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Yeast Meiotic Mutants Proficient for the Induction of Ectopic Recombination

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ABSTRACT

A screen was designed to identify *Saccharomyces cerevisiae* mutants that were defective in meiosis yet proficient for meiotic ectopic recombination in the return-to-growth protocol. Seven mutants alleles were isolated; two are important for chromosome synapsis (*RED1*, *MEK1*) and five function independently of recombination (*SPO14*, *GSG1*, *SPOT8/MUM2*, *3*, *4*). Similar to the *spoT8-1* mutant, *mum2* deletion strains do not undergo premeiotic DNA synthesis, arrest prior to the first meiotic division and fail to sporulate. Surprisingly, although DNA replication does not occur, *mum2* mutants are induced for high levels of ectopic recombination. *gsg1* diploids are reduced in their ability to complete premeiotic DNA synthesis and the meiotic divisions, and a small percentage of cells produce spores. *mum3* mutants sporulate poorly and the spores produced are inviable. Finally, *mum4-1* mutants produce inviable spores. The meiotic/sporulation defects of *gsg1*, *mum2*, and *mum3* are not relieved by *spo11* or *spo13* mutations, indicating that the mutant defects are not dependent on the initiation of recombination or completion of both meiotic divisions. In contrast, the spore inviability of the *mum4-1* mutant is rescued by the *spo13* mutation. The *mum4-1 spo13* mutant undergoes a single, predominantly equational division, suggesting that *MUM4* functions at or prior to the first meiotic division. Although recombination is variably affected in the *gsg1* and *mum* mutants, we hypothesize that these mutants define genes important for aspects of meiosis not directly related to recombination.

MEIOSIS enables diploid organisms to reproduce sexually by generating haploid gametes through two successive divisions. At meiosis I, the reductional division, homologous chromosomes disjoin from each other. At meiosis II, as at mitosis, sister chromatids separate and move to opposite poles. The meiotic divisions, in turn, are tightly linked to gamete differentiation. Fusion of gametes at fertilization restores the diploid chromosome number and initiates zygotic development.

Once meiosis is initiated, DNA replication occurs. Several differences have been observed between premeiotic and mitotic DNA synthesis. For instance, in *Triturus vulgaris*, *Mus musculus,* and *Saccharomyces cerevisiae*, the length of premeiotic S phase is significantly longer than mitotic S (Callan 1972; Kofman-Alfaro and Chandley 1970; Williamson *et al.* 1983). The basis for the difference between premeiotic and mitotic DNA synthesis is unknown; in fact, studies in *S. cerevisiae* have shown that the DNA replication rate and the origins of replication used are the same in both replication modes (Johnston *et al.* 1982; Collins and Newlon 1994).

Premeiotic DNA synthesis is followed by a lengthy prophase in which homologous chromosomes pair and synapse and high levels of recombination occur. Chromosome pairing refers to the presynaptic alignment of the homologous chromosomes; this is temporally and genetically distinct from chromosome synapsis (Loidl *et al.* 1994; Weiner and Kleckner 1994). Chromosome synapsis is the intimate association of homologous chromosomes in the context of the synaptonemal complex (SC). The SC is a meiosis-specific structure that is elaborated along the lengths of the chromosomes during prophase (reviewed in von Wettstein *et al.* 1984). Although the relationship between chromosome pairing, synapsis, and recombination has not been precisely delineated, there is convincing evidence that recombination is initiated before the SC is fully formed; however, mature recombinants do not appear until after chromosome synapsis is complete (Padmore *et al.* 1991; Goyon and Lichten 1993). Reciprocal crossing over occurring in the context of the SC establishes physical connections between homologous chromosomes (Engebrecht *et al.* 1990). These connections, termed chiasmata, ensure proper spindle attachment and alignment on the metaphase plate (reviewed in Hawley 1987). At the end of prophase, the SC breaks down; however, the connections between homologous chromosomes are presumably maintained until anaphase when homologous chromosomes segregate to opposite poles.

At meiosis II, sister chromatids segregate from each other. The second meiotic division differs from mitosis,

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most basically, in that it follows meiosis I and is not directly preceded by a round of DNA replication. This requires that the mitotic cell-cycle controls that tightly regulate the ordered occurrence of S phase and chromosome segregation be significantly altered in meiosis (McCarroll and Esposito 1994). Finally, the two meiotic divisions must be temporally controlled and, in turn, coordinated with gamete differentiation.

Genetic analysis of meiosis in the yeast *S. cerevisiae* has significantly advanced our understanding of meiotic chromosome behavior (reviewed in Roeder 1995; Kleckner 1996). Meiotic mutants that perturb premeiotic DNA synthesis without affecting mitotic S phase have been isolated (*mei1*, *mei2,3*, Roth 1973; *spo7*, *spo9*, Esposito and Esposito 1974a; *spoT1-spoT11*, Tsuboi 1983); however, the functions of the corresponding gene products have not been elucidated. Consequently, little is known about the unique aspects of premeiotic DNA synthesis. In contrast, there are a large number of meiotic mutants that define genes required for chromosome synapsis and recombination (reviewed in Kupiec *et al.* 1997). These mutants have been identified in genetic screens that look for the production of inviable meiotic products or defects in meiotic recombination by using specially marked strains (reviewed in Loidl *et al.* 1997). Molecular and cytological analyses of the corresponding gene products have shown that they encode structural proteins of the SC and components of the recombination machinery (reviewed in Kupiec *et al.* 1997). Recently, a number of mutants have been identified that arrest prior to the meiosis I division (*ndt80*, Xu *et al.* 1995; *com1/sae2*, Prinz *et al.* 1997; McKee and Kleckner 1997a; *sae1*, *sae3*, McKee and Kleckner 1997b). Except for *ndt80*, the meiotic arrest observed in these mutants is dependent on the initiation of recombination, suggesting that these mutations define genes important for the completion of meiotic recombination.

We initiated a mutant hunt to isolate recombination-proficient meiotic yeast mutants by examining the induction of recombination between an endogenous locus and an artificial insert placed at a nonhomologous site in the genome (ectopic recombination). Previous studies in yeast have suggested that ectopic recombination is mechanistically similar to allelic recombination (Jinks-Robertson and Petes 1986; Lichten *et al.* 1987; Haber *et al.* 1991; Steele *et al.* 1991), although the frequency of ectopic recombination is influenced by the locations of the recombining sequences (Goldman and Lichten 1996). We reasoned that a subset of meiotic mutants proficient for the induction of ectopic recombination would define genes required for processes other than recombination and chromosome synapsis such as premeiotic DNA synthesis, chromosome pairing and coordination of meiosis and spore formation. In addition to isolating alleles of previously characterized genes that function independently of recombination (*SPO14*, Honigberg *et al.* 1992; Rose *et al.* 1995) and are important for chromosome synapsis (*RED1*, Rockmill and Roeder 1990; Smith and Roeder 1997; *MEK1*, Rockmill and Roeder 1991; Leem and Ogawa 1992), mutations in *GSG1* (Kaytor and Livingston 1995), *SPOT8/MUM2* (Tsuboi 1983), *MUM3*, and *MUM4* (*MU*ddled *M*eiosis) were identified and characterized.

MATERIALS AND METHODS

Yeast strains and genetic manipulations: Yeast media were prepared and genetic methods were carried out as described by Rose *et al.* (1990). YPAD is YEPD medium supplemented with 100 μM adenine. YPAD medium containing 0.02% MMS (methyl methanesulfonic acid; Sigma) was used to determine MMS sensitivity of candidate mutants. Yeast strains were transformed using the lithium acetate procedure of Ito *et al.* (1983). All integrative transformants were verified by Southern blot analysis (Southern 1975). All gene disruptions were performed by the one-step gene replacement described by Rothstein (1991).

The genotypes of yeast strains used in this study are listed in Table 1. JB128 was obtained from Jaya Bhargava, BR2171-7B from Beth Rockmill, and MTM-964 from Craig Giroux (Wayne State University, Detroit).

spo11::TRP1 (Y344) and *spo13::URA3* (Y686) derivatives of strain Y315 were made by transforming the haploid parents with pGB324, obtained from Craig Giroux, and pNKY58 (Alani *et al.* 1987), respectively, and then mating the transformants. Strains Y366, Y369, Y693, and Y1069 for the physical recombination assay were constructed by introducing *Xba*I-*Bgl*II digested pJH118 and pJH119 (Borts *et al.* 1986) into the corresponding haploids and mating the transformants.

Plasmid constructions: The original *GSG1*-complementing plasmid was designated pME556. The *gsg1::LYS2* allele was constructed in two steps. First, the *Bam*HI-*Pvu*II fragment from pME556 was inserted into *Hin*dIII-*Bam*HI digested pHSS6 after the *Hin*dIII end was filled in using the Klenow fragment of DNA polymerase I. The resulting plasmid, pME699, was restricted with *Kpn*I, followed by removal of the single-stranded ends with T4 DNA polymerase, and *Xba*I; the *Pvu*II-*Xba*I *LYS2* fragment from pDP6 (Fleig *et al.* 1986) was then inserted to create pME704. This plasmid was digested with *Not*I prior to transformation into yeast.

The original *MUM2*-complementing plasmid was designated pME759. A 3.5-kb *Bgl*II-*Sal*I fragment from pME759 was subcloned into the *Bam*HI-*Sal*I sites of pUN55 (Elledge and Davis 1988), generating pME897. A 2.5-kb *Hin*dIII-*Sal*I fragment from pME759 was ligated to *Hin*dIII-*Sal*I digested pUN105 (Elledge and Davis 1988) to create pME898. *MUM2* was moved into a *TRP1 CEN* vector, pUN15 (Elledge and Davis 1988), by inserting the 2.5-kb *Not*I-*Sal*I fragment from pME898 into the corresponding sites of pUN15; the resulting plasmid was designated pME1141. To create the *mum2::LEU2* allele in pME917, pME897 was digested with *Eco*RI and *Cla*I, followed by filling in the ends with the Klenow fragment of DNA polymerase I, and the *Hpa*I-*Nar*I *LEU2* fragment from YEp351 (Hill *et al.* 1986), whose ends had been filled in with the Klenow fragment of DNA polymerase I, was inserted. The *mum2::LYS2* allele in pME1026 was constructed by inserting the *Pvu*II-*Xba*I *LYS2* fragment from pDP6, whose ends had been filled in with the Klenow fragment of DNA polymerase I, into the *Eco*RI and *Cla*I sites of pME897, whose ends had also been filled in with the Klenow fragment of DNA

TABLE 1

Yeast Strains

Strain	Genotype
JB128	MATa leu2-3,112 ura3-Stu @ HIS4 HO trp1-1 ura3-1 thr1-4 ade2-1 lys2-1 MATα leu2-3,112 ura3-Stu @ HIS4 HO trp1-1 ura3-1 thr1-4 ade2-1 lys2-1
Y17	Same as JB128 but homozygous for red1-12
Y207	Same as JB128 but homozygous for mek1-10
YKR125	Same as JB128 but homozygous for spo14-3
Y195	Same as JB128 but homozygous for mum1-1/gsg1-2
YKR137	Same as JB128 but homozygous for mum2-2
YKR150	Same as JB128 but homozygous for mum3-1
YKR132	Same as JB128 but homozygous for mum4-1
JE626-19B	Same as JB128 but homozygous for gsg1::LYS2
Y601	Same as JB128 but homozygous for mum2::LEU2
Y901	Same as JB128 but homozygous for mum2::LYS2
Y1002	Same as JB128 but homozygous for mum3::LEU2-765
Y1078	Same as JB128 but homozygous for mum3::LYS2
BR2171-7B	MATa leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2-1 MATα leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2-1
KR1-20A	MATa leu2 HO trp1-1 ura3-1 thr1-4 ade2 lys2 spo14-3 MATα leu2 HO trp1-1 ura3-1 thr1-4 ade2 lys2 spo14-3
Y264	MATa leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2-1 gsg1-2 MATα leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2-1 gsg1-2
Y279	MATa leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2 lys2-1 mum2-2 MATα leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2 lys2-1 mum2-2
MTM4-964	MATa HO trp1 ura1 ade1 spoT8-1 MATα HO trp1 ura1 ade1 spoT8-1
Y278	MATa leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2-1 lys2 mum3-1 MATα leu2 HO trp1-1 ura3-1 arg4-8 thr1-4 ade2-1 lys2 mum3-1
Y133	MATa leu2 HO trp1-1 ura3-1 thr1-4 ade2 lys2-1 mum4-1 MATα leu2 HO trp1-1 ura3-1 thr1-4 ade2 lys2-1 mum4-1
Y315	MATa leu2-27 his4-260 trp1-1 ura3-1 ADE2 thr1-4 lys2 MATα leu2-3,112 his4-280 trp1-1 ura3-1 ade2 THR1 lys2
Y316	Same as Y315 but homozygous for gsg1::LYS2
Y690	Same as Y315 but homozygous for mum2::LEU2
Y792	Same as Y315 but homozygous for mum2::LYS2
Y1004	Same as Y315 but homozygous for mum3::LEU2-765
Y1041	Same as Y315 but homozygous for mum3::LYS2
Y344	Sams as Y315 but homozygous for spo11::TRP1
Y398	Same as Y344 but homozygous for gsg1::LYS2
Y692	Same as Y344 but homozygous for mum2::LEU2
Y1007	Same as Y344 but homozygous for mum3::LEU2-765
Y686	Same as Y315 but homozygous for spo13::URA3
Y896	Same as Y686 but homozygous for gsg1::LYS2
Y691	Same as Y686 but homozygous for mum2::LEU2
Y1010	Same as Y686 but homozygous for mum3::LEU2-765
Y366	Same as Y315 but MATa URA3 leu2-KpBR322 MATa / MATα URA3 leu2-RpBR322 MATα
Y369	Same as Y366 but homozygous for gsg1::LYS2
Y693	Same as Y366 but homozygous for mum2::LEU2
Y1069	Same as Y366 but homozygous for mum3::LYS2
J114	MATa CDC10 leu2 his4 / MATα cdc10 LEU2::pNH18 HIS4 trp1 ura3 can1 cyh2 ade2-1 spo13-1 sap3 lys2-99
Y302	Same as J114 but gsg1::LYS2
Y897	Same as J114 but mum2::LYS2
Y1037	Same as J114 but mum3::LYS2
Y1047	MATa leu2-3,112 HIS4 trp1-1 ura3-1 SPO13 thr1-4 ADE2 lys2-1 MUM4 MATα leu2-3,112 HIS4 trp1-28 ura3-1 spo13::URA3 THR1 ade2 lys2-1 mum4-1
Y1048	MATa leu2-3,112 HIS4 trp1-1 ura3-1 SPO13 thr1-4 ADE2 lys2-1 mum4-1 MATα leu2-3,112 HIS4 trp1-28 ura3-1 spo13::URA3 THR1 ade2 lys2-1 mum4-1

(continued)

TABLE 1

Continued

Strain	Genotype
Y1079	*MAT**a** LEU2 his4 trp1-1 ura3-1 spo13::URA3 thr1-4 ADE2 lys2-1 MUM4*
	MATα leu2-3,112 HIS4 trp1-28 ura3-1 spo13::URA3 THR1 ade2 lys2-1 mum4-1
Y1080	*MAT**a** LEU2 his4 trp1-1 ura3-1 spo13::URA3 thr1-4 ADE2 lys2-1 mum4-1*
	MATα leu2-3,112 HIS4 trp1-28 ura3-1 spo13::URA3 THR1 ade2 lys2-1 mum4-1

The following strains have been described previously: JB128 (Bhargava *et al.* 1992), BR2171-7B and J114 (Engebrecht and Roeder 1989), KR1-20A (Rose *et al.* 1995), MTM4-964 (Tsuboi 1983). Y17, Y207, YKR125, Y195, YKR137, YKR150, and YKR132 are mutant derivatives of JB128. JB128, JE626-19B, Y601, Y901, Y1002, and Y1078 are isogenic diploids. Y264, Y279, Y278, and Y133 are mutant segregants derived from crosses between Y195, YKR137, YKR150, and YKR132 to BR2171-7B, respectively. Y315, Y316, Y792, Y690, Y1004, Y344, Y398, Y692, Y686, Y896, Y691, Y366, Y369, Y693, and Y1069 are isogenic diploids derived by mating the corresponding haploids or transformants of the haploids. J114, Y302, Y897, and Y1037 are isogenic disomes. Y1047, Y1048, Y1079, and Y1080 are congenic diploids derived by mating the corresponding haploids.

polymerase I. Plasmids ME917 and ME1026 were digested with *Pvu*II to allow substitution of the *MUM2* locus in yeast.

The original *MUM3*-complementing plasmid contained a 11.5-kb *Sau*3A partial yeast genomic fragment at the *Bam*HI site of YCp50 and was designated pKR588. A 5-kb *Cla*I fragment that retained complementing activity was inserted into the *Cla*I site of pHSS6 (Seifert *et al.* 1986) to generate pKR718. This plasmid was subjected to transposon mutagenesis (Seifert *et al.* 1986) and a plasmid containing a transposon marked with the *LEU2* gene inserted at the 5′-end of the *MUM3* ORF, pKR765, was isolated. Plasmid KR765 was digested with *Not*I prior to transformation into yeast to allow substitution of the *MUM3* locus. The *mum3::LYS2* disruption allele was created in two steps. First, the 1.7-kb *Eco*RI-*Sal*I fragment from pKR765 was inserted into the corresponding sites in Bluescript SK+ (Stratagene) to create pME1133. The *LYS2* gene on a *Xba*I fragment, whose ends had been filled in with the Klenow fragment of DNA polymerase I, was inserted at the *Bgl*II site, whose ends had also been filled in with the Klenow fragment of DNA polymerase I, at position 851 of the *MUM3* ORF to create pME1138. Plasmid ME1138 was digested with *Apa*I and *Not*I prior to transformation into yeast.

Mutant screen protocol: Spores from JB128 were treated with 10 mm DTT and 1 mg/ml Zymolyase 100T for approximately three hours to remove the ascus cell wall from greater than 90% of the tetrads. The spores were collected, washed with water, resuspended in 1 ml of 0.01% Nonidet P-40 and sonicated on ice for 30 sec using a sonicator equipped with a microtip. The haploid spores were plated for single colonies onto solid YPAD medium and the plates immediately exposed to UV using a 8 watt long wave ultraviolet light source (350–400 nm). Exposure to UV was calibrated to obtain approximately 50% viability (5 sec of exposure at a distance of 12 inches from the source). Approximately 50,000 mutagenized colonies were replica-plated to sporulation medium to induce sporulation and after 3 to 4 days replica-plated to YPAD medium and immediately exposed to ether vapors as described by Rockmill and Roeder (1988). Briefly, the bottom of the YPAD plates were inverted over glass petri lids containing Whatman #1 filters soaked with ether (approximately 0.75 ml) for 15 min in a fume hood. Ether was reapplied to the filter in the lids and the cells were exposed for an additional 15 min. The bottom of the plates were then removed and placed ajar on their own lids for 30–60 min to allow the ether to dissipate. The plates were incubated overnight at 30° and the colonies were scored for growth the next day. Ether-sensitive mutants were streaked for single colonies and retested for ether-sensitivity after being induced for sporulation. Candidate mutants were analyzed by phase-contrast microscopy and/or tetrad dissection; 108 mutants were recovered. Ten strains were sensitive to MMS and not further characterized. The remaining mutants were assayed for commitment to meiotic ectopic recombination; twelve mutants displayed at least 50% of wild-type levels of ectopic recombination. These mutants were analyzed for meiotic progression as described below; five mutants appeared to undergo the meiotic divisions at normal levels and with normal kinetics yet failed to produce spores and were not further characterized.

Doubling times: Overnight cultures were diluted 1:200 in YPAD at a starting density of $\sim 5 \times 10^5$ cells/ml and grown with aeration at 30°. After 4 hr of growth, aliquots were removed every 2 hr and fixed with 3.7% formaldehyde. Each sample was counted three times in a hemocytometer and the mean cell density determined. Doubling times were calculated between two points in mid-log with the following formula: elapsed time divided by [log (fold increase)/log 2].

Flow cytometric analysis: Cells were grown to saturation in YPAD medium, collected by centrifugation, washed once, and resuspended in 2% potassium acetate medium at a cell density of $1–2 \times 10^7$ cells/ml. Cells were prepared for flow cytometric analysis as described (Sazer and Sherwood 1990; with modifications: http://flosun.salk.edu/fcm/protocols/ycc.html). At 5-hr intervals, 5 ml samples were collected, washed with dH_2O, resuspended in 70% ethanol and incubated for at least 12 hr at 4°. The cells were washed and resuspended in 50 mm sodium citrate containing 0.1 mg/ml RNase A and incubated for 8 hr at 34°. One volume of 50 mm sodium citrate containing 20 μg/ml propidium iodide was added and incubated overnight at 4°. The cells were sonicated prior to flow cytometry, which was performed on a Becton Dickinson FACScan. At least 8000 cells were analyzed per sample.

β-galactosidase assays: Assays were performed as described by Hollingsworth and Ponte (1997) using the *HOP1-LacZ* fusion constructed by Vershon *et al.* (1992).

Analysis of the meiotic divisions: Cells were grown and sporulated as described above. Aliquots from duplicate cultures were removed and fixed with 3.7% formaldehyde at the indicated times after transfer to sporulation medium. After 1 hr, the fixed cells were washed and resuspended in SHA (1 m sorbitol, 0.1 m HEPES, 5 mm NaN_3) buffer. The cells were stained with 4′,6-diamidino-2-phenyl-indole (DAPI) and analyzed by fluorescence microscopy as described (Rose *et al.* 1995). A minimum of 600 cells were examined at each time point.

Recombination frequencies: Yeast strains were grown to saturation in YPAD, and aliquots of each culture were plated onto YPAD medium and synthetic media lacking the appro-

priate amino acids to determine the mitotic prototroph frequencies. The values are the median frequency ± the standard deviation.

Meiotic recombination frequencies were determined by taking 1.5 ml from each saturated culture, washing once with dH_2O, resuspending the cells in 10 ml of 2% potassium acetate and incubating them at 30° with aeration. Aliquots of the sporulating cultures were removed after 15 hr for return-to-growth experiments or after 2 days (completion of sporulation) for plating onto YPAD and synthetic media lacking the appropriate amino acids. Prototroph formation was assayed in triplicate, and the mean meiotic frequencies ± the standard deviation were determined. The percent sporulation was monitored after 2 days in all cultures to ensure that meiosis had progressed as expected.

Physical recombination assay: Strains Y366, Y369, Y693, and Y1069 were used to perform the physical recombination assay (Borts *et al.* 1986). Sporulation, DNA isolation and analysis were performed as described (Sym *et al.* 1993). Cells were allowed to grow for 24 hr in medium lacking leucine after induction of meiosis for the return-to-growth samples. Audioradiography was quantitated on a Bio-Rad densitometer. The relative levels of the recombinant 16.2-kb band were calculated as follows: The volume of the recombinant band was divided by the volume of the corresponding parental band (P_1). The wild-type number was set at 1.0 and the level of mutant recombinants expressed as a fraction or percentage of wild type.

RESULTS

Isolation of mutants defective in meiosis: Yeast mutants defective in meiosis were isolated using a variation of a screen described by Rockmill and Roeder (1988). Meiotic defects in DNA synthesis, chromosome pairing, synapsis, recombination or segregation should result in either failure to complete meiosis or the random segregation of chromosomes resulting in lethality due to aneuploidy. Therefore, yeast mutants defective in meiosis should either fail to sporulate or produce inviable spores. However, mutants that fail to sporulate could also define genes required for entry into meiosis or for packaging the meiotic products into spores. To eliminate these latter classes from consideration, Rockmill and Roeder (1988) only examined mutants that produced inviable spores.

To identify novel genes required for meiosis, we screened for mutants that failed to complete sporulation or produced inviable spores, but were proficient for the induction of meiotic ectopic recombination using a specially marked strain. We reasoned that a subset of mutants identified in this screen would define genes important for aspects of meiosis other than recombination, while at the same time eliminate any candidate mutant that failed to enter meiosis. In addition, we examined the meiotic divisions in strains that failed to sporulate to eliminate mutants that completed meiosis normally yet were unable to package the meiotic products into spores.

The homothallic (*HO*) strain used for the mutagenesis, JB128, contains a mutant allele of the *URA3* gene (*ura3-1*) at its normal location on chromosome *V* and a different mutant allele (*ura3-Stu*) at the *HIS4* locus on chromosome *III* (Bhargava *et al.* 1992); ectopic recombination between these sequences results in the production of Ura prototrophs and provides an assay for meiotic recombination (Jinks-Robertson and Petes 1986; Lichten *et al.* 1987; Goldman and Lichten 1996). Induction of meiosis in this strain resulted in an approximately 50-fold increase in Ura prototrophs (7×10^{-5}) over mitotic levels (Table 2).

Spores from JB128 were mutagenized with ultraviolet light to 50% survival. The spores were allowed to germinate, undergo mating-type switching, and mate to form diploid colonies homozygous for any induced mutations. These colonies were sporulated and then exposed to ether vapors. Vegetative cells are more sensitive to ether vapors than spores (Dawes and Hardie 1974); therefore mutants that fail to sporulate or produce inviable spores are ether-sensitive. The sporulation defect was verified by phase-contrast microscopy and/or tetrad dissection. Of approximately 50,000 colonies screened, 108 mutants were recovered.

Many mutants defective in DNA repair produce inviable spores (*e.g.*, the *RAD50* epistasis group; Game 1983). These mutants are sensitive to gamma rays and MMS because they are unable to repair the DNA damage induced by these agents. To eliminate these genes from consideration, candidate mutants were tested for sensitivity to MMS; 98 strains that displayed wild-type resistance to MMS were further characterized.

Because the mutants we isolated either failed to sporulate or produced inviable spores, induction of meiotic ectopic recombination was assayed by examining Ura prototrophy in return-to-growth experiments (Esposito and Esposito 1974b). These experiments are performed by permitting yeast cells to initiate meiosis and then returning them to vegetative growth medium prior to the completion of the first meiotic division; the resulting cells are referred to as meiotOtic cells (Esposito and Esposito 1974b). This procedure induces meiotic levels of recombination; however, the mutants are returned to vegetative growth prior to the manifestation of the meiotic-lethal phenotype. Twelve mutants were induced to at least 50% of wild-type levels for meiotic recombination and were selected for further study.

To eliminate mutants that were defective in packaging the meiotic products into spores, we analyzed the meiotic divisions in candidate mutants that failed to sporulate by staining meiotic cultures with DAPI and examining the cells by fluorescence microscopy. Only mutants that displayed a defect in either the number of cells that were able to undergo the meiotic divisions or appeared to arrest prior to the completion of meiosis I or meiosis II were further characterized. Seven recombination-proficient mutants defective in meiosis were recovered. Figure 1 summarizes the strategy and results of the mutant screen.

TABLE 2

Phenotypes of meiotic mutants

Strain	Relevant genotype	Percent sporulation[a]	Percent spore viability[b]	Ura prototrophs ($\times 10^7$)	
				Mitotic[c]	Meiototic[d]
JB128	Wild type	65.1	96	8.6 ± 1.3 (1.0)	720 ± 210 (1.0)
Y17	*red1-12*	33	2	7.2 ± 1.6 (0.8)	390 ± 62 (0.5)
Y201	*mek1-10*	42	9	6.9 ± 1.5 (0.8)	410 ± 100 (0.6)
YKR125	*spo14-3*	<0.1	NA	9.7 ± 1.7 (1.1)	810 ± 206 (1.1)
Y195	*mum1-1/gsg1-2*	4.5	74	8.9 ± 0.9 (1.0)	370 ± 61 (0.5)
JE626-19B	*gsg1::LYS2*	3.2	75	7.6 ± 1.6 (0.9)	230 ± 75 (0.3)
YKR137	*mum2-2*	3.0	NA	6.7 ± 1.5 (0.8)	470 ± 91 (0.7)
Y601	*mum2::LEU2*	<0.1	NA	4.8 ± 1.2 (0.5)	240 ± 90 (0.3)
Y901	*mum2::LYS2*	<0.1	NA	5.0 ± 1.3 (0.6)	220 ± 73 (0.3)
YKR150	*mum3-1*	6.4	38	5.9 ± 1.0 (0.7)	410 ± 56 (0.6)
Y1002	*mum3::LEU2-765*	8.8	33	9.3 ± 2.2 (1.1)	600 ± 120 (0.8)
Y1078	*mum3::LYS2*	7.2	37	7.1 ± 0.8 (0.8)	580 ± 61 (0.8)
YKR132	*mum4-1*	27.0	33	5.7 ± 1.1 (0.7)	710 ± 148 (1.0)

[a] Percent sporulation was determined by phase-contrast microscopy; at least 300 cells were examined.
[b] Percent spore viability was determined by dissecting a minimum of 100 spores.
[c] Mitotic recombination frequencies ± SD represent the median frequency of nine independent cultures for strains JB128, YKR137, and Y901 and the median frequency for three independent cultures from strains Y195, JE626-19B, Y601, YKR150, Y1002, and YKR132. Numbers in parentheses are the frequency normalized to the frequency of the wild-type strain, JB128.
[d] The meiototic cultures were plated after 15 hr in sporulation medium and represent the mean value ± the SD from three independent cultures. JE626-19B, Y601, Y901, and Y1002 are transformants of JB128. The numbers in parentheses are the frequency normalized to the frequency of the wild-type strain, JB128.
NA = not applicable.

Complementation analysis of recovered mutants: To determine if the meiotic phenotypes of these strains were due to single mutations, sporulated cultures from the mutants were mated to spores from a wild-type *HO* strain, BR2171-7B and the heterozygotes selected for on appropriate medium. Although the mutants sporulated poorly and/or produced inviable spores, the ability to mate large numbers of cells allowed for the selection of the rare spores that were produced and viable. Multiple heterozygotes from each cross were sporulated and tetrads dissected. In all cases, the heterozygotes sporulated well, indicating that the mutations were recessive; however, spore viability varied among the different heterozygotes derived from the same mutant. This is most likely due to aneuploidy in the mutant spores that were crossed to the wild-type strain. The individual diploid spore colonies from crosses that exhibited high spore viability were sporulated and tested for ether sensitivity. A minimum of 10 four-spore-viable tetrads were examined from each cross and in every tetrad ether-sensitivity segregated 2:2, indicating that single mutations are responsible for the sporulation defects in these mutants.

To determine whether the mutations in these strains were allelic with previously identified genes, plasmid rescue experiments were performed by transforming the mutant strains with centromere plasmids carrying known meiotic genes that one might expect to find in this screen. We tested the ability of *HOP1* (Hollingsworth and Byers 1989), *RED1* (Rockmill and Roeder 1988), *MEK1* (Rockmill and Roeder 1991; Leem and Ogawa 1992), *DMC1* (Bishop *et al.* 1992), *ISC10* (Kobayashi *et al.* 1993), *MSH4* (Ross-Macdonald and Roeder 1995), *MSH5* (Hollingsworth *et al.* 1995), *ZIP1* (Sym *et al.* 1993), and *ZIP2* (P. Chua and G. S. Roeder, personal communication) to rescue the meiotic-lethal phenotype of the mutant strains. Strains harboring mutations in all of these genes either produce no or inviable spores and are induced to varying extents for meiotic intragenic recombination. One mutant, Y17, was rescued by *RED1* and another mutant, Y207, was rescued by *MEK1*; linkage studies confirmed that the corresponding mutations are alleles of *RED1* and *MEK1* (data not shown) and consequently were not further characterized. The wild-type gene responsible for the mutant phenotype of strain KR1-20A was identified; restriction map and complementation analyses demonstrated that it is the *SPO14* gene (Honigberg *et al.* 1992). Characterization of this allele, *spo14-3*, has been described (Rose *et al.* 1995).

Haploid segregants of each of the remaining mutants were generated by crossing sporulated cultures of each mutant to a haploid strain and selecting heterozygotes on the appropriate medium. The resulting diploids were sporulated and tetrads dissected. *HO* segregants were sporulated directly and subjected to ether

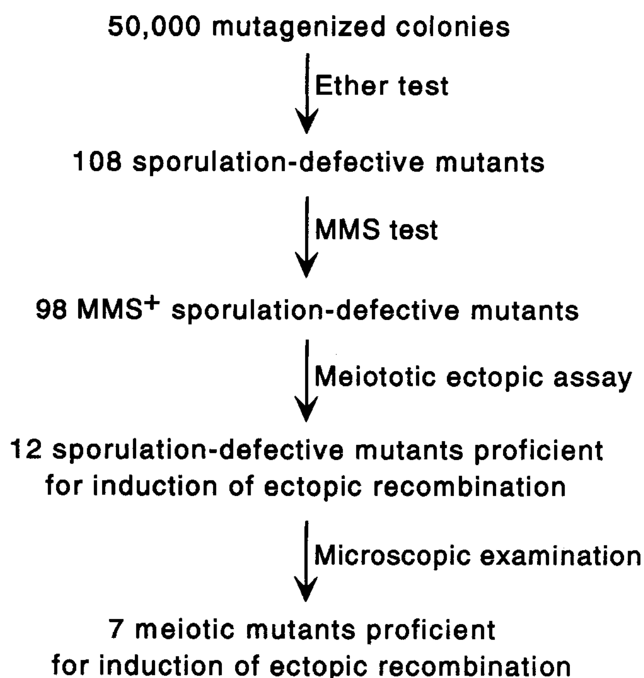


Figure 1.—Strategy and summary of mutant screen. The flow chart indicates the number of mutants at each stage of analysis. Meiototic refers to the return-to-growth protocol.

tests to determine if they harbored the mutation. Pairwise crosses were performed with the remaining haploid segregants and the resulting diploids were sporulated and subjected to ether tests to determine which haploids carried the mutation. Complementation tests were then performed with haploid mutant segregants; in all cases, the mutations complemented each other, indicating that four complementation groups are represented. We have designated the genes responsible for the mutant phenotypes *MUM1, 2, 3, 4* (for *MU*ddled *M*eiosis). The phenotypes of the mutants isolated in this screen are shown in Table 2. These mutants are proficient for meiototic ectopic recombination and either fail to sporulate or produce spores with a reduced frequency and display varying degrees of spore inviability.

***mum1-1* is an allele of *GSG1*:** MUM1 was cloned from a yeast genomic library in a centromere plasmid (Rose *et al.* 1987) by complementation of the ether-sensitivity of strain Y264. Five Ura$^+$ transformants, of approximately 5000 screened, produced spores at high frequency. Spontaneous mitotic Ura$^-$ segregants no longer produced spores. The same plasmid (pME556) was recovered from all transformants and shown to be responsible for the rescue of the meiotic defect. Subclone analysis localized the complementing activity to a 3-kb *Bam*HI-*Xba*I fragment. Strains carrying deletion alleles of these sequences (see below) were mated to a strain containing the original mutant allele, *mum1-1*; the resultant diploid failed to sporulate, indicating that the sequences represent the *MUM1* gene.

Sequence analysis revealed that the *MUM1* gene is identical to the chromosome *IV* ORF, YDR108w, identified in the *S. cerevisiae* genome project. The 2094-bp open reading frame encodes a 77-kDa protein with no homology to any known proteins. In the course of this study, *MUM1* was independently identified and named *GSG1* (Kaytor and Livingston 1995). Consequently, we have designated our original UV-induced allele *gsg1-2* and will hereafter refer to *MUM1* as *GSG1.*

Kaytor and Livingston (1995) reported that a homozygous *GSG1* deletion strain has no noticeable growth defect but fails to sporulate. Furthermore, premeiotic DNA synthesis is delayed and reduced in *gsg1* mutants and the sporulation defect is not rescued by the introduction of the *spo13* mutation (Kaytor and Livingston 1995). However, meiotic progression and recombination were not monitored in these *gsg1* strains.

We constructed a deletion allele of *GSG1* marked with the *LYS2* gene *in vitro* using the cloned gene (pME704; see materials and methods). Similar to what was observed by Kaytor and Livingston (1995), strains homozygous for *gsg1::LYS2* grew with rates similar to an isogenic wild-type strain (doubling times: *gsg1*, 98 min; wild type, 99 min). While the ability of *gsg1* strains to sporulate was greatly impaired (3.2% for *gsg1 vs.* 65.1% sporulation for the isogenic wild-type strain; Table 2), spores were produced and viable (75% for *gsg1 vs.* 96% spore viability in the isogenic wild-type strain; Table 2). Consistent with the analysis of our original mutant isolate, the *gsg1* deletion strain was induced for meiotic ectopic recombination (Table 2). The difference observed in the ability of *gsg1* deletion mutants to sporulate between this and the previous study (Kaytor and Livingston 1995) most likely reflects strain background differences.

***mum2-1* is an allele of *SPOT8*:** *MUM2* was cloned from a yeast genomic library in a centromere plasmid (Rose *et al.* 1987) by complementation of the sporulation defect of strain Y279. One Ura$^+$ transformant of approximately 5000 screened produced spores at high frequency. Spontaneous mitotic Ura$^-$ segregants no longer produced spores. A single plasmid (pME759) was recovered and shown to be responsible for the rescue of the meiotic defect. Sequences derived from this plasmid were subcloned and targeted for integration into the yeast genome (Rothstein 1991). Spores from a *HO* strain marked with *URA3* at these sequences were mated to a sporulated culture from a *HO mum2-1* strain and heterozygotes selected. The resulting diploid was sporulated and tetrads dissected. All 15 four-spore-viable tetrads displayed a parental configuration for Ura prototrophy and ether-sensitivity, indicating that the marked sequences are tightly linked to the *mum2-1* mutation.

Subclone and sequence analysis demonstrated that the *MUM2* gene corresponds to YBR057c on the right arm of chromosome *II* (Feldmann *et al.* 1994). This lo-

cation places *MUM2* in proximity to the *spoT8-1* mutation on the genetic map. The *spoT8-1* strain was isolated as a sporulation-deficient mutant that fails to undergo premeiotic DNA synthesis (Tsuboi 1983). Similar to *spoT8-1*, analysis of premeiotic DNA synthesis revealed that *mum2* mutants failed to replicate their DNA during meiosis (see below). Therefore, we tested whether the *MUM2* gene on a *CEN* plasmid could rescue the sporulation defect of the *spoT8-1* temperature sensitive mutant, MTM-964. Approximately 50% of the cells produced spores at the restrictive temperature in the *spoT8-1* strain harboring the *MUM2* plasmid when induced for meiosis compared with no spores in the same strain harboring the vector alone. Taken together, these data indicate that *MUM2* is the gene responsible for the mutant phenotype of the *spoT8-1* strain. As *spoT* does not conform to standard nomenclature we have adopted *MUM2* as the locus designation.

The *MUM2* gene contains a 1098-bp ORF that encodes a 41-kD protein. The C terminus of the protein is predicted to form a coiled coil based on the Coils Program (Lupas *et al.* 1991).

MUM2 deletions marked with *LEU2* (pME917) and *LYS2* (pME1026) were constructed *in vitro* using the cloned gene. A strain homozygous for the *mum2::LEU2* deletion grew at rates similar to an isogenic wild-type strain (doubling times: *mum2*, 103 min; wild type, 99 min). Unlike the original UV induced allele, *mum2* deletion strains did not produce any spores (Table 2), suggesting that *mum2-1* is a leaky allele. In addition, while ectopic recombination was stimulated to wild-type levels in the *mum2-1* strain, ectopic recombination was reduced approximately threefold relative to wild type in the corresponding *mum2* deletion strains (Table 2).

Identification of the *MUM3* gene: *MUM3* was cloned from a yeast genomic library in a centromere plasmid (Rose *et al.* 1987) by complementation of the sporulation defect of strain YKR150. One Ura^+ transformant of approximately 10,000 screened produced spores at high frequency. Spontaneous mitotic Ura^- segregants no longer produced spores. A single plasmid (pME588) was recovered and shown to be responsible for the rescue of the sporulation defect. Sequences derived from this plasmid were subcloned and targeted for integration into the yeast genome (Rothstein 1991). Spores from a *HO* strain marked with *URA3* at these sequences were mated to spores from a *HO mum3-1* strain and heterozygotes selected. The resulting diploid was sporulated and tetrads dissected. Of 17 four-spore-viable tetrads, all displayed a parental configuration for Ura prototrophy and ether-sensitivity, indicating that the marked sequences are tightly linked to the *mum3-1* mutation.

Subclone and sequence analysis demonstrated that the *MUM3* gene corresponds to YOR298w on chromosome *XV*. YOR298w does not correspond to any previously identified gene; the sequence does not display similarity to proteins in the Genbank Database.

Disruptions of the *MUM3* gene marked with *LEU2* (pME765) and *LYS2* (pME1138) were constructed. Strains homozygous for the *mum3* disruption alleles grew at rates similar to an isogenic wild-type strain (doubling times: *mum3*, 95 min; wild type, 99 min). When transferred to sporulation medium, *mum3* mutants sporulated poorly and produced inviable spores (Table 2). In addition, ectopic recombination was stimulated to the same extent as wild type in strains carrying the different *mum3* alleles (Table 2).

Complementation tests with *mum4-1*: Several genomic libraries have been screened for sequences that complement the spore inviability of *mum4-1* mutants; however, to date, no complementing plasmids have been identified. Therefore, we determined if the spore inviability defect of a *mum4-1* mutant could be rescued with plasmids that contain additional yeast genes known to be important for meiosis. Centromere plasmids containing *MER1*, *MER2*, *REC102*, *XRS2*, *MEI4*, *SPO11*, *SPO13*, and *SPO12* failed to rescue the spore inviability of *mum4-1*, indicating that *mum4-1* is not an allele of these genes. Although the phenotype of a *mum4-1* mutant suggests it is not an allele of *REC103*, *REC104*, *REC114* (Malone *et al.* 1991), *MRE2*, *MRE11* (Ajimura *et al.* 1993), *COM1/SAE2* (Prinz *et al.* 1997; McKee and Kleckner 1997a), *SAE1*, *SAE3* (McKee and Kleckner 1997b), *NDT80* (Xu *et al.* 1995), *NDJ1/TAM1* (Conrad *et al.* 1997; Chua and Roeder 1997), or *MEI5* (Giroux *et al.* 1993), we can not presently rule out this possibility.

Premeiotic DNA synthesis: To determine if *gsg1* or the *mum* mutants undergo premeiotic DNA synthesis, DNA content of yeast cells induced for sporulation was analyzed by flow cytometry. Wild-type yeast cells entered meiosis with a G1 content of DNA, corresponding to the 2N peak (Figure 2). After approximately 10 hr in sporulation medium in this strain background, there was an increase in the 4N peak, corresponding to cells that had undergone premeiotic DNA synthesis. The number of cells with a 4N content of DNA continued to increase until 20 hr after transfer to sporulation medium. As shown in Figure 2, premeiotic DNA synthesis was variably affected in the mutants. In comparison to wild type, only a small percentage of *gsg1* cells underwent premeiotic DNA synthesis. Consistent with the analysis of *spoT8-1*, *mum2* deletion mutants failed to replicate their DNA. The number of *mum4-1* cells that underwent premeiotic DNA synthesis was slightly reduced, while *mum3* mutants replicated their DNA as well as wild type.

The failure of the *mum2* mutant to undergo premeiotic DNA synthesis raised the possibility that meiosis is not initiated in these strains. To eliminate this possibility, we performed two experiments. First, we analyzed DNA content and induction of ectopic recombination from the same culture; ectopic recombination was induced even though DNA replication did not occur (data not shown). Second, we examined the expression

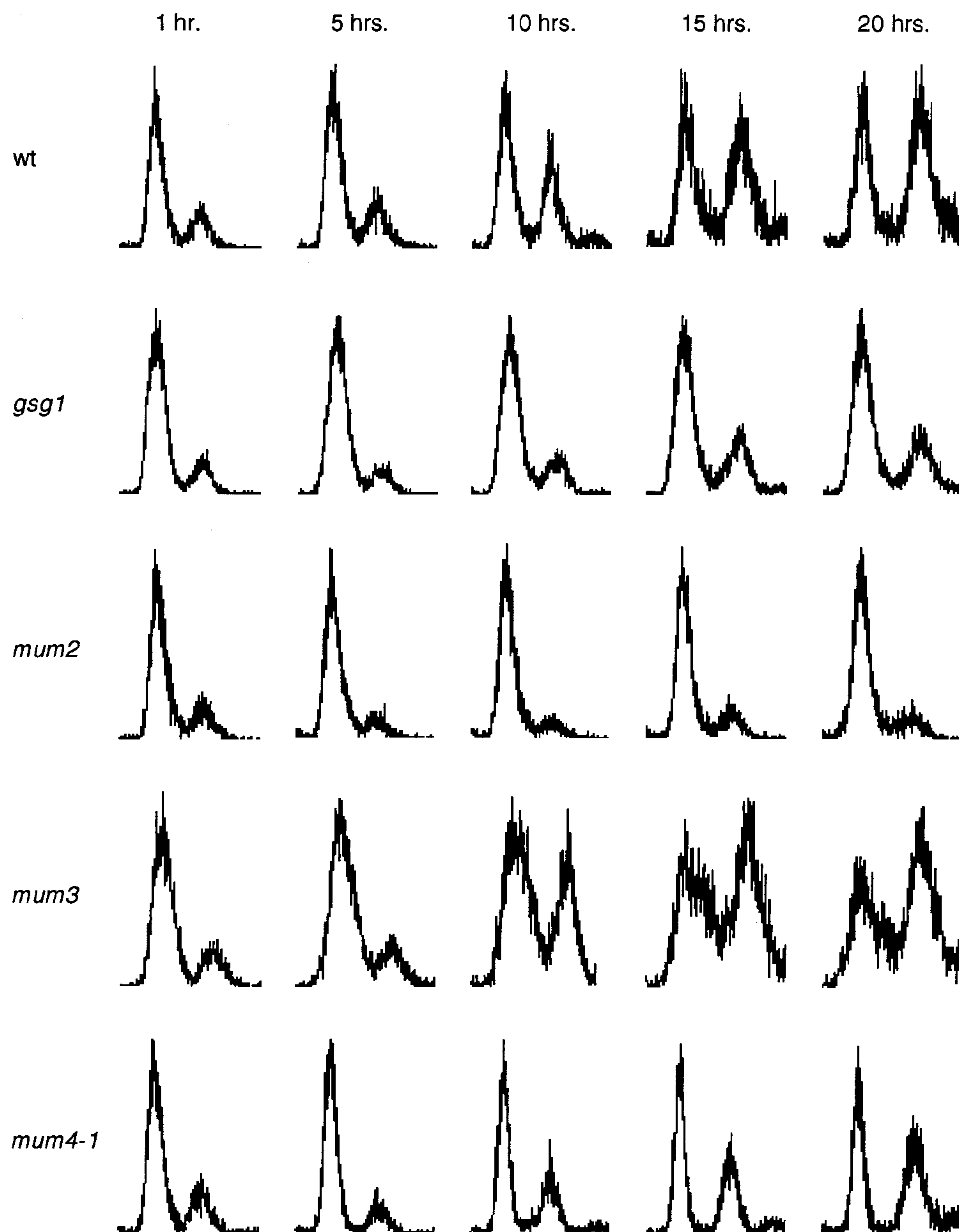
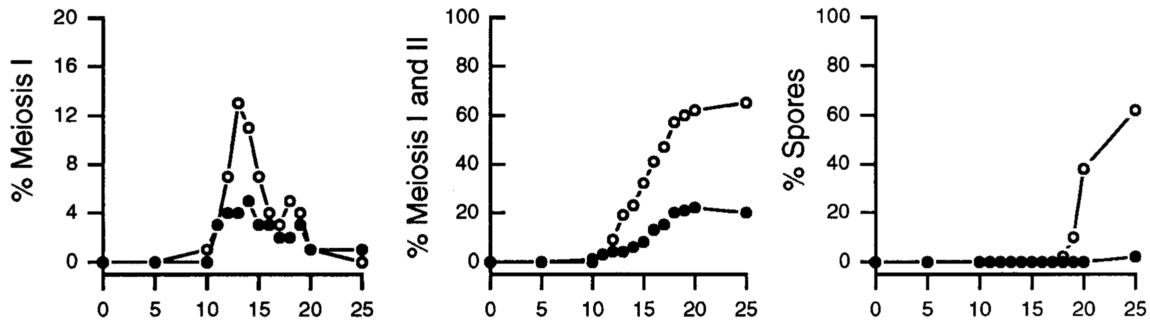
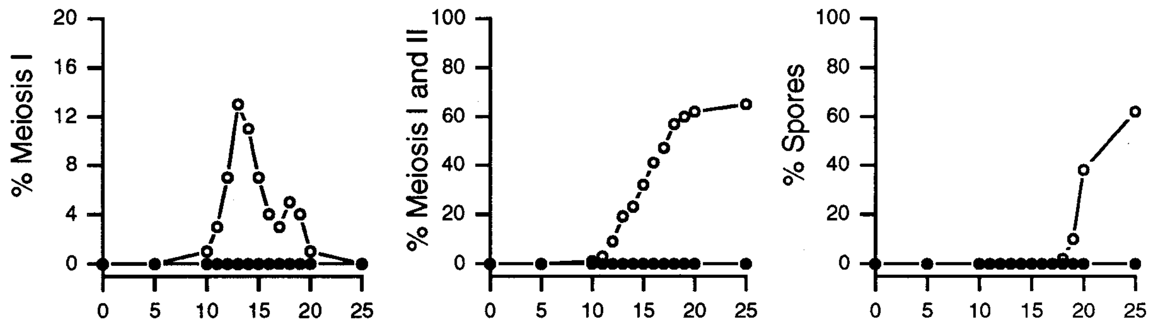


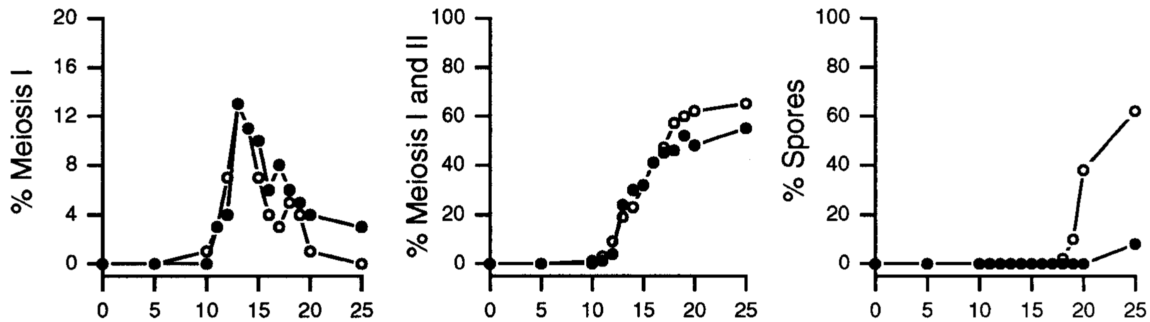
Figure 2.—Premeiotic DNA synthesis in wild-type and homozygous *gsg1::LYS2*, *mum2::LYS2*, *mum3::LEU2-765*, and *mum4-1* mutants. Cellular DNA content was assayed by flow cytometry at the indicated times after induction of sporulation. Left and right peaks of each histogram represent cells with 2N and 4N DNA content, respectively.

of the *HOP1* gene in a *mum2* mutant. *HOP1* encodes a meiosis-specific component of the SC; *HOP1* is not expressed in vegetative cells but is specifically induced in prophase of meiosis I (Hollingsworth *et al.* 1990; Smith and Roeder 1997; Vershon *et al.* 1992). Wild-type and *mum2* strains harboring a *HOP1-LacZ* fusion on a 2-μ plasmid (Vershon *et al.* 1992) were assayed for β-galactosidase activity in vegetative cells and upon transfer to sporulation medium. As expected, no activity was detected in vegetative cells; however, β-galactosidase activity was induced in both wild-type and *mum2* cells transferred to sporulation medium (data not shown). Taken together, these results indicate that meiosis is induced in *mum2* mutants.

Meiotic progression: To examine the meiotic divisions in *gsg1* and *mum* mutants, diploids were induced to undergo meiosis and at various time-points, cells were fixed, stained with the DNA-specific dye, DAPI, and examined by fluorescense microscopy. Spore formation was simultaneously monitored by phase contrast microscopy. At 10 hr after transfer to sporulation medium, binucleate cells were observed in wild-type cells, representing the completion of the first meiotic division. The level of binucleated cells peaked at 13 hr and then began to decline. Tri- and tetranucleated cells, representing the completion of the second meiotic division, were observed at 13 hr after transfer to sporulation medium and the levels continued to rise







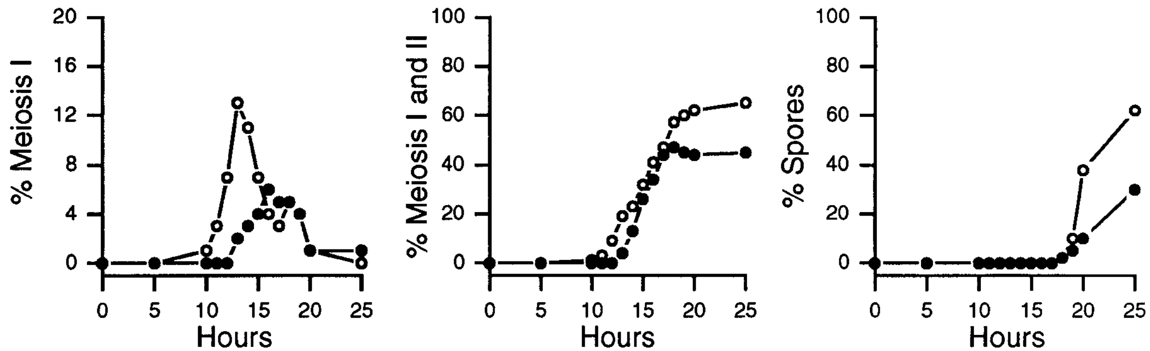


Figure 3.—Meiotic progression in homozygous *gsg1::LYS2* (A), *mum2::LYS2* (B), *mum3::LEU2-765* (C) and *mum4-1* (D) mutants. The left graph of each panel shows the average % of cells from two independent cultures completing meiosis I, monitored by fluorescence microscopy, in mutant (closed circles) and the isogenic wild-type strain (opened circles) plotted against time (Hours) in sporulation medium. The middle graph shows the sum of the average % of cells from two independent cultures completing meiosis I and meiosis II, monitored by fluorescence microscopy, in mutant (closed circles) and the isogenic wild-type strain (opened circles) plotted against time. The graph on the right shows the average % of asci, monitored by phase microscopy in mutant (closed circles) and wild type (open circles) plotted against time. At least 600 nuclei were counted at each time point.

throughout the experiment. At 18 hr, tetrads were observed by phase contrast microscopy (Figure 3). In *gsg1* mutants induced for meiosis, there was a reduction in the number of cells that progressed through the first meiotic division, a further reduction in the number of cells able to progress through the second meiotic division and only a small fraction formed mature asci (Figure 3A). In contrast, only uninucleated cells were observed in *mum2* mutants, indicating that *mum2* cells arrest prior to the meiosis I division (Figure 3B). The timing and levels of the meiotic divisions appeared to occur normally in *mum3* mutants although there was a reduction in the number of spores that were packaged (Figure 3C). Finally, *mum4-1* mutants were delayed approximately 3 hr relative to wild type for the meiosis I division. The second meiotic division and spore formation appeared to occur with normal kinetics in *mum4-1* but at a reduced frequency relative to wild type (Figure 3D).

Epistasis analysis: To further define the stage at which these genes function, epistasis analysis was undertaken with meiotic mutations that define genes required for recombination and one of the meiotic divisions. *spo11* mutants sporulate; however, the spores produced are inviable due to severe defects in recombination (Klapholz *et al.* 1985). A subset of meiotic mutants fail to sporulate due to a cell cycle checkpoint that monitors recombination (Lydall *et al.* 1996). These mutants are able to sporulate in the presence of a *spo11* mutation since meiotic recombination is not initiated (*i.e.*, *dmc1*; Bishop *et al.* 1992). The sporulation defect of *gsg1*, *mum2*, or *mum3* mutants was not relieved by introduction of a *spo11* mutation (Table 3). In addition, the meiotic divisions in the double mutants look similar to the single mutants (Table 3). These results suggest that *GSG1*, *MUM2*, and *MUM3* function is not dependent on the initiation of meiotic recombination.

spo13 mutants perform only a single division resulting in the production of two diploid spores (Klapholz and Esposito 1980); in our strain background, this single division is predominately equational (Engebrecht and Roeder 1989). Many mutants defective in processes necessary for the proper completion of the first reductional division produce viable spores in a *spo13* background (*i.e.*, *spo11*, *rad50*, *red1*, *hop1*; reviewed in Petes *et al.* 1991). Analysis of *gsg1 spo13* and *mum3 spo13* strains revealed that these mutants sporulated as inefficiently as *gsg1 SPO13* and *mum3 SPO13* strains and viability among the rare spores was not improved (Table 3). The introduction of a *spo13* mutation did not allow *mum2* mutants to divide or sporulate (Table 3), indicating that in the absence of *MUM2* neither of the meiotic divisions can occur.

In contrast to the other double mutants, *mum4-1 spo13* mutants produced viable spores (Table 3). Analysis of the products of the *mum4-1 spo13* meiosis revealed that they primarily undergo equational divisions (see below; Table 6), indicating that *MUM4* functions at or before the first meiotic division.

Meiotic recombination: We examined meiotic recombination using a number of different assays in strains harboring *gsg1*, *mum2*, and *mum3* alleles. The effect of a

TABLE 3

Epistasis analysis

Strain	Relevant genotype	Percent meiotic divisions[a]	Percent sporulation[b]	Percent spore viability[c]
Y315	*GSG1, MUM*	71	87	97
Y344	*spo11::TRP1*	60	52	1
Y686	*spo13::URA3*	62	56	75
Y316	*gsg1::LYS2*	23	4	71
Y389	*gsg1::LYS2 spo11::TRP1*	25	3	<1
Y896	*gsg1::LYS2 spo13::URA3*	7	2	35
Y690	*mum2::LEU2*	<0.1	<0.1	NA
Y692	*mum2::LEU2 spo11::TRP1*	<0.1	<0.1	NA
Y691	*mum2::LEU2 spo13::URA3*	<0.1	<0.1	NA
Y1004	*mum3::LEU2-765*	50	7	32
Y1007	*mum3::LEU2-765 spo11::TRP1*	43	8	<1
Y1010	*mum3::LEU2-765 spo13::URA3*	44	6.5	24
Y1047	*MUM4 SPO13*	57	69	92
Y1048	*mum4-1 SPO13*	48	48	10
Y1079	*MUM4 spo13::URA3*	29	68	83
Y1080	*mum4-1 spo13::URA3*	41	73	71

[a] Percent meiotic division includes bi-, tri-, and tetranucleated cells and was determined by fixing cells after 20 hr in sporulation medium, staining with DAPI, and examining by fluorescence microscopy. A minimum of 300 cells were examined.

[b] Percent sporulation was determined by phase-contrast microscopy; at least 300 cells were counted.

[c] Percent spore viability was determined by dissecting a minimum of 100 spores.

NA = not applicable.

TABLE 4

Allelic recombination in *gsg1*, *mum2* and *mum3* mutants

Strain	Relevant genotype	His prototrophs (×10⁴)		Leu prototrophs (×10⁵)	
		Mitotic[a]	Meiototic[b]	Mitotic[a]	Meiototic[b]
Y315	*GSG1, MUM*	3.4 ± 1.3 (1.0)	84.0 ± 15 (1.0)	1.4 ± 0.7 (1.0)	690.0 ± 110 (1.0)
Y316	*gsg1::LYS2*	2.1 ± 0.9 (0.6)	34.0 ± 11 (0.4)	1.5 ± 0.8 (1.1)	200.0 ± 71 (0.3)
Y792	*mum2::LYS2*	0.8 ± 0.3 (0.2)	4.8 ± 1.4 (0.06)	0.4 ± 0.1 (0.3)	1.7 ± 1.2 (0.002)
Y1004	*mum3::LYS2*	2.7 ± 1.2 (0.8)	65.0 ± 14 (0.8)	1.1 ± 0.6 (0.8)	610.0 ± 110 (0.9)

His and Leu prototrophs were selected in diploids carrying heteroalleles at the *HIS4* and *LEU2* loci.

[a] Mitotic recombination frequencies are median frequencies ± SD derived from three independent cultures for strains Y316 and Y1004 and nine independent cultures from strains Y315 and Y792. Numbers in parentheses are the mitotic frequency normalized to the wild-type strain, Y315.

[b] The meiotic frequency represents the mean frequency from three independent cultures plated after 15 hr in sporulation medium. Numbers in parentheses are the meiototic frequency normalized to the wild-type strain, Y315. Y316, Y792, and Y1004 are transformants of Y315.

gsg1, *mum2*, or *mum3* mutation on allelic intragenic recombination was examined in diploid strains that carry heteroalleles at the *HIS4* and *LEU2* loci; His and Leu prototrophic recombinants result primarily from gene conversion (Fogel and Hurst 1967). Wild-type and mutant cells were transferred to sporulation medium and then returned to vegetative medium in return-to-growth experiments. As shown in Table 4, induction of meiotic prototroph formation in *gsg1* mutants was close to the wild-type levels (a two- to threefold decrease is observed) and occurred at wild-type levels in *mum3* mutants.

The mitotic frequency of His and Leu prototrophs was significantly lower in the diploid *mum2* Y792 strain compared to the isogenic wild-type strain. Meiotic prototroph formation in *mum2* mutants was reduced 18-fold at the *HIS4* locus and 406-fold at the *LEU2* locus compared to the isogenic wild-type strain (Table 4) at 15 hr after transfer to sporulation medium. The decrease in meiotic recombination in these strains was not simply due to the reduced basal levels, since the fold induction was also reduced [His prototrophs: 25-fold (wt) *vs.* 6-fold (*mum2*) over the mitotic frequency; Leu prototrophs: 492-fold (wt) *vs.* 5-fold (*mum2*) over the mitotic frequency]. Thus, allelic recombination is perturbed in *mum2* mutants.

The effect of a *gsg1*, *mum2*, or *mum3* mutation on induction of meiotic intrachromosomal recombination was measured in *spo13* haploid strains disomic for chromosome *III* using the assay developed by Hollingsworth and Byers (1989). One of the chromosome *III* homologs contains a duplication of 11.4 kb of DNA between *HIS4* and *LEU2*; these repeats flank inserted vector DNA including the *URA3* and *CYH2* genes. Cells that have lost the *CYH2* marker as a result of reciprocal recombination between the duplicated elements can be selected on medium containing cycloheximide, due to a recessive mutation conferring cycloheximide-resistance at the *CYH2* locus on chromosome *VII*. As *spo13* does not rescue the sporulation defect of these mutants, induction of intrachromosomal recombination was also measured in return-to-growth experiments. In this assay, the *gsg1::LYS2*, *mum2::LYS2*, and *mum3::LYS2* strains displayed a level of recombination that was indistinguishable from wild type (Table 5).

Reciprocal recombination in the *gsg1*, *mum2*, and *mum3* mutants was examined by monitoring the physical exchange of DNA molecules using the system developed by Borts *et al.* (1986). In this assay, diploids carrying restriction site polymorphisms generate novel restriction fragments as a result of reciprocal exchange (Figure 4A). Genomic DNA was isolated from wild-type and mutant cells before entry into meiosis and 24 hr after induction of meiosis. DNA samples were then digested and analyzed by Southern blot hybridization using a probe that detects both parental and recombinant fragments. The recombinant 29-kb fragment is not resolved from the

TABLE 5

Intrachromosomal recombination in *gsg1*, *mum2* and *mum3* mutants

Strain	Relevant genotype	Cyh^R frequency (×10⁴)	
		Mitotic[a]	Meiototic[b]
J114	*GSG1, MUM*	4.2 ± 0.3 (1.0)	12 ± 2.3 (1.0)
Y302	*gsg1::LYS2*	3.5 ± 0.7 (0.8)	13 ± 2.1 (1.1)
Y897	*mum2::LYS2*	4.0 ± 0.7 (0.9)	12 ± 1.7 (1.0)
Y1037	*mum3::LYS2*	3.0 ± 0.2 (0.7)	12 ± 3.5 (1.0)

Cyh^R recombinants were selected on medium as described by Hollingsworth and Byers (1989).

[a] Mitotic frequencies of Cyh^R recombinants are the median ± SD from three independent cultures for strains Y302 and Y1037 and from nine independent cultures for strains J114 and Y897. Numbers in parentheses are the frequency normalized to the wild-type strain, J114.

[b] The meiototic frequencies of Cyh^R recombinants are the mean frequency ± SD from three independent cultures plated at 15 hr after induction of meiosis. Numbers in parentheses are the frequency normalized to the wild-type strain, J114. Y302, Y897, Y1037 are transformants of J114.

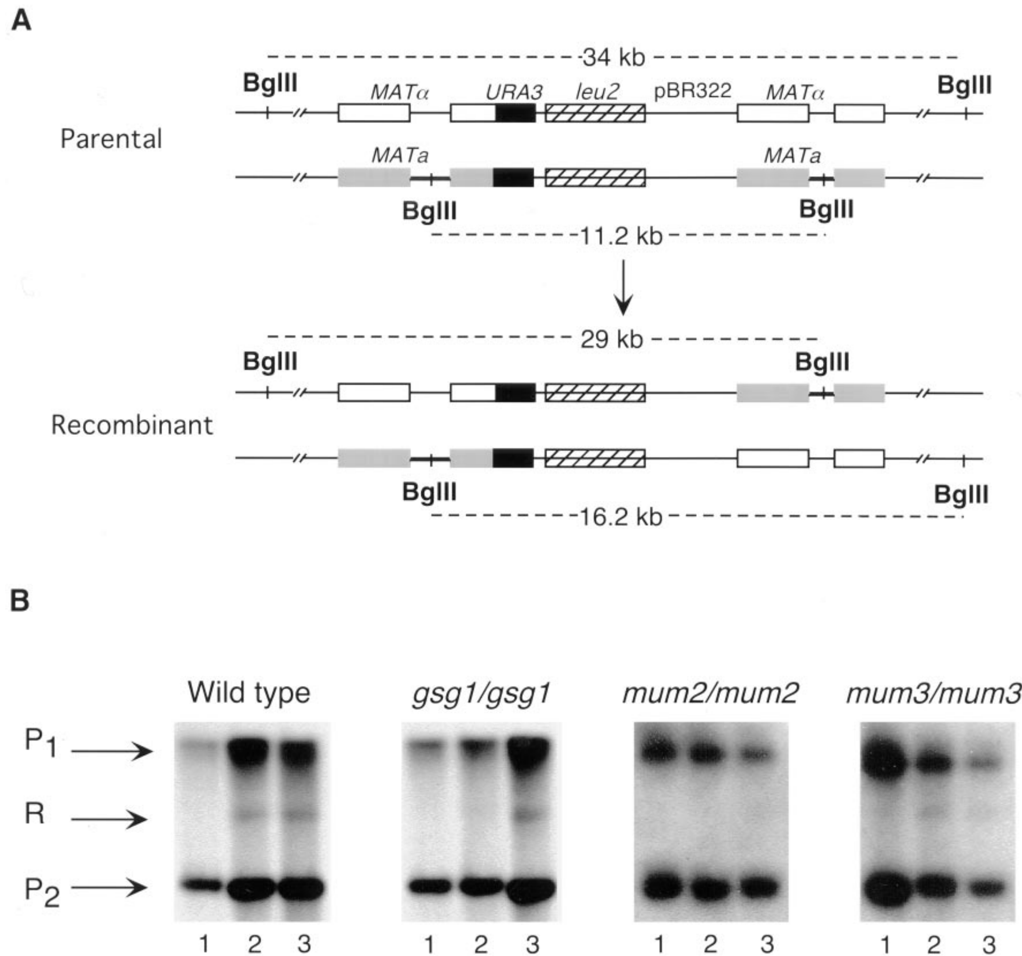


Figure 4.—Reciprocal recombination in wild-type, *gsg1::LYS2*, *mum2::LYS2*, and *mum3::LYS2* mutants. (A) Diagramatic representation of parental and recombinant chromosomes in strains used for the physical detection of exchange (Borts *et al.* 1986). The *Bgl*II (BglII) sites and predicted sizes of the *Bgl*II fragments are indicated. *MAT*α (open rectangles), *URA3* (black rectangles), *leu2* (hatched rectangles), and *MAT***a** (grey rectangles) are shown. (B) Autoradiograph of a Southern blot. P_1 (34 kb) and P_2 (11.2 kb) indicate the position of the parental bands and R (16.2 kb) indicates the recombinant band (the 29 kb recombinant band is not resolved from the parental 34 kb fragment). Lane 1: DNA isolated from vegetative cells; lane 2: DNA isolated from meiotic cells and lane 3: DNA isolated from cells that had been induced to undergo meiosis and then returned to vegetative growth for 24 hrs before DNA isolation.

34-kb parental fragment; consequently, the appearance of recombined DNA is monitored by the appearance of the 16.2-kb fragment (Borts *et al.* 1986; Figure 4). A meiotic recombinant band was present in wild-type and *mum3* cells at similar levels (1.0 *vs.* 0.93 for wild type and *mum3*, respectively); however, reciprocal recombinants were reduced in *gsg1* cells (0.4) and not detectable in *mum2* cells at this time in meiosis (Figure 4B, lane 2).

Meiotic intragenic and intrachromosomal recombination in these mutants had been measured in return-to-growth assays (see above). Therefore, DNA was also isolated from cells that had been induced in meiosis and returned to mitotic growth before DNA isolation. This experiment revealed that physical recombinants were observed at 70% and 83% of the wild-type level upon return to mitotic growth in *gsg1* and *mum3* strains, respectively (Figure 4B, lane 3). A recombinant band was detected in DNA isolated from *mum2* cells that had been allowed to return to growth at approximately 10% of the wild-type level (Figure 4B, lane 3).

Analysis of meiotic recombination in *mum4-1* mutants was made possible by the high spore viability exhibited by *mum4-1* strains in the presence of the *spo13* mutation. Congenic *spo13* strains homozygous or heterozygous for *mum4-1* were used to assay gene conversion at the *TRP1* locus by random spore analysis and crossing over on chromosome *III* by dyad dissection. Mitotic and meiotic levels of Trp prototrophs were determined and the results presented in Table 6. Similar to ectopic recombination between the *URA3* alleles (Table 2), allelic recombination at the *TRP1* locus occurred at the wild-type level in *mum4-1* mutants.

Reciprocal crossing over on chromosome *III* occurred at close to wild-type levels in *mum4-1* mutants (Table 6). The map distances for wild-type and *mum4-1* homozygous diploids were 37 cM and 32 cM for the *LEU2-MAT* interval and 16 cM and 16 cM for the *LEU2-HIS4* interval, respectively. In addition, analysis of the segregation pattern of the centromere linked marker, *TRP1*, indicated that *mum4-1 spo13* strains primarily undergo equational divisions. Given the *spo13* rescue of *mum4-1*, these data suggest that *MUM4* acts at or before the first meiotic division but independently of recombination.

DISCUSSION

In most organisms, recombination is essential for proper chromosome segregation at the meiosis I divi-

TABLE 6

Recombination in *mum4-1 spo13* strains

A. Allelic recombination

Strain	Relevant genotype	Trp prototrophs (×10^6) Mitotic[a]	Trp prototrophs (×10^6) Meiotic[b]
Y1079	*MUM4 spo13::URA3*	6.8 ± 0.9 (1.0)	130 ± 36 (1.0)
Y0180	*mum4-1 spo13::URA3*	6.3 ± 0.7 (0.9)	160 ± 27 (1.2)

B. Reciprocal recombination

		Chromosome *III*		Chromosome *IV*
Strain	Relevant genotype	*MAT-LEU2* distance (cM)[c]	*HIS4-LEU2* distance (cM)[d]	Percent equational segregation[e]
Y1079	*MUM4 spo13::URA3*	37	16	89
Y1080	*mum4-1 spo13::URA3*	32	16	90

[a] Mitotic recombination frequencies are median frequencies ± SD derived from three independent cultures. Numbers in parentheses are the mitotic frequency normalized to the wild-type strain, Y1079.

[b] The meiotic recombination frequency ± SD represent the mean frequency from three independent cultures plate after 48 hr in sporulation medium. Numbers in parentheses are the meiotic frequency normalized to the wild-type strain, Y1079.

[c] Single crossover recombinant dyads for the *MAT-LEU2* interval had the following phenotypes: a mater Leu+: α mater Leu$^+$ and nonmater Leu$^-$: nonmater Leu$^+$. The dyads with the phenotype a mater Leu$^-$: α mater Leu$^+$ were scored as four-strand double crossovers. Dyads in which *MAT* and *LEU2* have retained the parental configuration of markers probably arose from a reductional division that had not undergone exchange in this interval. The alternative possibility is that these dyads arose from a two-strand double crossover followed by equational division. If this were true, then an equal number of dyads displaying four-strand double crossovers and equational division would be predicted. There were significantly fewer of these types (1 *vs.* 10). Therefore, many of these dyads are probably the products of reductional chromosome segregation.

[d] Single crossovers in the *LEU2-HIS4* interval produced dyads with the following phenotypes: Leu$^-$ His$^+$: Leu$^+$ His$^+$ and Leu$^+$ His$^-$: Leu$^+$ His$^+$. Dyads exhibiting a mater: nonmater or α mater: non mater were scored as nondisjunctants (Klapholz and Esposito 1980) and were not included in the calculation of map distances. Map distance was calculated by using a derivation of Perkins' formula (Perkins 1949) as follows: map distance = [single crossovers + 6(4-strand double crossovers)/total] × 100. Scoring was as follows: 107 dyads were scored for Y1079 and 96 dyads were scored for Y1080.

[e] Equational segregation was determined by examining the Trp phenotype of the dyad spore colonies as follows: equational segregation = Trp^{pap}: Trp^{pap}; reductional segregation = Trp$^-$: Trp$^-$; aberrant segregation = Trp^{pap}: Trp$^-$. Seven percent of dyads from Y1079 and four percent of dyads from Y1080 exhibited aberrant segregation.

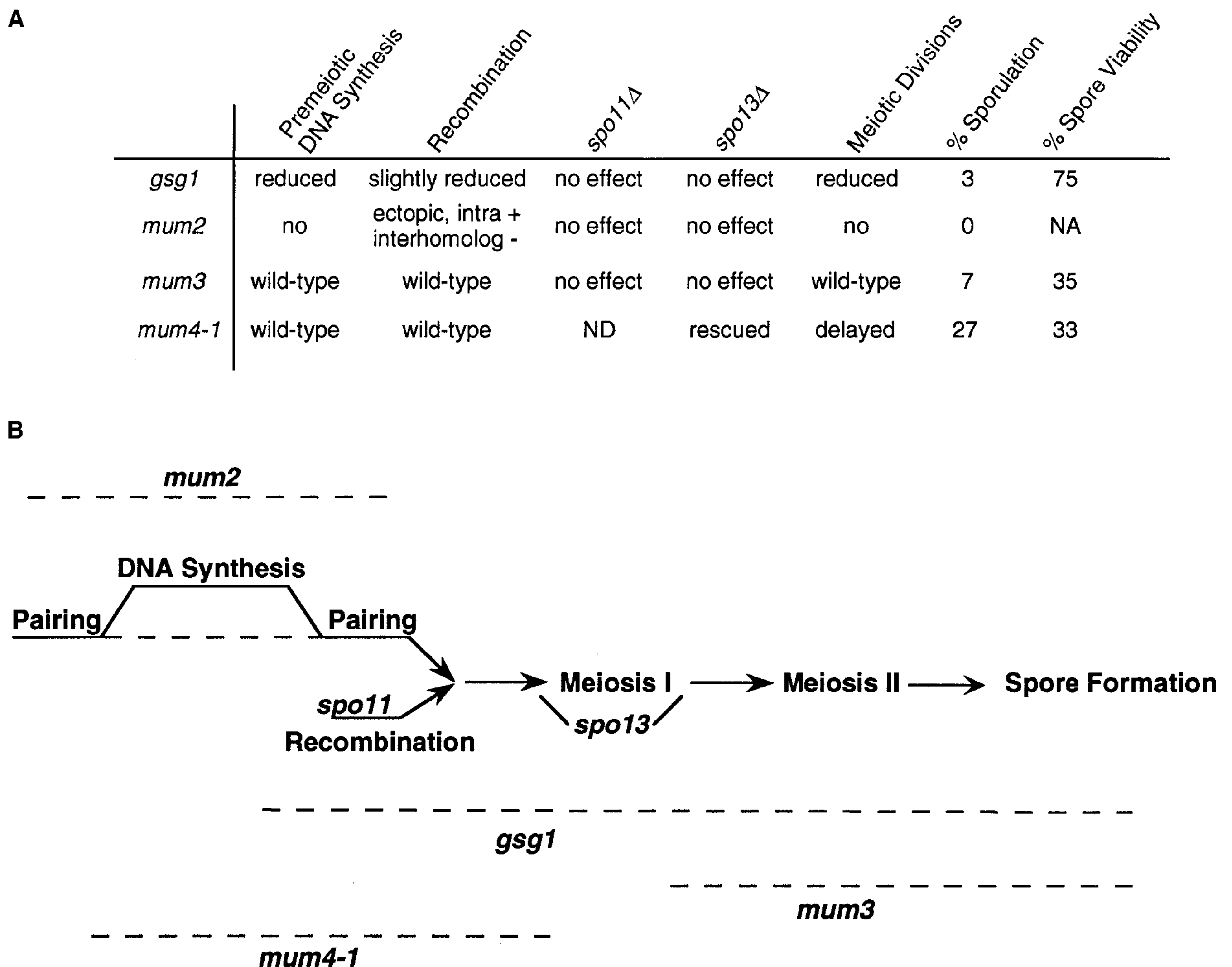
sion (reviewed in Hawley 1987). In *S. cerevisiae*, genetic screens and selections have been designed to identify meiotic mutants that, for the most part, perturb meiotic recombination. Consequently, most of these mutants define genes important for meiotic recombination and chromosome synapsis. To identify other processes important for meiosis, we initiated a mutant hunt to identify genes required for meiosis that function independently of recombination. The screen was designed to isolate mutants that either failed to sporulate or produced inviable spores, but were proficient for the induction of meiotic ectopic recombination. Of the seven mutants isolated, five appear to define genes important for processes distinct from genetic recombination (*SPO14*, Honigberg *et al.* 1992; Rose *et al.* 1995; *GSG1*, *MUM2*, *MUM3*, *MUM4*, Figure 5A). The other two mutants we identified carry alleles of *MEK1* (Rockmill and Roeder 1991; Leem and Ogawa 1992) and *RED1* (Rockmill and Roeder 1988). *RED1* encodes a component of the SC (Smith and Roeder 1997) and *MEK1* encodes a protein kinase that displays genetic interactions with *RED1* and *HOP1* (Rockmill and Roeder 1991; Hollingsworth and Ponte 1997), another SC component (Hollingsworth *et al.* 1990). Strains harboring *red1* and *mek1* mutations display substantial levels of meiotic recombination; therefore it is not surprising that alleles of these genes were isolated.

Recently, genetic screens to identify mutants whose meiotic arrest is dependent on the initiation of recombination have been designed and genes important for completion of recombination identified (McKee and Kleckner 1997a; Prinz *et al.* 1997; McKee and Kleckner 1997b). Similar to this class of mutants, *gsg1*, *mum2*, and *mum3* mutants fail to sporulate or sporulate very poorly; however, in contrast to these mutants, their defect in meiotic progression is not relieved by the in-

troduction of the *spo11* mutation. Spo11p catalyzes meiosis-specific double strand breaks, the initiators of recombination (Keeney *et al.* 1997). The failure of the sporulation defect in these mutants to be rescued by the introduction of a *spo11* mutation suggests that these mutations define genes important for processes independently of recombination (Figure 5).

Mutants specifically defective in premeiotic DNA synthesis have been previously isolated in yeast (Roth 1973; Esposito and Esposito 1974a; Tsuboi 1983); however, the corresponding gene products have either not been identified or remain uncharacterized. One of these, *spoT8-1*, is allelic with *mum2*. Genetic analysis of *spoT8-1* demonstrated that these mutants fail to undergo premeiotic DNA synthesis, the meiotic divisions and spore formation (Tsuboi 1983).

Strains harboring *mum2* null alleles are induced for meiosis yet fail to replicate their DNA and arrest prior to the first meiotic division. Recombination between chromosomes is reduced in both vegetative and meiotic cells, while intrachromosomal recombination occurs at wild-type levels in *mum2* deletion strains. Furthermore, in the assays examined, allelic recombination is more perturbed than ectopic recombination although we cannot eliminate the possibility that this is due to allele or strain differences. Interestingly, the observed induction of recombination in *mum2* mutants indicates that the initiation of meiotic recombination is not dependent on the completion of premeiotic DNA synthesis (Figure 5). However, failure to undergo premeiotic DNA synthesis does prevent the meiotic divisions from occurring.



A

	Premeiotic DNA Synthesis	Recombination	spo11Δ	spo13Δ	Meiotic Divisions	% Sporulation	% Spore Viability
gsg1	reduced	slightly reduced	no effect	no effect	reduced	3	75
mum2	no	ectopic, intra + interhomolog -	no effect	no effect	no	0	NA
mum3	wild-type	wild-type	no effect	no effect	wild-type	7	35
mum4-1	wild-type	wild-type	ND	rescued	delayed	27	33

Figure 5.—The effect of mutations of genes isolated in this screen on the major landmarks of meiosis. (A) Summary of mutant phenotypes. The chart shows the phenotypes of *gsg1*, *mum2*, *mum3*, and *mum4-1* with respect to premeiotic DNA synthesis, recombination, *spo11* and *spo13* epistasis, the meiotic divisions, sporulation and spore viability. ND = not determined; intra = intrachromosomal; NA = not applicable. (B) Based on genetic and epistasis analyses, *mum2* mutants perturb the early events of chromosome pairing and premeiotic DNA replication. In contrast, *gsg1* and *mum3* mutants affect multiple processes independent of recombination, while the *mum4-1* mutant affects a process at or before the first meiotic division.

mum2 mutants grow at a rate indistinguishable from wild type, suggesting that mitotic DNA synthesis occurs normally. However, the decrease in mitotic recombination between homologous chromosomes in *mum2* mutants indicates that *MUM2* functions in vegetative cells. The ability to replicate DNA during the mitotic cycle and undergo normal levels of intrachromosomal recombination imply that *mum2* mutants are not defective in either DNA replication or the recombination machinery *per se* and suggest that *MUM2* functions in a process that affects both premeiotic DNA synthesis and recombination between chromosomes.

Cytological analysis of yeast chromosomes have revealed that homologous chromosomes are paired before meiosis; chromosome pairing is lost during premeiotic DNA synthesis and reestablished after the completion of DNA replication (Weiner and Kleckner 1994). Weiner and Kleckner (1994) argue that mitotic and meiotic pairing are mechanistically similar. As *mum2* mutants are perturbed for mitotic interchromosomal recombination as well as premeiotic DNA synthesis and meiotic interchromosomal recombination, we favor the hypothesis that *MUM2* is important for both chromosome pairing and premeiotic DNA synthesis. However, our genetic data cannot distinguish between a direct or indirect role for *MUM2* in either of these processes. Examination of chromosome behavior in *mum2* mutants and cytological analysis of Mum2p should help clarify the role of this protein in the early events of chromosome pairing and premeiotic DNA synthesis.

In contrast to *mum2*, strains harboring mutations in *GSG1* and *MUM3* do not display an uniform arrest but affect multiple processes required for the proper formation of viable spores. Furthermore, epistasis analysis suggests that the *GSG1* and *MUM3* gene products function independently of recombination and a single meiotic division.

During the course of these experiments, *GSG1* was independently identified as a secondary mutation in a strain carrying a suppressor of *rad52* (Kaytor and Livingston 1995). *RAD52* is important for DNA repair and both mitotic and meiotic recombination (Game 1983), consequently, *rad52* mutants produce inviable spores. Kaytor and Livingston (1995) reported that *gsg1* mutants are delayed for premeiotic DNA synthesis and do not sporulate. In addition, the *GSG1* gene is expressed in a similar manner to *RAD52* in that *GSG1* RNA is present in vegetative cells and RNA levels increase approximately twofold during meiosis (Kaytor and Livingston 1995). As both *GSG1* and *RAD52* are required for meiosis and display a similar expression pattern, Kaytor and Livingston (1995) suggested that there is a functional link between these two genes. However, our epistasis studies and analysis of recombination in *gsg1* mutants makes it unlikely that *GSG1* plays a direct role in meiotic recombination.

Genetic analysis of the *mum4-1* mutant suggests that *MUM4* is important for the successful completion of the first meiotic division. *mum4-1* mutants are rescued by the introduction of the *spo13* mutation and the double mutant undergoes a primarily equational division. Until the corresponding gene has been identified, we can not rule out the possibility that *mum4-1* is an allele of a previously characterized gene. However, the phenotype of this mutant allele is distinct from mutations in other genes that function at or before the first meiotic division. Most notably, at all loci examined, *mum4-1* mutants appear to undergo wild-type levels of meiotic recombination. Although we cannot exclude the possibility that the *mum4-1* mutation is a leaky allele and that strains that carry a deletion of the gene will display effects on recombination, it seems likely that *MUM4* may mediate a process distinct from recombination that is required for proper chromosome segregation at the meiosis I division.

Given the spectrum of the phenotypes of the mutants isolated, it is not surprising that this screen was not saturated. We did not isolate multiple alleles of any of the genes we identified nor alleles of genes that we predict should have been isolated. For instance, *zip1* (Sym *et al.* 1993), *zip2* (Chua and Roeder, personal communication), *msh4* (Ross-Macdonald and Roeder 1995), and *msh5* (Hollingsworth *et al.* 1995) mutants display close to wild-type levels of intragenic recombination and fail to sporulate (*zip1* and *zip2*) or reduce the number of viable spores (*msh4* and *msh5*) but were not isolated in this screen.

While this screen did not isolate mutations in a specific aspect of meiosis, analysis of the mutants identified suggests that early events in meiotic chromosome metabolism, independent of the initiation of meiotic recombination, play key roles in meiosis. Furthermore, processes are occurring parallel to the initiation of meiotic recombination and the reductional division that are important for the production of viable meiotic products. Molecular and cytological analyses of the genes and corresponding gene products identified in this screen should help define the roles they play in meiosis.

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