Copyright © 1989 by the Genetics Society of America

MEI4, a Yeast Gene Required for Meiotic Recombination

Thomas M. Menees and G. Shirleen Roeder

Department of Biology, Yale University, New Haven, Connecticut 06511-8112

Manuscript received May 31, 1989
Accepted for publication August 24, 1989

ABSTRACT

Mutants at the *MEI4* locus were detected in a search for mutants defective in meiotic gene conversion. *mei4* mutants exhibit decreased sporulation and produce inviable spores. The spore inviability phenotype is rescued by a *spo13* mutation, which causes cells to bypass the meiosis I division. The *MEI4* gene has been cloned from a yeast genomic library by complementation of the recombination defect and has been mapped to chromosome *V* near *gln3*. Strains carrying a deletion/insertion mutation of the *MEI4* gene display no meiotically induced gene conversion but normal mitotic conversion frequencies. Both meiotic interchromosomal and intrachromosomal crossing over are completely abolished in *mei4* strains. The *mei4* mutation is able to rescue the spore-inviability phenotype of *spo13 rad52* strains (*i.e.*, *mei4 spo13 rad52* mutants produce viable spores), indicating that *MEI4* acts before *RAD52* in the meiotic recombination pathway.

MEIOSIS is a form of eukaryotic cell division in which a diploid cell divides to yield four haploid progeny. Cells entering meiosis undergo DNA synthesis followed by an extensive prophase, during which homologous chromosomes pair and recombine. Pairing is mediated by a structure called the synaptonemal complex, which assembles and then disassembles in prophase I (for a review, see VON WETTSTEIN, RASMUSSEN and HOLM 1984). Recombination in meiosis occurs at a frequency 100- to 1000-times higher than in mitosis and is thought to take place during prophase when chromosomes are paired. The meiosis I (reductional) division ensues, in which homologous chromosomes segregate from each other. This is followed by the meiosis II (equational) division, in which sister chromatids separate and segregate from each other to complete meiosis.

In organisms that undergo meiotic chromosome pairing and recombination, these processes are required for the proper segregation of chromosomes at the first division (BAKER *et al.* 1976). *Drosophila melanogaster c(3)G* mutants (GOWEN 1933) lack synaptonemal complexes and produce inviable gametes due to nondisjunction at meiosis I (HALL 1972). Drosophila mutants *mei-41* and *mei-218* alter the distribution and frequency of recombination events in meiosis and undergo nondisjunction at meiosis I (BAKER *et al.* 1976). Plant mutants that fail to pair (asynaptic) or recombine homologs, or terminate pairing prematurely (desynaptic), have severe defects in the meiosis I division and concomitant reductions in fertility (BAKER *et al.* 1976).

A number of mutants defective in meiotic recombination have been identified in the yeast, *Saccharomyces cerevisiae*. These mutants have come from many sources including screens for mutations affecting spore viability (*mer1*) (ENGEBRECHT and ROEDER 1989), meiotic recombination (*hop1*) (HOLLINGSWORTH and BYERS 1989), sporulation (*spo11*) (ESPOSITO and ESPOSITO 1969), radiation sensitivity (*rad50, rad52, rad57*) (GAME 1983), gene conversion (*con, rec*) (RODARTE-RAMON and MORTIMER 1972; ROTH and FOGEL 1971), and various mitotic cell functions (JOHNSON and NASMYTH 1978; KASSIR and SIMCHEN 1978; LIRAS *et al.* 1978; SIMCHEN 1974). In cases where it has been examined (*mer1, hop1, spo11, rad50, rad52, rad57*), these mutants produce inviable spores, consistent with the hypothesis that recombination is required for proper chromosome disjunction at meiosis I.

These yeast mutants can be classified into groups according to the stages in the meiotic recombination pathway at which they are believed to act. For simplicity, meiotic recombination can be considered to occur in three stages: the pairing of homologous chromosomes, the initiation of recombination and the resolution of recombination intermediates. Whether pairing precedes the initiation of exchange or these events are mutually dependent is unknown. The determination of the stage in which a mutant is defective is based on the severity of the phenotype, the types of recombination affected and the interactions of the mutant with other meiotic mutants.

The *spo13* mutation (KLAPHOLZ and ESPOSITO 1980) is particularly useful in classifying recombination mutants. In meiosis, *spo13* diploids undergo pairing and recombination followed by a single meiosis II (equational) division to form two diploid spores. Many recombination-defective mutants produce viable, al-

The publication costs of this article were partly defrayed by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

beit nonrecombinant, spores in a *spo13* background (see below). Apparently, mutants blocked early in the recombination pathway can produce viable spores when the meiosis I division is bypassed because recombination is not required for meiosis II chromosome segregation. Mutants blocked later in the recombination pathway produce inviable spores in a *spo13* background, perhaps because recombination has introduced breaks that cannot be repaired in the absence of the defective gene product. Alternatively, recombination may have established connections that cannot be resolved; subsequent attempts at chromosome segregation either destroy the integrity of the chromosomes or result in massive nondisjunction, producing inviable spores.

The *spo11* (KLAPHOLZ, WADDELL and ESPOSITO 1985) and *rad50* (GAME *et al.* 1980; WAGSTAFF, GOTTLIEB and ESPOSITO 1986) mutants are completely defective in both meiotic inter- and intrachromosomal recombination. The double mutants, *spo11 spo13* and *rad50 spo13*, produce viable, nonrecombinant spores (MALONE 1983). Neither *spo11* nor *rad50* null mutants form synaptonemal complexes (DRESSER, GIROUX and MOSES 1986; FARNET *et al.* 1988). Thus, *spo11* and *rad50* are presumed to be defective in the initiation of all forms of meiotic exchange.

Mutants at the *HOP1* locus (HOLLINGSWORTH and BYERS 1989) undergo meiotic interchromosomal recombination at 10% of the wild-type level. Strains containing both *hop1* and *spo13* produce viable spores, suggesting that *hop1* mutants are defective early in the recombination pathway. These mutants do, however, undergo wild-type levels of meiotic intrachromosomal recombination between repeated sequences on the same chromosome. Meiotic recombination between, but not within, chromosomes is thought to be dependent on homologous pairing (HOLLINGSWORTH and BYERS 1989). Thus, *hop1* has been classified as a pairing-defective mutant (HOLLINGSWORTH and BYERS 1989).

Mutants at the *MER1* locus (ENGEBRECHT and ROEDER 1989) undergo meiotic inter- and intrachromosomal recombination at 10% of wild-type levels. The *mer1 spo13* double mutant produces viable spores. The *MER1* gene product may be required for initiation of the major pathway of meiotic recombination. The residual recombination in *mer1* mutants presumably represents a minor pathway, which is not sufficient to ensure proper chromosome segregation at meiosis I.

Mutations in *RAD52* and *RAD57* have severe effects on meiotic interchromosomal recombination (GAME *et al.* 1980). The spore-inviability phenotype of these mutants is not overcome by a *spo13* mutation (RESNICK 1987). Presumably, recombination initiates in *rad52* and *rad57* mutants, leaving the chromosomes either broken or inseparably joined. Introduction of a mutation that blocks the initiation of recombination (*e.g.*, *spo11* or *rad50*) into *rad52 spo13* or *rad57 spo13* strains restores spore viability. Using a physical assay, *rad52* and *rad57* mutants were shown to form recombined molecules in meiosis (BORTS, LICHTEN and HABER 1986), which is consistent with the hypothesis that these mutants are competent in the initiation of exchange.

A search for additional yeast genes involved in meiotic recombination was initiated by screening for recombination-defective mutants. By expanding the catalog of genes required for meiotic recombination and examining the relationships between them, it is hoped that a better understanding of meiotic exchange will emerge. This study focuses on the isolation and characterization of a new gene, called *MEI4*, which is required for meiotic recombination in yeast.

MATERIALS AND METHODS

Strains: Yeast strains used for this study are shown in Table 1; karC2-4 was obtained from NANCY HOLLINGSWORTH (HOLLINGSWORTH and BYERS 1989), PLM39 from PATTY MINEHART and BORIS MAGASANIK and NKY611 from NANCY KLECKNER. *Escherichia coli* strains DH1 and YCM10 *recA* were used, as well as strains for the shuttle mutagenesis system of SEIFERT *et al.* (1986).

Genetic manipulations: Yeast media and genetic methods are described by SHERMAN, FINK and HICKS (1986). SC is synthetic complete medium, which can also be prepared lacking specific amino acids (*e.g.*, SC-histidine). Cycloheximide was added at a concentration of 2 μg/ml. Rich medium is YPD. Yeast transformations were carried out using the lithium acetate procedure (ITO *et al.* 1983).

Transposon mutagenesis was carried out in strains and plasmids specified for that purpose (SEIFERT *et al.* 1986). Individual transposon insertions were introduced into the yeast strain, Y20, by transformation with linear fragments (ROTHSTEIN 1983). Substitutions of transposon-containing fragments for yeast chromosomal sequences were selected by virtue of the *URA3* gene carried on the transposon. Transformants were patched and their recombination phenotypes assessed by the number of His^+ and Leu^+ papillae appearing following sporulation.

Deletion alleles of *MEI4* were also introduced into yeast by transformation with linear fragments (ROTHSTEIN 1983). Southern blot analysis (SOUTHERN 1975) of DNA isolated from yeast transformants confirmed the presence of the deletion alleles on the chromosome.

Mutant screen: Strain Y17 (see Figure 1) was used in the screen for yeast mutants defective in meiotic recombination. This strain was plated on rich medium (200 colonies per plate) and mutagenized with UV light to 50% killing. Plates were incubated in the dark at 30° for 4 days. Survivors were patched to rich medium, grown at 30° and then replica-plated to sporulation medium. Following sporulation at 30°, patches were replica-plated to SC-histidine and SC-leucine to select His^+ and Leu^+ recombinants. Patches that contained fewer recombinants than wild type (as indicated by the number of His^+ and Leu^+ papillae) were examined for sporulation-competence. Dyads were dissected in order to assess spore viability.

MMS sensitivity: The sensitivity of the *mei4-1* mutant to the radiomimetic drug, methylmethane sulfonate (MMS; Kodak, Rochester, NY), was assessed in a plate assay. The mutant and a wild-type control were patched onto rich

TABLE 1

Yeast strains

Strain	Genotype
Y17	MATa CRY1^s leu2-112 his4-260,39 / MATα cry1^r leu2-3 his4-280 ura3 trp1-H3 spo13::TRP1
Y21	MATa CRY1^s leu2-112 his4-260,39 / MATα cry1^r leu2-3 his4-280 ura3 mei4-1 trp1-H3 spo13::TRP1
TM18-52B	MATa leu2 his4 cyh10 lys2-1 ura3 ade2-1 trp1-H3
Y63	MATa CRY1^s leu2 his4-260,39 trp1 spo13::TRP1 ADE2 lys2-1 cyh10^r ura3 mei4-1 / MATα cry1^r leu2 his4-280 trp1 SPO13 ade2 LYS2 cyh10^r ura3 mei4-1
Y20	MATa CRY1^s leu2-112 his4-260,39 / MATα cry1^r leu2-3 his4-280 ura3 trpl-H3 spo13::TRP1 ade2-1 lys2-1 cyh10^r
Y33	MATa CRY1^s leu2-112 his4-260,39 / MATα cry1^r leu2-3 his4-280 ura3 mei4::ADE2 trpl-H3 spo13::TRP1 ade2-1 lys2-1 cyh10^r
Y34	MATa CRY1^s leu2-112 his4-260,39 / MATα cry1^r leu2-3 his4-280 ura3 mei4::URA3 trpl-H3 spo13::TRP1 ade2-1 lys2-1 cyh10^r
NKY611	MATa leu2::hisG ho::LYS2 lys2 ura3 / MATα leu2::hisG ho::LYS2 lys2 ura3
Y64	MATa leu2::hisG ho::LYS2 lys2 ura3 mei4::URA3 / MATα leu2::hisG ho::LYS2 lys2 ura3 MEI4
Y50	MATa leu2::hisG ho::LYS2 lys2 ura3 mei4::URA3 / MATα leu2::hisG ho::LYS2 lys2 ura3 mei4::URA3
Y58	MATa leu2 his4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 ade2-1 trpl-H3 ura3 / MATα leu2 HIS4 LYS2 CYH10 spol3::TRP1 arg4-8 thr1-4 ade2-1 trpl-H3 ura3
Y59	MATa leu2 his4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 ade2-1 trpl-H3 ura3 mei4::URA3 / MATα leu2 HIS4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4 ade2-1 trpl-H3 ura3 mei4::URA3
Y60	MATa leu2 his4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 ade2-1 trpl-H3 ura3 rad52::ADE2 / MATα leu2 HIS4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4 ade2-1 trpl-H3 ura3 rad52::ADE2
Y61	MATa leu2 his4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 ade2-1 trp1-H3 ura3 mei4::URA3 rad52::ADE2 / MATα leu2 HIS4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4 ade2-1 trp1-H3 ura3 mei4::URA3 rad52::ADE2
karC2-4	MATa CDC10 leu2 his4 / MATα cdc10-2 LEU2::pNH18-1 HIS4 ura3 can1 trp1 cyh2^r ade2-1 spo13-1 sap3
Y56	MATa CDC10 leu2 his4 / MATα cdc10-2 LEU2::pNH18-1 HIS4 mei4::ADE2 ura3 can1 trp1 cyh2^r ade2-1 spo13-1 sap3
PLM39	MATa leu2-3,112 ade2-101 ura3-52 gln3::LEU2

Y21 is a mutant derivative of Y17. Y33 and Y34 are transformants of Y20. Y59, Y60 and Y61 are transformants of Y58. Y56 is a transformant of karC2-4 and Y64 is a transformant of NKY611. Y50 was constructed by mating two meiotic Ura$^+$ segregants of Y64. pNH18-1 refers to the plasmid containing *URA3* and *CYH2* which was integrated into chromosome *III* to create an 11.4-kbp duplication of sequences that lie between *HIS4* and *LEU2* (HOLLINGSWORTH and BYERS 1989).

medium and then replica-plated to rich medium containing 0.02% MMS. Following an overnight incubation at 30°, patches were replica-plated to rich medium and scored for MMS sensitivity after 24 hr.

Complementation testing: The ability of *mei4-1* to be complemented by *MER1*, *SPO11*, and *RAD50* was tested by transforming a *mei4-1* mutant strain (Y21) with plasmids containing wild-type copies of these genes (see section below on plasmid constructions). To test *mei4-1* for its ability to complement *hop1*, a *hop1* haploid was mated to a mating-competent derivative of Y21. Y21 is heterozygous at *CRY1*, with a recessive cryptopleurine-resistant allele linked to the *MATα* locus. Selection for resistance to cryptopleurine (Chemasea, Sydney, Australia) detects primarily crossover events that render homozygous the right arm of the chromosome *III* homolog containing *cry1* and *MATα* (see Figure 1).

Determination of recombination frequencies: Mitotic intragenic recombination frequencies were determined by growing three independent cultures to saturation in rich medium and plating on SC-histidine, SC-leucine (to select for recombinants) and SC (to determine the viable cell titer). The median frequency was used as the measure of the mitotic recombination frequency. Cells were washed, transferred to sporulation medium, and incubated for four days at 30° with shaking. Meiotic recombination frequencies were determined by plating sporulated cultures on SC-histidine, SC-leucine and SC. The average of the meiotic frequencies for the three cultures was used as the measure of the meiotic recombination frequency. Cultures containing mitotic jackpots were not considered for either frequency determination. Meiotic frequencies were also determined by plating isolated spores (LAMBIE and ROEDER 1988). Intrachromosomal recombination frequencies were determined in a similar fashion except that cells were plated

onto SC-histidine-leucine+cycloheximide to identify recombinants (HOLLINGSWORTH and BYERS 1989).

Plasmid constructions: All plasmid constructions were carried out using standard methods (MANIATIS, FRITSCH and SAMBROOK 1982). The yeast genomic library was provided by FORREST SPENCER and PHIL HIETER and comprised *Sau*3A partial digest fragments, 9–12 kbp in size, inserted into a *CEN* vector derived from YCp50 (PARENT, FENIMORE and BOSTIAN 1985), in which the *URA3* gene has been replaced with *LEU2*. The *MEI4* clone originally isolated from the yeast genomic library was designated pTM11.

The following *MEI4* plasmids were constructed. pTM12 was made by cloning the 3.8-kbp *MEI4*-complementing *Sal*I fragment from pTM11 (see Figure 2) into the *Sal*I site of YCp50 (PARENT, FENIMORE and BOSTIAN 1985). A *MEI4* plasmid used in shuttle mutagenesis (SEIFERT *et al.* 1986), was constructed in two steps. First, the 3.8-kbp *Sal*I fragment of pTM11 was cloned into the *Sal*I site of the polylinker on pATH3 (obtained from ALEX TZAGALOFF). Second, a 3.8-kbp *Eco*RI-*Bgl*II fragment from this construct, containing the original 3.8-kbp *Sal*I fragment carrying *MEI4*, was inserted into *Eco*RI-*Bgl*II digested pHSS6 (SEIFERT *et al.* 1986) to form pTM13. A series of transposon insertions was generated in pTM13. Insertions that gave a Mei$^-$ phenotype were given *MEI4* allele names according to the numbers assigned to the insertions (*e.g.*, transposon insertion #17 gave rise to *mei4::T17*). Plasmids containing *mei4* deletion/disruption alleles were derived from pTM14, which carries *mei4::T17* on pHSS6. The *mei4::URA3* deletion/disruption was made by cloning the 1.9-kbp *Bgl*II-*Sal*I fragment of YRp10 (PARENT, FENIMORE and BOSTIAN 1985), containing the *URA3* gene, into *Bam*HI-*Xho*I digested pTM14, to generate pTM6. The *Bam*HI site in pTM14 lies at one end of the transposon sequences; thus, this construction resulted in replacement by *URA3* of approximately 750 bp of *MEI4* and all the transposon sequences, except one of the 38 bp repeats (see Figure 2). The *mei4::ADE2* deletion/disruption allele was constructed in two steps. First, pTM14 was digested with *Stu*I (which cuts in the transposon) and *Sna*BI and religated. This results in removal of approximately one third of the transposon sequences and 500 bp of *MEI4*. Second, the resulting plasmid was cut at the *Bam*HI site in the remaining transposon sequences and a 3.6 kbp *Bam*HI fragment of Yp3.6 ADE (obtained from JEFF LEMONTT), containing the *ADE2* gene, was ligated into this site to generate pTM7 (see Figure 2).

pTM1, a plasmid carrying a *TRP1* disruption of *SPO13*, was constructed by cloning the 1.5-kbp *Nae*I fragment of YRp7 (PARENT, FENIMORE and BOSTIAN 1985), containing the *TRP1* gene, into the *Stu*I site of p(*spo13*)16 (WANG *et al.* 1987). pTM4, a *CEN* plasmid carrying the *RAD50* gene, was constructed by cloning the 4.3-kbp *Sal*I-*Hin*dIII fragment from pMK50-1 (KUPIEC and SIMCHEN 1984) into *Sal*I-*Hin*dIII digested YCp50. pTM5, a plasmid carrying an *ADE2* disruption of *RAD52*, was constructed in two steps. First, the *LEU2* gene was removed from pSM20 (obtained from DAVID SCHILD), a plasmid carrying a *LEU2* disruption of *RAD52*, by digestion with *Bgl*II and religation. The resulting plasmid was redigested with *Bgl*II and the 3.6-kbp *Bam*HI fragment, containing *ADE2*, was inserted to generate pTM5. YCp50 plasmids containing wild-type copies of *MER1* and *SPO11* were obtained from JOANNE ENGEBRECHT and CRAIG GIROUX, respectively.

RESULTS

Isolation and characterization of the *mei4-1* mutant: A search for recombination-defective mutants was carried out using a strain in which the frequency of meiotic gene conversion could easily be assessed. Y17, the starting strain for the mutagenesis, is disomic for chromosome *III* and heterozygous for mating type, and carries the *spo13* mutation (Figure 1). This strain undergoes meiosis in a manner characteristic of *spo13* strains; pairing and recombination are followed by a single equational division, resulting in the formation of two disomic spores. Recessive mutations can be recovered on all chromosomes except *III*. The chromosome *III* homologs carry heteroalleles at *HIS4* and *LEU2* so that recombination to yield His$^+$ and Leu$^+$ prototrophs can be assayed. Finally, the strain is heterozygous for the *MAT*-linked cryptopleurine-resistance mutation (*cry1^r*), allowing mating-competent derivatives to be selected (see MATERIALS AND METHODS). This scheme is essentially similar to that described by ROTH and FOGEL (1971), except that the starting strain carries the *spo13* mutation.

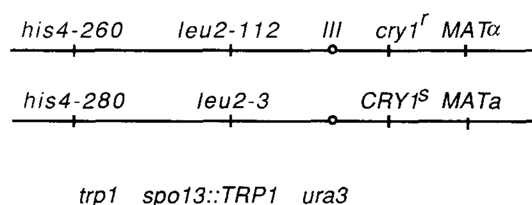

FIGURE 1.—Starting strain for mutant screen. Y17 is disomic for chromosome *III* but otherwise haploid. See text for an explanation of the genetic markers.

Y17 was mutagenized with UV light to 50% killing; 1440 survivors were patched to rich medium, replica-plated to sporulation medium and then to SC-histidine and SC-leucine to assess meiotic recombination. Meiotic recombination in a patch of the wild type, Y17, resulted in confluent growth of His$^+$ and Leu$^+$ recombinants. One mutant (Y21) was identified that demonstrated no meiotic induction of heteroallelic recombination (see below) and produced viable spores in the *spo13* starting strain.

A cryptopleurine-resistant derivative of Y21 that behaved as an α-mater (see MATERIALS AND METHODS) was crossed to a wild-type strain, TM18-52B. Pairwise crosses of segregants of this cross indicated that the mutation affecting meiotic recombination was recessive and segregated 2$^+$:2$^-$. The mutation was designated *mei4-1* (for *mei*osis-specific).

Yeast mutants affected in the repair of x-ray induced DNA damage (*rad50* series) are also sensitive to the radiomimetic drug, methylmethane sulfonate (MMS). Many of these radiation-sensitive mutants also are defective in meiotic recombination. Strains bearing the *mei4-1* mutation demonstrate wild-type sensitivity to MMS and lack any other obvious mitotic defects. Thus, *mei4-1* does not belong to the *rad50* series of mutants.

The *mei4-1* mutant was tested for allelism with other mutants that are deficient in meiotic recombination and *spo13*-rescued. The *mei4-1* mutation is not an allele of *SPO11*, *RAD50*, *MER1*, or *HOP1*.

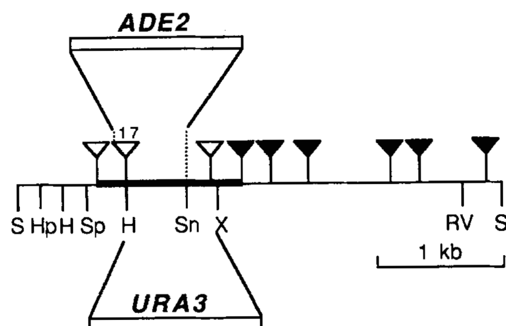

FIGURE 2.—Restriction map of the *MEI4* region. Filled triangles indicate transposon insertions that confer a Mei$^+$ phenotype; open triangles indicate transposon insertions that confer a Mei$^-$ phenotype. The position of transposon insertion #17 is indicated. The minimum size of *MEI4* is indicated by the thick line on the restriction map. The two deletion/disruption alleles of *MEI4* (*mei4::ADE2* and *mei4::URA3*) are shown above and below the restriction map with the deletion endpoints noted. S, *Sal*I; Hp, *Hpa*I; H, *Hind*III; Sp, *Ssp*I; Sn, *Sna*BI; X, *Xho*I; RV, *Eco*RV.

Cloning of *MEI4*: The *MEI4* gene was cloned from a yeast genomic library by complementation of the meiotic recombination defect. A *mei4-1* diploid strain (Y63) carrying *his4* heteroalleles and *leu2* was transformed with a yeast genomic library carried on a *CEN* plasmid marked with the *LEU2* gene. Transformants were patched to SC-leucine and then replica-plated to sporulation medium. Following sporulation, transformants were replica-plated to SC-histidine to select recombinants. One transformant, out of approximately 400 screened, underwent wild-type levels of meiotic recombination as determined by the number of His$^+$ prototrophs. Spontaneous mitotic Leu$^-$ segregants were unable to recombine at wild-type levels, indicating that the complementing activity was plasmid-borne. Total yeast DNA from the transformant was used to transform *E. coli* and a plasmid with a 9.2-kbp insert of yeast DNA was recovered. When this plasmid was reintroduced into a *mei4-1* strain (Y21), the resulting transformants displayed wild-type levels of recombination. Subcloning delimited the complementing region to a 3.8-kbp *Sal*I fragment, a restriction map of which is shown in Figure 2.

Further localization of the *MEI4* complementing activity was accomplished by transposon mutagenesis of the *MEI4* region, employing mini-*Tn3* (SEIFERT *et al.* 1986). Transposon insertions into the 3.8-kbp *Sal*I fragment were introduced into yeast and the phenotypes of the transformants assessed as described in MATERIALS AND METHODS. The results delimit the gene to a 2-kbp region (Figure 2).

To analyze the phenotype of *mei4* null mutants, deletion alleles were constructed (see MATERIALS AND METHODS). For the *mei4::URA3* allele, approximately 750 bp of *MEI4* was deleted and replaced with *URA3*. For the *mei4::ADE2* allele, approximately 500 bp of *MEI4* was deleted and replaced with *ADE2*. The two deletion alleles studied are diagrammed in Figure 2. Strains carrying these deletion alleles failed to complement the *mei4-1* mutant, indicating that the gene cloned from the yeast library is the wild-type *MEI4* gene.

TABLE 2

***mei4* is a meiotic-lethal mutation that is *spo13*-rescued**

Strain	Relevant genotype	Spore viability (%)
Y64	*MEI4* / *mei4::URA3*	93
Y50	*mei4::URA3* / *mei4::URA3*	0
Y58	*MEI4 spo13* / *MEI4 spo13*	61
Y59	*mei4::URA3 spo13* / *mei4::URA3 spo13*	80

Tetrads were dissected for strains Y64 (45 tetrads) and Y50 (42 tetrads). Dyads were dissected for strains Y58 (177 dyads) and Y59 (175 dyads).

***mei4* is a meiotic-lethal mutation which is rescued by *spo13*:** The *mei4::URA3* mutation was introduced into a wild-type diploid (NKY611) by substitutive transformation (ROTHSTEIN 1983). Tetrad dissection of a sporulated transformant resulted in two Ura$^+$ and two Ura$^-$ spores indicating that the *MEI4* gene is not essential for mitotic growth. Matings of segregants from this tetrad produced a homozygous *mei4::URA3* diploid. This mutant diploid (Y50) and the original heterozygous transformant (Y64) were sporulated and tetrads dissected to assess spore viability. The homozygous *mei4* diploid produced inviable spores, indicating that *mei4* is a meiotic-lethal mutation (Table 2).

Isogenic *spo13* diploids, homozygous for *MEI4* or *mei4::URA3*, were constructed and sporulated and dyads were dissected to assess spore viability. The *mei4 spo13* diploid produced viable spores, indicating that the *mei4* mutation is *spo13*-rescued (Table 2).

***mei4* is defective in meiotic interchromosomal recombination:** Meiotic recombination between two different mutant alleles (heteroalleles) of a gene to generate a wild-type gene generally occurs by gene conversion (HURST, FOGEL and MORTIMER 1972; FINK and STYLES 1974). Interchromosomal gene conversion was measured in isogenic *spo13* strains carrying either *MEI4*, *mei4::URA3* or *mei4::ADE2*, as well as heteroalleles at *HIS4* and *LEU2*, as described in MATERIALS AND METHODS. As shown in Table 3A, strains carrying either *MEI4* disruption allele fail to undergo meiotic induction of interchromosomal gene conversion. Mitotic recombination frequencies are unaffected by *mei4* mutations.

Isogenic *MEI4* and *mei4::URA3* diploid strains were constructed and used to measure reciprocal crossing over. These diploids are heterozygous for pairs of markers that define intervals on three different chromosomes. The three intervals in which recombination

TABLE 3

Recombination in *mei4* mutants

A. Intragenic recombination in *spo13* disomic strains

Strain	Relevant genotype	Mitotic recombination		Meiotic recombination		Fold meiotic induction	
		His^+	Leu^+	His^+	Leu^+	His^+	Leu^+
Y20	*MEI4*	1.5×10^{-4}	9.5×10^{-6}	5.5×10^{-3}	1.3×10^{-3}	37	137
Y21	*mei4-1*	9.3×10^{-5}	1.1×10^{-5}	1.1×10^{-4}	6.2×10^{-6}	1.2	0.6
Y33	*mei4::ADE2*	2.8×10^{-4}	5.7×10^{-6}	7.0×10^{-5}	7.3×10^{-6}	0.3	1.3
Y34	*mei4::URA3*	2.0×10^{-4}	7.4×10^{-6}	1.1×10^{-4}	7.8×10^{-6}	0.6	1.1

B. Intergenic recombination in *spo13* diploid strains

Strain	Relevant genotype	Intergenic distances (cM)[a]			Percent spore viability	Percent aberrant segregation[b]
		CYH10-LYS2	*HIS4-MAT*	*ARG4-THR1*		
Y58	*MEI4* / *MEI4*	38.0	57.1	19.6	61	15
Y59	*mei4::URA3* / *mei4::URA3*	<0.4	0.4	<0.4	80	0

C. Intrachromosomal recombination in *spo13* disomic strains

Strain	Relevant genotype	Recombination		Fold meiotic induction[c]	Meiotic chromosome segregation		
		Mitotic	Meiotic		Percent equational	Percent reductional	Percent aberrant[b]
karC2-4	*MEI4*	5.8×10^{-4}	2.1×10^{-3}	20	18	70	12
Y56	*mei4*	8.1×10^{-4}	8.2×10^{-4}	1	91	0	9

[a] One-hundred sixty-three two-spore-viable dyads were scored for strain Y58 and 263 for strain Y59. Dyads classified as recombinants for the *ARG4-THR1* interval displayed either $2^+:0^-$ segregation for *ARG4* and $1^+:1^-$ segregation for *THR1* or $2^+:0^-$ segregation for *THR1* and $1^+:1^-$ segregation for *ARG4*. Dyads classified as recombinants for the *HIS4-MAT* interval displayed either $1^+:1^-$ segregation for *HIS4* and 2 nonmater:0 mater segregation for *MAT or 1a*:1α segregation for *MAT* and $2^+:0^-$ segregation for *HIS4*. Four-strand double crossovers in the *HIS4-MAT* interval displayed $\alpha His^+:aHis^-$ segregation. Dyads classified as recombinants for the *CYH10-LYS2* interval fell into two classes: (1) those in which either *LYS2* segregated $1^+:1^-$ and both spore clones were Cyh^s but generated Cyh^r mitotic recombinants and (2) those in which *LYS2* segregated $2^+:0^-$ with one segregant being Cyh^r and the other being Cyh^s and unable to generate Cyh^r mitotic recombinants. Map distances were calculated using a derivation of PERKIN's (1949) formula as follows: map distance = [single crossovers + 6(4-strand double crossovers)/total] × 100. Four-strand double crossovers in the *ARG4-THR1* and *CYH10-LYS2* intervals could only be detected when followed by a reductional division; thus, the map distances for these intervals are underestimates. See KLAPHOLZ, WADDELL and ESPOSITO (1985), ROCKMILL and ROEDER (1988) and ENGEBRECHT and ROEDER (1989) for details on calculating map distances from dyad data.

[b] Dyads showing 1 nonmater:1 mater segregation were scored as aberrant segregations for chromosome *III*.

[c] The fold meiotic induction of His^+ Leu^+ Cyh^r recombinants over mitotic levels was normalized for the amount of equational division by multiplying the uncorrected fold meiotic induction by (100%/% equational segregation). The amount of equational segregation for chromosome *III* was determined by the segregation of the centromere-linked *cdc10-2* mutation, which is heterozygous in karC2-4 and its derivatives. The *cdc10-2* allele confers a temperature-sensitive lethal phenotype.

was measured are *CYH10-LYS2* on chromosome *II*, *HIS4-MAT* on chromosome *III* and *ARG4-THR1* on chromosome *VIII*. The diploids were sporulated and dissected and dyads were scored for recombination. As shown in Table 3B, *mei4* abolishes meiotic intergenic crossing over in all three regions.

As has been shown for *spo11* (KLAPHOLZ, WADDELL and ESPOSITO 1985), *mei4* improves the spore viability of *spo13* strains (Table 3B). Furthermore, the amount of aberrant segregation observed for chromosome *III* is greatly reduced by the *mei4* mutation (Table 3B). It has been suggested that recombination causes a decrease in the fidelity of chromosome segregation in a *spo13* meiosis because chromosomal associations (chiasmata) resulting from recombination are not effectively processed on the meiosis II spindle (KLAPHOLZ, WADDELL and ESPOSITO 1985). Defects in the initiation of recombination eliminate these chromosomal associations.

***mei4* is defective in meiotic intrachromosomal recombination:** Meiotic intrachromosomal recombination was measured in a *mei4 spo13* background using the karC2-4 strain (HOLLINGSWORTH and BYERS 1989). This strain has a duplication of an 11.4-kbp segment of DNA between *LEU2* and *HIS4* on one of the chromosome *III* homologs. The intervening vector sequences contain *CYH2*; crossing over between the repeats results in excision of the plasmid and subsequent loss of the *CYH2* marker. Recombinants that have lost the *CYH2* marker can be selected by plating on medium containing cycloheximide because the strain carries a recessive mutation conferring drug resistance at the *CYH2* locus. Selection for His^+ Leu^+ Cyh^r derivatives allows the recovery of intrachromosomal recombinants (for details, see HOLLINGSWORTH and BYERS 1989). A *mei4* mutation abolishes meiotic recombination in this assay (Table 3C).

As discussed above, chromosomes usually undergo a single equational division during meiosis in most *spo13* strains. In contrast, the chromosome *III* homo-

logs of the disomic strain, karC2-4, most often undergo a single reductional division during meiosis. As has been shown for *hop1* (HOLLINGSWORTH and BYERS 1989) and *mer1* (ENGEBRECHT and ROEDER 1989), the presence of *mei4* in the karC2-4 background results in primarily equational segregation of both copies of chromosome *III* (see Table 3C).

***mei4* is epistatic to *rad52*:** The epistatic relationship between *mei4* and *rad52* was examined by assessing the spore viability of multiply mutant strains. The *rad52 spo13* strain (Y60) produced no viable spores out of 34 examined. In contrast, the *mei4 rad52 spo13* strain (Y61) produced 34 viable spores out of 44 examined (77%), which is comparable to the viability observed in *mei4 spo13* strains (see Table 2). This result is consistent with *MEI4* acting before *RAD52* in the meiotic recombination pathway.

***MEI4* maps to chromosome *V*:** Radiolabeled *MEI4* DNA was hybridized to a Southern blot of whole yeast chromosomes that had been separated on a CHEF gel (CHU, VOLLRATH and DAVIS 1986). The *MEI4* probe hybridized to chromosome *V* (data not shown). Tetrad analysis indicates that *MEI4* maps 2.5 cM centromere-distal of *GLN3* (77 PD: 0 NPD: 4 T) and 8.2 cM centromere-proximal of *HOM3* (148 PD: 0 NPD: 29 T).

DISCUSSION

A new yeast gene that is required for meiotic recombination has been identified. Mutations in *MEI4* abolish meiotic inter- and intrachromosomal recombination; mitotic recombination is unaffected. The *mei4* mutant produces inviable spores, a phenotype expected for a mutant defective in meiotic recombination.

The rescue of *mei4* spore-inviability in *spo13* strains suggests that *MEI4* acts early in the meiotic recombination pathway. Furthermore, the *mei4* mutation rescues the spore-inviability phenotype of *rad52 spo13* strains, indicating that *MEI4* acts before *RAD52*. It is likely that the lethal recombination intermediates generated in *rad52* strains do not form in the presence of the *mei4* mutation because recombination is blocked at an earlier, *MEI4*-dependent stage.

The meiotic phenotype of *mei4* is indistinguishable from that of *spo11* or *rad50*. It is conceivable that the *MEI4*, *SPO11* and *RAD50* gene products act at the same step in recombination, possibly as part of a complex. Alternatively, these gene products may act at different steps, which cannot be distinguished with current methods of analysis.

The possibilities for the function of *MEI4* are numerous. For example, the *MEI4* gene product may function in the search for homology between chromosomes early in prophase I. This search may involve interactions between proteins bound at specific sites or direct interactions between the DNA molecules themselves. It has been proposed that such DNA-DNA interactions involve late-replicating DNA, such as the zygotene DNA observed in meiocytes of Lilium (STERN and HOTTA 1987). Strand exchange has also been proposed as a mechanism for identifying homology (CARPENTER 1987). Homologous sequences could be joined by strand exchange while the extent of homology is assessed. Based on this assessment, the chromosomes may then engage in full synapsis or disengage. These exchange events could be responsible for ectopic recombination observed between homologous sequences on nonhomologous chromosomes (JINKS-ROBERTSON and PETES 1985, 1986; LICHTEN, BORTS and HABER 1987).

A second possibility is that the *MEI4* gene product is a structural component of the synaptonemal complex, and therefore required for the synapsis of homologs. The fact that *mei4* does not have the same phenotype as *hop1* could reflect differences in the phenotypes of various classes of pairing mutants.

A third possibility is that the *MEI4* gene product may play a role in the initiation of recombination events that depend on the effective pairing of homologs (VON WETTSTEIN, RASMUSSEN and HOLM 1984). *MEI4* may encode an enzyme required for initiation of recombination, such as an endonuclease that introduces single-strand nicks or double-strand breaks. Alternatively, the *MEI4* gene product may be an enzyme involved in strand-transfer. Such a model requires that the search for homology that precedes synapsis not involve recombination or that such recombination events are independent of the *MEI4* gene product.

A final possibility is that the *MEI4* gene product regulates the activity of one or more proteins involved in synapsis and exchange but does not play a direct role in either process. The *MEI4* gene product could regulate the transcription of other meiotic genes or modify gene products post-transcriptionally (*e.g.*, by phosphorylating proteins or by increasing the stability of their mRNAs).

Sequence analysis of *MEI4* is in progress with the hope that insight into the function of this gene will emerge. In addition, *mei4* mutants will be examined for the presence of synaptonemal complexes. Antibodies against the *MEI4* gene product may be useful in determining the location of the *MEI4* protein within meiotic cells. Elucidation of the function of *MEI4* may lead to a better understanding of meiotic recombination in yeast and of the relationship between recombination, pairing and disjunction.

This work was supported by National Institutes of Health grant GM 28904. T.M. is supported by Public Health Service training grant 5 T32 GM07499 from the National Institutes of Health. We wish to thank JAYA BARGAVA, JOANNE ENGEBRECHT, BRAD OZENBERGER, BETH ROCKMILL, MIKE SNYDER and SUE STEWART for critical reading of the manuscript.

LITERATURE CITED

BAKER, B. S., A. T. C. CARPENTER, M. S. ESPOSITO, R. E. ESPOSITO and L. SANDLER, 1976 The genetic control of meiosis. Annu. Rev. Genet. **10:** 53–134.

BORTS, R. H., M. LICHTEN and J. E. HABER, 1986 Analysis of meiosis-defective mutations in yeast by physical monitoring of recombination. Genetics **113:** 551–567.

CARPENTER, A. T. C., 1987 Gene conversion, recombination nodules, and the initiation of meiotic synapsis. BioEssays **6:** 232–236.

CHU, G., D. VOLLRATH and R. W. DAVIS, 1986 Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:** 1582–1585.

DRESSER, M. E., C. N. GIROUX and M. J. MOSES, 1986 Cytological analysis of meiosis using synaptonemal complexes in spread preparations of yeast nuclei. Yeast **2:** S96.

ENGEBRECHT, J., and G. S. ROEDER, 1989 Yeast *mer1* mutants display reduced levels of meiotic recombination. Genetics **121:** 237–247.

ESPOSITO, M. S., and R. E. ESPOSITO, 1969 The genetic control of sporulation in Saccharomyces. I. The isolation of temperature-sensitive sporulation-deficient mutants. Genetics **61:** 79–89.

FARNET, C., R. PADMORE, L. CAO, E. ALANI and N. KLECKNER, 1988 The *RAD50* gene of *S. cerevisiae*, pp. 201–215 in *Mechanisms and Consequences of DNA Damage Processing*, edited by E. FRIEDBERG and P. HANAWALT. Alan R. Liss, New York.

FINK, G. R., and C. A. STYLES, 1974 Gene conversion of deletions in the *HIS4* region of yeast. Genetics **77:** 231–244.

GAME, J. C., 1983 Radiation-sensitive mutants and repair in yeast, pp. 109–137 in *Yeast Genetics*, edited by J. F. T. SPENCER, D. M. SPENCER and A. R. W. SMITH. Springer-Verlag, New York.

GAME, J. C., T. J. ZAMB, R. J. BRAUN, M. RESNICK and R. M. ROTH, 1980 The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94:** 51–68.

GOWEN, J. W., 1933 Meiosis as a general character in *Drosophila melanogaster*. J. Exp. Zool. **65:** 83–106.

HALL, J., 1972 Chromosome segregation influenced by two alleles of the meiotic mutant *c(3)G* in *Drosophila melanogaster*. Genetics **71:** 367–400.

HOLLINGSWORTH, N. M., and B. BYERS, 1989 *HOP1*: a yeast meiotic pairing gene. Genetics **121:** 445–462.

HURST, D., S. FOGEL and R. K. MORTIMER, 1972 Conversion-associated recombination in yeast. Proc. Natl. Acad. Sci. USA **69:** 101–105.

ITO, H., Y. FUKADA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JINKS-ROBERTSON, S., and T. D. PETES, 1985 High-frequency meiotic gene conversion between repeated genes on nonhomologous chromosomes in yeast. Proc. Natl. Acad. Sci. USA **82:** 3350–3354.

JINKS-ROBERTSON, S., and T. D. PETES, 1986 Chromosomal translocations generated by high-frequency meiotic recombination between repeated yeast genes. Genetics **114:** 731–752.

JOHNSON, L. H., and K. A. NASMYTH, 1978 *Saccharomyces cerevisiae* cell cycle mutant *cdc9* is defective in DNA ligase. Nature **274:** 891–893.

KASSIR, Y., and G. SIMCHEN, 1978 Meiotic recombination and DNA synthesis in a new cell cycle mutant of *Saccharomyces cerevisiae*. Genetics **90:** 49–68.

KLAPHOLZ, S., and R. E. ESPOSITO, 1980 Recombination and chromosome segregation during the single division meiosis in *spo12-1* and *spo13-1* diploids. Genetics **96:** 589–611.

KLAPHOLZ, S., C. S. WADDELL and R. E. ESPOSITO, 1985 The role of the *SPO11* gene in meiotic recombination in yeast. Genetics **110:** 187–216.

KUPIEC, M., and G. SIMCHEN, 1984 Cloning and mapping of the *RAD50* gene of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **193:** 525–531.

LAMBIE, E. J., and G. S. ROEDER, 1988 A yeast centromere acts in *cis* to inhibit meiotic gene conversion of adjacent sequences. Cell **52:** 863–873.

LICHTEN, M., R. H. BORTS and J. E. HABER, 1987 Meiotic gene conversion between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. Genetics **115:** 233–246.

LIRAS, P., J. MCCUSKER, S. MASCIOLI and J. E. HABER, 1978 Characterization of a mutation in yeast causing nonrandom chromosome loss during mitosis. Genetics **88:** 651–671.

MALONE, R. E., 1983 Multiple mutant analysis of recombination in yeast. Mol. Gen. Genet. **189:** 405–412.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

PARENT, S. A., C. M. FENIMORE and K. A. BOSTIAN, 1985 Vector systems for the expression, analysis and cloning of DNA sequences in *S. cerevisiae*. Yeast **1:** 83–138.

PERKINS, D. D. 1949 Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34:** 607–627.

RESNICK, M. A., 1987 Investigating the genetic control of biochemical events in meiotic recombination, pp. 157–210 in *Meiosis*, edited by P. B. MOENS. Academic Press, New York.

ROCKMILL, B., and G. S. ROEDER, 1988 *RED1*: a yeast gene required for the segregation of chromosomes during the reductional division of meiosis. Proc. Natl. Acad. Sci. USA **85:** 6057–6061.

RODARTE-RAMON, U., and R. K. MORTIMER, 1972 Radiation-induced recombination in Saccharomyces: isolation and genetic study recombination-deficient mutants. Radiat. Res. **49:** 133–147.

ROTH, R., and S. FOGEL, 1971 A selective system for yeast mutants defective in meiotic recombination. Mol. Gen. Genet. **112:** 295–305.

ROTHSTEIN, R., 1983 One step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SEIFERT, H. S., E. Y. CHEN, M. SO and F. HEFFRON, 1986 Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83:** 735–739.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics: Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SIMCHEN, G., 1974 Are mitotic functions required in meiosis? Genetics **76:** 745–753.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

STERN, H., and Y. HOTTA, 1987 The biochemistry of meiosis, pp. 303–331 in *Meiosis*, edited by P. B. MOENS. Academic Press, New York.

VON WETTSTEIN, D., S. W. RASMUSSEN and P. B. HOLM, 1984 The synaptonemal complex in genetic segregation. Annu. Rev. Genet. **18:** 331–413.

WAGSTAFF, J. E., S. GOTTLIEB and R. E. ESPOSITO, 1986 The role of *RAD50* in meiotic intrachromosomal recombination, pp. 75–83 in *Mechanisms of Yeast Recombination*, edited by A. KLAR and J. STRATHERN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

WANG, H., S. FRACKMAN, J. KOWALISYN, R. E. ESPOSITO and R. ELDER, 1987 Developmental regulation of *SPO13*, a gene required for separation of homologous chromosomes at meiosis I. Mol. Cell. Biol. **7:** 1425–1435.

Communicating editor: N. KLECKNER