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Cell Biology: An Open Solution for Closed Mitosis

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At the end of mitosis, cells must remodel their nuclear envelope to produce two identical daughter nuclei. Two new studies using *Schizosaccharomyces pombe* provide insight into how compartmentalized nuclear pore complex disassembly allows cells that undergo closed mitosis to achieve nuclear division.

The double-membrane nuclear envelope (NE) forms the boundary of the nucleus in all eukaryotic cells. Although it has a unique identity, the NE forms a continuous membrane system with the endoplasmic reticulum. The inner (INM) and outer nuclear membranes connect at the nuclear pores occupied by the nuclear pore complexes (NPCs). The NE must be remodeled in mitosis to allow chromosome segregation by the microtubule-based mitotic spindle. Distinct NE remodeling strategies have arisen in evolution, ranging from fully 'open' to fully 'closed' mitosis, with many variations in between [1]. Somatic cells of higher eukaryotes tend to undergo complete nuclear envelope breakdown (NEBD) at mitotic entry. Several mitotic kinases phosphorylate the NPC components, leading to NPC disassembly and NE fenestration and remodeling [2–4]. At the end of this 'open' mitosis, the cytoplasmic spindle disassembles, and the NE reforms around the segregated genomes. On the opposite side of the spectrum, in the 'closed' mitosis of many lower eukaryotes, the nuclei remain compartmentalized and chromosomes are segregated within the confines of the NE. To achieve this, cells must import spindle components from the cytoplasm to the nucleus, assemble the spindle pole bodies (SPBs), specialized structures for microtubule nucleation and anchorage at the NE, and expand the NE to allow spindle elongation. Finally, they must accomplish the topological feat of remodeling the mother nucleus into the two daughters without ever losing nucleocytoplasmic compartmentalization [5]. Two recent complementary studies, including one in this issue of *Current Biology*, provide fascinating insights into this ultimate NE fission event in the model yeast *Schizosaccharomyces pombe* [6,7].

In closed mitosis, the nucleus divides through a dumbbell-shaped intermediate, where the NE surrounds the segregated chromosomes and aligns tightly with the central part of the extending spindle (Figure 1). Daga and colleagues have previously discovered that the NE is differentiated along the length of this bridge. The NPCs, normally distributed throughout the NE, become excluded from most of the bridge, except for the domain overlying the spindle midzone [8]. This is a region where the antiparallel interpolar microtubules overlap, preventing spindle collapse, and where the force for spindle elongation by kinesin motors is generated [9]. The nucleocytoplasmic transport through the bridge NPCs mediated by the importin α-containing complexes has been linked to spindle disassembly at the end of mitosis [8]. Now, in two new studies, this so-called midzone membrane domain is shown to be the site where the NPCs undergo stepwise disassembly, starting from the peripheral nucleoporins and ending with the structural components [6,7] (Figure 1). This results in local NE fenestration and fission, shown in beautiful electron microscopy images by Dey *et al.* [7].

At each end, the midzone membrane domain is defined by 'stalks' enriched in the INM proteins, including the paralogous Les1 and Ish1, and also Lem2 [6,7]. Based on the *les1* deletion phenotype, Dey *et al.* propose that this protein plays a direct role in defining the site of eventual NE fission, by 'corralling' the NPCs to the middle of the bridge. In the absence of Les1, the NPCs and, hence NE fenestration, are not restricted to the central domain [7]. Establishing the place of Les1 within the hierarchy of players organizing the NE in anaphase and uncovering its molecular function will be an interesting direction for future studies. The INM proteins mentioned above are initially distributed throughout the bridge, and their enrichment at the 'stalks' is coincident with the enrichment of the NPCs at the midzone membrane domain. Suggesting that sorting of NE components into large lateral domains along the bridge occurs on a broader scale, yet another INM protein, Ima1, was previously shown to localize to the central part of the bridge, and together with Lem2, promote timely NE fission [10]. It remains to be seen if such stereotypic spatial organization of NE bridge components is regulated by signals emanating from the spindle midzone and/or the two daughter nuclei. Indeed, Exposito-Serrano *et al.* show that these large domains are not properly defined in the absence of a well-organized spindle midzone [6]. The architecture of the bridge is also disturbed by changes in NE lipid composition [6]. Given strong negative genetic interactions between *les1* and the genes encoding ESCRT-III machinery [11], Dey *et al.* [7] hypothesize that proper organization of the bridge could be important for keeping the daughter nuclei sealed off before the ESCRT-III/Vps4 machinery recruited by Lem2 repairs the NE openings [12,13].

Interestingly, the molecular composition of the NPCs at the midzone membrane domain is different from the nascent daughter nuclei to start with — the NPCs entering the bridge lack the central basket components, the Tpr (translocated promoter region) proteins Nup211 and Alm1 [6] (Figure 1). Whether these more transiently associated NPC components are stripped off when

entering the 'stalk', or only already basketless NPCs can partition to that highly curved spindle-associated nuclear membrane, remains unclear. The Tpr protein network restricts NPC mobility and distribution and promotes NE integrity [14]. The absence of the Tpr proteins may thus increase lateral mobility of the NPCs within the bridge, facilitating NPC clustering at the midzone membrane domain, and setting the stage for their spatially restricted disassembly.

Once the site is determined, how are NPC disassembly and NE fission initiated? Unlike in open forms of mitosis [1], the stepwise removal of the nucleoporins at the midzone membrane domain in *S. pombe* occurs at mitotic exit. At this stage, the activities of mitotic kinases phosphorylating nucleoporins for open mitosis (e.g., CDK1, NIMA and Polo [2–4]) are low. What is clear is that NE fission requires the function of the importin α Imp1. Cells lacking Imp1 fail to remove the structural NPC components, resulting in persistent NE bridges between the daughter nuclei [6]. Understanding how Imp1 functions in NPC disassembly will require further investigation, and in particular, the introduction of approaches to manipulate it locally. Imp1 is distributed throughout the NE of the daughter nuclei, in addition to its localization to the midzone membrane domain [6,8], and its post-mitotic phenotype may reflect defects at earlier stages of the cell cycle. Imp1 likely drives the nucleocytoplasmic transport of yet unknown regulators of NPC disassembly, although Exposito-Serrano *et al.* also envisage other possibilities [6].

The peripheral nucleoporin Nup60 is removed on time in Imp1-deficient cells, indicating that other pathway(s) likely collaborate with Imp1-dependent trafficking to promote NPC disassembly specifically at the midzone membrane domain. Daga and colleagues have previously shown that spindle disassembly is critically delayed in the absence of Imp1. Nuclear delivery of the AAA-ATPase Cdc48 was suggested to be critical for this process by promoting the removal of the spindle midzone bundler Ase1 [8]. It remains to be seen if the lack of spindle disassembly in Imp1-deficient cells is a defect parallel to the failure of NE fission or if these two events are in fact linked functionally. Curiously, the

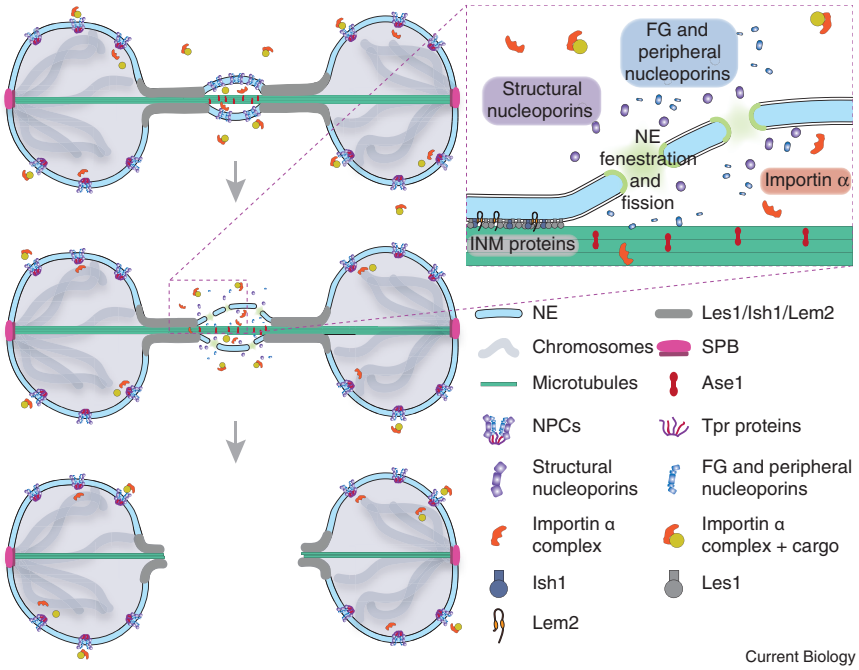

Figure 1. Nuclear envelope remodeling at mitotic exit in *S. pombe*.
A cartoon depicting the current model of how compartmentalized NPC disassembly close to the spindle midzone drives NE fission in closed mitosis. Pictorial legend is included.

S. pombe NE can divide without the spindle in the poorly characterized and slow process enabled by NE expansion and powered by tight association of chromosomes with the SPBs [15]. This again suggests that multiple pathways likely converge to drive the individuation of the daughter nuclei in closed mitosis.

In retrospect, it is perhaps not so unlikely that closed mitosis in fission yeast involves localized NE disassembly. *S. pombe* has the machinery to remodel both NE membranes to form openings for the SPBs at mitotic entry, and promote SPB extrusion to the cytoplasmic side of the NE at mitotic exit [5]. It undergoes a curious 'virtual' NEBD during the anaphase of meiosis II [16,17], which has been proposed to enable chromatin restructuring required for gamete formation and survival [18]. Finally, its close relative, *Schizosaccharomyces japonicus*, does not expand the NE during mitosis, and overtly breaks it in anaphase [19,20].

The discovery of localized NPC disassembly, which enables NE fission without the loss of nucleocytoplasmic compartmentalization in closed mitosis, raises a host of fascinating questions to be tackled in the future. It underscores the structural vulnerabilities of the NE common to all eukaryotes and points at the ways to regulate them for NE division. Finally, it suggests that massive differences in mitotic strategies of eukaryotes observed at the cytological level can be achieved through fairly minor tinkering of the toolbox supporting NE integrity.

REFERENCES

1. Makarova, M., and Oliferenko, S. (2016). Mixing and matching nuclear envelope remodeling and spindle assembly strategies in the evolution of mitosis. Curr. Opin. Cell Biol. *41*, 43–50.

2. Laurell, E., Beck, K., Krupina, K., Theerthagiri, G., Bodenmiller, B., Horvath, P., Aebersold, R., Antonin, W., and Kutay, U. (2011). Phosphorylation of Nup98 by multiple kinases is crucial for NPC disassembly during mitotic entry. Cell *144*, 539–550.

3. Linder, M.I., Kohler, M., Boersema, P., Weberruss, M., Wandke, C., Marino, J., Ashiono, C., Picotti, P., Antonin, W., and Kutay, U. (2017). Mitotic disassembly of nuclear pore complexes involves CDK1- and PLK1-mediated phosphorylation of key interconnecting nucleoporins. Dev. Cell *43*, 141–156.e147.

4. Martino, L., Morchoisne-Bolhy, S., Cheerambathur, D.K., Van Hove, L., Dumont, J., Joly, N., Desai, A., Doye, V., and Pintard, L. (2017). Channel nucleoporins recruit PLK-1 to nuclear pore complexes to direct nuclear envelope breakdown in C. elegans. Dev. Cell *43*, 157–171.e157.

5. Zhang, D., and Oliferenko, S. (2013). Remodeling the nuclear membrane during closed mitosis. Curr. Opin. Cell Biol. *25*, 142–148.

6. Expósito-Serrano, M., Sánchez-Molina, A., Gallardo, P., Salas-Pino, S., and Daga, R.R. (2020). Selective nuclear pore complex removal drives nuclear envelope division in fission yeast. Curr. Biol. *30*, 3212–3222.

7. Dey, G., Culley, S., Curran, S., Henriques, R., Kukulski, W., and Baum, B. (2019). Closed mitosis requires local disassembly of the nuclear envelope. bioRxiv, 779769.

8. Lucena, R., Dephoure, N., Gygi, S.P., Kellogg, D.R., Tallada, V.A., Daga, R.R., and Jimenez, J. (2015). Nucleocytoplasmic transport in the midzone membrane domain controls yeast mitotic spindle disassembly. J. Cell Biol. *209*, 387–402.

9. Khmelinskii, A., and Schiebel, E. (2008). Assembling the spindle midzone in the right place at the right time. Cell Cycle *7*, 283–286.

10. Hiraoka, Y., Maekawa, H., Asakawa, H., Chikashige, Y., Kojidani, T., Osakada, H., Matsuda, A., and Haraguchi, T. (2011). Inner nuclear membrane protein Ima1 is dispensable for intranuclear positioning of centromeres. Genes Cells *16*, 1000–1011.

11. Frost, A., Elgort, M.G., Brandman, O., Ives, C., Collins, S.R., Miller-Vedam, L., Weibezahn, J., Hein, M.Y., Poser, I., Mann, M., *et al*. (2012). Functional repurposing revealed by comparing S. pombe and S. cerevisiae genetic interactions. Cell *149*, 1339–1352.

12. Pieper, G.H., Sprenger, S., Teis, D., and Oliferenko, S. (2020). ESCRT-III/Vps4 controls heterochromatin-nuclear envelope attachments. Dev. Cell *53*, 27–41.e26.

13. von Appen, A., LaJoie, D., Johnson, I.E., Trnka, M.J., Pick, S.M., Burlingame, A.L., Ullman, K.S., and Frost, A. (2020). LEM2 phase separation promotes ESCRT-mediated nuclear envelope reformation. Nature *582*, 115–118.

14. Niepel, M., Molloy, K.R., Williams, R., Farr, J.C., Meinema, A.C., Vecchietti, N., Cristea, I.M., Chait, B.T., Rout, M.P., and Strambio-De-Castillia, C. (2013). The nuclear basket proteins Mlp1p and Mlp2p are part of a dynamic interactome including Esc1p and the proteasome. Mol. Biol. Cell *24*, 3920–3938.

15. Castagnetti, S., Oliferenko, S., and Nurse, P. (2010). Fission yeast cells undergo nuclear division in the absence of spindle microtubules. PLoS Biol. *8*, e1000512.

16. Arai, K., Sato, M., Tanaka, K., and Yamamoto, M. (2010). Nuclear compartmentalization is abolished during fission yeast meiosis. Curr. Biol. *20*, 1913–1918.

17. Asakawa, H., Kojidani, T., Mori, C., Osakada, H., Sato, M., Ding, D.Q., Hiraoka, Y., and Haraguchi, T. (2010). Virtual breakdown of the nuclear envelope in fission yeast meiosis. Curr. Biol. *20*, 1919–1925.

18. Flor-Parra, I., Iglesias-Romero, A.B., Salas-Pino, S., Lucena, R., Jimenez, J., and Daga, R.R. (2018). Importin alpha and vNEBD control meiotic spindle disassembly in fission yeast. Cell Rep. *23*, 933–941.

19. Yam, C., He, Y., Zhang, D., Chiam, K.H., and Oliferenko, S. (2011). Divergent strategies for controlling the nuclear membrane satisfy geometric constraints during nuclear division. Curr. Biol. *21*, 1314–1319.

20. Aoki, K., Hayashi, H., Furuya, K., Sato, M., Takagi, T., Osumi, M., Kimura, A., and Niki, H. (2011). Breakage of the nuclear envelope by an extending mitotic nucleus occurs during anaphase in Schizosaccharomyces japonicus. Genes Cells *16*, 911–926.

Connectomics: Bringing Fly Neural Circuits into Focus

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Three new studies use a whole adult brain electron microscopy volume to reveal new long-range connectivity maps of complete populations of neurons in olfactory, thermosensory, hygrosensory, and memory systems in the fly *Drosophila melanogaster*.

Neural systems need to organize diverse sensory information to direct appropriate actions and form appropriate memories. Maps of the brain circuits that implement these processes have remained blurry: they lack the resolution and completeness necessary to resolve comprehensive connectivity within and between networks. Three studies [1–3] reported in this issue of *Current Biology* now bring the organization of sensory and memory systems in the brain of the adult fruit fly, *Drosophila melanogaster*, into sharp focus.

Densely Mapping Long-Distance Projections

Mapping complete populations of neurons and synapses is no easy task. Determining precise neuron morphology and synapse locations requires the nanometer resolution currently only afforded by electron microscopy (EM). Because connected brain regions can sit on opposite sides of the brain, EM volumes must be big enough to include them all. Moreover, tracing and proofreading of neural reconstructions is labor intensive (although increasing automation promises to reduce this burden [4]). These challenges have historically constrained connectomics studies in the adult fly, such that mapping often focuses on relatively sparse tracing of long-range projections to a handful of neurons [5] or dense tracing of complete populations without reconstructing long-range projections [6].

The studies of Bates and Schlegel *et al.* [1], Marin *et al.* [2], and Otto *et al.* [3] now provide complete maps of large neural populations and their long-range axonal or dendritic projection patterns in the adult fly. All three trace many new neurons and their synaptic partners from an EM volume of an entire adult female brain [7]. Their findings provide a level of detail about fly circuits that has previously only been available in larvae [8].

Intermingling of the Odor Code

Bates and Schlegel *et al.* [1] investigate the fly olfactory system, focusing on second order projection neurons (PNs) of