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Review

Maintaining soluble protein homeostasis between nuclear and cytoplasmic compartments across mitosis

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The nuclear envelope (NE) is central to the architecture of eukaryotic cells, both as a physical barrier separating the nucleus from the cytoplasm and as gatekeeper of selective transport between them. However, in open mitosis, the NE fragments to allow for spindle formation and segregation of chromosomes, resulting in intermixing of nuclear and cytoplasmic soluble fractions. Recent studies have shed new light on the mechanisms driving reinstatement of soluble proteome homeostasis following NE reformation in daughter cells. Here, we provide an overview of how mitotic cells confront this challenge to ensure continuity of basic cellular functions across generations and elaborate on the implications for the proteasome – a macromolecular machine that functions in both cytoplasmic and nuclear compartments.

Highlights

How dividing cells reinstate protein homeostasis following intermixing of nuclear and cytosolic components in open mitosis is a critical aspect of cell biology.

Nuclear exclusion of (large) cytosolic proteins during late mitosis is the result of physical constraints on the genome that include chromatin condensation (alongside with swelling of the nuclear envelope (NE) formed around the decondensing chromatin) and chromosome clustering, both of which prevent aberrant inclusion of cytosolic molecules inside newly formed nuclei.

After nuclear exclusion in late mitosis, nuclear protein complexes such as proteasomes, are swiftly reimported into the newly formed nucleus.

Nuclear import of the proteasome is facilitated by AKIRIN2 and the NE transport machinery. Targeting protein homeostasis during mitosis could be a promising target for therapy.

Mammalian cell division in brief

The mammalian cell cycle lasts roughly 24 h and comprises two distinct parts: a longer **interphase** (see Glossary) (18–22 h) and a shorter **mitotic phase** (1–2 h), with their precise duration depending on the cell type [1]. One mitotic cycle generates two nearly identical daughter cells needed for organismal growth and tissue renewal. In interphase cells, the nucleus houses the genome and serves as the site of DNA replication and RNA synthesis, while the cytoplasm manages protein translation and signal transduction, as well as accommodates the cytoskeleton and various organelles [1]. As proliferating cells progress through the cell cycle, they increase protein synthesis (initial growth phase, G1) and duplicate their genomic DNA (S phase), followed by a second growth phase (G2) that ensures sufficient quantity of organelles and expands the overall cell volume. Now, mitosis can be initiated, propelling the mother cell into sequential steps of prophase, prometaphase, metaphase, anaphase, and telophase that culminate in the physical separation between the nascent daughters (i.e., cytokinesis) (Figure 1) [1]. During prophase, chromatin condenses into individual chromosomes, centrosome duplication occurs and the **nuclear envelope (NE)** fragments to make way for spindle formation and chromosome segregation. In prometaphase, chromosomes become attached to spindle microtubules radiating from centrosomes, which have by now migrated to the opposing maternal poles [2]. Membrane-enclosed organelles like the **endoplasmic reticulum (ER)**, Golgi, mitochondria, and endosomes also fragment or otherwise rearrange at this time to accommodate the spindle apparatus and prepare for their own inheritance [3,4]. In metaphase, chromosomes align and are cleared from ER fragments [5]. Once the mother cell certifies metaphase completion, anaphase is initiated and chromosomes are allowed to move apart along microtubule tracks towards their respective spindle poles [6]. Like the chromosomes, organelles also partition at this time, and the cleavage furrow forms to mark the future site of abscission [3,4]. Finally, in telophase, the NE

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reconstitutes around the progeny's nuclei and chromosomes begin to decondense. Also, the ER and Golgi reassemble, and designated endosomes travel towards the now narrowed intercellular bridge to provide force and membranes for cytokinesis [4,7]. The two newly independent daughter cells now embark on their own journeys through the cell cycle. Although the above steps are general across multicellular organisms, some have evolved unique variations that are detailed in Box 1.

As gleamed from the above overview, the majority of mitotic research to date has focussed on genetic and organellar inheritance, with little attention paid to the soluble fraction partitioned between the nucleus and cytosol. This is surprising, given that soluble proteins and macromolecular complexes account for a substantial part of the total cellular weight. Thus, continuity of molecular processes across generations must also rely on the propagation of the soluble proteome separated by the nucleoplasmic barrier of the NE. In the following sections we discuss compartmentalisation of the soluble proteome in eukaryotes and delve into the mechanisms of its dynamic reorganisation across mitosis.

Soluble proteome dichotomy

The eukaryotic soluble proteome is partitioned between cytoplasmic and nuclear fractions by the NE double membrane barrier [8]. Correspondingly, cellular functions fulfilled by soluble proteins and macromolecular complexes are predicated on their localisation relative to the NE, and nuclear import and export must therefore be tightly controlled. Because protein synthesis occurs in the cytosol, nuclear residents including histones [9], transcription factors [10], and protein complexes such as RNA polymerase [11] require active nuclear transport to access their site of function through mechanisms detailed in the upcoming sections. Additionally, macromolecular machines that contain both protein subunits (translated in the cytosol) and nucleic acid components (transcribed in the nucleus) undergo a complex choreography of import and export across the NE. For example, spliceosomes that orchestrate maturation of mRNAs assemble in the cytoplasm, where newly translated small nuclear ribonucleoprotein particles (snRNPs) and accessory protein subunits encounter exported small nuclear RNAs (snRNAs), followed by import of fully assembled and functional spliceosomes into the nucleus [12]. Conversely, assembly of ribosomes that mediate protein synthesis in the cytosol starts in the nucleoli, where ribosomal subunits imported from the cytoplasm meet ribosomal RNAs prior to their export into the cytosol [13]. Finally, the mammalian 26S proteasome, a 2.5-MDa macromolecular machine [14], catalyses degradation of proteins in both nuclear and cytosolic compartments [15]. Remarkably, nuclear import of proteasomes in interphase cells is negligeable [16,17], and recent findings reveal that partitioning of these complexes across the NE occurs primarily at and immediately following mitotic exit [18,19].

At the onset of **open mitosis**, cells fragment their NE to facilitate spindle formation and chromosome segregation [1,7]. Consequently, soluble cytosolic and nuclear proteomes intermix and must be reinstated after the NE reforms around the nascent nuclei of daughter cells. The nature of soluble proteome's inheritance was first studied back in 1987 using fibroblasts loaded with fluorescent dextrans of various sizes [20]. This study, later corroborated by others [21,22], demonstrated that soluble molecules lacking nuclear targeting signals and sized above the free diffusion cutoff across the NE are excluded from newly formed nuclei. Since then, a wealth of experimentation ranging from traditional microscopic, biochemical, and genetic methods to modern genome editing approaches and imaging techniques allowing high spatial and temporal resolution has elaborated on the mechanisms underlying this exclusion and revealed how selective cargo transport across the NE membrane facilitates timely reinstatement of the nuclear proteome postmitosis. Our current understanding of soluble proteome's inheritance is discussed

Glossary

Endoplasmic reticulum (ER): a network of membranous tubules and sheets in the cytoplasm of eukaryotic cells, continuous with the NE. The ER oversees protein folding, lipid synthesis, calcium storage, and protein transport via the biosynthetic route, as well as communicates with other intracellular organelles.
Inner nuclear membrane (INM): encloses the nucleoplasm and is connected to the DNA via lamins and other INM proteins. The INM stabilises the NE and is involved in chromatin organisation.
Interphase: phase of the cell cycle characterised by cell growth (G1 phase), DNA replication (S phase), and expansion to prepare for mitosis (G2 phase). Rapidly dividing cells spend the majority of their lifespan in interphase.
Ki-67: protein that forms brush-like protein structures on the surface of mammalian chromosomes.
Mitosis/mitotic phase: phase of the cell cycle during which a single mother cell divides its replicated chromosomes, organelles, and soluble nuclear and cytoplasmic proteomes between the two (nearly) identical daughter cells.
Nuclear envelope (NE): a selectively permeable lipid bilayer, also known as the nuclear membrane, that surrounds the genomic content of the cell, separating its nucleus from the cytosol.
NE breakdown (NEBD): process in higher eukaryotes wherein the NE detaches from chromatin and fragments during open mitosis to allow separation of the genome over two newly formed daughter cells.
Nuclear pore complex (NPC): a multi-subunit protein complex inserted into the NE that forms a selectively permeable transport channel between the nucleus and the cytoplasm.
Nucleocytoplasmic compartmentalisation: separation between nuclear and cytoplasmic compartments by the NE, allowing for specialisation and regulation of cellular tasks in space and time.
Open mitosis: refers to the fate of NE breakdown and reformation during mitosis in somatic cells of higher eukaryotes. In open mitosis, the NE disassembles and is removed from chromatin to allow proper attachment of kinetochores to the condensed chromosomes.

in the following sections, where an overview of NE architecture in interphase cells and its remodelling during mitosis are followed by a discussion on how mammalian cells achieve propagation of their cytosolic and nuclear proteomes across generations.

Outer nuclear membrane (ONM): the outer membrane from the NE facing the cytoplasm. The ONM is continuous with the ER.

Q15: anilinoquinazoline 15 (Q15), a condensin inhibitor that targets human CAP-G2 (also known as NCAPG2), a subunit of condensin II, thereby preventing proper condensation of chromosomes during mitosis.

NE architecture in interphase cells

During interphase, the NE functions as a selectively permeable barrier between the nuclear interior and the surrounding cytoplasm and thus enables establishment and maintenance of their distinct proteomes [8]. The NE is formed by two closely juxtaposed lipid bilayers: the **outer nuclear membrane (ONM)**, continuous with the ER network facing the cytoplasm, and the **inner nuclear membrane (INM)**, enclosing the nuclear content (Figure 2A). On the nuclear side, the NE is structurally supported by A- and B-type nuclear lamins; a network of intermediate filaments. Additional mechanical support is provided by connections between the NE proteins and the cytoskeleton (Figure 2B). For instance, the LINC complex formed by Sad1/UNC84(SUN) domain-containing proteins at the INM and spectrin-repeat proteins (nesprins) in the ONM links the nuclear lamina to the cytoskeleton [23]. Also, various integral membrane proteins connect the INM to the heterochromatin through interactions with lamina-associated domains (LADs) that contribute to chromosomal organisation [24]. Among these, LAP2, emerin and MAN1 (LEM)-domain proteins interact with the chromosome-associated barrier-to-autointegration factor (BAF), and lamin B receptor (LBR) connects to chromosomes through interactions with the heterochromatin protein (HP)1 and modified histones (Figure 2B) [8]. Even though these different architectural support elements interact tightly with one another, their connections are dynamic and can be remodelled to accommodate NE fragmentation and reformation taking place during cell division, as well as in response to mechanical cues or ruptures [25,26]. In the following sections we provide an overview of the NE's semipermeable nature and delve into its remodelling across mitotic phases.

NPCs as a selectively permeable barrier

The semipermeable barrier function of the NE tolerates passive diffusion of small proteins and other soluble substances (<30 kDa or 5 nm) and actively facilitates carrier-mediated passage of larger molecules into and out of the nucleus [27,28]. To accommodate these functions, the NE

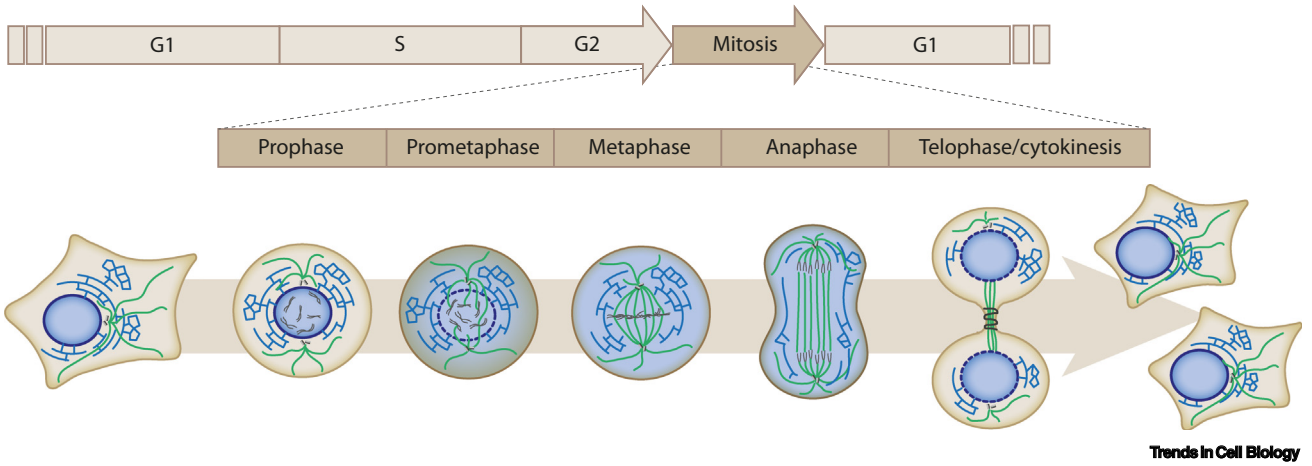

Figure 1. The mammalian cell cycle. The mammalian cell cycle comprises two distinct parts: a longer interphase and a much shorter mitotic phase. The cell cycle begins with a growth phase (G1), followed by duplication of genomic material (S) and a second growth phase (G2) needed to prepare the mother cell for the upcoming division into two nearly identical daughter cells. The process of division, termed mitosis, is characterised by five sequential steps. Firstly, during prophase, chromatin condenses and the nuclear envelope fragments. This enables chromosomes to encounter and attach to the mitotic spindle in prometaphase. Next, in metaphase, chromosomes align, instigating promotion of anaphase during which sister chromatids are segregated towards opposing spindle poles. In telophase, the nuclear envelop is reformed around the two newly inherited genomes, and cytokinesis takes place, completing physical separation between the daughters.

Box 1. Mitosis across the eukaryotic kingdom: a shared concept with variations

Eukaryotes are defined by the presence of an NE, which serves as a physical barrier between the nucleus and the cytosol. However, during cell division this membrane brings with it a logistic challenge when replicated genomic content must be divided over two daughter cells. To overcome this problem, nature has evolved two main strategies known as open and closed mitosis [89]. In open mitosis, the maternal NE is fragmented and subsequently reconstructed around the DNA of nascent daughter cells. This system mainly occurs in mammals, other Metazoa, and land plants. Conversely, in closed mitosis, DNA is duplicated and divided into two parts postmitosis, while the NE stays intact. This strategy is mainly used by certain unicellular eukaryotes, such as yeast [89]. In yeast mitosis, the NE simply expands and is later cleaved into two membrane-encircled nuclei. To divide the condensed chromatin between the daughters, yeast utilises a duplicated centriolar structure (the spindle pole body) inside the closed NE. While yeast equally distributes old nuclear pore proteins, as well as most organelles, such as the mitochondria and peroxisomes, over its progeny, newly made nuclear pore proteins and the spindle pole body are unequally inherited by the daughters [90].

Although open and closed mitoses are exploited by many eukaryotes, variations between these strategies exist in different organisms. In *Dictyostelium*, for example, the NE is not fragmented, but tubulin dimers and other proteins are allowed to diffuse across the NE through a process called semiclosed or semiopen mitosis. To assemble the spindle apparatus for proper segregation of chromatin, the centrosome is inserted into the NE through a yet unrevealed mechanism. Furthermore, in *Aspergillus nidulans,* the NPC is partly degraded to allow diffusion of materials between cytosolic and nuclear compartments [91]. In plants, an additional mitotic phase termed the preprophase exists, characterised by the formation of a dense microtubule band under the plasma membrane. This pre-prophase band marks the plane of future division between the daughters. Unlike animal cells, plant cells lack centrioles to organise their mitotic spindle. Instead, this function of the microtubule organising centre is executed by the NE itself [92]. These examples illustrate how different eukaryotes have evolved creative solutions enabling them to either maintain or reinstate soluble proteome homeostasis across the NE barrier during and after mitosis. While some eukaryotes avoid mixing their cytosolic and nuclear contents, others have found ways to overcome the danger of cytosolic matter getting caught within the *de novo* assembled nuclear space.

harbours numerous aqueous channels termed **nuclear pore complexes (NPCs)** (Figure 2A–C). NPCs are large ring-shaped structures weighing in at ~120 MDa in human cells that integrate 500–1000 nucleoporins (NUPs) stratified into nearly 30 different types [29]. The central channel of the NPC is lined by NUPs featuring intrinsically disordered domains rich in phenylalanine-glycine residue repeats (FG-NUPs) (Figure 2C) [30]. Proteins destined for NPC-facilitated transport into the nucleus feature nuclear localisation signals (NLS), while those seeking to leave are marked with nuclear export sequences (NESs). Some large cargos, including preribosomal subunits, even contain multiple NLS/NES signals for efficient targeting across the NE [31,32]. Both NLS and NES are recognised by FG-NUP-binding nuclear transport receptors (NTRs) [31]; many of which belong to the karyopherin-family of importins, exportins and bidirectional transporters [33]. Given the wide variety of soluble cargoes, certain NTRs can facilitate cargo transport independently of NLS/NES, as exemplified by importin-9 [34].

Interactions between karyopherins and their cargoes are typically regulated by the small GTPase Ran that cycles between GDP- and GTP-bound states. Ran GTPase activity is modulated by the nuclear guanine nucleotide exchange factor (RanGEF, also known as RCC1), whose function encourages Ran to bind GTP, and the cytoplasmic Ran GTPase-activating protein (RanGAP)1 that stimulates GTP hydrolysis. Collectively, these regulators sustain a RanGTP/GDP gradient along the NE (Figure 2D) [35]. Once karyopherin–NLS–cargo complexes, which form in the cytosol, cross the NE via the NPC, they encounter nuclear RanGTP and are forced to dissociate [35]. Conversely, binding of RanGTP to karyopherin–NES–cargo complexes facilitates nuclear export [35]. In the cytoplasm, Ran-bound GTP is hydrolysed causing disassembly of the carrier complex. Released RanGDP then returns to the nucleus with the help of its dedicated non-karyopherin carrier Ntf2 [36], where it can once again be loaded with GTP for the next round of transport. While these mechanisms are central to the maintenance of nuclear and cytosolic proteomes, noncanonical modes of facilitated movement across the NE have also been reported, including cytoskeleton-assisted nuclear cargo transport, direct binding of cargo to FG-NUPs, cargo 'piggybacking' on another NLS/NES-containing protein, and export based on budding

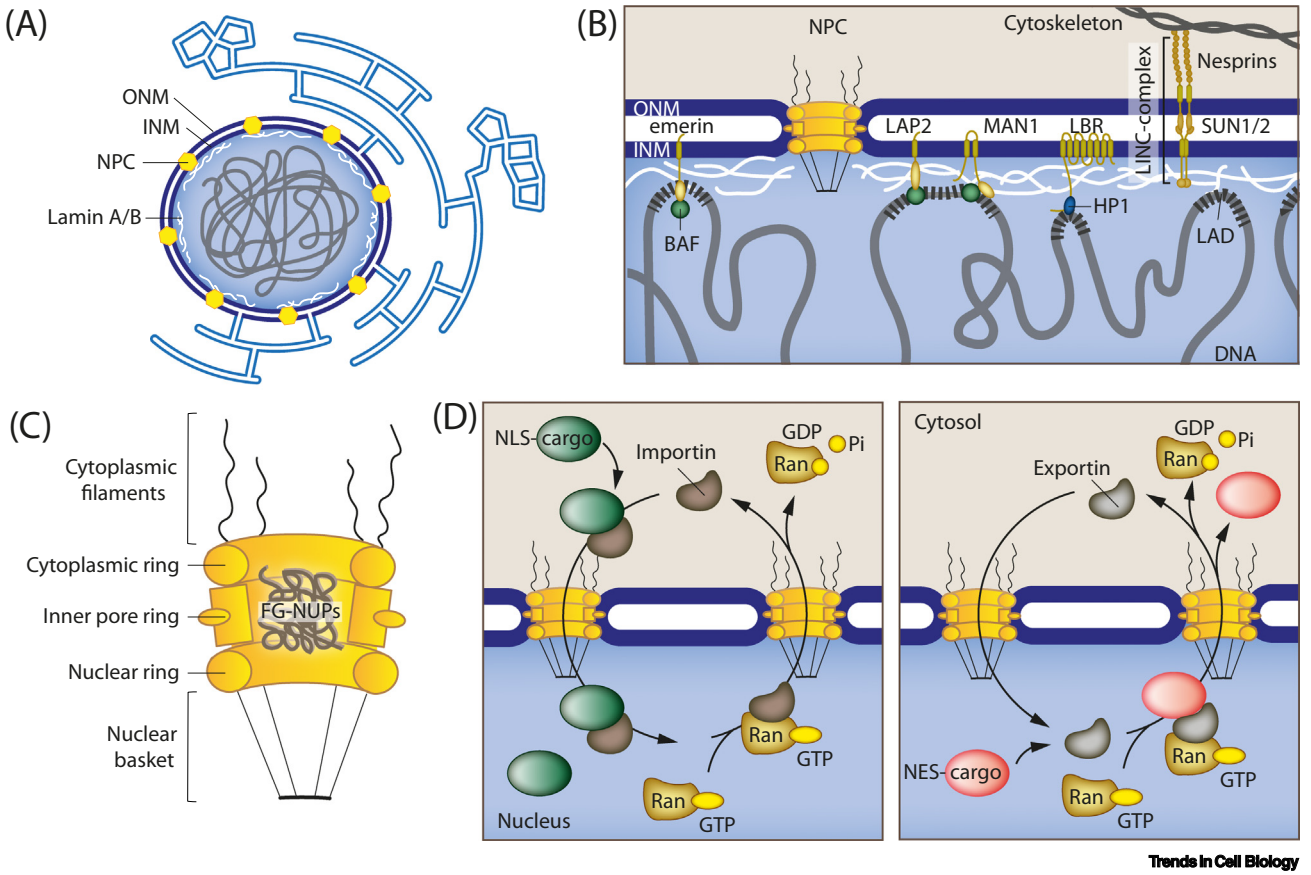

Figure 2. Nuclear envelope as a selectively permeable barrier. (A) The NE forms a selective barrier between the cytosol and nucleoplasm. It is composed of two closely juxtaposed lipid bilayers: INM that encloses the cell's genomic material, and ONM that is continuous with the endoplasmic reticulum. INM and ONM come in contact with one another via NPCs – large ring-like pores that bisect the two membranes and support passive diffusion as well as active transport of molecules across the NE. Physical integrity of the NE is supported by intermediate filaments lamin A and B. (B) Transmembrane proteins (e.g., LAP2, emerin, MAN1, LBR, and SUN1/2) residing in INM and ONM connect either to the cytoskeleton via nesprins or to chromatin via DNA-binding proteins (BAF and HP1) that recognise LADs, thereby providing mechanical support. (C) The NPC consists of three superimposed ring-shaped entities that form a central channel lined by FG-NUPs. The NPC channel is decorated with cytoplasmic filaments and a nuclear basket facing the cytosol and nucleoplasm, respectively. (D) Active transport through the NPC is facilitated by importins and exportins cooperating with the small GTPase Ran. Importins recognise NLS sequences on cargo proteins for transport from the cytoplasm into the nucleus, where they dissociate upon encounter with Ran-GTP (enabled by RanGEF). Exportins interact with NES-sequences and Ran-GTP to facilitate nuclear export. Once in the cytoplasm, GTP hydrolysis (induced by RanGAP) disassembles the complex in the cytoplasm, releasing Ran-GDP. Abbreviations: BAF, barrier-to-autointegration factor; FG-NUPs, phenylalanine–glycine residue repeats; HP1, heterochromatin protein 1; INM, inner nuclear membrane; LAD, lamina-associated domain; LAP2, lamina-associated polypeptide 2; LBR, lamin B receptor; NE, nuclear envelope; NES, nuclear export sequence; NLS, nuclear localisation signal; NPC, nuclear pore complex; NUPs, nulceoporins; ONM, outer nuclear membrane; SUN, Sad1/UNC84.

from the NE [31]. Furthermore, in addition to carrier-based transport, NPC dilation has also been proposed to mediate movement of (particularly large) cargoes across the NE membrane [37], which may be induced by mechanical forces transmitted through the LINC complex [38,39]. The abundance of mechanisms responsible for nucleocytoplasmic cargo exchange underscores the importance of soluble proteome homeostasis. Challenges and solutions associated with NE breakdown and reformation encountered by mitotic cells with respect to their soluble proteomes are discussed in the following sections.

Breakdown and reformation of the NE during mitosis

Critical functions of the NE operational during interphase become temporarily discontinued when cells enter open mitosis. A decisive point in **NE breakdown (NEBD)** is the initiation of NPC

dissociation where key contacts between NUPs are disrupted and connections between INM proteins and chromatin become destabilised by a series of phosphorylation events [25,40]. Key kinases responsible include Aurora kinase (AURK)B and cyclin-dependent kinase (CDK)1 that drive dissociation of HP1 and LBR from chromatin [41,42], and vaccinia-related kinase (VRK)1 that releases BAF from DNA and LEM-containing proteins (Figure 3) [43,44]. Disruption of these interactions leads to integration of NE fragments within the ER [45]. Nuclear lamina components lamin A and B depolymerise after phosphorylation by CDK1 [46]. Once chromosomal segregation is complete, NE reformation can be initiated to restore **nucleocytoplasmic compartmentalisation**. Firstly, NE components embedded in the ER re-engage chromatin in response to changes in phosphorylation (Figure 3) [47]. For instance, dephosphorylation of BAF by protein phosphatase (PP)2A and its cofactor LEM-4/ANKLE2 [48,49] allows binding of BAF to chromosomes, thereby bridging distant DNA sites to create a single unified nucleus [50]. NUPs also become dephosphorylated, which causes their release from inhibitory importins in a RanGTP-dependent manner [51] and initiates ordered reassembly of the NPC [40]. Finally, re-emerging NE fragments are sealed to from a continuous NE membrane through a process coordinated by the ESCRT III system [52–54]. Then, as the nuclear volume expands due to chromatin decondensation [55], small proteins and metabolites diffuse in, and NLS-containing proteins benefit from active transport via the NPC. Meanwhile, proteins sized above the free-diffusion cutoff and lacking nuclear targeting signals remain in the cytosol, concluding restoration of soluble proteome homeostasis.

Managing loss of NE barrier function

Complete disassembly and subsequent reformation of the NE in open mitosis challenge cells to accurately and efficiently reinstate their nuclear and cytosolic proteomes for basic cellular functions, including transcription and RNA processing in the nucleus, protein synthesis in the cytoplasm, and degradation in both compartments. Upon mitotic entry, transcription [56,57] and RNA processing [58] are strongly downregulated, and chromatin condensation takes place [59,60]. Although condensed chromosomes have long been thought to contain mostly chromatin [61], recent evidence indicates that nearly half of their volume is occupied by other entities [62]. Indeed, RNAs [63], transcription factors [64,65], and other proteins have been

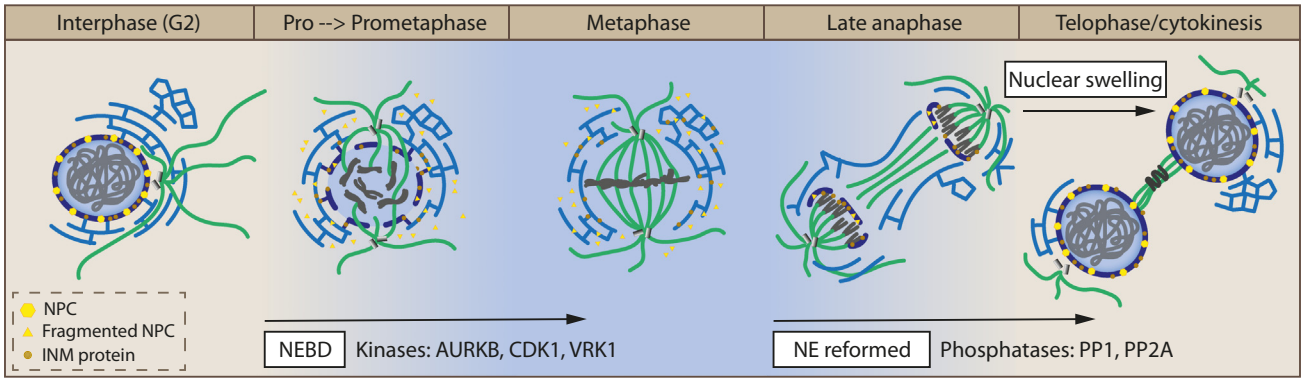

Figure 3. Nuclear envelope remodelling in open mitosis. In open mitosis, regulated NE break down (NEBD) takes place during prophase, allowing condensed chromosomes to access the mitotic spindle. NEBD is initiated by a series of phosphorylation events (mediated by kinases AURKB, CDK1, and VRK1) in prophase, which lead to NPC dissociation and disruption of stabilising connections between NE membrane proteins, chromatin, and the cytoskeleton. These disruptions lead to the integration of NE and NPC fragments within the ER, as well as induce NPC subunit dispersion through the cytosol. During NE reformation initiated in telophase, phosphatases such as PP1 and PP2A counteract the aforementioned phosphorylation events, enabling re-emergence of NE components from the mitotic ER and supporting NPC reassembly toward reconstitution of the nucleocytoplasmic barrier. Abbreviations: AURKB, Aurora kinase B; CDK1, cyclin-dependent kinase 1; ER, endoplasmic reticulum; INM, inner nuclear membrane; NE, nuclear envelope; NPC, nuclear pore complex; PP, protein phosphatase; VRK1, vaccinia-related kinase 1.

found to coat the chromosomal periphery in mitosis [66,67]. Retention of mitotic proteins on chromosomes was reaffirmed in a recent comparative study examining behaviours of 28 different endogenous proteins in dividing cells [68]. Among the peripheral proteins associated with condensed chromosomes is **Ki-67** [69,70], which is thought to form brush-like structures that function as biological surfactants aiding separation between individual chromosomes and faithful partitioning of the genome [71] (Figure 4A).

In contrast to the designated chromosomal volume constituents above, the bulk of the soluble proteome of both nuclear and cytoplasmic origin is actively excluded from condensed chromatin during NE reformation. This exclusion appears to prevent stochastic trapping of cytoplasmic components inside postmitotic nuclei, as demonstrated by aberrant capture of cytosolic proteins within newly formed nuclei of dividing cells treated with the chromatin condensation inhibitor **Q15** (Figure 4A,B) [18]. Also, chromosome clustering induced by orderly collapse of Ki-67 surfactant shields in telophase was shown to prevent contamination of the nucleoplasm with cytoplasmic residents (Figure 4A,B) [72]. Therefore, exclusion of soluble proteins from condensed chromosome clusters essentially results in *ab initio* exclusion from newly formed nuclei. This is crucial because the majority of cytoplasmic residents do not possess NES signals and, if trapped inside a sealed nucleus, have no easy way out. Conversely, NLS-containing proteins can be efficiently imported into the nucleus once the NE regains its semipermeable barrier function in late mitosis. Timely initiation of nuclear import at mitotic exit is ensured by RanGEF RCC1, which associates with mitotic chromatin and commences reinstatement of RanGDP/GTP gradient as soon as NE reformation is completed [73].

A recent study exploiting an incremental ladder of GFP multimers (ranging from mono- to hexa-GFP) demonstrated that exclusion of proteins from condensed chromatin during NE reformation is not size specific, but rather affects all GFP-ladder proteins [18]. However, only larger GFP-ladder proteins were found to be excluded from fully formed nuclei, unless coupled to NLS [18], implying that reinstatement of soluble protein homeostasis postmitosis takes place in accordance with the limits of diffusion through the NPCs. These findings echo seminal studies on NPC size exclusion that examined postmitotic distribution of differently sized dextran molecules or gold particles coated with polyethylene glycol (PEG) [20,22,74]. Real-time exclusion from newly forming nuclei was also visualised for genetically encoded multimeric nanoparticles (GEMs) with a diameter of 41 nm as well as fluorescently tagged ribosomes [72]. Although postmitotic nuclear import is typically NLS dependent, some large molecules without NLS or chromosome binding sequence (as shown for the tetrameric complex KiKGR, 210 kDa) can still diffuse across the NE immediately after cytokinesis [75]. This suggests that the NE is either less restrictive directly after mitosis than during the rest of interphase, or that dedicated nuclear import systems exist for such proteins.

The proteasome problem

Crossing the newly formed NE barrier creates a pressing problem for macromolecular complexes such as the proteasome whose steady-state movement across the NE barrier is exceedingly slow [15–17]. Continuity of proteasome function is crucial to cellular fitness, and accumulation of unprocessed substrates can lead to proteotoxic stress and cell death [76]. Strikingly, nuclear import of the proteasome in late mitosis takes place even faster than free diffusion of GFP molecules (Figure 4C) and is terminated roughly 20 min post-NE reformation to yield relatively stable nuclear and cytosolic proteasome contingents derived from a single homogeneous maternal pool [18]. Because proteasome function is required in both nuclear and cytoplasmic compartments, how cells determine when enough proteasomes have entered the nucleus remains to be addressed. Although relative expression levels of proteasome importers could offer a viable

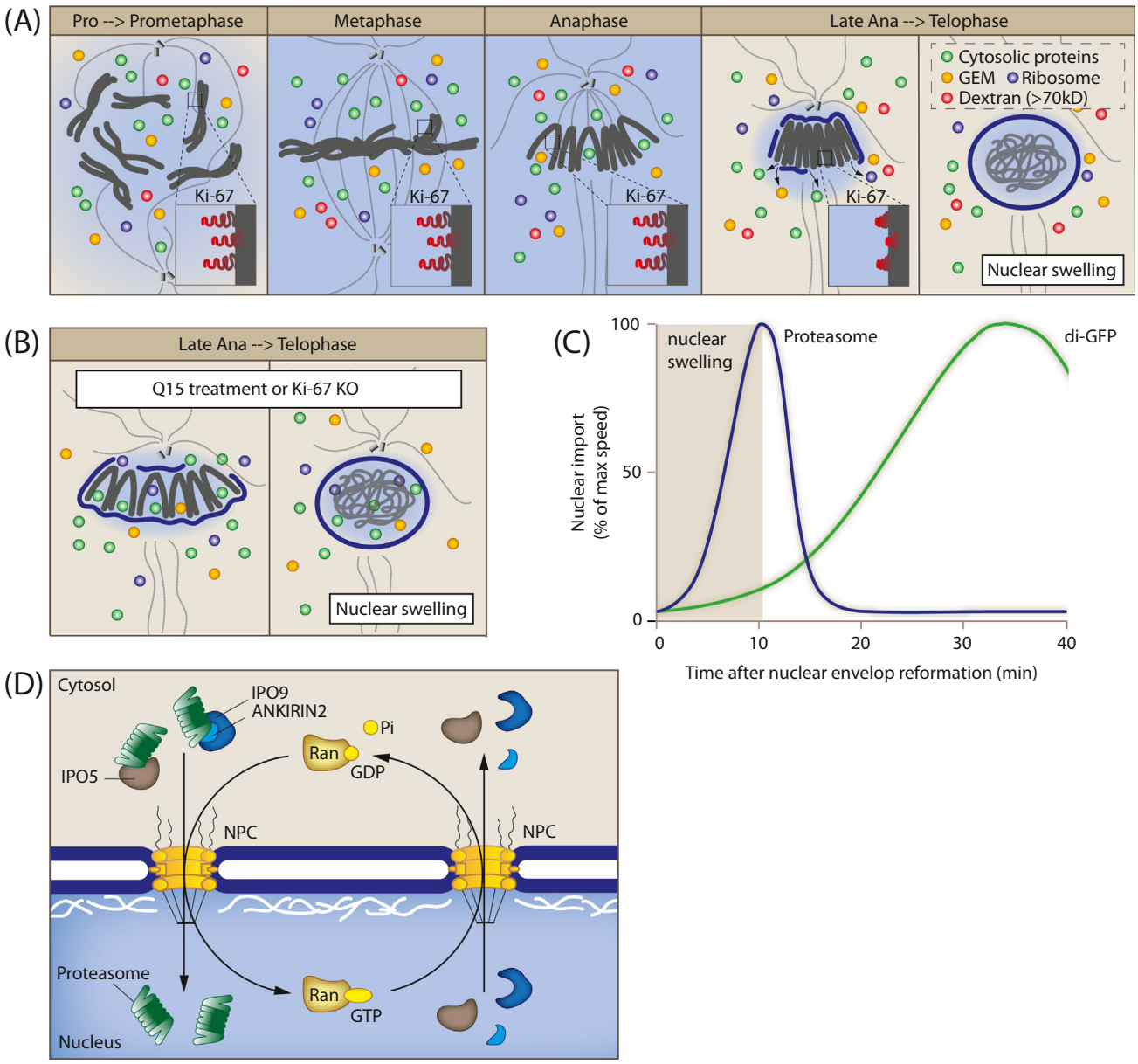

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Figure 4. Maintaining protein homeostasis during mitosis. (A) NE fragmentation in early mitosis causes cytosolic and nuclear contents to intermix. Protein Ki-67, which localises at the chromosomal periphery to form brush-like structures that act as biological surfactants, facilitates physical separation between chromosomes and thus segregation of sister chromatids. In the absence of the NE, soluble proteins (green), macromolecular complexes ribosomes (blue) and inert molecules such as GEMs (yellow) and dextran (red) diffuse freely between condensed chromosomes. In late mitosis (e.g., late anaphase/telophase), Ki-67 brushes collapse, resulting in tight clustering of chromosomes during NE reformation, which prevents stochastic trapping of cytosolic residents in the nucleus. (B) Treatment with the condensin inhibitor Q15 or genetic ablation of Ki-67 (knockout) results in aberrant enclosure of cytosolic components inside the newly formed nuclei. (C) At mitotic exit, proteasomes are swiftly imported into the nucleus to enable degradation of nuclear proteins during interphase. This import is terminated shortly (~20 min) after NE reformation to ensure sufficient proteasome activity in the cytosol. (D) Proteasome transport across the NE is facilitated by importins IPO5 and IPO9 in collaboration with ANKIRIN2, regulated by the classical Ran-GTP/GDP cycle. Abbreviations: GEM, genetically encoded multimeric nanoparticles; IPO, importin; KO, knock out; NE, nuclear envelope.

explanation [18], how their concerted actions are mobilised in time is presently unclear. Thus far, two karyopherin-mediated nuclear import mechanisms for the proteasome in mammalian cells have been identified that may be interchangeable between cell types [77,78]. First, an siRNA approach to target 17 different karyopherin-α and -β family members implicated importin (IPO) 5 as a key player in nuclear import of the 20S proteasome (Figure 4D) [18]. Soon thereafter, an elegant temporally controlled CRISPR/Cas9 screen identified AKIRIN2, a highly conserved but understudied protein, as a central mediator of nuclear import for mammalian proteasomes. In the cytosol, AKIRIN2 was found to form homodimers and use its C-terminal SYVS motif to directly bind fully assembled proteasomes and mediate their nuclear import [19]. In addition, the import receptor IPO9 was identified as a collaborator of AKIRIN2, suggesting formation of a ternary complex with the proteasome bound AKIRIN2 to achieve timely import of this proteolytic machine through the NPC (Figure 4D) [19]. These data are in line with previous observations implicating IPO9 in proteasomal import in *Drosophila* [79]. Furthermore, AKIRIN2 mirrors the function of Sts-1, a protein mediating nuclear import of the 26S proteasome in yeast that acts by binding the nuclear import factor Srp1 [80]. Yet, AKIRIN2 and Sts-1 are evolutionary unrelated, suggesting different mechanisms of action consistent with their different modes of interacting with proteasomes. While Sts-1/Srp1 binds to RPN11, the catalytically active subunit of the 26S proteasome lid [80,81], AKIRIN2 engages the 20S core particle to facilitate transport [19]. In addition to AKIRIN2, other thus far unidentified chaperoning proteins could regulate mitotic import of macromolecular machines into (and out of) the nascent nucleus.

Because proteasome homeostasis is an active area of research with strong therapeutic implications [76], manipulation of proteasomal import has the potential to be exploited towards cell killing. Here, interfering with AKIRIN2 function may be of interest, since its loss appears to elevate levels of proapoptotic cleaved caspase-3. However, resolving whether swift postmitotic nuclear import afforded by the AKIRIN2/IPO9 route can also be used by other cargos is key to defining the scope of NE carrier specificity and understanding potential consequences of interference with this pathway.

Concluding remarks

In interphase cells, the semipermeable NE barrier maintains distinct nuclear and cytoplasmic proteomes required for the execution of specialised cellular tasks, such as transcription, translation, and proteolysis. However, during open mitosis, the NE fully fragments allowing genomic inheritance to take place. Due to NE loss, soluble nuclear content intermixes with that of the cytosol and must be reinstated following NE reformation in daughter cells. Here, we have covered recent advances in our understanding of soluble proteome inheritance through concepts such as chromatin condensation and chromosome clustering that help avoid aberrant nuclear capture of cytosolic matter during NE reformation. Important remaining unknowns in this arena include the error rate associated with physical exclusion of molecules from the condensed chromatin space and consequences of protein mislocalisation postmitosis (see Outstanding questions).

Import and export of cargos into and out of newly formed nuclei at mitotic exit may seem simple, given the vastness of existing knowledge on the NPC and its associated professional carriers. However, a closer look at the problem reveals that much remains to be learned about recompartmentalisation of the soluble proteome, particularly with regards to macromolecular complexes. Recent findings on proteasome dynamics and homeostasis in dividing cells covered here exemplify the exciting research opportunities in the field of nanomachine inheritance. This applies for instance also to the spliceosome and ribosome, both macromolecular complexes requiring coassembly of nuclear and cytoplasmic components. *De novo* ribosome production cannot explain swift initiation of protein synthesis in nascent daughter cells [82], and controlled

Outstanding questions

How is proteasomal nuclear import temporally controlled?

Does a general mechanism exist allowing (large) protein complexes with nuclear localisation to be reintroduced into the nucleus at mitotic exit?

How is homeostasis of macromolecular complexes, such as the spliceosome or RNA polymerase II, maintained across mitosis?

What is the error rate in the physical exclusion of molecules from the condensed chromatin space?

What is the fate of cytosolic proteins ending up in the nucleus?

segregation is therefore likely to occur. The prevailing dogma in the field is that segregation of maternal ribosomes is driven by an ordered stochastic process tending towards equal inheritance [83]. By contrast, in the case of spliceosomes, early observations paint a picture of highly dynamic behaviour across the NE [84–86]. For instance, nuclear speckles containing snRNPs disassemble and disperse in mitosis (from prophase till late anaphase), yet some spliceosome components locate to the periphery of condensed chromosomes [85]. Then, in early telophase, nuclear speckles reassemble, suggesting immediate nuclear entry of snRNPs upon NE sealing. While numerous components of spliceosome assembly and transport have been successfully defined in interphase cells [12], whether the same molecular players operate during and immediately after cell division remains to be resolved.

Because much of what we know regarding the mitotic behaviour of ribosomes and spliceososmes predates the availability of modern genetic and microscopic techniques, this warrants re-examination with modern technologies. Growing availability of CRISPR/Cas-mediated genome editing for ablation and fluorescent tagging of endogenous proteins [87], in combination with advancements in screening methods are likely to have a profound impact on the field [88]. Furthermore, advancements in time-lapse imaging enabling fast iterative image acquisition at high resolution offer new possibilities to study (endogenous) protein behaviour at times of drastic cellular rearrangements. As our view of protein homeostasis throughout the cell cycle continues to expand, future manipulations of soluble proteome inheritance and homeostasis may yield new ways to control viability of actively dividing cells.

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Declaration of interests

The authors declare no competing interest.

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