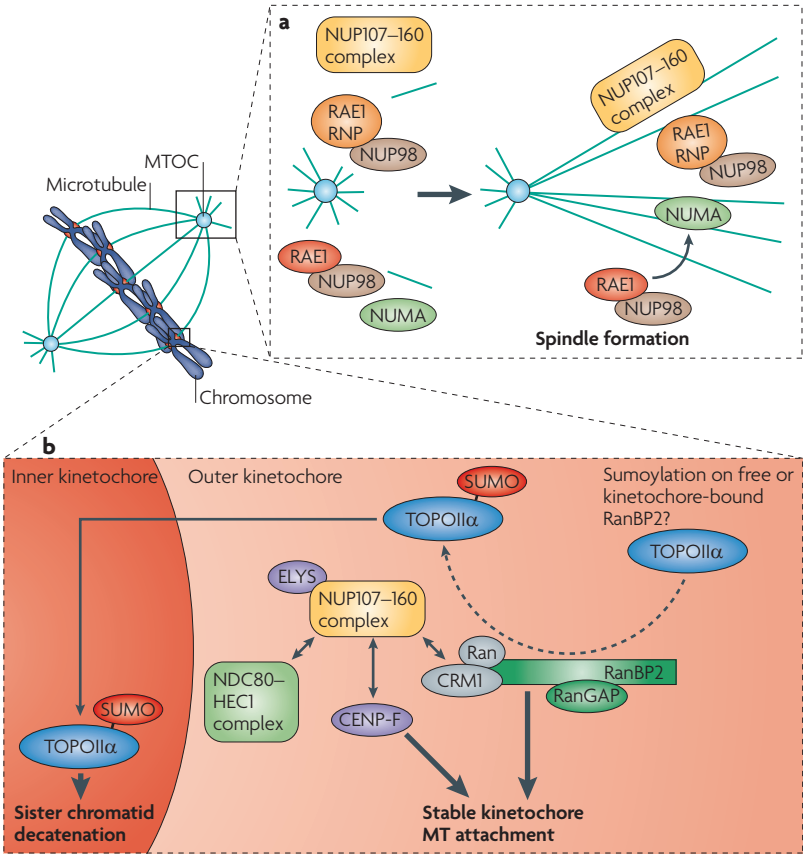

Figure 4 | **Nucleoporins in spindle assembly and kinetochore function.** **a** | The nucleoporin NUP107–160 complex and RNA export 1 (RAE1)-containing ribonucleoproteins (RNPs) localize to spindle microtubules and are required for bipolar spindle formation. RAE1 is also involved in microtubule bundling by the microtubule-associated protein nuclear mitotic apparatus protein (NUMA). The balance of RAE1 and NUMA levels is crucial for correct spindle assembly. Whether RAE1 functions in these processes alone or in conjunction with its binding partner NUP98 is unclear. **b** | The NUP107–160 complex in conjunction with its chromatin-targeting factor ELYS (MEL-28 in *Caenorhabditis elegans*)[80,132–134] is recruited to kinetochores primarily through the NDC80–HEC1 complex[84], a major building block of outer kinetochores. The presence of the NUP107–160 complex at kinetochores influences the attachment of kinetochore microtubules (MTs), both by recruitment of Ran-binding protein 2 (RanBP2; also known as NUP358) and Ran GTPase-activating protein 1 (RanGAP1), and by stabilizing the attachment of centromere protein F (CENP-F), a protein that is required for kinetochore–microtubule interactions. RanBP2 and RanGAP1 recruitment to the kinetochore depends on the formation of a complex with the exportin CRM1 (also known as XPO1) and RanGTP. How RanBP2 and RanGAP1 affect kinetochore MT attachment is not known. In addition, RanBP2 is required for sumoylation of topoisomerase IIα (TOPOIIα) and its targeting to the inner kinetochore, where TOPOIIα functions in sister chromatid decatenation. Whether TOPOIIα is sumoylated (SUMO) by free or kinetochore-bound RanBP2 is not known. MTOC, microtubule-organizing centre.

NUP107–160 complex
An essential, multimeric nucleoporin subcomplex that consists of ~10 subunits. It is the major constituent of the central nuclear pore complex (NPC) scaffold and it symmetrically localizes to both sides of the NPC.

In addition to RAE1, the NUP107–160 nucleoporin subcomplex has been implicated in spindle formation using *X. laevis* egg extracts[79], in which it evenly decorates *in vitro* assembled spindles. Depletion of the NUP107–160 complex compromises spindle assembly, either by the absence of the complex or indirectly by the co-depletion of associated factors.

In mitotic mammalian cells, the NUP107–160 complex can also be detected at spindle poles and proximal microtubules during a narrow time window in prometaphase[79]. But intriguingly, a prominent fraction of the NUP107–160 complex localizes to kinetochores in both *C. elegans* embryos[80] and mammalian cells[81–83]. Cells that are depleted of the NUP107–160 complex display a prolonged prometaphase, defects in chromosome congression and a delay in anaphase onset[84]. The absence of the NUP107–160 complex at kinetochores causes defects in chromosome attachment and in the formation of stable microtubule–kinetochore interactions. The molecular mechanism of the underlying defect is not completely clear, but centromere protein F (CENP-F), a protein that is involved in microtubule attachment, is more easily stripped from kinetochores in the absence of the NUP107–160 complex[84].

The NUP107–160 complex also helps to attract other nucleoporins to kinetochores, namely the Ran-binding protein 2 (RanBP2; also known as NUP358)–RanGAP1 complex. RanBP2 has SUMO E3 ligase activity and forms a stable complex with sumoylated RanGAP1 throughout the cell cycle[85,86]. Furthermore, the exportin CRM1 (also known as XPO1) binds to kinetochores in a NUP107–160 complex-dependent manner, suggesting that this nucleoporin subcomplex has a central scaffolding role at both NPCs and kinetochores[84,87]. Whereas NUP107–160 and CRM1 are found at kinetochores that are already in prophase, RanBP2 and RanGAP1 are not recruited until prometaphase[88]. Their association coincides with the establishment of stable microtubule–kinetochore attachments and depends on microtubules and CRM1 (REFS 87,89). The depletion of RanBP2–RanGAP1 leads to defects in bipolar spindle formation, the accumulation of unaligned chromosomes and a lack of cold-stable microtubules[88,89]. It will be interesting to see if the enzymatic activities of RanBP2 and RanGAP1 in protein sumoylation and in the stimulation of the GTPase activity of Ran, respectively, are required for normal kinetochore function.

The intriguing connection between NPC components and spindle assembly raises the question of how NPC disassembly and microtubule–kinetochore attachment are linked. One factor that is connected to both kinetochores and the NUP107–160 complex is CENP-F, which interacts with the NUP133 subunit of the NUP107–160 complex on the one hand[84], and is required for kinetochore–microtubule interactions through the NDEL1–NDE1–LIS1–dynein pathway on the other[90]. Thus, understanding the spatial and temporal dynamics of the protein interaction network around CENP-F might be a promising avenue to follow.

Nucleoporins and the spindle assembly checkpoint. The correct bipolar attachment of kinetochores to the mitotic spindle is monitored by the spindle assembly checkpoint (SAC; BOX 1). Mitotic arrest deficient 1 (MAD1) and MAD2, which are key regulators of the SAC, are associated with NPCs during interphase[91]. As cells enter prophase, MAD1 and MAD2 accumulate on unattached kinetochores[92–94] and monitor microtubule occupancy to prevent premature anaphase onset. MAD1 is responsible for targeting of the MAD1–MAD2 complex to both NPCs and kinetochores. Two additional regulators of

MAD1–MAD2, namely p31^{comet} (an inhibitor of MAD2 activation) and MPS1 (a kinase that is required for targeting of MAD1 and MAD2 to kinetochores), also localize to NPCs[95,96]. Not only in mammalian cells, but also in budding yeast the Mad1–Mad2 complex is associated with NPCs[97] and partially relocalizes from NPCs to kinetochores on activation of the spindle checkpoint[97,98]. Thus, key members of the SAC pathway are kept at the nuclear pore during interphase. Whether NPC association helps to keep them in an inhibited state is unknown. Furthermore, it has not been addressed whether the association of MADs with NPC components outside kinetochores persists during mitosis in mammalian cells and whether this affects the spindle checkpoint response.

The ties between the SAC pathway and nucleoporins extend beyond MAD1–MAD2, as the SAC protein BUB3 shows homology to the nucleoporin RAE1 (REF. 99). Both haploinsufficient *Bub3*$^{+/-}$ and *Rae1*$^{+/-}$ mice show mitotic defects, such as increased missegregation of chromosomes and a failure to arrest in mitosis after the induction of spindle damage[100], suggesting that RAE1, like BUB3, could have a function in the spindle checkpoint. Indeed, RAE1, in complex with its partner NUP98, interacts with a CDH1 (also known as FZR1)-associated fraction of anaphase-promoting complex/cyclosome (APC/C) during prometaphase (see REF. 101) (BOX 1). CDH1 is an activator of APC/C and confers substrate specificity to it. RAE1–NUP98 specifically inhibits APC/C^{CDH1}-mediated ubiquitylation of securin *in vitro*[102]. Consistently, *Rae1*$^{+/-}$ *Nup98*$^{+/-}$ mice show premature securin degradation, leading to precocious anaphase onset and increased aneuploidy[102,103]. These data indicate that the RAE1–NUP98 heterodimer assists in regulating APC/C^{CDH1} during early mitosis. Although APC/C^{CDH1} complex formation is assumed to be prevented by CDK-dependent phosphorylation of CDH1 until anaphase onset[104], an additional mechanism that involves the RAE1–NUP98 heterodimer might be in place to inhibit premature APC/C activation by CDH1. Notably, RAE1 has also been implicated in the negative regulation of APC/C^{CDH1} activity during cell cycle exit from G1 in differentiating neuroblastoma cells[105]. Collectively, these findings suggest an intimate link between RAE1 and APC/C^{CDH1} activity. How RAE1 association with APC/C^{CDH1} is controlled throughout the cell cycle deserves to be the subject of future studies.

RanBP2 and resolution of sister chromatids. After all chromosomes have been faithfully captured by microtubules, anaphase is initiated and sister chromatids are separated. This requires decatenation of sister chromatids at centromeres by TOPOIIα. The recruitment of TOPOIIα to centromeres requires its sumoylation, which is promoted by RanBP2 in mammalian cells[106]. Strikingly, mice that express low levels of RanBP2 display defects in chromosome separation owing to the impaired association of TOPOIIα with centromeres[106]. Similar to TOPOIIα inhibition, reduced levels of RanBP2 lead to the formation of anaphase bridges, and consequently, mice with reduced levels of RanBP2 develop severe aneuploidy and are prone to spontaneous tumour formation[106]. This makes RanBP2 the first example of a nucleoporin that has tumour-suppressing activity.

NE assembly

The NE starts to reform around each segregated mass of chromatin in late anaphase, and nuclear reassembly is completed in telophase. In each of the future daughter cells, the reassembly of the NE must ensure the enclosure of the whole set of chromosomes into a single nucleus. This requires coordination between processes that affect chromatin status on the one hand, and recruitment of membranes and NPC insertion on the other. The attraction of NE components back to chromatin is controlled in a spatially and temporally defined fashion. An important spatial cue originates from the production of RanGTP on

Box 1 | The spindle assembly checkpoint

The onset of anaphase is tightly regulated by the spindle assembly checkpoint (SAC). The SAC ensures that sister chromatid separation is initiated only after all chromosomes have established correct bipolar attachment.

Sister chromatid separation requires the degradation of securin, an inhibitor of the cohesin-cleaving enzyme separase. Securin degradation depends on the activity of the anaphase-promoting complex/cyclosome (APC/C), a multisubunit E3 ubiquitin ligase (for reviews, see REFS 101,104). Cell division cycle 20 (CDC20) and CDH1 (also known as FZR1) are non-core subunits of the APC/C that are responsible for activation and substrate specificity of the complex. Whereas CDC20 functions early during mitosis, it is exchanged for CDH1 during anaphase. The SAC specifically inhibits CDC20 and thereby blocks APC/C-dependent ubiquitylation of cyclin B and securin, which is required to promote anaphase. Members of the SAC include mitotic arrest deficient 1 (MAD1), MAD2, MPS1, BUB1, BUB3 and BUBR1 (Mad3 in yeast). MAD2 and BUBR1 directly interact with CDC20 and are thought to be the main mediators of APC/C^{CDC20} inactivation. Notably, BUB3 is a co-inhibitor of BUBR1 and displays sequence similarity to the nucleoporin RNA export 1 (RAE1).

In prophase, different SAC members, including MAD1 and MAD2, accumulate on unattached kinetochores. MAD1 and MAD2 form a stable complex throughout the cell cycle, with MAD1 responsible for the targeting of the complex to kinetochores and nuclear pore complexes (NPCs). Furthermore, the kinases MPS1 and BUB1 are required at kinetochores to load the MAD1–MAD2 complex. Whereas all MAD1 is found in complex with MAD2, MAD2 itself is also present as free protein. It can adopt an open (O-MAD2) and a closed (C-MAD2) conformation. Only C-MAD2 can bind MAD1 or CDC20, and the transformation of O-MAD2 to C-MAD2 is thought to be the rate-limiting step of inhibitory CDC20 complex formation[156]. The MAD2 template model predicts that transformation of O-MAD2 to C-MAD2 is catalysed on MAD1–C-MAD2 at unattached kinetochores, thereby generating CDC20–C-MAD2 complexes that function as a diffusible signal to inhibit APC/C (for a discussion, see REF. 101). It is not clear whether the NPC association of MAD1–C-MAD2 has any functional significance in the context of the SAC.

Centromere
A specialized heterochromatin region on each chromosome where sister chromatids are held together and on which the kinetochore is formed during mitosis.

RanBP2
(Ran-binding protein 2). The major constituent of the cytoplasmic filaments of the nuclear pore complex. RanBP2 has small ubiquitin-like modifier (SUMO) E3 ligase activity and forms a stable complex with sumoylated Ran GTPase-activating protein 1 (RanGAP1) throughout the cell cycle. Furthermore, it contains four Ran-binding domains, which facilitate the disassembly of RanGTP-containing export complexes.

SUMO
(Small ubiquitin-like modifier). A ubiquitin-like polypeptide that can be covalently attached to Lys residues on target proteins by an enzyme cascade. Unlike ubiquitin, it does not target proteins for proteasomal degradation. Attachment of SUMO to Ran GTPase-activating protein 1 (RanGAP1) is required for the localization of RanGAP to nuclear pore complexes in metazoan cells.

CRM1
A RanGTP-binding nuclear transport receptor that mediates the export of proteins that have Leu-rich nuclear export signals.

Cold-stable microtubules
Microtubules that resist depolymerization at low temperatures.

Haploinsufficient
A gene is haploinsufficient in a diploid organism when one functional allele is insufficient to maintain a wild-type state.

Securin
An inhibitor of the enzyme separase that cleaves the sister chromatin cohesion protein 1 (SCC1) subunit of cohesin to allow for sister chromatid separation.

Aneuploidy
Having too many or too few copies of a chromosome.

Sister chromatid
Two linked copies of a replicated chromosome that are the product of DNA replication. They are separated during the metaphase–anaphase transition of mitosis and then segregated into the two daughter cells.

Decatenation
The process in which rings or chains are untangled from each other. DNA sister chromatids become entangled or catenated as a consequence of DNA replication and must be decatenated to allow for sister chromatid separation and segregation during mitosis.

Anaphase bridge
Chromatin fibres that connect the two separating chromosome masses during anaphase. Anaphase bridges can lead to chromosome amplifications, translocations or deletions and are considered a hallmark of genomic instability.

Chromokinesin
A subgroup of molecular motors belonging to the kinesin family that associate with chromosome arms during mitosis.

Micronucleus
A nuclear envelope-enclosed chromosome (or group of chromosomes) that is not incorporated with most of the chromosomes into the newly formed nucleus during cell division.

AAA+ ATPase
(ATPase associated with various cellular activities). A protein that contains one or two ATP-binding domains, and that forms ring-like oligomers and functions in conformational remodelling of macromolecules.

Aurora B
A member of the Aurora family of Ser/Thr protein kinases. Aurora B is a component of the chromosome passenger complex and is required for several aspects of mitosis, such as kinetochore function, the spindle assembly checkpoint and cytokinesis.

the surface of mitotic chromatin that helps to liberate NPC components from inhibitory complexes with importins close to chromatin[11,107–109] (see below). Furthermore, changes in chromatin structure and composition that occur during its decondensation might allow for the timely reassociation of NE constituents. These steps are temporally guided by the overall orchestration of mitotic exit. Dephosphorylation of nucleoporins, chromatin-associated factors and NE membrane proteins requires the inactivation of mitotic kinases, such as CDK1, and the action of protein phosphatases. The best-understood contribution to this stems from protein phosphatase 1, which, by use of specific targeting subunits, controls anaphase chromosome structure[110], chromatin decondensation[111] and lamin B dephosphorylation[112].

Preparation of chromatin for NE reassembly. The formation of a compact cluster of neighbouring chromosomes in late anaphase is one mechanism to ensure the reassembly of the NE around the entire chromatin mass. In fact, the volume occupied by chromatin is the smallest at this stage of mitosis[110,113]. The principle that underlies the formation of a compact cluster of neighbouring chromosomes is based on both bringing individual chromosomes into close proximity during poleward movement and compacting individual chromosomes by axial shortening (FIG. 5a,b). Chromosome compaction is dependent on Aurora kinase activity as well as on microtubule dynamics[113]. This microtubule dependency has recently been accentuated by the discovery that the chromokinesin KID (also known as KIF22 and kinesin 10) contributes to anaphase chromatin compaction in both HeLa cells and mouse zygotes[114]. KID is a DNA-binding, plus-end-directed motor that localizes to chromosome arms and to spindle microtubules, and is known to have an earlier role during mitosis, such as in prometaphase chromosome movements towards the spindle equator[115]. The contribution of KID to proper nuclear assembly during later steps of mitosis is most striking in mouse oocytes and early embryos, in which the loss of KID-mediated anaphase chromosome compaction leads to a high frequency of micronuclei and multinucleated cells, causing embryonic death[114]. By contrast, neither male meiosis nor somatic mitosis is affected by KID deficiency. It is unclear why KID deficiency is more deleterious in mouse early embryos than later in development — either there is a redundant activity to KID in somatic cells, or an intrinsic feature makes maternally controlled nuclear divisions more susceptible to the loss of this chromokinesin.

Another aspect in the preparation of chromatin for NE assembly has been unravelled through studies on the role of the AAA+ ATPase and ubiquitin-dependent chaperone p97 (also known as Cdc48 and VCP) in NE formation. In *X. laevis* egg extracts, p97, in conjunction with its heterodimeric ubiquitin-binding cofactor Ufd1–Npl4, is needed for the formation of a closed NE around partially decondensed chromatin[116]. But how can the requirement for p97–Ufd1–Npl4 be explained in molecular terms? Recent work has revealed that p97–Ufd1–Npl4 promotes NE formation by extracting polyubiquitylated Aurora B from chromatin, both during nuclear assembly in the *X. laevis* nuclear assembly system *in vitro* and during the first divisions of the *C. elegans* early embryo *in vivo*[117]. Persistence of active Aurora B kinase on chromatin has an inhibitory effect on both proper chromatin decondensation and NE reformation in these embryonic systems. Consistently, the requirement for p97 in nuclear assembly *in vitro* can be overcome by inhibiting Aurora B kinase activity. Understanding whether and how the inhibition of chromatin decondensation and NE reformation by Aurora B are linked will require the identification of the Aurora B substrates that exert these effects. Known Aurora B targets, such as subunits of the condensin I complex[118] and histone H3 (REF. 119), are prime candidates for such substrates. Interestingly, changes in post-translational modifications of H3, including its phosphorylation and dephosphorylation during mitotic entry and exit, respectively, correlate with chromatin arm dissociation and re-association of heterochromatin protein 1 (HP1)[120–122]. HP1, in turn, might contribute to NE assembly — for example, by aiding the recruitment of INM proteins[123], such as its interaction partner LBR[124,125].

Although it is unclear whether the role of p97 in NE formation is conserved in somatic cells, it is intriguing that ubiquitylation of Aurora B has also been implicated in the control of the dynamic association of Aurora B with chromatin in HeLa cells. The dissociation of Aurora B from chromosomes and its accumulation at the spindle midzone following anaphase onset are dependent on the E3 ubiquitin ligase cullin 3 (CUL3) and its substrate-specific adaptor proteins KLH3 and KLH9 (REF. 126). In cells that are depleted of CUL3 or KLH3 and KLH9, Aurora B fails to dissociate from segregating chromosomes and remains active, resulting in prolonged histone H3 phosphorylation. It has not been addressed, however, whether this causes a delay in chromatin decondensation and/or NE reformation in somatic cells.

NPC reassembly. An early event in NE reformation is the initiation of NPC assembly during late anaphase (FIG. 5b), which is marked by chromatin recruitment of so-called NPC 'prepores'[127] that are composed of the NUP107–160 complex[13,82,128]. Depletion of the NUP107–160 complex leads to a reduction in NPC number in somatic cells[128,129] and results in the formation of pore-free nuclei in a *X. laevis* nuclear reformation assay[82,128].

At least three mechanisms control the binding of the NUP107–160 complex to chromatin, and these might act in concert to guide the assembly of a chromatin-associated prepore in a spatially and temporally controlled manner. First, in the mitotic cytoplasm, the NUP107–160 complex is chaperoned by importin-β. The binding of importin-β to RanGTP, which is generated by the chromatin-bound RanGEF RCC1, induces the dissociation of importin-β from the NUP107–160 complex, thereby liberating the complex for chromatin binding[130,131]. Some form of temporal regulation must support this spatial control, as RanGTP production around chromatin persists throughout mitosis. This second mechanism might involve dephosphorylation of the NUP107–160 complex

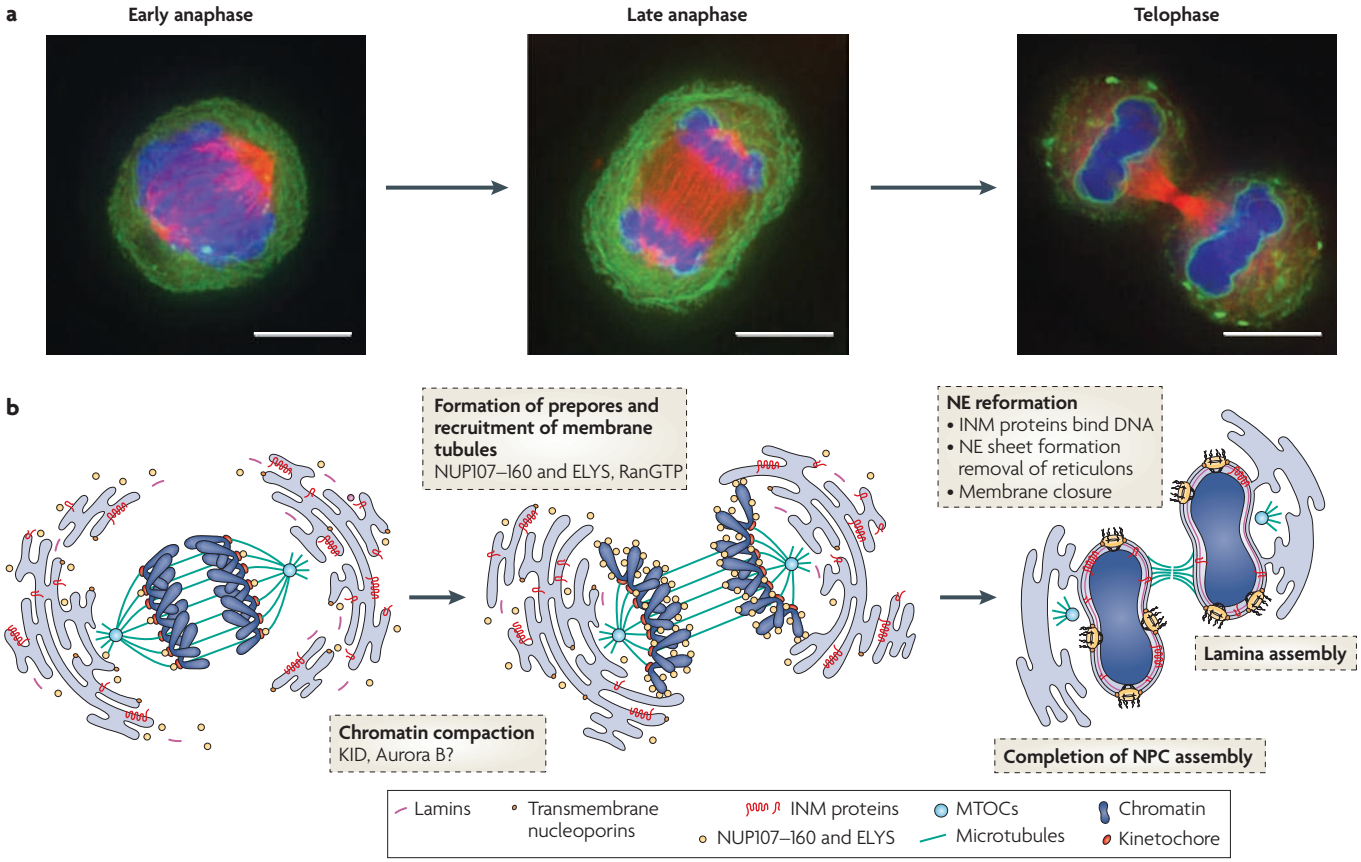

Figure 5 | **Nuclear envelope reassembly after mitosis. a** | The images show HeLa cells in which the inner nuclear membrane (INM, green; stained by green fluorescent protein fused to lamina-associated protein 2β (LAP2β)), DNA (blue; stained with Hoechst) and microtubules (red; stained by red fluorescent protein–α-tubulin) are visualized in early anaphase, late anaphase and telophase. Scale bars, 10 μm. **b** | In anaphase, separating chromatin masses are compacted by the action of the DNA-binding chromokinesin KID (also known as KIF22 and kinesin 10). At this stage of mitosis, INM proteins, such as LAP2β, are still dispersed in the tubular mitotic endoplasmic reticulum (ER). Nuclear pore complex (NPC) assembly is initiated during anaphase by the recruitment of nucleoporin NUP107–160 complexes through ELYS (MEL-28 in *Caenorhabditis elegans*) to chromatin, resulting in the formation of chromatin-bound 'prepores'. This requires the RanGTP-dependent liberation of NUP107–160 complexes from an inhibitory association with importin-β. During late anaphase, ER membrane tubules start binding to the chromatin surface. What mediates the initial attachment of the tubules to chromatin is unknown. During telophase, the retraction of membrane-bending proteins (reticulons) into the peripheral ER allows the remodelling of ER tubules into flattened membrane sheets on the chromatin surface. Binding of INM proteins to DNA/chromatin supports the attachment of membrane sheets to chromatin. The first traces of lamins can be detected on chromatin at this stage. NPC formation is completed by the step-wise recruitment of further NPC constituents and the nuclear envelope (NE) is sealed. Finally, transport-competent NPCs allow for the nuclear import of lamins to complete the assembly of the nuclear lamina. MTOC, microtubule-organizing centre.

E3 ubiquitin ligase
The last enzyme in a cascade of enzymes (E1, E2 and E3) that mediates the attachment of mono- or polyubiquitin to target proteins. E3 enzymes are binding platforms for E2 ligases and substrate proteins and thereby confer specificity to the ubiquitylation reaction.

Annulate lamellae
Stacks of membrane cisternae, usually localized in the cytoplasm, that are densely packed with nuclear pore complexes.

during anaphase, although the exact timing and the phosphatases that are responsible for this are unknown. A third contribution to the control of chromatin association of the NUP107–160 complex has been revealed by the discovery of its chromatin-targeting factor ELYS (MEL-28 in *C. elegans*), which is required to confine postmitotic NPC assembly to the surface of chromatin[80,132,133]. Consistently, RNA interference-mediated knockdown of vertebrate ELYS results in a reduction in the number of NPCs in the NE accompanied by an increase in ectopic NPC assembly in annulate lamellae as a result of uncoupling NPC assembly from chromatin[132,133]. Likewise, mutations in MEL-28 lead to defects in NE morphology and NPC assembly in *C. elegans*[80,134].

The binding of ELYS to chromatin depends on an AT-hook DNA-binding motif in the carboxyl terminus of ELYS and occurs at AT-rich chromatin regions[135,136]. But what controls ELYS association with chromatin? Again, several mechanisms can be envisioned to collectively control this reaction. Spatial control is brought about by the RanGTP-dependent recruitment of ELYS to chromatin[133,134]. Temporal regulation might depend on the dephosphorylation of ELYS or chromatin proteins during anaphase as well as on the timely exposure of AT-rich DNA regions during chromatin decondensation.

Subsequent events in NPC assembly include the association of membranes and other soluble nucleoporins with the chromatin-associated prepore, to produce a

closed NE that contains transport-competent NPCs (see below). Live-cell imaging of somatic cells shows that NPC assembly is not just the reversal of its early mitotic breakdown and that NPC components join in a defined order[13]. Membrane recruitment is manifested by the association of the transmembrane nucleoporins POM121 (pore membrane of 121 kDa) and probably NDC1 with the chromatin-associated prepore. This is followed by the attachment of other central NPC subcomplexes, such as the NUP93, NUP98 and NUP62 complexes, the recruitment of which correlates with the gain of nuclear import activity.

Starting NE membrane reformation. The NE reforms from the mitotic ER by a process that involves the attraction of INM proteins to the surface of decondensing chromatin[137,138], membrane-remodelling events that allow for the formation of a uniformly spaced double membrane[139] and the generation of pores that house NPCs. Different NE membrane proteins probably orchestrate distinct steps in the membrane recruitment and remodelling process, which has been proposed to be accomplished by a massive reorganization of the tubular mitotic ER network on the chromatin surface[140].

The first step in NE membrane reformation is the binding of the tips of ER tubules to chromatin[141]. Proteins that are known to eventually reside in curved parts of NE membranes, such as the pore membrane proteins NDC1 (REFS 33,142) and POM121 (REF. 143), the NPC-associated protein SUN1 (REF. 144) or the membrane-coating NUP107–160 complex[145,146], might be present on these tips and mediate chromatin association. The recruitment of ER tubules is followed by the attraction of more membrane material in-between the initial contact sites, finally leading to the formation of expanded, flattened NE patches on chromatin[140]. The conversion of chromatin-associated tubules to membrane sheets is kinetically controlled by removal of ER-tubule-forming proteins of the reticulon family from the reforming NE[141]. Consistently, reticulon overexpression delays NE formation, whereas reticulon depletion by RNA interference speeds it up[141].

DNA-binding INM proteins might stabilize the association of membrane sheets with chromatin at this point, as exogenous DNA inhibits flat sheet formation[140]. In support of this idea, it has been shown that several INM proteins can directly bind to DNA and that DNA can compete for membrane recruitment to chromatin during NE assembly *in vitro*[147]. Furthermore, ~50% of mammalian INM proteins show a striking enrichment in basic, extralumenal domains[147], suggesting that the binding of several different INM proteins to DNA could together account for the efficient recruitment of membranes to chromatin.

Besides a direct contribution of DNA, changes in chromatin assist membrane protein recruitment. In *C. elegans*, BAF is essential for NE reformation, presumably by triggering the recruitment of INM proteins that have LEM domains to chromatin[148]. Surprisingly, the depletion of vaccinia-related kinase 1 (VRK1), which mediates the phosphorylation of BAF and thereby causes its chromatin dissociation, also compromises NE formation[148]. Depletion of VRK1 causes LEM proteins to remain bound to chromatin during mitosis, which might in turn inhibit postmitotic NE assembly.

During early telophase, different regions of chromatin initially attract distinct sets of NE proteins. Whereas LBR, LAP2β and lamin B bind to more peripheral chromatin regions, LAP2α, BAF, emerin and traces of A-type lamins localize to so-called 'core' regions that face the spindle midzone and spindle poles[149,150]. Although some association of nuclear lamins is observed during early stages of NE reformation[150,151], the bulk of nuclear lamins are reassembled into the lamina only after the nuclei have regained competence for nuclear import[152].

Coordination of closed NE formation and NPC insertion. A crucial step in nuclear reformation is the closure of the NE to re-establish its boundary function. At the same time, pores that are occupied by NPCs must be generated in the assembling double membrane. Therefore, a mechanism must exist to coordinate membrane closure and NPC insertion. The deposition of a chromatin-associated prepore built by the NUP107–160 complex during anaphase is a crucial event, because depletion of its chromatin anchor ELYS leads to the formation of a pore-free NE *in vitro*[133].

But how are membrane pores generated at the sites of prepore deposition? Two different models have been proposed to explain this. The first model proposes that pores are inserted into patches of flattened double membranes on the surface of chromatin[153]. This would require a membrane fusion event between the ONM and INM that is not understood mechanistically. Such fusion could be linked to the site of prepore deposition by the recruitment of the membrane fusion machinery to the chromatin-bound NUP107–160 complex. The paradigm of this mode of NPC insertion is found in NPC assembly in yeasts with closed mitosis and in metazoan cells during interphase. Another model suggests that the chromatin-associated prepore[128] attracts membranes from aside, such that membranes would encircle the chromatin-associated prepore[140] and render membrane fusion unnecessary for pore formation. Clearly, to distinguish between these two possibilities, a molecular understanding of membrane fusion during interphase NPC assembly is required to be able to test whether factors that trigger pore formation during interphase NPC assembly also affect postmitotic NPC assembly.

A final step in NE assembly is the sealing of the NE. This requires both the formation of a continuous sheet of double membrane around chromatin and the closing of remaining holes that connect the INM and ONM by annular membrane fusion[8]. Annular fusion involves the constriction of a membrane ring, which results in the separation of two membranes by an unknown mechanism. If the second of the aforementioned models holds true, membrane remodelling around reassembling NPCs could be a way to overcome a need for such annular fusion events by filling the remaining holes with NPCs[140].

Is, then, membrane fusion at all required for the establishment of a closed NE from the mitotic ER network? Notably, *in vitro* nuclear assembly experiments have shown that NE reformation requires SNARE-mediated fusion of chromatin-associated NE membrane patches[154]. However, a functional NE can also be reformed *in vitro* under conditions that are assumed to block SNARE-mediated membrane fusion, but only if a reconstituted ER network is used as source of membranes[140]. Thus, the SNARE-dependent reformation of the NE on sperm chromatin *in vitro*[154] might represent an ER reformation step that is coupled to chromatin. Likewise, the transition from mitotic to interphase ER organization might also occur in part on the chromatin surface *in vivo*.

During NE reformation, it might be necessary to prevent the precocious formation of a closed NE by uncontrolled membrane closure uncoupled from NPC insertion. One possibility to account for such a control mechanism is derived from *in vitro* studies that suggest that the transmembrane nucleoporin POM121, together with the NUP107–160 complex, is part of a potential checkpoint to coordinate NPC and membrane assembly[155]. *In vitro*, depletion of POM121 from membranes causes a block in membrane fusion after vesicles have docked to chromatin. Co-depletion of the NUP107–160 complex overrides the fusion defect, leading to the formation of a closed NE that is devoid of NPCs[155]. It will be important to understand the underlying molecular mechanism of this postulated checkpoint and to unravel its significance to NE reformation *in vivo*. Alternatively, the cell might just rely on a defined kinetic pathway for nuclear assembly, which ensures that the deposition of the NUP107–160 complex on chromatin is tightly coordinated with membrane recruitment, assembly of complete NPCs and membrane closure.

Conclusions and perspectives

During cell division, dynamic changes in the organization of cellular compartments occur not only to guarantee the faithful transmission of the genetic material but also to maintain proper compartmentalization of daughter cells. The disassembly and reassembly of the nucleus during open mitosis is intimately linked to the overall orchestration of mitosis by kinases and the pathways that regulate their timely activation and inactivation. Although progress in the field has been rapid over the past few years, major challenges remain to gain an even deeper molecular understanding.

Phosphorylation events are crucial for NEBD, but the causal relationship between kinase activity and the specific steps in NEBD are largely unknown. The functional consequences of phosphorylation have been assessed in only a few kinase targets. In addition to protein phosphorylation, other post-translational modifications of NE components, such as sumoylation or ubiquitylation, might also have relevance for NE dynamics during mitosis. Clearly, we still do not know the complete repertoire of components that are involved in nuclear disassembly. Although roles of the microtubule cytoskeleton and of ER dynamics in NEBD have emerged, intriguing questions remain. For example, how are molecular motors attached to the NE to facilitate NEBD? Do factors that are involved in mitotic remodelling of the ER have a direct role in NE disassembly? Another poorly explored area is the role of NE membrane lipids and lipid signalling in mitotic NE and ER organization — a role that is expected to be analogous to the function of lipids in membrane dynamics of other organelles. Collectively, our efforts to identify and characterize the machinery that is involved in nuclear disassembly are still in their infancy, but might hopefully bring up new candidate factors that could also serve as potential drug targets in cancer treatment.

Box 2 | **NPC biogenesis and ageing**

In dividing metazoan cells, nuclear pore complexes (NPCs) are assembled during interphase and after mitosis. However, recent work has discovered a lack of NPC assembly in postmitotic, differentiated cells[157]. The expression of scaffold nucleoporins is downregulated and pre-existing scaffold nucleoporins are not exchanged in both somatic cells of adult worms and differentiated mouse myotubes. If there is no NPC biogenesis in quiescent cells, the accumulation of damage in old NPCs might cause changes in NPC transport or barrier properties. Strikingly, nuclei that are isolated from non-proliferative brain regions of old rats tend to be more leaky compared with those of young animals. Thus, the lack of NPC turnover in differentiated cells correlates with age-related defects in the NE permeability barrier.

Ageing is not limited to metazoan organisms, as unicellular species also age. Asymmetric division of budding yeast generates two cells with different life expectations — whereas mother cells age, daughter cells rejuvenate and regain a preset replicative life span. This phenomenon is attributed to the asymmetric distribution of ageing factors during cell division, including the accumulation of extrachromosomal ribosomal DNA circles (ERCs) in mother but not daughter cells.

How ERCs are confined to mother cells has recently found an explanation buried in the NE[158]. Anaphase yeast cells establish a lateral diffusion barrier in the NE that ensures that most pre-existing NPCs are retained in the mother. These pre-existing NPCs are suggested to tether ERCs, thereby preventing their inheritance to the daughter. Indeed, the association of reporter DNA circles with NPCs is abrogated in cells that lack the NPC basket components myosin-like protein 1 and 2 (Mlp1 and Mlp2, respectively), leading to randomized segregation of DNA circles. Furthermore, a non-functional diffusion barrier that is generated by the deletion of budneck-associated factor 6 (Bud6) affects ageing. Δ*bud6* mothers age slower, whereas their daughter cells display premature ageing, which correlates with the deficiency of mother cells to retain old NPCs and DNA circles.

Although there is no evidence for an involvement of ERCs in human ageing, it is noteworthy that stem cells also divide asymmetrically, giving rise to self-renewing and differentiating daughter cells. By analogy to budding yeast, it will be interesting to determine how cellular membrane systems might contribute to the asymmetric inheritance of cell fate determinants in organisms that are undergoing 'open' mitosis.

SNARE
(Soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor). A large protein family of membrane-anchored coiled-coil proteins that contribute to the specificity of membrane trafficking and promote membrane fusion.

ERC
(Extrachromosomal ribosomal DNA circle). A self-replicating, non-centromeric plasmid that is generated sporadically by homologous recombination in chromosomal repeats of ribosomal DNA.

Compared with the role of kinases in NEBD, our knowledge of protein phosphatases in nuclear reassembly is mediocre at best. This area of research will benefit from deciphering the role of protein phosphatases in mitotic exit in general. Recent discoveries provide the first insights into how changes in chromatin impinge on NE reassembly. But how is chromatin decondensation linked in molecular terms to the attraction of NPC components and the re-establishment of the NE? Progress on this subject will probably depend on advances in our understanding of mitotic chromosome organization and dynamics.

Notably, recent data have revealed unexpected links between NPC assembly and ageing (BOX 2) — a connection that will provoke new interests in understanding the regulation of NPC biogenesis during development.

Last, but not least, the NE is not a passive element in mitotic progression. Many NE components have active roles in the control of cell division. A number of nucleoporins influence spindle assembly, kinetochore function and chromosome segregation. Conversely, proteins that are involved in the SAC are found at NPCs during interphase. It seems that there are close ties between NPCs on the one hand and kinetochore proteins or SAC factors on the other. We anticipate that more of these links will be revealed and expect that mechanistic studies will lead to a more integrated picture of how mitotic events, from NPC disassembly over spindle formation and chromosome segregation to the final steps in cell division, are connected.

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DATABASES

UniProtKB: http://www.uniprot.org
Aurora A | BAF | BUB3 | CDK1 | ELYS | GP210 | KID | LAP2α | LAP2β | LBR | MAD1 | MAD2 | NDC1 | NimA | NUMA | NUP53 | NUP98 | NUP107 | p97 | PLK1 | POM121 | RanBP2 | RanGAP1 | RAE1 | RCC1 | TOPOIIα | YOP-1

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