

Forespore membrane assembly in yeast: coordinating SPBs and membrane trafficking

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Summary

In the yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, sporulation involves de novo synthesis of forespore membrane (FSM) within the cytoplasm of mother cells. The FSM ultimately becomes the plasma membrane of the developing ascospores. Several protein components of the FSM have been identified. Visualization of these proteins has demonstrated the dynamic nature of the genesis and development of the FSM. It begins to develop at the differentiated outer plaque of the spindle pole bodies (SPBs) and extends outwards, encapsulating each of the haploid nuclei produced by meiosis. Several coiled-coil proteins are specifically recruited to the SPBs and play indispensable roles in FSM assembly. Temporal and spatial coordination of meiotic

nuclear divisions and membrane assembly is of special importance. Comparison of the processes of FSM assembly in these yeasts shows that the basic mechanism has been conserved, even though the individual proteins involved are often different. Understanding these dynamic aspects of yeast sporulation will help to elucidate a general mechanism for the cellularization of cytoplasm containing multiple nuclei.

Movie available online

Key words: Forespore membrane, Meiosis, Spindle, Spindle pole body, Sporulation, Yeast

Introduction

Sporulation in yeast is the process of gametogenesis. Following meiotic nuclear divisions, a diploid mother cell differentiates into an ascus containing four haploid ascospores. Yeast sporulation provides a suitable model system for studying genome-wide control of gene expression during cell differentiation. Whole-genome microarray analyses have, for example, established that gene expression profiles are extensively altered during sporulation (Chu et al., 1998; Primig et al., 2000; Mata et al., 2002). Sporulation is also attractive to cell biologists because it represents a mode of cell division that is different from typical mitotic cell division; namely, plasma membranes for four daughter cells (haploid spores) are constructed within the mother cell cytoplasm (Shimoda and Nakamura, 2004; Moreno-Borchart and Knop, 2003).

Yeast sporulation has been intensively studied in both *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* (Esposito and Klapholz, 1981; Kupiec et al., 1997; Yamamoto et al., 1997). Two groups originally defined sporulation-specific (*spo*) genes by analysis of sporulation-deficient mutants (e.g. Bresch et al., 1968; Esposito and Esposito, 1969), and the genes were subsequently cloned by phenotypic complementation (e.g. Shimoda and Uehira, 1985; Shimoda et al., 1987). Completion of the genome sequences of the two yeasts has accelerated molecular studies (Goffeau et al., 1996; Wood et al., 2002). In addition, systematic gene disruption and whole-genome expression experiments have defined other sporulation-related genes (Rabitsch et al., 2001; Mata et al., 2002; Enyenihi and Saunders, 2003). Comparative genomics provides a molecular basis for

considering yeast sporulation in the broader context of eukaryotic gametogenesis.

Here, I discuss the molecular mechanisms shared by fission and budding yeast sporulation. In particular, I focus on de novo synthesis of the spore plasma membrane, which is originally constructed as a double membrane called the forespore membrane (FSM), and the role of spindle pole bodies (SPBs) as FSM-organizing centers. The interplay between SPBs and the FSM could provide important lessons applicable to broader studies of membrane trafficking and microtubule-organizing centers.

Sporulation as a morphogenetic process

Vegetative cells of *S. pombe* are generally haploid, whereas those of *S. cerevisiae* are diploid. Mating occurs only in the absence of nitrogen sources in the case of haploid *S. pombe* cells, whereas *S. cerevisiae* haploid cells conjugate in rich growth medium to generate diploid cells. Despite such differences in their life cycles, sporulation can be triggered by nitrogen starvation in both yeasts. Diploid vegetative *S. cerevisiae* cells undergo sporulation in media containing only non-fermentable carbon sources. In nitrogen-depleted medium, haploid *S. pombe* cells conjugate to form zygotes, which then undergo sporulation.

The morphogenetic stages of meiosis and sporulation are shown in Fig. 1. Like many fungi, budding and fission yeasts undergo closed mitosis, in which the nuclear envelope remains intact throughout mitosis and meiosis. In *S. cerevisiae*, the nuclear envelope is continuous until the end of meiosis II; the

four haploid sets of chromosomes are distributed into lobes of the nucleus, which then separate (Byers, 1981). By contrast, two discrete nuclei form after meiosis I in *S. pombe*.

In both cases, after meiosis II, each haploid nucleus is encapsulated by a double membrane, which is termed the FSM in *S. pombe* and the prospore membrane in *S. cerevisiae* (Tanaka and Hirata, 1982; Neiman, 1998); here, the term FSM is used for both. The term 'prespore' is used here to refer to the fragile, newly formed spore precursors, which are bounded by the FSM and bear one haploid nucleus, a set of organelles and some cytosol. Prespores mature through spore wall synthesis. Spore wall components are deposited in the luminal space between the outer and inner leaflets of the FSM (Yoo et al., 1973; Lynn and Magee, 1970). The inner leaflet becomes the plasma membrane of the spore. The outer leaflet covers the spore walls during early sporulation, but its fate is not known after spore maturation.

The *S. cerevisiae* spore wall consists of four layers: two inner polysaccharide layers composed of β -glucan and α -mannan, a central chitosan layer and an outermost layer of crosslinked dityrosine (Briza et al., 1986; Briza et al., 1988). Dityrosine is synthesized by an enzyme encoded by two late-

sporulation-specific genes, *DIT1* and *DIT2* (Briza et al., 1994). Electron microscopy has revealed that the *S. pombe* spore wall consists of an inner, electron-transparent layer and an outer, electron-dense layer (Johnson et al., 1982). The inner layer is probably composed of polysaccharides similar to the wall components of vegetative cells. When a germinated spore commences polarized growth, the outer, electron-dense layer is locally disrupted, and the inner layer protrudes, which indicates that the inner layer is structurally related to the vegetative cell wall and that the outer layer is spore specific. Because the genes *bgs2⁺* and *chs1⁺*, which encode a β -glucan synthase and a chitin synthase, respectively, are required for formation of viable spores, β -glucan and chitin must be necessary components of spore walls (Martin et al., 2000; Liu et al., 2000; Arellano et al., 2000). *S. pombe* spore walls also contain a major polypeptide of 23 kDa, although its function is not yet defined (Shimoda, 1983).

Roles of SNARE proteins in FSM assembly

The most intriguing feature of yeast sporulation is the formation of the FSM within the cytoplasm of mother cells.

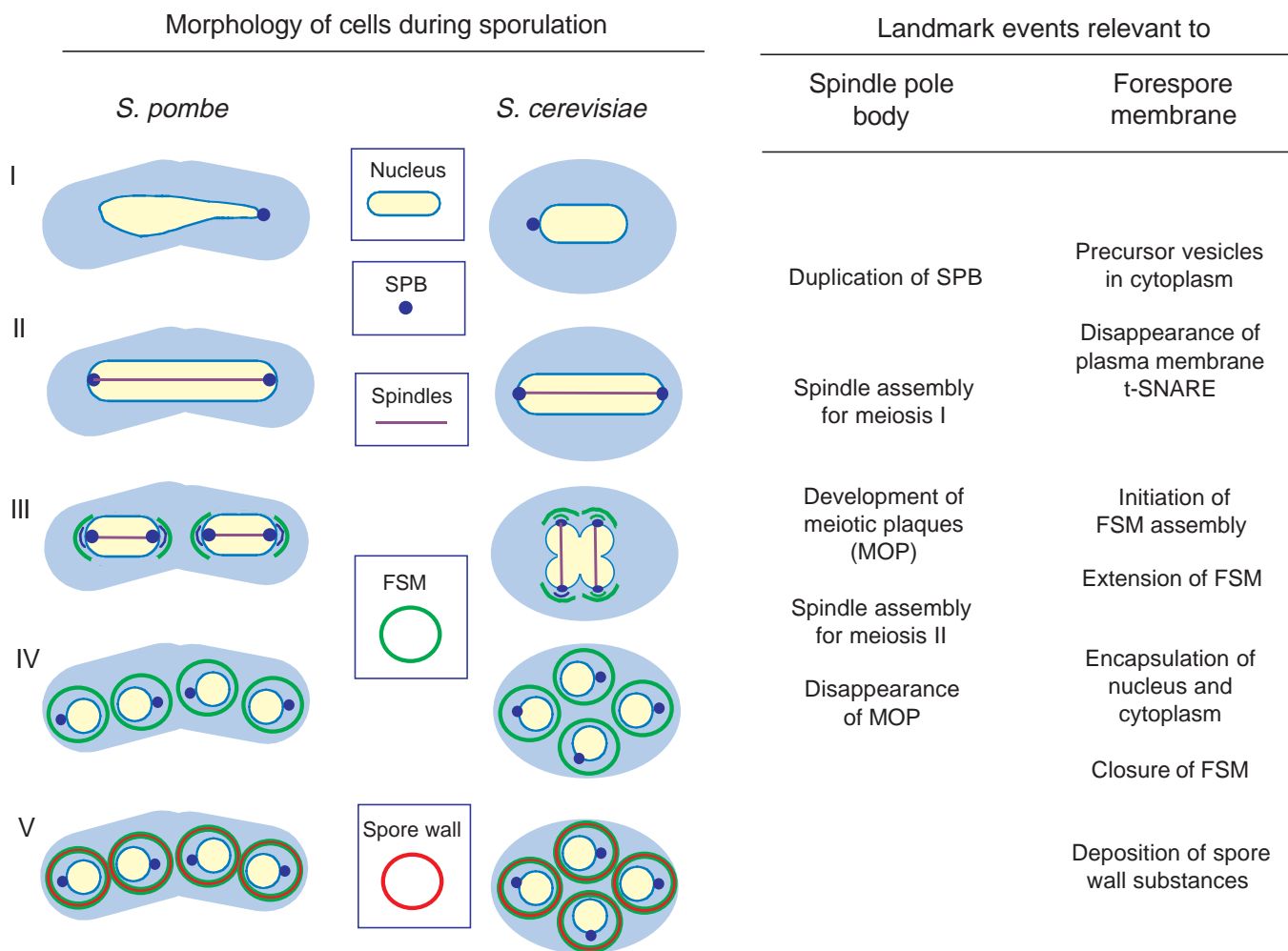


Fig. 1. Diagrammatic representation of sporulation stages in the yeasts *S. pombe* and *S. cerevisiae*. Stage I, meiotic prophase I. Stage II, meiotic metaphase I/anaphase I. Stage III, meiotic metaphase II/anaphase II; formation of the FSM. Stage IV, completion of FSM assembly. Stage V, spore wall synthesis. Landmark events relevant to the SPB and the FSM are described with reference to cell morphology.

Previously, the FSM had been observable only by electron microscopy (Yoo et al., 1973; Tanaka and Hirata, 1982). Recently, two FSM proteins, SpSpo3* and Psy1, were identified in *S. pombe*. Tagging of these proteins with green fluorescent protein (GFP) has enabled us to trace the dynamic behavior of the nascent FSM by fluorescence microscopy (Nakamura et al., 2001). GFP-tagged SpSpo3 or Psy1 appears first as two pairs of bright arcs (membranes) near the SPB at metaphase II. The two cup-shaped membranes face each other and surround one of the anaphase II nuclei. The opening of the growing FSM eventually closes and the resulting sac completely engulfs the haploid nucleus.

Because the *S. pombe spo3* mutant produces no spores, SpSpo3 must be essential for FSM formation (Nakamura et al., 2001). In fact, the FSM is aberrant in this mutant, as revealed by another FSM marker, GFP-Psy1. *Spspo3*⁺ is transcribed when cells are induced to undergo meiosis. SpSpo3 shows no apparent sequence similarity with other known proteins, although it contains a potential membrane-spanning domain at its extreme N-terminus, which may anchor it to the FSM. *psy1*⁺ was isolated as a high-copy-number suppressor of sporulation defects in *Spspo3* mutants. This gene is transcribed constitutively in vegetative cells, and is further induced in cells undergoing meiosis. GFP-Psy1 localizes to the plasma membrane in mitotic cells. Interestingly, *psy1*⁺ encodes a *S. pombe* homolog of the mammalian t-SNARE protein syntaxin-1A, which localizes to the plasma membrane.

The SNARE hypothesis predicts that the docking/fusion of small membrane vesicles with their target membranes is mediated by the membrane-associated proteins termed SNAREs (soluble NSF attachment protein receptors). Vesicles and target membranes carry v-SNAREs and t-SNAREs, respectively. The specific interaction between v- and t-SNAREs might facilitate both docking and fusion steps. The SNARE proteins have been identified as essential components for membrane trafficking: synaptobrevin family proteins as v-SNAREs, and syntaxin family proteins and SNAP-25 as t-SNAREs (Bennett et al., 1992) (for reviews, see Gotte and von Mollard, 1998; Weimer and Jorgensen, 2003). Like other t-SNAREs, Psy1 contains two coiled-coil regions and one C-terminal transmembrane domain. In common with other t-SNAREs, the coiled-coil domain near its C-terminus might interact with the coiled-coil domains of a second t-SNARE (SNAP-25) and the v-SNARE (e.g. synaptobrevin) on another membrane (reviewed by Gotte and von Mollard, 1998). The *S. pombe* genome contains one synaptobrevin v-SNARE homolog (*Syb1*) and one SNAP-25 homolog (encoded by *SPBC26H8.02c*). *Syb1* is required for proliferation and localizes to the plasma membrane in the medial region and cell ends during vegetative growth (Edamatsu and Toyoshima, 2003), and to the FSM during sporulation (Y. Maeda, personal communication). Temperature-sensitive mutations in *psy1* lead to defective FSM assembly even at the permissive temperature (Y. Maeda, personal communication). The membrane fusion process mediated by this SNARE complex thus seems to be necessary for correct FSM formation.

GFP-Psy1 shows a localization pattern similar to that of GFP-Spo3 during meiosis and sporulation, but the signal

persists after spore maturation – unlike that of GFP-Spo3, which disappears after spore maturation (Nakamura et al., 2001). Psy1 localizes to the plasma membrane of mother cells before completion of meiosis I. Immediately after exit from meiosis I, it suddenly disappears from the plasma membrane. It reappears at the cytoplasmic face of the SPB between meiotic metaphase II and anaphase II (Nakamura et al., 2001). Translocation of Psy1 from the plasma membrane to the FSM might be a key event for the initiation of FSM assembly, although the mechanism remains to be investigated.

Neiman reported that sporulation in *S. cerevisiae* requires the late-acting secretion proteins Sec1, Sec4 and Sec8, and proposed that FSM formation is mediated by fusion of secretory vesicles (Neiman, 1998). These Sec proteins are involved in exocytosis: Sec1 is a SNARE-complex-binding protein (Carr et al., 1999), Sec4 is a Rab-family small GTPase (Goud et al., 1988), and Sec8 is a component of the exocyst complex (TerBush et al., 1996). Intriguingly, Sec9 – a SNAP-25 homolog – is essential for growth but dispensable for sporulation. *S. cerevisiae* contains another SNAP-25 homolog, ScSpo20, which is required for sporulation but not for growth. ScSpo20 and Sec9 form complexes with the same SNARE partners (*Sso1* and *Sso2*), but only SpSpo20 is functional in sporulating cells (Neiman et al., 2000). This pioneering work suggests that redirection of vesicles from the secretory pathway towards the FSM partly depends on use of a different version of SNAP-25.

S. cerevisiae has a pair of redundant genes encoding syntaxin-1/Psy1 homologs, *SSO1* and *SSO2*, which perform an essential role (Aalto et al., 1993). However, they do show a functional difference. A homozygous Δ *Sso1* diploid mutant exhibits defective sporulation, whereas a Δ *Sso2* homozygote can sporulate. This indicates that *Sso1* is more profoundly involved in sporulation (Jantti et al., 2002). The *S. cerevisiae* genome also contains two duplicated genes encoding v-SNARE synaptobrevin/*Syb1* homologs, *SNC1* and *SNC2* (Protopopov et al., 1993). Temperature-sensitive *sso1* or *sso2* mutants are suppressed by overproduction of either *Snc1* or *Snc2* (Jantti et al., 2002), as predicted by the SNARE hypothesis (Sollner et al., 1993).

Two other *S. pombe spo* genes encode homologs of *S. cerevisiae* Sec proteins (components of the secretory pathway): SpSpo14 and SpSpo20. SpSpo14 is a homolog of Sec12, a GDP/GTP exchange factor for the Sar1 GTPase that is necessary for budding of vesicles from the endoplasmic reticulum (ER) (Nakano and Muramatsu, 1989; Barlowe and Schekman, 1993; Nakamura-Kubo et al., 2003). The *S. pombe spo14* mutant is suppressed by *SEC12* of *S. cerevisiae*. In *Spspo14* mutant cells, ER-like membranes accumulate. These observations suggest that SpSpo14 plays a similarly indispensable role in the secretory pathway. SpSpo20 is a homolog of Sec14, a phosphatidylinositol/phosphatidylcholine (PI/PC)-transfer protein essential for formation of Golgi-derived vesicles (Bankaitis et al., 1989; Bankaitis et al., 1990; Nakase et al., 2001). In *S. pombe spo14* and *spo20* mutants, the FSM begins to form but does not fully develop, probably because the supply of membrane vesicles for FSM assembly is limited in these mutants. SpSpo20 is present on the plasma membrane, especially at the cell tips and septum (Nakase et al., 2001). It is puzzling that SpSpo20 accumulates in the nucleus when meiosis is induced by nitrogen starvation. This

*To avoid confusion, the *spo* genes or their products from *S. pombe* and *S. cerevisiae* are named with Sp or Sc, respectively. For example, the *S. pombe spo3*⁺ gene is referred to as *Spspo3*⁺, and the *S. cerevisiae SPO20* gene is *ScSpo20*.

unexpected observation suggests that it plays additional, unknown roles during meiosis and sporulation. The studies with the fission yeast also support the notion that vesicle trafficking machinery is crucial for FSM assembly, the FSM growing through fusion with ER/Golgi-derived vesicles.

Leading edge proteins are involved in the initiation and development of the FSM

How does the FSM extend and encapsulate each daughter nucleus? There are at least two different regions in the FSM: the SPB attachment site and the leading edge. Once the prespore is completed, the FSM is released from the SPB. The mechanism by which the FSM is anchored to the SPB is not known. It is thought that the leading edge is coated with leading edge proteins (LEPs) in *S. cerevisiae* (Moreno-Borchart et al., 2001). The LEP coat might play an important role in morphology of the FSM. Three LEPs have been identified: Don1, Ssp1 and Ady3. No homologous proteins are found in *S. pombe*, although Meu14 was reported to localize to the leading edge of the *S. pombe* FSM (Okuzaki et al., 2003). *SSP1* is essential for sporulation in *S. cerevisiae*, whereas *DON1* is not. *ady3* mutants sporulate very poorly and frequently produce asci containing only one or two spores (Moreno-Borchart et al., 2001; Nickas and Neiman, 2002). Ady3 is required for the later stage of sporulation: closure of the FSM and maturation of the spores.

Knop and Strasser found that Don1-GFP first appears in the cytoplasm as small bright spots, which then accumulate near the SPB (Knop and Strasser, 2000). These might represent cytoplasmic precursor vesicles, to which other LEPs (Ssp1 and Ady3) localize (Moreno-Borchart et al., 2001). These vesicles undergo homotypic fusion to construct the FSM precursor, which is anchored to the SPB. As discussed previously, the t-SNARE proteins Psy1 and Sso1/2 might be recruited to the FSM precursor, which is probably anchored to the SPB through interactions between LEPs and SPB component(s). Ady3 physically interacts both with SPB components, such as Mpc54, and with other LEPs, such as Don1. In *S. cerevisiae*, spore wall synthesis is triggered by the closure of the FSM. Ady3 might thus play a part in spore wall synthesis by controlling closure of the FSM (Nickas and Neiman, 2002). LEPs therefore probably play pivotal roles not only in leading the FSM edge but also in initiation and closure of the FSM.

Septins comprise a family of filament-forming proteins that play important roles in cytokinesis and bud site determination (for reviews, see Chant, 1996; Field and Kellogg, 1999). *S. cerevisiae* has seven septins (Longtine and Bi, 2003). Three *S. cerevisiae* septins (Cdc3, Cdc11 and Spr3) localize to the FSM, preferentially to its leading edge (Fares et al., 1996). Such sporulation-specific septin organization is controlled by the Gip1-Glc7 protein phosphatase complex (Tachikawa et al., 2001). *S. pombe* has seven septin genes, and meiosis-specific transcription has been reported for at least two of them (Abe and Shimoda, 2000; Watanabe et al., 2001; Mata et al., 2002). Presumably, septin proteins facilitate construction of the FSM. However, the functional relationship between LEPs and septins is still unknown.

The dynamics of FSM growth have been analyzed by time-lapse microscopy of live *S. pombe* cells expressing GFP-Psy1 (T. Nakamura, personal communication). These studies

showed that the FSM is fragile, because rapid elongation of spindles at anaphase II remarkably distorts the shape of the FSM. Newly formed prespores form elongated ovals, then gradually become spherical. Perhaps this shaping depends on spore wall synthesis. A movie of the FSM formation in live *S. pombe* cells is available online (<http://jcs.biologists.org/supplemental/>).

SPBs as FSM-organizing centers

Electron and fluorescence microscopy studies reveal that FSM formation starts at the SPB during the second meiotic division, which suggests that SPBs serve as the FSM-organizing centers (Tanaka and Hirata, 1982; Hirata and Shimoda, 1994; Knop and Strasser, 2000; Nakamura et al., 2001). SPBs, which are yeast equivalents of mammalian centrosomes, function primarily as microtubule-organizing centers (for reviews, see Byers, 1981; Adams and Kilmartin, 2000). The SPB of *S. cerevisiae* is a complex proteinaceous structure composed of three layers. The central plaque is embedded in the nuclear envelope, connecting the outer and inner plaques, which are responsible for the organization of cytoplasmic microtubules and spindle microtubules, respectively. The *S. pombe* SPB is located in the cytoplasm very close to the nuclear envelope during interphase, but becomes embedded in the nuclear envelope when cells enter mitosis (Ding et al., 1997). At anaphase, a larger part of the SPB is released from the nuclear envelope, probably leaving the other part of the SPB containing a γ -tubulin complex on the nuclear envelope. The SPB is probably embedded in the nuclear envelope during meiosis and is then released into the cytoplasm near the nuclear envelope (Hirata and Shimoda, 1994).

During the second meiotic division, the SPB is structurally modified both in *S. cerevisiae* and in *S. pombe*. The outer plaque of the *S. cerevisiae* SPB enlarges (Byers, 1981), acquiring several novel components, such as Mpc54 and SpSpo21/Mpc70 (Knop and Strasser, 2000). These proteins are produced in a sporulation-specific manner and recruited to the outer plaque, where they replace one of the mitotic outer plaque components, Spc72. This specialized outer plaque is designated as the meiotic outer plaque (MOP). The MOP also contains Ady4 and ScSpo74 (Nickas et al., 2003). SpSpo21/Mpc70 is essential for MOP formation and for FSM assembly (Bajgier et al., 2001). The *ADY1* gene was identified in a systematic screening of nonessential mutants obtained by genome-wide deletion mutagenesis (Winzeler et al., 1999). The *ady1*-null mutant frequently produces non-sister dyads (Deng and Saunders, 2001). In $\Delta ady1$ cells, Mpc54 is located at only one of the sister SPBs in meiosis II nuclei. This mutant phenotype strongly supports the notion that the MOP is responsible for assembly of the FSM.

In *S. pombe*, a few layers of the outer plaque are formed before the second meiotic division (Tanaka and Hirata, 1982; Hirata and Shimoda, 1994). The nascent FSM contacts the outermost layer (Fig. 2A and 2B) of the SPB, which can be visualized by immunofluorescence microscopy using antibodies against SPB components, such as Sad1 (Fig. 2C). The immunostained SPB is usually visible first as a small compact dot, which transforms into a crescent prior to formation of the FSM (Hagan and Yanagida, 1995). The structural relationship between images obtained by electron

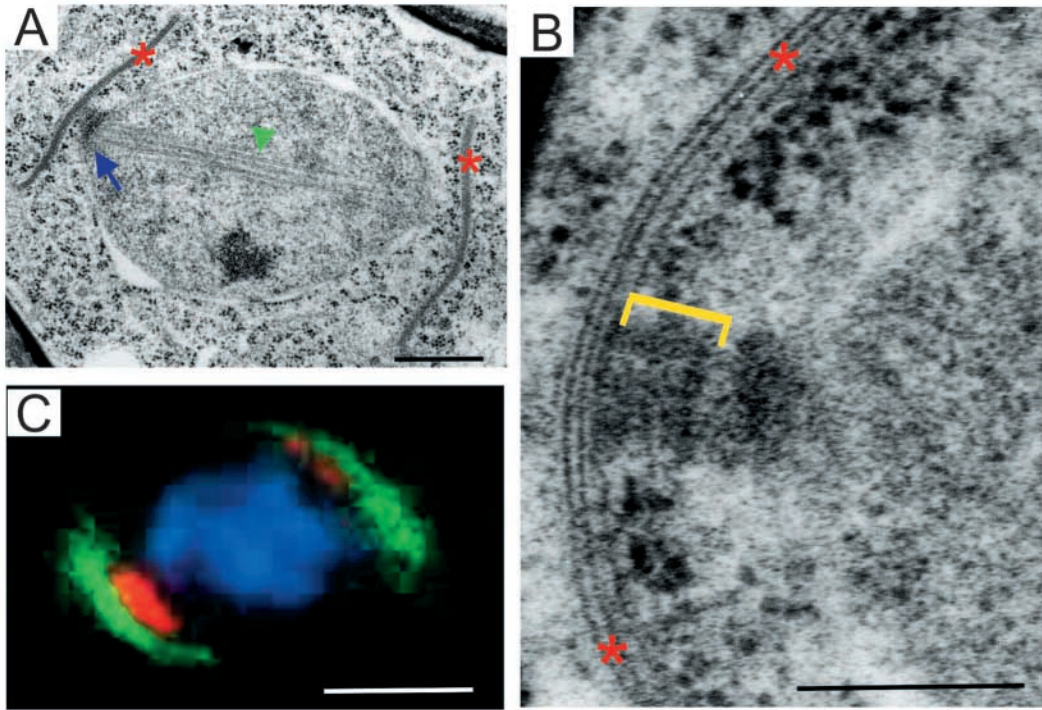


Fig. 2. Electron and fluorescence photomicrographs showing FSM formation near the outer plaque of the SPB in *S. pombe*. (A and B) Electron microscopy. A blue arrow and a green arrowhead represent SPBs and spindles, respectively. Red asterisks show the FSM. The outer plaques are indicated by a yellow bar. Bar, 0.5 μm . Courtesy of A. Hirata, University of Tokyo, Japan. (C) Fluorescence microscopy. Green, GFP-Psy1 (FSM); Red, Sad1 (SPB); Blue, DNA. Bar, 2 μm . Courtesy of Y. Nakase, Osaka City University, Japan.

microscopy and fluorescence microscopy is not well understood.

Among the *S. pombe spo* gene products, two proteins (SpSpo13 and SpSpo15) localize to the SPB (Ikemoto et al., 2000) (Y. Nakase, personal communication). SpSpo13 and SpSpo15 are coiled-coil proteins like most other SPB components in yeasts. SpSpo15 is expressed constitutively, although the *spo15*-null mutant is viable and exhibits no apparent defects in cell proliferation (Ikemoto et al., 2000). By contrast, SpSpo13 is expressed only during meiosis; *Spspo13* mutants appear to have no defects during vegetative growth (Y. Nakase, personal communication). Notably, the modification of the SPB to form a crescent is completely abolished in *Spspo15* mutants and is partially blocked in *Spspo13* mutants. FSM formation is defective in both mutants. SpSpo13 cannot localize to the SPB in the absence of SpSpo15. Immunofluorescence microscopy revealed that SpSpo13 resides on the cytoplasmic side of the SPB in close contact with the nascent FSM, supporting the notion that SpSpo13 is a component of the outer plaque. The presence of SpSpo13 might allow the SPB to begin assembly of the FSM. Whereas the SPB modification takes place only at metaphase II, SpSpo13 is recruited to the SPB before meiosis I. Furthermore, ectopic overexpression of SpSpo13 in vegetative cells can cause neither the formation of an FSM nor the structural transition of the SPB to a crescent morphology, although it does promote recruitment of SpSpo13 to the SPB. Recruitment of SpSpo13 to the SPB per se is thus not sufficient for either. Other processes such as post-translational modification of SpSpo13 or recruitment of additional components to the outer plaque must occur.

Coordination of FSM assembly with meiosis

In the mitotic cell cycle, nuclear division and cytokinesis must

be coordinated. Similarly, the spatiotemporal coordination of meiotic nuclear division and FSM formation is essential for accurate distribution of the genome into four haploid spores. Given that two rounds of consecutive nuclear divisions occur in meiosis, the coordination mechanism seems more complex than mitotic cell division. FSM formation in wild-type cells is strictly controlled so that it starts only after completion of meiosis I. The SPB is a key element for this coordination: spatially it serves as the organizing center so that the FSM is assembled in the vicinity of nuclei, and temporally – through modification of the outer plaque – it determines the timing of FSM assembly during meiosis II.

Mutants that produce two diploid spores are known. In these mutants, the SPB is likely to be prematurely activated in meiosis I, causing the precocious assembly of the FSM. In *S. pombe*, the temperature-sensitive *cdc2* mutant forms such diploid spores at permissive temperatures (Nakaseko et al., 1984). Observations of *cdc2* mutant cells show that SPB modification occurs at metaphase I and the FSM is formed during meiosis I (Y. Nakase, personal communication). Interestingly, *S. pombe* Cdc2 localizes to the SPB and spindles during meiosis (Decottignies et al., 2001). Higher cyclin-dependent kinase activity at meiosis I might prevent the activation of the SPB, and the reduction in enzyme activity at meiosis II could then de-repress the structural modification of the SPB and in turn induce FSM assembly. Once a diploid nucleus is encapsulated by the FSM, further nuclear divisions appear to be repressed. This phenomenon cannot currently be explained.

The *Scspo13* mutation was first found in a natural *S. cerevisiae* isolate that forms two-spored asci (Klapholz and Esposito, 1980). The ScSpo13 protein, which is different from SpSpo13 protein, is thought to provide meiotic cohesion at centromeres (Shonn et al., 2002). *Scspo13* mutants exhibit defective sister chromatid cohesion during meiosis I. The two-

spored asci phenotype depends on the operation of the spindle checkpoint mediated by Mad2. The fact that sporulation starts before meiosis II in *Scspo13* mutants implies that a putative regulator for timing of FSM assembly must work independently of meiotic progression. Mutations in genes encoding components of the mitotic apparatus also induce two-spored ascus formation in *S. pombe* (K. Takahashi, personal communication). *S. cerevisiae cdc5* and *cdc14* mutants also form two-spored asci (Schild and Byers, 1980). It will be important to determine whether this aberrant phenotype requires a spindle checkpoint control or not.

A working model of FSM assembly

The studies of sporulation in *S. pombe* and *S. cerevisiae* discussed above are consistent with the following model of ascospore production in the mother cell (Fig. 3). Prior to meiosis II, membrane vesicles carrying several FSM components are formed within the cytoplasm of mother cells. At meiosis II, the outer plaque of the SPB differentiates, recruiting several novel proteins. Vesicles then gather on the

surface of the plaques and fuse to form a precursor to the FSM. t-SNAREs are also recruited, and the FSM grows continuously by fusion with vesicles derived from the ER/Golgi, developing a cup-like structure. The leading edge of the FSM is coated with LEPs. Chromosome segregation is coordinated with the growth of the FSM so that the divided nucleus is encapsulated by the FSM. Cytosol and a set of organelles for the ascus are also incorporated, either actively or passively. The FSM eventually closes to form prespores. This triggers the synthesis of spore wall components, which accumulate in the luminal space of the FSM.

Concluding remarks

The de novo assembly of a unit membrane in the mother cell cytoplasm is a unique feature of sporulation. Similar phenomena exist in other biological systems: for example, the formation of the autophagosome in yeasts (reviewed by Noda et al., 2002), the formation of phragmoplasts in higher plants (reviewed by Nacry et al., 2000), and the cellularization of syncytial blastoderm in *Drosophila* (reviewed by Mazumdar

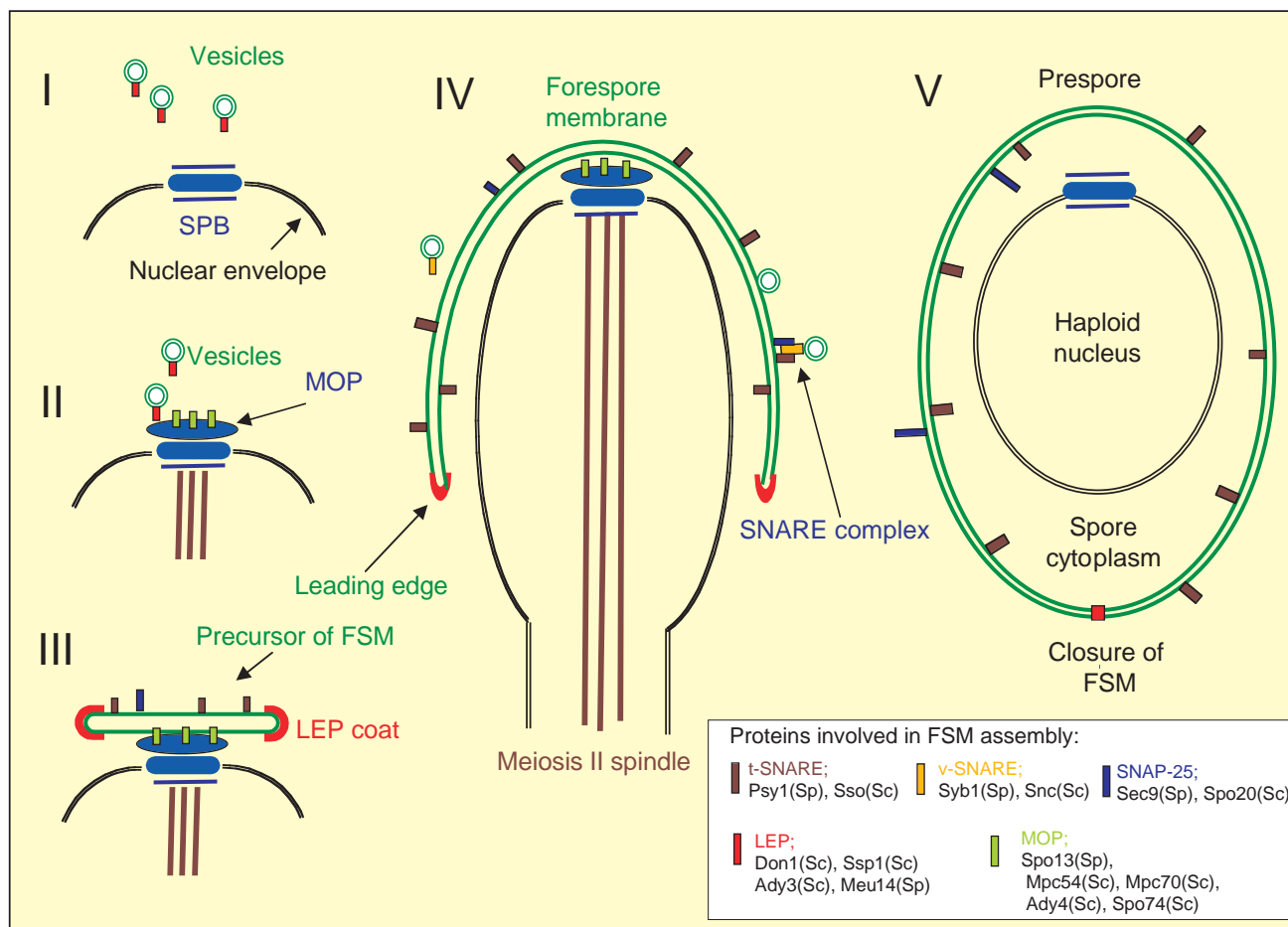


Fig. 3. A model for the initiation and development of the FSM on the outer plaque of the SPB. (I) The nucleus contains a three-layered, unmodified SPB. Cytoplasmic vesicles, which carry components of LEPs, are observable. (II) These vesicles gather on the outer plaque of SPBs. Remarkably, the outer plaque develops to be a meiotic outer plaque (MOP), which contains several specific proteins. (III) A FSM precursor is formed by fusion of vesicles. t-SNAREs are recruited to the FSM precursor. A LEP complex is formed at the leading edge of the FSM. (IV) Cells enter anaphase II. The FSM grows by fusion with cytoplasmic vesicles that carry v-SNARE proteins. (V) The FSM engulfs the nucleus and a part of the cytoplasm. Closure of the FSM forms a membrane compartment called the prespore, which contains a haploid nucleus and part of the mother cell cytoplasm.

and Mazumdar, 2002). Studies of yeast sporulation should facilitate understanding of a common mechanism for the de novo assembly of biological membranes within the cytoplasm. Several questions remain to be addressed: (1) Which component(s) of the SPB serves as a nucleation site for new membranes? (2) What is the identity of the precursor membrane from which the FSM forms? (3) How are the t-SNARE proteins translocated from the plasma membrane of the mother cell to the FSM? (4) How does the growing membrane curve to form a sac?

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