Divergence of mitotic strategies in fission yeasts

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Abbreviations: ER, endoplasmic reticulum; NE, nuclear envelope; INM, inner nuclear membrane; NEBD, nuclear envelope breakdown; NPC, nuclear pore complex; SPBs, spindle pole bodies; SIN, septation initiation network; MEN, mitotic exit network

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The aim of mitosis is to produce two daughter nuclei, each containing a chromosome complement identical to that of the mother nucleus. This can be accomplished through a variety of strategies, with “open” and “closed” modes of mitosis positioned at the opposite ends of the spectrum and a range of intermediate patterns in between. In the “closed” mitosis, the nuclear envelope remains intact throughout the nuclear division. In the “open” division type, the envelope of the original nucleus breaks down early in mitosis and reassembles around the segregated daughter genomes.

In any case, the nuclear membrane has to remodel to accommodate the mitotic spindle assembly, chromosome segregation and formation of the daughter nuclei. We have recently shown that within the fission yeast clade, the mitotic control of the nuclear surface area may determine the choice between the nuclear envelope breakdown and a fully “closed” division. Here we discuss our data and argue that comparative cell biology studies using two fission yeast species, Schizosaccharomyces pombe and Schizosaccharomyces japonicus, could provide unprecedented insights into physiology and evolution of mitosis.

Cytology of Mitosis in Schizosaccharomyces japonicus

Initial sequence of events during mitosis in S. japonicus is fairly similar to that in S. pombe. The mitotic microtubule-based spindle assembles inside an intact nucleus at the duplicated spindle pole bodies (SPBs) that reside at the cytoplasmic face of the NE throughout interphase but become embedded into the NE upon mitotic commitment.⁵ The spindle rapidly extends to “metaphase” length spanning the nuclear diameter.¹ Both yeasts have three similarly sized chromosomes⁴ and the chromosomal dynamics can be monitored using both kinetochore⁴ and chromatin marker protein.⁵ Kinetochore microtubules rapidly capture and align chromosomes equidistantly from the spindle poles forming a metaphase plate. Upon anaphase A onset, the kinetochore...
pairs split and the sister chromosomes travel to the poles of the mitotic spindle that at this stage remains short. Immediately after that, cells enter anaphase B that is characterized by rapid spindle elongation and changes in the nuclear shape.

At this stage, the mitotic programs in two yeasts begin to differ. In *S. pombe*, the spindle remains straight throughout anaphase and the nucleus divides into two through a dumbbell shaped intermediate. On the other hand, the elongating spindles buckle severely inside *S. japonicus* nuclei, which in turn, assume a diamond and often, a bow shape. Since the outer NE is continuous with the endoplasmic reticulum (ER), the behavior of the nuclear membrane at this stage is best visualized by imaging the bright artificial marker GFP-AHDL that diffuses throughout the lumen of the entire ER (Fig. 1), or endogenous membrane-spanning markers of the cisternal ER, such as Sec63-GFP or Ost1-GFP. The dynamic process of nuclear elongation into a diamond shape with its frequent bending into a bow culminates in a dramatic equatorial rupture of the NE followed by virtually instantaneous intermixing between the nuclear and the cytosolic components. The physical breakage of the NE was confirmed at an ultra-structural level by Niki and colleagues. A single expanding tear appears to relieve the strain and the NE relaxes into a more rounded conformation. Simultaneously, the mitotic spindle straightens abruptly, indicating that it had been experiencing the compressive stress when confined in the nucleus. The NE resolves into three parts—the two daughter nuclei and a short-lived medial compartment. The mitosis is completed when the nuclear integrity is restored, the nucleoplasmic markers are reimported into the newly formed daughter nuclei and the spindle disassembles. Thus, *S. japonicus* undergoes mitosis of what could be classified as a "semi-open" type, where the spindle is assembled in a "closed" manner but the NE still fragments late in anaphase. This mode of mitosis is unusual for the fission yeast clade since not only *S. pombe* but also *Schizosaccharomyces octosporus* (S. octosporus) maintain the nuclear integrity throughout division (Fig. 2).

Another curious aspect of the mitotic division in *S. japonicus* is unusual dynamics of the nuclear pore complexes (NPCs). Shortly after the onset of anaphase B spindle elongation, the NPC markers (Cut11, Nup85, Nup132 and Nup189), exhibit a net motion toward the mitotic poles, effectively clearing the nuclear equator. Tts1, a transmembrane ER protein that localizes preferentially to the curved membrane regions including the nuclear pores, exhibits a similar behavior. Three-dimensional reconstructions of the anaphase nuclei clearly show an increase in the nuclear pore density.

![Figure 1](https://example.com/image1.png) **Figure 1.** *S. japonicus* and *S. pombe* exhibit distinct nuclear envelope dynamics during mitosis. Time-lapse single plane images of mitotic *S. japonicus* (A) and *S. pombe* (B) cells expressing the artificial ER marker GFP-AHDL. Note the abrupt rupture of the nuclear envelope (at time point 52") in *S. japonicus*. The anaphase nucleus in *S. pombe* divides through a dumbbell shaped intermediate. Time is in minutes and seconds. Scale bars represent 5 μm.

![Figure 2](https://example.com/image2.png) **Figure 2.** *S. octosporus* undergoes "closed" mitosis. Time-lapse maximum projection images of a mitotic *S. octosporus* cell expressing the nuclear marker Nhp6-GFP. Note that Nhp6-GFP is restricted to the nucleus throughout mitosis. Similar to *S. pombe*, the mitotic *S. octosporus* nucleus divides through a dumbbell shaped intermediate. Time is in minutes. Scale bar represents 2.5 μm.
at the poles of the dividing nucleus as compared with the medial portion. We did not observe mitosis-specific dissociation of the core nucleoporins from the nuclear envelope. Interestingly, throughout the cell cycle in S. pombe, the nuclear basket proteins, Mlp homologs Nup211 and Alm1 exhibit a diffuse nucleoplasmic localization in addition to their association with the nuclear pores (our unpublished data). This is in contrast to the situation in S. pombe and budding yeast where the Mlp proteins localize exclusively to the nuclear rim. The nucleoplasmic pool is released into the cytoplasm upon the NE breaking and it is reimported into the nucleus when mitosis is complete (our unpublished data). So far, this is the only observed reimportation of nuclear materials during mitosis in eukaryotes, the nucleolus in S. pombe and S. japonicus.

However, since the degree of Mlp association with the nuclear pores does not change during mitosis, we do not believe that their differential localization could be directly responsible for the variance in mitotic scenarios. The NPC “sliding” occurs concurrently with the bulk movement of the mitotic chromosomes toward the spindle poles, which suggests a possible association between the chromatin and the nuclear pores. During anaphase, chromosomes move toward the SPBs and the general shape of the chromatin correlates with the distribution of the core nucleoporins. It is worth mentioning that the anaphase chromatin in S. japonicus appears less condensed than in S. pombe, based on imaging of GFP-tagged histone H3. This provides an unprecedented opportunity to visualize chromosome segregation in the yeast system using light microscopy. The nucleolus does not persist through mitosis in S. japonicus, as it does in S. pombe and other organisms undergoing “closed” nuclear division. Imaging of the nucleolar markers (the ribosome biogenesis proteins Erd11 and Fkh12, and transmission electron microscopy) reveals that this organelle stretches in anaphase B following mitotic spindle elongation in S. japonicus. Upon NE rupture, the stretched mother nucleolus breaks into three compartments. Each of the daughter nuclei inherits one of the smaller polar nuclear fragments likely associated with the nucleolar-organizing regions on the chromosomes (rDNA arrays). The largest medial compartment partially surrounded by the NPC-free NE is discarded in the cytoplasm in between the segregated daughter genomes. It rapidly disperses, concomitantly with a visible increase in the size of the daughter nucleoli, suggesting that the protein components of the mother nucleolus are recycled to build the daughters. Thus, similar to the higher eukaryotes, the nucleolus in S. japonicus exhibits a dispersive behavior during mitosis, although the nuclear disassembly occurs in late anaphase rather than in prophase. Similar nuclear dynamics have been observed in the filamentous Ascomycete Aspergillus nidulans. To conclude, the fission yeast S. japonicus exhibits the following cytological features that set it apart from the related, commonly studied species S. pombe and could be exploited to gain important insights into mechanisms underlying mitotic division and structuring the mitotic nucleus in eukaryotes. First, and perhaps most important, the NE breaks in anaphase to be reassembled after mitosis. Second, the bulk of the nuclear material disperses upon the nuclear envelope breaking and the daughter nucleoli must be built de novo. Third, the sheer size of an S. japonicus cell (the linear dimensions of which are approximately twice as large as in S. pombe) and the less compacted chromatin allow for detailed observations of the chromosome and the nuclear envelope dynamics.

**Geometry and Mechanics of the Nuclear Division**

A “closed” division mode implies constancy of the nuclear volume during mitosis. Since both volume and surface area depend on the radius of a sphere with volume scaling to the power of 3 and the surface area scaling to the power of 2, the surface area of the dividing “closed” nucleus must increase in order to accommodate its division. Indeed, the dividing S. pombe nucleus increases its surface area by approximately 30% within a fairly short time it takes to complete anaphase. We have found that there is no addition of new membranes to the dividing nucleus in S. japonicus. Therefore, the only remaining alternative is to reduce the nuclear volume by NE breakage to form smaller daughter nuclei. This is exactly what we observed—the experimentally determined values of combined volume of the two daughter nuclei in S. japonicus are approximately 70% that of the mother nucleus. The ER is continuous with the NE and therefore, could potentially serve as a considerable membrane reservoir in any dividing cell. So why does the dividing S. japonicus nucleus fail to draw on this resource? One possible explanation could be the presence of a physical barrier between the NE and the peripheral ER that is abrogated during mitosis in S. pombe but not S. japonicus. Whether there indeed exists a junction-like barrier between the outer NE and the ER or it is simply the matter of structuring the inner NE, for instance by modifying its association with chromatin and therefore controlling the nuclear surface area, could involve an upregulation of the membrane biosynthesis just before mitosis and assembly of a specific ER compartment that could serve as a source of membranes for the dividing nucleus in S. pombe and other organisms with “closed” mitosis. Supporting this hypothesis, inhibition of membrane biosynthesis by treating S. pombe cells at G2/M boundary with cerulenin, an inhibitor of fatty acid synthesis, results in failure of nuclear division. An important aspect of the “closed” mitosis is intranuclear assembly and elongation of the mitotic spindle. Elongating spindles produce pushing force that acts on the NE and are capable of deforming it when the mitotic spindle minus end directly encounters the membrane instead of being properly anchored at the SPBs. Since the SPBs reinforce the NE, the elongating spindle will also experience compressive stress along the longitudinal direction when pushing against the NE. When the NE surface area increases in

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parallel with spindle elongation, like in S. pombe, the compressive stress is negligible. However, when the nuclear surface area does not increase, as in S. japonicus or in cerulein-treated S. pombe cells, the compressive stress leads to spindle buckling that could potentially trigger spindle collapse. In S. japonicus, this problem is ultimately resolved by the NE breakdown that allows the severely bent spindle to straighten and complete chromosome segregation. However, since S. pombe lacks the mechanism to break down the NE, anaphase spindles indeed collapse and chromosome segregation fails when the membrane reservoir is not available. This underscores the relative fragility of the mitotic spindle in relation to the NE. It would be of interest, for instance, to compare its mechanical properties including microtubule cross-linking requirements for spindle mid-zone construction in two yeasts.

In summary, we propose that scaling considerations dictate either nuclear surface expansion or the NE breakdown during mitosis. It is tempting to speculate that they could have played a central role in emergence of distinct mitotic strategies across eukaryotes.

**Nuclear Envelope Breakdown in Schisosaccharomyces japonicus**

As discussed above, in the absence of the membrane reservoir S. japonicus appears to have evolved a mechanism to break down the NE in late anaphase. Importantly, it is an active mechanism rather than the passive tearing of the membrane by the spindle forces: when cells undergo mitosis in the absence of the mitotic spindle, the NE still breaks down. This occurs however, with a slightly slower kinetics—instead of a virtually instantaneous rupture of the NE, the nuclear markers now take longer time to redistribute throughout the cellular volume. It is likely that in the presence of the elongating spindle, the first tear in the NE becomes the only, rapidly expanding one, while in the absence of the spindle force, the NE still gets permeabilized but in a slowly de novo-like manner. This requirement for microtubule forces to trigger the timely and efficient NE breakdown is reminiscent of the situation in other experimental systems. In a mammalian model of the NE breakdown (NEBD), the partial tearing of the membrane by centrosomal microtubules is followed by a rapid collapse of the entire NE, including NPC disassembly and depolymerization of the nuclear lamina. Although the NEBD is still able to occur when the microtubule network is compromised, it is delayed significantly. In the Basidiomycete fungus Ustilago maydis the pulling forces generated by microtubules also appear to mediate the NE rupture. Interestingly, while the NE morphology appears normal in the absence of microtubules, the NE permeabilization, as judged by the loss of compartmentalization of nuclear resident proteins, still occurs. Thus, microtubule-based forces appear to facilitate the NE breakdown but are not essential for this process.

In higher eukaryotes, the changes in NPC permeability mark the first step in NEBD and partial disassembly of the NPCs precede disassembly of lamins and dissociation of the inner nuclear membrane (INM) proteins. The nuclear pore is a massive and highly organized protein complex that contains over a hundred nucleoporins. FxFG, GLGF and WD-40 repeat-containing and non-repeat nucleoporin types play distinct functional roles, either in mediating recognition and interaction with the transported cargo, or in maintaining the INM protein Lem2, exhibits precocious mitosis-specific dissociation from the NPCs and initiate the disassembly process of the remaining core nucleoporins during prophase. Similarly, phosphorylation of nucleoporins by Cdk1 and NIMA kinases seems to occur during semi-open mitosis in the filamentous fungus Aspergillus nidulans, with the structural components of NPC remaining at the NE throughout mitosis but with the FG-repeat nucleoporins dispersing in the cytoplasm already in prophase. While we did not observe any mitosis-specific dissociation of core nucleoporins from the NPCs in S. japonicus, it would be interesting to test if the peripheral FG-repeat nucleoporin Nup98 and its interacting protein, the WD-repeat homolog Rae1/Gle2 shown to reside at the vertebral NPC disassembly in higher eukaryotes, remain associated with the nuclear pores throughout nuclear division.

In higher eukaryotes, the Ran-GTP gradient that drives the nucleocytoplasmic transport, is maintained across the NE during interphase by localizing RanGAP to the cytoplasm and activating RanGAP in the nucleus (for review see ref. 29). While the nucleocytoplasmic trafficking is modified during “closed” nuclear division to allow mitosis-specific trafficking events to take place, the overall Ran system remains functional. Interestingly, during mitosis II in S. pombe, RanGAP enters the nucleus that coincides with the nuclear marker dispersal, despite the seemingly intact NE and the NPC structure (“virtual” NEBD). However, in S. japonicus mitosis, the specific nuclear and cytoplasmic markers remain compartmentalized and RanGAP is excluded from the nucleus right up to the point of the NE breakage (our unpublished data), suggesting that the Ran system is likely downstream of the NE breakdown machinery.

Possible mechanisms of the NE breakage could involve mitosis-specific modifications of the INM proteins modulating the NE strength and rigidity. Supporting this hypothesis, S. japonicus cells lacking the INM protein Lem2, exhibit precious loss of the nucleocytoplasmic compartmentalization immediately after onset of anaphase B spindle elongation. The vertebrate homolog of Lem2 was also implicated in maintaining NE structural integrity. Most likely, Lem2 also participates in restoring the NE function after mitosis—the post-mitotic recruitment of nuclear markers is delayed in lem2A cells and Lem2 itself is enriched at the interphase between the spindle and the NE in the rescaling daughter nuclei. It is possible that in fungi, a lineage that lacks lamins, a network of the INM proteins could function in supporting the nuclear membrane. The vesicular trafficking events or ER membrane remodeling could also provide...
a potential mechanism for regulating the NE-breakdown during mitosis in S. japonicus. For instance, reversing the direction of ER-Golgi vesicle transport delays the nuclear marker dispersion during mitosis in S. pombe, suggesting that limitation of membrane components during forespore membrane formation may account for the NE permeability changes. Functional links between the vesicular trafficking and the NE morphology were also reported in budding yeast. Finally, NE permeabilization could be facilitated by mitosis-specific restructuring of the outer NE mediated by ER remodeling proteins including the homotypic membrane fusion machinery (for review see ref. 30).

Any of the possible mechanisms of NE-breakdown must be entwined into the cell cycle machinery to ensure that the NE breakage occurs only at a specific stage of mitosis. In S. japonicus, the NE rupture takes place in late anaphase B and it is of immediate interest to identify the exact signaling pathway that triggers the NE permeabilization. As discussed above, in mammalian cells and Apergillus nidulans, Cdc1 and NIMA kinases control the NPC disassembly in prophase. In the “virtual” NEBD occurring in S. pombe mitosis II, it is the sequestration initiation kinase network (SIDN) that appears to control the permeabilization of the nuclear membrane at metaphase to anaphase transition. Dissipation of the SIN-related mitotic exit network (MEN) in Ustilago maydis leads to incomplete NEBD. Thus, it appears that signaling inputs from various cell cycle regulators might co-evolve with effectors at the nuclear periphery to achieve timely nuclear envelope breakdown in a variety of physiological contexts.

In conclusion, the distinct features of mitotic division in S. japonicus, a genetically tractable unicellular model organism, offer an exciting opportunity to address fundamental questions pertaining to the mitotic nuclear envelope breakdown and reassembly in eukaryotes. Using this system it will be possible to understand how chromosome segregation, NPC dynamics and nuclear membrane remodeling are coordinated during the NE-breakdown and how the nucleoporin integrity is reestablished following mitosis. On the other hand, comparative and synthetic studies using the related fission yeast species, S. japonicus and S. pombe, will hopefully shed light on the evolution of physiologically diverse modes of nuclear division.

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28. De Souza CP, Osmani AH, Hashmi SB, Osmani SA. Partial nuclear pore complex disassembly during closed mitosis in Aspergillus nidulans. Curr Biol 2004; 14:1973-84; http://dx.doi.org/10.1016/j.cub.2004.10.059

29. Clarke PR, Zheng C. Spatial and temporal coordination of mitosis by Ran GTPase. Nat Rev Mol Cell Biol 2008; 9:464-77; http://dx.doi.org/10.1038/nrm2419

30. Anni K, Zate M, Tanaka K, Yamamoto M. Nuclear compartmentalization is abolished during fission yeast mitosis. Curr Biol 2010; 20:1913-8; http://dx.doi.org/10.1016/j.cub.2010.09.094

31. Asakawa H, Kojidani T, Mori C, Osakada H, Sato M, Ding DQ, et al. Virtual breakdown of the nuclear envelope in fission yeast meiosis. Curr Biol 2010; 20:1919-25; http://dx.doi.org/10.1016/j.cub.2010.09.078

32. Ulbert S, Antonin W, Platani M, Mattaj IW. The inner nuclear membrane protein Lem2 is critical for normal nuclear envelope morphology. PLoS Lett 2006; 500:4955-41; http://dx.doi.org/10.1016/j.modar.2006.10.060

33. Hibiya Y, M salmona H, Asakawa H, Chikashige Y, Kojidani T, Osakada H, et al. Inner nuclear membrane protein Ima1 is dispensable for centromere positioning in fission yeast. Gene Cells 2011; 16:1000-11; http://dx.doi.org/10.1111/j.1365-2443.2011.01544.x

34. Gonzalez Y, Saito A, Sazer S. Fission yeast Lem2 and Man1 perform fundamental functions of the animal cell nuclear lamina. Nucleus 2012; 3.

35. Webster MT, McCaffery JM, Cohen-Fix O. Vesicle trafficking maintains nuclear shape in Saccharomyces cerevisiae during membrane proliferation. J Cell Biol 2012; 195:1079-88; http://dx.doi.org/10.1083/jcb.201206055

36. Hu J, Reiner WA, Kiriyu TA. Wearing the Web of ER Tubules. Cell 2011; 147:1226-31; http://dx.doi.org/10.1016/j.cell.2011.11.022