A Genome-Wide Screen for Sporulation-Defective Mutants in Schizosaccharomyces pombe

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ABSTRACT Yeast sporulation is a highly regulated developmental program by which diploid cells generate haploid gametes, termed spores. To better define the genetic pathways regulating sporulation, a systematic screen of the set of ~3300 nonessential Schizosaccharomyces pombe gene deletion mutants was performed to identify genes required for sporulation. A high-throughput genetic method was used to introduce each mutant into an h90 background, and iodine staining was used to identify sporulation-defective mutants. The screen identified 34 genes whose deletion reduces sporulation, including 15 that are defective in forespore membrane morphogenesis. In S. pombe, the total number of sporulation-defective mutants is a significantly smaller fraction of coding genes than in S. cerevisiae, which reflects the different evolutionary histories and biology of the two yeasts.

KEYWORDS knockout collection erp2 erp5 forespore membrane

Ascospore formation in yeast is a response to nutrient deficiency (Tomar et al. 2013). In Schizosaccharomyces pombe, cells exit mitosis to differentiate into spores when they encounter the lack of a nitrogen source (Tanaka and Hirata 1982; Egel 1989; Shimoda and Nakamura 2004b). First, haploid cells of opposite mating types fuse to form diploid zygotes. These diploids then immediately undergo meiosis to generate four haploid nuclei. During the course of meiosis, these nuclei become packaged into daughter cells, termed spores. Spores are created by a specialized form of cell division that occurs without cleavage of the mother cell (Shimoda 2004a). Each of the four haploid nuclei produced by meiosis are packaged into daughter cells by envelopment within newly synthesized membranes called forespore membranes (Yoo et al. 1973; Shimoda and Nakamura 2004b). Forespore membrane formation initiates on meiotic spindle pole bodies (SPBs) early in meiosis II and as meiosis proceeds, each forespore membrane expands to engulf the associated nucleus (Shimoda 2004a; Nakase et al. 2008). Closure of the forespore membrane around a nucleus completes cell division, and these cells then mature into spores by deposition of spore wall material (Yoo et al. 1973). All of these events occur within the cytoplasm of the original mother cell, which is referred to as the ascus.

Mutants defective in meiosis and sporulation have been identified in S. pombe in a number of different screens. Originally spo mutants were found by direct screening for sporulation defects (Bresch et al. 1968; Kishida and Shimoda 1986). More recently targeted mutagenesis of genes whose expression is sporulation-induced has identified additional genes involved in both processes (Gregan et al. 2005; Martin-Castellanos et al. 2005). Although these screens have defined many genes involved in sporulation, these screens were not saturating and so additional genes likely remain to be identified.

The process of sporulation is similar in S. pombe and in the budding yeast Saccharomyces cerevisiae, although there appears to be only limited conservation of the specific genes involved in the process (Shimoda 2004a). Systematic screening of the S. cerevisiae knockout collection has proven to be a valuable approach, identifying hundreds of genes required for sporulation (Deutschbauer et al. 2002; Eyenhi and Saunders 2003; Marston et al. 2004). Sporulation-defective mutants in S. cerevisiae can be divided into several broad categories: (1) genes required for aspects of cell physiology necessary to support sporulation, for example mitochondrial function or autophagy; (2) genes required for progression through meiotic prophase to the initiation of spore development; and (3) genes required for spore assembly, per se, for instance genes involved in growth of the prospore membrane (the S. cerevisiae equivalent of the forespore membrane) or for spore wall formation (Neiman 2005).

To obtain a more comprehensive list of genes required for sporulation in S. pombe, we undertook a genome-wide systematic screen of the S. pombe haploid deletion set (~3300 strains in total).
In *S. pombe*, nitrogen starvation induces haploid cells of opposite mating types (h+h- and h-h-) to mate and then undergo meiosis and spore formation. Strains that carry the h90 allele at the mat1 locus are homothallic, meaning the cells switch mating types during mitotic growth so that both the h+ and h- mating types are present in colonies originally derived from a single cell. Diploids generated by h90 strains are therefore completely homozygous because they are a result of self-mating. This greatly simplifies the detection of meiotic and sporulation mutants because meiosis is normally induced only in diploid cells. The haploid deletion set was constructed in an h+ mating type background. Therefore, it was necessary to introduce h90 into each deletion strain to enable the creation of homozygous mutant diploids. After these mutants were exposed to conditions that promote sporulation, iodine staining was used as an initial screen to determine whether spores were present (Garcia et al. 2006). Secondary screens included direct observation of ascid by phase contrast microscopy and examination of fluorescent markers for the forespore membrane and SPBs. Our screen identified >90% of the previously known sporulation-defective mutants present in the collection, suggesting that the screen has identified the majority of non-essential genes required for spore formation. Among the novel sporulation genes are membrane trafficking proteins, signaling proteins, transcription factors, and metabolic enzymes. These results have a wealth of information for future investigations.

**MATERIALS AND METHODS**

**Yeast strains and culture**

Standard media and growth conditions were used unless otherwise noted (Forsburg and Rhind 2006). For synthetic medium containing G418, pombe glutamate medium (PMG) was used (Sabatinos and Forsburg 2010). Genotypes of the strains used in this study are listed in Table 1. Strain EAP20, which was used to introduce h90, as well as genes encoding tagged versions of *psy1* and *sid4* (markers for the forespore membranes and SPBs, respectively) into the knockout collection, was constructed in several steps. First, a spontaneous cycloheximide resistant mutant of strain JLP18 (EAP3) was selected by plating cells on YES plates containing 10 mg/L of cycloheximide (Sigma-Aldrich Co.). EAP11 was generated by transforming EAP3 with SpI-I-digested pEA4, which targets integration of the *S. cerevisiae* URA3 gene adjacent to the his5+ locus. his5+ is tightly linked to mat1, which contains the h90 allele, and the Ura+ phenotype can then be used to follow the h90 allele in subsequent crosses. Next, an allele of the SPB gene *sid4* fused to a gene encoding the fluorescent protein tdTomato (sid4+-tdTomato::hphMX6) was introduced by crossing EAP11 with strain 843 (Doyle et al. 2009) to generate EAP16. To introduce a marker for the forespore membrane, a strain [FY12295; (Nakase et al. 2008)] carrying a green fluorescent protein (GFP)-tagged allele of *psy1* was crossed to EAP16, generating EAP19. Finally, EAP19 was backcrossed to EAP16 to generate a segregant, EAP20, which carries the marked h90 locus, both fluorescent protein gene fusions, and cycloheximide resistance.

**Plasmids**

pEA4, which contains the *S. pombe* his5+ gene in pRS306 (Sikorski and Hieter 1989), was constructed by polymerase chain reaction (PCR) amplification of a 1.3-kb fragment including his5+ and its 5’ and 3’ regions from genomic DNA using EAO11 (5’-GGTCTCTGGTACAGCCATGTCGAGAGGGAA-3’) and HJO274 (5’-GGTGTTCGAAATTTTTTCTTCTGGGCGCGCCGTCCACACTTGTAGCACCACACCTTGG-3’) oligonucleotides. The PCR product was engineered to contain Kan and EcoRI sites at its 5’ and 3’ ends, respectively, and was cloned into similarly digested pRS306.

pEA18, which expresses wsc1+-mTagBFP under control of the spo13 promoter, was constructed in three steps. First, a yeast codon-optimized form of mTagBFP without a stop codon was PCR amplified from pRS426 Spowsc1+-mTagBFP (Lin et al. 2013) using EAO44 (5’-GGTCTTCTCTATGGTTGACCCAACTTTG-3’) and EAO46 (5’-GGTGTTCGACAGCCATGTCGAGAGGGAA-3’) and cloned into similarly digested pRS424 (Forsburg 1993) creating pEA17. Second, overlap PCR was used to construct a Psps13-wsc1+ fusion. A ~500-bp fragment of the spo13 promoter region and the wsc1+ open reading frame lacking the stop codon were amplified using the oligonucleotide pairs EAO47 (5’-GGTCTCTGGTACAGCCATGTCGAGAGGGAA-3’) and EAO48 (5’-GGGGAATTTAAAAGACCATATGGTTGACCCAACTTTG-3’) and cloned into NdeI/EcoRI sites in pRS306. Finally, EAP18 was digested with PstI and NotI and cloned into similarly digested pEA13 to replace the nmt1 promoter of pREP42x in front of mTagBFP creating pEA17. Finally, pEA18 was created by amplifying mTagBFP with its stop codon from pRS426 Spowsc1+-mTagBFP using EAO44 and EAO45 to yield a ~1.6-kb spo13pr-wsc1+ fusion fragment. This product was digested with PstI and NotI to yield a ~1.6-kb spo13pr-wsc1+ fusion fragment. This product was digested with PstI and NotI and cloned into similarly digested pEA13 to replace the nmt1 promoter of pREP42x in front of mTagBFP creating pEA17. Finally, pEA18 was created by amplifying mTagBFP with its stop codon from pRS426 Spowsc1+-mTagBFP using EAO44 and EAO45 to yield a ~1.6-kb spo13pr-wsc1+ fusion fragment. This product was digested with PstI and NotI and cloned into similarly digested pEA13. This study

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**Table 1 Strains used in this study**

| Name             | Genotype                                      | Source                  |
|------------------|-----------------------------------------------|-------------------------|
| JLP18            | h90 ura4-D18 leu1-32 his3-127                 | This study              |
| EAP3             | h90 ura4-D18 leu1-32 his3-127 cyhR            | This study              |
| EAP11            | h90 his5::URA3::his5+ ura4-D18 leu1-32 his3-127 cyhR | This study              |
| 843              | h90 myoS1+-GFP::kanMX6 sid4+-tdTomato::hphMX6 ura4-D18 leu1-32 | (Doyle et al. 2009)    |
| EAP16            | h90 his5::URA3::his5+ sid4+-tdTomato::hphMX6 ura4-D18 leu1-32 | This study              |
| FY12295          | h90 spo15::ura4+ ura4-D18 leu1+::GFP::psy1+   | (Nakase et al. 2008)    |
| EAP19            | h90 leu1::GFP::psy1+ LEU2 sid4+-tdTomato::hphMX6 ura4-D18 leu1-32 | This study              |
| EAP20            | h90 his5::URA3::his5+ leu1+::GFP::psy1+ sid4+-tdTomato::hphMX6 ura4-D18 leu1-32 cyhR | This study              |
| Bioneer deletion set deletion mutants after outcrosses | h+ ade6-M210 ura4-D18 leu1-32 geneX::kanMX4 | (Kim et al. 2010)       |
|                  | h90 his5::URA3::his5+ leu1+::GFP::psy1+ sid4+-tdTomato::hphMX6 ura4-D18 leu1-32 cyhR geneX::kanMX4 | This study              |
Genetic screen

The haploid S. pombe deletion mutant library was purchased from Bioneer (South Korea). The knockouts are in an h* strain background (h* ade6-M210 ura4-D18 leu1-32). To examine sporulation, each mutant was crossed to strain EAP20 and a modified form of the synthetic gene array method was used to introduce the knockout alleles into an h* background (Tong et al. 2001; Baryshnikova et al. 2010). The steps in this process are outlined in Figure 1. First, strains containing individual geneX::kanMX4 deletions were grown in liquid YES medium in microtiter dishes. To each well was then added 1/10th volume of a saturated culture of EAP20 grown in YES and the mixed cultures were pinned onto ME plates, allowing the cells to grow, mate, and sporulate (Forsburg and Rhind 2006). Use of ME at this step produced higher efficiency sporulation than other media (data not shown). The patches were then replica plated to YES plates supplemented with 200 mg/L Geneticin (G418; US Biological Life Sciences, Salem, MA) and 10 mg/L cycloheximide. Combined, these drugs select for recombinant haploids from the cross. Geneticin selects for the knockout marker. Cycloheximide resistance is a recessive trait, and cycloheximide therefore selects against both the original knockout strain and any diploid cells created by mating of EAP20 with the deletion strain. Inclusion of this step is essential to prevent a background of diploids heterozygous for the knockout allele from contaminating the patches (Baryshnikova et al. 2010). After 3 d incubation at 31°C, patches were replica plated to EMM2 plates with 200 mg/L Hygromycin B (Calbiochem, Merck KGaA, Darmstadt, Germany). This medium selects for both uracil and leucine prototrophy, which are linked to h* and the forespore membrane marker GFP-psy1* , respectively, and for the SPB marker sid4*-tdTomato::hphMX6. In addition it also selects against the ade6-M210 allele found in the deletion set. It is important that the resulting strain be ade6* as the red pigment created by the ade6-M210 mutant complicates both the subsequent iodine staining and fluorescence analyses. We found that removal of the G418 selection at this step allowed the growth of cells lacking the knockout allele and so an additional replica plating to PMG plates with 200 mg/L G418 was performed. G418 selection is more efficient on PMG than EMM2 (Benko and Zhao 2011). The resulting patches consist of h* haploid deletion mutants harboring sid4*-tdTomato::hphMX6 and GFP-psy1* . These patches were then replica plated to SPA plates to induce sporulation, incubated at 25°C for 3 d, and then inverted over a Petri dish of iodine crystals for 2-3 min (Sabatinos and Forsburg 2010). Staining of mature spore wall with iodine vapor produces a dark brown color (Meade and Gutz 1975). Patches displaying absent or reduced staining with iodine were scored as sporulation-defective candidates.

Candidates were picked from the PMG plates to a fresh PMG plate with 200 mg/L G418, replica plated to SPA, and then restested for iodine staining. Cells from patches that failed to exhibit good iodine staining after the restest were then directly analyzed by light microscopy for the presence of spores and by fluorescence microscopy of the Sid4-tdTomato and GFP-Psy1 markers to evaluate progression through meiosis and forespore membrane formation.

Barcode sequencing

In construction of the knockout collection, each knockout incorporated “uptag” and “downtag” sequences that provide a unique barcode for each knockout (Kim et al. 2010). To confirm the identity of the mutants identified in our screen, we amplified the uptag region for each one. This PCR product was then sequenced using EAO62 (5’-GGGGGAGCAGTGACATCATGCG3' ) and the results were compared with the list of uptag sequences given by Kim et al. (2010). In addition, the meu14 and mfr1 deletions were also analyzed by PCR-amplification of the loci with flanking primers to distinguish the knockout and wild-type alleles.

![Figure 1 Outline of generation of homothallic mutant strains and the screen for sporulation defective mutants. Genotypes of cells at each stage are boxed. Blue indicates genes from the deletion set strains, and red indicates genes derived from EAP20. These two strains were first mixed in liquid and then spotted onto ME plates to allow mating and sporulation. Cells were then replica plated to plates containing G418 and cycloheximide to select for recombinant progeny containing both the geneX::kanMX4 and the cyh* alleles. These haploids were transferred to minimal medium containing hygromycin to select for those segregants that also carry the h* mating type (linked to URA3, indicated by the underline) and harbor markers for the forespore membrane (GFP-psy1*) and the SPB (sid4*-tdTomato::hphMX6). A final transfer to minimal medium containing G418 ensures that the deletion alleles are still present. Meiosis and sporulation were induced by replica plating the patches to SPA medium and spore formation was assayed by exposure of the cells to iodine vapor, which causes spores to exhibit a red colony color. An example of sporulation proficient and defective patches is shown.](https://example.com/example.png)
Microscopy
Images were collected on a Zeiss Observer Z.1 microscope and processed using Zeiss Axiovision or Zen software.

Acetone resistance assays
Spore wall function was tested using an acetone resistance assay modified from (Smith 2009). Wild-type spores are resistant to acetone, whereas unsporulated cells or cells with defective spore walls are killed. The wild-type and the knockout strains were first incubated on PMG plates with 200 mg/L G418 at 31°C for 2 d. Cells were then replica plated to SPA plates and incubated at 25°C for 3 d to allow for mating and sporulation, and then replica-plated onto YES plates. An acetone-soaked filter paper (Whatman #3, 1003-090) was placed on a glass Petri dish and inverted above the YES plate to expose the patches to acetone vapor for 15 min. These were then incubated at 31°C for 3 d before being photographed.

RESULTS AND DISCUSSION
Isolation of sporulation-defective mutants
Using a series of selective steps diagrammed in Figure 1, we constructed h+0 homothallic derivatives of each deletion strain in the Bioneer S. pombe haploid deletion collection, at the same time introducing fluorescent markers for the SPBs and the forespore membrane. Because h+0 strains are able to undergo mating type switching, h+0 cells can be induced to self-mate and create homozygous diploids that then proceed through meiosis and sporulation. The ability of the strains in the deletion set to form spores was then assayed by exposure to iodine, which produces a dark brown stain in patches containing spores.

Eighty-five candidates passed the initial screen as well as a retest. In addition to sporulation-defective mutants, the assay of decreased iodine staining might also identify knockouts that cause h- specific mating defects, that is, mutants that are unable to mate with h+ haploids. The deletion strain background is h+ and these cells are therefore able to mate with h+ cells present in the h+0 background in the initial cross. However, these cells will be unable to self-mate once in the h+0 background and so will not produce spores. Similarly, as the URA3 marker is integrated approximately 10 cM from the mat1 locus containing the h+0 allele (Egel 2004), recombination between URA3 and the mat locus can produce URA3 h+0 haploids that would slip through the selection procedure and these would also fail to sporulate.

To test for such false positives, the 85 strains were assayed for their mating types by replica-plating to h+ and h- tester strains followed by iodine staining to examine whether diploids formed that could sporulate. Strains that are h+0 are expected to mate to both h+ and h- cells. Three of the candidates mated only to the h+ tester strains, and thirty-three mutants mated only to the h- tester, demonstrating that a mating defect is indirectly responsible for the absence of spores. Of the strains that mated only to the h- tester, two were deletions in mam1 (M-factor transporter) and mam2 (P-factor receptor), both of which are known to produce an h- specific sterility (Kitamura and Shimoda 1991; Christensen et al. 1997). The remaining mutants we suspect were simply h+ strains that leaked through the selection process. The strains with mating defects were not analyzed further.

The remaining candidates were sporulated and examined by phase contrast microscopy to determine the frequency of spore formation in the culture. Those strains in which no spores were detected were also examined by fluorescence microscopy of the Sid4-tomato and GFP-Psy1 markers to look at progression through meiosis and forespore membrane formation, respectively. Based on these microscopy assays the mutants can be divided into four classes: (1) reduced frequency of zygotes, suggesting that the sporulation defect is secondary to a mating defect; (2) near wild-type frequency of zygotes and spores, suggesting a defect in formation of the iodine-reactive layer of the spore wall; (3) no spores and no forespore membrane formation; and (4) no spores with abnormal forespore membrane formation (Table 2).

To confirm the identity of the deleted gene in the knockout strains, we used PCR to amplify the unique uptag region for many of the deletions (Kim et al. 2010). These PCR products were then sequenced and compared with the published lists to confirm the identity of the knockouts. For 48 knockouts for which we obtained sequences, 32 matched the published barcodes. The knockouts that did not produce the expected barcode sequence are listed in Table 3. In three cases, the barcode sequence found corresponded to that of known sporulation-defective mutants, suggesting that the identification by the barcode sequence, rather than position in the collection, is correct. In all cases of misidentification, the expected knockout and the actual one are found in different plates within the collection. These errors are, therefore, unlikely to have been caused by cross-contamination during our handling of the collection as different plates were processed at different times. Although this is a small sample, the surprisingly high error rate (33%) highlights the need for confirmation of knockout identity when using this collection.

To test the effectiveness of our screen, we culled from the literature a list of previously identified mutants that block spore formation. Several of the original spo mutants proved to be hypomorphic alleles of essential genes (Nakase et al. 2001; Nakamura-Kubo et al. 2003) and so are not present in our deletion set; however we identified 13 known sporulation-defective mutants listed as present in the collection (Table 4). Amplification and barcode sequencing confirmed the presence of nine of these at the correct location in the collection and another two mutants were identified at different locations. Of these 11 mutants, 10 were identified in the screen. This yield suggests that the screen has identified ~90% of the sporulation-defective mutants present in the collection.

Classes of genes required for positive iodine staining phenotype

Genes required for zygote formation: For mutants that formed some level of visible spores, the frequency of zygote formation and of spore formation were examined by light microscopy (Table 5). Mutants that display bilateral mating defects, that is, are able to mate with the h+ and h- tester strains but are unable to self-mate to produce zygotes would pass the mating tests described above and show reduced sporulation. For seven mutants, zygote formation was reduced greater than threefold from that seen in a wild-type h+0 strain, indicative of a bilateral mating defect. Thus, the primary defect in these mutants is likely to be in the mating process or response to nitrogen starvation rather than in spore formation, per se. The two genes in this class with the strongest phenotype were prn1+ and cyp9+. Consistent with our interpretation, prn1+ encodes an integral membrane protein recently shown to be necessary for conjugation (Sun et al. 2013; Curto et al. 2014). These results reveal a previously unknown role for cyp9+ in the mating reaction.

Genes required for spores to be iodine-reactive: Mutants in seven additional genes formed zygotes at near normal frequency and displayed at most modestly reduced spore formation relative to wild type. Because strains in this class form significant numbers of spores,
their loss of staining may reflect defects in generation of the iodine reactive alpha-glucan component of the spore wall (Garcia et al. 2006). It is noteworthy that a number of mutants known to disrupt assembly of the beta-glucan or chitosan layers of the spore wall were present in the collection but were not found in our screen, probably because those mutants that effect beta-glucan or chitosan do not alter iodine staining (Liu et al. 2000).

Two of the genes in this class, \( \text{php}^3^+ \) and \( \text{php}^5^+ \), encode subunits of the CCAAT-binding transcription complex (McNabb et al. 1997; Mercier et al. 2006). Although previous reports have implicated this complex in induction of transcription during nitrogen starvation and in the activity of meiotic recombination hotspots, no requirement for these genes in spore formation has been reported (Nakashima et al. 2002; Steiner et al. 2011). This work suggests that transcriptional induction by this complex of as yet unidentified genes is important for proper spore formation.

**Genes required for entry into meiosis or for the initiation of forespore membrane assembly:** The five genes identified in this class were previously known. Three of the genes are required for entry into meiosis. \( \text{mei}^2^+ \) encodes an RNA-binding protein required for meiosis (Watanabe et al. 1988; Watanabe and Yamamoto 1994). \( \text{mei}^3^+ \) is essential for the initiation of meiosis since it encodes a protein that binds and inhibits the meiosis-inhibitory protein kinase Pat1 during sporulation (McLeod and Beach 1988). The transcription factor that is encoded by \( \text{mei}^4^+ \) is

### Table 2: Genes identified in the sporulation-defective screen

| Class 1. Genes required for zygote formation (n = 7) | Gene ID | Comments |
|-------------------------------------------------|---------|----------|
| \( \text{atg}^9^+ \) | SPAC1783.06c | Autophagy-associated ubiquitin-like modifier |
| \( \text{cyp9}^+ \) | SPCC553.04 | Predicted cyclophilin family peptidyl-prolyl cis-trans isomerase |
| \( \text{mmd}^1^+ \) | SPAC30C2.02 | Predicted deoxyxypusine hydroxylase |
| \( \text{prm}^1^+ \) | SPAP7G5.03 | Integral membrane protein important for cell—cell fusion |
| \( \text{fsc}^1^+ \) | SPBC1711.12 | Predicted oxidized protein hydrolase |
| \( \text{lcf}^2^+ \) | SPBC18E5.08 | Predicted N-acetyltransferase |
| \( \text{rik}^1^+ \) | SPBC146.02 | Sequence orphan |

| Class 2. Genes required for spores to be iodine-reactive (n = 7) | Gene ID | Comments |
|-------------------------------------------------|---------|----------|
| \( \text{fsc}^1^+ \) | SPAC22H12.05c | Fasciclin domain protein |
| \( \text{mam}^3^+ \) | SPBP4H10.11c | Long-chain-fatty-acid-CoA ligase |
| \( \text{mcl}^1^+ \) | SPAP11E10.02c | Cell agglutination protein |
| \( \text{php}^3^+ \) | SPAP11E10.02c | DNA polymerase alpha accessory factor |
| \( \text{php}^5^+ \) | SPBC38B.02 | CCAAT-binding factor complex subunit |
| \( \text{rik}^1^+ \) | SPCC11E10.08 | CCAAT-binding factor complex subunit |

| Class 3. Genes required for entry into meiosis or for the initiation of forespore membrane assembly (n = 5) | Gene ID | Comments |
|-------------------------------------------------|---------|----------|
| \( \text{mei}^2^+ \) | SPAC27D7.03c | RNA-binding protein required for meiosis |
| \( \text{mei}^3^+ \) | SPBC119.04 | Required for the initiation of meiosis |
| \( \text{mei}^4^+ \) | SPBC32H8.11 | Transcription factor regulating meiotic gene expression |
| \( \text{mug}^7^9^+ \) (spo7^+ | SPAC6G9.04 | Meiotic spindle pole body component |
| \( \text{sbo}^15 \) | SPAC1F3.06c | Meiotic spindle pole body component |

| Class 4. Genes that are essential for the proper formation and the maturation of the forespore membrane (n = 15) | Gene ID | Comments |
|-------------------------------------------------|---------|----------|
| \( \text{csm}^1^+ \) | SPBC15.03c | COP9/signalosome complex subunit |
| \( \text{csm}^2^+ \) | SPAP17E12.04c | COP9/signalosome complex subunit |
| \( \text{csm}^3^+ \) | SPAC17H9.19c | COP9/signalosome associated factor |
| \( \text{csm}^4^+ \) | SPAC17A5.08 | ER exit receptor for secretory cargo |
| \( \text{csm}^5^+ \) | SPBC16E9.09c | ER exit receptor for secretory cargo |
| \( \text{mes}^1^+ \) | SPAC5D6.08c | Meiotic APC/C regulator |
| \( \text{spe}^2^+ \) | SPBP4H10.05c | S-adenosylmethionine decarboxylase/proenzyme |
| \( \text{spe}^3^+ \) | SPBC12C2.07c | Predicted spermidine synthase |
| \( \text{spn}^2^+ \) | SPBC82.06 | Septin |
| \( \text{spo}^3^+ \) | SPAC607.10 | Required for spore formation |
| \( \text{spo}^4^+ \) | SPBC21C3.18 | Kinase required for spore formation |
| \( \text{spo}^5^+ \) | SPBC29A10.02 | Meiotic RNA-binding protein |
| \( \text{tpp}^1^+ \) | SPAC9G12.15c | Trehalose-6-phosphate phosphatase |
| \( \text{tpp}^2^+ \) | SPAC6C3.06c | Predicted P-type phospholipid flippase |
| \( \text{tpp}^3^+ \) | SPCC1739.04c | Sequence orphan |

ER, endoplasmic reticulum; APC/C, Anaphase Promoting Complex/Cyclosome.

a Descriptions are based on PomBase (Wood et al. 2012) (www.pombase.org).
b Knockout not confirmed by barcode sequencing.
a regulator necessary for the expression of many sporulation-induced
genes (Horie et al. 1998). The remaining two genes in this class, 
mug79*/spo7* and spo15*, both encode components of the meiotic SPB
necessary for the SPB to catalyze the coalescence of secretory vesicles into
a forespore membrane (Ikemoto et al. 2000; Nakamura-Kubo et al. 2011).

**Genes that are essential for the proper formation and the
maturation of the forespore membrane:** Mutants in Class 4 genes
progress through meiosis and initiate forespore membrane growth,
but the membranes display morphological defects and no spores are
visible by light microscopy. There were 15 genes identified in this
category, of which five (spo3*, spo4*, spo5*, mes1*, spo2*) were pre-
viously shown to be required for sporulation (Nakamura et al. 2001,
2002; Izawa et al. 2005; Kasama et al. 2006; Onishi et al. 2010).
Among the 10 genes in this class not previously associated with spor-
ulation defects, two encode subunits of the COP9 signalosome (csn1+
and csn2+) and one encodes a reported interacting partner of the
signalosome (ctd2+) (Mundt et al. 1999; Liu et al. 2005). Several other
COP9 subunits are present in the collection but were not found to be
iodine-negative in our screen. Thus, the Csn1 and Csn2 subunits of
the signalosome may be specifically required for sporulation. A similar
difference in function between Csn1/Csn2 and other COP9 subunits
in sensitivity to DNA damage has been noted previously (Mundt et al.
2002). Also in this class are spe2* and SPBC12C2.07c (spe3*), genes
that encode enzymes involved in consecutive steps in spermidine
synthesis (Tabar and Tabor 1985; Chattopadhyay et al. 2002). This
pathway has also been shown to be required for sporulation in
*S. cerevisiae* (Cohn et al. 1978), suggesting a conserved requirement
for spermidine for spore formation in fungi.

Three of the mutants in this class have predicted functions within
the secretory pathway. SPAC6C3.06c encodes a predicted phospho-
lipid flippase orthologous to the *NEO1* gene of *S. cerevisiae*. *Neol*
is localized to the endosome and to the Golgi and has been implicated
in membrane trafficking (Hua and Graham 2003; Wicky et al. 2004).
The *erp2* and *erp5* genes encode two S. *pombe* members of the p24
protein family. The p24 proteins are a highly conserved family of
integral membrane proteins that act as cargo receptors and shuttle
between the endoplasmic reticulum (ER) and the Golgi (Strating and
Martens 2009). In particular, they play an important role in cargo
selection and packaging into COPII vesicles at ER exit sites (Strating
and Martens 2009). Consistent with the similar phenotypes of both
*erp2* and *erp5* deletions, studies in *S. cerevisiae* suggest that the four

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**Table 3 Gene deletions that do not have the correct barcode**

| Gene       | Gene ID          | Barcode Information                          |
|------------|------------------|---------------------------------------------|
| atg15Δ     | SPAC23C4.16c     | Matches with spo5                           |
| atp10Δ     | SPAC4G8.11c      | No match                                   |
| atp14Δ     | SPBC29A3.10c     | No match                                   |
| ctf1Δ      | SPBC38B9.11c     | No match                                   |
| lsk1Δ      | SPAC2F3.15       | Matches with mei4                           |
| mei4Δ      | SPBC32H8.11      | No match                                   |
| mfr1Δ      | SPBC119H.12      | Matches with SPAC17H9.14c mfr1 knockout is not present as determined by PCR with flanking primers, |
| scd1Δ      | SPAC16E8.09      | Matches with mei4                           |
| spo5Δ      | SPBC29A10.02     | No match                                   |
| spo6Δ      | SPBC1778.04      | No match                                   |
| spo15Δ     | SPBC15C4.06c     | No match                                   |
| apg12Δ     | SPBC428.04       | Matches with cyp9                           |
|            | SPBC21H7.06c     | Matches with cyp9                           |
|            | SPAC139.01c      | Matches with nrd1                           |
|            | SPBC23G7.06c     | Matches with nrd1                           |
|            | SPBC1711.08      | Matches with nrd1                           |

Table 4 Known sporulation-defective genes listed in the *S. pombe* haploid deletion set

| Gene   | Comment                        | Phenotype in Our Study |
|--------|--------------------------------|------------------------|
| spo3*  | Confirmed by barcode sequence  | Sporulation defect     |
| spo4*  | Confirmed by barcode sequence  | Sporulation defect     |
| spo5*  | Knockout found at different position in the collection* | Sporulation defect |
| spo6*  | Not present*                   | n.d.                   |
| mug79*/spo7* | Confirmed by barcode sequence | Sporulation defect |
| spo15* | Confirmed by barcode sequence  | Sporulation defect     |
| mei2*  | Confirmed by barcode sequence  | Sporulation defect     |
| mei3*  | Confirmed by barcode sequence  | Sporulation defect     |
| mei4*  | Knockout found at different position in the collection* | Sporulation defect |
| mes1*  | Confirmed by barcode sequence  | Sporulation defect     |
| mfr1*  | Not present*                   | n.d.                   |
| mei14* | Knockout is present as determined both by PCR with flanking primers and by barcode sequence | Normal sporulation |
| cdt2*  | Confirmed by barcode sequence  | Sporulation defect     |

n.d., not determined; PCR, polymerase chain reaction.

* See Table 3.
family members function in a single complex (Hirata et al. 2013). Knockouts of the other family members in S. pombe, emp24 (SPCC24B10.17.1) and ern25+ (SPAC23H4.03c.1), were not present in the collection, though we expect mutants in these genes would display a similar sporulation defect. We predict that the p24 family is necessary for the exit of some protein(s) from the ER so that the cargo protein(s) can be transported through the secretory pathway to the forespore membrane and contribute to proper membrane growth.

erp2 and erp5 mutants do not cause a general block to ER exit

In S. cerevisiae, a different class of ER cargo receptor, encoded by the ERV14 and ERV15 genes, is required for proper formation of the prospore membrane (the budding yeast equivalent of the forespore membrane) during sporulation (Powers and Barlowe 1998; Nakanishi et al. 2007). Although these genes are not essential for vegetative growth, their deletion creates a general block to ER exit of integral membrane proteins during sporulation (Nakanishi et al. 2007). Because ERV14 deletion mutants have small, abnormal forespore membranes similar to erp2Δ and erp5Δ mutants, we hypothesized that, parallel to the S. cerevisiae ER cargo receptors, erp2+ and erp5+ might become essential for ER exit of integral membrane proteins in S. pombe sporulation. The GFP-Psy1 reporter is localized to the forespore membrane in erp2Δ and erp5Δ cells, however this does not provide a strong test of a role for erp2+ and erp5+ in ER exit as Psy1 is relocated from the plasma membrane to the forespore membrane via the endosome (Kashiwazaki et al. 2011). Therefore, to test a possible general role for erp2+ and erp5+ in ER exit, the strains were transformed with a plasmid carrying an integral plasma membrane protein, wsc1+, fused with mTagBFP and placed under control of the sporulation-specific protein spo13 promoter (Nakase et al. 2008). When expressed in a wild-type strain, Wsc1-mTagBFP localized to the forespore membrane (Figure 2). In erp2Δ and erp5Δ mutants Wsc1-mTagBFP colocalized with GFP-Psy1 in the abnormal forespore membranes and no additional BFP fluorescence from the ER was seen, indicating that transport of Wsc1-mTagBFP is unaffected in the mutants (Figure 2). If loss of erp2 or erp5 cause forespore membrane defects indirectly by limiting the exit of some cargo from the ER, this is likely an effect on some specific cargo protein(s) and not due to a more general block in transport.

Table 5 Mating and sporulation efficiency of different mutants

| Gene          | Gene ID   | Class | % of Zygotes a (SD) | % of Sporulation a (SD) |
|---------------|-----------|-------|--------------------|-------------------------|
| WT            |           |       |                    |                         |
| cyp9Δ         | SPCC553.04| 1     | 67.0 (4.0)         | 76.7 (5.5)              |
| prm1Δ         | SPAP7G5.03| 1     | <0.5               | n.d.                    |
| atg12Δ        | SPAC1783.06c| 1 | 19.0 (8.6)         | 47.7 (6.8)              |
| mmd1Δ         | SPAC30C2.02| 1     | 21.5 (11.2)        | 71.0 (8.9)              |
| fsc1Δ         | SPCC22H12.05c| 2 | 27.3 (12.6)        | 52.0 (4.4)              |
| mcl1Δ         | SPAPB1E7.02c| 2     | 38.5 (8.3)         | 56.3 (12.5)             |
| php3Δ         | SPAC23C11.08| 2 | 38.5 (6.6)         | 43.0 (6.6)              |
| mam3Δ         | SPAP11E10.02c| 2 | 39.5 (4.8)         | 76.3 (6.7)              |
| lcf2Δ         | SPBP4H10.11C| 2   | 43.8 (11.5)        | 58.5 (6.1)              |
| rik1Δ         | SPCC11E10.08| 2   | 57.8 (7.1)         | 85.3 (3.9)              |

SD, standard deviation; n.d., not determined.

a Class 1 = Genes required for zygote formation; Class 2 = Genes required for spores to be iodine-reactive.

b The average of at least three experiments. At least 100 cells were counted in each experiment.

c The average of at least three experiments. At least 100 asci were counted in each experiment.

The lcf2+ and mcl1+ gene products may contribute to spore wall function

The spore wall provides the cell with resistance to environmental stresses such as acetone vapor (Egel 1977). To examine spore wall function we tested mutants in Class 2 for resistance to acetone (Smith 2009). Two of the mutants, lcf2Δ and mcl1Δ, showed strong sensitivity to acetone exposure (Figure 3). This stress-sensitivity is striking as these mutants show near-normal levels of sporulation. This result suggests a structural defect in the spore walls of these mutants, presumably in the alpha-glucan component of the spore wall. The mcl1+ gene encodes a polymerase alpha accessory protein, so its effect on the spore wall is likely indirect (Williams and McIntosh 2005). lcf2+...
encodes a predicted fatty-acyl CoA ligase, which could influence the composition of cellular membranes (Fujita et al. 2007). The stress sensitivity and iodine staining defects in these cells may reflect an influence of \( lcf2 \) on the activity or delivery of the integral membrane Mok14 alpha-glucan synthase responsible for synthesis of the iodine-reactive polymer (Garcia et al. 2006).

**Sporulation genes in budding and fission yeast**

In this screen we have produced the first survey of the nonessential knockout collection of *S. pombe* for sporulation defective mutants. One of the most striking results is the relatively small number of mutants that displayed a sporulation defect. In all, only \( \sim 1\% \) of the *S. pombe* collection showed loss or reduction of spores. This low number is not due to poor recovery in our screen, as we identified known mutants with \( \sim 90\% \) efficiency. By contrast, comparable screens of the knockout collection in *S. cerevisiae* found that more than 10% of the knockouts produced a sporulation defect (Enyenih and Saunders 2003; Marston et al. 2004). In part, the reasons for this difference reflect the different biology of these two yeasts. Whole categories of genes essential for sporulation in *S. cerevisiae* are not found in our screen. For example, *S. cerevisiae* is a petite-positive yeast that can grow in glucose medium without functional mitochondria (Kominsky and Thorsness 2000). However, sporulation is an obligatorily aerobic process. Therefore, any mutations that impair respiration are viable but sporulation defective. This accounts for more than a quarter of the sporulation-defective mutants in *S. cerevisiae* (Neiman 2005). In contrast, *S. pombe* cannot grow mitotically without mitochondrial function, and so most of the orthologous genes should be essential in fission yeast (and therefore absent from the haploid deletion set). Another significant fraction of sporulation-defective genes in budding yeast are involved in autophagy, either directly or through effects on the different evolutionary histories of the yeasts. The whole-genome duplication that occurred during the evolution of *Saccharomyces* allowed for the emergence of distinct sporulation- and vegetative-specific isozymes (Wolfe and Shields 1997). For example, the t-SNAREs *psy1* and *sec9* are both essential genes in *S. pombe* that are also essential for forespore membrane growth (Nakamura et al. 2005; Maeda et al. 2009). In *S. cerevisiae*, gene duplication has produced two versions of each gene, *SSO1/SSO2* for *psy1* and *SEC9/SPO20* for *sec9*. In each case, one paralog is specifically required for sporulation (*SSO1* and *SPO20*) (Neiman 1998; Jantti et al. 2002). In the absence of such extensive gene duplication, there has been less opportunity for sporulation-specific functions to evolve in *S. pombe* and as a result, we expect that more essential genes play “double duty” in both vegetative growth and sporulation.

Even when we accounted for the absence of these three categories of mutants, there appear to be fewer nonessential genes required for spore assembly in *S. pombe* than in *S. cerevisiae*. This finding probably reflects the different evolutionary histories of the yeasts. The whole-genome duplication that occurred during the evolution of *Saccharomyces* allowed for the emergence of distinct sporulation- and vegetative-specific isozymes (Wolfe and Shields 1997). For example, the t-SNAREs *psy1* and *sec9* are both essential genes in *S. pombe* that are also essential for forespore membrane growth (Nakamura et al. 2005; Maeda et al. 2009). In *S. cerevisiae*, gene duplication has produced two versions of each gene, *SSO1/SSO2* for *psy1* and *SEC9/SPO20* for *sec9*. In each case, one paralog is specifically required for sporulation (*SSO1* and *SPO20*) (Neiman 1998; Jantti et al. 2002). In the absence of such extensive gene duplication, there has been less opportunity for sporulation-specific functions to evolve in *S. pombe* and as a result, we expect that more essential genes play “double duty” in both vegetative growth and sporulation.

In summary, we report the results of a systematic screen through the *S. pombe* haploid deletion set for mutants displaying spore formation defects. The genes identified provide multiple new avenues for investigation into spore differentiation. These include a role for the COP9 signalosome in forespore membrane formation, sporulation-specific requirements for the p24 family of ER export cargo receptors, and the possible function of fatty acid metabolism in regulation of spore wall assembly.

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**LITERATURE CITED**

Baryshnikova, A., M. Costanzo, S. Dixon, F. J. Vizeacoumar, C. L. Myers et al., 2010 Synthetic genetic array (SGA) analysis in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Methods Enzymol. 470: 145–179.

Benko, Z., and R. Y. Zhao, 2011 Zeocin for selection of bleMX6 resistance in fission yeast. Biotechniques 51: 57–60.

Bresch, C., G. Muller, and R. Egel, 1968 Genes involved in meiosis and sporulation of a yeast. Mol. Gen. Genet. 102: 301–306.

Chattopadhyay, M. K., C. W. Tabor, and H. Tabor, 2002 Absolute requirement of spermidine for growth and cell cycle progression of fission yeast (*Schizosaccharomyces pombe*). Proc. Natl. Acad. Sci. USA 99: 10330–10334.

Christensen, P. U., J. Davey, and O. Nielsen, 1997 The *Schizosaccharomyces pombe* mam1 gene encodes an ABC transporter mediating secretion of M-factor. Mol. Gen. Genet. 255: 226–236.

Cohn, M. S., C. W. Tabor, and H. Tabor, 1978 Isolation and characterization of *Saccharomyces cerevisiae* mutants deficient in S-adenosylmethionine decarboxylase, spermidine, and spermine. J. Bacteriol. 134: 208–213.

Curto, M. A., M. R. Sharifmoghadam, E. Calpena, N. De Leon, M. Hoya, et al., 2002 Parallel phenotypic analysis of sporulation and postgermination growth in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 99: 13030–13034.

Delgato, A., R. Martin-Garcia, A. T. Coulton, S. Bagley, and D. Mulvhill, 2009 Fission yeast *M myositis* is a meiotic spindle pole body component.
with discrete roles during cell fusion and spore formation. J. Cell Sci. 122: 155–170.

Kominsky, D. J., and P. E. Thorsness, 2000 Expression of the Saccharomyces cerevisiae gene YME1 in the petite-negative yeast Schizosaccharomyces pombe converts it to petite-positive. Genetics 154: 147–154.

Lin, C. P., C. Kim, S. O. Smith, and A. M. Neiman, 2013 A highly redundant gene network controls assembly of the outer spore wall in S. cerevisiae. PLoS Genet. 9: e1003700.

Liu, C., M. Poteleaa, A. Watson, S. H. Yoshida, C. Shimoda et al., 2005 Transactivation of Schizosaccharomyces pombe cdt2+ stimulates a Pcr4-Ddb1-DSN ubiquitin ligase. EMBO J. 24: 3940–3951.

Liu, J., X. Tang, H. Wang, and M. Balasubramanian, 2000 Bgp2p, a l,3-beta-glucan synthase subunit, is essential for maturaion of ascospore wall in Schizosaccharomyces pombe. FEBS Lett. 478: 105–108.

Maeda, Y., J. Kashiwazaki, C. Shimoda, and T. Nakamura, 2009 The Schizosaccharomyces pombe syntaxin 1 homolog, Psyl, is essential in the development of the forespore membrane. Biosci. Biotechnol. Biochem. 73: 339–345.

Marston, A. L., W. H. Tham, H. Shah, and A. Amon, 2004 A genome-wide screen identifies genes required for cenotermere cohesion. Science 303: 1367–1370.

Martin-Castellanos, C., M. Blanco, A. E. Rozalen, P. Perez-Hidalgo, A. I. Garcia et al., 2005 A large-scale screen in S. pombe identifies seven novel genes required for critical meiotic events. Curr. Biol. 15: 2056–2062.

McLeod, M., and D. Beach, 1988 A specific inhibitor of the ran1+ protein kinase regulates entry into meiosis in Schizosaccharomyces pombe. Nature 332: 509–514.

McNabb, D. S., K. A. Tseng, and L. Guarente, 1997 The Saccharomyces cerevisiae Hap5p homolog from fission yeast reveals two conserved domains that are essential for assembly of heterotetrameric CCAAT-binding factor. Mol. Cell. Biol. 17: 7008–7018.

Meade, J. H., and H. Gutz, 1975 A new type of mutation in Schizosaccharomyces pombe: vegetative iodine reaction. Genetics 80: 711–714.

Mercier, A., B. Pelletier, and S. Labbe, 2006 A transcription factor cascade involving FeP1 and the CCAAT-binding factor PhP4 regulates gene expression in response to iron deficiency in the fission yeast Schizosaccharomyces pombe. Eukaryot. Cell 5: 1866–1881.

Mundt, K. E., J. Porte, J. M. Murray, C. Brikos, P. U. Christensen et al., 1999 The COP9/signalosome complex is conserved in fission yeast and has a role in S phase. Curr. Biol. 9: 1427–1430.

Mundt, K. E., C. Liu, and A. M. Carr, 2002 Deletion mutants in COP9/ signalosome subunits in fission yeast Schizosaccharomyces pombe display distinct phenotypes. Mol. Biol. Cell 13: 493–502.

Nakamura, T., M. Nakamura-Kubo, A. Hirata, and C. Shimoda, 2001 The Schizosaccharomyces pombe spo+ gene is required for assembly of the forespore membrane and genetically interacts with psyl(+)-encoding syntaxin-like protein. Mol. Biol. Cell 12: 3955–3972.

Nakamura, T., M. Nakamura-Kubo, T. Nakamura, and C. Shimoda, 2002 Novel fission yeast Cdc7-Dbf4-like kinase complex required for the initiation and progression of meiotic second division. Mol. Cell. Biol. 22: 309–330.

Nakamura, T., J. Kashiwazaki, and C. Shimoda, 2005 A fission yeast SNAPP-25 homologue, Spsec9, is essential for cytokinesis and sporulation. Cell Struct. Funct. 30: 15–24.

Nakamura-Kubo, M., T. Nakamura, A. Hirata, and C. Shimoda, 2003 The fission yeast spo+ gene encoding a functional homologue of budding yeast Sce12 is required for the development of forespore membranes. Mol. Biol. Cell 14: 1109–1124.

Nakamura-Kubo, M., A. Hirata, C. Shimoda, and T. Nakamura, 2011 The fission yeast pleckstrin homology domain protein Sp07 is essential for initiation of forespore membrane assembly and spore morphogenesis. Mol. Biol. Cell 22: 3432–3455.

Nakanishi, H., Y. Suda, and A. M. Neiman, 2007 Erv14 family cargo receptors are necessary for ER exit during sporulation in Saccharomyces cerevisiae. J. Cell Sci. 120: 908–916.

Nakase, Y., T. Nakamura, A. Hirata, S. M. Routt, B. S. McNeil et al., 2001 The Schizosaccharomyces pombe spo20+ gene encoding a homologue of a nitrogen source that drives adaptation processes. Genes Cells 12: 155–170.
Saccharomyces cerevisiae Sec14 plays an important role in forespore membrane formation. Mol. Biol. Cell 12: 901–917.

Nakase, Y., M. Nakamura-Kubo, Y. Ye, A. Hirata, C. Shimoda et al., 2008 Meiotic spindle pole bodies acquire the ability to assemble the spore plasma membrane by sequential recruitment of sporulation-specific components in fission yeast. Mol. Biol. Cell 19: 2476–2487.

Nakashima, A., M. Ueno, T. Ushimaru, and M. Uritani, 2002 Involvement of a CCAAT-binding complex in the expression of a nitrogen-starvation-specific gene, tp6+, in Schizosaccharomyces pombe. Biosci. Biotechnol. Biochem. 66: 2224–2227.

Neiman, A. M., 1998 Prospre membrane formation defines a developmentally regulated branch of the secretory pathway in yeast. J. Cell Biol. 140: 29–37.

Neiman, A. M., 2005 Ascospore formation in the yeast Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 69: 565–584.

Onishi, M., T. Koga, A. Hirata, T. Nakamura, H. Asakawa et al., 2010 Role of septins in the orientation of forespore membrane extension during sporulation in fission yeast. Mol. Cell. Biol. 30: 2057–2074.

Perez-Hidalgo, L., S. Moreno, and P. A. San-Segundo, 2003 Regulation of meiotic progression by the meiosis-specific checkpoint kinase Mek1 in fission yeast. J. Cell Sci. 116: 259–271.

Powers, J., and C. Barlowe, 1998 Transport of Axl2p depends on Erv14p, an ER-vesicle protein related to the Drosophila cornichon gene product. J. Cell Biol. 142: 1209–1222.

Roeder, G. S., and J. M. Bailis, 2000 The pachytene checkpoint. Trends Genet. 16: 395–403.

Sabatinos, S. A., and S. L. Forsburg, 2010 Molecular genetics of Schizosaccharomyces pombe, pp. 759–795 in Methods in Enzymology, edited by J. Weissman, C. Guthrie, and G. R. Fink. Academic Press, San Diego.

Shimada, M., K. Nabeshima, T. Tougan, and H. Nojima, 2002 The meiotic recombination checkpoint is regulated by checkpoint rad+ genes in fission yeast. EMBO J. 21: 2807–2818.

Shimada, C., 2004a Forespore membrane assembly in yeast: coordinating SPBs and membrane trafficking. J. Cell Sci. 117: 389–396.

Shimoda, C., and T. Nakamura, 2004b Control of late meiosis and ascospore formation, pp. 311–326 in The Molecular Biology of Schizosaccharomyces pombe: Genetics, Genomics and Beyond, edited by R. Egel. Springer-Verlag, Berlin.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19–27.

Smith, G. R., 2009 Genetic analysis of meiotic recombination in Schizosaccharomyces pombe. Methods Mol. Biol. 557: 65–76.

Steiner, W. W., P. A. Davidson, and A. T. Bagshaw, 2011 Important characteristics of sequence-specific recombination hotspots in Schizosaccharomyces pombe. Genetics 187: 385–396.

Strating, J. R., and G. J. Martens, 2009 The p24 family and selective transport processes at the ER-Golgi interface. Biol. Cell. 101: 495–509.

Sun, L. L., M. Li, F. Suo, X. M. Liu, E. Z. Shen et al., 2013 Global analysis of fission yeast mating genes reveals new autophagy factors. PLoS Genet. 9: e1003715.

Tabor, C. W., and H. Tabor, 1985 Polyamines in microorganisms. Microbiol. Rev. 49: 81.

Tanaka, K., and A. Hirata, 1982 Ascospore development in the fission yeasts Schizosaccharomyces pombe and S. japonicus. J. Cell Sci. 56: 263–279.

Tomar, P., A. Bhatia, S. Ramdas, L. Diao, G. Bhanot et al., 2013 Sporulation genes associated with sporulation efficiency in natural isolates of yeast. PLoS ONE 8: e69765.

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader et al., 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294: 2364–2368.

Watanabe, Y., and M. Yamamoto, 1994 S. pombe mei2+ encodes an RNA-binding protein essential for premeiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. Cell 78: 487–498.

Watanabe, Y., Y. Lino, K. Furuhata, C. Shimoda, and M. Yamamoto, 1988 The S. pombe mei2+ gene encoding a crucial molecule for commitment to meiosis is under the regulation of cAMP. EMBO J. 7: 761–767.

Wicky, S., H. Schwarz, and B. Singer-Kruger, 2004 Molecular interactions of yeast Neo1p, an essential member of the Drs2 family of aminophospholipid translocases, and its role in membrane trafficking within the endomembrane system. Mol. Cell. Biol. 24: 7402–7418.

Williams, D. R., and J. R. McIntosh, 2005 Mcl1p is a polymerase alpha replication accessory factor important for S-phase DNA damage survival. Eukaryot. Cell 4: 166–177.

Wolfle, K. H., and D. C. Shields, 1997 Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387: 708–713.

Wood, V., M. A. Harris, M. D. McDowall, K. Rutherford, B. W. Vaughan et al., 2012 PomBase: a comprehensive online resource for fission yeast. Nucleic Acids Res. 40: D695–D699.

Yoo, B. Y., G. B. Calleja, and B. F. Johnson, 1973 Ultrastructural changes of the fission yeast (Schizosaccharomyces pombe) during ascospore formation. Arch. Microbiol. 91: 1–10.

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