**Slk1 is a meiosis-specific Sid2-related kinase that coordinates meiotic nuclear division with growth of the forespore membrane**

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**Summary**

Septation and spore formation in fission yeast are compartmentalization processes that occur during the mitotic and meiotic cycles, and that are regulated by the septation initiation network (SIN). In mitosis, activation of Sid2 protein kinase transduces the signal from the spindle pole body (SPB) to the middle of the cell in order to promote the constriction of the actomyosin ring. Concomitant with ring contraction, membrane vesicles are added at the cleavage site to enable the necessary expansion of the cell membrane. In meiosis, the forespore membrane is synthesized from the outer layers of the SPB by vesicle fusion. This membrane grows and eventually engulfs each of the four haploid nuclei. The molecular mechanism that connects the SIN pathway with synthesis of the forespore membrane is poorly understood. Here, we describe a meiosis-specific Sid2-like kinase (Slk1), which is important for the coordination of the growth of the forespore membrane with the meiotic nuclear divisions. Slk1 and Sid2 are required for forespore membrane biosynthesis and seem to be the final output of the SIN pathway in meiosis.

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Key words: Meiosis, Forespore membrane, Sporulation, SIN, Sid2, Slk1

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**Introduction**

Membrane biosynthesis needs to be highly coordinated with cell division. During mitosis, the plasma membrane of the mother cell is inherited by the two daughter cells. By contrast, in meiosis, the formation of germ cells requires the de novo synthesis of plasma membranes within the mother-cell cytoplasm. In fission yeast, this process is called sporulation and is initiated during the second nuclear division by the formation of a double-layered membrane, termed the forespore membrane. Synthesis of the forespore membrane is closely related – both temporally and spatially – to the second meiotic nuclear division and is initiated from the spindle pole bodies (SPBs). In meiosis II, at the metaphase-to-anaphase transition, SPBs undergo a transient change in shape from a dot into a crescent (Hagan and Yanagida, 1995). The cytoplasmic face of SPBs differentiates into multilayered plaques (Hirata and Shimoda, 1994; Shimoda and Nakamura, 2004; Tanaka and Hirata, 1982); the inner side of these plaques forms the meiotic spindle, whereas their outer side serves as a platform for the assembly of the forespore membrane (Hirata and Shimoda, 1994; Tanaka and Hirata, 1982). The forespore membrane grows by vesicle fusion and eventually encapsulates each of the four haploid nuclei. Finally, spore walls are synthesized by the accumulation of wall materials – lipids and polysaccharides – between the inner and outer membranes of the forespore (Hirata and Shimoda, 1994; Tanaka and Hirata, 1982).

The septation initiation network (SIN) is an SPB-associated signal-transduction pathway that regulates cytokinesis during the mitotic cell cycle. The SIN consists of the Spg1 GTPase; the downstream kinases Cdc7, Sid1 and Sid2; and the associated proteins Cdc14 (in complex with Sid1) and Mob1 (in complex with Sid2) (Fankhauser and Simanis, 1994; Guertin et al., 2000; Hou et al., 2000; Salimova et al., 2000; Schmidt et al., 1997; Sparks et al., 1999). Sid4 and Cdc11 form an assembly platform for the SIN components at the SPB (Chang and Gould, 2000; Krapp et al., 2001; Tomlin et al., 2002). Activation of Sid2 is the key output of the SIN pathway that presumably transmits the signal from the SPBs to the medial ring, where it activates actomyosin-ring contraction and septation (Sparks et al., 1999).

The role of the SIN in meiosis, in which the cell does not form a contractile ring or a division septum, has only been clarified recently (Krapp et al., 2006). Most SIN genes, with the interesting exception of sid2, are highly transcribed during meiosis, peaking at meiosis II (Mata et al., 2002). Mutants in SIN components can complete the meiotic nuclear divisions but cannot form spores. Therefore, the SIN pathway seems to be essential for proper forespore membrane formation around the haploid nuclei (Krapp et al., 2006).

Here, we describe Slk1, which is a Sid2 parologue that is only expressed in meiosis, in which it is required to couple the growth of the forespore membrane to the meiotic nuclear division. We propose that Slk1, together with Sid2, could be the main output of the SIN pathway in meiosis.

**Results**

*slk1* is a meiosis-specific *sid2* parologue

The *slk1* (*mug27*) gene was identified in a large-scale-deletion screening of meiotically upregulated genes (Martin-Castellanos et al., 2005). Viable *mug* mutants (167 in total) were systematically...
analyzed after mating and sporulation for spore number and morphology, as well as for the number and relative size of the nuclei after DAPI staining. Deletion of mug27, also known as ppk35, showed asci with abnormally small spores (Martin-Castellanos et al., 2005) (Fig. 3A). The mug27 gene encodes a paralogue of Sid2, a conserved serine-threonine protein kinase that belongs to the Ndr subfamily of the AGC group of kinases (Tamaskovic et al., 2003) (Fig. 1A). Dbf2 and Dbf20 in Saccharomyces cerevisiae, Ndr1 and Ndr2 in metazoans, and Orb6 in Schizosaccharomyces pombe are members of this group of kinases (Fig. 1B,C). Accordingly, we renamed Mug27 as Slk1, for Sid2-like kinase 1.

We studied the expression of slk1 in the diploid strain h+/hr pat1-114/pat1-114, containing a thermosensitive mutation for the pat1 gene. Exponentially growing cells (Fig. 2, exp) were pre-synchronized in G1 by nitrogen starvation at 25°C for 14 hours (Fig. 2, t=0 hours). Nitrogen was reintroduced and the cultures were incubated at 34°C to inactivate the Pat1 kinase (Bahler et al., 1991). Under these conditions, the cells underwent a synchronous meiosis (Fig. 2C,D): slk1 mRNA was not transcribed in exponentially growing cells; it was expressed during prophase and peaked between meiosis I and meiosis II, according to the microarray expression data (Mata et al., 2002) (Fig. 2A). To analyze Slk1 protein levels, we constructed the diploid strain h+/h– pat1-114/pat1-114 slk1-GFP/slk1-GFP. These cells contain a functional version of Slk1 tagged at the C-terminus with the green fluorescent protein (GFP). Slk1 protein levels followed those of the mRNA and remained high up to the second meiotic nuclear division (Fig. 2B). This experiment indicates that slk1 mRNA and protein are meiosis-specific.

The slk1 mutant is defective in spore formation
To investigate the function of Slk1, the slk1 deletion was examined. Haploid cells deleted for slk1 (slk1Δ) showed no apparent growth or cell cycle defects (Fig. S1A,B in the supplementary material) and they were able to mate with the same efficiency as wild-type cells (data not shown), suggesting that slk1 has no obvious function in the mitotic cell cycle. This is consistent with the fact that slk1 is expressed only during meiosis.

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The slk1 mutant encodes a serine-threonine protein kinase similar to S. pombe Sid2. (A) Schematic representation of the Slk1 domains. The kinase domain is represented in black and the AGC-kinase C-terminal domain is represented in grey. The amino acid positions of these domains are indicated. (B) Sequence comparison of Slk1 and related proteins. Sequence alignment was generated using CLC Free Workbench 4.0.3 software (CLC Bio, Aarhus, Denmark). Shading was performed with the Boxshade 3.21 program at the http://www.ch.embnet.org/ server. Identical amino acids are highlighted in black and similar amino acids are highlighted in grey. Sp, Schizosaccharomyces pombe; Sc, Saccharomyces cerevisiae; Hs, Homo sapiens. (C) The phylogenetic tree was constructed using CLC Free Workbench 4.0.3 software according to the UPGMA algorithm.
To study the function of Slk1 in meiosis, homothallic h90 slk1Δ mutants were mated. In fission yeast, mating is followed by meiosis and sporulation. These cells were able to complete both meiotic nuclear divisions and show four spores or less, which were smaller than those of the wild-type control (Fig. 3A). The spore viability of four-spore asci was analyzed by tetrad dissection, and no differences were observed between wild-type and slk1Δ cells (data not shown). To analyze meiotic cell cycle progression in the slk1Δ mutant, we induced synchronous meiosis in pat1-114 strains. As shown in Fig. 3B, slk1Δ cells proceed through meiotic divisions with kinetics identical to that of the wild type.

Sporulation in S. pombe requires the de novo synthesis of plasma membrane within the mother-cell cytoplasm. This process is initiated during meiosis II, at the metaphase-to-anaphase transition, from the cytoplasmic face of the SPBs by the formation of a double-layered membrane, termed the forespore membrane (Shimoda and Nakamura, 2004). Psy1 is a t-SNARE syntaxin 1A that localizes to the plasma membrane in vegetative cells and that, in meiosis, relocates to the nascent forespore membrane (Nakamura et al., 2001), where presumably it is required for vesicle fusion. The forespore membrane can be visualized by using the fusion protein Psy1-GFP. In metaphase II, Psy-GFP staining is shaped as an arc that then develops into a cup-like structure by extension of the membrane, which eventually closes to form a double-layered...
suggested a role of Slk1 in the growth of the forespore membrane and engulfment of the nuclei. In the slk1 mutant, the forespore
membrane initiated growth around the four nuclei but in some cases failed to engulf one or more nuclei (Fig. 4C, arrow). A closer
examination of forespore membrane formation in wild-type and slk1A cells by video microscopy revealed that the initial stages –
the formation of the two pairs of bright arcs near the SPBs at metaphase II – took place normally in both the wild type and in
the slk1A mutant (Fig. 5A, t=0-22 minutes; see Movies 1,2 in the supplementary material). However, growth of the forespore
membrane after anaphase II decelerated in the slk1A mutant (Fig. 5A,B, t=24-40 minutes), indicating that the expansion of the
forespore membrane after anaphase II does not take place in the absence of Slk1 and, as a consequence, that the forespore
membrane closes with a smaller size, resulting in small spores.

Slk1 is localized at the spindle pole body and the forespore membrane

In order to examine the role of Slk1 in the formation of the forespore membrane, we looked for genetic interactions with Spo3, a
membrane component required for the assembly of the forespore membrane. Cells deleted for spo3 completely fail to form the
forespore membrane (Nakamura et al., 2001). However, the spo3-
S3 mutant formed small spores, similar to the slk1A mutant (Fig.
6A). Interestingly, the growth of the forespore membrane in spo3-
S3 mutant cells is also severely impaired after anaphase II (Taro
Nakamura, personal communication). We constructed a spo3-S3
slk1Δ double mutant and found that these cells were unable to form
spores (Fig. 6A,B). However, they were able to form forespore
membranes that failed to encapsulate the haploid nuclei (Fig. 6C).
Similar phenotypes were found in the double mutant spo3-GFP slk1
deletion, in which the Spo3 function was slightly compromised (Fig.
6A,B,D). These results indicate a genetic interaction between spo3
and slk1.

Slk1 is highly related to Sid2, which is a mobile component of
the SIN pathway that transduces the signal from the SPB to the
division site to induce actomyosin-ring contraction and septation
(Sparks et al., 1999). The SIN proteins Sid1, Cdc7, Sid2 and Mob1
associate with the SPB during meiosis II, when forespore membrane
biosynthesis begins (Krapp et al., 2006). We found that Slk1 was
also localized to the SPB during metaphase II and anaphase II; after
anaphase II, Slk1 was also localized at the forespore membrane
(Fig. 7A,B).

Krapp et al. have shown that the SIN pathway plays a key role in
spore formation during meiosis (Krapp et al., 2006). In the absence of SIN components, meiosis takes place normally but the
nuclei are not encapsulated by the forespore membrane. This
phenotype is similar to the one we found for the spo3-S3 slk1Δ
double mutant (Fig. 6A,C). We also looked for genetic interactions
between sid2 and slk1 during meiosis. The temperature-sensitive
sid2-250 mutant was able to carry out meiosis and sporulation at
25°C and 34°C, whereas the double mutant sid2-250 slk1Δ was
completely unable to sporulate, even at 25°C (Fig. 8A,B). A similar
genetic interaction was found between cdc7-24 and slk1Δ (Fig.
8A,B), indicating that Slk1 is absolutely required for spore
formation when the SIN pathway is slightly compromised by a
temperature-sensitive mutation. In addition, we observed that, in
some sid2-250 slk1Δ cells, the forespore membrane cut the
nucleus, resulting in several masses of DNA (Fig. 8C,D, arrows).
Interestingly, the SIN pathway activated normally in the slk1Δ
mutant, because Sid1 and Cdc7 recruitment to the SPBs, which

Fig. 4. slk1 is required for proper engulfment of the nuclei by the forespore membrane at the end of meiosis. Homothallic h90 strains S1478 (wild-type) and S1883 (slk1Δ) carrying the plasmid pREP81-Psy1-GFP were sporulated on MEA plates at 25°C. (A) A percentage of slk1Δ cells show defects in nucleus engulfment by the forespore membrane, as revealed by Psy1-GFP fluorescence. Three representative cells are shown. Left panel: bright-field images. Central panels: Hoechst and GFP fluorescence. Right panel: merge. (B) Frequency of abnormal forespore membrane formation in the slk1Δ mutant. Cells are classified according to the number of defective spores. In class I, all nuclei are engulfed by the forespore membranes; class II, one forespore membrane fails to engulf a nucleus; class III, there are two defective forespore membranes; class IV, there are three defective forespore membranes; class V, there are four defective forespore membranes; class VI, there are fewer than four forespore membranes; and class VII, forespore membrane formation is incomplete. Means and standard deviations of three independent experiments are presented. In each experiment, at least 200 cells were counted. (C) Time-lapse experiment showing forespore membrane growth in a slk1Δ strain (S1883) carrying the plasmid pREP81-Psy1-GFP. Cells were sporulated on MEA plates and DNA was stained with Hoechst. Stacks of five images separated by 1 μm were taken every 5 minutes. GFP (green) and Hoechst (red) merged images were generated with ImageJ. Arrows mark the abnormal engulfment of DNA by the forespore membrane in one spore. Scale bars: 10 μm.
signals SIN activation, occurs at meiosis II in slk1Δ as in the wild type (Krapp et al., 2006) (see Fig. S2 in the supplementary material). These results indicate that Slk1 and Sid2 protein kinases are the final output of the SIN pathway in meiosis and are required for the correct engulfment of the haploid nuclei by the forespore membrane.

Sid2 overexpression rescues the phenotype of the slk1Δ mutant

Sid2 and Slk1 are highly related protein kinases that might perform redundant functions in meiosis. To test whether they were functional paralogues, we overexpressed the sid2 gene under the control of the nmt1 promoter (P41nmt1 version) under repressed

![Fig. 5: Forespore membrane growth is defective in the slk1Δ mutant. (A) Time-lapse experiments showing forespore membrane growth in wild-type (S1478) and slk1Δ (S1883) strains carrying the plasmid pREP81-Psy1-GFP. Cells were sporulated on MEA plates and DNA was stained with Hoechst (blue). Stacks of eight images separated by 0.5 μm were taken every 2 minutes. Deconvolution of images and maximal projections were obtained using Deltavision software. GFP and Hoechst merged images were generated with ImageJ. Scale bars: 4 μm. (B) Forespore membrane (FSM) growth stops prematurely in slk1Δ cells. The FSM length of wild-type and slk1Δ spores shown in A was measured, and means and standard deviations are represented.](image-url)
Fig. 7. Slk1-GFP localizes to the spindle pole body and the forespore membrane during meiosis. (A) A homothallic strain harbouring the slk1-GFP allele under its own promoter (S1717) was incubated on MEA for 24 hours at 25°C. Meiotic cells were observed under the fluorescence microscope and photographed. Stacks of four images were taken every 2.5 minutes. Deconvolution of images and maximal projections were obtained using Deltavision software. Notice that Slk1 (arrow) moves from the spindle pole body to the forespore membrane (t=0-10 minutes). After 12.5 minutes, Slk1-GFP localizes to the forespore membrane. (B) A homothallic strain expressing Slk1-GFP under the nmt1(41) promoter (S1931) was incubated on MEA for 24 hours at 25°C and photographed. (Ba) Slk1 (arrow) localizes to the spindle pole body in metaphase II cells. (Bb, Bc) At anaphase II, Slk1 extends from the spindle pole body to the forespore membrane. (Bd) After anaphase II, Slk1 localizes to the forespore membrane. Note that, in the mitotic cells shown in the fields photographed, Slk1 localizes to the nucleolus when overexpressed. Scale bars: 4 μm.

Fig. 6. Genetic interaction between slk1 and spo3 alleles. (A) spo3-S3 and spo3-GFP alleles enhance the sporulation defect caused by the deletion of slk1. Homothallic wild-type (S1478), slk1Δ (S1883), spo3-S3 (S1734), slk1Δ spo3-S3 (S1889), spo3-GFP (S1458) and slk1Δ spo3-GFP (S1888) strains were sporulated on MEA plates, and DIC images were taken after 2 days of incubation at 25°C. (B) MEA plates from the experiment shown in A were stained with iodine vapour after incubation at 25°C for 3 days. Staining is slightly reduced in the slk1Δ single mutant, but it is completely abolished in the double mutants. (C) Psy1-GFP fails to encapsulate the nuclei in slk1Δ spo3-S3 cells. Mutant cells carrying the plasmid pREP81-Psy1-GFP were sporulated on MEA plates at 25°C, stained with Hoechst and photographed. Merged images are shown in the right column. (D) Forespore membranes are unable to engulf the nuclei in slk1Δ spo3-GFP cells. Forespore membranes were visualized by Spo3-GFP fluorescence and DNA was stained with Hoechst. Merged images are shown in the right column. Scale bars: 10 μm.
Role of Slk1 in meiosis and sporulation

(+thiamine) or derepressed (–thiamine) conditions; sid2 overexpression was able to fully rescue the slk1Δ phenotype (Fig. 9). This result indicates that Sid2 and Slk1 are functionally redundant in meiosis. Interestingly, when a weaker version of the nmt1 promoter was used (P81nmt1), nearly no asci were formed under repressed conditions, resembling the phenotype of the slk1/H9004 sid2-250 double mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown). Expression of slk1 in the mitotic cell cycle was unable to rescue the cytokinesis defect of the sid2-250 mutant (data not shown).

Fig. 9. sid2+ overexpression rescues the sporulation defect caused by deletion of slk1. (A) Schematic representation of the P41nmt1-sid2 construction. Endogenous sid2 was under the control of the P41nmt1 promoter, repressed by thiamine. (B) Homothallic strain slk1Δ P41nmt1-sid2 (S1890) was spotted onto MEA (sid2 expression high) and MEA+thiamine (sid2 expression low). DIC images of asci were taken after 2 days of incubation at 25°C. As controls, images of wild-type (S1478) and slk1Δ (S1883) asci are shown. Note that sid2+ overexpression improves the sporulation of slk1Δ, but sid2 shut-off does not enhance the sporulation defect of the slk1Δ mutant, indicating that sid2+ is still expressed in the presence of thiamine. Scale bars: 10 μm. (C) The MEA plates of the experiment shown in B were stained with iodine vapour after 2 days of incubation at 25°C.

Discussion

Role of Slk1 in sporulation

The de novo biosynthesis of the plasma membrane of prespores within the cytoplasm of the mother cell is one of the most intriguing features of sporulation. In mitotic cell division, the plasma membrane of the daughter cells is produced by an extension of the plasma membrane of the mother cell. By contrast, in meiosis, the plasma membrane precursor of the spore is assembled by de novo
biosynthesis. Here, we show that Slk1 plays an important role in spore formation. The \textit{slk1}Δ mutant forms small spores owing to a defect in the degree of extension of the forespore membrane. A similar phenotype has been described for mutants defective in components of the machinery of membrane trafficking and vesicle fission (Nakamura-Kubo et al., 2003; Nakase et al., 2001). We also observed an additive defect when the deletion of \textit{slk1} was combined with a mutation in \textit{spo3}. Although the exact molecular function of \textit{Spo3} is not known, \textit{Spo3} is a component of the forespore membrane that is essential for its assembly and stability, and it probably acts in collaboration with the t-SNARE protein Psy1 and the SNAP-25 protein Sec9 (Nakamura et al., 2005; Nakamura et al., 2001). Our experiments indicate that forespore membrane development initiates normally in the four haploid nuclei but decelerates after anaphase II. A similar phenotype has been observed in the \textit{spo3}-S3 mutant (T. Nakamura, personal communication), suggesting that both \textit{Slk1} and \textit{Spo3} might participate in the same step during sporulation (i.e. vesicle fusion and/or forespore membrane stability). Membrane trafficking could be defective in \textit{slk1}-deleted cells, with a smaller amount of membrane reaching the forespore membrane, causing its slower growth. It is noteworthy that, during septation, the redirection of endocytic vesicles to the division site requires a functional SIN pathway (Gachet and Hyams, 2005). In addition, several lines of evidence in higher eukaryotes suggest that SIN-MEN (mitotic exit network)-related proteins regulate the localization of SNARE complexes at the site of cell division. First, human centrinol, which shares homology to the fission yeast Cdc11 and the budding yeast Nud1, is required for exocyst and SNARE-complex localization at the site of abscission, and its disruption causes defects in cytokinesis (Gromley et al., 2005). Second, in Xenopus, upregulation of Cdc14A, which is homologous to the downstream component of the SIN-MEN pathway in yeast, Flp1/Cdc14, prevents targeting of the exocyst and SNARE complexes to the midbody (Krasinska et al., 2007).

How does the SIN-MEN pathway regulate membrane trafficking during septation and sporulation? Much research has been directed towards uncovering the substrates of the most downstream kinases of the SIN-MEN pathway (i.e. \textit{Sid2}-Mob1 in fission yeast, and Dbf2-Mob1 in budding yeast). Interestingly, protein analyses aimed at identifying in vitro substrates of Dbf2-Mob1 yielded, among others, a protein involved in the endocytic pathway (Vps27/Sst4), suggesting that Dbf2 might phosphorylate proteins involved in endocytosis and protein sorting (Ma et al., 2005). This could be the case for Slk1 during sporulation. Alternatively, Slk1 could phosphorylate meiosis-specific proteins involved in forespore membrane growth, such as \textit{Spo3}, or proteins at the leading edge of the forespore membrane, such as \textit{Meu14} (Ozukazi et al., 2003). Although Meu14 was recruited to the leading edge of the forespore membrane in the \textit{slk1}Δ mutant as in the wild type (data not shown), the leading-edge complex plays an important role in the growth and shaping of the prospore membrane in \textit{S. cerevisiae} and, therefore, its defective regulation might be behind the abnormal growth of spores in the \textit{slk1}Δ mutant (Moreno-Borchard et al., 2001; Neiman, 2005). These analyses will be addressed in future studies and could shed light on targets of the SIN pathway during sporulation and septation in fission yeast, as well as on the process of abscission in higher eukaryotes.

Another possibility is that \textit{slk1}Δ could be defective in coordinating the exit from meiosis II with sporulation. This is the case of the budding yeast \textit{CDC15} (homologous to \textit{cdc7} in fission yeast), mutants of which result in defects in the disassembly of anaphase II spindles and of the meiotic outer plaque of SPBs, leading to a sporulation defect similar to that described in SIN mutants; that is, the inability of forespore membranes to properly engulf haploid nuclei (Pablo-Hernando et al., 2007). Interestingly, the function of Cdc15 in sporulation seems to be independent of MEN and Cdc14 functions in meiotic divisions. In fission yeast, \textit{slk1}Δ, similar to other SIN mutants, is not defective in meiotic divisions nor in the assembly and disassembly of the meiotic spindles (Krappe et al., 2006) (Fig. S4 in the supplementary material). However, because a certain redundancy exists between \textit{Sid2} and \textit{Slk1} in sporulation (see below), spindle and SPB dynamics should be analyzed in double mutants in order to avoid any compensation effects. It is also interesting to note that the severe segregation defects observed in \textit{slk1}Δ \textit{sid2}-250 and \textit{slk1}Δ \textit{cdc7}-24 mutants, in which about 50% of the asci contained more than four DAPI-stained bodies (Fig. 8C,D, and L.P.-H. and S.M., unpublished observations), seem to be due to the aberrant dynamics of forespore membrane biosynthesis. In some cases, uncoordinated growth and closure of the forespore membrane resulted in a meiotic ‘cut’ phenotype (Fig. 8C, Fig. 8C,D). ‘Cut’ nuclei were less frequent in the double mutant \textit{slk1}Δ \textit{spo3}-S3, which does not form spores, suggesting that, in double mutants \textit{slk1}Δ \textit{sid2}-250 and \textit{slk1}Δ \textit{cdc7}-24, forespore membrane defects are more severe.

\textit{Slk1}: a meiotic \textit{Sid2} parologue

\textit{Slk1} and \textit{Sid2} perform redundant functions in sporulation, as shown by the fact that the thermosensitive allele of \textit{sid2}, \textit{sid2}-250, decreases the sporulation defect of \textit{slk1}Δ even at the permissive temperature. Conversely, increased expression of \textit{sid2} in meiosis suppresses the sporulation defect of \textit{slk1}Δ. The role of \textit{Sid2} in sporulation has not been shown until now because available \textit{sid2} mutants do not have apparent sporulation defects. The existence of a meiosis-specific \textit{Sid2}-like kinase in fission yeast explains the lack of sporulation phenotype of \textit{sid2} alleles. In budding yeast, a function of the MEN downstream kinases Dbf2 and Dbf20 in sporulation has not been reported. Blast searches failed to reveal the existence of a meiosis-specific homologue. However, a role for Dbf2 and/or Dbf20 in sporulation cannot be ruled out, because some MEN components have been shown to play a role in spore morphogenesis (Gordon et al., 2006; Kamieniecki et al., 2005; Pablo-Hernando et al., 2007). Dbf20 expression peaks later than Dbf2 during meiosis, suggesting that Dbf20 could be a better candidate to perform a role in sporulation than Dbf2 (Chu et al., 1998).

Two additional intriguing aspects connect \textit{Slk1} with \textit{Sid2} and the SIN pathway. First, during the mitotic cycle, \textit{Sid2} initially localizes to the SPBs and moves to the medial ring upon SIN activation. Similarly, \textit{Slk1} first localizes to the SPBs and then moves to the forespore membrane. It remains to be tested what the signals are that regulate this translocation. Second, in the \textit{slk1}Δ mutant, the forespore membrane growth decelerates concurrently with the onset of anaphase B and Cdc2 inactivation (Fig. 5), coinciding in time with SIN activation in meiosis (Krapp et al., 2006). Given the conservation of the \textit{Sid2} family of protein kinases in mammalian cells, it will be worth studying the function of these proteins in cytokinesis and membrane biosynthesis at the end of mitosis and meiosis.

Materials and Methods

Yeast strains and methods

Strains used in this study are listed in Table S1 in the supplementary material. Fission yeast cells were grown and manipulated according to standard protocols (Moreno et al., 1991). Genetic crosses were done on malt extract agar plates (MEA). Cells were grown in yeast extract with supplements (YES) or Edinburgh minimal medium (EMM).
at the appropriate temperatures. To repress expression from the nmt1 promoter, 5 μg/ml of thiamine was added to minimal medium.

Diploid pat1-114 strains were generated by protoplast fusion (Sipiczki and Ferenczy, 1977). Meiotic time-course experiments were done as described previously (Blanco et al., 2001). Briefly, pat1-114 diploid cells were grown in YEL until the exponential phase and then transferred to EMM supplemented with leucine at 100 μg/ml (Pat1+). In the exponential phase, cells were washed and transferred to EMM-N (+leucine at 50 μg/ml). After 14 hours, cells were induced to enter meiosis by shifting the temperature to 34°C. Nitrogen was reintroduced (0.5 g/l of NH4Cl) and an additional supplement of leucine was added (50 μg/ml).

Slk1 GFP-tagging and P41nmt-sid2 construction

Slk1 was C-terminally tagged with the GFP epitope using the PCR-based method described previously (Bahler et al., 1998). Oligonucleotides with 80 bases of homology to regions flanking the slk1 stop codon were used to amplify the GFP and kanMX6 sequence from plasmid pAA6-GFP-kanMX6. This PCR product was used to transform fission yeast cells. Transformation was performed following the lithium acetate protocol. Correct GFP integration was checked by PCR. A similar protocol was followed to insert the P41nmt1 promoter at the sid2 locus, in this case with oligonucleotides with homology to regions around the sid2 initiation codon.

Construction of slk1-containing plasmids

The slk1+ cDNA was amplified by PCR using cDNA obtained from a 4-hour pat1 meiotic culture with primers slk1-N 5′-TTTTCCTAGGAGCATGACTTCA-CTGGGCTTAAG-3′ (BamHI site, underlined; XhoI site, italized) and slk1-C 5′-GGTGCCTGTGAGAAGAAATATACAGCTG-3′ (Smal site, underlined; an added stop codon, italized). This PCR product was digested with BamH and Smal and cloned into a similarly digested PREP3X vector (Forsburg, 1993), producing plasmid pREP3X-slk1+. This plasmid was transformed into E.coli DH5α and used to transfect fission yeast cells. Transformation was performed following the lithium acetate protocol. Correct GFP integration was checked by PCR. A similar protocol was followed to insert the P41nmt1 promoter at the sid2 locus, in this case with oligonucleotides with homology to regions around the sid2 initiation codon.

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