Physical breakdown of the nuclear envelope is not necessary for breaking its barrier function

Haruhiko Asakawa,1 Yasushi Hiraoka1-3 and Tokuko Haraguchi1-3,*
1Graduate School of Frontier Biosciences; Osaka University; Suita; 2Advanced ICT Research Institute Kobe; National Institute of Information and Communications Technology; Nishi-ku, Kobe; 3Graduate School of Science; Osaka University; Toyonaka, Japan

During mitosis in higher eukaryotic cells, nuclear envelope breakdown (NEBD) occurs and leads to the disassembly of the nuclear membrane and nuclear pore complexes (NPC). This brings about a mixing of nuclear and cytoplasmic macromolecules (open mitosis). On the other hand, in many fungi, mitosis occurs without NEBD (closed mitosis). In a recent study, we reported a novel phenomenon in a closed mitosis organism, Schizosaccharomyces pombe: mixing of nuclear and cytoplasmic proteins occurred in meiosis without breakdown of the nuclear membrane or disassembly of nuclear pore complexes. We designated this event virtual nuclear envelope breakdown (V-NEBD). The key event in V-NEBD is nuclear translocation of Rna1, a RanGAP1 homologue in S. pombe. This leads to collapse of the Ran-GTP gradient across the nuclear envelope (NE) and occurs coincidently with V-NEBD. Thus, the barrier function of the NE can be abated without its physical breakdown through modulation of the Ran-GTP gradient.

One of the core regulators of nucleocytoplasmic transport is Ran, a small Ras-like GTPase. Ran exists in two nucleotide-bound forms: GDP-bound and GTP-bound. Ran-GDP is converted from Ran-GTP by the function of Ran GTPase activating protein1 (RanGAP1) together with Ran binding protein 1, RanBP1.1,2 Ran-GTP is converted from Ran-GDP by the function of Ran guanine nucleotide exchange factor (RanGEF/RCC1). RanGAP1 is localized in the cytoplasm during interphase while RanGEF/RCC1 is localized on the chromatin. This asymmetric localization of Ran regulators generates enrichment of Ran-GTP in the nucleus, thereby generating a difference in Ran-GTP concentration between the nucleus and the cytoplasm. This subcellular gradient of Ran-GTP across the NE defines the directionality of nucleocytoplasmic transport.3-8 In open mitosis, NEBD leads to collapse of the Ran-GTP gradient and mixing of nuclear and cytoplasmic macromolecules (Fig. 1A). In contrast, in closed mitosis, observed in many fungi, the NE remains intact throughout the mitotic cell cycle (Fig. 1B). The fission yeast Schizosaccharomyces pombe is a closed mitosis organism.

In a recent study, we and another group reported an intriguing phenomenon which occurred in S. pombe: nuclear proteins diffused into the cytoplasm transiently in anaphase of the second meiotic division (anaphase II) as if NEBD had occurred in this closed mitosis organism.9-11 To determine whether the NE actually remained intact during anaphase II, we examined the NE by transmission electron microscopy of fixed cells undergoing anaphase II and by fluorescence microscopy imaging of GFP-tagged nuclear pore proteins (nucleoporins) in living cells. The transmission electron microscopy of serial sections of S. pombe revealed an intact continuous nuclear membrane, and none of the 31 nucleoporins tested disassembled from the NPC during anaphase II (see Fig. S2 in ref. 9).

The fact that the nuclear membrane and the NPC remained intact during meiosis...
proteins into the cytoplasm (see Fig. 4 in ref. 9), we speculated that the nuclear localization of RanGAP1 triggered diffusion of nuclear proteins out of the nucleus during anaphase II.12

How does the nuclear translocation of RanGAP1 occur? One factor is that S. pombe RanGAP1 is not targeted to the NE. SUMOylation is a well-known post-translational modification of RanGAP1 in metazoans. Human RanGAP1 can be modified with SUMO1 at its C-terminal domain by RanBP2/Nup358, an E3 SUMO-protein ligase, and SUMOylation targets human RanGAP1 to the NPC.13-15 The plant Arabidopsis thaliana RanGAP1 lacks the C-terminal SUMOylation domain, instead it has an additional plant-specific WPP domain at its N-terminus. The WPP domain interacts with NPC-localizing WIP proteins, targeting A. thaliana RanGAP1 to the NPC (Fig. 2).16,17 The majority of closed mitosis organisms, on the other hand, have neither a SUMO1 attachment domain nor an alternative domain for NPC localization in their RanGAP1 proteins (Fig. 2). This molecular feature is consistent with the fact that fungus RanGAP1 does not show significant localization at the NE and this allows for translocation of fungal RanGAP1 across the NE. This idea is supported by the fact that the RanGAP1 homologue in Saccharomyces cerevisiae contains both an NLS and an NES signal motif by which nuclear/cytoplasmic localization can be switched.18

The filamentous fungus Aspergillus nidulans undergoes semi-open mitosis: the nucleoporins, except for a core scaffold subcomplex, are disassembled upon mitosis.19,20 In this process, a mitotic kinase NimA is implicated in the phosphorylation of nucleoporins.19,21 This suggests the possibility that S. pombe NimA may regulate nuclear translocation of RanGAP1 by phosphorylating nucleoporins. S. pombe RanGAP1 also has a NimA kinase target site (consensus, F-x-x-S/T; Table 1). Therefore, in V-NEBD in S. pombe, NimA kinase might regulate NPC and/or RanGAP1 function. To test this possibility, we constructed a mutant RanGAP1 whose potential NimA target threonine residue was replaced with unphosphorylatable alanine (A) or phosphomimic aspartic
acid (D). Both mutant proteins, however, were translocated to the nucleus from the cytoplasm upon anaphase II, similarly to the wild-type protein. Furthermore, RanGAP1 nuclear translocation and V-NEBD occurred during meiosis II in cells deficient in Fin1, the S. pombe ortholog of NimA kinase (Asakawa H, unpublished results). These results suggest NimA kinase and its possible target amino acid residue on RanGAP1 is dispensable for RanGAP1 nuclear translocation and V-NEBD.

Considering that the V-NEBD occurs specifically in meiosis, we speculate that other meiosis-specific kinase(s) might be involved in V-NEBD. Spore formation proceeds coordinately with the second meiotic division and is consequently temporally linked to V-NEBD. Spo5 is an RNA binding phosphoprotein,22,23 and Spo4-Spo6 is a kinase complex whose activity is required for initiation of spore formation.24,25 Cells deleted for spo5, spo4 or spo6 underwent meiosis without RanGAP1 nuclear translocation or V-NEBD, suggesting that spo5+, spo4+ and spo6+ were involved in V-NEBD, either directly or indirectly (Fig. 3).9,10 We examined whether RanGAP1 was directly phosphorylated by Spo4-Spo6 in vitro and found that the RanGAP1 was not phosphorylated by the Spo4-Spo6 kinase (Asakawa H, unpublished results). S. pombe RanGAP1 has other potential sites for phosphorylation; however, it remains unknown whether such residues are phosphorylated and responsible for V-NEBD (Table 1). We speculate that transient nuclear translocation of the S. pombe RanGAP1, Rna1, occurs through as yet unknown modifications of Rna1.

V-NEBD is a novel phenomenon which does not involve physical disassembly of the NE or NPC. Although its molecular mechanism remains unclear, RanGAP1 is likely to be a key molecule that causes V-NEBD by virtue of its translocation into the nucleus. Further investigation will be needed to determine whether RanGAP1 translocation requires specific modifications of RanGAP1 itself or, alternatively, of one or more nucleoporins. In higher eukaryotic open mitosis, NEBD allows nuclear and cytoplasmic components to come into contact with each other. In S. pombe, on the other hand, NEBD does not occur, but RanGAP1 translocates from the cytoplasm to the nucleus and brings about V-NEBD, which also allows nuclear and cytoplasmic components to come into contact with each other. This fact implies that NEBD is just a way to translocate proteins across the NE, and that if the cell acquires other means to do so, NEBD may be dispensable. Clarifying the molecular mechanism of V-NEBD will reveal not only a novel regulation of RanGAP1 and/or nucleoporins but also the evolution of the modes of mitosis in eukaryotes.

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Table 1. Possible posttranslational modification sites of S. pombe RanGAP1

| Definitions | Possible modification sites | Point mutations introduced | Phenotypes of replaced mutants | Nuclear localization |
|-------------|----------------------------|---------------------------|-------------------------------|---------------------|
| similar to SUMOylation consensus (x-K-x-E) | K76 | R | lethal | yes |
| similar to Nim A kinase consensus (F-x-x-S/T) | T109 | A | lethal | yes |
| possible phosphorylation site1, conserved in closed mitosis organisms | S175 | D | viable | yes |
| possible phosphorylation site2, conserved in eukaryotes | S5, T72, T347, T349 | not tested | not tested | not tested |
| phosphorylation sites3 (S. pombe specific sites) | S10, S18, S33, S58, T1094, Y128, S242, S269, T278, T293, S316, S330, S360, S362, T367, S368 | not tested | not tested | not tested |

These phosphorylation sites were predicted by NetPhos 2.0 server program (http://www.cbs.dtu.dk/services/NetPhos/). Mutant genes were integrated to an additional chromosomal locus of diploid strain, whose authentic RanGAP1 gene was heterozygously deleted. The diploid cells were induced to meiosis and sporulation, and spores were dissected. Progenies which had the mutant RanGAP1 gene and did not have endogenous RanGAP1 gene were tested for their phenotypes. Mutant proteins fused to GFP were expressed from an additional chromosomal locus in cells harboring authentic RanGAP1 gene. T109 and its adjacent sites are similar to NimA kinase target consensus.
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