Chapter 10
NPCs in Mitosis and Chromosome Segregation

Masaharu Hazawa, Akiko Kobayashi and Richard W. Wong

Abstract In eukaryotic cells, the nuclear envelope (NE) separates thousands of genes and RNAs inside the nucleus from the rest of the cell. The NE consists of two separate membranes, the inner nucleoplasm-facing nuclear membrane and the outer cytoplasm-facing nuclear membrane, which is continuous with the endoplasmic reticulum (ER). These membranes are separated by the perinuclear luminal space. Transport between the cytoplasm and the nucleus occurs through dedicated, cylindrical holes that are present at sites where the inner and outer nuclear membranes connect together. These holes are filled with macromolecular gates termed nuclear pore complexes (NPCs), which are the only gateway between the nucleus and the cytoplasm. In recent years, several nucleoporins have been shown to play important roles in mitosis. In this chapter, we summarize the recent progress in our understanding of the roles of different nuclear pore components at different stages of mitosis, with a focus on their functions within the mitotic machinery and in the inhibition of tumorigenesis.

Keywords Mitosis · NPC · nucleoporin · chromosomes · cancer

10.1 NPC Structure and Composition

The structure of the human NPC was initially determined using transmission electron microscopy, subsequently scanning electron microscopy and most recently cryo-electron tomography (for review, see (Sakiyama et al. 2017)) and atomic

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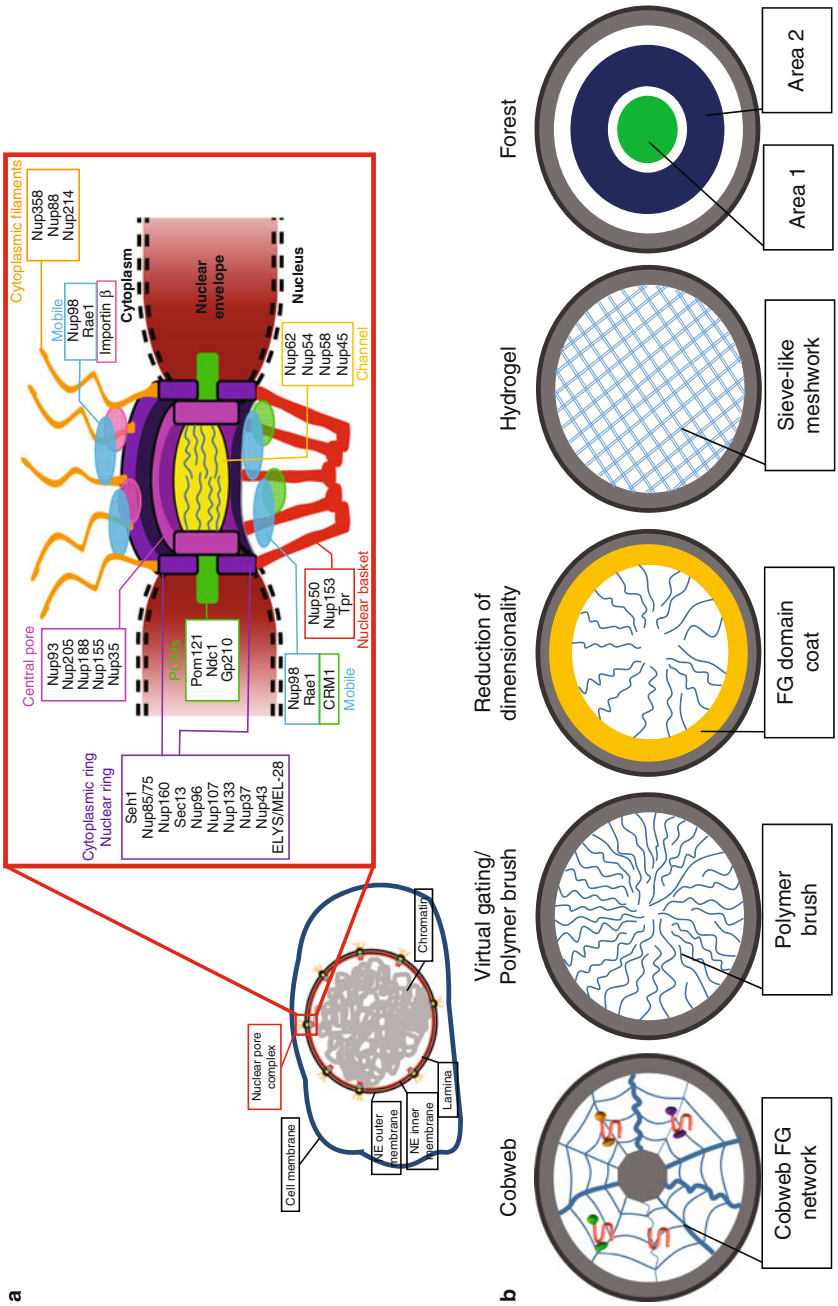

Fig. 10.1 (a) Structure and composition of the vertebrate NPC. (b) A schematic illustration of various Nucleocytoplasmic transport models

force microscopy (AFM) (Dufrene et al. 2017; Liashkovich and Shahin 2017; Stanley et al. 2017; Mohamed et al. 2017). NPCs are large multiprotein channels of around 120 MDa and about 80-120 nm in diameter in vertebrates (Blobel 2010; Doucet and Hetzer 2010). They have eightfold rotational symmetry and comprise multiple copies of ~30 different proteins called nucleoporins or Nups (Fig. 10.1a) (Sakuma and D'Angelo 2017; Hayama et al. 2017; Wong 2015; Nakano et al. 2011). The structures of NPCs consist of a spoke-ring complex, a central transporter, cytoplasmic and nucleoplasmic rings, kinetically movable transporters (e.g., Rae1 and Nup98), attached cytoplasmic filaments and a nuclear basket (Sakuma and D'Angelo 2017). Nups (labeled "Nup" followed by their expected molecular weight) are segmental proteins with a limited number of structural motifs (coiled-coils, α-solenoids, β-propellers) that are used repetitively to build the symmetrical NPC structure. Nearly one-third of Nups consist phenylalanine–glycine (FG) domain motifs interspersed with spacer sequences (Wong 2015; Nakano et al. 2011) (Fig. 10.1b). These FG domains are intrinsically disordered polypeptide chains and serve as interaction sites for transport receptors (karyopherins) that escort cargo through the pore (Sakuma and D'Angelo 2017). These FG-Nups form a selective barrier allowing passive diffusion and active transport with the assistance of transport receptors. Several FG-Nup trafficking models have been proposed (Fig. 10.1b). Recently, by directly observing native nuclei of colon cancer cells via HS-AFM, we found that FG-Nups are short, stiff, hair-like, twisted ropes that together form a broken spider's web pattern (Mohamed et al. 2017) (Fig. 10.1b).

Lately, growing appreciation of the role of NPCs in cell division has emerged. When mitosis starts, the NE and NPCs are disassembled and nucleoporins are detected either alone or in subcomplexes/small building blocks after nuclear envelope breakdown (NEBD) (Webster et al. 2009). Nups are associated with the kinetochore, spindle and centrosome functions. At the end of telophase, Nup building blocks reassemble sequentially and are finally brought to the NPC within NE. Here, we describe the recent progress in research on various NPC components in mitosis.

10.2 Function of NPC Components in Chromosome Segregation and Mitotic Apparatus Orchestration During Mitosis

The precise capture of mitotic chromosomes by spindle microtubules is critical for accurate cell division. During mitosis, microtubules are assembled such that the minus ends are focused into two poles at centrosomes, while the plus ends interact with chromosomes via kinetochores and align them on the metaphase plate. Spindle reorganization is assisted not only by centrosomes and chromatin, but also by microtubule-binding proteins, such as molecular motors (Guttinger et al. 2009; Nakano et al. 2011). Higher eukaryotes form a cytoplasmic spindle, which incorporates NEBD to allow the contact of spindle microtubules to kinetochores in the

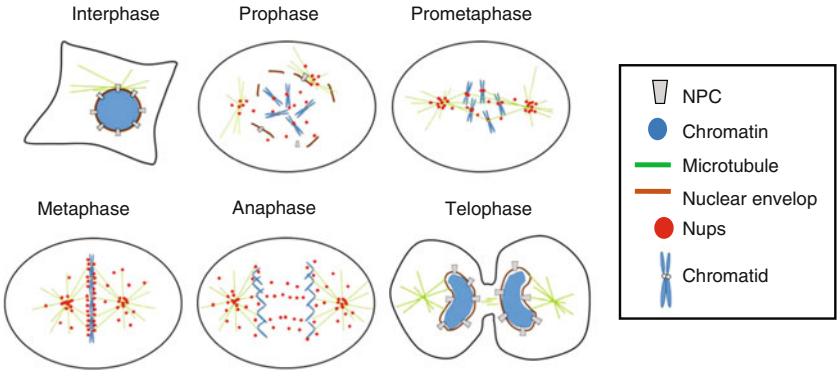

Fig. 10.2 Schematic model of NPC proteins/Nups and chromosome-spindle interactions during the cell cycle

early stage of mitosis. NEBD results in the removal of NE membranes and the disassembly of large macromolecular complexes, such as the lamina and NPCs. After late anaphase, NE is restructured around each mass of chromatin in the daughter cells to reform the nuclear boundary (Guttinger et al. 2009). The most impressive change of the mammalian NPC takes place at the beginning of cell division, when individual Nups become heavily phosphorylated and NPCs are taken into pieces (Tran and Wente 2006; D'Angelo and Hetzer 2008; Guttinger et al. 2009; Strambio-De-Castillia et al. 2010; Wozniak et al. 2010; Hetzer 2010; Ibarra and Hetzer 2015; Wong and D'Angelo 2016). Later, NPC subunits relocate to mitotic substructures, mainly the kinetochores, and many of them show mitotic-specific functions in spindle assembly or anaphase onset (Wozniak et al. 2010). During exit from mitosis, NPCs reassemble into the reforming NE, and their number is approximately doubled during interphase (Guttinger et al. 2009; Nakano et al. 2011). NPC components are also involved in the dynamics of S phase and contribute to the maintenance of genome integrity, preventing the accumulation of DNA lesions (Hetzer 2010; Ibarra and Hetzer 2015) (Fig. 10.2). In the text below, we summarize how these nucleoporins coordinate cell cycle progression in mammalian cells.

10.2.1 Nup358/RanBP2 Subcomplex in Mitosis

Nup358/RanBP2, a Ran binding protein with four Ran binding domains, is an utmost cytosolic component of filaments derived from the cytoplasmic ring of the NPC (Matunis and Pickart 2005; Wu et al. 1995) (Fig. 10.1a). This large protein (3,224 residues in humans) can be divided into several domains: an N-terminal TPR domain, an α-helical region, four Ran-binding domains, eight tandem zinc fingers, a SUMO E3 ligase domain, FG and FxFG repeats that act as binding surfaces for transport receptors, and a C-terminal domain that displays sequence homology to cyclophilins (Fig. 10.1) (Wong and D'Angelo 2016; Hashizume et al. 2013). Surprisingly, Nup358/RanBP2 has recently been shown also to play an

unexpected role in Y-complex oligomerization, a finding that blurs the conventional boundary between scaffold and transport-channel Nup building blocks (von Appen et al. 2015). Nup358/RanBP2 function was initially linked to nucleocytoplasmic transport; however, consistent with its multidomain configuration, Nup358/RanBP2 has been shown to have more pleiotropic functions. This large Nup is now recognized as a regulator of numerous cellular processes. In particular, RanBP2–RanGAP1*SUMO1/Ubc9 was identified as a multisubunit SUMO E3 ligase (Werner et al. 2012). Remarkably, RanBP2 has also been implicated in the delivery and integration of the genomic material of HIV-1 (Wong et al. 2015).

At the beginning of mitosis when the NE breaks down and NPCs disassemble, RanBP2–RanGAP1–SUMO1–Ubc9 subcomplexes diffuse into the mitotic cytosol and gather at the plus ends of free spindle microtubules and at kinetochores of chromosomes that have been captured by spindle microtubules (Hashizume et al. 2013; Joseph et al. 2002, 2004). The nuclear export receptor Crm1 is responsible for kinetochore targeting of RanBP2–RanGAP1–SUMO1–Ubc9 (Arnaoutov et al. 2005). In HeLa and RGG cells, knockdown of RanBP2 causes several mitotic abnormalities, including misalignment of the chromosome in metaphase, mislocalization of several kinetochore-associated proteins and formation of multipolar spindles (Hashizume et al. 2013; Joseph et al. 2004; Salina et al. 2003). When anaphase starts, sister chromatids are disconnected and separated. This comprises decatenation of sister chromatids at centromeres by Topoisomerase II (TOPOII) (Guttinger et al. 2009). Nup358/RanBP2 was found to enrol TOPOII to centromeres by its sumoylation in mammalian cells (Dawlaty et al. 2008), and similar to TOPOII depletion, knockdown of Nup358/RanBP2 enhanced the formation of anaphase bridges (Dawlaty et al. 2008). As a consequence, mice with reduced levels of Nup358/RanBP2 exploit serious aneuploidy phenotypes and are predisposed to cancer (Dawlaty et al. 2008). These findings suggest that Nup358/RanBP2 functions as a tumor suppressor (Guttinger et al. 2009). Moreover, using RanBP2 conditional knockout mouse embryonic fibroblasts and a series of mutant constructs, Hamada et al. showed that transport, rather than mitotic, functions of RanBP2 are required for cell viability (Hamada et al. 2011).

Interestingly, Nup358/RanBP2 has been associated with cancer in different, and possibly contradictory, manners. On the one hand, its downregulation induces G_2/M phase arrest, impairs chromosomal alignment and results in mitotic catastrophe and cell death. Consistent with this, Nup358/RanBP2 participation in chromosomal translocations that result in hematological malignancies suggests its potential oncogenic role in patients. Vecchione et al. (2016) discovered that increased expression of Nup358/RanBP2 protects a subgroup of colorectal cancer (CRC) cells from undergoing mitotic cell death, which is consistent with an oncogenic function. They studied an important subset of CCs that carry the BRAF mutation V600E; this mutation occurs in about 8%–10% of CRC patients and is associated with a poor prognosis, particularly in the metastatic setting (Wong and D'Angelo 2016). Vecchione et al. (2016) discovered that the suppression of Nup358/RanBP2 is selectively lethal to colon cancers having a BRAF-like signature (Fig. 10.3). Consistent with previous findings (Hashizume et al. 2013), they also found that the depletion of Nup358/RanBP2 causes defective kinetochore structure

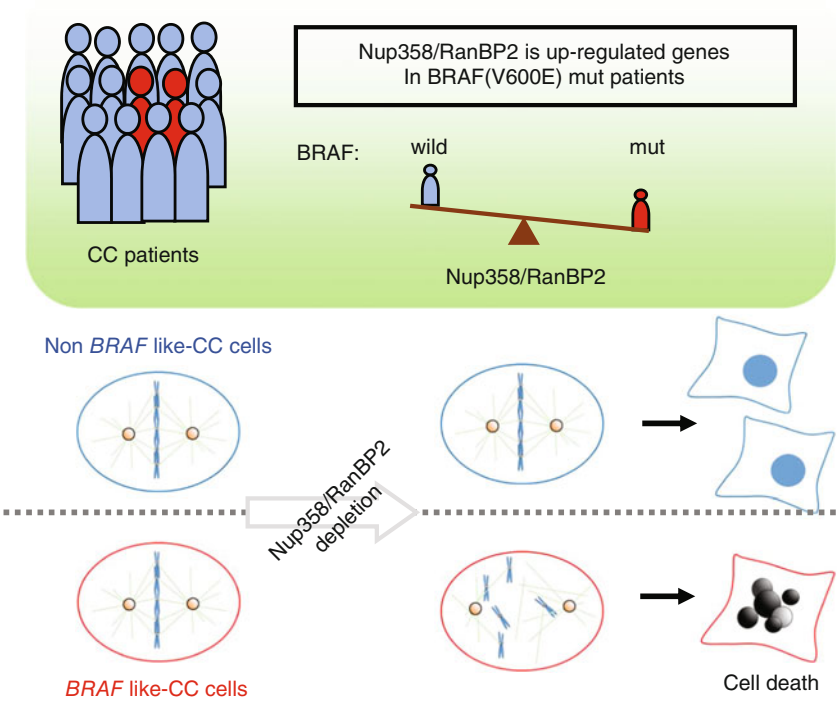

Fig. 10.3 A Model for Nup358/RanBP2 in mitotic progression and faithful chromosomal segregation

and composition, abnormal mitotic progression and abnormal chromosome segregation. BRAF-like CC cells depleted of Nup358/RanBP2 showed prolonged mitosis or mitotic arrest, which eventually triggered mitotic cell death (Hashizume et al. 2013). They also revealed that BRAF-like CC cell lines are defective in kinetochore microtubule outgrowth and that Nup358/RanBP2 depletion further potentiates this abnormality, leading to mitotic cell death (Fig. 10.3). These recent findings prompted the proposal that BRAF-like CC cells depend on Nup358/RanBP2 expression to tolerate the defects in mitosis (Wong and D'Angelo 2016).

10.2.1.1 Nup88

Nup88 localizes between Nup358 and Nup214 and physically interacts with them (Hashizume et al. 2010). Alterations in the expression of Nup88 in mice (Naylor et al. 2016) or cells (Hashizume et al. 2010) enhanced the multinucleation of cells and multipolar spindle formation, leading to aneuploidy and enhanced genomic instability (Hashizume et al. 2010). A potential explanation for the appearance of these cell populations is that disruption of the normal Nup88 expression levels

(by overexpression or depletion strategies) leads to a failure in the kinetochore–spindle microtubule interactions to capture chromosomes, ultimately leading to mitotic exit and nuclear envelope (NE) reformation around dispersed chromosomes or groups of chromosomes. It is enticing to speculate that Nup88 might be involved in cell cycle checkpoints to prevent aneuploidy. Naylor et al. also reported that the Nup88–Nup98–Rae1–APC/Cdh1 axis contributes to aneuploidy. They found that Nup88 overexpression did not alter global nuclear transport, but was a potent inducer of aneuploidy and chromosomal instability in mice (Naylor et al. 2016). The sequential molecular mechanisms underlying Nup and APC (Cdc20–Cdh1) signaling pathways are still not fully understood.

10.2.1.2 Nup214

Another cytoplasmic filament nucleoporin, Nup214/CAN, is a proto-oncogene implicated in leukemia (Saito et al. 2016). RNAi-mediated knockdown of Nup88 disrupted Nup214 expression and localization and caused multipolar spindle phenotypes (Hashizume et al. 2010). The abolition of Nup214 from mitotic spindles results in chromosome separation defects and aneuploidy with multinucleated cells. Similarly, the downregulation of Nup214 by miR-133b, which has been described as a tumor suppressor in esophageal squamous cell carcinoma (SCC) (Bhattacharjya et al. 2015), results in chromosome defects, with some cells appearing like "flowers" with greatly lobulated nuclei (multipolarization and multilobulation of cells) (Bhattacharjya et al. 2015).

10.2.2 Nup62 Subcomplex in Mitosis

The mammalian Nup62 subcomplex assembles from 4 O-glycosylated nucleoporins Nup62, 58, 54 and 45. The 62-kDa component of this complex, Nup62, contains three domains: N-terminal FG-repeat, central threonine/alanine-rich linker and C-terminal α-helical coiled-coil (Guan et al. 1995; Wong 2015). During interpahse, NUP62 as a gatekeeper regulates p63 nuclear transport and cell fate of squamous cell carcinoma (Hazawa et al 2018). During mitosis, Nup62 has been shown to play a novel role in centrosome integrity (Hashizume et al. 2013). Knockdown of Nup62 induces mitotic arrest in the G_2/M phase and mitotic cell death. Depletion of Nup62 also results in abnormal centriole synthesis and maturation, defective centrosome segregation, formation of multipolar centrosomes, dramatic spindle orientation defects, centrosome component rearrangements that impair cell bipolarity and multinucleated cells (Fig. 10.4) (Hashizume et al. 2013). Consistent with these functions, a GFP–Nup62 fusion protein was reported to accumulate at centrosomes (Hashizume et al. 2013) and spindle-like structures, identified by costaining with α-tubulin antibody (Wu et al. 2016). Consistent with this intimate link between centrosomes and cilia, nucleoporins have also been detected in the base of the cilia. Takao and Verhey (2016) developed a system to

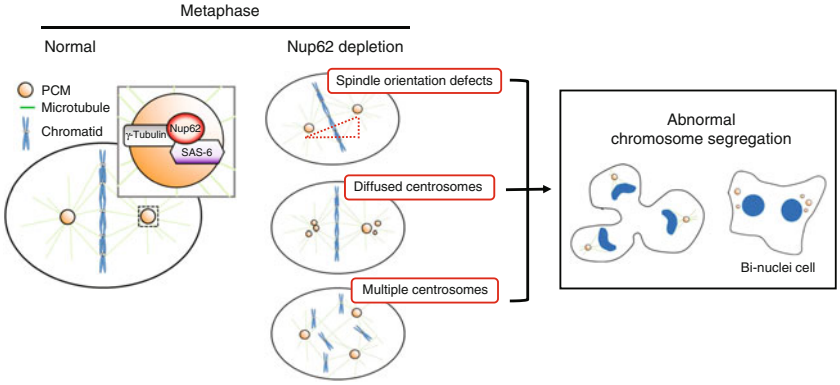

Fig. 10.4 Orchestration of the Nup62 inner ring subcomplex dynamics at the centrosomes

clog the pore by inhibiting Nup62 function via forced dimerization, and showed that forced dimerization of Nup62-Fv attenuated the kinesin-2 motor KIF17 (Wong et al. 2002) into the ciliary compartment, proposing the existence of a "ciliary pore complex" (Takao and Verhey 2016). Whether this model will stand the test of time is yet to be determined (Del Viso et al. 2016), but it offers an interesting mechanistic possibility. The mitotic functions of other members (Nup54, Nup58 or Nup45) of the Nup62 subcomplex remain to be established.

10.2.3 Nup107–Nup160 Subcomplex in Mitosis

Several lines of evidence support the localization of vertebrate Nup107–Nup160 complex (yeast Nup84 complex) at the kinetochores and spindles during mitosis (Belgareh et al. 2001; Loiodice et al. 2004). This subcomplex contains at least 10 members (Nup160, Nup133, Nup107, Nup96, Nup85, Nup43, Nup37, Sec13, Seh1 and ELYS/MEL-28) (Resendes et al. 2008). It is also worth noting that phosphorylation controls the breakdown of the NE at mitosis and the disassembly of the NPC into different subcomplexes. The Nup107–160 subcomplex was shown to interact with CENP-F. Partial depletion of the components ELYS or Nup133 in human cells did not alter spindle assembly or chromosome segregation, but induced cytokinesis defects (Rasala et al. 2008). By combining in vivo and in vitro studies, Mishra et al. (2010) further showed that the Nup107–160 complex promotes spindle assembly through Ran-GTP-regulated nucleation of microtubules by c-TuRC at kinetochores (Wozniak et al. 2010) (Fig. 10.5). Bolhy et al. (2011) also showed that Nup133 exerts this function through an interaction chain via CENP-F and NudE/EL. This molecular network is critical for maintaining centrosome association with the NE at mitotic entry. Moreover, depletion of one of the subunits, Seh1, induced a mitotic delay (Zuccolo et al. 2007). Seh1 also interacts with Mio, a key member of the SEACAT complex in both interphase and

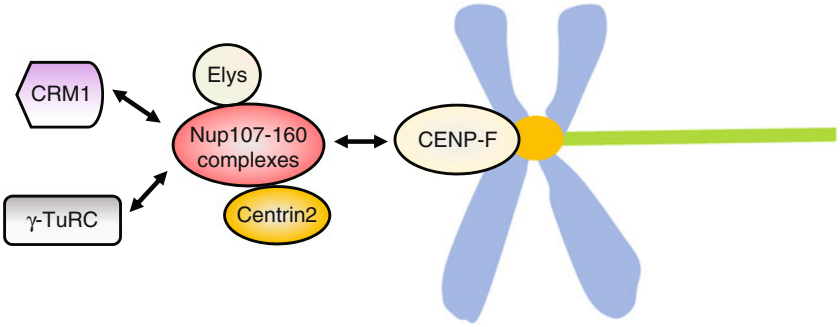

Fig. 10.5 Orchestration of the Nup107-160 core ring subcomplex dynamics in mitosis

mitosis. Mio plays a critical role in activation of the essential mitotic kinases, Aurora A and Plk1, at spindle poles/centrosomes (Platani et al. 2015). In addition, Clever et al. (2011) showed that ELYS/Mel28 plays a role in NE subdomain formation in late mitosis. The depletion of ELYS/Mel28 also accelerates the entry into cytokinesis after the recruitment of emerin to chromosomes. Moreover, Yokoyama et al. (2014)showed that, upon mitotic NPC disassembly, MEL-28 dissociates from chromatin and relocalizes to spindle microtubules and kinetochores. It then directly binds microtubules in a Ran-GTP-regulated manner via its C-terminal chromatin-binding domain. Supporting this notion, Gómez-Saldivar et al. (2016) identified functional domains responsible for NE and kinetochore localization, chromatin binding, mitotic spindle association and chromosome segregation. Phylogenetic profile analysis also suggested that Nup107–160 subcomplex proteins may function in the SAC and that they potentially interact with Mad2 and MadBub/Bub3 (van Hooff et al. 2017).

10.2.4 Tpr–Nup153 Subcomplex in Mitosis

Mitotic arrest deficient 1 (Mad1) and Mad2 proteins, which are critical regulators of the spindle assembly checkpoint (SAC), interact with another Nup, Tpr (Mlp1 and 2 in yeast). Once the cells enter prophase, Mad1 and Mad2 gather on unattached kinetochores and monitor microtubule occupancy to avoid the premature onset of anaphase (Guttinger et al. 2009). Mad1 is accountable for targeting of the Mad1–Mad2 complex to both NPCs during interphase and kinetochores during mitosis (Rao et al. 2009). Tpr, Mad1 and Mad2 coprecipitate in mitotic-enriched HeLa cell extracts, which lack microtubules and intact nuclear pores (Lee et al. 2008; Lince-Faria et al. 2009; Nakano et al. 2010). Tpr-depleted cells show chromosome segregation defects similar to those seen in cells depleted of Mad1 and Mad2 (Nakano et al. 2010). The functional significance of the connection between the basket and the SAC was further emphasized by experiments demonstrating that Tpr is liable for faithful chromosome segregation during mitosis through its

association with the dynein light chain (DLC) (Nakano et al. 2010). Tpr acts as a spatial and temporal regulator of the SAC, maintaining the efficient recruitment of Mad1 and Mad2 to the molecular motor dynein to promote correct anaphase progression (Nakano et al. 2010). When Tpr levels are reduced, many cells show abnormal spindle polarity, bending chromosomes and chromosome lagging (Nakano et al. 2010). These phenotypes suggest a direct role for Tpr in forming spindle structures. Nakano et al. performed a series of assays to rescue the chromosome-lagging defects in cells in which Tpr had been knockdown by siRNA, to confirm the functional role of the Tpr–dynein interaction with the mitotic spindle (Rodriguez-Bravo et al. 2014) (Nakano et al. 2010) (Fig. 10.6). Tpr also associates with A-Kinase Anchoring Protein 95 (AKAP95) during mitosis. AKAP95-depleted cells display more rapid prometaphase-to-anaphase transition, escape from nocodazole-induced mitotic arrest and show partial delocalization from kinetochores of the SAC component Mad1 {Lopez-Soop, 2017 #1950}. Kobayashi et al. (2015) also reported that Tpr depletion enhances the rate of tetraploidy and polyploidy. Mechanistically, Tpr interacts, via its central domain, with Aurora A but not Aurora B kinase. In Tpr-depleted cells, the expression levels, spindle pole/centrosomal localization and phosphorylation of Aurora A were all found to be reduced (Kobayashi et al. 2015). Remarkably, an Aurora A inhibitor, Alisertib (MLN8237), also disrupted the centrosomal localization of Tpr and induced cell death in a time- and dose-dependent manner (Fig. 10.7).

Schweizer et al. (2013) showed that Tpr was normally undetectable at kinetochores and dispensable for the kinetochore localization of Mad1, but not of Mad2, which suggests that SAC robustness depends on Mad2 levels at kinetochores. In addition, Rodriguez-Bravo et al. showed that Mad1–Mad2 complexes tethered to the nuclear basket, which activated soluble Mad2 as a binding partner and inhibitor of Cdc20 in the cytoplasm. Displacing Mad1–Mad2 from nuclear pores hastened the onset of anaphase, prevented the effective correction of merotelic errors and increased the threshold of kinetochore-dependent signaling needed to halt mitosis in response to spindle poisons (Rodriguez-Bravo et al. 2014). They suggested that both nuclear pores and kinetochores emit “wait anaphase” signals that preserve genome integrity (Rodriguez-Bravo et al. 2014). Moreover, Rajanala et al. found that Tpr is phosphorylated at the S2059 residue by CDK1 and the phosphorylated form clearly localizes with chromatin during telophase. Abrogation of S2059 phosphorylation abolishes the interaction of Tpr with Mad1, thus compromising the localization of both Mad1 and Mad2 proteins, resulting in cell cycle defects (Rajanala et al. 2014). It is tempting to surmise that Tpr function impacts on the progression out of metaphase–anaphase transition and/or chromosome segregation itself, and the lack of coordination that follows results in aberrant chromatin morphology (Nakano et al. 2010) (Fig. 10.8). In this context, it is worth mentioning that the Tpr–Met oncogene, a carcinogen-induced chromosomal rearrangement resulting in fusion of a protein dimerization of Tpr to the receptor tyrosine kinase domain of Met, has been described (Peschard and Park 2007). In particular, the N terminus of Tpr undergoes frequent rearrangement with Met, Trk and Raf in gastric and thyroid cancers, resulting in hyperactive tyrosine kinase

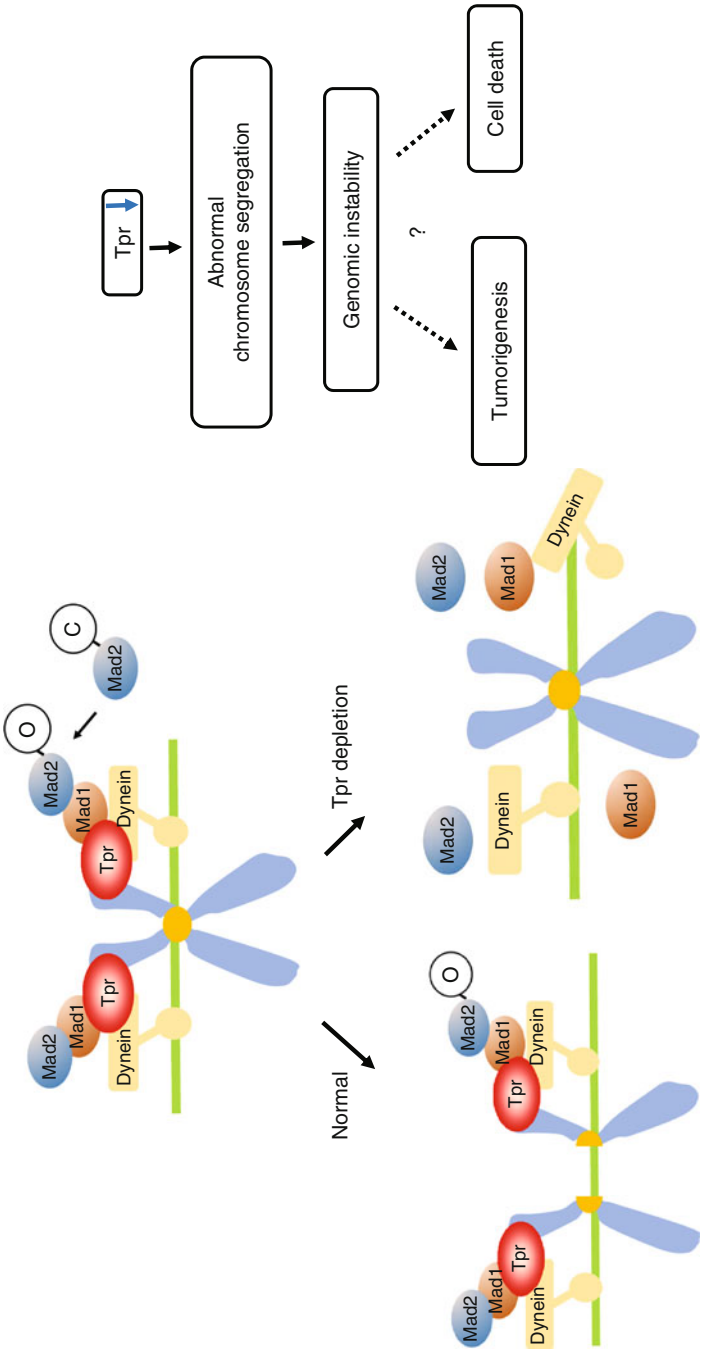

Fig. 10.6 Orchestration of the Tpr-Nup153 nuclear basket subcomplex dynamics in mitosis

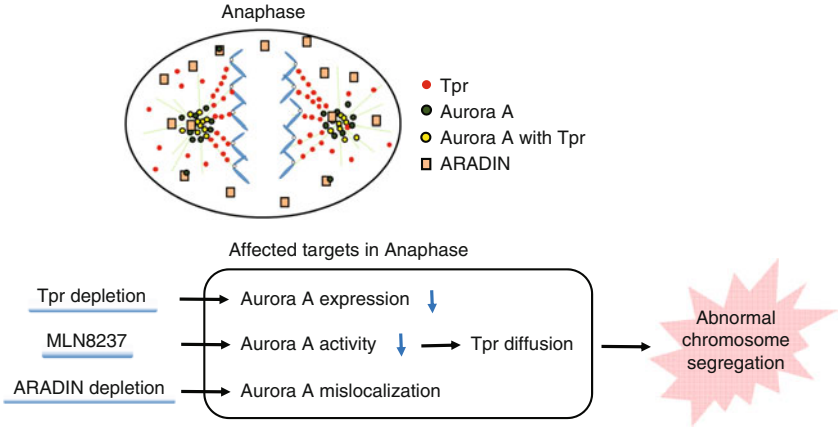

Fig. 10.7 Orchestration of the Tpr-Aurora A subcomplex dynamics in mitosis

fusions that are mislocalized to the cytoplasm (Kohler and Hurt 2010). This segment of Tpr also induces lagging chromosomes when expressed on its own (Nakano et al. 2010), suggesting that these translocations fuel carcinogenesis through increased tyrosine kinase signaling and by subversion of NPC-based defenses against chromosome instability. Given that the data shows chromosome lagging and congregation defects at the metaphase–anaphase transition (Lee et al. 2008; Nakano et al. 2010), one might assume that chromosomal rearrangement of Tpr could lead to chromosomal instability in certain tumors.

10.2.4.1 Nup153

Tpr interacts with another nuclear basket protein, Nup153. Makay et al. (2009) demonstrated that two different phenotypes result from the knockdown of Nup153 to different levels and that rescue of these phenotypes involves distinct domains within Nup153. They suggested that the FG-rich region of Nup153 plays a critical role in mitosis. Besides, when Nup153 levels are reduced further, many cells show abnormal, multilobed nuclei. This phenotype might indicate at a direct role for Nup153 in formation of the nuclear structure (Mackay et al. 2009). By image analysis of live cells in which Nup153 had been knocked down, they also showed that significant delays occur early in mitosis (Mackay et al. 2009; Lussi et al. 2010). Ullman's group also indicated that Nup153 function influences the active state of the Aurora B-mediated abscission checkpoint during cell division (Mackay et al. 2015). Finally, both nuclear basket proteins Tpr and Nup153 were reported to play a role in genome integrity. For example, Tpr siRNA treatment impaired cell growth and proliferation compared with those in control siRNA-treated cells. In Tpr-depleted cells, the levels of p53 and p21 proteins were also increased (Funasaka et al. 2012). Moreover, Tpr depletion increased the nuclear accumulation of p53 and facilitated autophagy (Funasaka et al. 2012).

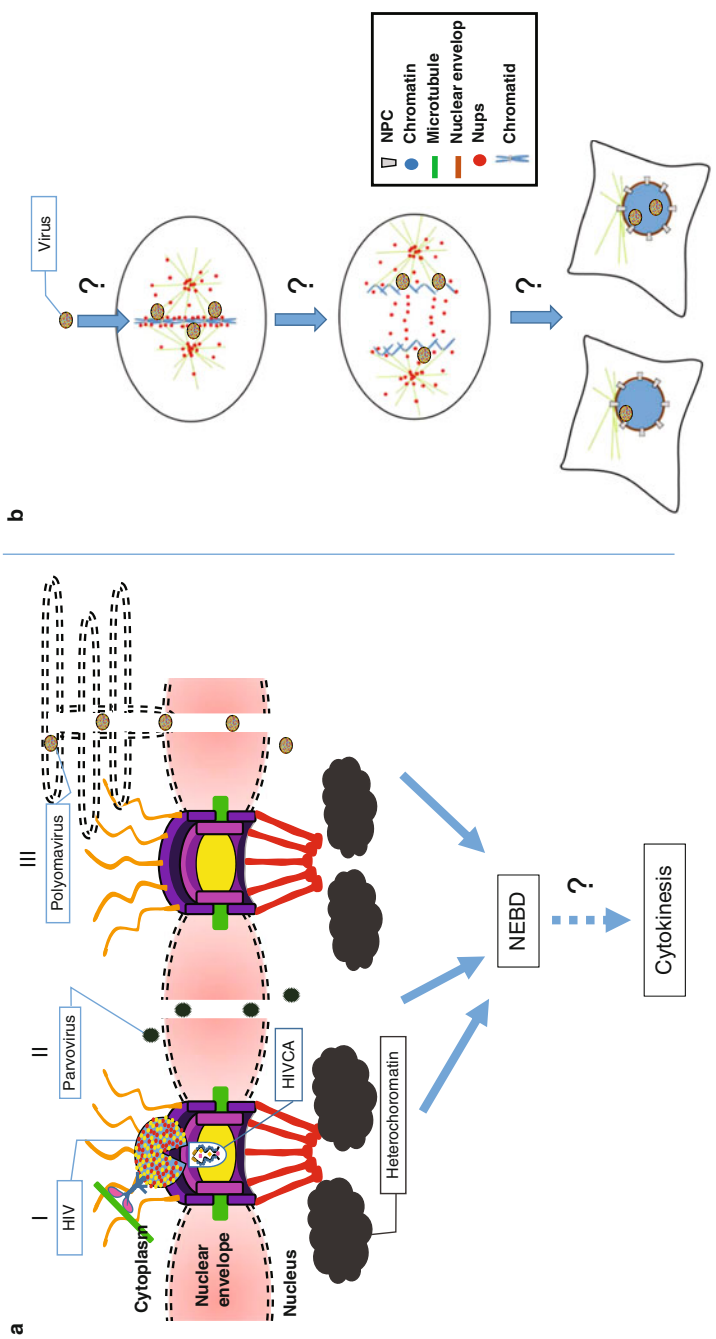

Fig. 10.8 A model for viral (a) nuclear import, integration and (b) mitosis with nucleoporins

10.2.5 Rae1–Nup98 Subcomplex in Mitosis

Another Nup that has been related with spindle assembly is RNA export 1 (Rae1/GLE2/mRNP41). Rae1 has also been linked to the pathophysiology of breast cancer (Chin et al. 2006). Rae1 is a tryptophan–aspartic acid (WD) repeat β propeller protein that is kinetically distributed in NPCs during interphase (Wong et al. 2006). Rae1 forms a complex with Nup98, and both are implicated in RNA export during interphase (Pritchard et al. 1999; Ren et al. 2010; Tran and Wente 2006). Rae1 has been showed to bind to Nup98 and the mitotic checkpoint kinase Bub1 through their Gle2-binding site (GLEBS) domains and to function with Nup98 in securin degradation. Rae1–Nup98 complex has also been reported to inhibit the formation of the anaphase-promoting complex (APC) in a mouse model system (Jeganathan et al. 2005). Several studies have also reported that Rae1 binds to microtubules (MT) (Kraemer et al. 2001; Wong et al. 2006). Using *Xenopus* egg (Blower et al. 2005) and HeLa cell (Wong and Blobel 2008) extract systems for mitotic spindle formation, Rae1 was identified as a essential component for the promotion of microtubule assembly. It was also found to interact and colocalize with nuclear mitotic apparatus protein (NUMA), a microtubule-associated protein that supports microtubule bundling at spindle poles (Wong and Blobel 2008; Wong et al. 2006). Moreover, Rae1 interacts with a subunit of cohesin, SMC1947-967, and it was shown that binding to Rae1 only occurred after the phosphorylation of Ser957 and Ser966 by the spindle pole-localized kinase ATM (Ataxia Telangiectasia Mutated) (Wong and Blobel 2008). Imbalances in SMC1 or Rae1 reactions were also found to cause the formation of multipolar spindles (Wong 2010a,b; Wong and Blobel 2008). In this context, the following question arises: Which signaling pathways regulate the mobile nucleoporin Rae1 in the cell cycle? Jahanshahi et al. (2016) identified that the Hippo pathway targets Rae1 to regulate mitosis and organ size, and provides feedback to regulate the upstream components merlin, hippo and warts. Rae1 loss restricts cyclin B levels and organ size, while Rae1 overexpression has the opposite effect, similar to Hippo pathway overactivation or loss of function, respectively (Jahanshahi et al. 2016). Future work should also define how Rae1 acts in a feedback circuit to regulate pathway homeostasis in cancer cells.

10.2.5.1 Nup98

Chromosomal translocations involving chimeric fusions of the nucleoporin Nup98 protein have often been described in acute myelogenous leukemia (AML). All of the fusion proteins have an identical Nup98 N terminus, which includes the GLEBS motif for interaction with Rae1 and FG repeats that associate with the transcription factors HDAC1 and p300 (Funasaka and Wong 2011). Nup98 RNAi caused severe chromosome segregation defects and disrupted Rae1 but not HDAC1 expression and localization (Funasaka et al. 2011). Interestingly, the wild

type Nup98 and the leukemogenic fusion protein Nup98–HOXA9 behave differently during the cell cycle. In mitosis, only Nup98–HD fusions were found to be concentrated on chromosomes (Funasaka et al. 2011). In Nup98–HOXA9-transfected cells, Rae1 protein is also decreased and mislocalized. These findings were confirmed in Nup98–HOXA9 transgenic mice and a Nup98–HOXA9 AML patient (Funasaka et al. 2011). Moreover, Nup98 stability was shown to be controlled by a PEST sequence, absent in NUP98 oncoproteins, whose deletion reproduced the aberrant chromosome segregation activity of Nup98 oncoproteins (Salsi et al. 2014, 2016).

10.2.5.2 Nup188

Nup188 is a component of the Nup93 subcomplex. Itoh et al. showed that Nup188 localizes to spindle poles during mitosis, through the C-terminal region of Nup188. In Nup188-depleted mitotic cells, chromosomes fail to align to the metaphase plate, which induces mitotic arrest due to the SAC. Nup188 also associates with NuMA, which plays an instrumental role in focusing microtubules at centrosomes, and NuMA localization to spindle poles is disturbed in Nup188-depleted cells (Itoh et al. 2013). Following this line of evidence, del Viso found that Nup188 localized at the bases of cilia that extend from centrioles (Del Viso et al. 2016).

10.3 Viral Nups in Mitosis

Viruses use several strategies to deliver their genomes into the host nucleus (Fig. 10.8). One involves nuclear entry during mitosis, when the NE is disassembled; an example of this is found in gammaretroviruses, the replication of which is dependent on the passage of target cells through mitosis, at which point they are believed to obtain access to chromosomes when the NE dissolves for mitosis (Matreyek and Engelman 2013). Another mechanism is viral genome release in the cytoplasm, followed by entry of the genome through the nuclear pore complex (NPC); an example of this occurs in lentiviruses such as HIV-1, which infect nondividing cells and are believed to enter the nucleus by passing through the NPC. Recent evidence has highlighted the importance of the HIV-1 capsid in this process. Furthermore, the capsid was found to be responsible for the viral requirement of various nucleoporins Tpr, Nup153 and Nup358, during infection (Matreyek and Engelman 2013; Wong et al. 2015). Which mechanism a particular virus uses may depend on its size and structure, the cellular cues that it uses to trigger capsid disassembly and genome release, as well as the phase of the cell cycle (Matreyek and Engelman 2013; Wong et al. 2015) (Fig. 10.8).

10.4 ESCRT-III in NE/NPC Sealing at the End of Mitosis

Recent work has also shed light on the NPC and ESCRT (endosomal sorting complex required for transport)-III membrane remodeling machinery in this process (Vietri et al. 2015) (Fig. 10.9). It has been proposed that ESCRT-III, VPS4 and spastin cooperate to coordinate NE sealing and spindle disassembly at NE–microtubule intersection sites during mitotic exit to ensure nuclear integrity and genome safeguarding, with a striking mechanistic parallel to cytokinetic abscission (Vietri et al. 2015).

10.5 Concluding Remarks

While the functions of NPCs in transport are well established, coupling of the nuclear transport machinery to processes that regulate chromosome segregation during mitosis is still an emerging area of investigation. Our current understanding of this issue can be summarized as follows: (1) during the early stage of mitosis, the cytoplasmic NPC component Nup358 facilitates centrosome anchoring by its association with the molecular motors dynein and kinesin, which localizes at the NPC during G2. (2) Upon NEBD (complete disassembly of the NPC), Nups start to be relocated to the kinetochore, spindles and centrosomes. The NE and NPCs disassemble in prophase, which is most likely triggered by the phosphorylation of NE and NPC proteins. Nucleoporins usually remain in subcomplexes and are found dispersed in the mitotic cytoplasm or associated with mitotic structures, such as the spindle or kinetochores.

How Nups adapt their functions during mitosis and whether their phosphorylation by mitotic kinases is linked to that adaptation remain to be investigated. Moreover, Nups are directly implicated in cancer in several ways: changes in nucleoporin protein expression levels during the cell cycle, single point variants and chromosomal translocations generating fusion proteins. The accurate regulation of Nup levels and functions seems crucial to avoid the accumulation of DNA lesions and aneuploidy and probably to prevent malignant transformation in proliferating cells (Ibarra and Hetzer 2015; Nakano et al. 2011).

A challenging issue to be addressed in the near future involves elucidation of the individual steps, both spatially and temporally, taken by nucleoproteins after the NE breaks down in mitosis. Resolving this issue will involve a combination of developing extremely high-resolution real-time single-molecule imaging microscopy or HS-AFM techniques along with techniques in the fields of biochemistry, genetics, bioinformatics and structural biology. These approaches have provided and should continue to provide intriguing advances in our understanding of the roles of nucleoporins during mitosis. From a clinical perspective, it will also be important to further address the exact contributions of individual nucleoporins to various stages of carcinogenesis.

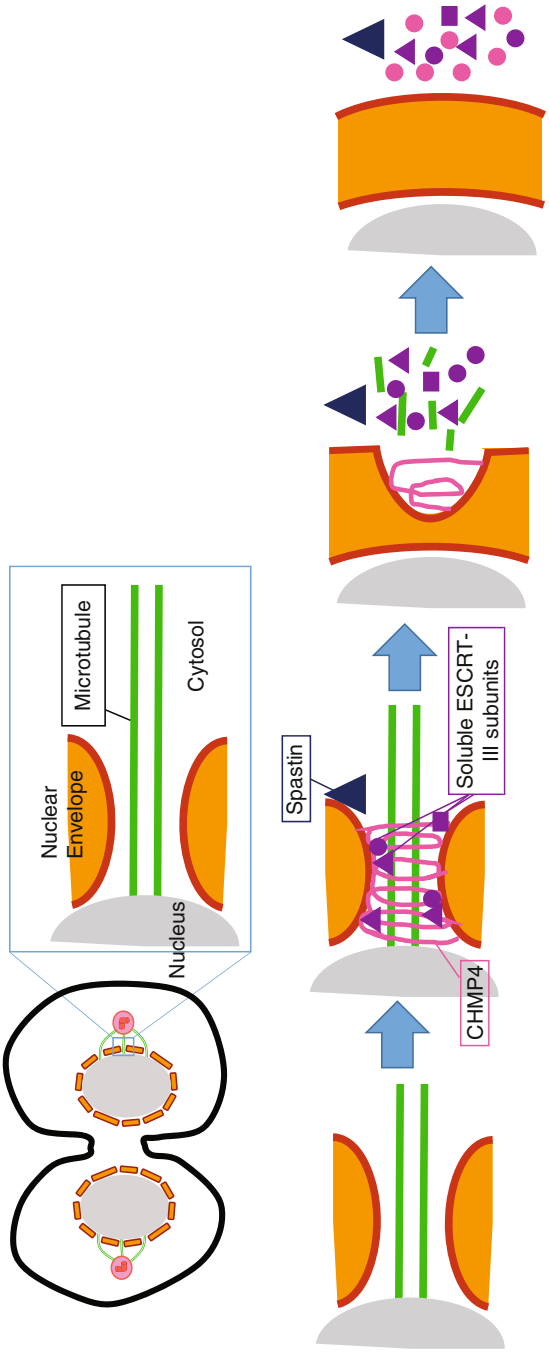

Fig. 10.9 A model for ESCRT-III in NE/NPC sealing at the end of mitosis

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