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Remodeling the nuclear membrane during closed mitosis

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The mitotic spindle assembly and chromosome segregation in eukaryotes must be coordinated with the nuclear envelope (NE) remodeling. In a so-called 'open' mitosis the envelope of the mother nucleus is dismantled allowing the cytoplasmic spindle microtubules to capture the chromosomes. Alternatively, cells undergoing 'closed' mitosis assemble the intranuclear spindle and divide the nucleus without ever losing the nucleocytoplasmic compartmentalization. Here we focus on the mechanisms underlying mitotic NE dynamics in unicellular eukaryotes undergoing a closed nuclear division, paying specific attention to the emerging roles of the lipid biosynthesis machinery in this process. We argue that lessons learned in these organisms may be generally relevant to understanding the NE remodeling and the evolution of mitotic mechanisms throughout the eukaryotic domain.

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Introduction

Invention of the nucleus segregated from the cytoplasm by a selectively permeable membrane barrier has been a fundamental step in the advent of eukaryotic life. This called for integration of the nuclear membrane dynamics within the cellular physiology framework and in particular, its coordination with inheritance of the genetic material. Eukaryotes segregate duplicated chromosomes during mitosis using the microtubule-based spindle apparatus. This poses a spatial quandary since microtubules are normally located in the cytoplasm, where they function in intracellular transport and polarity establishment. Thus, either the nuclear membrane must break down early in mitosis to allow microtubules to capture the chromosomes or cells have to assemble an intranuclear mitotic spindle by importing the microtubule subunits and microtubule-associated proteins into an intact nucleus. The modern eukaryotes use both strategies – known as the open and the closed mitosis – and a host of variations in between. This raises the questions of specific functional adaptations required for different types of mitoses, possible physiological advantages of distinct division modes and the degree of plasticity in this integral cell biological mechanism.

The nuclear envelope (NE) is an elaboration of the endomembrane system that consists of two lipid bilayers called the outer (ONM) and the inner nuclear membrane (INM). The ONM is continuous with the endoplasmic reticulum (ER), suggesting a close relationship between the NE and the general ER dynamics. The INM is occupied by a network of proteins interacting with the chromatin and contributing to the nuclear structure and function. The two NE membranes are fused at the nuclear pores, the sites of nucleocytoplasmic exchange decorated with multisubunit nuclear pore complexes (NPCs) [1]. Unlike the complex NE dynamics during open mitosis that involves NPC and nuclear lamina disassembly, release of the nuclear proteins and dispersal of the NE membranes into the ER, followed by their reassembly into the daughter nuclei [1,2], the closed nuclear division is essentially, a study in membrane remodeling. The major steps of this process include insertion of the microtubule-organizing centers (MTOCs) into the NE, anaphase nuclear membrane expansion and the fission of the nuclear membrane to yield two independent daughter nuclei. In this review, we will discuss mostly the membrane events preceding daughter nuclei individuation.

Membrane remodeling at the spindle pole body-nuclear envelope insertion sites

The spindle pole bodies (SPBs) serve as the MTOCs for the mitotic spindle in many lower eukaryotes. The SPB is a morphologically stratified structure duplicating once in each cell cycle [3,4]. Its inner plaque faces the nuclear interior and nucleates and anchors the spindle microtubules, whereas the outer plaque projects into the cytoplasm and nucleates the astral microtubule arrays. The central core ensures structural integrity of the SPB and anchors it within the NE plane. In budding yeast, the mother SPB localizes at the NE throughout the cell cycle and the newly formed daughter structure is inserted into the NE following the conservative SPB duplication in the G1 phase of the cell cycle [3,5,6]. The daughter SPB insertion is driven by a concerted action of the SPB–NE tethering Mps3-Mps2-Bbp1-Nbp1 protein network [7–11] and the transmembrane nucleoporin Ndc1 that, incidentally, also contributes to the NPC anchorage at the NE [12]. Similarly to the NPC biogenesis, the SPB insertion machinery appears to function through the

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membrane-bending and membrane-anchoring properties of its constituents [11] although the molecular details of their action remain unknown.

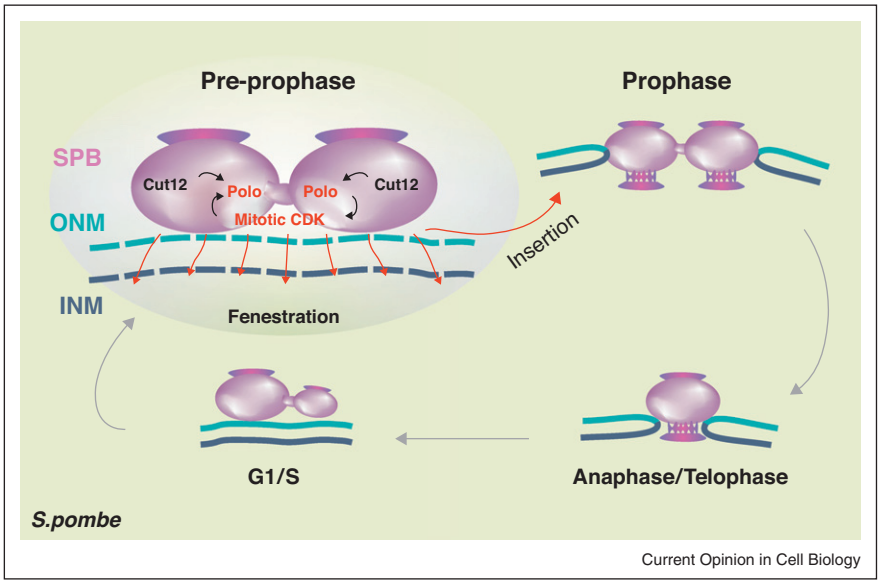
Conceivably, the fusion between the INM and ONM resulting in the formation of a SPB pore, or 'fenestra', could also be promoted by local alterations in the spontaneous membrane curvature, for instance, by modulating distribution of lipids with fusogenic properties within the lipid bilayers [13]. The fission yeast *Schizosaccharomyces pombe* (*S. pombe*) perhaps provides a naturally sensitized system to study the SPB pore formation. Unlike in budding yeast, the *S. pombe* SPBs briefly settle within the NE during mitosis but are extruded to the outer nuclear face throughout the rest of the cell cycle [14] (Figure 1).

The NE fenestration and SPB insertion are well coordinated and a tight nucleocytoplasmic barrier is maintained throughout mitosis [15,16]. In cells carrying mutations in gene *brr6* the SPBs fail to properly anchor within the NE and the presumptive fenestration site remains agape [17••]. *Brr6* encodes a member of the transmembrane NE protein family restricted to the organisms assembling the NE-spanning MTOCs and undergoing a 'closed' nuclear division. The Brr6 protein also appears to promote nuclear membrane remodeling during SPB extrusion at a later stage of mitosis.

In budding yeast, Brr6 and a functionally related protein Apq12 have been implicated in the NPC insertion into the NE, possibly through maintaining cellular lipid homeostasis and affecting the nuclear membrane flexibility [18,19•,20], although genetic interactions data suggest that these proteins may also function during the assembly of a 'closed' spindle [21]. Interestingly, treatment with chemicals that increase membrane fluidity alleviates the abnormal NE dynamics in cells lacking Apq12 [18,19•] but severely aggravates the Brr6-related phenotypes [19•]. Moreover, Apq12 and Brr6 mutants exhibit opposite patterns of sensitivity to growth at low and high temperatures. Since temperature shifts are known to trigger profound changes in the membrane fluidity through alterations in the length and the saturation status of the membrane phospholipid fatty acyl chains [22,23], Brr6 and Apq12 could function by either effecting the changes in membrane properties or possibly, sensing them. The recruitment of Brr6 to the mitotic SPBs that correlates with the SPB insertion and extrusion cycle, could perhaps argue for a direct, localized role of Brr6 in membrane lipid remodeling.

The precise molecular functions of Brr6 and Apq12 remain presently unknown but in light of the above observations, it would be of interest to see if direct manipulations of the fatty acid chain length and saturation status could affect the NE pore formation, during either the SPB or the NPC insertion events. The steady state fatty acid chain length distribution depends on the activity of the acetyl-CoA carboxylase that generates malonyl-CoA, the two-carbon donor in the synthesis of long-chain and very long-chain fatty acids. In budding yeast, the specific function of the acetyl-CoA carboxylase,

Figure 1



SPB-associated NE remodeling during mitosis in *S. pombe*. ONM, outer nuclear membrane; INM, inner nuclear membrane; CDK, cell cycle-dependent kinase. The resident SPB protein Cut12 boosts activity of Polo and CDK kinases specifically at the mitotic SPBs through amplification of the positive feedback loop. High activity of mitotic kinases in turn could promote localized remodeling of the NE and allow the SPB insertion. The red arrows indicate the presumptive signal for the NE fenestration.

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Acc1, in the synthesis of very long-chain fatty acids is required for the normal NE structure [24,25]. Provocatively, Acc1 inactivation leads to a marked increase in the distance between the ONM and INM and a failure to properly insert the nuclear pores [24], possibly owing to defects in NE membrane fusion or pore maintenance. The *S. pombe* mutants of acetyl-CoA carboxylase exhibit severe spindle malfunction and fail to form equally sized daughter nuclei [26]. Reexamining the acetyl-CoA carboxylase mutants for the SPB insertion related phenotypes and testing the mutants that specifically affect synthesis of very long-chain fatty acids could prove useful in dissecting the NE membrane-shaping pathways. Similarly, the fatty acid saturation status could be modulated through the activity of the fatty acid desaturases and it would be of interest to examine possible effects of these enzymes on the SPB–NE interaction. Synthetic genetic interaction arrays and other currently available high-throughput methodologies should afford a systematic analysis of the functions of various lipid biosynthesis pathways in mitotic NE remodeling.

Cell cycle control of the SPB–NE insertion in fission yeast

The failure to integrate the SPBs in the *S. pombe* Brr6 mutants appears to parallel the consequences of mutating the Ndc1 ortholog Cut11 [27] and the mitotic regulator Cut12 [16,28]. While the effect of Cut11 deficiency is probably owing to a physical failure in anchoring the SPB within a fenestra, similar to its function in the NPC insertion, the reasons for the Cut12-related phenotype are less clear. Cut12 is an essential protein that lacks the membrane association domains; instead, it localizes to the inner edge of the SPB, facing the NE [28]. In cells carrying conditional loss-of-function alleles of *cut12*, the NE breaks down in the vicinity of the old, mother SPB but remains intact underneath the daughter SPB born in the previous interphase [16]. Thus, the old and the new SPBs apparently exhibit inherent differential sensitivity to the defects in the Cut12 function. Curiously, in spores germinating in the absence of Cut12, both SPBs fail to insert themselves into the NE or nucleate spindle microtubules [28].

A major biochemical role of Cut12 appears to promote mitosis through boosting the mitotic cyclin-dependent kinase (CDK) activity by amplification of the Polo kinase-driven positive feedback loop [29]. Both Polo [30,31] and the CDK complex [32] associate with the mitotic SPBs suggesting that the local activation of these kinases (through either a series or a parallel circuit) could promote localized NE fenestration and SPB insertion. Arguing for the involvement of the Polo kinase, conditional mutation in the fission yeast pericentrin Pcp1 leads to delocalization of Polo from the SPBs and a failure to fenestrate the NE in spite of an otherwise normal mitotic progression [33•]. Furthermore, fenestra closure and the SPB relocalization to the outer nuclear surface at the end of anaphase coincide in time with the disappearance of Polo from the SPBs [30,31]. Evocative of the NE disassembly in higher eukaryotes, one could consider the SPB insertion in fission yeast essentially a localized NE breakdown triggered by activity of the mitotic kinases (Figure 1). Although the exact mechanisms relaying cell cycle signals to the SPB insertion remain to be elucidated, we predict that these may involve highly localized modulation of membrane properties leading to stabilization of the NE fenestra and the SPB anchorage by integral membrane proteins.

Scaling considerations in closed nuclear division

To accommodate spindle elongation and a closed division of the spherical mother nucleus into two daughters, the nuclear surface area increases during anaphase and it does so in a non-scalable manner [34]. For instance, a dividing *S. pombe* nucleus increases its surface area by approximately 30% by the end of mitosis while the nuclear volume remains constant [34,35••]. This is not a simple issue of passively drawing the membrane material from the peripheral ER by the elongating nucleus. Arguing for a mitosis-specific mechanism of the NE growth, both *S. cerevisiae* and *S. pombe* cells deficient in spindle function and delaying in metaphase undergo extensive NE proliferation [36••,37]. In cells with very little peripheral ER, such as a flagellate protist Euglena, the NE massively expands in preparation for mitosis, forming protuberances and invaginations that eventually resolve during nuclear division and formation of two spherical daughter nuclei [38].

The lipin family proteins that control the endomembrane growth appear to function at the crux of the matter (Figure 2). Interphase yeast cells lacking the lipin proteins (called Pah1 in *S. cerevisiae* and Ned1 in *S. pombe*) overproliferate both nuclear and peripheral ER membranes [39,40]. Enzymatically, lipins are Mg^{2+}-dependent phosphatidate phosphatases that hydrolyze phosphatidic acid (PA) to diacylglycerol (DAG) [41]. Since triglycerides (TAG), the neutral storage lipids, are synthesized from DAG, the lack of Pah1 leads to a marked decrease in the cellular TAG levels [42]. In the absence of Pah1 function, PA can be rerouted through the CDP-DAG synthase pathway to yield the CDP-DAG, a key metabolite in the phospholipid production. In addition to its enzymatic function, Pah1 is involved in transcriptional repression of genes encoding the key phospholipid biosynthesis enzymes [39,43,44]. As a result, cells lacking Pah1 show a pronounced increase in steady state levels of structural phospholipids [42]. Modulating Pah1 activity can therefore balance the production of structural vs. storage lipids in many physiological scenarios, including the cell cycle progression. Indeed, Pah1 activity is subject to inhibitory phosphorylation by the cyclin-dependent

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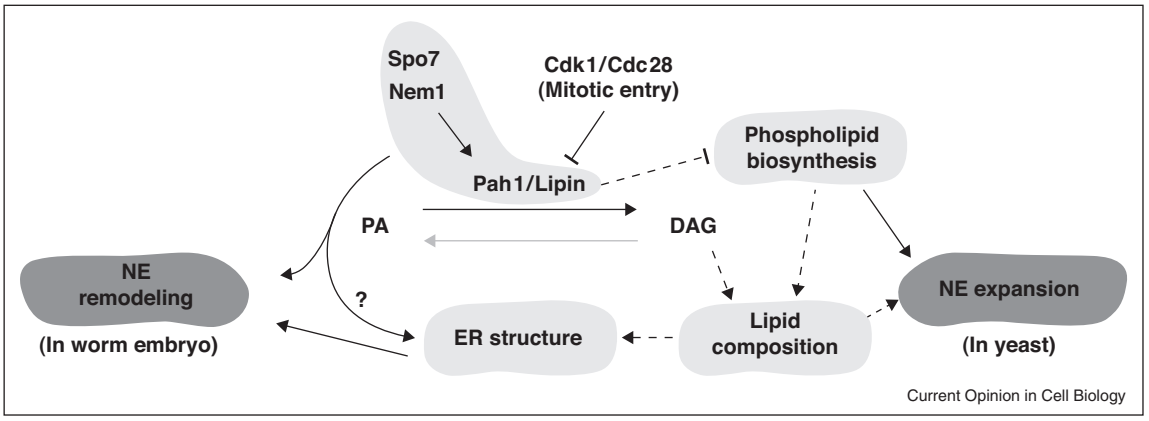
Figure 2



Potential mechanistic links between lipin functions and the NE dynamics in yeast cells and the round worm, *C. elegans*. Solid lines indicate direct functions; dotted lines suggest indirect contributions. PA: phosphatidic acid; DAG: diacylglycerol.

kinases, including the mitotic CDK [45,46•]. The ER-localized Nem1/Spo7 phosphatase complex reverts the modification and promotes Pah1 activation [39,47,48]. Since the activity of CDK is highest at mitotic entry, the inhibition of Pah1 at this time point could account for a burst in phospholipid biosynthesis and assembly of a membrane reservoir. Curiously, lipins also function in NE remodeling during the open mitosis of the nematode *C. elegans* although the mechanisms underlying this phenomenon remain unclear [49–51].

Unlike the interphase cells lacking lipin function, the mitotic yeast cells do not overproliferate the entire ER network [36••]. The preferential growth of the NE suggests the existence of a mechanism for mitosis-specific regulation of the lipid flow within the endomembrane system. One possibility could be a relatively localized assembly of the ER membranes competent for incorporation into the dividing nucleus. At least in case of *S. pombe*, cells appear to assemble the ER compartment enriched in proteins involved in membrane shaping and lipid metabolism at the equatorial region apposing the dividing nucleus ([52] and our unpublished data). It is also possible that the mitotic stage of the cell cycle is favorable for the growth of large ER sheets constituting the NE rather than the highly tubulated peripheral ER. The Ran GTPase system [15] and the NIMA kinase [53] have been implicated in regulating the NE integrity and/or expansion but the exact mechanisms underlying their functions remain unknown.

A pertinent question is where exactly the new membranes are inserted during the nuclear division. Studies in budding yeast suggest that the nuclear membrane adjacent to the nucleolar, chromatin-free region serves as an intrinsically expandable NE domain during non-scalable surface area increase [54]. The reasoning is that the massive influx of new membranes throughout the NE could disrupt the complex organization of the chromosomal domains at the INM and adding a membrane 'sink' in a chromatin-free area could solve such problems [36••]. However, the precise location of the 'membrane injection' sites in dividing yeast nuclei still awaits direct experimental characterization.

Striking diversity of mitotic mechanisms

The importance of assembling a membrane reservoir to enable the closed nuclear division is underscored by the unusual mitotic strategy of the fission yeast *Schizosaccharomyces japonicus* (*S. japonicus*) [35••,55•]. *S. japonicus* builds the intranuclear spindle and initiates the closed type of mitosis in a manner similar to its relative *S. pombe*. However, the *S. japonicus* nucleus fails to divide through the typical dumbbell intermediate. Instead, it elongates into diamond-like and bow-like shapes and spectacularly ruptures in late anaphase (Figure 3). The growing spindles initially buckle inside the intact nuclei but straighten upon the NE breakdown, suggesting that they can be constrained by the NE. Notably, the NE surface area in *S. japonicus* does not increase, and consistent with the simple scaling argument, the combined volume of the two re-formed daughter nuclei drops to approximately 70% of the mother [35••]. NE rupture in *S. japonicus* is driven by the cell cycle machinery rather than by a mechanical force produced by the elongating spindle. When the membrane reservoir is made unavailable in *S. pombe* cells that do not have an intrinsic mechanism for the NE breakdown, the spindle buckles and breaks under the compressive stress and the diamond shaped mitotic nucleus collapses back to a single sphere of the original diameter [35••]. Thus, it appears that at least within the fission yeast clade, the NE breakdown could have evolved to allow anaphase chromosome segregation and formation of two daughter nuclei in the absence of the mitotic NE growth. Whether

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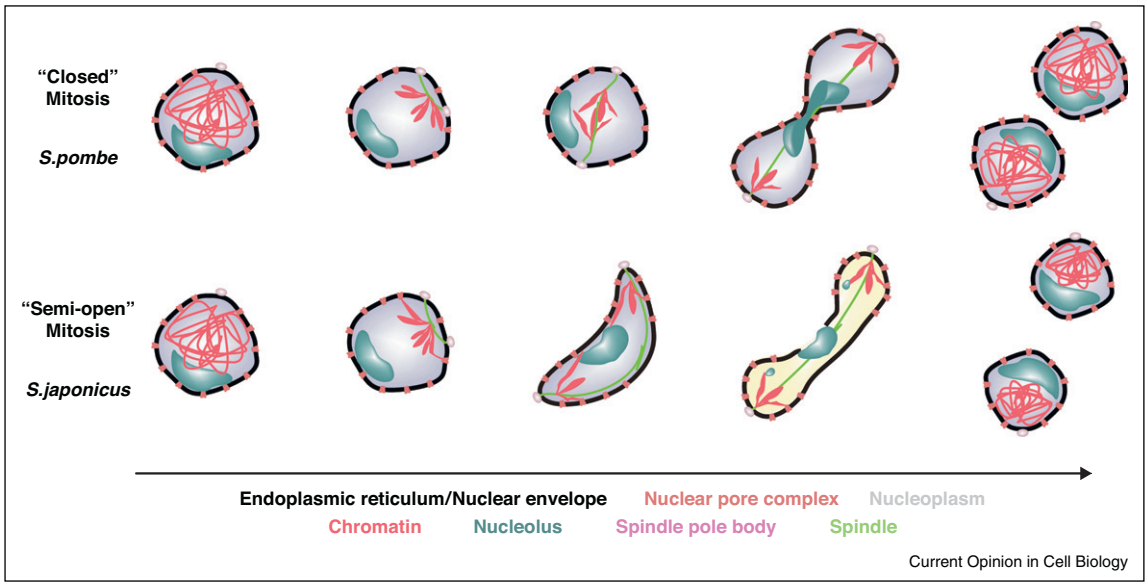
Figure 3



Nuclear envelope dynamics during mitotic progression in fission yeasts. A cartoon depicting the differences in the mitotic NE dynamics in *S. pombe* and *S. japonicus*.

such scaling considerations apply to evolution of mitotic mechanisms in all eukaryotes remains an open question.

The striking difference between the two related fission yeast species argues that invention of the variant mitotic mechanisms may require rather limited tinkering with the available cell physiology toolbox. This view is consistent with the unusual switch in mitotic NE behavior during development of *Physarum polycephalum*. During the amoeba stage, this slime mold assembles the centriole-based spindle and undergoes a typical open mitosis. However, upon commitment to the multinucleated plasmodium it undergoes a gradual transition to a fully closed mitosis [56].

What could be the physiological significance to the different types of NE behavior? Closed mitosis comes at the cost of producing a massive membrane reservoir in preparation for nuclear division and could possibly slow down the rates of spindle assembly and kinetochore capture in larger cells, owing to the necessity to import the entire microtubule machinery inside the intact nuclei. However, keeping the NE intact could prevent spindle entanglement, chromosome mis-segregation and uncontrolled nuclear fusion in a context of a multinucleated cell. This argument would fit well with the persistent nuclear membrane during mitosis in many filamentous ascomycetes and the early embryonic divisions in worms and flies that occur in syncytia. The dumbbell morphology of the dividing closed nucleus has been proposed to facilitate asymmetric segregation of nucleoplasmic proteins [57••] and circular DNA episomes [58••] possibly contributing to functional differentiation within colonies of unicellular organisms. Finally, keeping the mitotic spindle – that helps to position the future cell division site in metazoans – within the enclosed compartment may provide a means for uncoupling chromosome segregation from cellular division plane specification.

Understanding the origins and functions of mitotic variation requires extending mechanistic analyses to multiple experimental systems, beyond the conventional few. A wealth of older ultrastructural records [59], combined with burgeoning genome sequence data, comprehensive phylogenetic analyses and the relative ease of experimentation, makes fungi especially attractive for this purpose. Establishing the rules and pathways for generating phenotypic diversity should provide unprecedented insights into the fundamental aspects of nuclear evolution.

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- •• of outstanding interest

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