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Mps1p Regulates Meiotic Spindle Pole Body Duplication in Addition to Having Novel Roles during Sporulation

Paul D. Straight, Thomas H. Giddings, Jr., and Mark Winey*

Molecular, Cellular, and Developmental-Biology, Porter Biosciences, University of Colorado, Boulder, Colorado 80309

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Sporulation in yeast requires that a modified form of chromosome segregation be coupled to the development of a specialized cell type, a process akin to gametogenesis. Mps1p is a dual-specificity protein kinase essential for spindle pole body (SPB) duplication and required for the spindle assembly checkpoint in mitotically dividing cells. Four conditional mutant alleles of *MPS1* disrupt sporulation, producing two distinct phenotypic classes. Class I alleles of *mps1* prevent SPB duplication at the restrictive temperature without affecting premeiotic DNA synthesis and recombination. Class II *MPS1* alleles progress through both meiotic divisions in 30–50% of the population, but the asci are incapable of forming mature spores. Although mutations in many other genes block spore wall formation, the cells produce viable haploid progeny, whereas *mps1* class II spores are unable to germinate. We have used fluorescently marked chromosomes to demonstrate that *mps1* mutant cells have a dramatically increased frequency of chromosome missegregation, suggesting that loss of viability is due to a defect in spindle function. Overall, our cytological data suggest that *MPS1* is required for meiotic SPB duplication, chromosome segregation, and spore wall formation.

INTRODUCTION

Gamete formation in sexually reproducing organisms consists of meiotic chromosome segregation to generate haploid cells coupled with a developmental pathway to generate specialized cell bodies equipped for fertilization. Meiotic cell division in the budding yeast *Saccharomyces cerevisiae* is initiated when a diploid cell of a/α mating type is starved for a fermentable carbon source and nitrogen. Starvation of yeast initiates a transcriptional program that controls meiotic DNA synthesis, recombination, and chromosome segregation and coordinates these events with the generation of spore bodies (Malone, 1990; Chu *et al.*, 1998). Successful sporulation in yeast requires the proper coordination of meiotic spindle formation and segregation of chromosomes into the four developing spore bodies.

Two significant distinctions between meiotic and mitotic chromosome segregation include the duplication of the spindle pole body (SPB; the yeast equivalent of the centrosome) and the formation and function of the meiotic spindles. During meiotic chromosome segregation, two rounds of spindle formation occur after a single round of DNA synthesis, resulting in a reduction of the chromosome number by half in the progeny spores. Presumably, the physical duplication of the SPB requires the same fundamental assemblage of components during meiosis and mitosis; however, regulation of the process must differ significantly because of the requirement of two successive duplications of the meiotic SPBs. Furthermore, the SPBs become the site of prospore wall initiation after a modification to the cytoplasmic face of this organelle at the time of the second duplication (Moens and Rappaport, 1971). Not only are the spindle poles required to build spindles for chromosome segregation, as they are in mitosis, but they are also modified for spore formation, a process unique to meiosis. These observations suggest that the SPB may be an organelle of central importance in the coordination of meiosis and spore formation.

A host of meiosis-specific genes are required for spore wall formation and could potentially affect SPB function because of its role in prospore wall initiation. A meiosis-specific gene that has a role in SPB duplication is *SPO1* (Moens *et al.*, 1974; Tevzadze *et al.*, 2000). The *spo1* mutation causes a largely monopolar phenotype in sporulating yeast, and sequence analysis revealed strong similarity to the phospholipase B–encoding gene *PLB1* (Tevzadze *et al.*, 1996). A better characterized set of genes regulate and carry

* Corresponding author. E-mail address: mark.winey@colorado.edu.
Abbreviations used: CCD, charge-coupled device; DIC, differential interference contrast; GFP, green fluorescent protein; SC, synthetic complete; SPB, spindle pole body.

out spore wall formation. A sporulation-specific MAPK, *SMK1*, was demonstrated to regulate spore formation (Krisak *et al.*, 1994). Two upstream kinases, *SPS1* and *CAK1*, modulate the function of Smk1p, although *CAK1* is not a meiosis-specific gene (Wagner *et al.*, 1997). Also, the recently discovered gene *SWM1* is a regulator of spore wall formation (Ufano *et al.*, 1999). *SMK1*, *SPS1*, *CAK1*, and *SWM1* control spore wall formation through transcriptional regulation of a set of effector genes represented by *DIT1*, *DIT2*, *SPS100*, and others. Double mutant analysis has demonstrated that *SMK1* and *SPS1* function in a pathway separate from *SWM1* (Ufano *et al.*, 1999). Furthermore, Sps1p may have a meiotic function in addition to its proposed role as a Smk1p upstream activating kinase (Friesen *et al.*, 1994), suggesting that a complex signaling network regulates spore wall initiation and assembly.

Many of the genes required during the vegetative cell cycle are also used in the context of meiosis and spore formation (Simchen, 1974). The effects of two mutations that disrupt the process of SPB duplication in mitotic cells have been studied in meiotic cells. In vegetatively growing cells, both *cdc31-1* and *ndc1-1*, which define the early and late steps, respectively, of SPB duplication, prevent mitotic SPB duplication and trigger cell cycle arrest by activating the spindle assembly checkpoint (Winey *et al.*, 1993; Weiss and Winey, 1996). The meiotic effects of the *cdc31-1* and *ndc1-1* mutations result in viable dyads (two-spored asci) as a result of the failure in the second of the two rounds of SPB duplication (Byers, 1981; Thomas and Botstein, 1986). Predominantly, the chromosomes undergo reductional segregation of paired homologues, indicating a frequent failure of meiosis II. Nonetheless, strains carrying either mutation execute one of two rounds of SPB duplication during meiosis and form viable diploid spores.

An intermediate step of mitotic SPB duplication is revealed by mutation of the *MPS1* gene (Winey *et al.*, 1991). *MPS1* encodes an essential dual-specificity kinase that performs two known roles during mitosis (Lauzé *et al.*, 1995). Mps1p is a positive-acting regulator of SPB duplication and a component of the spindle assembly checkpoint (Weiss and Winey, 1996). Recently, a set of conditional mutations in the kinase domain of the *MPS1* gene was analyzed (Schutz and Winey, 1998). All of the mutant alleles of *MPS1* disrupt both mitotic functions at the restrictive temperature, producing a loss of viability associated with monopolar mitosis in the absence of a checkpoint. Here we report our phenotypic analysis of four *mps1* mutant alleles that form two phenotypic classes during meiotic chromosome segregation and spore formation.

MATERIALS AND METHODS

Strain Construction

Yeast strains used are listed in Table 1. Original temperature-sensitive strains were constructed by the two-step allele replacement method (Scherer and Davis, 1979). *URA3*-marked integrative (pRS306-derived) plasmids, containing each *mps1* allele, were cut with the *Mro*I restriction enzyme to direct integration at the endogenous *MPS1* locus. DNA fragments were transformed with the use of the EZ Transformation kit (Zymo Research, Orange, CA). Ura^+ transformants were selected and subsequently streaked to synthetic complete (SC)-ura plates containing 5-FOA (1 g/l) to select for strains having excised part of the integrated DNA by homologous recombination. Temperature-sensitive colonies, having retained the mutation contained in the integrated allele, were selected by failure to grow at 37°C and subsequently tested for complementation of the *mps1-1* allele by mating. Noncomplementing strains were used for further study according to the integrated allele of *mps1*. The majority of experiments presented here were done with SK-1–derived strains, but the sporulation phenotypes as described by light microscopic analysis (phase and fluorescence data) were recapitulated in W303-derived strains.

Yeast Cultures and Sporulation

Yeast culture and genetic techniques were as described by Rose *et al.* (1990) with the following exceptions. Synchronous sporulation in liquid medium was performed as described by Alani *et al.* (1990) with modifications for use of temperature-sensitive strains. Cultures were grown overnight in 5 ml of YPD to stationary phase. Cells were diluted in YEPA (1% potassium acetate) to an OD_{600} of 0.30–0.35 and allowed to grow at room temperature for 13.5 h. Cultures of density $> OD_{600} = 1.2$ ($\sim 3 \times 10^7$ cells/ml) were then shifted to sporulation medium (0.3–1% potassium acetate) at the restrictive temperature, maintaining the same cell density. Medium was supplemented with amino acids to cover auxotrophies at one-fourth the concentration recommended for synthetic medium (Rose *et al.*, 1990). Can/Cyh assays were performed with the use of SC medium without arginine containing 3 μg/ml cycloheximide and 60 μg/ml L-canavanine.

Light Microscopy

Fluorescence imaging consisted of visualization of DNA with the use of 1 μg/ml DAPI, tubulin in a green fluorescent protein GFP–*TUB1* fusion (Straight *et al.*, 1996), or centromere-proximal marked chromosomes with the use of a GFP–*LacI* repressor fusion (Straight *et al.*, 1996). Microscopy was performed on a Leica DMRXA/RF4/V fluorescence microscope (Leica, Solma, Germany) equipped with a Cooke Sensicam charge-coupled device (CCD) camera (Tonawanda, NY) and a motorized stage for three-dimensional imaging. Image processing was performed with the use of the Slidebook program by Intelligent Imaging Innovations (Denver, CO). All images were subjected to deconvolution with the use of the nearest-neighbors algorithm. Sporulated cells were either visualized live as a wet mount in 1% potassium acetate on poly-L-lysine–coated microscope slides (Polysciences, Warrington, PA) or fixed in 70% ethanol for 15 min to 1 h and resuspended in PBS containing 0.1 μg/ml DAPI. Cells used for DAPI exclusion assays were fixed in a 3.7% formaldehyde solution for 15 min, stained with 1 μg/ml DAPI for <5 min, resuspended in PBS, and placed on poly-L-lysine–coated slides.

Dityrosine Fluorescence Assay

Assays for spore wall fluorescence were performed with the use of a method similar to that described by Esposito *et al.* (1991). Patches of cells were sporulated on nitrocellulose filters (Schleicher & Schuell, Keene, NH) incubated at 23 and 34°C. Filters were subsequently treated with lysis buffer (350 μl of 0.1 M Na-citrate, 0.01 M EDTA, pH 5.8, 70 μl of Glusulase [DuPont NEE-154], 15 μl of β-mercaptoethanol) at 37°C for 4 h and then treated with 300 μl of concentrated ammonium hydroxide. Fluorescence was detected by excitation under UV light (UVP, San Gabriel, CA), and images were captured with the use of a digital camera with a blue filter (Wratten #98, Kodak, Rochester, NY).

Electron Microscopy

Samples for electron microscopy were prepared with the use of a modification of the method used by Winey *et al.* (1995). At selected times, 5- to 10-ml aliquots were removed from a synchronously sporulating culture, and cells were collected by vacuum filtration to form a yeast paste. The cell paste was cryofixed in a BAL-TEC

Table 1. Yeast strains

Strain	Genotype	Source
S490	*MATα, ura3, leu2Δ (asp718-ecoR1), arg4-bgl, ade2-bgl*	N. Hollingsworth
S483	*MATa, ura3, leu2Δ (asp718-ecoR1), arg4-nsp, ade2-bgl*	N. Hollingsworth
RKY1145	*MATa, ura3, leu2Δ::hisG, his4-X*	M. Lichten
RKY1146	*MATα, ura3, leu2Δ::hisG, his4-B*	M. Lichten
MAS119	*MATa, ho::LYS2, lys2, ura3, leu2::hisG*	M. Shonn
MAS140	*MATα, LacOpr:LEU2, Cyc1pLacI-GFP13:URA3, ho::LYS2*	M. Shonn
MAS142	*MATa, LacOpr:LEU2, ho::LYS2*	M. Shonn
YUMY069	*MATa/α, mps1-1/mps1-1, ura3/ura3, HIS4/his4-X, ADE2/ade2-B, ARG4/arg4-B*	This study
YUMY070	*MATa/α, mps1-3796/mps1-3796, ura3/ura3, leu2Δ/leu2Δ, arg4-N/arg4-B, ade2-B/ade2-*	This study
YUMY071	*MATa/α, mps1-1237/mps1-1237, ura3/ura3, leu2::hisG/leu2Δ, HIS4/his4-X, ARG4/arg4-B, ADE2/ade2-B*	This study
YUMY072	*MATa/α, mps1-412/mps1-412, ura3/ura3, leu2::hisG/leu2Δ, ARG4/arg4-N, ADE2/ade2-B, HIS4/his4-B*	This study
YUMY1D1	*MATa, ura3, leu2Δ (asp718-ecoR1), arg4-bgl, ade2-bgl*	This study
YUMY102	*MATα, mps1-1237, Cyc1pLacI-GFP13:URA3, LacOpr:LEU2, arg4-N*	This study
YUMY104	*MATa, mps1-1237, Cyc1pLacI-GFP13:URA3, LacOpr:LEU2, arg4-B*	This study
YUMY110	*MATa, mps1-1237, ura3, leu2Δ (asp718-ecoR1), arg4-B*	This study
YUMY119	*MATα, mps1-1237, ura3, leu2Δ (asp718-ecoR1), arg4-nsp*	This study
YUMY120	*MATa, mps1-1237, ura3, leu2Δ (asp718-ecoR1), arg4-bgl*	This study
YUMY125	*MATα, ura3, leu2Δ (asp718-ecoR1), arg4-bgl*	This study
YUMY126	*MATa, ura3, leu2Δ (asp718-ecoR1), arg4-nsp*	This study
YUMY131	*MATα, ura3, leu2Δ (asp718-ecoR1), arg4-nsp, his4-bgl*	This study
YUMY1980	*MATa/α, mps1-412/mps1-412, ura3/ura3, leu2D/leu2D, arg4-N/arg4-B, ade2-B/ade2-B*	This study
YUMY3E3	*MATa/α, MPS1/MPS1, ura3/ura3, ho::LYS2/ho::LYS2, leu2D/leu2D, arg4-N/arg4-B, ade2-B/ade2-B*	This study
YUMY3I1	*MATa/α, mps1-1237/mps1-1237, ura3/ura3, ho::LYS2/ho::LYS2, leu2D/leu2D, arg4-N/arg4-B, ade2-B/ade2-B*	This study
YUMY3I8	*MATa/α, mps1-3796/mps1-3796, ura3/ura3, ho::LYS2/ho::LYS2, leu2D/leu2D, arg4-N/arg4-B, ade2-B/ade2-B*	This study
YUMY4B1	*MATa/α, MPS1/MPS1, GFP-TUB1:URA3/GFP-TUB1:URA3, leu2Δ (asp718-ecoR1)/leu2-K, HIS4/his4-X, ARG4/arg4-N*	This study
YUMY4B5	*MATa/α, mps1-412/mps1-412, GFP-TUB1:URA3/GFP-TUB1:URA3, leu2D::hisG/leu2D(asp718-ecoR1), ADE2/ade2-B, arg4-N/arg4-N, HIS4/his4-X*	This study
YUMY4C2	*MATa/α, mps1-3796/mps1-3796, GFP-TUB1:URA3/GFP-TUB1:URA3, leu2D/leu2D, arg4-N/arg4-B, ade2-B/ade2-B*	This study
YUMY4C6	*MATa/α, mps1-1237/mps1-1237, GFP-TUB1:URA3/GFP-TUB1:URA3, leu2-R/leu2Δ (asp718-ecoR1), arg4-N/arg4-N*	This study
YUMY4C9	*MATa/α, mps1-1/mps1-1, GFP-TUB1:URA3/GFP-TUB1:URA3, leu2Δ (asp718-ecoR1)/leu2-K, HIS4/his4-X, ARG4/arg4-N*	This study
YUMY4F4	*MATa/α, MPS1/MPS1, ura3/ura3, leu2::hisG/leu2::hisG, his4-X/his4-B*	This study
TM002[a]	*MATa/α, MPS1/MPS1, ura3-1/ura3-1, leu2-3, 112/leu2-3, 112, his3-11/his3-11, ade2-1/ade2-1, trp1-1/trp1-1, can^r/CAN^s, cyh^r/CYH^s*	T. McKenna
TM0019[a]	*MATa/α, mps1-3796/mps1-3796, ura3-1/ura3-1, leu2-3, 112/leu2-3, 112, his3-11/his3-11, ade2-1/ade2-1, trp1-1/trp1-1, can^r/CAN^s, cyh^r/CYH^s*	T. McKenna
TM027[a]	*MATa/α, mps1-1237/mps1-1237, ura3-1/ura3-1, leu2-3, 112/leu2-3, 112, his3-11/his3-11, ade2-1/ade2-1, trp1-1/trp1-1, can^r/CAN^s, cyh^r/CYH^s*	T. McKenna

[a] These strains are W303 background; all others are SK-1.

(Balzers, Liechtenstein) HPM-010 high-pressure freezer. The samples were then processed by freeze-substitution in 2% osmium tetroxide and 0.1% uranyl acetate in acetone at −80°C for 3 d, followed by equilibration to room temperature and embedding in Spurr's resin. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips (Eindhoven, the Netherlands) CM10 electron microscope. Images were captured with the use of a CCD camera (Gatan, Pleasanton, CA) and processed with Digital Micrograph version 2.5 (Gatan, Pleasanton, CA).

Commitment to Recombination

Assays for commitment to recombination were performed with the use of strains heteroallelic for different point mutations at *arg4* (Wu and Lichten, 1995). Wild-type (YUMY3E3), *mps1-1237* (YUMY3I1), and *mps1-3796* (YUMY3I8) strains were synchronously sporulated at the restrictive temperature, and aliquots were removed at 2-h time points, diluted in 1% potassium acetate, and plated to SC for viability and to SC-arginine for commitment to recombination. Viability was measured as colony-forming units (CFU) at each time point per CFU at time zero. Interhomologue recombination frequency is expressed as the number of Arg^+ colonies per total CFU. Each sample was plated in triplicate.

Chromosome Segregation Assays

Chromosome III was marked at the *LEU2* locus by integration of pAFS59, containing 256 tandem repeats of the *LacO* (Lac operator)

(Straight *et al.*, 1996). Visualization of the marked chromosome was made possible by integration at the *URA3* locus of a GFP–*LacI* repressor fusion. Diploid strains were made both homozygous and hemizygous for the *LacO* array by mating marked to marked, and marked to unmarked, a and α haploids, respectively. Homozygous strains were generated by crossing wild-type strains (MAS140 $\times$ MAS142) and *mps1-1237* strains (YUMY102 $\times$ YUMY104). The hemizygous strains were used for microscopy and quantitation of the phenotype in this study. Marked *MPS1* (MAS140 $\times$ MAS119) and *mps1-1237* (YUMY102 $\times$ YUMY110) strains were induced to undergo synchronous sporulation as described previously. Samples were removed at 1-h time points from 6–12 h after initiation of sporulation. Cells were visualized either live (GFP/differential interference contrast [DIC] panels) or after fixation in 70% ethanol (GFP/DAPI). Several planes of a whole field of cells were captured with the use of a motorized stage and a CCD camera. Digital images were then used to perform data acquisition.

Northern Analysis

Total RNA was isolated from synchronously sporulating cultures of the *mps1-1237/mps1-1237* (YUMY119 $\times$ YUMY120) and *MPS1/MPS1* (YUMY125 $\times$ YUMY126) strain backgrounds according to yeast RNA isolation procedures from Ausubel *et al.* (1997). Twenty micrograms of RNA was loaded per lane in a 1% agarose–formaldehyde gel. rRNA was stained with ethidium bromide (25 μg/ml) and photographed before transfer of RNA to Hybond-N by a capillary blotting procedure according to the manufacturer's specifications (Amersham International, Buckingham, United Kingdom). RNA was UV cross-linked to the membrane with the use of a UV Stratalinker (Stratagene, La Jolla, CA), and the blots were dried at 80°C for 1 h. DNA probes were labeled with [α-^{32}P]dCTP with the use of the Prime-It II kit (Stratagene). PCR products, used as templates, were generated for *SMK1*, *SWM1*, *SPS100*, and *ACT1* by amplification from isolated SK-1 genomic DNA. Blots were stripped between probing by boiling in 10 mM Tris (pH 7.5), 1 mM EDTA, and 0.1% SDS and allowing the solution to cool to room temperature.

RESULTS

Loss of Sporulation in Temperature-sensitive mps1 Strains

To study the sporulation phenotypes caused by mutation in *MPS1*, several mutant alleles of the gene were introduced into the efficiently sporulating strain SK-1 (Kane and Roth, 1974) and the W303 strain of *Saccharomyces cerevisiae* by two-step integration (see MATERIALS AND METHODS). To confirm proper integration of the mutant alleles, strains were assayed mitotically for their ability to complement the *mps1-1* mutation. With this method, strains of both a and α mating type were constructed that contain the following conditional alleles of *mps1*: *mps1-1*, *mps1-412*, *mps1-1237*, and *mps1-3796*. All of the alleles tested have mutations that fall within the kinase domain of *MPS1*. Molecular characterization of the mitotic phenotypes of these strains has been described (Schutz and Winey, 1998). In vegetatively growing cells, all four alleles of *mps1* cause a failure of SPB duplication and are unable to activate the spindle assembly checkpoint at the restrictive temperature. Homozygous diploid (W303 and SK-1) strains for each allele of *mps1* were made and assayed for their ability to sporulate at 33°C (Table 1). Also, the minimal restrictive temperature for all four strains during vegetative growth was determined. All four strains are completely inviable at 33°C in mitotic culture, indicating that 33°C is a fully restrictive temperature for the meiotic experiments (our unpublished results).

A role for Mps1p during meiosis was suspected based on a report that the expression of *MPS1*, constant with respect to vegetative growth, is enhanced specifically during meiosis (Poch *et al.*, 1994). Furthermore, the upstream activating sequence (UAS) of *MPS1* contains two meiosis specific element (MSE) elements used for meiosis-specific up-regulation of transcription (Chu and Herskowitz, 1998). Initially, the ability of the *mps1* mutant strains to form spores at the restrictive temperature was assessed by phase microscopy. All of the alleles of *MPS1* prevented wild-type levels of spore formation at this temperature, reducing the number of asci to between 1 and 30% of the population (our unpublished results). Furthermore, the extent of the defect depends on the allele present in the strain. Strains homozygous for the *mps1-412* and *mps1-1237* alleles produce a higher percentage of two- to four-spored asci (~30–50% of the population) than strains of the *mps1-1* (~0–2%) and *mps1-3796* (~5–15%) types. However, all of the spores that could be dissected did not form viable haploid colonies. Germination in the absence of cell wall digestive enzymes was checked by isolation of whole asci on rich medium. Spores bearing *mps1* mutations were unable to form colonies on rich medium, indicating that passage through the events of meiosis and spore formation under conditions of compromised Mps1p function is lethal to the cells (*mps1-1237:* 2 of 30 germinated, 1 of 30 colony forming; *mps1-412:* 3 of 30 germinated, 0 of 30 colony forming).

Loss of Mps1p Function Is Lethal in the Presence and Absence of Spore Formation

Strains carrying *mps1* mutations were sporulated at the restrictive temperature and at times tested during the course of 12 h to assess viability in the population. Whereas wild-type strains maintained high viability during the experiment, cells containing the mutant alleles of *MPS1* lost viability dramatically (Figure 1A). Extending the analysis of viability to 24–48 h demonstrated that the loss of viability in *mps1* strains was nearly complete (<1% colony forming; our unpublished results). To demonstrate that any viable cells left in *mps1* cultures were not from strains that carry out sporulation, viability was tested with the use of the highly sensitive Can^r, Cyh^r haploidization assay (Figure 1C). Homozygous *mps1-3796* strains that are heterozygous for two recessive alleles conferring resistance to canavanine and cycloheximide are unable to produce any Can^r, Cyh^r progeny, indicating that these strains produce no viable haploid spores (Hollingsworth and Byers, 1989). Similar strains homozygous for *mps1-1237* produce very few papillae within a patch, indicating a severe loss of viability in these strains.

Mutations in genes required for recombination during meiosis, such as *RAD50* and *SPO11*, are often lethal (Klapholz *et al.*, 1985; Alani *et al.*, 1990). Because mutation in *mps1* results in a loss of viability, commitment to recombination was measured in diploid strains homozygous for *mps1-1*, *mps1-1237*, and *mps1-3796* mutations. Strains mutant for *mps1* and heteroallelic at the *ARG4* locus were sporulated at the restrictive temperature and plated to SC-arginine or SC to assess production of Arg$^+$ recombinants (Figure 1B). This assay measures the level of commitment to recombination within the sporulating population (Esposito and Esposito, 1974). Cells that have initiated

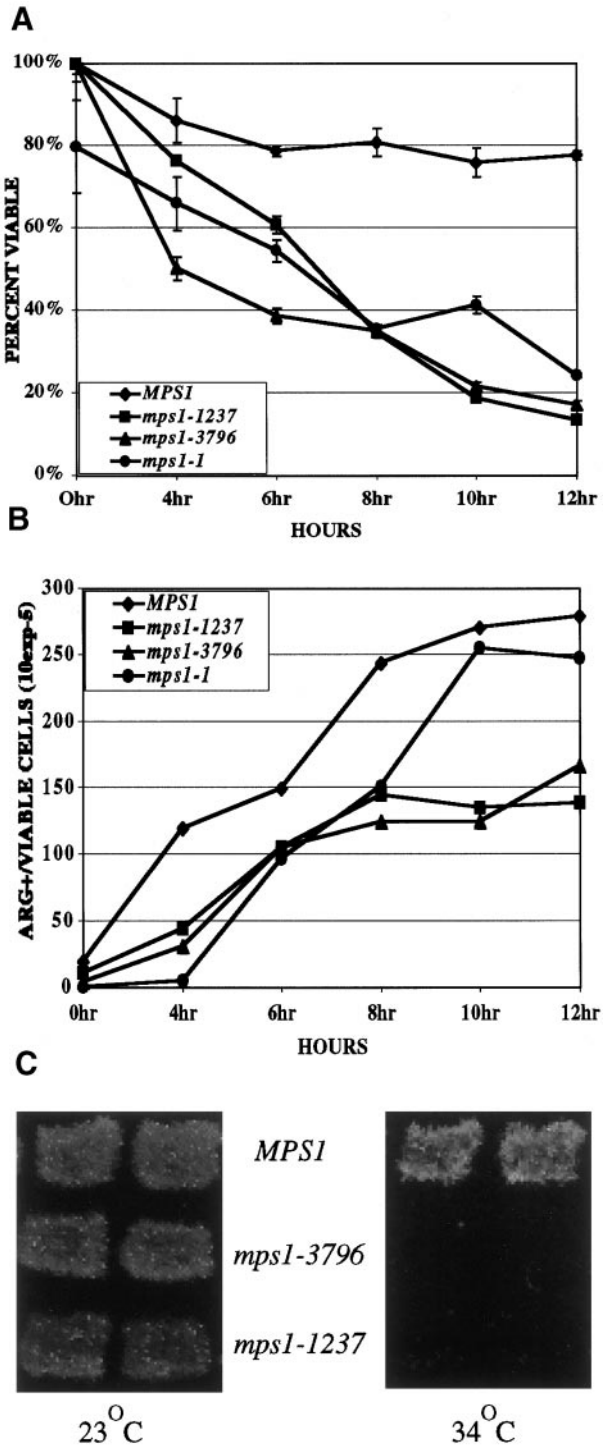

Figure 1. Return-to-growth assays were done to determine viability (A) and commitment to recombination (B) with the use of wild-type (YUMY3E3), *mps1-1* (YUMY131 × YUMY1D1), *mps1-1237* (YUMY3I1), and *mps1-3796* (YUMY3I8) homozygous diploid mutant strains. Strains were synchronously sporulated, and aliquots of cells were plated to SC or SC-arginine medium at the times indicated. Viability was measured as the number of CFU at each time point as a percentage of CFU at 0 h. Commitment to recombination was determined with the use of *arg4* heteroallelic diploids and plating to SC-arginine medium. Recombinant frequency is expressed as Arg^+ colonies per CFU at each time point according to the method described by Esposito and Esposito (1974). Recombination assays were performed three times with triplicate samples. One representative example is shown. (C) Viability of cells sporulated at the restrictive temperature was assessed with the use of Can/Cyh resistance generated by haploidization of two recessive drug-resistance markers. Wild-type (TM002), *mps1-3796* (TM019), and *mps1-1237* (TM027) strains were sporulated at room temperature and 34°C. Patches were replica plated to medium containing cycloheximide and L-canavanine (see MATERIALS AND METHODS) and incubated at room temperature for 2–5 d to assay viable haploid cell production caused by haploidization of both *can1-100* and cyh^r markers.

meiotic recombination can complete recombination and revert to vegetative growth as diploids when returned to glucose-containing medium before anaphase I. Using this assay, we can measure the timing and level of entry into the meiotic recombination process in our strains. The *mps1* mutant strains are competent to become committed to recombination, because they produced Arg^+ colonies with a slight reduction compared with *MPS1*. The loss of viability and the absence of recombination checkpoint arrest in these strains suggest that the *mps1* phenotypes are not attributable to a defect in recombination.

Mutation in MPS1 Produces Two Phenotypic Classes

We performed a cytological analysis of the mutant strains to characterize the physiological defect caused by mutation of *mps1* in sporulating cells. Strains containing mutant alleles of *mps1* were transformed with a GFP-tagged copy of the α-tubulin gene (*TUB1*) integrated at the *URA3* locus for visualization of spindle formation. Cells containing the GFP–*TUB1* fusion gene were induced to undergo sporulation synchronously at the restrictive temperature (Alani *et al.*, 1990). Aliquots were removed from the sporulation cultures, fixed, and stained with DAPI to monitor DNA segregation. Flow cytometry demonstrates that in all *mps1* mutant strains, premeiotic DNA synthesis is completed with similar timing as in wild-type strains (our unpublished results). After 12 h of sporulation at the restrictive temperature, wild-type strains showed ~80% completion of meiosis and many mature ascospores, e.g., 161 of 200 three- to four-spored asci. Spindle formation during meiosis I and meiosis II was clearly visible in these strains with the use of the GFP–Tub1p chimera (Figure 2). A distinction could be made between two levels of progression through sporulation in strains carrying different mutant alleles of *mps1*. Cells harboring the *mps1-3796* and *mps1-1* alleles failed to form spindles (e.g., ~1–5% spindle formation, 12 of 200 three- to four-spored asci) or segregate DNA during meiosis, as seen by DAPI staining (Figure 2; our unpublished results). We define these cells as exhibiting the class I meiotic phenotype of *mps1*. On the other hand, cells containing the *mps1-1237* and *mps1-412* alleles revealed spindle formation at times between 6 and 12 h in a significant proportion (e.g., 30–50%, 56 of 200 two- to four-spored asci) of cells in the culture (Figure 2; our unpublished results). The timing of spindle formation was similar to that of wild-type cells, with the first

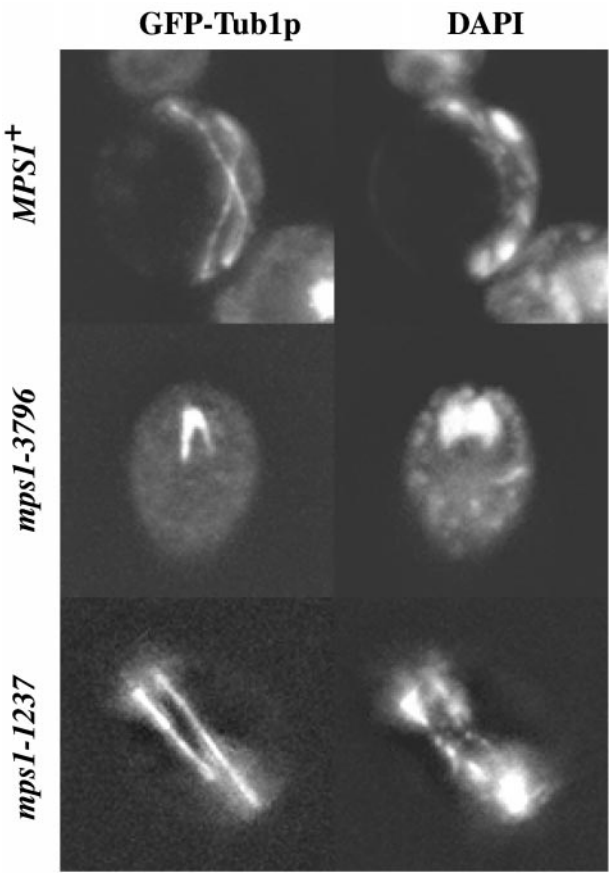

Figure 2. Cytological analysis of *mps1* mutants during meiosis at the restrictive temperature. GFP–Tub1p and DAPI staining of DNA demonstrate allele-dependent phenotypes. Cultures were sporulated at 33°C for 8 h (*MPS1*, YUMY4B1; *mps1-1237*, YUMY4C6) or 12 h (*mps1-3796*, YUMY4C2), fixed with 3.7% formaldehyde for up to 1 h, and stained with 1 μg/ml DAPI for 5 min. Wild-type cells proceed through two rounds of spindle formation and chromosome segregation, as seen by GFP and DAPI, respectively. The *mps1-3796* strain fails to form spindles over the course of meiosis and remains mononucleate. However, ~30% of cells harboring the *mps1-1237* allele successfully complete spindle formation and carry out chromosome segregation.

meiotic spindles formed roughly 6 h after introduction into sporulation medium. Therefore, the *mps1-1237* and *mps1-412* alleles result in the class II phenotype. The observation of phenotypic differences between alleles may reveal two distinct roles for the kinase during meiosis and spore formation.

Mps1p Is Required for Meiotic SPB Duplication

The class I phenotype caused by *mps1* mutation in sporulating cells suggests a conserved requirement for Mps1p in SPB duplication during meiosis. Figure 3B shows an electron micrograph of a nucleus in a *mps1-3796* cell after 12 h of incubation in sporulation medium at 33°C. Whereas wild-type cells duplicate their SPBs early in meiosis (Figure 3A), near the onset of premeiotic DNA synthesis, the *mps1-3796*

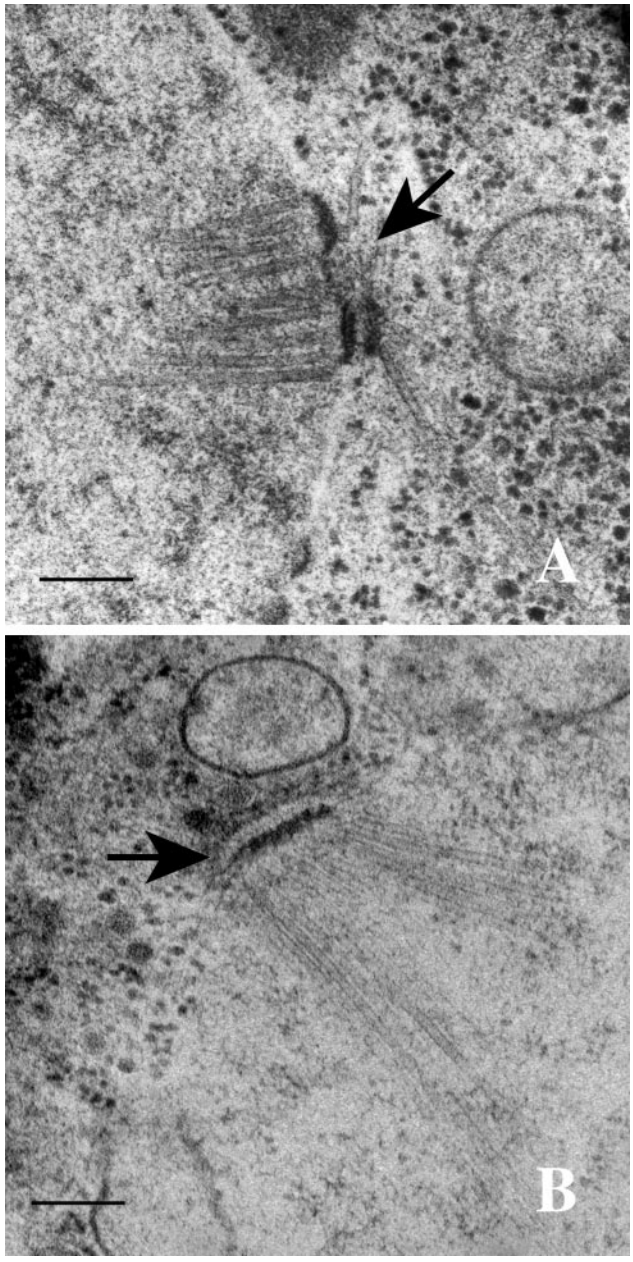

Figure 3. Thin-section electron microscopy of *MPS1* (YUMY4F4) and *mps1-3796* (YUMY070) strains during meiosis at the restrictive temperature reveals a SPB duplication defect in the *mps1* mutant strain. (A) *MPS1* strain after 6 h at 33°C in sporulation medium. Prophase meiotic nuclei have duplicated side-by-side SPBs (arrow) in the nuclear envelope. (B) *mps1-3796* strain after 12 h at 33°C. Spindle pole bodies fail to duplicate during meiosis in both the *mps1-3796* and *mps1-1* (YUMY069) strains, leading to the class I phenotype. Bars, 0.2 μm.

and *mps1-1* strains maintain a single SPB after sufficient time for cells to complete sporulation has passed. To demonstrate that the mononucleate cells in the culture did not represent only cells that failed to enter the sporulation process, several

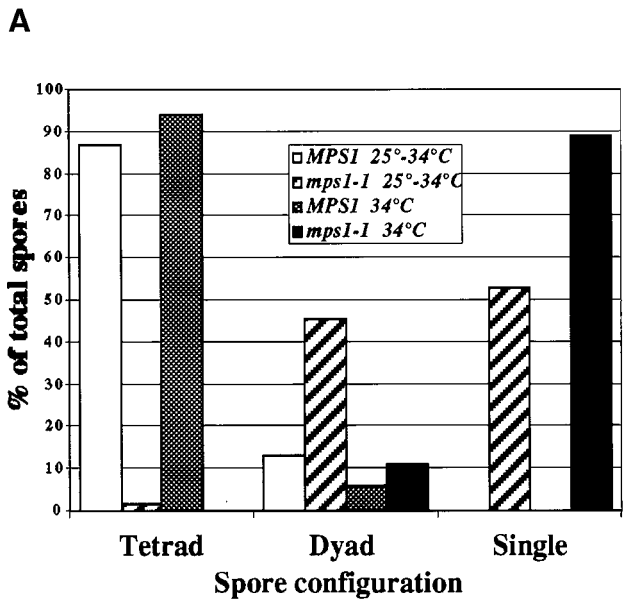

Figure 4. Timed temperature shift of an *mps1-1* strain produces two-spored asci. Wild-type (YUMY4B1) and *mps1-1* (YUMY4C9) strains were sporulated at 34 or 25°C for 6 h and then shifted to 34°C. Spore morphology was counted for 200 cells of each culture after 24 h of sporulation. White bars, wild-type, 25–34°C; striped bars, *mps1-1*, 25–34°C; gray bars, wild-type, 34°C; black bars, *mps1-1*, 34°C. The chart shows the percentages of cell morphology within the spore-forming population of the culture; n = 200 for each culture. Sporulation efficiency (two or more spores per ascus) was as follows: wild-type, 25–34°C = 91%; *mps1-1*, 25–34°C = 29.5%; wild-type, 34°C = 78%; *mps1-1*, 34°C = 2%.

cells from an earlier time (8 h) were examined by electron microscopy. Prophase meiotic nucleoplasm can be distinguished visually from mitotic nucleoplasm by a variety of meiosis-specific characteristics with the use of electron microscopy (Moens and Rappaport, 1971; Zickler and Olson, 1975). Wild-type meiotic prophase I nuclei, in addition to duplicated side-by-side SPBs, typically contain synaptonemal complex and polycomplex. The SPBs in meiotic prophase nuclei of class I phenotype were examined by serial section electron microscopy. The entire nucleus was examined to account for all SPBs. In every nucleus of a *mps1-3796* strain (n = 16) and a *mps1-1* strain (n = 10), only a single SPB was found within the nuclear envelope. Therefore, we conclude that the major defect in strains exhibiting the class I phenotype appears to be the failure to duplicate the SPBs.

We hypothesized that if both meiosis I and meiosis II SPB duplications require Mps1p, it would be possible to disrupt the second duplication event specifically with the use of a timed shift to the restrictive temperature. Cells of the *mps1-1* genotype and wild-type cells were sporulated synchronously at 34 or 25°C for 6 h and then shifted to 34°C for the remainder of the 24-h time course (Figure 4). After 6 h in sporulation medium, the cells are nearing the end of prophase and contain duplicated SPBs (Alani *et al.*, 1990). The *mps1-1* cells that were shifted to 34°C after 6 h produced nearly 50% two-spored asci, indicating successful completion of the first SPB duplication and failure in the second SPB duplication. Less than 2% three- to four-spored asci were detected in the *mps1-1* strain, revealing a requirement for Mps1p after the shift to higher temperature. Because dyads are formed with a timed temperature shift, we conclude that Mps1p is required for the completion of both meiosis I and meiosis II SPB duplication events.

Mps1p Is Required for Normal Spore Wall Formation

Meiotically dividing cells homozygous for *mps1-412* or *mps1-1237* mutations display the second class of mutant phenotypes, involving progression through sporulation. DAPI staining of DNA and GFP–Tub1p autofluorescence of microtubules reveal that these cells are capable of chromosome segregation by meiotic spindles (Figure 2). However, DAPI-treated mutant cells could be distinguished from wild-type cells by the ability of the stain to penetrate the interior of the spore (Figure 5). We used wild-type asci, fixed for 15 min with formaldehyde and then stained for 3 min with DAPI, to demonstrate that the mature spore walls are resistant to the permeation of DAPI (Figure 5A). In contrast, the interiors of spores from either *mps1-1237*– or *mps1-412*–containing strains are readily penetrated by DAPI, resulting in stained nuclear material (Figure 5, B and C). This difference between spores in wild-type and *mps1* strains suggested a defect in spore wall assembly.

We used electron microscopy to examine the sporulation products of diploid cells homozygous for the *mps1-1237* (Figure 5, F–H) or *mps1-412* alleles (our unpublished results). A mature wild-type spore wall is composed of four layers: the prospore membrane, the electron-translucent second layer, the chitin/chitosan layer, and the outer darkly staining dityrosine-containing layer (Briza *et al.*, 1986). Whereas wild-type spores contain all four spore wall layers in their proper organization (Figure 5F), *mps1-1237* (Figure 5G) and *mps1-412* spore walls are defective, lacking the uniform four-layered structure seen in wild-type spores. This phenotype is similar to that of homozygous *smk1* and *sps1* strains (Friesen *et al.*, 1994; Krisak *et al.*, 1994). Also, similar spore wall defects are detected in *mps1-1* and *mps1-3796* (class I) strains in a small percentage of the cells (up to 15%) that attempt to form an aberrant single spore (Figure 5, D, E, and H).

In *mps1* homozygous mutant strains, the following defects are detected: some of the spores contain fewer than four layers, the ordering of the component layers is frequently abnormal, and the overall thickness of the mutant spore walls is variable. The electron-dense outer layer of the spore walls is often missing, and when present it fails to develop the uniformity of the wild-type outer layer. Furthermore, the electron-dense material adjoining individual spores in a wild-type ascus is disorganized and fragmented in the *mps1* mutant strains, and the ascus fails to condense during maturation. However, unlike the spore walls in *smk1*Δ strains, the defective spores formed in *mps1* strains often contain spore wall structures detectable by phase microscopy (our unpublished results), indicating a different type of defect. To determine if a gross structural defect in SPB morphology could be the cause of deficient spore production, we examined several SPBs from homozygous *mps1-1237* and *mps1-*

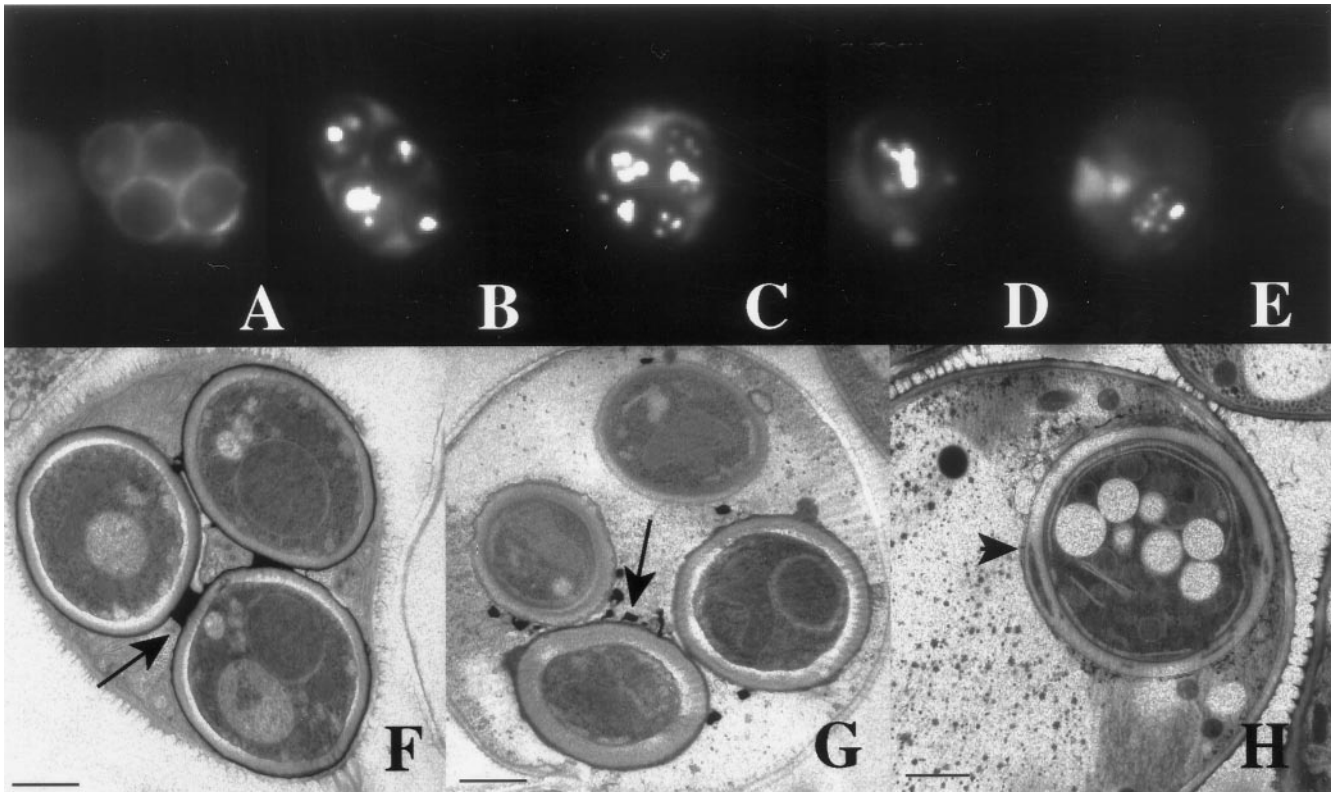

Figure 5. DAPI staining and electron microscopy of wild-type and *mps1* mutant strains reveals a spore wall defect. Cells were fixed for 15 min with 3.7% formaldehyde and stained with 1 μg/ml DAPI for <5 min (see MATERIALS AND METHODS). DAPI permeation into the interior of the spore is retarded by mature spore walls in wild-type *MPS1* (YUMY4B1) (A). Lack of complete spore wall formation in the *mps1* mutant strains allows DAPI to readily stain DNA within individual spores of *mps1-1237* (YUMY4C6) (B), *mps1-412* (YUMY4B5) (C), *mps1-1* (YUMY4C9) (D), and *mps1-3796* (YUMY4C2) (E). Note that in D and E, meiotic chromosome segregation fails as expected, and the cells build defective spore walls. Also note the fragmentation of DAPI-staining material within the mutant spores. Thin-section electron microscopy of *MPS1* (YUMY4F4) (F), *mps1-1237* (YUMY071) (G), and *mps1-3796* (YUMY070) (H) strains displays the heterogeneous nature of the spore wall defect caused by *mps1* mutation. (F) Wild-type spores have a four-layered structure that is relatively uniform in thickness. Mature spores are tightly associated in a tetrahedral array within a condensed ascus. Darkly staining material defines the outer, dityrosine-containing layer of the spore wall, and electron-dense material is seen adjoining individual spores (arrow). (G) Two to four spores are produced in *mps1-1237* strains, and individual spores lack the organization of the spore wall seen in wild-type. Spore wall thickness is more variable than in wild-type, with component layers of spore wall material not organized as in wild-type. The electron-dense outer layer of the mutant spores is often missing, and the asci tend not to condense around tightly adherent spores. Note that the very electron-dense material connecting the *MPS1* spores is disrupted in the *mps1-1237* ascus (arrow). Up to 15% of cells containing the *mps1-3796* allele eventually form an aberrant single spore body (H, arrowhead). The spore produced in these cells reveals similar structural defects as in a *mps1-1237* strain. Bars, 0.7 μm

412 strains. No visible defect in SPB morphology was detected in either strain (our unpublished results).

Genes required for spore wall formation are regulated by a sporulation-specific transcriptional program (Chu *et al.*, 1998). To investigate the role of Mps1p in spore wall formation, we performed Northern analysis to determine if mutations in *MPS1* affected induction of genes involved in the regulation or structural composition of developing spores. A strain containing the *mps1-1237* allele, which produces the class II phenotype, was sporulated at the restrictive temperature. Transcriptional induction of midlate genes such as *SMK1*, *SWM1* (Ufano *et al.*, 1999), and *DIT1* was assayed as well as the late spore wall–specific gene *SPS100* (Figure 6A). Analysis of the abundance of these transcripts in sporulating cells of the *mps1-1237* genotype revealed no dramatic change in the expression of *SMK1* or *SWM1* (our unpublished results), but the abundance of the *SPS100* transcript was reduced dramatically at the late times. The level of *DIT1* transcript was consistently higher in the *mps1-1237* strain than in wild type, similar to the effect of *sps1* mutation (Friesen *et al.*, 1994). Furthermore, these strains were assayed for dityrosine production with the use of a dityrosine fluorescence assay (Esposito *et al.*, 1991) and found to produce a fluorescent signal similar to wild type, indicating that the function of the *DIT1* gene is intact (Figure 6B). Therefore, we conclude that the defects in spore wall assembly seen in *mps1* mutant strains are independent of Smk1p kinase and Swm1p but may exert their effect through Sps1p or some other mechanism.

Finally, to determine if the class II phenotype results from the *mps1-1237* or *mps1-412* alleles being hypomorphic, we assayed the ability of a centromeric plasmid containing the

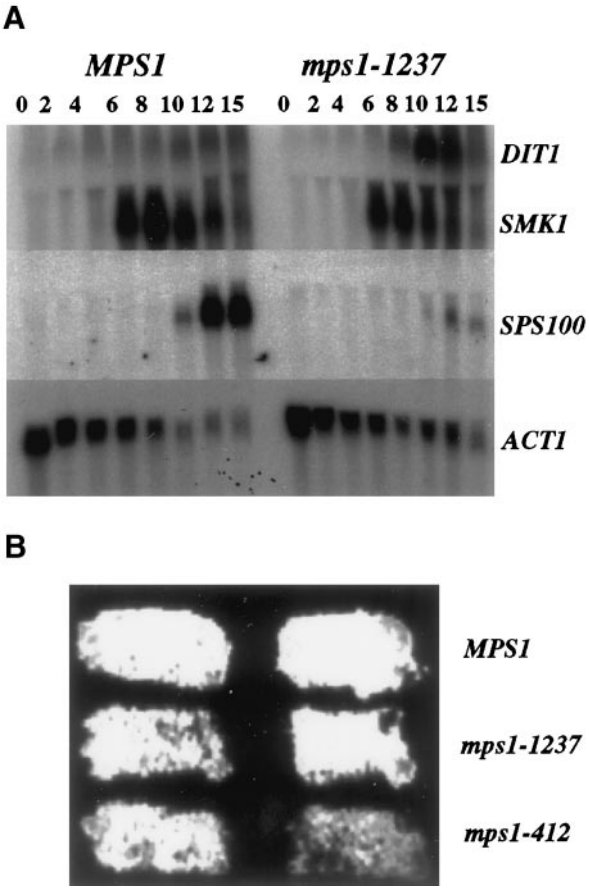

Figure 6. Northern analysis of sporulation-specific gene expression and dityrosine fluorescence in *mps1* mutant strains. (A) *MPS1* (YUMY125 × YUMY126) and *mps1-1237* (YUMY119 × YUMY120) strains were synchronously sporulated, and aliquots were removed for RNA isolation. Filters were probed for expression of three mid-late sporulation-specific genes, *SMK1*, *SWM1*, and *DIT1*, the late spore wall–specific gene *SPS100*, and the *ACT1* gene as a loading control. Expression of *SMK1* and *SWM1* (our unpublished results) is unaffected in the *mps1-1237* strain compared with wild type. The expression of the *SPS100* gene is severely diminished in the mutant strains, whereas *DIT1* expression is enhanced above wild-type levels. (B) Dityrosine fluorescence is detected in both *mps1* mutant strains producing two- to four-spored asci. Patches of *MPS1* (YUMY3E3), *mps1-1237* (YUMY3I1), and *mps1-412* (YUMY 1980) were sporulated at the restrictive temperature and then treated to activate dityrosine fluorescence. The presence of fluorescent material in wild-type and *mps1* mutant strains indicates that insoluble fluorescent dityrosine is produced efficiently in both strain types.

mps1-1237 allele to rescue the *mps1-1237* sporulation phenotype. Two independent experiments were carried out with the use of a *mps1-1237* homozygous strain (YUMY119 × YUMY120; Table 1) transformed with either p*MPS1-1237* containing the *mps1-1237* allele (Schutz and Winey, 1998) or the pRS316 empty vector. The strains were sporulated at the restrictive temperature for 48 h, fixed, and examined for spore wall integrity with the use of the DAPI permeation assay. Neither plasmid was capable of rescuing the phenotype (*mps1-1237*, 6.3% normal spores, number of DAPI-resistant per total in two experiments: 0 of 52 and 5 of 23; vector, 7.3% normal spores, number of DAPI-resistant per total in two experiments: 1 of 34 and 5 of 42). On the other hand, a plasmid carrying wild-type *MPS1* recovered the DAPI resistance of spores (72%, 36 of 50). Extra copies of the *mps1-1237* allele did not rescue the mutant phenotype, suggesting that this is not a hypomorphic allele of *MPS1*.

Chromosome Missegregation Is Increased in MPS1 Mutant Strains

The high viability of spores with mutations in any one of several late-sporulation genes involved specifically in spore wall formation demonstrates that disruption of spore wall production is not an inherently lethal event (Krisak *et al.*, 1994). The defective spores produced in many of these strains, for example *smk1*, are hypersensitive to environmental assault such as heat shock, Glusulase, or ether (Krisak *et al.*, 1994). Nonetheless, the spores are capable of germinating at near wild-type levels in the absence of external perturbation. In the case of class II *mps1* mutant strains, the spores produced at the restrictive temperature show low viability under all circumstances. An early observation with the use of DAPI staining (Figure 5) indicated that chromosomes in the *MPS1* mutant strains may be unequally partitioned to individual spores during meiosis.

To test directly the hypothesis that mutation in *MPS1* causes increased levels of chromosome missegregation in meiotically dividing strains, we used a system of chromosome III marked with a Lac operator array near the centromere and visualized by fluorescence microscopy with the use of a GFP–*LacI* repressor fusion (Straight *et al.*, 1996). As the homozygous *mps1-1237* mutant strains progressed through meiosis, it became evident that in many cases the labeled centromeres failed to segregate properly (Figure 7, A and B). By means of a combination of a single marked centromere of chromosome III and marked copies of both centromeres on homologous pairs of chromosome III, missegregation of chromosomes at either meiosis I (Figure 7A) or meiosis II (Figure 7B) was observed. Cells progressing through the first meiotic division are seen as binucleates by DAPI staining. GFP marking of both chromosome III homologues in binucleate cells demonstrated that meiosis I often segregated homologues to a single pole. Meiosis I and II missegregation, resulting in all four chromatids segregated to a single spore, was detected in *mps1-1237* cells, as shown in GFP/DIC panels (Figure 7A). To obtain a quantitative assessment of the level of missegregation in these cells, we used a single marked chromosome to monitor meiosis II specifically (Figure 7, B and C). More than half (62%) of the *mps1-1237* mutant cells that carry out both meiosis I and meiosis II and show at least four DAPI masses within the ascus inappropriately segregate sister chromatids during meiosis II (Figure 7B). In many cases, DAPI staining revealed chromosomal material outside the developing spore body. Moreover, in some of these cells, spore formation excluded the marked chromosomes (Figure 7, far right), leaving one or more GFP foci in the ascal or "epiplasmic" space. Therefore, it is probable that missegregation and misincorporation of chromosomes into spore bodies contributes to the loss of viability in cells harboring mutations at the *MPS1* locus.

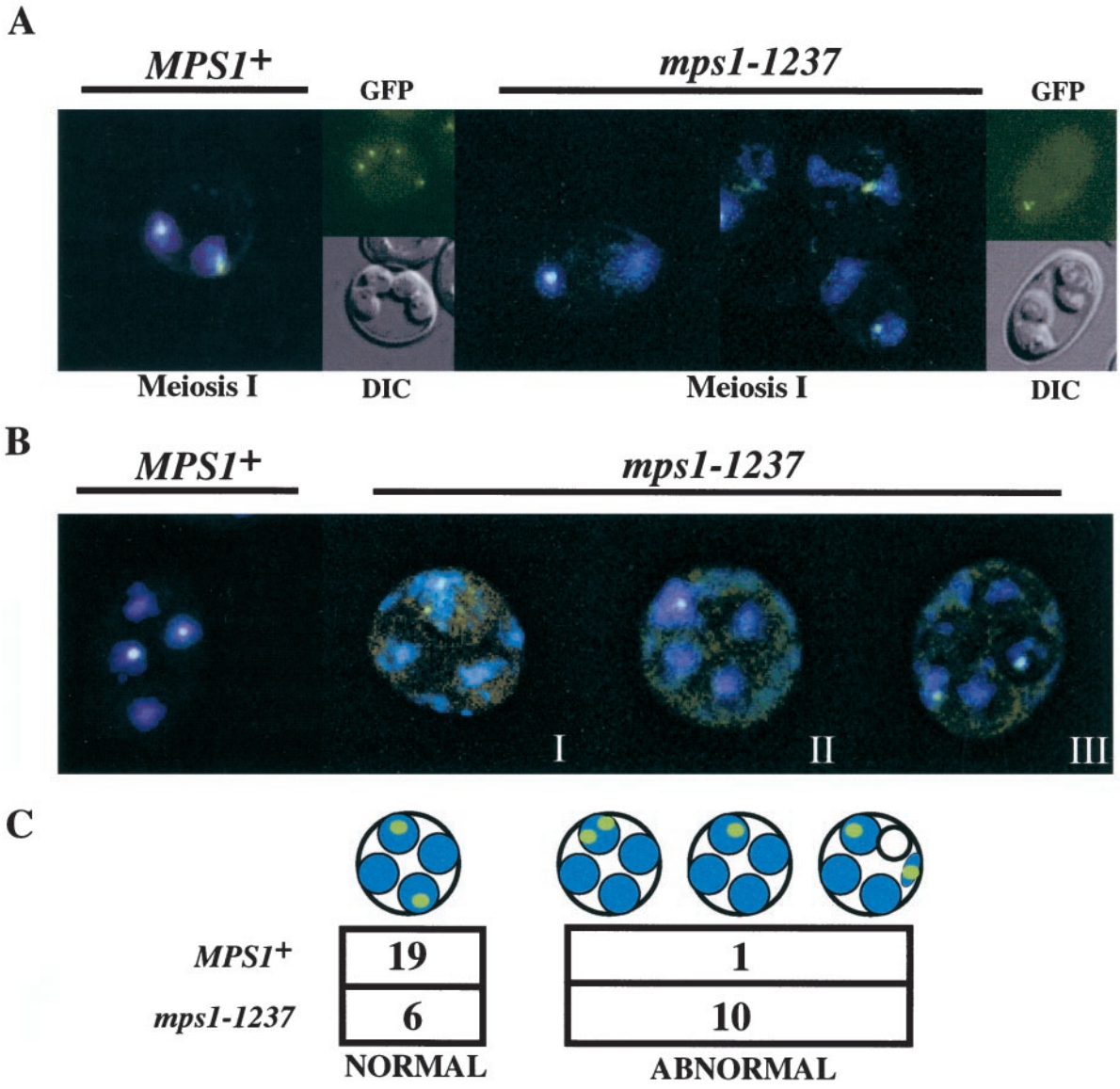

Figure 7. Loss of chromosome segregation fidelity in the class II *mps1* mutant strains. Chromosome segregation was monitored in *mps1-1237* and wild-type strains with the use of *LacO*pr-marked centromere III (*LEU2*) visualized with a GFP–LacI repressor protein fusion (Straight *et al.*, 1996). (A) Chromosome segregation during meiosis I was monitored with the use of marked homologous chromosomes (four chromatids). Binucleate cells in a sporulating population were examined for segregation of paired homologues to opposite poles. Left panels, wild-type binucleate (GFP/DAPI) and four-spored (GFP/DIC) asci; right panels, examples of meiosis I missegregation in *mps1-1237* binucleates (GFP/DAPI), which occurred in up to 80% of cells that entered meiosis I in a given experiment, and a four-spored ascus (GFP/DIC), showing no meiosis I or meiosis II segregation. (B and C) A single copy of chromosome III was marked to follow meiosis II (sister chromatid separation) specifically. Wild-type *MPS1* strains show four DAPI-staining spore nuclei, two of which contain copies of the marked chromosome III (left panel). Right panels, *mps1-1237* cells display a variety of aberrant segregation patterns. Three examples are shown. (I) Both marked centromeres reside in one of four detectable spore bodies. DAPI staining is seen within and outside of spore bodies. (II) A single GFP signal is associated with one of four DAPI-staining foci, suggesting that sister chromatids remain paired and unresolvable. (III) Many instances of DAPI staining outside of nascent spore bodies can be seen in *mps1* cells. In some cases, one of the marked chromatids is found outside any of the four spores. To quantitate missegregation in these cells, we restricted our analysis to the few mutant cells with a single pair of marked chromatids and at least four visible DAPI foci. This restriction allowed us to unambiguously assign normal versus abnormal segregation, but it also resulted in a small number of scoreable cells due to the percentage of segregation in the population and weakened expression of GFP in sporulating (autofluorescent) cells. Nonetheless, the difference between *MPS1* and *mps1-1237* is statistically significant (C; $p < 0.005$ by χ^2 analysis).

Table 2. Mutant alleles of *MPS1*

Allele	Subdomain[a]	Mutation[b]	Restrictive temperature		Phosphorylation[b,d]	Kinase activity[b,e]	Mitotic[b]		Meiotic[c]	
			S288c[b]	SK-1[c]			SPB duplication	Checkpoint	SPB duplication	Spore walls[c]
1	XI	C696Y	30°C	30°C	Reduced	Reduced	Fails	Fails	Fails	Defect
6	IX	C642Y	30°C	Lethal	Slight reduction	WT	Fails	Fails	N/D	N/D
412	VI	E546K	34°C	33°C	WT	Reduced	Fails	Fails	OK	Defect
737	III	E486K	34°C	Lethal	Slight reduction	WT	Fails[f]	Fails	N/D	N/D
1237	VIII	P608S	34°C	33°C	Slight reduction	WT	Fails	Fails	OK	Defect
3796	V	E517K	34°C	30°C	WT	Reduced	Fails	Fails	Fails[g]	Defect

WT, wild type; N/D, not detected.

[a] Hanks SK, Quinn AM, and Hunter T (1988).

[b] Schutz and Winey (1998).

[c] This study.

[d] Data from Western analysis of Mps1p banding patterns corresponding to phosphorylated forms of GST-Mps1p.

[e] In vitro kinase activity was monitored by autophosphorylation of GST-Mps1p.

[f] A distinct step of SPB duplication; see Schutz and Winey (1998).

[g] Intermediate phenotype.

DISCUSSION

The essential Mps1p kinase is a regulator of SPB duplication during mitosis (Winey *et al.*, 1991) and a component of the spindle assembly checkpoint in budding yeast (Hardwick *et al.*, 1996; Weiss and Winey, 1996). We have shown that mutation in the Mps1p kinase prevents wild-type levels of spore formation. Four alleles of *mps1*, previously described in the context of their mitotic phenotypes (Schutz and Winey, 1998), were studied, and their effects on meiosis revealed multiple phenotypes, indicating more than one function for the kinase during sporulation (Table 2). The multiple requirements for Mps1p during sporulation reflect functions either shared with mitosis (SPB duplication) or unique to sporulation (chromosome segregation and spore formation). The *mps1* mutations have no effect on premeiotic DNA synthesis, and the *mps1* strains show meiotic commitment to recombination. Nonetheless, Mps1p kinase is essential to the completion of meiosis and spore formation, as demonstrated by the severe loss of viable progeny in temperature-sensitive strains sporulated at the restrictive temperature.

The role of Mps1p in SPB duplication is retained during sporulation. The class I phenotypes resulting from the *mps1-3796* and *mps1-1* mutations are caused by a failure in SPB duplication early during meiosis. The end product of sporulation in this case is a mononucleate cell that proceeds through prophase I but fails to segregate chromosomes because of a lack of spindle formation. This phenotype contrasts with that of mutations in other known SPB duplication genes, such as *cdc31-1* and *ndc1-1* (Byers, 1981; Thomas and Botstein, 1986), that result in the production of viable dyad asci. It is possible that this phenotype is unique to the experimental conditions or the particular alleles of *cdc31* and *ndc1* used. In fact, cells harboring the *mps1-1* allele, when shifted to the restrictive temperature after 6 h of sporulation at the permissive temperature, generate many normal dyad asci, demonstrating a requirement for Mps1p during the second SPB duplication event. In addition to *CDC31*, *NDC1*, and *MPS1*, a meiosis-specific gene that plays a role in SPB duplication is *SPO1*. Deletion of the *SPO1* gene produces a predominantly monopolar phenotype during sporulation (Moens *et al.*, 1974; Tevzadze *et al.*, 2000). Unlike *MPS1*, *SPO1* is not essential for vegetative growth. Therefore, Mps1p is the only protein known to regulate mitotic SPB duplication that is also required during both meiotic SPB duplication events.

An unexpected second class of sporulation phenotypes arises in homozygous *mps1-1237* and *mps1-412* strains. In these strains, 30–50% of the cells in the population progress through both meioses, and the initiation of the spore wall formation process appears to occur normally. However, electron microscopy analysis showed that in these strains the morphology of the spore walls is abnormal, suggesting a defect in the assembly and maturation of spore wall components. A mid-meiotic function for Mps1p was predicted from the meiosis-specific transcriptional induction of *MPS1* (Poch *et al.*, 1994; Chu *et al.*, 1998). Furthermore, the abnormal spores produced are inviable because of extensive missegregation of chromosomes, indicating a role for Mps1p in spindle function during meiosis.

The morphological characteristics of the *mps1* class II spore defects are shared with several known regulators of spore wall formation. Northern analysis demonstrated that the *mps1* class II phenotype does not involve a delay or reduction in the expression of either of the key regulators, *SMK1* or *SWM1*. However, the abundance of the late meiotic marker transcript *SPS100* is dramatically reduced, suggesting that the Mps1p spore wall function is downstream or in a separate pathway from *SMK1* or *SWM1*. In addition to loss of *SPS100* transcription, the mid-late meiotic marker transcript *DIT1* is consistently more abundant in the *mps1-1237* strain than it is in wild-type strains, both phenotypes shared

with the *sps1* mutation. *SPS1* encodes a Ste20p kinase homologue that functions upstream of the Smk1p MAPK, but Sps1p is thought to have a Smk1p-independent function based on the observed loss of *DIT1* regulation (Friesen *et al.*, 1994; Krisak *et al.*, 1994). Therefore, the *mps1* class II defect reveals that Mps1p may function in regulating the late events of spore wall maturation in a pathway shared with Sps1p.

Disruption of spore wall formation is accompanied by a loss of viability in the class II *mps1* strains. Fluorescence microscopy of GFP-marked chromosomes demonstrated that the fidelity of chromosome segregation is compromised as a result of defective Mps1p. Chromosome missegregation at the level of a single pair of sister chromatids was quantitated as both chromatids segregated to a single pole during meiosis II. Also, aberrant patterns of chromosome segregation were detected during meiosis I. Extreme chromosome missegregation, revealed by marked chromosomes outside of spore bodies, indicates either lagging chromosomes during spore wall formation or a loss of coordination between spindle function and spore formation. The consequence of this disruption is a failure to position chromosomes in close proximity to the SPBs as spores are formed. These observations indicate a defect in spindle function that compromises the fidelity of chromosome segregation in class II strains. The genetic interaction of *MPS1* with *DAM1*, a spindle-associated protein that does not show a synthetic interaction with other spindle checkpoint genes, hints at a role for Mps1p in spindle function (developed further by Jones *et al.*, 1999). In the context of meiosis, this putative Mps1p function may be essential, a hypothesis requiring further experimentation.

The defects in chromosome segregation seen in *mps1* mutant strains are likely to be the primary cause of lethality in these strains, because spore wall defects do not result in severe loss of viability (Krisak *et al.*, 1994). The second mitotic function of Mps1p, a component of the spindle assembly checkpoint, could become an essential function during meiosis. A *MAD2*-dependent checkpoint monitors meiosis I specifically during sporulation (Shonn and Murray, 2000). In fact, cells lacking Mad2p undergo increased levels of meiosis I nondisjunction without spindle perturbation, indicative of an enhanced requirement for checkpoint function during meiosis I (Shonn and Murray, 2000). This first demonstration of the Mad2p-dependent checkpoint during meiosis reveals that the checkpoint is important but not absolutely required for all meioses. The meiotic checkpoint function of Mps1p cannot be tested with the existing alleles of *mps1* that exhibit multiple defects. However, we can conclude that Mps1p has a role in meiotic chromosome segregation separate from the Mad2p checkpoint based on the severity of chromosome missegregation during both meiosis I and meiosis II in *mps1* class II strains.

The two classes of *mps1* phenotypes demonstrate that Mps1p is an important regulator of multiple meiosis and spore formation processes: SPB duplication, high-fidelity chromosome segregation, and spore wall assembly. Although all three processes involve the SPB at some level, they are likely to require Mps1p independently. We have demonstrated that the class II mutant phenotype is not the result of a hypomorphic allele of *mps1*, and Northern data implicate Mps1p in transcriptional regulation of spore wall assembly. Furthermore, the spore wall defect is not likely a consequence of chromosome missegregation, because other mutations that lead to missegregation, such as recombination minus (rec$^-$) mutants (e.g., *rad50*Δ), produce normal spore walls, as does a *mad2*Δ strain. However, using the mutant alleles at hand, we cannot determine whether Mps1p carries out its spore wall function early in meiosis at the time of SPB duplication or if Mps1p interacts directly with the regulatory networks that control spore wall maturation late during sporulation. We favor the second model because the *MPS1* transcript accumulates during mid-meiosis, and our Northern data reveal a phenotype similar to *sps1*. Further experimentation examining the interaction of Mps1p with the late sporulation gene products (e.g., Sps1p, Smk1p) and possibly the generation of meiosis-specific separation-of-function alleles of *mps1* will likely resolve this issue.

In summary, this study demonstrates that the essential regulatory kinase Mps1p, which is required for mitotic SPB duplication and the activation of the spindle assembly checkpoint, is also essential for meiosis and spore formation. Furthermore, the protein is necessary for completion of multiple processes during sporulation, including SPB duplication, spore formation, and the fidelity of meiotic chromosome segregation. Future study of Mps1p function during meiosis may enhance our understanding of the regulatory networks that ensure that properly segregated chromosomes are incorporated into the specialized cell types of spores in yeast and gametes in other eukaryotes.

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