

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

Haruhiko Asakawa,¹ Yasushi Hiraoka¹⁻³ and Tokuko Haraguchi^{1-3,*}

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

Keywords: RanGAP1, RanGTP gradient, nuclear envelope breakdown, nuclear pore complex, open mitosis, closed mitosis, meiosis

Abbreviations: LRR, leucine rich region; NE, nuclear envelope; NEBD, nuclear envelope breakdown; NES, nuclear export signal; NLS, nuclear localization signal; NPC, nuclear pore complex; MTOC, microtubule organizing center; RanGAP1, RanGTPase activating protein1; RanGEF, Ran guanine nucleotide-exchange factor; SUMO, small ubiquitin-related modifier; V-NEBD, virtual nuclear envelope breakdown; WIP, WPP-domain-interacting protein; WPP, tryptophan-proline-proline

Submitted: 04/18/11

Revised: 06/23/11

Accepted: 07/04/11

<http://dx.doi.org/10.4161/nucl.2.6.16117>

*Correspondence to: Tokuko Haraguchi;
Email: tokuko@nict.go.jp

Extra View to: 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; PMID: 20970342; DOI:10.1016/j.cub.2010.09.070.

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 coincidentally 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

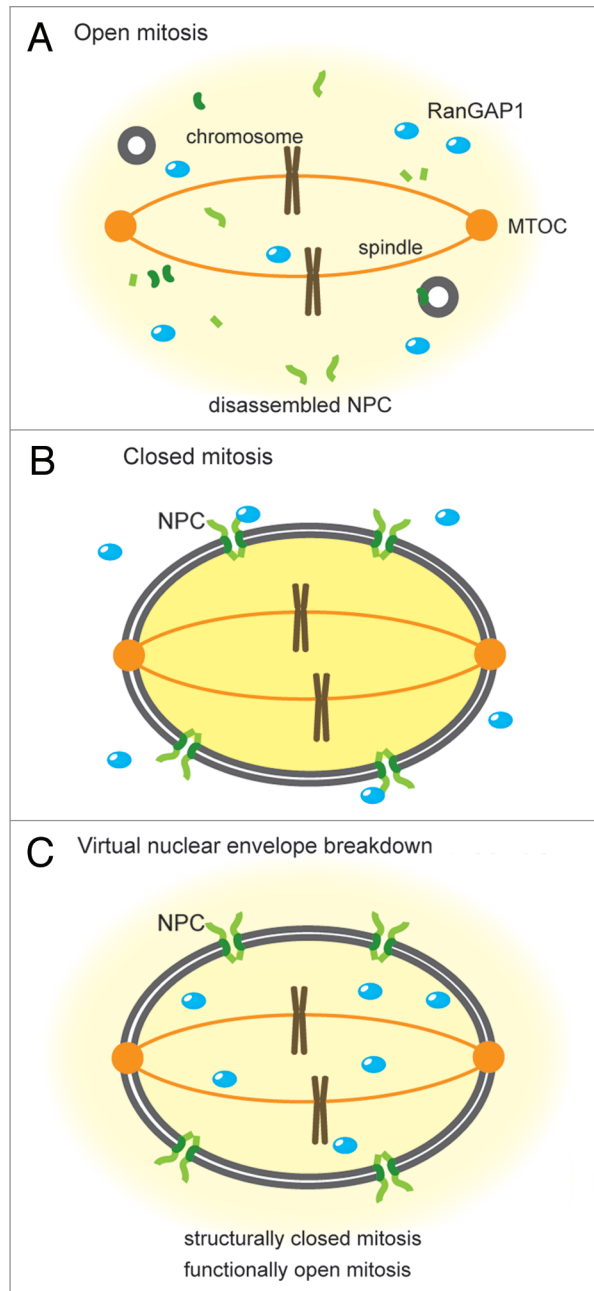


Figure 1. Virtual nuclear envelope breakdown (V-NEBD). (A) In higher eukaryotes, both the nuclear membrane and the NPCs disassemble at the beginning of mitosis. (B) Many lower eukaryotes, like fungi, undergo closed mitosis. During closed mitosis both the nuclear membrane and the NPCs remain intact, RanGAP1 remains localized in the cytoplasm, and the Ran-GTP gradient across the NE remains intact. (C) During meiosis II in the fission yeast *S. pombe*, both the nuclear membrane and the NPCs remain intact but RanGAP1 enters into the nucleus, resulting in V-NEBD.

indicated that the NE remained structurally closed but functionally open during anaphase II (Fig. 1C). Thus, we designated this event virtual nuclear envelope breakdown (V-NEBD).^{9,11}

Our study also revealed that RanGAP1 changed its localization from the cytoplasm to the nucleus at the onset of

anaphase II in exact correlation with the timing of nuclear protein diffusion into the cytoplasm.⁹ Because the timing of the nuclear localization of RanGAP1 coincided with the diffusion of nuclear proteins out of the nucleus and because ectopic expression of NLS-conjugated RanGAP1 induced diffusion of nuclear

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 metazoa. 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.

Acknowledgments

We thank D.B. Alexander for critical reading of the manuscript. This work was supported by grants from the Japan Science and Technology Agency (to T.H.) and the Ministry of Education, Culture, Sports, Science and Technology of Japan (to T.H. and Y.H.).

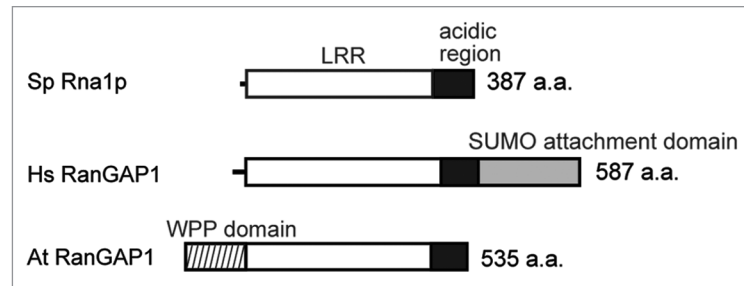


Figure 2. Molecular structure of RanGAP1 in eukaryotes. Schematics of RanGAP1 in eukaryotes are shown. Leucine rich region (LRR) (white box), acidic region (black box), SUMO attachment domain (gray box) and WPP domain (shaded box) are indicated. Sp, *S. pombe*; Hs, *Homo sapiens*; At, *Arabidopsis thaliana*. *Saccharomyces cerevisiae* and *Aspergillus nidulans* RanGAP1 belong to the Sp type; *Xenopus laevis* RanGAP1 belongs to the Hs type.

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

Definitions	Possible modification sites	Point mutations introduced	Phenotypes of replaced mutants ^b	Nuclear localization ^c
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
		D	viable	yes
possible phosphorylation site ^a , conserved in closed mitosis organisms	S175	A	viable	yes
		D	lethal	yes
phosphorylation sites ^a (conserved in eukaryotes)	S5, T72, T347	not tested	not tested	not tested
phosphorylation sites ^a (<i>S. pombe</i> specific sites)	S10, T18, S33, S58, T109 ^d , Y128, S242, S269, T278, T293, S316, S330, S360, S362, T367, S368	not tested	not tested	not tested

^aThese phosphorylation sites were predicted by NetPhos 2.0 server program (<http://www.cbs.dtu.dk/services/NetPhos/>). ^bMutant 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. ^cMutant proteins fused to GFP were expressed from an additional chromosomal locus in cells harboring authentic RanGAP1 gene. ^dT109 and its adjacent sites are similar to NimA kinase target consensus.

References

- Bischoff FR, Krebber H, Smirnova E, Dong W, Ponstingl H. Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. *EMBO J* 1995; 14:705-15.
- Seewald MJ, Kraemer A, Farkasovsky M, Körner C, Wittinghofer A, Vetter IR. Biochemical characterization of the Ran-RanBP1-RanGAP system: are RanBP proteins and the acidic tail of RanGAP required for the Ran-RanGAP GTPase reaction? *Mol Cell Biol* 2003; 23:8124-36.
- Izaurrealde E, Kutay U, von Kobbe C, Mattaj IW, Görlich D. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J* 1997; 16:6535-47.
- Takeda E, Hieda M, Katahira J, Yoneda Y. Phosphorylation of RanGAP1 stabilizes its interaction with Ran and RanBP1. *Cell Struct Funct* 2005; 30:69-80.
- Joseph J. Ran at a glance. *J Cell Sci* 2006; 119:3481-4.
- Ciciarello M, Mangiacasale R, Lavia P. Spatial control of mitosis by the GTPase Ran. *Cell Mol Life Sci* 2007; 64:1891-914.
- Clarke PR, Zhang C. Spatial and temporal coordination of mitosis by Ran GTPase. *Nat Rev Mol Cell Biol* 2008; 9:464-77.
- Güttinger S, Laurell E, Kutay U. Orchestrating nuclear envelope disassembly and reassembly during mitosis. *Nat Rev Mol Cell Biol* 2009; 10:178-91.
- 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.
- Arai K, Sato M, Tanaka K, Yamamoto M. Nuclear compartmentalization is abolished during fission yeast meiosis. *Curr Biol* 2010; 20:1913-8.
- Sazer S. Nuclear membrane: nuclear envelope PORosity in fission yeast meiosis. *Curr Biol* 2010; 20:923-5.
- Asakawa H, Hiraoka Y, Haraguchi T. Nuclear translocation of RanGAP1 coincides with virtual nuclear envelope breakdown in fission yeast meiosis. *Commun Integr Biol* 2011; 4:312-4.
- Matunis MJ, Coutavas E, Blobel G. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J Cell Biol* 1996; 135:1457-70.
- Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 1997; 88:97-107.
- Saitoh H, Pu R, Cavenagh M, Dasso M. RanBP2 associates with Ubc9p and a modified form of RanGAP1. *Proc Natl Acad Sci USA* 1997; 94:3736-41.
- Rose A, Meier I. A domain unique to plant RanGAP is responsible for its targeting to the plant nuclear rim. *Proc Natl Acad Sci USA* 2001; 98:15377-82.
- Xu XM, Meulia T, Meier I. Anchorage of plant RanGAP to the nuclear envelope involves novel nuclear-pore-associated proteins. *Curr Biol* 2007; 17:1157-63.
- Feng W, Benko AL, Lee JH, Stanford DR, Hopper AK. Antagonistic effects of NES and NLS motifs determine *S. cerevisiae* Rna1p subcellular distribution. *J Cell Sci* 1999; 112:339-47.
- 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.
- Osmani AH, Davies J, Liu HL, Nile A, Osmani SA. Systematic deletion and mitotic localization of the nuclear pore complex proteins of *Aspergillus nidulans*. *Mol Biol Cell* 2006; 17:4946-61.
- De Souza CP, Osmani SA. Double duty for nuclear proteins—the price of more open forms of mitosis. *Trends Genet* 2009; 25:545-54.

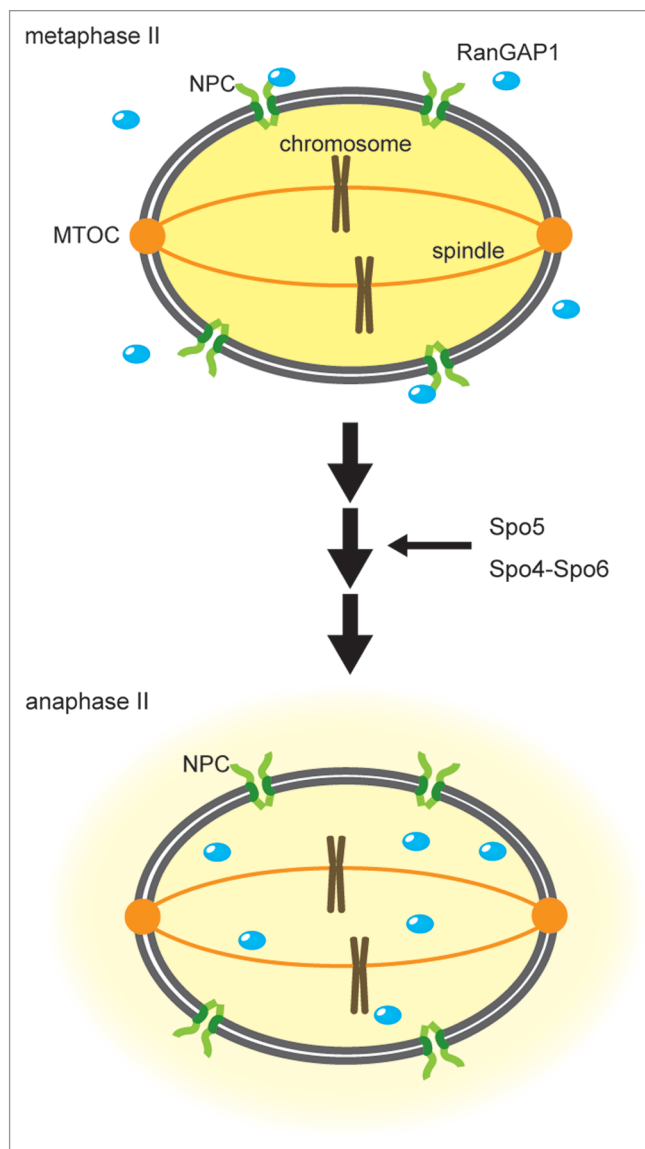


Figure 3. Involvement of Spo4-Spo6 kinase in V-NEBD. In *S. pombe*, both the nuclear membrane and the NPCs remain intact throughout the life cycle. However, when a cell proceeds from metaphase to anaphase during meiosis II (metaphase II and anaphase II, respectively), RanGAP1 enters into the nucleus, resulting in V-NEBD. Meiosis-specific regulators (an RNA-binding protein Spo5 and Spo4-Spo6 kinase) are involved in this process even though RanGAP1 is not a direct target of the Spo4-Spo6 kinase.

- Kasama T, Shigehisa A, Hirata A, Saito TT, Tougan T, Okuzaki D, et al. Spo5/Mug12, a putative meiosis-specific RNA-binding protein, is essential for meiotic progression and forms Mei2 dot-like nuclear foci. *Eukaryot Cell* 2006; 5:1301-13.
- Okuzaki D, Kasama T, Hirata A, Ohtaka A, Kakegawa R, Nojima H. Spo5 phosphorylation is essential for its own timely degradation and for successful meiosis in *Schizosaccharomyces pombe*. *Cell Cycle* 2010; 9:3751-60.
- Nakamura T, Kishida M, Shimoda C. The *Schizosaccharomyces pombe spo6⁺* gene encoding a nuclear protein with sequence similarity to budding yeast Dbf4 is required for meiotic second division and sporulation. *Genes Cells* 2000; 5:463-79.
- Nakamura T, Nakamura-Kubo M, Nakamura T, Shimoda C. Novel fission yeast Cdc7-Dbf4-like kinase complex required for the initiation and progression of meiotic second division. *Mol Cell Biol* 2002; 22:309-20.