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Importin α and vNEBD Control Meiotic Spindle Disassembly in Fission Yeast

Graphical Abstract

Authors

Ignacio Flor-Parra,
Ana Belén Iglesias-Romero,
Silvia Salas-Pino, Rafael Lucena,
Juan Jimenez, Rafael R. Daga

Correspondence

jjimmar@upo.es (J.J.),
rroddag@upo.es (R.R.D.)

In Brief

vNEBD is a phenomenon described in the fission yeast second meiotic division (MII), which resembles the nuclear envelope breakdown that characterizes the mitotic phase in eukaryotes. Flor-Parra et al. describe a biological role of vNEBD in spindle disassembly.

Highlights

- Imp1-dependent transport drives spindle dissolution in *S. pombe* meiosis I
- vNEBD short-circuits the Imp1-dependent disassembly, shortening maximum spindle length
- Imp1-dependent and vNEBD spindle disassembly systems are distinct but exchangeable
- Sequential Imp1- and vNEBD-driven spindle disassembly controls spore order and ploidy

Importin α and vNEBD Control Meiotic Spindle Disassembly in Fission Yeast

Ignacio Flor-Parra,^{1,2} Ana Belén Iglesias-Romero,^{1,2} Silvia Salas-Pino,¹ Rafael Lucena,^{1,3} Juan Jimenez,^{1,4,*} and Rafael R. Daga^{1,*}

¹Centro Andaluz de Biología del Desarrollo. Universidad Pablo de Olavide/Consejo Superior de Investigaciones Científicas, 41013 Sevilla, Spain

²These authors contributed equally

³Present address: Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA

⁴Lead Contact

*Correspondence: jjimmar@upo.es (J.J.), rroddag@upo.es (R.R.D.)
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SUMMARY

In metazoans, the nuclear envelope (NE) breakdown (NEBD) occurs during “open” mitosis and meiosis. In the fission yeast *Schizosaccharomyces pombe*, the mitosis and the first meiotic division (MI) are “closed,” during which the NE is maintained. Intriguingly, during the second meiotic division (MII), the NE is also maintained, but nuclear and cytoplasmic molecules are mixed similarly to open mitosis, a phenomenon of unknown biological significance called “virtual” NEBD (vNEBD). Here, we show that importin- α -dependent nucleocytoplasmic transport regulates spindle disassembly late in anaphase B at MI, as previously reported for mitosis. At MII, however, spindle dissolution is triggered by vNEBD early in anaphase B, a mechanism that short-circuits the nucleocytoplasmic transport system. We demonstrate that the sequential action of these two spindle disassembly systems regulates the spatiotemporal order and ploidy of the meiotic products.

INTRODUCTION

In eukaryotic cells, the nuclear envelope (NE) encloses the chromosomes and acts as a selective barrier between the nucleoplasm and the cytoplasm (Güttinger et al., 2009). During interphase, transport of macromolecules between these compartments takes place across the NE through nuclear pore complexes (NPCs). Nuclear transport is carried out by specialized transporter proteins known as importins and depends on the nucleocytoplasmic gradient generated by the small GTPase Ran. The GTP-bound form of Ran (Ran-GTP) is predominantly nuclear, while the hydrolyzed Ran-GDP form is enriched in the cytoplasm. Cargo protein binds importin β in the cytoplasm (directly or through importin α adaptors) in the presence of Ran-GDP and is released in the nucleoplasm when bound to Ran-GTP (Lange et al., 2007; Okada and Sato, 2015).

In metazoan cells, the NE breakdown (NEBD) occurs and the NPCs are disassembled during DNA segregation both in mitotic

and meiotic cycles, which allows free nucleocytoplasmic diffusion of molecules at this cell-cycle phase (open mitosis) (Güttinger et al., 2009). In contrast, many fungi, including fission yeast, undergo mitosis without NEBD (closed mitosis). In these cells, chromatids segregate while maintaining NE integrity and nuclear transport activity during the entire cell cycle (Zhang and Olfierenko, 2013). During the closed mitosis of the fission yeast *Schizosaccharomyces pombe*, the spindle pole bodies (SPBs) (equivalent to metazoan centrosomes) are embedded in the NE and nucleate the mitotic spindle inside of the nucleus (Hagan, 1998). Insertion of SPBs into the NE locally removes the nucleocytoplasmic barrier, but this removal is transient and rapid, as there is no detectable leakage of the nucleoplasm into the cytoplasm (Tallada et al., 2009).

The mitotic spindle is a bipolar array of microtubules (MTs), in which the minus ends are at SPBs, whereas the plus ends overlap at the central spindle, a region known as the spindle midzone. During anaphase, sister chromatids segregate toward SPBs by shortening kinetochore-bound MTs (anaphase A), and chromatid segregation was completed by spindle elongation (anaphase B) driven by MT-MT sliding at the spindle midzone (Fu et al., 2009; Khodjakov et al., 2004; Tolić-Nørrelykke et al., 2004). Spindle disassembly is the final step that marks the end of genome segregation, a process that is initiated late in anaphase B at the spindle midzone (Glötzer, 2009). In the closed mitosis of *S. pombe*, access of disassembly factors to the spindle midzone requires importin α Imp1 and nucleocytoplasmic transport through NPCs at the NE region surrounding the midzone (named “midzone membrane domain”; MMD). Imp1-dependent nucleocytoplasmic transport is, therefore, a key step in triggering spindle disassembly late in anaphase B in *S. pombe* cells (Lucena et al., 2015).

During fission yeast meiosis, the NE and NPCs remain assembled throughout the first and the second meiotic divisions, meiosis I (MI) and meiosis II (MII), respectively, although nuclear proteins diffuse into the cytoplasm transiently at the onset of anaphase B of MII. The NE and NPCs are maintained at this particular meiotic phase; however, nuclear and cytoplasmic molecules are mixed in a process resembling an open mitosis, a phenomenon called “virtual” NEBD (vNEBD) (Arai et al., 2010; Asakawa et al., 2010). The occurrence of vNEBD at anaphase B during MII indicates that opening the gate between

Figure 1. Meiotic Spindle Dynamics in *S. pombe* Cells

(A) Time-lapse microscopy images of a representative *h⁹⁰* WT cell expressing GFP-Atb2 (an MT marker) or Sid2-Tom (SPB marker) throughout MI and MII.

(B) Maximum spindle length (in microns) during MI (n = 13) and MII (n = 28) in *h⁹⁰* WT *GFP-atb2 sid2-Tom* cells.

(C) Microscopy images (representative cells) of MI and MII spindles of *h⁹⁰* *ark1-as3* mutant cells expressing GFP-Atb2 (MT marker) in the presence of 0.5 mM 1-NM-PP1 (hyper-elongated spindles) or DMSO (control).

(D) Maximum spindle length (in microns) in *h⁹⁰* *ark1-as3* *GFP-atb2* cells during MI (n = 11) and MII (n = 32) with 0.5 mM 1-NM-PP1 ATP analog (DMSO as control; MI n = 15, and MII n = 28). Significant differences are indicated (p values). Scale bars, 5 μ m.

round of DNA replication. The MT-based spindle is the key structure that ensures the correct segregation of chromosomes within each meiotic division. Homologous chromosomes segregate to opposite poles during MI, while sister chromatids segregate during MII (Hillers et al., 2015). *S. pombe* follows a haploid life cycle, in which haploid cells of opposite mating types (*h⁺* and *h⁻*) conjugate to produce a diploid zygote, which undergoes meiosis to produce four haploid meiotic products cooperatively with spore formation (ascospores). The use of *h⁹⁰* *S. pombe* strains, in which mating-type switching occurs in every cell cycle, facilitates zygote formation under

the nucleus and the cytoplasm at this meiotic window is a key event for *S. pombe* meiosis, but the biological significance of this phenomenon is yet unknown (Asakawa et al., 2016). In this study, we analyzed spindle disassembly during both MI and MII and found that, while spindle disassembly, as in mitosis, is triggered by Imp1-dependent transport activity in MI, vNEBD is the mechanism that drives this step in MII. The biological role that vNEBD plays in spindle dissolution and its implication in the spatiotemporal control of meiosis during ascospore development are discussed.

RESULTS AND DISCUSSION

Meiotic Spindle Dynamics in *S. pombe* Cells

Sexual reproduction in all eukaryotic organisms requires the production of haploid gametes with only one copy of each chromosome. This reduction in genetic content is accomplished during meiosis, a specialized cell division in which the diploid genome is reduced to a haploid state through a sequence of two meiotic cell divisions, called MI and MII, following a single

appropriate growth conditions and the analysis of meiotic events (Klar, 2007). To investigate the mechanisms that drive spindle disassembly during meiosis in *S. pombe* cells, we first expressed GFP-Atb2 (an MT marker) and Sid2-Tomato (a protein kinase located at the SPB and the division site) in *h⁹⁰* wild-type (WT) cells, and the *in vivo* dynamics of MTs and SPBs were followed by time-lapse microscopy. As shown in Figure 1A, the spindle assembled as zygotic cells entered into MI and elongated until disassembly was triggered late in anaphase B, before MII was initiated. Once homologous chromosomes were segregated and the MI spindle dissolved, the MII spindle was assembled, elongated during anaphase, and finally disassembled followed by spore differentiation. During anaphase B, the spindle elongates at a constant rate and then disassembles rapidly (Figure S1A); therefore, the time in anaphase B when maximum spindle length is observed can be used to estimate the timing of spindle disassembly initiation (Lucena et al., 2015). We found that spindle elongation rates were similar in MI and MII (0.95 ± 0.14 versus 0.84 ± 0.25 μ m/min; Figure S1A), but maximum spindle length at MII was about 40% the

Figure 2. Imp1 Is Required for Spindle Disassembly in MI, but Not in MII

(A) Time-lapse fluorescence images of a representative *h⁹⁰* WT cell (left panel) and *h⁹⁰ imp1Δ* cell (right panel) expressing GFP-Atb2 (Spindle) or Sid2-Tom (SPB). Remnant MI spindle during MII is indicated (asterisks). Scheme represents cell at 65 min with simultaneous MI and MII spindles (arrow).

(B) Maximum spindle length (in microns) during MI and MII in *h⁹⁰ GFP-atb2* (data from Figure 1A) or *h⁹⁰ GFP-atb2 imp1Δ* cells (MI n = 25; MII n = 54). Significant (p values) and non-significant (n.s.) differences are indicated.

(C) Fluorescence images of the *h⁹⁰ imp1Δ* cell (dashed white line) expressing mCherry-Atb2 (spindle) and Mis6-GFP (centromere) during MI and during anaphase A and anaphase B at MII (from Figure S2B). *S. pombe* chromosomes segregate normally in MI, but under persistent MI spindles (asterisk), sister chromatids missegregate during MII (arrows 1 and 2) (see schematic representation), driving to spore number/ascus (see spores) and spore size (dotted line) alterations.

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maximum length reached at MI (Figure 1B), suggesting that spindle disassembly in MII is likely triggered early in anaphase B.

During mitosis, spindle disassembly is initiated by dissolution of the spindle midzone, the central spindle region where the anti-parallel interpolar MTs (ipMTs) interdigitate at their plus ends (Daire and Poüs, 2011; Woodruff et al., 2010). This process is achieved by a number of overlapping subprocesses largely driven by Aurora B kinase and Kinesin-8. Among other functions, Aurora B phosphorylates the spindle stabilizer Mal3/EB1, thereby inactivating this MT growth factor to arrest ipMT growth, at the time that Kinesin-8 family members actively depolymerize spindle MTs to prevent their regrowth (Daire and Poüs, 2011; Woodruff et al., 2010). To check whether these universal regulators were also involved in meiotic spindle disassembly, we used the Aurora B kinase mutant *ark1-as3*, in which the kinase domain has been modified to allow inhibition with the ATP analog 1-NM-PP1 (Zhang et al., 2005). This mutant is particularly useful for analyzing phenotypic defects separately in the two meiotic divisions (Aoi et al., 2014). By adding the analog and analyzing cells that immediately entered either MI or MII after addition, we determined that *ark1-as3* inactivation leads to hyper-extended spindles in both cases (Figures 1C and 1D), indicating, as expected, that the role of Aurora B kinase in spindle disassembly is likely conserved in mitosis, MI, and MII. Similar results were obtained when using cells depleted for the ipMT-depolymerizing Klp5 Kinesin-8 (Figure S1B).

Imp1-Dependent Spindle Disassembly at MI

In addition to the aforementioned regulators, spindle disassembly in *S. pombe* cells is triggered by cytoplasmic factors (such as the AAA-ATPase Cdc48 or the proteasome component Rpn8) that are transported to the midzone by the importin α Imp1 (Lucena et al., 2015). To check whether Imp1-dependent transport is also required for meiotic spindle disassembly, we analyzed the *in vivo* dynamics of MTs in *h⁹⁰* WT and *h⁹⁰ imp1 Δ* cells during MI and MII. During MI, we found that spindle elongation rates were similar in both strains, but in *imp1 Δ* cells, spindles persisted for longer than in WT controls (Figure S1C). Accordingly, MI spindles were consistently longer in Imp1-depleted cells than in MI of WT cells (Figures 2A and 2B), indicating that, as described for mitosis (Lucena et al., 2015), spindle disassembly in MI is Imp1 dependent too. However, elongation rates and average spindle length during MII were similar in both strains (see Figures 2B and S1C), suggesting that disassembly at MII is triggered via a distinct Imp1-independent mechanism.

Wild-type *S. pombe h⁹⁰* cells normally produce four equal-sized spores per ascus (Figure S2A). However, we observed that, in *h⁹⁰ imp1 Δ* zygotic cells, only 74% were four-spored

asci (including a significant fraction of unequal-sized spores), the remaining being asci developing two (2.5%) or three (23.5%) spores (Figure S2A). Strikingly, we determined that, in 83% ($n = 54$) of these *imp1 Δ* zygotic cells, spindles of MII were assembled before the first one was disassembled, leading to the temporal coexistence, for several minutes, of MI and MII spindles in the same nucleus (Figure 2A). Therefore, fission yeast cells unlikely monitor MI spindle disassembly as a prerequisite to initiate the assembly of spindles in MII. By using the fission yeast Mis6/CENP-I (GFP-tagged) kinetochore marker (Takahashi et al., 2000), we observed that, under MI and MII spindle coexistence, sister chromatids remained together longer, and about 41% ($n = 34$) of the MII spindles failed to fully segregate them into separated nuclei (Figure 2C), resulting in ploidy alterations and in the development of asci with only two or three spores or four unequal-size spores (Figure S2B). Similar results were obtained by using the Cnp1/CENP-A (Tom-tagged) centromere marker (Lando et al., 2012; Figure S2B). It is, therefore, remarkable that proper Imp1-dependent spindle disassembly during MI is essential to guarantee the formation of haploid spores in the fission yeast.

During mitosis, as cells proceed through anaphase B, the spindle elongates with the two segregating nuclei linked by an inter-nuclear membrane bridge (Gonzalez et al., 2009). Imp1 transiently localizes at this mitotic inter-nuclear bridge at the MMD, where disassembly factors are directed to the spindle midzone (Lucena et al., 2015). We found that Imp1 also localized at the MMD during late anaphase B at MI (Figure 2D), which confirms that, in MI, as in mitosis (Lucena et al., 2015), Imp1-dependent nucleocytoplasmic transport at the MMD plays a role in spindle disassembly. In agreement with the functional analysis in *imp1 Δ* cells (Figure 2A), Imp1 was not observed in the inter-nuclear bridge during anaphase B at MII (Figure 2D), which is consistent with the hypothesis that spindle disassembly at MII might be triggered by an Imp1-independent mechanism (Video S1).

vNEBD Controls Spindle Disassembly at MII

In *S. pombe* cells, MI is closed similarly to mitosis. However, as cells enter into anaphase B of MII, the vNEBD allows cytoplasmic and nuclear molecules to mix as in open mitosis (Asakawa et al., 2016). Contrarily to MI, our aforementioned results indicate that spindle dissolution is triggered at anaphase B in MII by an Imp1-independent mechanism (Figures 2A and 2B). Since both mitotic (Lucena et al., 2015) and MI spindle disassembly (Figures 2A–2D) require cytoplasmic factors to be transported to the spindle midzone, we hypothesized that, at MII, such factors might gain access to the midzone via vNEBD.

(D) Time-lapse fluorescence images of a representative *h⁹⁰* WT cell expressing GFP-Atb2 (spindle) or Imp1-Tom (nucleoplasm and NE pore marker) during MI and MII. Imp1-Tom localizes at the MMD during MI (asterisk). Nucleocytoplasmic diffusion of Imp1-Tom (dashed arrows) marks vNEBD during MII (black arrow).

(E) Kymograph of *h⁹⁰* WT GFP-*atb2 sid2*-Tom representative cells (one nucleus per cell) during MII. Dashed line and arrow mark the initiation of vNEBD.

(F) Nuclear/cytoplasmic Imp1-Tom fluorescence throughout MII of individual cells ($n = 7$). Normalized data, related to the initial nuclear/cytoplasmic fluorescence intensity value in each cell, are represented (colored lines; each color represents an individual cell). Average values and SD are indicated (black lines). Spindle disassembly initiation is indicated (red dashed line).

(G) Average time ($\pm$ SD) from nucleoplasm Imp1-Tom signal dim to spindle disassembly.

Scale bars, 5 μ m.

See also Figure S2B.

Figure 3. Effects of Altered vNEBD in Spindle Disassembly

(A) Time-lapse fluorescence images of meiotic division in a representative *h90 spo5Δ* cell expressing GFP-Atb2 (spindle) or Sid2-Tom (SPB). At 64 min, hyper-elongated MII spindles overlap (asterisk). A schematic representation of this cell is shown.

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Imp1-Tomato is useful in following nucleocytoplasmic transport activity, but it is also appropriate for visualizing the vNEBD phenomenon (Figure S3A). By using Imp1-Tomato as a vNEBD marker and GFP-Atb2 to follow spindle dynamics, we analyzed individual cells and found that timing of vNEBD and spindle disassembly were highly correlated (Figure 2D). As shown in Figure 2D, Imp1-Tomato was found in the cytoplasm, but more intensely in the nucleoplasm and the NE throughout meiosis. As cells entered anaphase B, vNEBD took place and nucleocytoplasmic Imp1-Tomato underwent sudden mixing (see arrow in Figures 2D and 2E). In our experimental conditions, spindle dissolution was systematically initiated around 5 min (5.47 ± 1.3 min) after vNEBD was visualized (Figures 2F and 2G). By using the cytoplasmic Rpn8 factor (GFP tagged), which requires Imp1-dependent transport for midzone nucleoplasm localization (Lucena et al., 2015), we determined that this protein also localized throughout the cell following vNEBD at anaphase B in MII, thereby reaching midzone nucleoplasm in an Imp1-independent fashion (Figure S3B). Therefore, our results suggest that, during MII, cytoplasmic disassembly factors may target the midzone in anaphase B via vNEBD (Imp1 independent). Since vNEBD occurs early in anaphase B, this disassembly system may be responsible for shortening maximum spindle length in MII, as compared to those produced in MI, which are disassembled via Imp1-dependent transport late in anaphase B (Figures 1A and 1B).

Imp1-Dependent Transport and vNEBD Are Distinct but Functionally Exchangeable Systems to Trigger Spindle Disassembly

A number of *S. pombe* genes have been identified that are involved in the progression of the second meiotic division (Kovachikova et al., 2013). Among them, *spo5* encodes an RNA-binding phosphoprotein that promotes spore formation by regulating Cdc2/cyclin Cdc13 activity (Arata et al., 2014). Cells deleted for the *spo5* gene fail to develop spores (Nakamura et al., 2002; Togashi et al., 2014), but, interestingly, these cells undergo MII without vNEBD (Asakawa et al., 2016). MII spindle elongation rate in the *spo5* mutant strain was similar to that described in WT controls (Figure S1D). However, as shown in Figure 3A, we observed that abnormally elongated MII spindles were obtained in *spo5* mutant cells, supporting the idea that proper spindle length in MII is timely regulated by vNEBD-driven spindle disassembly. These abnormally long spindles frequently overlapped, thereby exchanging nuclear positions (Figure 3A). Therefore, shortening spindle length by vNEBD is likely required for the spatiotemporal regulation of MII segregation.

Surprisingly, *Spo5*-depleted cells lacking vNEBD accumulated Imp1-GFP at the central spindle late in anaphase B during MII, generating a MMD at this phase (see asterisks in Figure 3B). Moreover, depletion of Imp1 further delayed MII spindle dissolution in *spo5*Δ cells (Figures 3C, 3D, and S1D), indicating that this MMD assembled late in anaphase B in the absence of vNEBD is functional. Therefore, we conclude that the occurrence of vNEBD in WT cells, which takes place early in anaphase B, actually short-circuits the Imp1-dependent spindle disassembly system that operates late in this phase.

Conversely, in *twi1* mutant cells, which harbor a meiosis-specific mutation of the Cdc2 kinase that produces mature asci containing two diploid spores (Grallert and Sipiczki, 1990), vNEBD is ectopically induced in MI (Asakawa et al., 2016). We observed that, in these *twi1* cells, midzone dissolution occurred prematurely, shortening spindle length in MI (Figures 3E and S1D). Furthermore, deletion of *imp1* in *twi1* cells had no effect in MI spindle length (Figure 3F). Thus, spindle disassembly in *twi1* MI is likely triggered by the ectopic vNEBD, which becomes Imp1 independent. Overall, these results indicate that Imp1 dependent and vNEBD driven are distinct but functionally exchangeable systems to trigger spindle disassembly.

vNEBD Directly Triggers Spindle Dissolution

Induction of vNEBD accompanies the spore developmental program during MII in fission yeasts. However, we observed that, apart from the sporulation pathway, vNEBD occasionally occurred spontaneously (svNEBD) early in anaphase B during MI, at a rate of about 6% of MI nuclei (Figure 4A). To further confirm that ectopic vNEBD is involved in an Imp1-independent spindle disassembly system, we measured maximum spindle length during MI in the *h⁹⁰ imp1*Δ strain and determined that cells displaying svNEBD actually triggered premature spindle disassembly (Figure 4A). Interestingly, svNEBD was also occasionally observed in one or in both nuclei during MII in *h⁹⁰ spo5*Δ zygotic cells (see kymographs in Figure 4B). The former case makes it possible to compare nuclei undergoing svNEBD and no vNEBD in the same cell. By measuring maximum spindle length in this subset of *h⁹⁰ spo5*Δ zygotic cells, we determined that svNEBD reverted long MII spindles to the WT length in this strain (Figure 4B). Therefore, aside from other possible effects that opening the nuclear compartment may have in spore development (Asakawa et al., 2016), we conclude that vNEBD directly acts during meiosis as an Imp1-independent spindle disassembly system.

In fission yeast cells, vNEBD is preceded by nuclear localization of the Ran GTPase-activating protein (RanGAP1), Rna1

(B) Time-lapse fluorescence images of MI and MII in a representative *h⁹⁰ spo5*Δ cell expressing Sid2-Tom (SPB) and Imp1-GFP. Asterisks indicate the assembly of functional MMDs both in MI and MII, as reported by Imp1-GFP.

(C) Fluorescence microscopy images of MI and MII hyper-elongated spindles of a representative *h⁹⁰ spo5*Δ *imp1*Δ double-mutant cell expressing GFP-Atb2 (spindle) and Sid2-Tom (SPB).

(D) Maximum spindle length (in microns) in *h⁹⁰* GFP-Atb2 cells in the indicated genetics background during MI and MII (with ns ranging from 7 to 34). Relevant significant (**p < 0.001; ***p < 0.0001) and non-significant (n.s.) differences are indicated.

(E) Time-lapse fluorescence images of MI in representative *h⁹⁰* WT and *h⁹⁰ twi1* mutant cells expressing GFP-Atb2 (spindle).

(F) Maximum spindle length (in microns) in *h⁹⁰* WT (n = 18), *h⁹⁰ imp1*Δ (n = 7), *h⁹⁰ twi1* (n = 16), and *h⁹⁰ imp1*Δ *twi1* (n = 14) cells during MI. Significant (p value) and non-significant (n.s.) differences are indicated.

Scale bars, 5 μm.

Figure 4. Ectopic vNEBD in *S. pombe* Meiosis and Mitosis

(A) Time-lapse fluorescence images of a representative *h⁹⁰ imp1Δ GFP-atz2 RFP-NLS* cell without vNEBD during MI (upper panel) and another one where an ectopic vNEBD event (asterisk) takes place spontaneously at this phase (lower panel). svNEBD occurred in 6.25% (n = 64) of MI nuclei. Maximum MI spindle lengths (in microns) in cells with no vNEBD (n = 29) and those suffering svNEBD (n = 4) are indicated (right panel). p value is also indicated.

(B) Kymographs of representative *h⁹⁰ spo5Δ GFP-atz2 RFP-NLS* cells where no vNEBD occurs, or where svNEBD occurs (asterisk) in one or both nuclei during MII (upper panels) are presented. Time-lapse fluorescence images are shown of a representative *h⁹⁰ spo5Δ GFP-atz2 RFP-NLS* cell spontaneously recovering the vNEBD in only one nucleus during MII (asterisk) (lower panel, left). Maximum mitotic spindle lengths (in microns) in MII nuclei in this subset of zygotic cells (in which svNEBD and no vNEBD take place in the same cell) (lower panel, right; n = 7) are indicated. p value is also indicated.

(C) Time-lapse fluorescence images of representative *h⁹⁰ imp1Δ nmt:ra1-GFP-NLS mCherry-atz2* cells during mitosis, under *nmt* promoter repression (OFF; n = 10) and expression (ON; n = 16) (asterisk indicates the occurrence of vNEBD). Maximum mitotic spindle length (in microns) in both conditions is indicated (right panel). p value is indicated.

Scale bars, 5 μm.

(Asakawa et al., 2014). In fact, it has been shown that expression of an Rna1-GFP-NLS construct directly leads to the induction of vNEBD in mitotically growing cells (Asakawa et al., 2010). To check whether ectopic vNEBD is able to drive Imp1-independent spindle disassembly in mitosis too, we expressed this Rna1-GFP-NLS construct in *imp1Δ* cells and measured spindle length in this strain. As previously reported (Asakawa et al., 2010), we observed that Rna1-GFP-NLS expression provoked vNEBD early at anaphase B in mitotic *imp1Δ* cells (Figure 4C, asterisk). Concomitant with this ectopic vNEBD, the mitotic spindle rapidly disassembled (Figure 4C), suppressing the appearance of long fishhook spindles characteristic of *imp1Δ* cells (Lucena et al., 2015). Therefore, vNEBD also short-circuits the importin- α -dependent spindle disassembly system during mitosis.

In conclusion, here, we show that Imp1-dependent nucleocytoplasmic transport and vNEBD are distinct but functionally

exchangeable mechanisms for timely control of spindle disassembly in the fission yeast meiosis. In *S. pombe*, spindle length scales with cell size (Hagan et al., 1990). During MI, as in mitosis, the Imp1-dependent system initiates spindle dissolution late in anaphase B, yielding long spindles that push the genetic material up to the zygote ends (Figures 1A and 1B). During MII, however, two spindles coexist in the zygotic cell without further cell-size increase (Figure 1A). Thus, MII, but not MI, has been likely subjected to selection pressure for shorter spindle in relation to cell volume. The occurrence of vNEBD early in anaphase B short-circuits the Imp1-dependent transport system to trigger earlier spindle dissolution, which, in turn, results in two short spindles, each of which fit into half of the zygote volume (Figures 1A, 1B, and 3A). Functional alterations on these disassembly mechanisms indicate that, in *S. pombe* cells, the sequential action of importin- α - and vNEBD-dependent spindle disassembly systems is required for proper spatiotemporal genome segregation (Figures 2A, 3A, and 3C) and accurate production of haploid spores at the end of meiosis (Figures 2C and S2B).

Little is known about the factors that have driven the transition under closed and open mitosis during evolution. In the true

open mitosis of metazoans, NEBD occurs during pro-metaphase, but in a number of species (including *S. japonicus* and *Caenorhabditis elegans* embryos), mitotic breakage of the NE takes place only at anaphase (Boettcher and Barral, 2013). If opening the nuclear compartment during anaphase, as occurs with vNEBD in *S. pombe*, reflects an intermediate evolutionary position between closed and open mitosis, the role of vNEBD in fitting spindle length to a limited volume may cast some light on the nature of these factors.

Over eukaryotic cell evolution, both nuclear volume and spindle length scale linearly with increasing cell size, but spindle length will increase faster than the nuclear radius. Thus, the spindle would not be able to fit into the nucleus once eukaryotic cells reached a critical size. This is known as the “incompatible allometries” hypothesis (Sazer et al., 2014). According to the suggested role of vNEBD in shortening spindle length, we speculate that, in the evolution of mitosis, triggering earlier spindle disassembly by NEBD might also shorten maximum spindle length, allowing the spindle to fit into the nucleus (and, eventually, into the cell) above this critical cell size. Interestingly, the cell size of *S. japonicus* is several-fold bigger than that of *S. pombe* (Klar, 2013). Both yeasts assemble a mitotic spindle within an intact NE, but in *S. japonicus*, contrarily to *S. pombe*, NEBD takes place as the spindle elongates during anaphase (Makarova et al., 2016). Future studies will determine whether NEBD influences the timing of spindle disassembly in *S. japonicus* cells. However, according to the role of vNEBD proposed here, and on the assumption that early eukaryotic cells were small cells with a closed mitosis (Devos et al., 2014; Sazer et al., 2014), we hypothesize that NEBD could have arisen as an adaptive response to overcome incompatible allometries regarding nuclear volume versus spindle length, generated after reaching a critical cell size during eukaryote cell evolution.

EXPERIMENTAL PROCEDURES

S. pombe Strain Construction

Standard methods for *S. pombe* growth and genetics were used (Moreno et al., 1991). In general, strains expressing GFP-, mCherry-, or Tomato-tagged proteins, as well as deletions, were constructed and confirmed by PCR as described previously (Bähler et al., 1998). Double or multiple mutants were generated by crosses and tetrad analyses. Strains are described in Table S1. Conditional expression of Rna1 was achieved under the regulation of a thiamine-repressible promoter (*nmt41*), and expression was induced by the removal of thiamine from the growth media (Asakawa et al., 2010).

Imaging *S. pombe* Cells

For live-cell imaging, cells were grown in yeast extract with essential supplements (YES) plates at 25°C for 18–24 hr. Cells were transferred to sporulation plates and incubated at 30°C for 5–7 hr until a good amount of cells mating was observed. Cells were placed on microslide wells (Ibidi, #80821) coated with soybean lectin (Sigma, #1395). Images were acquired using a spinning-disk confocal microscope (IX-81, Olympus; CoolSNAP HQ2 camera, Plan Apo-chromat 100X, 1.4 NA objective, Roper Scientific). Temperature was stably controlled at 25°C during imaging, unless otherwise indicated. Time-lapse images were taken every 2 or 5 min with 18 Z sections of 0.5 µm for a total time of 8–10 hr.

Image Analysis and Measurement of MT Dynamics

Image stacks were analyzed using ImageJ (Schneider et al., 2012). Maximum intensity projections were used for analysis and for generating kymographs.

Spindle elongation (in microns per minute) was determined at its maximum rate during anaphase B.

Statistical Analysis

Graphs were created and statistical analyses were performed with Prism 5.0 (GraphPad Software) and Microsoft Excel. Unless otherwise stated, graphs represent mean, and error bars represent SD; n is the total number of cells scored from at least three independent experiments. Statistical comparison between two groups was performed by unpaired Student's t test, considering non-significant (n.s.) two-tailed p values exceeding 0.05.

Aurora Kinase Inhibition

Aurora kinase inhibition was performed using the *ark1*-as3 analog sensitive allele (Hauf et al., 2007), which allows rapid and specific inactivation of Aurora kinase using the ATP analog 1-NM-PP1 (Santa Cruz Biotechnology). Cells were mounted on microslide wells, and 0.5 mM of the ATP analog 1-NM-PP1 was added to the media immediately before time-lapse imaging. Those cells that, at the time of 1-NM-PP1 addition, entered MI or MII were followed for analysis.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and one video and can be found with this article online at <https://doi.org/10.1016/j.celrep.2018.03.073>.

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AUTHOR CONTRIBUTIONS

J.J. and R.R.D. designed experiments, analyzed the data, and wrote the paper. I.F.-P. designed and conducted experiments and analyzed the data. R.L., A.B.I.-R., and S.S.-P. conducted experiments.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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