Identification of New Genes Required for Meiotic Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

Mutants defective in meiotic recombination were isolated from a disomic haploid strain of *Saccharomyces cerevisiae* by examining recombination within the *leu2* and *his4* heteroalleles located on chromosome *III*. The mutants were classified into two new complementation groups (*MRE2* and *MRE11*) and eight previously identified groups, which include *SPO11*, *HOP1*, *REC114*, *MRE4/MEK1* and genes in the *RAD52* epistasis group. All of the mutants, in which the mutations in the new complementation groups are homozygous and diploid, can undergo premeiotic DNA synthesis and produce spores. The spores are, however, not viable. The *mre2* and *mre11* mutants produce viable spores in a *spo13* background, in which meiosis I is bypassed, suggesting that these mutants are blocked at an early step in meiotic recombination. The *mre2* mutant does not exhibit any unusual phenotype during mitosis and it is, thus, considered to have a mutation in a meiosis-specific gene. By contrast, the *mre11* mutant is sensitive to damage to DNA by methyl methanesulfonate and exhibits a hyperrecombination phenotype in mitosis. Among six alleles of *HOP1* that were isolated, an unusual pattern of intragenic complementation was observed.

DURING meiosis of the yeast, *Saccharomyces cerevisiae*, duplicated chromosomes are distributed into four haploid progeny as a result of two meiotic divisions (meiosis I and II). During meiosis I, the reductional division, homologous chromosome pairing and recombination occur with subsequent segregation of the homologs to opposite poles of the spindle. Recombination is required for completion of this process (BAKER *et al.* 1976). Cytological events related to recombination have been characterized in higher eukaryotes and in yeast (VON WETTSTEIN, RASMUSSEN and HOLM 1984). During the pachytene stage of meiosis I, condensed chromosomes (lateral elements) pair to form a synaptonemal complex (SC) in which recombination is probably mediated by recombination nodules. Many genes are involved in this process but little is known about the mechanism of recombination and its relationship to the meiotic structures.

The genetic control of meiotic recombination has been studied by two approaches. One approach has involved the analysis of recombination events between genetic markers in an attempt to determine the way in which the strands of genetic material are exchanged. This approach has been used, in particular in fungi, for construction of some plausible models that provide an interpretation of several steps in recombination, such as initiation events, establishment and resolution of intermediates and maturation of recombinant molecules (reviewed by SZOSTAK *et al.* 1983). Recently, a physical extension of this approach has revealed site-specific double-strand breaks in the meiotic chromosomes that might possibly represent early events in meiotic recombination in yeast (SUN *et al.* 1989; CAO, ALANI and KLECKNER 1990).

A second approach has involved the analysis of genes that control or carry out recombination (reviewed by BAKER *et al.* 1976; PETES, MALONE and SYMINGTON 1991). In several eukaryotes, a number of mutants defective in meiotic recombination have been isolated, and many meiosis-defective mutants have been isolated in *Drosophila melanogaster* in particular. Some mutations result in gamete aneuploidy due to nondisjunction of chromosomes during the meiotic divisions and several of the mutants are defective in recombination (reviewed by BAKER *et al.* 1976). In *S. cerevisiae*, a meiosis-specific, recombination-defective mutant (*spo11*) was identified among mutants that showed altered spore morphology (ESPOSITO and ESPOSITO 1969). Characterization of this mutant revealed that the absence of recombination in meiosis causes severe nondisjunction of homologs at meiosis I (KLAPHOLZ, WADDELL and ESPOSITO 1985). This absence of recombination results in aneuploid spores, most of which are not viable. Some other mutants of meiotic recombination in yeast have been identified on the basis of nonviability of spores [*red1*, ROCKMILL and ROEDER (1988); *mer1*, ENGEBRECHT and ROEDER (1989); *rec* mutations, MALONE *et al.* (1991); *mek1*, ROCKMILL and ROEDER (1991)]. Other types of mu-

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TABLE 1

Strains of *S. cerevisiae* used in this study

Strain	Genotype
116-7B	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2* / *MATα leu2-27 his4-290*
C239	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2 rad50-11* / *MATα leu2-27 his4-290*
K396-11A	*MAT***a** *ade1 leu2 ura3 his1 trp5 spo11-1 met3 lys7*
K396-22B	*MATα ade1 leu2 ura3 his1 trp5 spo11-1 met3 lys7*
M1584	*MAT***a** *rec102-1 lys2-1 hom3 leu1-c ade3 his5-2*
M1585	*MATα rec102-1 ade3 his5-2 leu1-c ura1*
M1586	*MAT***a** *rec104-1 lys2-1 ade3 leu1-c trp5-20 his5-2 ura1*
M1587	*MATα rec104-1 lys2-1 ade3 his5-2*
M1729	*MAT***a** *rec114-1 hom3 cyh2 leu1-c ade3 trp5-20 his5-2 ura1*
M1730	*MATα rec114-1 hom3 cyh2 leu1-c ade3 trp5-20 his5-2 ura1*
N7B-1	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2*
N7B-11	*MATα leu2-27 his4-290 trp1 met2 ade2*
N22D-1	*MAT***a** *leu2-27 his4-290 trp1 met2 ade2*
P7BA-1	*MAT***a** *leu2-1 his4-4 can1 ura3 cyh2 ade6 ade2*
P7BA-11	*MATα leu2-1 his4-4 can1 ura3 cyh2 ade6 ade2*
P7BAB	*MAT***a** *leu2-1 his4-4 TRP1 can1 ura3 cyh2 ade6 MET2 ade2* / *MATα leu2-27 his4-290 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2*
SAT174-7B	*MATα leu2 trp1 cyh2 his5 kar1 ade2*
XS2291-3	*MAT***a** *rad50 his1-1 leu1-1 trp5-18*
XS1492-1C	*MAT***a** *rad51 his1-1 leu1-1 trp5-18*
XS1882-4B	*MAT***a** *rad52 his1-1 leu1-1 trp5-18*
XS1873-5C	*MATα rad53 his1-1 leu1-1 trp5-18*
XS2454-1B	*MATα rad54 trp1 his3*
XS1918-1D	*MATα rad55 leu1-1 trp5-18*
XS2456-9B	*MAT***a** *rad56 trp1 his3*
XS1870-6A	*MAT***a** *rad57 his1-1 leu1-1 trp5-18*
KJC101	*MAT***a** *leu2 his4-4 lys2 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2* / *MATα leu2 his4-290 lys2 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2*
KJC102	*MAT***a** *leu2 his4-4 lys2 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2* / *MATα leu2 his4-4 lys2 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2*
KJC103	*MAT***a** *leu2 his4-290 lys2 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2* / *MATα leu2 his4-290 lys2 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2*
IMD501	*MAT***a** *lys2 ho::LYS2 ura3 spo13::hisG* / *MATα lys2 ho::LYS2 ura3 spo13::hisG*
NKY654	*MATα lys2 ho::LYS2 ura3 spo13::hisG rad50::hisG*
hop1	
C36	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2 hop1-11* / *MATα leu2-27 his4-290*
N36-1C	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2 hop1-11* / *MATα leu2-27 his4-290 trp1 met2 ade2 hop1-11*
P36-41	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 hop1-11*
P36AB	*MAT***a** *leu2-1 his4-4 TRP1 can1 ura3 cyh2 ade6 MET2 ADE2 hop1-11* / *MATα leu2-27 his4-290 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2 hop1-11*
N49A-1D	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2 hop1-12*
P49-41	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 hop1-12*
N60-11	*MATα leu2-27 his4-290 trp1 met2 ade2 hop1-13*
N60-2B	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2 hop1-13*
P60-41	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 hop1-13*
N144-1	*MAT***a** *leu2-1 his4-4 trp1 met2 ade2 hop1-14*
P144-2	*MATα leu2-27 his4-290 trp1 met2 ade2 hop1-14*
P144-41	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 hop1-14*
N176-2D	*MATα leu2-1 his4-4 trp1 met2 ade2 hop1-15*
P176-43	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 hop1-15*
N207-1A	*MATα leu2-1 his4-4 trp1 met2 ade2 hop1-16*
P207-43	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 hop1-16*

TABLE 1—*Continued*

Strain	Genotype
N60/144	*MATa leu2-1 his4-4 trp1 met2 ade2 hop1-13* *MATα leu2-27 his4-290 trp1 met2 ade2 hop1-14*
N60/144*	*MATa leu2-1 his4-4 trp1 met2 ade2 hop1-13* *MATa leu2-27 his4-290 trp1 met2 ade2 hop1-14*
mre2	
C40	*MATa leu2-1 his4-4 trp1 met2 ade2 mre2-1* *MATα leu2-27 his4-290*
N40-1A	*MATa leu2-1 his4-4 trp1 met2 ade2 mre2-1*
N40-11B	*MATα leu2-27 his4-290 trp1 met2 ade2 mre2-1*
N40-41	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 mre2-1*
P40AB	*MATa leu2-1 his4-4 TRP1 can1 ura3 cyh2 ade6 MET2 ade2 mre2-1* *MATα leu2-27 his4-290 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2 mre2-1*
IMH201	*MATa leu2 his4-4 lys2 ura3 ho::LYS2 trp1 ade6 ade2 mre2::LEU2*
IMD201	*MATa leu2 his4-4 lys2 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2 mre2::LEU2* *MATα leu2 his4-290 lys2 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2 mre2::LEU2*
IMD211	*MATa lys2 ho::LYS2 ura3 spo13::hisG mre2::LEU2* *MATα lys2 ho::LYS2 ura3 spo13::hisG mre2::LEU2*
rec114	
C192	*MATa leu2-1 his4-4 trp1 met2 ade2 rec114-11* *MATα leu2-27 his4-290*
N192A-4D	*MATa leu2-1 his4-4 trp1 met2 ade2 rec114-11*
N192B-11	*MATα leu2-27 his4-290 trp1 met2 ade2 rec114-11*
P192B-12	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 rec114-11*
P192AB	*MATa leu2-1 his4-4 TRP1 can1 ura3 cyh2 ade6 MET2 ade2 rec114-11* *MATα leu2-27 his4-290 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2 rec114-11*
IMH301	*MATa lys2 leu2 his4-4 ho::LYS2 trp1 ura3 ade6 ade2 rec114::URA3*
IMD301	*MATa leu2 his4-4 lys2 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2 rec114::URA3* *MATα leu2 his4-290 lys2 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2 rec114::URA3*
IMD311	*MATa lys2 ho::LYS2 ura3 spo13::hisG ade2 rec114::URA3* *MATα lys2 ho::LYS2 ura3 spo13::hisG ade2 rec114::URA3*
mre4/mek1	
C160	*MATa leu2-1 his4-4 trp1 met2 ade2 mre4-1* *MATα leu2-27 his4-290*
N160-11	*MATa leu2-1 his4-4 trp1 met2 ade2 mre4-1* *MATα leu2-27 his4-290 trp1 met2 ade2 mre4-1*
P160AB	*MATa leu2-1 his4-4 TRP1 can1 ura3 cyh2 ade6 MET2 ade2 mre4-1* *MATα leu2-27 his4-290 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2 mre4-1*
IMH403	*MATa leu2 his4-4 ura3 ho::LYS2 trp1 ade6 ade2 mre4::URA3*
IMD402	*MATa leu2 his4-4 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2 mre4::URA3* *MATα leu2 his4-290 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2 mre4::URA3*
IMD412	*MATa leu2 lys2 ho::LYS2 ura3 spo13::hisG mre4::URA3* *MATα leu2 lys2 ho::LYS2 ura3 spo13::hisG mre4::URA3*
mre11	
C8	*MATa leu2-1 his4-4 trp1 met2 ade2 mre11-1* *MATα leu2-27 his4-290*
N8A-2D	*MATa leu2-1 his4-4 trp1 met2 ade2 mre11-1*
N8B-11	*MATα leu2-27 his4-290 trp1 met2 ade2 mre11-1*
E8-1	*MATα trp1 can1 ura3 cyh2 ade6 ade2 mre11-1*
N8A/E8	*MATa leu2-1 his4-4 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2 mre11-1* *MATα LEU2 HIS4 trp1 can1 ura3 cyh2 ade6 MET2 ade2 mre11-1*
P8B-11	*MATα leu2-27 his4-290 can1 ura3 cyh2 ade6 ade2 mre11-1*
P8AB	*MATa leu2-1 his4-4 TRP1 can1 ura3 cyh2 ade6 MET2 ade2 mre11-1* *MATα leu2-27 his4-290 trp1 CAN1 URA3 CYH2 ADE6 met2 ade2 mre11-1*
KJC104	*MATa leu2 his4-4 ho::LYS2 trp1 CAN1 ura3 CYH2 ADE6 ade2 mre11::hisG* *MATα leu2 his4-290 ho::LYS2 trp1 can1 ura3 cyh2 ade6 ade2 mre11::hisG*
KJC106	*MATa lys2 ho::LYS2 ura3 spo13::hisG mre11::hisG* *MATα lys2 ho::LYS2 ura3 spo13::hisG mre11::hisG*

tants that are defective in meiotic recombination have been isolated from mutants that are sensitive to DNA damage and/or are defective in mitotic recombination (BAKER *et al.* 1976; GAME *et al.* 1980). The corresponding genes are thought to play some common roles in both mitotic and meiotic recombination.

More direct methods of screening for mutants that are defective in meiotic recombination in yeast have been developed. In one case, a *rad52 spo13* strain is used as the starting strain for the isolation of mutants in recombination. The *rad52 spo13* strain produces nonviable spores after meiosis. However, if a mutation, such as *rad50*, which is believed to block the initiation of recombination, is introduced, viable spores of the *rad50 rad52 spo13* mutant can be generated (MALONE 1983). Using this procedure, MALONE *et al.* (1991) isolated hundreds of mutants and five *REC* genes were identified. Another method involves use of a haploid strain that is disomic for chromosome *III* and carries genetic markers that can be exploited in screening for decreased meiotic recombination [ROTH and FOGEL (1971); *hop1*, HOLLINGSWORTH and BYERS (1989); *mei4*, MENEES and ROEDER (1989); *mre4*, LEEM and OGAWA (1992)]. Although such a strain is haploid, the heterozygous condition of the mating type locus (*MAT***a**/α) on the disome allows the initiation of meiosis and meiotic recombination. If such a strain carries of wild-type copy of the *SPO13* gene, it cannot complete the entire process of meiosis to generate viable spores because of its ploidy. However, after the commitment to recombination in meiosis, viable recombinants can be recovered by returning the cells to a vegetative growth medium (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974). This method has allowed isolation of meiotic mutants by monitoring recombination at heteroallelic markers (*leu2-1/leu2-27*) on the homologs of chromosome *III*. Although this method allows detection of mutations that affect meiotic recombination directly, a considerable fraction of the mutants isolated in this way are defective in the initiation of meiosis or in premeiotic synthesis of DNA, rather than in the recombination process itself.

In this study, we used the same strategy as ROTH and FOGEL (1971), but we performed large-scale screening for mutants that were defective in meiotic recombination. The mutants obtained were used to construct homozygous mutant diploids and those that were defective in the initiation of meiosis or in premeiotic synthesis of DNA were eliminated. Twenty-seven mutants were found to be defective in meiotic recombination. The corresponding mutations were classified into 11 complementation groups, two of which (*mre2* and *mre11*; *M*eiotic *RE*combination) represent new genes. The other mutations were located in previously identified genes that are involved in recombination, including *SPO11*, *HOP1*, *REC114*, *MRE4/MEK1*, and genes in the *RAD52* epistasis group. The properties conferred by the various mutations in these genes are described in this report.

MATERIALS AND METHODS

Strains: The strains of *S. cerevisiae* used in this study are listed, with their genotypes, in Table 1. *can1* and *cyh2* are recessive alleles that confer resistance to canavanine and cycloheximide, respectively. Cells with the *ade2* allele produce red colonies as a result of the accumulation of an intermediate in the biosynthesis of adenine, phosphoribosylaminoimidazole. The *ade6* allele blocks the pathway prior to the effect of *ade2* and, thus, it suppresses the formation of colored colonies by *ade2*.

The presence of *leu2* or *his4* heteroalleles for monitoring recombination was confirmed by the occurrence of enhanced mitotic gene conversion to prototrophy after exposure to a nonlethal dose of ultraviolet (UV) light (20 J/m^2).

Media and chemicals: The media used for isolation of mutants have been described elsewhere (ROTH and FOGEL 1971). The following media were also used (all solid media contained 1.5% agar): MYPD, 0.3% malt extract, 0.3% yeast extract, 0.5% polypeptone, 1.0% glucose; YPD, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% glucose; YPA, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% potassium acetate (pH 7.0); SD (synthetic complete media), 2% glucose, 0.67% yeast nitrogen base, with amino acid supplement; SPM, 0.25% Bacto-yeast extract, 0.1% glucose, 2% potassium acetate, with amino acid supplement; SPII, 2% potassium acetate (pH 7.0), 25% of the normal concentration of complete amino acid supplement. The composition of the complete amino acid supplement is described elsewhere (SHERMAN 1991). The canavanine (Can) supplement consisted of the complete amino acid supplement plus canavanine sulfate (30 mg/liter), instead of arginine, added after autoclaving. The cycloheximide (Cyh) supplement consisted of the complete amino acid supplement with cycloheximide (1 mg/liter) added after autoclaving. Medium with methyl methanesulfonate (MMS; obtained from Wako Fine Chemical, Co., Osaka, Japan) was made as follows. After MYPD medium had been autoclaved, it was allowed to cool to below 50° and just before pouring of plates, MMS at various concentrations was added to the medium with vigorous mixing. The MMS plates were used within 12 hr of preparation. A dilution buffer of 10 mM sodium phosphate buffer (pH 7.0) and 145 mM NaCl was used for diluting cultures of cells. 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI), methyl benzimidazole-2-yl-carbamate (MBC) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) were obtained from Sigma Chemical, Co., St. Louis, Missouri.

Mutagenesis and isolation of mutants: Logarithmically growing cells of the parental strain 116-7B were washed and resuspended in 50 mM Tris-malate (TM) buffer (pH 7.0) at a density of 5×10^7 cells/ml. Cells were exposed to NTG at 10 μg/ml for 60 min at 30°, and 70% of cells survived this treatment. The cells were washed twice with TM buffer and once with YPD and they were resuspended in YPD at a density of 2×10^7 cells/ml. Cells were then grown to stationary phase with shaking for 2 days at 24°. These cells were then plated onto MYPD at a density of about 100 survivors per plate. The effectiveness of mutagenesis was estimated by monitoring the increase in numbers of canavanine-resistant (Can^R) colonies. The frequency of Can^R colonies was approximately 2×10^{-3} after mutagenesis,

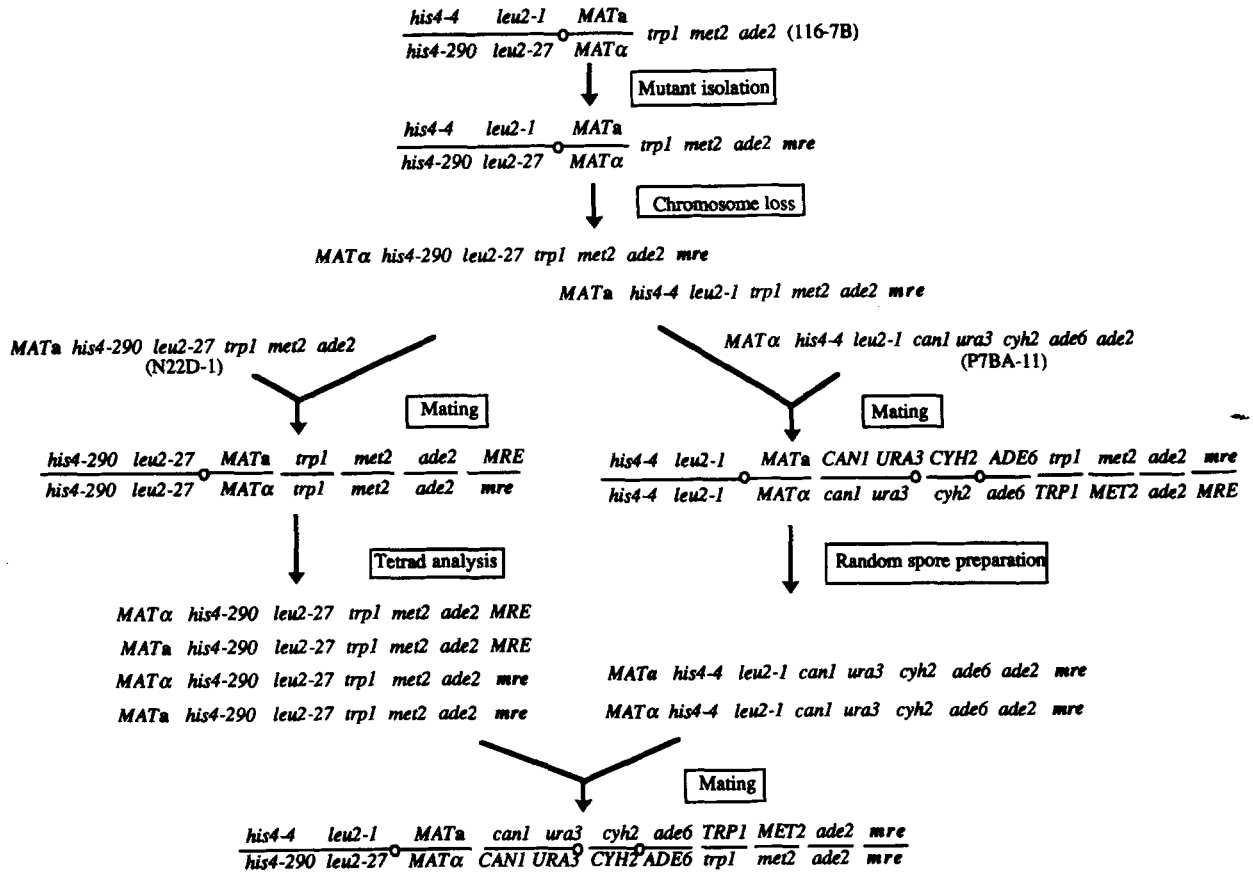

FIGURE 1.—Construction of diploid strains homozygous for mutations that are associated with defects in recombination. From the original disomic mutants (116-7B), two types of haploid were prepared by elimination of a chromosome (*MAT***a** and *MATα* segregants). A *MATα* segregant of each mutant was mated with a wild-type haploid (N22D-1) and the resultant diploid was sporulated and tetrads were dissected. The mutations in the spore clones were examined by monitoring meiotic recombination (*CAN1-URA3*) after mating with tester strains (below). *MAT***a** segregants were mated with another wild-type haploid (P7BA-11). The diploids were sporulated and random spore clones that contained markers for monitoring recombination (*CAN1-URA3*, *CYH2-ADE6*) were selected. These spores were mated with either *MAT***a** or *MATα* segregants and haploids that did not complement the defect in recombination were used as tester strains. The homozygous diploids arising from the crosses between spore clones and testers were examined for their meiotic phenotypes and the results are described in the text.

while the frequency of spontaneous mutations was about 1×10^{-6}.

The survivors were screened for defects in meiotic recombination by the replica-plating method of ROTH and FOGEL (1971). After primary screening, colonies that produced neither leucine nor histidine recombinant prototrophs were reexamined by a streak test. Cells of each mutant, at logarithmic phase in YPA, were washed once and resuspended in the same volume of SPII. After shaking of aliquots at both 24° and 34° for 8, 16, 24 and 48 hr, a small sample of each (10 μl) was withdrawn and streaked onto two plates: SD without leucine (SD-Leu) and SD without histidine (SD-His). Cells sporulated at 24° were allowed to form colonies at 24° and cells sporulated at 34° were grown at 34°. On these plates, a similar culture of wild-type cells (116-7B) yielded about 1000 prototrophs per streak. The mutants that yielded less than 100 prototrophs per streak were saved for further analysis.

Construction of strains: The parental strain (116-7B) used for isolation of mutants is a disomic haploid and, therefore, the original mutant strains could not be tested for their phenotypes in subsequent meiosis. Diploids homozygous for each mutation were constructed by the following manipulations (Figure 1).

Mutant haploid strains were obtained from the original mutant disomic haploids through elimination of one or other chromosomes *III*. MBC was used to stimulate loss of chromosomes (WOOD 1982), and haploids were identified by testing their mating abilities. A culture at stationary phase of mutant disomic haploid cells was diluted 100-fold into YPD medium that contained 50 μg/ml MBC and the culture was shaken for 16 hr at 30°. The cultures were then diluted and cells were plated on MYPD to yield 100–500 colonies per plate. After formation of colonies, each colony was tested for its mating ability by replica-plating onto two other MYPD plates. Each plate had previously been seeded with a lawn of either the *MAT***a** or *MATα* mating-type tester strain (K396-11A or K396-22B, respectively). Under these conditions, both the mutants and the tester strains form red colonies because they carry the *ade2* and *ade1* alleles, respectively. Mutant cells that lose one of their two chromosomes *III* will mate with one of the tester strains. The resulting diploids will be prototrophic for adenine and form white colonies. The mating-proficient segregants were formed mainly by loss of one chromosome *III*, as confirmed by the simultaneous loss of one of the two *leu2* heteroalleles.

Mutant haploid strains with markers for testing intergenic recombination (*can1-ura3* and *cyh2-ade6*) were constructed

from *MAT***a** segregants (Figure 1). *MAT***a** segregants (which have *leu2-1* and *his4-4* alleles) were crossed with P7BA-11. P7BA-11 has a set of markers that can be used for monitoring intragenic (*leu2-1*, *his4-4*) and intergenic (*can1-ura3*, *cyh2-ade6*) recombination. The diploids were sporulated and random spores carrying *ura3*, *can1*, *cyh2* and *ade6* were selected. These haploid clones were tested for the presence of mutant loci as follows. They were mated with the original *MAT***a** or *MAT*α segregants and the clones that did not complement the recombination-defective phenotypes (between *URA3* and *CAN1*) were collected as mutant tester strains. These mutant haploids were used both for analysis of the spore clones obtained by tetrad analysis, and for complementation tests.

Analysis of mutant loci: To establish whether the defect in recombination in each mutant was caused by a single mutation, tetrad analysis was performed with the *MAT*α segregants. *MAT*α segregants of each mutant were crossed with a haploid strain, N22D-1. N22D-1 is a wild-type *MAT***a** haploid strain that is isogenic with the parental disomic haploid (116-7B). It also carries *leu2-27* and *his4-290*, which are the same as the alleles of *leu2* and *his4* that are found in the *MAT*α segregants. The diploids were sporulated and tetrads were analyzed. Mutant loci in the spore clones were tested by use of the mutant tester strains described above. The replica-plating method was used and the spore clones patched on MYPD were mated with both *MAT***a** and *MAT*α mutant tester strains that had been seeded on MYPD plates as a lawn. The zygotes were obtained as Trp^+, Met^+ and Ura^+ cells by replica-plating onto synthetic selective SD-(Trp, Met and Ura) medium. The diploids were examined for their recombination abilities. Since all spore clones had *leu2-27* and *his4-290* alleles and all tester strains had *leu2-1* and *his4-4* alleles, the diploids were heteroallelic at both loci (*leu2-1/leu2-27* and *his4-4/his4-290*). These heteroalleles were used to monitor intragenic recombination. The diploids also had markers for measurements of intergenic recombination (*can1-ura3* and *cyh2-ade6*). The diploid strains described above were patched onto SPM plates. After formation of spores, recombination was examined by replica-plating onto SD-(Leu, Met, Trp and Ura), SD-(His, Met, Trp and Ura) or SD-(Met, Trp and Ura) + (Can and Cyh).

Complementation tests between different mutations were also carried out by the replica-plating method using both mating-competent segregants (*MAT*α) and tester strains (*MAT***a**).

Measurement of premeiotic synthesis of DNA: Cells grown to late-logarithmic phase in YPA were harvested, washed and resuspended in SPII at a density of 5×10^7 cells/ml. Cells were shaken at 30° (temperature-sensitive mutants were shaken at 24° and 34°) and, at intervals, an aliquot corresponding to 1×10^9 cells was withdrawn and cells were harvested by centrifugation and stored at −20°. Measurements of premeiotic synthesis of DNA were made basically as described by BOSTOCK (1970). After thawing of the pellet, cells were resuspended in 0.5 ml of 0.5 N NaOH and incubated at 32° for digestion of the spore wall, which is required for adequate extraction of DNA. After a 6-hr incubation, 0.5 ml of cold 2 N $HClO_4$ was added and the suspension of cells was immediately chilled on ice for 30 min. The mixture was then centrifuged (2,000 × *g*, 10 min) and the acid-insoluble fraction was collected and washed twice in cold 0.5 N $HClO_4$ and resuspended in 0.5 N $HClO_4$. DNA was hydrolyzed by heating at 70° for 20 min and then cooled to 0°. The acid-soluble hydrolysate was collected as the supernatant after centrifugation (2,000 × *g*, 10 min). The pellet was extracted once more as described above. DNA was quantitated by a modified version of the diphenylamine method (BURTON 1968).

Examination of spores: Spores were examined by phase-contrast microscopy or fluorescence microscopy after staining with DAPI. Meiotic cells were washed twice in H_2O and fixed in 70% ethanol. After standing for 30 min at room temperature, the cells were washed twice in H_2O and then resuspended in water that contained DAPI at 1 μg/ml. After standing for 20 min, cells were washed in H_2O and stored at 4° in 50% glycerol.

Observation of mitotic recombination: Cells carrying the heteroalleles *leu2-1/leu2-27* and *his4-4/his4-290* were grown in liquid YPD to stationary phase at 30° (at 24° and 34° in the case of ts mutants). Cells were washed and resuspended in dilution buffer, and then they were plated onto SD, SD-Leu or SD-His. The plates were irradiated with UV light at a dose of 1 J/m^2. The dosage was measured with a UV radiometer (UVP, San Gabriel, California).

Measurements of meiotic recombination ("return-to-growth" experiments): Cells grown in YPA to a density of 4×10^7 cells/ml were harvested by centrifugation, washed twice in H_2O and resuspended in SPII at the original density. At various times, aliquots were withdrawn, diluted and plated onto synthetic complete or synthetic selective medium.

Measurement of sensitivity to UV and MMS: Cells at stationary phase were washed and resuspended in dilution buffer. For measurements of sensitivity to UV, cells were plated on SD and the plates were irradiated with various doses of UV at a dose rate of 1 J/m^2/sec. For assessment of sensitivity to MMS, cells were plated onto MYPD plates that contained various concentrations of MMS.

Allelism test: In order to determine whether the *hop1-13* and *hop1-14* mutations are linked to each other, an allelism test was carried out. Sporulation of heterokaryons was necessary since the *hop1-13/hop1-14* heterozygote does not make tetrads that contain four viable spores at a sufficiently high frequency to permit a test of allelism. An aliquot of a culture of N60/144, the diploid resulting from the mating between *hop1-13* (N60-11) and *hop1-14* (N144-1), was plated on MYPD to yield 100–1000 colonies per plate. The plates were immediately irradiated with UV light at a dose of 5 J/m^2 to induce mitotic conversion between the *MAT* loci. After their formation, colonies were replica-plated onto MYPD plates covered with a lawn of the *ade1* mating tester strain (K396-22B: *MAT*α *ade1*). Since the diploid is homozygous for *ade2*, *MAT***a**/*MAT***a** convertants were apparent as white replica colonies. A convertant (N60/144*) colony identified in this way was mated with *kar1* cells (SAT174-7B) on MYPD. After a 16-hr incubation, mating mixtures were patched onto sporulation medium (SPM). After 3 days, six-spored asci were dissected and analyzed (KLAR 1980).

Cloning of the *MRE* genes: The *HOP1*, *MRE2* and *REC114* genes were cloned by screening for complementation of the mutant defects in meiotic recombination. The yeast library used to isolate the genes was composed of genomic DNA that has been partially digested with *Sau*3A and inserted into the YCp50 vector (supplied by D. BOTSTEIN). Yeast strains P36h1, P40 m2, and P192 m3 which are *ura3/ura3* derivatives of P36AB, P40AB and P192AB, respectively, were transformed with this library and about 20,000 Ura^+ transformants were obtained on SD-Ura plates for each strain. The transformants were replicated onto SPM plates. After 5 days at 30° on SPM plates, the transformants were replicated onto SD-His or SD-Leu or SD-His-Leu plates to screen for His^+ and/or Leu^+ recombinants and onto SD + Can or SD + Cyh or SD + Can + Cyh plates

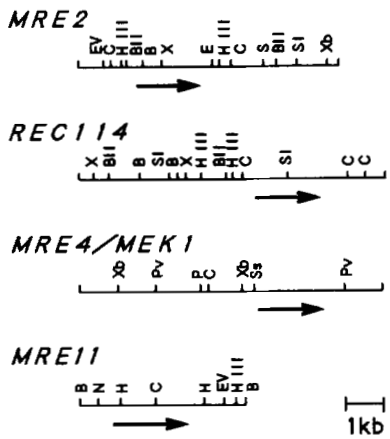

FIGURE 2.—Restriction maps of the *MRE* genes. The restriction maps of the *MRE2*, *REC114(MRE3)*, *MRE4/MEK1* and *MRE11* genes are shown. An arrow shows both the region and the direction of an open reading frame, as deduced from the nucleotide sequence. Abbreviations of restriction sites are as follows: B, *Bam*HI; BII, *Bgl*II; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; H, *Hpa*I; HIII, *Hin*dIII; N, *Nde*I; P, *Pst*I; Pv, *Pvu*II; S, *Sph*I; SI, *Sal*I; Ss, *Sst*I; X, *Xho*I; Xb, *Xba*I.

to screen for haploid Can^R and/or Cyh^R cells. The colonies that displayed wild-type levels of recombination and haploidization were selected and from them, plasmid DNA was recovered and retested for the complementation of the defects in meiotic recombination.

To clone the *MRE11* gene, the haploid *mre11* mutant strain, E8–1, was transformed with a genomic DNA library in YRp7 (ADZUMA, OGAWA and OGAWA 1984) and Trp$^+$ and MMS^R transformants were selected at 34° on SD-Trp plates that contained 0.01% MMS. After confirmation of cosegregation for both Trp$^+$ and MMS^R phenotypes, plasmid DNAs were purified from the transformants. The plasmids were used to transform a diploid *mre11-1* strain (N8A/E8) and tested for their ability to rescue the defect in meiotic recombination.

Construction of *mre* disruptants: Mutants with disruptions in the *mre* genes were constructed from the diploid strains KJC102 and KJC103 (LEEM and OGAWA 1992) by transformation and the one-step gene-disruption procedure (ROTHSTEIN 1983). The homozygous mutant diploid strains were prepared by mating between the haploid mutant strains obtained by dissecting the asci formed after the sporulation of the transformed diploids.

A subclone of the 3.4-kb *Cla*I fragment of the original isolate of the *MRE2* gene (Figure 2) was used for disruption of the *mre2* gene. A 2.2-kb *Xho*I-*Sal*I fragment containing the *LEU2* gene of YEp13 (ROSE and BROACH 1991) was inserted at the *Xho*I site in the *Cla*I subclone. The resultant 5.6-kb disrupted *Cla*I fragment was purified by gel electrophoresis and used to transform the diploid strains.

The *rec114::URA3* mutant strain was constructed by transformation of the diploid strains with the 3.9-kb disrupted *Cla*I fragment, in which the 1.2-kb *Hin*dIII fragment that contained the *URA3* gene of YEp24 (ROSE and BROACH 1991) was inserted at a *Sal*I site (Figure 2).

The *mre11::hisG* mutant strain was constructed as follows. The diploid strains were transformed with the 8.1-kb disrupted *Bam*HI fragment, in which the 3.8-kb fragment that contained the *hisG-URA3-hisG* genes (ALANI, CAO and KLECKNER 1987) was inserted at *Stu*I site of the 4.3-kb original isolate of the *MRE11* gene (Figure 2). From the Ura$^+$ transformants, Ura$^-$ segregants that occurred at high frequency by mitotic recombination between the *hisG* direct repeats to eliminate the *URA3* were isolated by selection of colonies resistant to 5-fluoro-orotic acid.

To confirm that the substitution with the disrupted gene had occurred at the correct chromosomal location as a result of transformation, genomic DNA from Leu$^+$ or Ura$^+$ transformants was examined by Southern blot analysis (SOUTHERN 1975). The procedure for the construction of the *mre4::URA3* disruptant has been described by LEEM and OGAWA (1992).

Construction of the *mre spo13* double mutants: The haploid strains carrying disruption mutations in the *mre* gene were mated with NKY654. The resultant diploids were sporulated. The tetrads were dissected and the *mre spo13* haploid segregants were mated to each other to generate *mre spo13* homozygous diploids. The genetic structure was confirmed by examining formation of dyads, and the structure of the disrupted gene in the chromosomal DNA was verified by Southern blot analysis.

RESULTS

Isolation of mutants defective in meiotic recombination: A disomic haploid strain, 116-7B, was used for screening of mutations. As described in MATERIALS AND METHODS, logarithmically growing cells were mutagenized with NTG, grown to stationary phase and plated onto MYPD. The colonies grown on MYPD were replica-plated onto sporulation medium, incubated for 4 days and then returned to leucine-free medium (SD-Leu) by a second round of replica plating, as described by ROTH and FOGEL (1971). Wild-type colonies treated in this way produced approximately 50–100 Leu$^+$ recombinant papillae on the second set of replica plates. Mutant colonies that produced few or no papillae were selected for further studies. In the primary screening, 248 colonies (mutants *#1–#248*) were identified among the 12,800 mutagenized colonies. The deficiency in recombination of these candidates was further analyzed by quantitative plating analysis; 118 colonies remained as candidates after this step. After exposure to liquid sporulation medium for two days, the frequency of recombination at *leu2* heteroalleles in wild-type cells increased approximately 10^3-fold, as compared with that of mitotic cells. While most of the candidate strains showed almost no increase in recombination, 10% of these strains exhibited residual levels of recombination at 1–10% of the wild-type level during meiosis.

Complementation and classification of mutants: Of 118 candidate strains, 49 were used to construct homozygous diploids (Figure 1, see MATERIALS AND METHODS). The remaining strains had defects in the mating type genes or in the leucine biosynthetic pathway, as determined by additional testing. Among the 49 mutant strains as diploids, 22 were defective in both premeiotic synthesis of DNA and formation of spores. These mutants were presumed to be defective in the initiation of meiosis or in premeiotic synthesis

TABLE 2

Classification of mutants defective in meiotic recombination

Gene	Mutants	New allele number
Class I (MMS^s, mitotic rec$^-$)		
RAD51	*#56*	*rad51-31*
	#189	*rad51-32*
	#195	*rad51-33*
	#197	*rad51-34*
RAD52	*#59*	*rad52-11*
	#93	*rad52-12*[a]
RAD54	*#147*	*rad54-11*
RAD57	*#57*	*rad57-11*[b]
Class II (MMS^s, mitotic rec$^+$)		
RAD50	*#139*	*rad50-11*
	#239	*rad50-12*
	#240	*rad50-13*
MRE11	*#8*	*mre11-1*[a]
Class III (MMS^r, mitotic rec$^+$)		
SPO11	*#154*	*spo11-11*
	#175	*spo11-12*
	#209	*spo11-13*
HOP1	*#36*	*hop1-11*
	#49	*hop1-12*
	#60	*hop1-13*
	#144	*hop1-14*
	#174	*hop1-15*
	#207b	*hop1-16*
MRE2	*#40*	*mre2-1*
	#55	*mre2-2*
	#77	*mre2-3*
	#222	*mre2-4*
REC114	*#192*	*rec114-11*
MRE4/MEK1	*#160*	*mre4-1/mek1*

Mutants are classified on the basis of their mitotic phenotypes. *a*, Heat-sensitive allele; *b*, cold-sensitive allele.

of DNA and were, therefore, set aside. The remaining 27 mutants were analyzed further and fell into 11 groups on the basis of complementation analysis with one another and also with mutants in the *RAD52* group (GAME and MORTIMER 1974), *spo11* (KLAPHOLZ, WADDELL and ESPOSITO 1985), *hop1* (HOLLINGSWORTH and BYERS 1989), *rec102*, *rec104*, *rec113* and *rec114* (MALONE *et al.* 1991; strains used are shown in Table 1). These 11 complementation groups can be divided into three classes on the basis of mitotic phenotypes (Table 2), as follows.

The first class is composed of eight mutants that are sensitive to a radiomimetic agent, MMS, and they are also defective in UV-induced mitotic recombination. All of the mutations were identified as mutations in one of four previously reported genes, (*RAD51*, *52*, *54* and *57*; GAME *et al.* 1980; HAYNES and KUNZ 1981) by complementation testing.

The second class contains four mutants. They are sensitive to MMS but show a hyperrecombination phenotype in mitosis and are able to carry out UV-induced recombination (as described in detail below). Three of these strains carried mutations in the *RAD50* gene. The remaining mutant (*#8*) did not complement any known *rad* mutation in the *RAD52* epistasis group at 34°. We called this mutation *mre11-1* and the mutated gene *MRE11*.

The third class contains 15 mutants that are defective in meiotic recombination but are capable of mitotic recombination. They exhibit no additional phenotype related to mitosis, such as an altered growth rate, a change in recombination frequency, or altered sensitivity to MMS or UV. These mutants fell into five complementation groups. Three groups correspond to the *SPO11*, *HOP1* and *REC114* genes. The other two groups appear to correspond to newly identified genes and were named *MRE2* and *MRE4*.

The complementation test between the *rec113* and the *rec114* mutants revealed that they could not complement each other. From this result and a personal communication from R. MALONE of University of Iowa, it appears that the *REC113* gene does not exist. We concluded that the *MRE3* and the *REC114* genes are the same gene by comparison of the restriction map of the *MRE3* gene with that of *REC114* (Figure 2 and R. MALONE, personal communication).

To confirm that the *MRE* genes are indeed new genes, all of the *MRE* genes were cloned (*MRE4*: LEEM and OGAWA 1992) and their restriction maps (Figure 2) were compared with those of the following identified and cloned genes: *MEI4* (MENEES and ROEDER 1989), *MER1* (ENGEBRECHT and ROEDER 1989), *MER2* (ENGEBRECHT, HIRSCH and ROEDER 1990), *RED1* (ROCKMILL and ROEDER 1988) and *MEK1* (ROCKMILL and ROEDER 1991). The comparisons showed that the *MRE2* and *MRE11* genes, but not the *MRE4* gene, were different from those genes. The nucleotide sequence of the *MRE4* gene was determined (LEEM and OGAWA 1992). The product of the *MRE4* gene was predicted to be a protein of 497 amino acids that contains a highly conserved amino acid sequence that is found in serine-threonine protein kinases. ROCKMILL and ROEDER (1991) isolated the *mek1* mutant as a sporulation-proficient and meiotic-lethal mutant. From a comparison of DNA sequences, we determined that *MEK1* is the same gene as *MRE4*.

All of the above-described 27 mutants were as efficient as the wild type in premeiotic synthesis of DNA, but each exhibited greatly diminished rates of meiotic recombination and haploidization. The results are presented in Table 3 for all mutants in the second and third classes discussed above.

Kinetics of premeiotic synthesis of DNA, formation of spores, haploidization and commitment to meiotic recombination in mutant diploids: Diploid strains homozygous for *hop1*, *mre2*, *rec114*, *mre4/mek1* and *mre11* were examined further to characterize meiotic phenotypes. These diploids have several genetic markers that permit monitoring of intragenic recombination (*leu2-1/leu2-27*, *his4-4/his4-290*), in-

TABLE 3

Properties of the *mre11* and class III mutant diploids with respect to meiosis

Strain	Relative increase in DNA content	Recombination		Haploidization	
		LEU2 ($\times 10^{-4}$)	*CAN1-URA3* (%)	Can^R (%)	Formation of spores (%)
Wild-type	1.66	172	30	73	60
hop1					
#36	1.49	0.80	0.34	0.52	46
#49	1.28	0.13	0.04	0.57	42
#60	1.48	0.44	0.62	1.85	49
#144	1.55	1.40	0.20	0.74	41
#176	1.42	0.04	0.01	0.01	43
#207b	1.85	0.02	0.04	0.15	18
mre2					
#40	1.56	0.09	0.02	0.23	7.9
#55	1.73	0.04	0.01	0.06	6.2
#77	1.46	0.01	0.04	0.04	22
#222	1.49	0.06	0.02	0.26	23
rec114					
#192	1.58	0.04	0.05	0.10	20
mre4/mek1					
#160	1.36	1.79	0.41	2.0	44
mre11					
#8	1.53	0.26	2.6	2.6	38

All meiotic events were examined 2 days after inoculation into sporulation medium (SPII). DNA content was measured as described in MATERIALS AND METHODS. Recombination was observed both between *leu2* heteroalleles and between *CAN1* and *URA3*; the values presented are given per 10^4 mitotic cells for the Leu$^+$ recombinants and as the percentage of recombinants for crossing over between *CAN1* and *URA3*. Haploidization was monitored by production of Can^R cells from heteroallelic (*CAN1/can1*) diploids. Formation of spores was examined under the light microscope as described in MATERIALS AND METHODS.

tergenic recombination (*can1-ura3*, *cyh2-ade6*), and haploidization (*can1*, *cyh2*). These diploids were sporulated and the time course of various meiotic events (premeiotic synthesis of DNA, haploidization, formation of spores and recombination) was monitored. The results of these experiments are shown in Figures 3 and 4. In each of the mutants for which results are shown in Figure 3 and Table 3, the rate of premeiotic synthesis of DNA was almost the same as that in the wild type. All *mre* mutants except *mre4/mek1* formed spores at the normal time, although the frequency of formation of spores was variable (Figure 3 and Table 3). In the *mre4/mek1* mutant, formation of spores was delayed by about 10-hr as compared to that in other strains. In the *mre2* and *rec114* mutants, the frequency of formation of spores was reduced to about one-third that of the wild type. The reduction in frequency was not specific to a particular mutant strain or mutant allele, since it was also observed with *mre2* mutants that carried other alleles (Table 3). The distribution of DNA in the spores was also examined after staining with DAPI. Analysis of a strain that carried an *mre4/mek1* disruption revealed that the DNA was distributed unequally among the spores (LEEM and OGAWA 1992). Similar results were obtained for other *mre* disruption mutants (our unpublished results). Although almost all the mutants formed spores at nearly wild-type levels, spore viability was below 4%. Haploidization was examined by monitoring the appearance of Can^R (or Cyh^R) colonies (GAME *et al.* 1980). Expression of Can^R represents either recombination between *CAN1* and *CEN5* or haploidization of chromosome *V*. None of the mutants produced Can^R or Cyh^R colonies after meiosis (Table 3 and Figure 4).

Commitment to meiotic recombination was examined by "return-to-growth" experiments (SHERMAN and ROMAN 1963; ESPOSITO and ESPOSITO 1974). After the initiation of meiosis by incubation in sporulation medium (SPII), cells were transferred back to vegetative growth medium at subsequent times. As shown in Figure 4, the frequencies of both intergenic recombination between *can1* and *ura3* (chromosome *V*) and intragenic recombination between the *leu2* heteroalleles were reduced in all mutants. The frequency of recombination in other regions (between *his4* heteroalleles or in the *cyh2-ade6* interval on chromosome *VII*) was also reduced in all mutants (data not shown). In *hop1*, *mre2* and *rec114* mutants, induction of meiotic recombination was almost completely blocked (the frequency was less than 0.01% of the wild-type frequency). In *mre4/mek1* diploids, recombination occurred at 2–7% of the wild-type level. The *mre4/mek1* mutant carrying a disrupted allele on the chromosome also exhibited a capacity for recombination at 2–5% of the wild-type level during meiosis (LEEM and OGAWA 1992). Although the mitotic frequency of recombination in the *mre11-1* diploid was higher than in the wild type, no additional induction of meiotic recombination was observed during meiosis. Therefore, all of the *MRE* genes are absolutely required for meiotic recombination.

***mre spo13* double mutants form viable spores but no recombinants:** We examined the viability of spores in the case of *mre2*, *rec114*, *mre4/mek1* and *mre11* disruption mutants in a *spo13* background (KLAPHOLZ and ESPOSITO 1980; MALONE 1983). The dyads of the double mutants were dissected and the frequency of viable spores was determined. As seen in Table 4, the frequencies in the double mutants (28–85%) were significantly higher than those in the respective *mre* single-disruption mutants (0.4–<0.3%). This result means that the nonviability of spores of all *mre* mutants is suppressed almost completely if the first meiotic division is bypassed by introduction of a *spo13* mutation.

The frequency of crossing over between *CEN3* and the *MAT* locus was examined for the dyads produced from the *mre spo13* double mutants. As shown in Table 4, it was 36.8% for the *spo13* single mutant and 0.8–2.5% for the double mutants. Thus the frequency

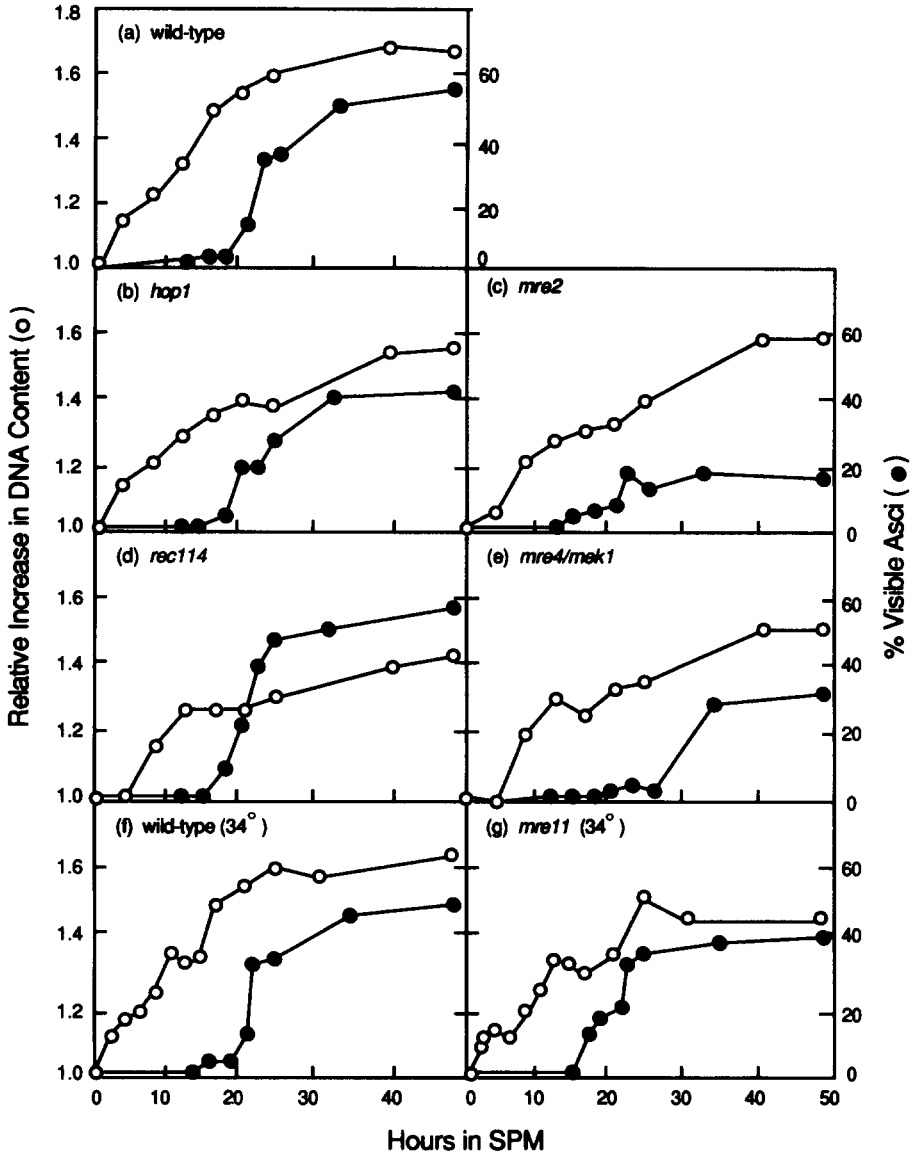

FIGURE 3.—Relative increase in DNA content and time course of formation of spores during meiosis in *mre11* and class III mutants. Diploid cells were inoculated into YPA and grown to a density of 2 × 10^7 cells per ml at 30°. Then the cells were collected and resuspended in SPII at a density of 5 × 10^7 cells per ml. *Measurements of rates of DNA synthesis:* After incubation in SPII at 30° for varying lengths of time, an aliquot of 4 × 10^9 cells was withdrawn and DNA content was measured as described in MATERIALS AND METHODS. *Observation of formation of spores:* After incubation in SPII at 30° for varying lengths of time, an aliquot of cells was withdrawn and formation of spores was examined under the light microscope as described in MATERIALS AND METHODS. Cells used in (f) and (g) were grown and sporulated at 34°. The strains used were as follows: (a) P7BAB, (b) P36AB, (c) P40AB, (d) P192AB, (e) P160AB, (f) P7BAB and (g) P8AB.

of meiotic recombination in the *mre* mutants was decreased to 2–7% of the wild-type frequency. These results show that all the *MRE* genes are actually involved in meiotic recombination.

The *mre11* mutation is pleiotropic: An *mre11-1* haploid strain (P8B-11) was crossed with a wild-type haploid (N7B-1) and the resultant diploid was subjected to tetrad analysis. The three mutant phenotypes of *mre11-1* at 34° namely, sensitivity to MMS, hyperrecombination during mitosis and a defect in recombination during meiosis, cosegregated 2:2 in all asci examined (8 asci dissected, data not shown), indicating that the pleiotropic phenotype of the original mutant was conferred by a single mutation.

Sensitivity to MMS and UV of the *mre11-1* mutant: To characterize the mitosis-related phenotype of *mre11-1* in more detail, sensitivity to MMS and UV and frequencies of mitotic recombination and mutation were determined in an *mre11-1* disomic haploid strain (C8). Since the *mre11-1* allele was associated with temperature-sensitivity to MMS, and temperature-dependent frequencies of recombination and mutation (data not shown), cultures of *mre11-1* were grown at both a permissive (24°) and a nonpermissive (34°) temperature to stationary phase, and both cultures were used for further analyses. Figure 5 shows the survival curves of *mre11-1* disomic haploid cells (C8) exposed to various concentrations of MMS on MYPD plates at the permissive (24°) and the nonpermissive (34°) temperature. Wild-type cells (116-7B) were resistant to MMS up to concentrations of 0.01% at both temperatures, whereas *mre11-1* cells grown at 34° showed a marked sensitivity (10^{-4} colony forming units at 0.01% MMS) to MMS similar to that seen with *rad50-11* (C239). We also tested the sensitivity to MMS of *mre11-1* by another method. Mutant cells grown at 34° were transiently exposed to a solution that contained a high concentration of MMS (0.1%) for up to 40 min and the survivors were monitored at 34° by plating onto MYPD plates after quenching of

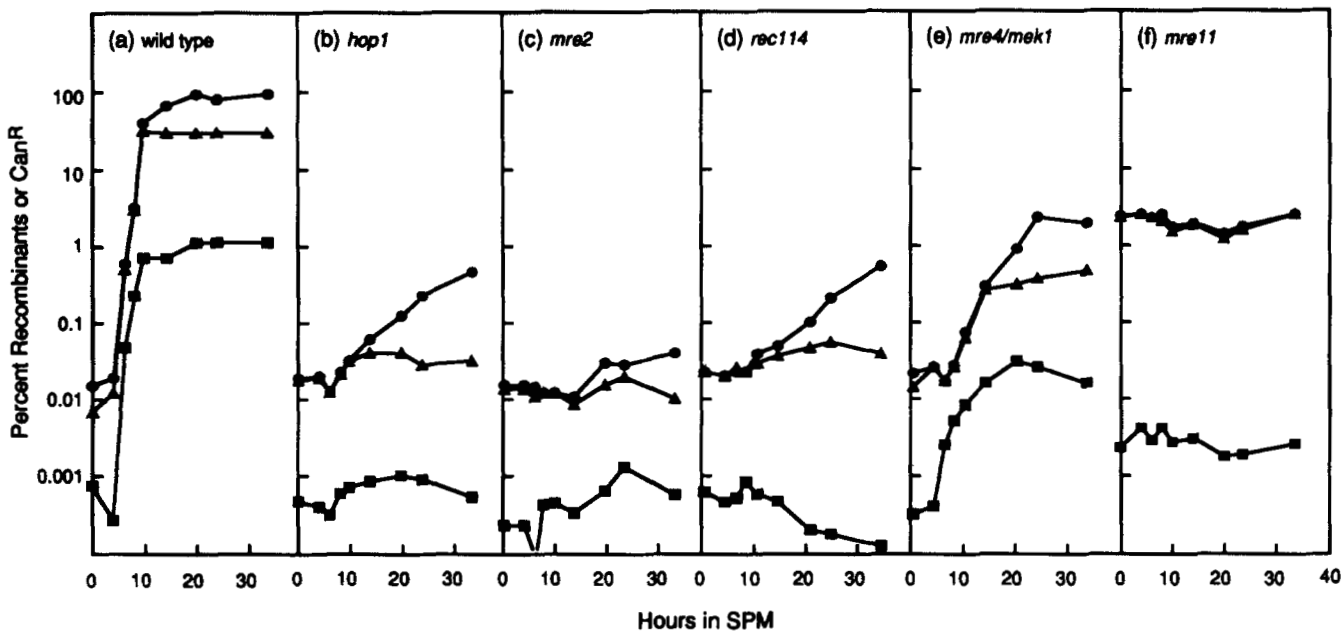

FIGURE 4.—Time course of haploidization and recombination in meiosis. Haploidization (Can^R) (●), recombination between *CAN1* and *URA3* (▲) and recombination between *leu2* heteroalleles (■) during meiosis were examined in "return-to-growth" experiments. Cells grown in YPA to 2×10^7 cells per ml at 30° or at 34° (for *mre11-1*) were collected and resuspended in SPII at the same density. After incubation in SPII at 30° or 34° (for *mre11-1*) for varying lengths of time, aliquots of cells were withdrawn and each meiotic event was examined by plating as described in MATERIALS AND METHODS. The strains used were as follows: (a) P7BAB, (b) P36AB, (c) P40AB, (d) P192AB, (e) P160AB and (f) P8AB at 34°

TABLE 4

Viability of spores and recombination in *mre* mutants and *mre spo13* double mutants

Strain	Genotype	Viability of spores (%) (viable spores/total spores)	Percent crossing over (recombinant dyads/total dyads)
KJC101	*MRE SPO13*	97.2 (171/176)	
IMD501	*MRE spo13*	70.5 (141/200)	36.8 (14/76)
IMD201	*mre2::LEU2 SPO13*	0.3 (1/352)	
IMD211	*mre2::LEU2 spo13*	85.4 (205/240)	0.94 (2/430)
IMD301	*rec114::URA3 SPO13*	<0.3 (0/352)	
IMD311	*rec114:URA3 spo13*	59.5 (100/168)	2.28 (2/176)
IMD402	*mre4/mek1::URA3 SPO13*	<0.3 (0/352)	
IMD412	*mre4/mek1::URA3 spo13*	28.5 (41/144)	2.5 (2/160)
KJC104	*mre11::hisG SPO13*	0.4 (1/224)	
KJC106	*mre11:hisG spo13*	58.3 (161/276)	<0.72 (0/276)

The double-mutant strains IMD211, IMD311, IMD412 and KJC106, and the *spo13* mutant strain IMD501 were sporulated as described in MATERIALS AND METHODS and dyads were dissected. To measure crossing over between *CEN3* and *MAT*, the spores from dyads that contained two viable spores were mated with *MAT***a** or *MATα* haploid tester strains. The dyads with one *MAT***a**/*MAT***a** and one *MATα*/*MATα* spore were scored as recombinants between *CEN3* and *MAT*. Percentage of crossing over was obtained by multiplying numbers of recombinant dyads by two, since only half of the recombination events between *MAT* and *CEN3* are detectable. However, the calculated values of crossover may be slight overestimates, especially for wild type because our calculation assumes 100% equational chromosome segregation. Reductional segregation without recombination can also lead to the same spore genotypes as those of the recombinants. For the *MRE spo13* strain, reduction segregation is expected to be more frequent than for the *mre spo13* double mutants.

MMS with sodium thiosulfate. The rate of decrease in viability of *mre11-1* cells was similar to that observed in the case of *rad50-11* (data not shown).

We examined the UV sensitivity of the *mre11-1* disomic haploid. The mutant had the same sensitivity to UV as the wild type (data not shown).

UV-induced mitotic recombination and mutagenesis in *mre11-1*: Figure 6 shows the increase in mitotic recombination after irradiation of *mre11-1* (C8), *rad50-11* (C239) and wild-type (116-7B) strains with UV light. Cells grown to stationary phase were collected and the frequency of recombination between *leu2* heteroalleles was measured after irradiation with UV light. In the wild type, Leu^+ recombinants were induced at a rate up to 100-fold greater than the rate of spontaneous induction, with the final level being proportional to the dose of UV light. Although the rates of spontaneous recombination in both the *mre11-*

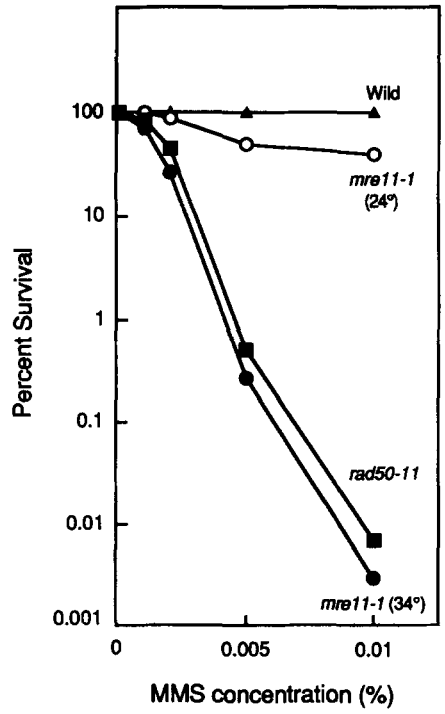

FIGURE 5.—Sensitivity to MMS of the *mre11-1* mutant. Sensitivity was measured on MMS plates. Wild-type (116–7B) and *rad50-11* (C239) mutant cells were grown to stationary-phase in YPD at 30° and plated on MYPD plates that contained various concentrations of MMS (MYPD-MMS plates). The plates were incubated at 30°. Stationary-phase cultures of the *mre11-1* mutant (C8) were generated in YPD at both 24° and 34°. The MYPD-MMS plates with cells grown at 24° and 34° were incubated at 24° and 34°, respectively. Colonies were counted after a 4-day incubation at either temperature.

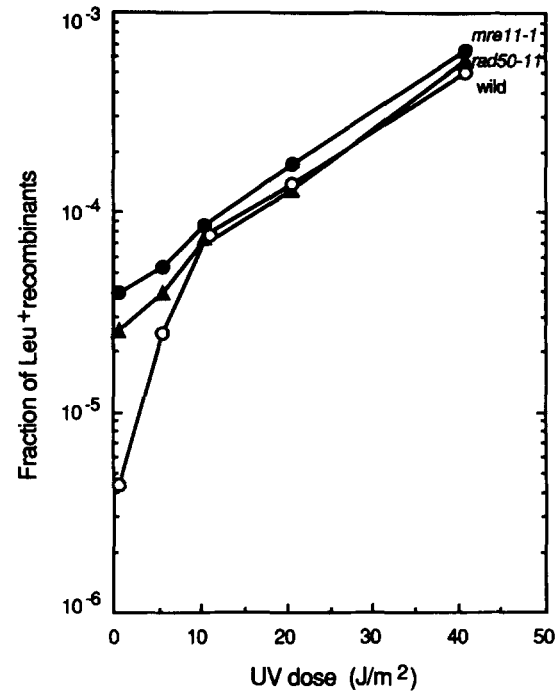

FIGURE 6.—Stimulation of recombination at *LEU2* by UV irradiation. Cells in stationary cultures in YPD of wild-type (116-7B, ○), *mre11-1* (C8, ●) and *rad50-11* (C239, ▲) strains were washed with dilution buffer and plated on SD and SD-Leu plates. The plates were irradiated with various doses of UV and incubated for 4 days. In the case of the *mre11-1* mutant, cells were grown and plates were incubated at 34°.

1 and *rad50-11* mutants were about 10 times higher than that in the wild type, the final numbers of UV-induced recombinants were similar to that observed with the wild type. Results similar to those described above were also obtained with another pair of heteroalleles (*his4-4/his4-290*) and by monitoring intergenic recombination between *CAN1* and *CEN5* (data not shown).

The frequency of mutation during mitosis was measured at the *CAN1* locus by monitoring the rate of the forward mutation (Can^S to Can^R; GAME *et al.* 1980). The *mre11-1* mutant was indistinguishable from the wild type with respect to spontaneous mutation at the *CAN1* locus and the increase in the rate of mutation as a function of the dose of UV irradiation. Frequencies of backward mutation at both *leu2-1/leu2-1* and *leu2-27/leu2-27* (Leu^- to Leu^+) were also examined in other *mre11-1* disomic haploid strains. The frequency of backward mutation, both spontaneously and after UV irradiation (40 J/m^2), at both *leu2* homoalleles was the same in the wild type and *mre11-1* strains (data not shown).

The mutant *mre11-1* was the only allele of the *MRE11* gene obtained in the screening described above. However, we subsequently obtained two other alleles of *mre11* (*mre11-2* and *-3*) by screening MMS-sensitive mutants for those that are defective in meiotic recombination but proficient in mitotic recombination (R. KATO, M. AJIMURA and H. OGAWA, unpublished results). These two new mutants do not have a temperature-dependent phenotype and their properties are the same as those of *mre11-1* at the nonpermissive temperature (34°). Therefore, the properties of *mre11-1* determined at 34° should be representative of properties of mutations in the *MRE11* gene.

Intragenic complementation among *hop1* mutants: As shown in Table 1, six *hop1* mutants were obtained by our screening procedure. Although the six corresponding alleles were classified into one group, unusual complementation was observed between some pairs of alleles. As shown in Table 5, three combinations of *hop1* alleles (*hop1-11/hop1-14*, *hop1-13/hop1-14* and *hop1-13/hop1-15*) resulted in a significant increase in recombination as compared with each homoallelic diploid. Quantitative analysis of the three diploids showed that recombination occurred at about 10% of the wild-type rate, but clearly at a rate higher than in the homoallelic diploids.

One possible explanation for this result is that the complementation is caused by a specific combination of mutant alleles within the *hop1* gene (intragenic complementation). To test this possibility, linkage be-

TABLE 5

Recombination in diploids heteroallelic at *HOP1*

	*MAT***a**				
MATα	*hop1-11*	*hop1-12*	*hop1-13*	*hop1-14*	*hop1-15*
hop1-11	0.8	3.2	1.7	12	4.4
hop1-12	4.6	1.3	6.2	5.5	2.6
hop1-13	1.6	17	11	220	47
hop1-14	35	9.4	160	1.4	4.8
hop1-15	17	6.6	56	4.2	2.5
hop1-16	0.6	7.7	2.3	3.2	3.6

Recombination was observed between *leu2* heteroalleles as described in MATERIALS AND METHODS. The values presented are the numbers of Leu$^+$ recombinants per 10^5 mitotic cells.

tween *hop1-13* and *hop1-14* was examined: a diploid obtained by mating *hop1-13* and *hop1-14* haploids was subjected to tetrad analysis to determine whether the two alleles always segregated in an allelic pattern (2 *hop1-13*: 2 *hop1-14*).

Two *hop1* haploid strains, N60-2B (*hop1-13*) and N144-2 (*hop1-14*), were mated with one another. Although the resultant diploid showed significant complementation for production of spores, the complementation was insufficient to allow production of four viable spores in an ascus. Therefore, four viable spores were obtained by the *kar1* meiosis method (KLAR 1980). As described in MATERIALS AND METHODS, a *MAT***a**/**a** convertant (N60/144*) of this diploid was selected after induction of conversion at the *MAT* locus by UV irradiation. The resulting *MAT***a**/*MAT***a** diploid was mated with a *MATα kar1* strain (SAT174-7B). The mated cells formed heterokaryons which included the *MATα* and *MAT***a**/**a** nuclei. Despite the failure of nuclear fusion, the information about mating type provided by the *kar1* nucleus via the cytoplasm is sufficient to allow the *MAT***a**/*MAT***a** nucleus to undergo meiotic divisions and sporulation. These heterokaryons produced asci that contained six spores. When these asci were dissected, only the four spores from the *hop1* diploid were viable, as determined by an analysis of genetic markers.

The spore clones from four asci were dissected and their *hop1* alleles were analyzed. They were mated with tester strains that carried the *hop1-13*, *hop1-14* or *hop1-16* allele. The resultant diploids were tested for their capacity for recombination between the *CAN1* and *URA3* loci. The results are shown in Table 6. All of the tetrads from the *hop1-13*/*hop1-14* diploid (N60/144*) appeared to have one or other of the two mutant alleles. When these spores were crossed with a haploid *hop1-16* tester strain, all the resulting diploids showed little or no meiotic recombination. This result suggests that *hop1-13* and *hop1-14* are allelic, because if *hop1-13* and *hop1-14* were unlinked, this type of segregation (parental ditype) would occur in only 1/6 of all tetrads. Moreover, as expected from

TABLE 6

Recombination between *CAN1* and *URA3* in *hop1* heteroallelic mutants

		Tester Strain (*hop1* allele)		
Tetrad[a]	*leu2*	P60-41 (*hop1-13*)	P144-41 (*hop1-14*)	P207-43 (*hop1-16*)
A	1	230	10	0.7
B	1	25	330	0.9
C	27	180	13	2
D	27	3	1500	2
A	27	7	220	0.4
B	1	3	180	0.6
C	1	400	17	3
D	27	320	14	2
A	1	58	17	1
B	27	4	410	3
C	27	7	420	2
D	1	320	11	4
A	1	7	750	2
B	27	450	7	2
C	27	5	280	0.5
D	1	180	28	0.9

Results of the allelism test for *hop1-13* and *hop1-14*. Spore clones from tetrads obtained from *hop1-13*/*hop1-14* diploids by the *kar1* meiosis method (see text) were mated with mutant tester strains P60-41, P144-41 and P207-43 for *hop1-13*, *hop1-14* and *hop1-16*, respectively. The resulting diploids were incubated in SPII for 2 days and recombination (*CAN1-URA3*) was monitored by plating on SD-Ura + Can plates. The values presented are experimental values multiplied by 10^{-4}. The wild-type level of recombination in the same experiment was 0.3 (*i.e.*, 3000×10^{-4}).

[a] All tetrads were *MAT***a**.

the original relationship between *hop1-13* and *hop1-14*, when a *hop1-13* haploid was used as the tester strain, all tetrads included two spores that complemented and two that failed to complement. When a *hop1-14* haploid was used as the tester strain, the two of the four spore clones that were not complemented by *hop1-13* were complemented. This pattern of segregation was consistently observed in all four tetrads. These results suggest that *hop1-13* and *hop1-14* are allelic and, therefore, the unusual pattern of complementation observed between mutants in *HOP1* is caused by intragenic complementation.

DISCUSSION

Using a disomic haploid strain, we isolated 27 mutants that are defective in meiotic recombination. These mutants fall into 11 complementation groups (Table 2). Two of these groups represent genes that have not been described elsewhere (*MRE2* and *MRE11*). With respect to mitosis, mutants in several complementation groups have additional phenotypes, such as the inability to repair DNA damage and/or some change in the capacity for mitotic recombination. Considering these phenotypes, we can divide our mutants roughly into three classes.

The genes in the first class (*RAD51*, *52*, *54* and *57*)

are involved in recombinational repair (*RAD52* epistasis group; reviewed by HAYNES and KUNZ 1981). The *rad52-1* mutant is sensitive to X-rays and MMS (RESNICK 1969). It exhibits a low level of spontaneous mitotic recombination and no X-ray- or UV-stimulated mitotic recombination (MALONE and ESPOSITO 1980). With respect to meiosis, spores made by the *rad52-1* mutant are not viable (GAME *et al.*, 1980; PRAKASH *et al.* 1980). Because this absence of viability is not rescued by a *spo13-1* but is rescued by *rad50-1 spo13-1*, *RAD52* is thought to be involved in an intermediate step in meiotic recombination (MALONE 1983).

The second group consists of *RAD50* (GAME *et al.* 1980) and *MRE11*. During mitotic growth, these mutants are sensitive to MMS but are capable of recombination. In fact, *rad50* and *mre11* mutants exhibit a mitotic hyperrecombination phenotype. In the case of both mutations, the inability to form viable spores is rescued by bypassing meiosis I by placing the mutations in a *spo13* background. This result suggests that both mutants are blocked at an early step in meiotic recombination. These two mutants are the only members of this group that we have been able to identify after an exhaustive search. We tried to isolate more mutants with this phenotype by screening for MMS-sensitive mutants. Among the MMS-sensitive mutants, a number of mutants defective in meiotic recombination and proficient in mitotic recombination were identified. Using this screening technique, we isolated two other mutant alleles of *mre11* and three other mutant alleles of *rad50*. Neither of the new mutant alleles of *mre11* (*mre11-2* and *mre11-3*) was associated with a temperature-dependent phenotype and the phenotypes were, in fact, the same as those of *mre11-1* at nonpermissive temperatures.

RAD50 is a member of the *RAD52* epistasis group (*RAD50-57*; reviewed in HAYNES and KUNZ 1981). Mutants in this group show similar responses to several kinds of damage to DNA and are very sensitive to X-ray and MMS, but they are only slightly sensitive to UV irradiation (GAME and MORTIMER 1974). A genetic analysis of *rad50 rad52* double mutants showed that the *RAD50* and *RAD52* genes participate in the same DNA-repair pathway. The *rad50* or *mre11* mutation causes a mitotic hyperrecombination phenotype, whereas other mutations within the *rad52* epistasis group result in a deficiency in spontaneous and induced homologous exchanges and mating-type switching (MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981). However, all these mutations abolish meiotic recombination. Thus, the behavior of these mutants provides evidence for the presence of common and unique gene functions that govern aspects of both mitotic and meiotic recombination.

There are two alternative ways of explaining the differences between both the *rad50* and *mre11* mutants and the other mutants in the *rad52* epistasis group. We can assume that the *RAD50* and *MRE11* genes are involved in the DNA-repair pathway alone and are not directly involved in the mitotic recombination pathway (although other genes in the *RAD52* group are involved in both pathways). We must also assume that the intermediate products, accumulated as a result of the blocking the repair of spontaneous damage to DNA by the *rad50* and *mre11* mutations, are recombinogenic. It then follows, that a high frequency of mitotic recombination in these mutants can be interpreted as a result of the accumulation of the recombinogenic intermediates generated by blocking the pathway for repair of spontaneous damage to DNA during mitosis. However, in meiosis, both the DNA-repair pathway and the recombination pathway in mitosis must be necessary for initiation or completion of meiotic recombination. The second explanation assumes that the *RAD50* and *MRE11* genes, are involved in both the DNA-repair and the recombination pathways. Then two ways of interpreting the results appear plausible. In mitosis, an unknown function might compensate for a defect in mitotic recombination but not for a defect in the repair process caused by the *rad50* or *mre11* mutation. During meiosis, such a function would not compensate for a defect in meiotic recombination. This compensatory function might result in recombination at a higher frequency in mitosis than that carried out by the *RAD52* recombination pathway. Another interpretation of the difference between the two mutants is that the *RAD50* and *MRE11* genes function differently in the two cell-division processes, mitosis and meiosis. In mitosis, these genes are involved only in DNA repair, while in meiosis they are involved only in recombination. This interpretation seems not implausible. In the case of *RAD50*, a mutant allele, *rad50S*, has been reported that confers resistance to DNA damage but results in a defect in meiotic recombination (ALANI, PADMORE and KLECKNER 1990). Thus, a separation of meiotic and mitotic functions may be possible for the product of a particular gene.

The newly isolated mutants alleles *mre2* and *mre4/mek1* can be placed in the third class with *spo11*, *hop1* and *rec114*. Because the corresponding mutants do not show any alterations in growth or abnormalities in aspect of mitosis such as recombination and repair, the alleles appear to be meiosis-specific in function. All of these mutants produce spores but the spores are not viable. In every case, nonviability of spores is overcome by the *spo13* mutation. Therefore, these genes may be involved in an early stage of meiotic recombination.

The Hop1 protein has been shown to be located along the length of meiotic chromosome. Thus, a

failure in the formation of synaptonemal complexes in diploids homozygous for *hop1* might result from a deficiency in a functional component of the chromosomes (HOLLINGSWORTH, GOETSCH and BYERS 1990). We found that three pairs of alleles within the *hop1* complementation group showed partial complementation of the recombination-defective phenotype. Tests for allelism between *hop1-13* and *hop1-14* showed that these mutant alleles are linked to each other. We assume that the partial complementation is intragenic in nature. The mechanism of the intragenic complementation could be explained in two ways. The *HOP1* gene might have two distinct activities, both of which are necessary for wild-type recombination. A mutant phenotype, caused by inactivation of only one of the two activities, can be complemented by another mutant that is inactive with respect to the second function. In yeast, such examples are found in the *HIS4* and *TRP5* genes (JONES and FINK 1982). Alternatively, the product of the *HOP1* gene might have only one activity. In this case, intragenic complementation could be caused by the interaction of two different mutant proteins encoded by the same gene. Genetic analysis does, in fact, suggest that products of the *HOP1* gene interact with each other as follows. Both *hop1-13* and *hop1-14* diploids showed residual levels of meiotic recombination. In a *hop1-16* diploid, recombination is blocked completely. Although *hop1-16* is not dominant with respect to the wild-type *HOP1* allele, recombination was completely blocked in *hop1-13/hop1-16* and *hop1-14/hop1-16* heteroallelic diploids. One interpretation of these results is that the *hop1-16* mutation abolishes recombination completely but the mutant protein still has the ability to interact with and eliminate the residual activity of the *hop1-13* and *hop1-14* mutant proteins (Table 6).

In this study, we tested complementation of all *mre* mutants with mutants in the *RAD52* group, *spo11-1*, *hop1* and the *rec* mutants. Three other mutants have been reported as being specifically defective in meiotic recombination. *mei4-1* (MENEES and ROEDER 1989) reduces the frequency of meiotic recombination to the mitotic background level. *red1-1* (ROCKMILL and ROEDER 1988) and *mer1-1* (ENGEBRECHT and ROEDER 1989) reduce the frequency of recombination about 10-fold. All of the corresponding mutants produce nonviable spores and the nonviability can be rescued by *spo13*. Thus, the corresponding genes are considered to act at an early stage of recombination. We have cloned all of the *MRE* genes on the basis of complementation of the mutant phenotypes (*MRE4/MEK1*: LEEM and OGAWA 1992; ROCKMILL and ROEDER 1991). Restriction maps of these *MRE* genes, when compared with those of the three above mentioned genes, revealed that the *MRE* genes are different from them.

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