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Double duty for nuclear proteins – the price of more open forms of mitosis

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During cell division, eukaryotic cells pass on their genetic material to the next generation by undergoing mitosis which segregates their chromosomes. During mitosis the nuclear envelope, nuclear pore complexes (NPCs) and nucleolus must also be segregated. Cells achieve this in a range of different forms of mitosis, from closed, in which these nuclear structures remain intact, to open, in which these nuclear structures are disassembled. In between lies a smorgasbord of intermediate forms of mitosis, displaying varying degrees of nuclear disassembly. Gathering evidence is revealing links exist between the extent of nuclear disassembly and the evolution of new roles for nuclear proteins during mitosis. We propose proteins with such double duties help coordinate reassembly of the nucleus with chromosomal segregation.

Mitotic variations: different means to the same end

Mitosis is the process by which eukaryotic cells segregate their genomes upon the mitotic spindle to form two equal daughter nuclei. The mitotic spindle forms when microtubules nucleated from the microtubule organizing centers (MTOCs) attach to the kinetochores of chromosomes. Once bipolar attachments to all chromosomes have been made, the spindle segregates the chromosomes. Although these are universal features of mitosis, several other aspects of mitosis do not occur in all organisms. Textbook descriptions of mitosis typically classify two extreme types: closed mitosis and open mitosis. This distinction refers to the behavior of the nuclear envelope which partitions the nucleus from the cytoplasm and itself must be segregated to daughter nuclei. During closed mitosis, the nuclear envelope remains intact, the spindle forms within the nucleus and fission of the nuclear envelope occurs following chromosomal segregation. An example of this occurs in *Saccharomyces cerevisiae* which can undergo closed mitosis because its MTOCs, the spindle pole bodies, are embedded within the nuclear envelope (Figure 1a). Contrasting this, the MTOCs of many organisms are cytoplasmic and in order to facilitate the interaction of microtubules with kinetochores, the nuclear envelope is broken down in what is termed an open mitosis (Figure 1e). During open mitosis in mammalian cells, the nuclear envelope is broken down during mitotic entry and its reassembly is regulated such that it begins following chromosomal segregation. However, the timing and extent of nuclear envelope breakdown varies considerably in different organisms and an array of variant mitotic modes exist in between the extremes of closed and open mitosis (Figure 1) [1,2]. For example, in *Drosophila* early embryos, the nuclear envelope initially breaks down only in the region of the centrosomes and does not completely breakdown until after the onset

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of chromosomal segregation [3,4](Figure 1d). Therefore in these cells mitosis is semi-open until after metaphase. In the basidiomycete *Ustilago maydis*, MTOCs embedded within the nuclear envelope are extracted to the cytoplasm during mitotic entry resulting in a form of open mitosis with a cytoplasmic spindle (Figure 1c)[5]. More subtle mitotic modification of the nuclear envelope occurs during the semi open mitosis of the filamentous ascomycete *Aspergillus nidulans* in which an intact nuclear envelope becomes permeable during mitosis as a result of the partial disassembly of its nuclear pore complexes (NPCs) [6,7](Figure 1b). NPCs are mega structures embedded in the nuclear envelope which function as the sole gateways for regulated nucleocytoplasmic transport (Box 1) [8–11]. Many NPC proteins (nucleoporins or Nups) locate to mitotic structures such as kinetochores during open, but not during closed mitosis [8,10]. Recent research has revealed that Nups have functions at these mitotic structures which contribute to mitotic fidelity. This suggests that in organisms which disassemble their NPCs during mitosis, Nups have evolved additional roles which help mitosis.

Box 1

The nuclear pore complex (NPC)

The nuclear envelope partitions the nucleoplasm from the cytoplasm and consists of an outer nuclear membrane, which is continuous with the endoplasmic reticulum (ER), and an inner nuclear membrane. NPCs perforate the nuclear envelope at regular intervals, where the inner nuclear membrane and outer nuclear membrane meet. Each NPC consists of multiple copies of ~ 30 different proteins (nucleoporins or Nups) arranged to form a donut shaped structure. NPCs are highly modular, consisting of distinct subcomplexes as shown schematically in Figure 1a[8,9]. The largest subcomplex is the Nup84 complex (yNup84 or vNup107–160 in vertebrates) which, together with the yNup170 complex, forms a core scaffold coating the curved surface of the nuclear envelope within each NPC. This core scaffold is thought to be anchored to the nuclear envelope by transmembrane Nups [8,9]. In the middle of the core scaffold is the central channel occupied by natively unfolded Nups containing phenylalanine-glycine (FG) repeats [86]. These FG Nups form a diffusion barrier and help mediate regulated nucleocytoplasmic transport [10,11,86]. FG Nups are tethered to the core scaffold by linker Nups [9]. Other peripheral Nups extend from the plane of the nuclear envelope forming cytoplasmic filamentous structures as well as a nuclear basket-like structure [8].

The best known function of NPCs is to help facilitate nuclear transport [8,10,11,87]. The specificity for transport comes from the binding of cargo to transport factors termed karyopherins (also termed Kaps, importins, exportins or transportins). FG Nups interact with karyopherin-cargo complexes which transit through NPCs in a manner requiring a high nuclear concentration of Ran-GTP generated by the chromatin association of the Ran-GEF, RCC1 [70].

New findings have challenged our understanding of the minimal requirements for a functional NPC. For example, *S. cerevisiae* NPCs remain functional following the deletion of over half the total mass of their FG domains [88]. In addition, *A. nidulans* remains viable when all three known fungal transmembrane Nups are simultaneously deleted [32]. This suggests that additional unknown transmembrane Nups might exist or that the proposed interactions between the Nup84 complex and the nuclear envelope [32,89–91] might be sufficient for stabilizing NPCs within the nuclear envelope.

During entry into open mitosis, the NPC is disassembled in a stepwise manner [18]. In *A. nidulans*, which undergoes a semi-open form of mitosis, FG Nups, peripheral Nups and linker Nups disperse but the Nups of the NPC core scaffold remain in the nuclear envelope during mitosis (Figure 1b)[6,7]. As highlighted in the cross section, this removes the diffusion barrier within the NPC central channel making the nuclear envelope permeable

during the semi-open mitosis of this organism. Similar partially disassembled NPC intermediates are transiently present during entry into open mitosis [18]. Such partially disassembled NPC structures likely have a role in the early nuclear entry of mitotic regulators.

(a) Interphase

(b) Mitotic Entry

Figure I.

In addition to NPCs, other nuclear structures, most notably the nucleolus, are disassembled during open mitosis but remain intact during closed mitosis. Nucleoli are the sites of ribosome biogenesis and are built around the nucleolar organizing regions (NORs), landmarks within the genome that encode the repeated rRNA genes [12]. As is the case for NPC proteins, many nucleolar proteins display distinctive localizations during open mitosis and recent findings have implicated some of these nucleolar proteins in mitotic regulation [13].

Although the spindle provides a universal mechanism to segregate chromosomes, mitosis becomes increasingly complicated as the degree of nuclear disassembly increases in variant mitoses between the closed and open form. Nuclear structures which are disassembled during mitotic entry must be correctly reassembled into daughter nuclear in a manner that is integrated with chromosome segregation. Many mitotically disassembled components of nuclear

structures don't simply disperse into the mitotic cytoplasm but reside at mitotic structures. Evidence is emerging that many of these proteins have taken on additional roles which are important for mitotic regulation. Here we discuss our current understanding of how the mitotic functions of disassembled nuclear proteins contribute to the proper orchestration of mitosis and suggest that we are only just beginning to define the full infantry of nuclear proteins with moonlighting functions during mitosis.

Mitotic regulation of nuclear transport and NPC segregation during closed mitosis

Many of the universal features of mitosis and its regulation have been defined using model genetic systems such as *S. cerevisiae* and *Schizosaccharomyces pombe* which undergo closed mitosis. In addition to the universal features of mitosis, unique mitotic events are required for closed mitosis such as the mitotic specific modification of nuclear transport. In some instances this occurs by mitotic phosphorylation of transport cargo. An example of this is the Siz1 SUMO ligase whose mitotic specific phosphorylation facilitates its nuclear export, providing it access to its mitotic substrates [14]. Nuclear transport has also been shown to be modified during closed mitosis by phosphorylation of NPC proteins. This is the case for the Kap121 nuclear transport pathway which is modified during mitosis by the mitotic specific phosphorylation of the Nup53 NPC protein [15,16].

Another requirement of closed mitosis is that NPCs within the nuclear envelope are segregated to daughter nuclei. Interestingly, in *S. cerevisiae* this is not a random process and NPCs are preferentially retained in the NE around the nucleus which remains in the mother bud while new NPCs form in the NE of the nucleus segregated to daughter bud [17]. Thus segregation of NPCs during closed mitosis, although a regulated process, does not appear to involve their disassembly.

NPC disassembly in open and semi-open forms of mitosis

In contrast to closed mitosis, the defining feature of open forms of mitosis is the disassembly of the nuclear envelope including NPC disassembly. This process occurs in a stepwise manner and in human cells and starfish oocytes one of the first steps is the dispersal of Nup98 from NPCs [18,19]. Other Nups then disassemble from the core NPC structure resulting in partially disassembled open NPC intermediates. The partial opening of NPCs increases the permeability of an otherwise intact NE [18] (Figure 2b). NPCs then continue to disassemble, along with the nuclear envelope, as cells progress further into mitosis. Following the initiation of NPC disassembly, the nuclear envelope breaks down completely as best demonstrated in starfish oocytes [18]. Several mechanisms contribute to nuclear envelope breakdown including the hyperphosphorylation of nuclear envelope associated proteins such as lamins and microtubule-mediated tearing of the nuclear envelope [20–23]. Interactions between the COPI (Coat protein complex) complex and the Nup153 and RanBP2 (also known as Nup358) NPC proteins might also play a role in breakdown of the nuclear envelope, perhaps by helping its reorganization [24]. As breakdown proceeds, the nuclear envelope and many of its protein constituents are redistributed to the endoplasmic reticulum (ER) [20,25], providing a source of membrane to rebuild the nuclear envelope during late mitosis.

It is known that mitotic NPC disassembly is regulated by mitotic specific Nup phosphorylation [6,26,27]. However, little is known about the key Nup phosphorylation sites or the kinases involved. Complicating the issue, Nups contain multiple sites of mitotic phosphorylation [26,27] suggesting that no single phosphorylation event is likely to trigger NPC disassembly. Instead multiple phosphorylations might weaken interactions between the NPC sub-

complexes, leading to their disassembly from each other. This likelihood makes mutational analysis of Nup phospho-sites a considerable challenge.

Mitotic specific Nup phosphorylation has also been implicated in regulating the partial disassembly of NPCs which occurs during mitosis in *A. nidulans*. The first Nups identified in this organism were isolated in a genetic screen for extragenic suppressors of a mutation in the *nimA* (never in mitosis) mitotic kinase [28,29]. Temperature sensitive mutations in this essential kinase (generated by similar strategies to the classical *cdc* mutant screens in *S. cerevisiae* and *S. pombe* [30]) cause a G2 arrest at restrictive temperature [31]. Mutations in either of two conserved Nups suppress the G2 arrest of the *nimA1* allele. The first mutant, *sonA1* (suppressor of *nimA1*), encoded an orthologue of the Rae1 (also known as Gle2) nucleoporin and relieved the requirement of NIMA1 activation for the nuclear localization of the Cdk1/Cyclin B kinase [29]. The second suppressor, *sonB1*, encoded an orthologue of the Nup98/Nup96 family of proteins, which are autoproteolytically cleaved to generate two distinct nucleoporins, Nup98 and Nup96 [28]. Nup98 is known to bind to Rae1 and the mutation within *sonB1*^{Nup98/Nup96} allele occurs in a domain required for this interaction and weakens the binding of SONB1^{Nup98} to SONA^{Rae1} [28]. Further study of *A. nidulans* NPCs during mitosis revealed that SONB^{Nup98} and SONA^{Rae1}, along with 13 other nucleoporins, disassembled from a core NPC structure which remained associated with the NE during mitosis [6,7,32,33] (Figure 1b, Box 1). The kinase activities of both Cdk1/Cyclin B and NIMA are required to initiate such NPC disassembly and SONB^{Nup98} undergoes both NIMA independent and dependent phosphorylations during mitotic entry [6]. NIMA is further implicated in regulating this process as it localizes to NPCs specifically at the G2/M transition [6]. Although it remains to be determined if one or more of the 11 human NIMA-like kinases (NEKs) [34] regulate NPC disassembly, NIMA induces NPC disassembly when ectopically expressed in HeLa cells [35]. Therefore, NEK phosphorylation of NPC substrates might initiate the early stages of NPC disassembly during open mitosis in a manner similar to the mechanism by which NIMA initiates partial NPC disassembly in *A. nidulans*. In both scenarios the resulting increased nuclear envelope permeability might serve the same function; to allow mitotic regulators access to the prophase nucleoplasm.

It therefore appears that mitotic specific phosphorylation of NPC components might help regulate both open and closed mitosis. During closed mitosis this phosphorylation causes changes in the transport properties of NPCs but does not lead to NPC disassembly as occurs in more open forms of mitosis.

Regulation of spindle assembly

Bipolar attachment of kinetochores to the mitotic spindle occurs during all forms of mitosis. In lower eukaryotes such as *S. cerevisiae* and *A. nidulans* kinetochores are maintained near the spindle pole bodies during interphase [33,36,37] likely increasing the efficiency of kinetochore capture to the spindle during early stages of mitosis. Contrasting this, in higher eukaryotes the cytoplasmic localization of centrosomes means that microtubule capture of kinetochores is probably less efficient and additional mechanisms help facilitate spindle assembly which begins as the nuclear envelope disassembles. Interestingly, some proteins which disassemble from the NPCs during mitotic entry have roles in regulating spindle assembly during open mitosis. For example, the NPC protein Rae1 (the orthologue of SONA^{Rae1}) has been purified as a spindle-assembly factor from *Xenopus* extracts and is part of a ribonucleoprotein complex that associates with the spindle [38]. Rae1 also associates with another spindle assembly factor, NuMA, suggesting that these two proteins act cooperatively to promote spindle assembly during open mitosis [39].

In addition to Rae1, the vNup107–160 complex partially localizes first to the mitotic spindle and then to mitotic kinetochores in organisms undergoing open mitosis [40–43]. This localization is not observed for the equivalent proteins during closed mitosis or during the semi-open mitoses of *U. maydis*, *A. nidulans* or *Drosophila* [5,32,44]. During open mitosis, the kinetochore localization of the vNup107–160 complex plays roles in spindle formation as depleting this complex from *Xenopus* extracts perturbs this process [42]. In addition, the vNup107–160 complex is required for the kinetochore recruitment of another Nup, RanBP2 (also known as Nup358), during open mitosis [41,43]. The function of RanBP2 is required for proper chromosome segregation and diminished levels of RanBP2 results in severe aneuploidy in a mouse model [41,43]. Recent work has demonstrated that the SUMO ligase activity of RanBP2 is required for the sumoylation and kinetochore recruitment of TopoII α [45]. This provides a locale pool of TopoII α at kinetochores which likely facilitates the locale decatenation of sister chromatids at centromeric DNA thus allowing chromosomes to segregate freely.

Coordinating spindle assembly with chromosome segregation

To help ensure mitotic fidelity, eukaryotic cells have evolved a mitotic monitoring system termed the spindle assembly checkpoint (SAC) [46]. This checkpoint is utilized during both open and closed mitosis to delay chromosome segregation until all kinetochores are correctly attached to kinetochore microtubules. Intriguingly, three SAC proteins Mps1 (monopolar spindle), Mad1 and Mad2 (mitotic arrest deficient) localize to NPCs during interphase but function at kinetochores during mitosis [33,36,37,47–50]. When activated, the SAC inhibits the anaphase promoting complex/cyclosome (APC/C), in large part by the inhibitory binding of Mad2 to the APC/C activator Cdc20. Analogously to Mad2, the Rae1/Nup98 NPC subcomplex binds and inhibits another APC activator, Cdh1 [51]. At present there is not consensus as to why SAC proteins localize to NPCs, however it is becoming clear that a subset of proteins which reside at interphase NPCs are involved in regulating different stages of mitosis.

In addition to its role in inhibiting Cdh1, the Rae1 nucleoporin has another connection to the SAC in that it displays a high degree of similarity (34% identity and 52% similarity in humans) with the Bub3 (budding uninhibited by benzimidazoles) SAC protein [29,52]. This relationship is not limited to sequence homology as when overexpressed Rae1 can correct defects caused by Bub3 haplo-insufficiency in mice [52]. Therefore, in addition to its roles in nuclear transport, Rae1 has multiple functions during mitosis and has been implicated in mitotic regulation of NPC disassembly [29], spindle assembly [38] and SAC function [51], [52].

Another NPC protein has recently been demonstrated to play a facilitatory role in the SAC response by mediating the localization of Mad1 and Mad2. During interphase, the NPC localization of Mad1 and Mad2 is mediated by members of the Mlp family of proteins (Figure 2; Box 2) [33,37,53]. In the closed mitosis of *S. cerevisiae*, Mlp proteins continue to localize Mad1 to NPCs but, if spindle formation is disrupted, Mad1 redistributes to unattached kinetochores to help activate the SAC [37,54] (Figure 2a). Recent studies indicate that Mlp proteins still help facilitate Mad1 and Mad2 localization during semi-open and open mitosis, but do so as part of a mitotic spindle matrix [53] (Box 2) (Figure 2). The mitotic spindle matrix localization of *Drosophila* Mlp (Mtor) has been known for some time [55] and Mlp proteins display a spindle like localization in other organisms (Box 2) [33,56,57]. Studies in *Drosophila*, *A. nidulans* and human cells have revealed that Mlp proteins, most likely as part of the spindle matrix, are required for the proper mitotic localization of Mad1 and Mad2 [33, 53,56]. This is important for SAC function as depletion of Mlp proteins weakens the checkpoint response [53,56]. Although this effect is not as strong as when the Mad2 SAC protein is depleted, depletion of Mlp proteins still results in defective chromosome segregation [53,56].

Together the data suggest that Mlp proteins act as a scaffold to locate SAC proteins during open, and semi-open mitosis as part of a spindle matrix (Figure 2).

Box 2

Mlp proteins and the spindle matrix

Mlp proteins are large ~250 kD proteins which are part of the NPC nuclear basket. Interestingly, Mlp proteins are excluded from NPCs in the region of the nucleolus in *S. cerevisiae* [92]. Mlp proteins have been implicated in diverse cellular processes. This is, in part, due to their role as a nuclear scaffold for chromosomal organization and to locate proteins to the inner nuclear membrane [8]. For example, Mlp proteins tether the SAC proteins Mad1 and Mad2 to the nuclear basket of NPCs (Figure 2) [33,37,53]. Recently the role of Mlp proteins to localize Mad1 and Mad2 has been extended to open and semi-open mitosis when Mlp proteins are disassembled from NPCs but locate to a mitotic spindle matrix (Table I) [33,53,56]. This pattern of localization is not unique among inner nuclear membrane proteins as *Xenopus* lamin B is also part of a mitotic spindle matrix [93], and in mammalian cells lamin B concentrates around the centrosomes in early mitosis [22]. Other proteins associated with the inner nuclear membrane including, *C. elegans* Titin and human Samp1 (equivalent to *S. pombe* Ima1), also display a mitotic spindle like localization [94, 95]. Together with the spindle matrix role for Mlp proteins, this begins to build a paradigm whereby proteins associated with the inner nuclear membrane during interphase associate with the mitotic spindle when the nuclear envelope breaks down. The potential that inner nuclear membrane proteins have mitotic roles is an interesting area for further studies.

The spindle matrix might also provide spatiotemporal regulation of the SAC during telophase as in both *A. nidulans* and *Drosophila*, Mad1 and Mad2 persist at the spindle matrix region located in between telophase nuclei [33,44]. Potentially Mad2 could locally inhibit APC/C activity in this region of the mitotic spindle which might then establish a gradient of APC/C activity along the mitotic apparatus (Figure 3b). The persistent localization of SAC proteins in the vicinity of the telophase spindle is also consistent with the notion that the SAC might continue to monitor mitotic functions after anaphase. Indeed experimental evidence indicates that the SAC can be reengaged after its initial fulfillment at the metaphase to anaphase transition [33,58,59].

Nuclear reassembly following open mitosis

Once the SAC has been satisfied, chromosomes segregate and during the late stages of open mitosis, a nuclear envelope containing functional NPCs is reassembled around segregated chromosomes. Mitotically disassembled Nups play pivotal roles in orchestrating the reformation of the nuclear envelope which initiates at the surface of mitotic chromatin. This process occurs in a stepwise process as highlighted in studies utilizing *in vitro* *Xenopus* nuclear assembly assays [43,60–64]. Reassembly of mammalian NPCs proceeds in a similar stepwise manner although, interestingly, the order of reassembly is not the reverse of disassembly [19]. One of the first steps is the chromatin association of ELYS, the most recently identified component of the vNup107–160 complex. ELYS then recruits the rest of the vNup107–160 complex forming a so called prepore at the surface of chromatin which recruits the remaining Nups to form NPCs [43,63]. It is also noteworthy that in addition to its role in initiating NPC reformation, ELYS has a more global role in cell cycle regulation as it has been implicated in DNA replication licensing; a cell-cycle mechanism that ensures a single round of replication follows each round of mitosis [65].

To regulate the association of Nups with chromatin following mitosis, cells utilize proteins which, during interphase, help regulate nuclear transport by binding to transport cargo [66] (Box 1). These proteins include the karyopherins transportin, importin α and importin β which negatively regulate post-mitotic NPC reformation by binding to certain Nups, inhibiting their association with chromatin [61,62,67–69]. The release of this Nup cargo from karyopherins specifically at chromatin is promoted by the high concentration of Ran-GTP around mitotic chromatin [70]. This is analogous to the role of Ran-GTP in promoting release of cargo from karyopherins during nuclear transport and spindle assembly [66]. At least in nuclear transport, cargo release from karyopherins is accelerated by Nup2/Nup50 at the nuclear face of NPCs [71,72]. Although it remains to be determined if Nup2/Nup50 similarly promote cargo release from karyopherins during spindle or NPC assembly, this has been suggested to occur in *A. nidulans* in which the chromatin localization of Nup2 is required for normal mitosis [7]. This function might be conserved in vertebrates as Nup50 also associates specifically with mitotic chromatin [19].

Post mitotic NPC reassembly is coordinated with nuclear envelope reformation and the details of how this occurs are beginning to be resolved [73]. During the association of preprores with the surface of chromatin, the ER membrane also associates with chromatin [74]. Recent studies support a model whereby multiple nuclear envelope proteins in the mitotic ER bind to chromatin and act in concert to drive the re-association and spreading of ER membranes to reform the NE [75]. The Ndc1 and Pom121 transmembrane Nups contribute to this process but how these proteins then associate with other Nups present in the preprore to form a closed system with intact NPCs is unknown. Interestingly, it has been proposed that Pom121 and the vNup107–160 complex are part of a checkpoint that ensures that the chromatin binding of Pom121 and the vNup107–160 complex occurs before sealing of the NE [64]. This is important because it prevents the formation of non-functional nuclei surrounded by nuclear envelope which does not contain NPCs [64,76].

It is also worth considering how nuclear envelope reformation is orchestrated such that a single nuclear envelope encases the complete genome of segregated chromosomes following open mitosis. This is in part achieved by the tight clustering of each set of chromosomes during telophase however other factors must also contribute this complicated reorganization of membrane. One factor is the amount of membrane available to form nuclear envelope around each daughter nucleus which, as proposed by the “limited flat membrane hypothesis” [73], must be tightly controlled. In addition, the affinity of nuclear envelope and NPC proteins for different regions of mitotic chromatin might also help ensure that a single nuclear envelope encases all chromosomes. For example the AT-hook motif on ELYS binds to AT-rich regions of chromatin to initiate NPC reassembly at these sites [63]. Whether other nuclear envelope or NPC proteins bind to distinct regions of chromatin, or if mitotic post-translational histone modifications direct this process remain open questions. Another possibility is that the portion of the vNup107–160 complex present at kinetochores during mitosis contributes to spatial and temporal regulation of NPC and nuclear envelope reformation. Clearly more research is required to define the mechanisms that encase multiple chromosomes within single functional daughter nuclei.

Contributions from the nucleolus to mitotic regulation

During closed mitosis in *S. cerevisiae* the nucleolus does not disassemble although the regulated release of the Cdc14 phosphatase from the nucleolus plays a crucial role in mitotic exit [77]. This process is not conserved in open mitosis during which nucleoli are disassembled. Notably, nucleolar disassembly and reassembly occurs in parallel with the mitotic disassembly and reassembly of the nuclear envelope and it has been suggested that parallel regulatory mechanisms might be involved [12]. As is the case for NPC proteins, several nucleolar proteins

associate with mitotic chromatin and are thought to act as the building blocks which then form functional nucleoli around the NORs [12,78–80]. Interestingly, several nucleolar proteins have been suggested to contribute to spindle assembly and chromosome segregation [13,81–83]. Extending the paradigm of how NPC proteins contribute to nuclear reassembly, it is tempting to speculate that nucleolar proteins might also help coordinate the complex series of events required to reassemble nuclear components into daughter nuclei.

Surprisingly, during the semi-open mitosis of *A. nidulans*, the nucleolus initially remains intact but is excluded to the cytoplasm. In this process the NORs are removed from the nucleolus which remains located between the reforming daughter nuclei during telophase-G1 [84] (Figure 3b). NE restriction and fission on both sides of the nucleolus then generates three nuclear envelope associated structures, the two daughter nuclei and a central nucleolar remnant [84]. During G1, the cytoplasmic nucleolar remnant undergoes sequential disassembly, releasing nucleolar proteins to the cytoplasm from where they are re-imported to daughter nuclei to form new nucleoli at the NORs. Therefore, although the nucleolus does disassemble as part of the mitotic process in the semi-open mitosis of *A. nidulans*, this disassembly occurs in G1 rather than at the G2/M transition as occurs in an open mitosis. It is currently unknown how this is regulated although the localization of mitotic regulators such as Mad1, Mad2 and Cyclin B to the spindle matrix region around the telophase nucleolus (Figure 3b) could potentially be involved [33,84]. Finally, the exclusion of the nucleolus to the cytoplasm does not require the forces of the mitotic spindle instead it has been suggested that the reorganization of the nuclear envelope may drive this process [84].

It is not known why *A. nidulans* disassembles its nucleolus in the cytoplasm during G1 although it has been suggested that the increased permeability of the nuclear envelope during its semi-open mitosis might be a factor [84]. This is because cytoplasmic contaminants can potentially enter the permeabilized nucleus and thus the exclusion of the nucleolar remnant to the cytoplasm followed by selective nuclear transport of its constituents to G1 nuclei might act to cleanse the nucleolus of cytoplasmic contaminants.

Specialized nuclear envelope and spindle matrix functions during syncytial mitoses

Numerous organisms and cell types maintain many nuclei in a common syncytial cytoplasm, presenting unique issues for mitotic regulation [1,2]. For example, if nuclei undergo mitosis synchronously in such syncytia, mechanisms are required to ensure that spindle microtubules attach to kinetochores of the appropriate nucleus. One way to achieve this is confine each mitotic apparatus within an individual nuclear envelope as occurs in the syncytial mitoses of *A. nidulans* [2]. However during the synchronous mitoses within the syncytia of *Drosophila* early embryos, the nuclear envelope must breakdown to allow centrosomal microtubules to interact with kinetochores (Figure 3a). Nevertheless, the nuclear envelope might still shield kinetochores from interaction with microtubules emanating from inappropriate centrosomes as nuclear envelope breakdown is restricted to the region of the centrosomes until after metaphase (Figure 3a). Another possibility is that the *Drosophila* Mlp spindle matrix might help direct microtubules nucleated from cytoplasmic MTOCs to the appropriate nucleus (Figure 3a). The Mlp spindle matrix could also be part of the mechanism that confines the SAC response to individual spindles [85]. Supporting this idea, *Drosophila* Mlp is unable to exchange between the spindle matrices of different nuclei in cells that have two spindles [56]. Thus both the nuclear envelope and NPC proteins might take on specialized functions to help facilitate waves of synchronous mitoses in syncytia.

Concluding remarks

Successful mitosis dictates not only the faithful segregation of chromosomes, but also that other nuclear structures are correctly segregated. As the nucleus becomes increasingly disassembled in different forms of mitosis, more regulation is needed to correctly rebuild daughter nuclei following mitosis. Open mitosis is a highly dynamic process in which elaborate nuclear structures including NPCs, the nucleolus and the nuclear envelope are dismantled as cells enter mitosis and then reassembled to form daughter nuclei. As we delve more into this process we are learning that constituents of these nuclear structures are not simply bystanders to the mitotic process but instead contribute to the proper completion of open mitosis. At least for NPC proteins, which are collectively required for interphase nuclear transport in all eukaryotes, the evidence is mounting that many have taken on additional duties to help regulate mitotic events, particularly during open mitosis. As the nuclear envelope and NPCs disassemble, some former NPC residents assist in the formation of a bipolar spindle while others help ensure that this process is completed before chromosomes segregate. Later in mitosis, NPC proteins not only form the seeds to rebuild NPCs during late mitosis, but are also part of the mechanisms which coordinate this process with reformation of the nuclear envelope, thereby ensuring that daughter nuclei are functional. Given this precedent, and that a growing number of nucleolar proteins have been implicated in mitotic regulation and localize to a variety of mitotic structures during open mitosis, we may just be scratching the surface of how nuclear proteins contribute to mitosis. One underlying problem that will be encountered when trying to decipher the potential mitotic functions of such proteins is how to differentiate between their interphase and mitotic functions. One key approach will be to generate separation-of-function alleles to distinguish between the interphase and mitotic functions. It is therefore advantageous that several model genetic organisms exist that undergo intermediate mitotic modes between the extremes of open and closed.

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Figure 1.

Variant forms of mitosis occur in different organisms. Although a spindle forms in all these forms of mitosis, other mitotic events are distinct. **(a)** During *S. cerevisiae* closed mitosis, the spindle forms within an intact nuclear envelope containing intact NPCs. **(b)** In *A. nidulans* semi-open mitosis, although the nuclear envelope remains intact, NPCs undergo a partial disassembly, permeabilizing the nuclear envelope. **(c)** In *Ustilago maydis*, the spindle pole bodies are extracted from the nuclear envelope resulting in a cytoplasmic spindle and a form of open mitosis. **(d)** During mitotic entry in *Drosophila* early embryos, partial breakdown of the nuclear envelope allows centrosomally nucleated microtubules to attach to kinetochores and at this stage mitosis is semi-open. The remaining nuclear envelope does not completely

breakdown until after metaphase. (e) Open mitosis in which the nuclear envelope completely breaks down prior to metaphase.

Figure 2.

Mad1 and Mad2 SAC protein localization by Mlp proteins in different forms of mitosis. Examples shown are for *S. cerevisiae*, *A. nidulans* and human cells. In all cases, during interphase Mlp proteins are part of the nuclear basket of NPCs and tether Mad1 and Mad2 to NPCs. **(a)** During the closed mitosis of *S. cerevisiae*, Mlp proteins continue to localize Mad1 and Mad2 to NPCs [36,37]. **(b)** During the semi-open mitosis of *A. nidulans*, the nuclear basket is disassembled and Mlp is part of a spindle matrix. Mlp localizes Mad1 and Mad2 to this spindle matrix which might serve to keep these SAC proteins in close proximity to the spindle [33]. **(c)** Prometaphase in the open mitosis of mammalian cells. Microtubules nucleated from the centrosomes are beginning to gain access to the kinetochores as the nuclear envelope disassembles. The SAC is active, as indicated by the kinetochore association of Mad1 and Mad2, as all bipolar attachments have yet to be accomplished. Mlp (Tpr in human cells) has a facilitatory role in SAC activation and displays a spindle like localization which is likely analogous to the *Drosophila* Mlp (Mtor) and *A. nidulans* spindle matrix [53,56,98].

(a) *Drosophila* early embryos (b) *A. nidulans* telophase **Figure 3.**

Specialized mitotic nuclear envelope and spindle matrix functions. **(a)** During entry into mitosis in *Drosophila* early embryos, localized nuclear envelope breakdown occurs in the region of the centrosomes allowing access of spindle microtubules to kinetochores. The remaining nuclear envelope persists until after bipolar spindle assembly in metaphase and contains partially disassembled NPC intermediates [3,4]. As many nuclei enter mitosis synchronously within the syncytium of early embryos, the late breakdown of the nuclear envelope provides a physical barrier which might help prevent microtubules interacting with kinetochores of inappropriate nuclei. The Mlp (Mtor) spindle matrix (purple) might also help guide spindle formation within individual nuclei during mitotic entry. **(b)** Schematic showing double restriction of the nuclear envelope during *A. nidulans* telophase [84]. Each nuclear envelope restriction occurs at the junctions between the nucleolus and the daughter nuclei. At this stage of *A. nidulans* mitosis, the NORs have been removed from the nucleolus. Nuclear envelope fission at the two sites of nuclear envelope restriction then results in the exclusion of the nucleolus to the cytoplasm [84]. Mlp in the telophase spindle matrix localizes the Mad1 and Mad2 SAC proteins in between the daughter nuclei [33]. The localization to Mad1 and Mad2 to this region of the mitotic apparatus might locally inhibit APC/C activation, potentially providing spatiotemporal regulation of mitosis.

Table I

Mlp proteins and their cell-cycle-dependent localizations.

Organism	Mlp protein	Location Interphase - Mitosis	Mitotic mode	Refs
Human	Tpr (Translocated promotor region)	NPC - dispersed but partially associated with the spindle	Open	[53,56,96]
<i>Arabidopsis thaliana</i>	NUA (Nuclear Pore Anchor)	NPC - dispersed but partially spindle associated in prometaphase	Open	[57]
<i>D. melanogaster</i>	Mtor/Megator/Bx34 antigen	NPC and intranuclear space - dispersed and spindle matrix	Semi-open ^a	[55,56]
<i>C. elegans</i>	Npp-21	NPC - Not determined	Semi-open ^a	[8]
<i>A. nidulans</i>	Mlp1 (Myosin-like protein)	NPC - dispersed and spindle matrix	Semi-open ^b	[33,33,56]
<i>S. cerevisiae</i>	Mlp1 and Mlp2 (Myosin-like protein)	NPC - NPC	Closed	[36,37,49]
<i>S. pombe</i>	Nup211	NPC - Not determined	Closed	[97]

^aIn early embryos, during mitotic entry, NPCs disassemble from the nuclear envelope, which undergoes limited breakdown in regions near centrosomes. Complete nuclear envelope breakdown occurs at later stages of mitosis.

^bNPCs undergo partial disassembly during mitosis, whereas the nuclear envelope remains intact.