The *rec102* Mutant of Yeast Is Defective in Meiotic Recombination and Chromosome Synapsis

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ABSTRACT

A mutation at the *REC102* locus was identified in a screen for yeast mutants that produce inviable spores. *rec102* spore lethality is rescued by a *spo13* mutation, which causes cells to bypass the meiosis I division. The *rec102* mutation completely eliminates meiotically induced gene conversion and crossing over but has no effect on mitotic recombination frequencies. Cytological studies indicate that the *rec102* mutant makes axial elements (precursors to the synaptonemal complex), but homologous chromosomes fail to synapse. In addition, meiotic chromosome segregation is significantly delayed in *rec102* strains. Studies of double and triple mutants indicate that the REC102 protein acts before the *RAD52* gene product in the meiotic recombination pathway. The *REC102* gene was cloned based on complementation of the mutant defect and the gene was mapped to chromosome *XII* between *CDC25* and *STE11*.

MEIOSIS is a special kind of cell division in which one round of DNA replication is followed by two rounds of chromosome segregation. After premeiotic DNA replication, homologous chromosomes synapse and undergo high levels of recombination. At the first meiotic division, homologous chromosomes move to opposite poles; at the meiosis II division, sister chromatids separate and segregate. Thus, meiosis results in the production of four haploid progeny from a single diploid cell.

Two events unique to meiosis are chromosome synapsis and high levels of genetic recombination. Synapsis is mediated by a proteinaceous structure called the synaptonemal complex (SC) (VON WETTSTEIN, RASMUSSEN and HOLM 1984) which assembles and then disassembles during prophase of meiosis I. The SC is a tripartite structure consisting of two lateral elements (each representing a pair of condensed sister chromatids) and a central element. Prior to their assembly into SC, the unpaired lateral elements are referred to as axial elements. Late in prophase I, the SC dissolves and homologous chromosomes remain connected at chiasmata, the physical manifestations of reciprocal crossover events. In the absence of genetic recombination and chiasma formation, homologous chromosomes separate from each other prematurely and therefore frequently nondisjoin at the first meiotic division.

In the yeast, *Saccharomyces cerevisiae*, numerous mutants defective in chromosome synapsis and/or genetic recombination have been isolated and characterized (*rad52*, *rad57*, GAME *et al.* 1980; *spo11*, KLAPHOLZ, WADDELL and ESPOSITO 1985; *red1*, ROCKMILL and ROEDER 1988, 1990; *hop1*, HOLLINGSWORTH and BYERS 1989; *mei4*, MENEES and ROEDER 1989; *mer1*, ENGEBRECHT and ROEDER 1989, 1990; *rad50*, ALANI, PADMORE and KLECKNER 1990; *mer2*, ENGEBRECHT, HIRSCH and ROEDER 1990). A valuable tool in the characterization of these mutants is the *spo13* mutation, which causes cells to bypass the meiosis I division and undergo a single round of predominantly equational chromosome segregation (KLAPHOLZ and ESPOSITO 1980). Recombination-defective mutants can be classified into two groups based on their behavior in a *spo13* background (MALONE and ESPOSITO 1981; MALONE 1983). Although all exchange-defective mutants produce inviable spores as single mutants, several of these, called class 1 mutants (PETES, MALONE and SYMINGTON 1991), produce viable meiotic progeny in a *spo13* background (*e.g.*, *spo11 spo13* strains produce viable spores). Other mutants, called class 2 mutants (PETES, MALONE and SYMINGTON 1991), produce dead spores even in *spo13* diploids (*e.g.*, *rad52 spo13* strains produce inviable progeny). It is generally assumed that Class 1 mutants are defective at early steps in recombination, such as initiation, and that class 2 mutants are defective at later stages. The latter class of mutants may accumulate recombination intermediates and/or broken chromosomes that cannot be resolved or repaired even in *spo13* strains. Consistent with this hypothesis, a class 1 mutation restores spore viability to *spo13* strains carrying a class 2 mutation (*e.g.*, *spo11 rad52 spo13* strains produce viable spores).

To understand better the molecular events in

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meiosis, mutants defective in meiotic chromosome segregation have been isolated (ENGEBRECHT and ROEDER 1989). In this paper, we describe the cloning and characterization of *REC102*, a yeast gene required for meiotic recombination and chromosome synapsis. The data presented indicate that *rec102* is a class 1 mutant, defective at an early step in the meiotic exchange pathway. At a late stage in this study, MALONE *et al.* (1991) reported the isolation of yeast mutants defective in meiotic recombination. Complementation tests revealed that our mutant is an allele of the gene previously designated *REC102* (MALONE *et al.* 1991).

MATERIALS AND METHODS

Strains: Yeast strains used in this study are listed in Table 1. Y35 and Y36 were obtained from TOM MENEES and RMA3R2a from ROBERT MALONE. PPA187 was obtained from KELLY TATCHELL (via BARBARA PAGE) and contains a *cdc25::URA3* disruption mutation that confers a temperature-sensitive growth defect. BR1824-3B, BR1373-6D(SF), and BR1919-8B(SF) were obtained from BETH ROCKMILL; BR1373-6D(SF) and BR1919-8B(SF) are *spo13::ura3-1* derivatives of BR1373-6D and BR1919-8B, respectively (ROCKMILL and ROEDER 1990). *Escherichia coli* XL1-Blue (Stratagene) was used for plasmid constructions. *E. coli* DH1 and YMC10 *recA* were used for transposon mutagenesis (SEIFERT *et al.* 1986).

Genetic manipulations: Yeast media and genetic methods used in this study are described by SHERMAN, FINK and HICKS (1986). Yeast strains were transformed using the lithium acetate procedure of ITO *et al.* (1983). Isogenic diploids used in assays of intragenic recombination and spore viability were constructed by transforming BR1373-6D(SF) and BR1919-8B(SF) with various plasmids and then mating the appropriate transformants to generate diploids. The isogenic diploids, JB266 and JB274, used for studies of intergenic recombination, were constructed by transforming the haploids, Y35 and Y36, and then mating the transformants. Transposon mutagenesis was carried out as described by SEIFERT *et al.* (1986). Transposon insertions and deletion-disruption mutations were introduced into yeast by the one-step gene disruption method of ROTHSTEIN (1983). All transformants were checked by Southern blot analysis (SOUTHERN 1975).

Mitotic and meiotic intragenic recombination frequencies were determined as described by MENEES and ROEDER (1989). Ectopic recombination frequencies (ROCKMILL and ROEDER 1990) were determined in return-to-growth experiments (ESPOSITO and ESPOSITO 1974). Tests of sensitivity to methylmethane sulfonate (MMS) were carried out as described by MENEES and ROEDER (1989).

The *rec102-11* mutant was one of 12 meiotic-lethal mutants isolated by ENGEBRECHT and ROEDER (1989) using the ether test described by ROCKMILL and ROEDER (1988). Spores of an *HO* strain of yeast were plated on rich medium and exposed to ultraviolet light to 50% survival. The spores grew up into diploid colonies due to HO-catalyzed mating-type switching. These were replica plated to sporulation medium for three days, then transferred to rich medium and briefly exposed to ether vapours to kill unsporulated diploid cells. Colonies in which most of the spores were inviable were ether-sensitive. Spore inviability was confirmed by tetrad dissection. The ether test was also used in cloning the *REC102* gene. A *rec102* strain was transformed with a yeast genomic library and ether-resistant transformants were identified.

Plasmid constructions: Plasmids were constructed by standard methods (MANIATIS *et al.* 1982). The *REC102* gene was isolated from a yeast genomic library provided by FORREST SPENCER and PHIL HIETER. The library consists of a *Sau*3A partial digest of yeast genomic DNA inserted into a derivative of YCp50 (PARENT, FENIMORE and BOSTIAN 1985) in which the *URA3* gene is replaced by *LEU2*. The original clone containing *REC102* sequences was designated pJB1. pJB5 was constructed by inserting a 2.5-kbp *Bam*HI fragment from pJB1 into pUN105 (ELLEDGE and DAVIS 1988). pJB3 was constructed by inserting the same fragment at the *Bam*HI site of pHSS6 (SEIFERT *et al.* 1986). A series of transposon insertion mutations was generated (SEIFERT *et al.* 1986) in pJB3 and these were assigned numbers as indicated in Figure 1. Insertion mutation #9 is designated as the *rec102-12::LEU2* allele. Derivatives of pJB3, carrying transposon insertions, were digested with *Not*I prior to transformation into yeast.

A *rec102* deletion mutation was constructed from the pJB3 derivatives carrying transposon insertions 9 and 19. Both plasmids were digested with *Sac*I and *Sna*BI; the *Sac*I site is located in the transposon to the right of the *LEU2* gene. The large *Sac*I-*Sna*BI fragment from insertion 9 was ligated to the small *Sac*I-*Sna*BI fragment from insertion 19. This results in deletion of the *REC102* sequences between the sites of the two insertions while retaining the *LEU2* gene. The resulting plasmid is designated pJB7 and the mutant allele is referred to as *rec102-13::LEU2*. pJB7 was digested with *Not*I prior to yeast transformation.

The *rad52::TRP1* plasmid (SCHILD *et al.* 1983) was targeted for substitutive transformation by digestion with *Bam*HI. A plasmid containing the *STE11* gene was obtained from BEVERLY ERREDE. The *Bam*HI-*Sal*I fragment containing *STE11* (CHALEFF and TATCHELL, 1985) was subcloned between the *Bam*HI and *Sal*I sites of YIp5 and targeted for integration by digestion with *Xho*I. Plasmid CM208, containing the wild-type *REC102* gene on YCp50, was obtained from ROBERT MALONE (MALONE *et al.* 1991).

Cytology: For cytological studies, cultures were grown at 30° in YPAD overnight and diluted 1:2 in YPAD 8 hr prior to transfer to sporulation medium. Meiotic nuclei were prepared and spread for examination in the electron microscope using the general protocol of DRESSER and GIROUX (1988) as modified by ENGEBRECHT and ROEDER (1990). Cells were stained with diamidino-2-phenyl-indole (DAPI) and examined in the fluorescence microscope as described by THOMPSON and ROEDER (1989).

RESULTS

Mutant isolation: A screen for yeast mutants that produce inviable spores was carried out by ENGEBRECHT and ROEDER (1989). One of the mutants recovered, J3, produced only 2% viable spores (Table 2). The rare viable spores from J3 were mated to spores from the wild-type strain, JB128; the resulting diploids (homozygous for *HO*) were sporulated and tetrads were dissected and analyzed in order to determine whether the meiotic-lethal phenotype is due to a single mutation. Spores from such tetrads grow into diploid colonies due to HO-catalyzed mating-type switching followed by mating within the colony; these colonies were sporulated and assayed for spore viabil-

TABLE 1

Yeast strains

Strain	Genotype
BR1373-6D (SF)	*MATa leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-1 cyh10^r*
BR1824-3B	*MATa HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 lys2* *MATα HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 lys2*
BR1919-8B (SF)	*MATα leu2-3,112 his4-280 ura3-1 spo13::ura3-1 thr1-4 trp1-289 ade2-1*
J3	*MATa HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 lys2 rec102-11 mut?* *MATα HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 lys2 rec102-11 mut?*
JE138-8D	*MATa leu2-27 his4-260 trp1-289 ura3-1 spo13::URA3 lys2*
J23-11A	*MATa leu2-27 his4-260 HO trp1 ura3-1 lys2 rec102-11* *MATα leu2-27 his4-260 HO trp1 ura3-1 lys2 rec102-11*
JB128	*MATa leu2-3,112 HIS4-ura3-Stu-his4-260 ura3-1 HO trp1-1 thr1-4 ade2-1 lys2-1* *MATα leu2-3,112 HIS4-ura3-Stu-his4-260 ura3-1 HO trp1-1 thr1-4 ade2-1 lys2-1*
JB247	*MATa leu2-3,112 HIS4-ura3-Stu-his4-260 ura3-1 HO trp1-1 thr1-4 ade2-1 lys2-1 rec102-12::LEU2* *MATα leu2-3,112 HIS4-ura3-Stu-his4-260 ura3-1 HO trp1-1 thr1-4 ade2-1 lys2-1 rec102-12::LEU2*
JB262	*MATa leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-1 cyh10^r rec102-12::LEU2*
JB266	*MATa leu2 HIS4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 ade2-1 trp1-H3 ura3 rec102-12::LEU2* *MATα leu2 his4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4 ade2-1 trp1-H3 ura3 rec102-12::LEU2*
JB268	*MATa leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-1 cyh10^r rec102-12::LEU2* *MATα leu2-3,112 his4-260 ura3-1 spo13::ura3-1 ARG4 thr1-4 trp1-289 ade2-1 CYH10 rec102-12::LEU2*
JB274	*MATa leu2 HIS4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 ade2-1 trp1-H3 ura3 REC102* *MATα leu2 his4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4 ade2-1 trp1-H3 ura3 rec102-12::LEU2*
JB276	*MATa leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-1 chy10^r REC102* *MATα leu2-3,112 his4-260 ura3-1 spo13::ura3-1 ARG4 thr1-4 trp1-289 ade2-1 CHY10 rec102-12::LEU2*
JB278	*MATa leu2-27 his4-280 ura3-1 spo13::ura3-1 arg4-8 thr1-1 trp1-1 ade2-1 cyh10^r rec102-13::LEU2* *MATα leu2-3,112 his4-260 ura3-1 spo13::ura3-1 ARG4 thr1-4 trp1-289 ade2-1 CHY10 rec102-13::LEU2*
JB289	*MATa leu2 HIS4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1trp1-H3 ade2-1 cyh10^r rec102-12::LEU2 rad52::TRP1* *MATα leu2 his4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4trp1-H3 ade2-1 CYH10 rec102-12::LEU2 rad52::TRP1*
JB290	*MATa leu2 HIS4 lys2-1 cyh10^r spo13::TRP1 ARG4 THR1 trp1-H3 ade2-1 cyh10^r REC102 rad52::TRP1* *MATα leu2 his4 LYS2 CYH10 spo13::TRP1 arg4-8 thr1-4 trp1-H3 ade2-1 CYH10 rec102-12::LEU2 rad52::TRP1*
JB294	*MATα leu2 his4 trp1 ade2 thr1 ura3::HIS3 STE11-YIp5*
PPA187	*MATa leu2 his4 ura3-52 HO CDC25* *MATα leu2 his4 ura3-52 HO cdc25::URA3*
RMA3R2α	*MATα rec102-1 ade3 leu1-c his5-2 cyh2^r*
Y35	*MATa leu2 lys2-1 cyh10^r spo13::TRP1 ade2-1 trp1-H3 ura3*
Y36	*MATα leu2 his4 spo13::TRP1 arg4-8 thr1-4 ade2-1 trp1-H3 ura3*

J3 is a mutant derivative of BR1824-3B. JB266 and JB274 are isogenic diploids, derived by mating Y35 and Y36 or transformants of these haploids. JB268, JB276, JB278, JB289 and JB290 are isogenic diploids derived by mating BR1373-6D(SF) and BR1919-8B(SF) or transformants thereof. JB262 is a transformant of BR1373-6D(SF).

ity. In 11 four-spore-viable tetrads scored, spore viability segregated 2+:2− (4), 1+:3− (3) and 0+:4− (4), indicating that J3 harbors at least two different mutations, each of which confers meiotic lethality.

Spores from J3 were also crossed to the *ho* haploid, JE138–8D. One of the *HO* segregants of this cross, JB23-11A, sporulated efficiently but produced inviable spores (Table 2). Spores from this diploid were mated to spores from an *HO* strain and the resulting diploid was sporulated and tetrads were dissected. In 13 four-spore-viable tetrads analyzed, the spore inviability phenotype segregated 2+:2−, indicating that JB23-11A carries a single mutation. This mutation was designated *rec102-11* (see below). From the cross between J3 and JE138-8D, a *ho* haploid strain carrying the *rec102-11* mutation was recovered. When this

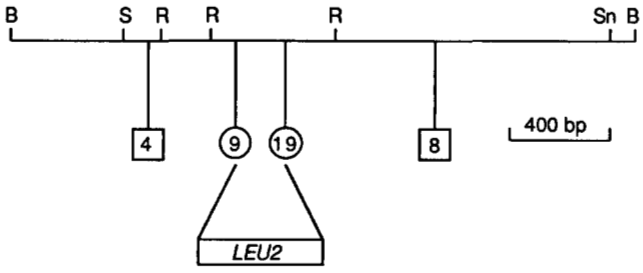

FIGURE 1.—Restriction map of the *REC102* gene. Circles indicate the sites of transposon insertions that confer a Rec⁻ phenotype; squares indicate insertions that confer a Rec⁺ phenotype. The numbers inside the circles and squares indicate the number of the insertion. Strains carrying transposon number 9 are designated *rec102-12::LEU2*. Shown below the restriction map are the position and extent of the *rec102-13::LEU2* deletion/disruption mutation. The *LEU2* gene is not drawn to scale. *Hin*dIII, *Bgl*II and *Xba*I sites were not found. B, *Bam*HI; S, *Sal*I; R, *Eco*RV; Sn, *Sna*BI.

haploid was mated to a wild-type haploid, the resulting diploid produced viable spores indicating that the *rec102-11* mutation is recessive.

The *rec102* mutant displays wild-type resistance to MMS: Many mutants that produce inviable spores are also defective in the repair of DNA damage (GAME 1983). These mutants (known as the *rad50* series) are sensitive to gamma rays and radiomimetic drugs such as MMS because they are unable to repair the DNA damage induced by these agents. Strain JB23-11A is resistant to concentrations of MMS that inhibit the growth of *rad52* strains (data not shown), indicating that the *rec102-11* mutation is not a member of the *rad50* series.

Cloning of the *REC102* gene: Strain JB23-11A was used to clone the *REC102* gene based on complementation of the spore inviability phenotype. JB23-11A was transformed with a yeast genomic library on a centromere-containing plasmid marked with the *LEU2* gene. The transformants were screened for the production of viable spores as described in the MATERIALS AND METHODS. Of approximately 2500 transformants screened, one produced viable meiotic progeny. The plasmid in this transformant (pJB1) was recovered in *E. coli* and found to contain a 12-kbp insert. Complementing activity was localized to a 2.5-kbp *Bam*HI fragment by subcloning; a restriction map of this fragment is shown in Figure 1.

The limits of the *REC102* gene were further localized by transposon mutagenesis (SEIFERT *et al.* 1986). Insertions of a transposon marked with the *LEU2* gene were generated in *E. coli* and then transformed into the *HO* diploid strain, JB128, by the one-step gene disruption method of ROTHSTEIN (1983). The resulting transformants, heterozygous for the insertion, were sporulated and tetrads dissected. Most tetrads contained four viable spores and the Leu⁺ phenotype segregated 2+:2−, indicating that the *REC102* gene product is not essential for mitotic growth. The Leu⁺ diploid colonies were sporulated and dissected to assess spore viability; this analysis of insertion mutants limited the *REC102* gene to an approximately 1-kbp region (Figure 1). One of the transposon insertions generated, called *rec102-12::LEU2* (Table 2), was used for most of the studies presented below.

A deletion mutant in which *REC102* sequences were replaced by the *LEU2* gene was also constructed. This null mutation, called *rec102-13::LEU2*, is described in the MATERIALS AND METHODS and diagrammed in Figure 1. A haploid strain carrying the *rec102-13::LEU2* deletion mutation was mated to a haploid carrying the original UV-induced mutation, *rec102-11*. The resulting diploid produced inviable spores indicating that the two mutations fail to complement each other. Thus, the gene cloned and disrupted is the gene defined by the original *rec102-11* mutation.

***rec102* spore inviability is *spo13* rescued:** To determine if *rec102* spore lethality is rescued by a *spo13* mutation, a diploid strain homozygous for a *spo13* mutation and the *rec102-12::LEU2* disruption was constructed and assayed for spore viability by dyad dissection. As shown in Table 2, the *rec102 spo13* strain produces viable spores, suggesting that the *REC102* gene product acts at or prior to the first meiotic division. In fact, the spore viability of the *rec102 spo13* strain is significantly higher than that of the isogenic *REC102 spo13* diploid ($P < 0.001$). An improvement in spore viability is also effected by the *rad50* (MALONE and ESPOSITO 1981), *spo11* (KLAPHOLZ, WADDELL and ESPOSITO 1985), *mer1* (ENGEBRECHT and ROEDER 1989), *mei4* (MENEES and ROEDER 1989) and *hop1* (HOLLINGSWORTH and BYERS 1989) mutations. Approximately a third of the spore colonies produced by *spo13* strains are small whereas spore colonies from *rec102 spo13* strains are large and uniform in size (Table 2).

***rec102* rescues the inviability of *rad52 spo13* strains:** To determine whether a *rec102* mutation restores viability to *rad52 spo13* strains, isogenic *rad52 spo13* and *rec102 rad52 spo13* diploids were constructed and analyzed for spore viability by dyad dissection. As shown in Table 2, a *rec102* mutation greatly improves the viability of *rad52 spo13* strains, indicating that the *rec102* mutation is epistatic to *rad52*. The viability of the triple mutant, *rec102 rad52 spo13*, is not as high as that of the double mutant, *rec102 spo13*. The reduced viability of the triple mutant is probably due to mitotic chromosome loss induced by the *rad52* mutation (SCHILD and MORTIMER 1985) and/or to the sensitivity of the *rad52* mutant to the enzymatic digestion used in dyad dissection. An isogenic *mei4 rad52 spo13* strain also displayed less than 60% spore viability. However, in a different

TABLE 2

Spore viability in *rec102* strains

Strain	Relevant genotype	Percent spore viability	Percent small colonies
BR1824-3B	*REC102* / *REC102*	89 (68/76)	ND
J3	*rec102-11 mut?* / *rec102-11 mut?*	2 (4/240)	NA
JB23-11A	*rec102-11* / *rec102-11*	0 (0/104)	NA
JB128	*REC102* / *REC102*	95 (155/164)	ND
JB247	*rec102-12::LEU2* / *rec102-12::LEU2*	0 (0/140)	NA
JB276	*REC102 spo13* / *rec102-12::LEU2 spo13*	65 (78/120)	30
JB268	*rec102-12::LEU2 spo13* / *rec102-12::LEU2 spo13*	93 (113/122)	2
JB289	*rec102-12::LEU2 spo13 rad52::TRP1* / *rec102-12::LEU2 spo13 rad52::TRP1*	56 (53/94)	20
JB290	*REC102 spo13 rad52::TRP1* / *rec102-12::LEU2 spo13 rad52::TRP1*	2 (1/50)	NA

Spore viability was determined by dissection of tetrads from *SPO13* strains and by dissection of dyads from *spo13* strains. BR1824-3B is the parent of J3. JB128 and JB247 are isogenic. JB276, JB268, JB289 and JB290 are isogenic. *mut?* refers to an additional meiotic-lethal mutation present (at an unidentified locus) in strain J3. ND, not determined; NA, not applicable.

strain background, the spore viability of the *mei4 rad52 spo13* triple mutant was as high as that of the *mei4 spo13* double mutant (77% for *mei4 rad52 spo13 vs.* 80% for *mei4 spo13*; MENEES and ROEDER 1989).

Intragenic recombination is not induced in *rec102* strains: Meiotic recombination includes both reciprocal crossing over and nonreciprocal events, called gene conversion. Gene conversion is most conveniently measured in auxotrophic diploids that carry two different mutant alleles at the same locus (*i.e.*, heteroallelic diploids). Intragenic recombination to produce prototrophic recombinants results most often from gene conversion (HURST, FOGEL and MORTIMER 1972; FINK and STYLES 1974).

The effect of the *rec102* mutation on gene conversion was examined in the spores produced by isogenic *rec102 spo13* and *REC102 spo13* strains carrying heteroalleles at the *HIS4*, *TRP1* and *THR1* loci. As shown in Table 3, the frequencies of His^+, Trp^+ and Thr^+ spores in the mutant were decreased relative to wild type. In fact, the frequencies of prototrophs in the *rec102* diploids were not increased above the mitotic background level, indicating that the *REC102* gene product is absolutely required for meiotically induced gene conversion. Both the *rec102-12::LEU2* insertion mutation and the *rec102-13::LEU2* deletion mutation decrease gene conversion to the same extent, suggesting that the *rec102-12::LEU2* allele is a null mutation. *rec102* mutations have no effect on mitotic gene conversion frequencies (Table 3).

Intergenic recombination is abolished in *rec102* mutants: The effect of a *rec102* mutation on reciprocal crossing over was measured by the dissection and analysis of dyads from isogenic *rec102 spo13* and *REC102 spo13* diploids. Crossing over was measured in three different intervals, *CYH10-LYS2* on chromosome *II*, *HIS4-MAT* on chromosome *III* and *ARG4-THR1* on chromosome *VIII*. As shown in Table 4, meiotic crossing over is completely eliminated by the *rec102-12::LEU2* mutation.

Although the predominant pattern of chromosome segregation in *spo13* diploids is equational, reductional and aberrant segregations also occur. The *rec102* mutation eliminates the reductional and aberrant segregations, resulting in exclusively equational chromosome segregation (Table 4).

Ectopic recombination is eliminated in *rec102* strains: Ectopic recombination (between homologous sequences at nonhomologous positions in the genome) is induced in meiosis (JINKS-ROBERTSON and PETES 1985; LICHTEN, BORTS and HABER 1987). Ectopic gene conversion was measured in *SPO13* strains containing the *ura3-1* allele at the *URA3* locus and a copy of the *URA3* gene carrying the *ura3-Stu* mutation inserted at the *HIS4* locus on chromosome *III* (ROCKMILL and ROEDER 1990). Cells were introduced into

TABLE 3

Intragenic recombination in *rec102 spo13* strains

Strain	JB276	JB268	JB278
Relevant genotype	*REC102* / *rec102-12::LEU2*	*rec102-12::LEU2* / *rec102-12::LEU2*	*rec102-13::LEU2* / *rec102-13::LEU2*
His prototrophs			
Mitotic	8.8×10^{-5}	6.4×10^{-5}	7.8×10^{-5}
Meiotic	8.9×10^{-3}	3.5×10^{-5}	2.9×10^{-5}
Fold decrease	1×	254×	306×
Trp prototrophs			
Mitotic	1.4×10^{-6}	1.4×10^{-6}	0.8×10^{-6}
Meiotic	1.1×10^{-4}	1.8×10^{-6}	1.4×10^{-6}
Fold decrease	1×	61×	78×
Thr prototrophs			
Mitotic	3.9×10^{-7}	4.5×10^{-7}	4.0×10^{-7}
Meiotic	2.0×10^{-4}	8.3×10^{-7}	6.5×10^{-7}
Fold decrease	1×	240×	307×

Frequencies refer to the number of prototrophic recombinants per viable spore. Mitotic refers to the mitotic recombination frequency and represents the median value obtained from three independent cultures. Meiotic refers to the meiotic frequency and represents the average obtained from three independent cultures. Fold decrease is calculated by dividing the mean meiotic frequency for wild type by the mean meiotic frequency for the mutant. JB276, JB268 and JB278 are isogenic diploids.

TABLE 4

Intergenic recombination in *rec102 spo13* strains

Strain	Relevant genotype	Intergenic distance (cM)			Percent spore viability	Percent aberrant segregation (*III*)	Segregation (*II*)		
		CYH10-LYS2	*HIS4-MAT*	*ARG4-THR1*			Percent equational	Percent reductional	Percent aberrant
JB266	*REC102* / *rec102-12::LEU2*	34.2	44.9	15.1	57.2 (228/398)	15.7	76.4	14.4	10.5
JB274	*rec102-12::LEU2* / *rec102-12::LEU2*	<0.4	<0.4	<0.4	69.0 (162/234)	0.0	100.0	0.0	0.0

JB266 and JB274 are isogenic diploids. The number of two-spore viable dyads scored was 83 for JB266 and 68 for JB274. Map distances were calculated using a derivation of PERKINS formula (1949) as follows: map distance = [single crossovers + 6(4-strand double cross overs)/ total] × 100. This equation accounts for the fact that half of the crossovers that are followed by equational segregation escape detection. Dyads displaying aberrant segregation were disregarded in calculating map distances. Recombinants in the *HIS4-MAT* and *CYH10-LYS2* intervals were identified as described by ROCKMILL and ROEDER (1990). Dyads classified as recombinants for the *ARG4-THR1* interval showed $2^+:0^-$ segregation for *ARG4* and $1^+:1^-$ for *THR1* or $2^+:0^-$ segregation for *THR1* and $1^+:1^-$ for *ARG4*. Four-strand double crossovers between *ARG4* and *THR1* resulted in dyads in which one spore was Arg^+ Thr^- and was Arg^- Thr^+ when recombination was followed by reductional segregation. The *ARG4-THR1* and *CYH10-LYS2* distances are underestimated because four-strand double crossovers could be detected only when followed by reductional segregation. Aberrant and reductional segregations for chromosomes *II* and *III* were identified as described by ROCKMILL and ROEDER (1990).

sporulation medium and then returned to growth medium, after the induction of meiotic recombination but prior to the commitment to meiotic chromosome segregation (ESPOSITO and ESPOSITO 1974). As shown in Table 5, the frequency of Ura^+ recombinants from the *rec102* diploid is 66-fold lower than the frequency in the isogenic wild-type strain, demonstrating that the *REC102* gene product is required for ectopic recombination in this assay. Although the frequency of Ura prototrophs is increased dramatically above the mitotic background for the wild type, there is no meiotic induction in the mutant (Table 5).

***REC102* is required for chromosome synapsis:** To examine the effect of the *rec102* mutation on SC assembly, chromosomes from isogenic Rec^+ and Rec^- strains were examined in spreads of meiotic nuclei (Figure 2). Cells were harvested and spread after 12

TABLE 5

Ectopic recombination in *rec102 spo13* strains

Strain	Relevant genotype	Ura prototrophs		
		Mitotic	Meiotic	Fold decrease
JB128	*REC102* / *REC102*	2.5×10^{-6}	1.0×10^{-4}	1×
JB247	*rec102-12::LEU2* / *rec102-12::LEU2*	1.9×10^{-6}	1.5×10^{-6}	66×

Meiotic frequencies of Ura prototrophs were measured after 15 hr in sporulation medium. The mitotic frequencies are the median values obtained from three independent cultures. Meiotic frequencies are the average of three cultures. The fold decrease was calculated by dividing the mean meiotic frequency of the wild type by that observed in the mutant.

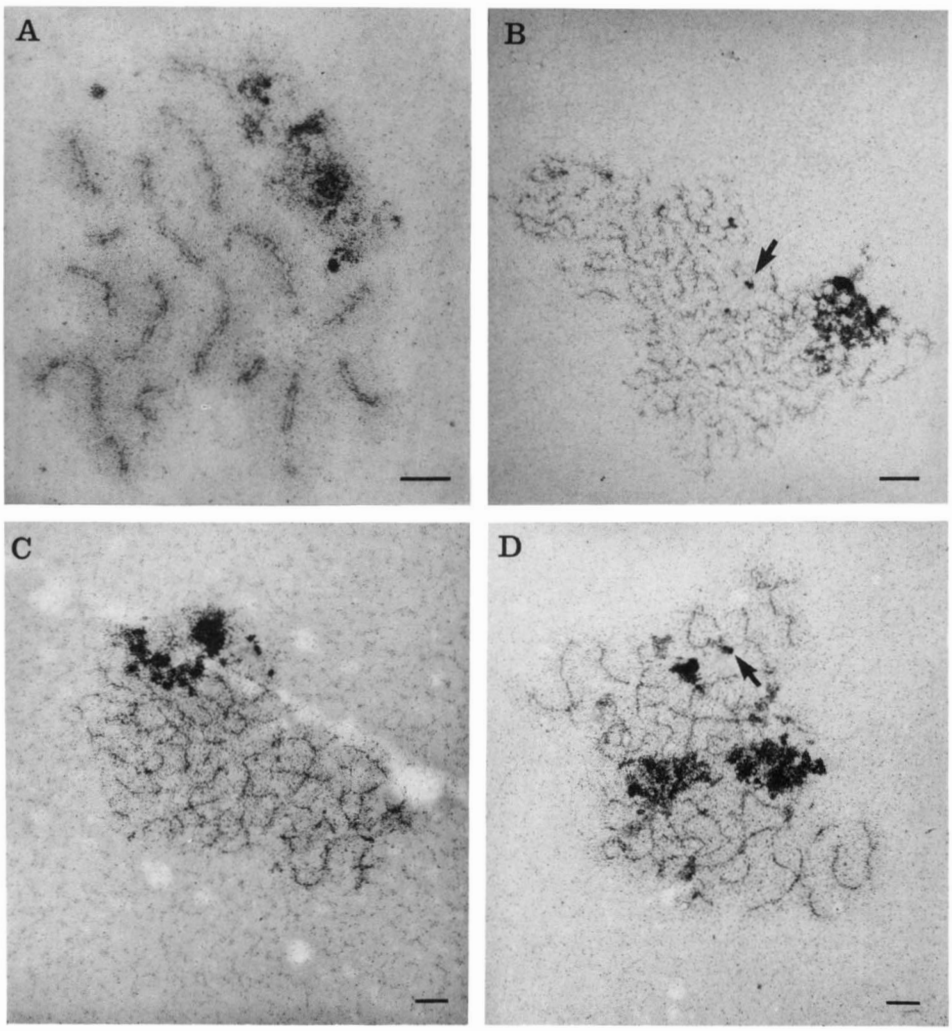

FIGURE 2.—Electron micrographs of meiotic nuclei from *REC102* and *rec102* strains. (A) Pachytene nucleus from a wild-type strain, JB128. Pairs of lateral elements can be seen along most chromosomes. (B–D) Meiotic nuclei from the *rec102* strain, JB247. Spindle pole bodies are indicated by arrows. The large, darkly staining structures are nucleoli. Bar = 1 μm.

TABLE 6

Effect of *rec102* on SC assembly

Strain	Relevant genotype	Time	Percent of nuclei (no./total)				
			Homogeneous	Axial	SC	Meiosis I	Meiosis II
JB128	*REC102*/*REC102*	12	69 (34/49)	0 (0/49)	29 (14/49)	2 (1/49)	0 (0/49)
		15	40 (20/50)	0 (0/50)	38 (19/50)	18 (9/50)	4 (2/50)
JB247	*rec102-12::LEU2*/*rec102-12::LEU2*	12	78 (40/51)	20 (10/51)	0 (0/51)	2 (1/51)	0 (0/51)
		15	63 (44/70)	34 (24/70)	0 (0/70)	3 (2/70)	0 (0/70)

Nuclei classified as homogeneous show uniform staining and contain no evidence of SC or axial elements. Nuclei classified as axial contain multiple short stretches of axial elements or apparently full-length axial elements. Nuclei classified as SC contain fully synapsed chromosomes. In most of the homogeneous, axial and pachytene nuclei, duplicated but unseparated spindle pole bodies are apparent. Meiosis I nuclei contain two separated spindle pole bodies and no SC; meiosis II nuclei contain four separated spindle pole bodies and no SC. JB128 and JB247 are isogenic.

and 15 hr in sporulation medium. At 12 hr, 29% of the wild-type nuclei were in the pachytene stage of prophase I in which chromosomes are fully synapsed (Table 6). By 15 hr, 38% of the nuclei contained SC. A typical pachytene nucleus from a wild-type cell is shown in Figure 2A; on most chromosomes, the two parallel lateral elements can be distinguished. Unsynapsed axial elements were never observed in spreads of wild-type nuclei.

SC was not observed in meiotic nuclei from the *rec102* strain (Table 6). In some preparations, the nuclei with the greatest degree of structure contained numerous, short stretches of axial elements (*e.g.*, Figure 2B). In other preparations, there were many nuclei in which the axial elements were almost or completely full-length (*e.g.*, Figure 2, C and D). The fact that full-length axial elements were observed only in some preparations suggests that these structures are easily fragmented during spreading. Even after 17 hr in sporulation medium, SC was not observed in nuclei

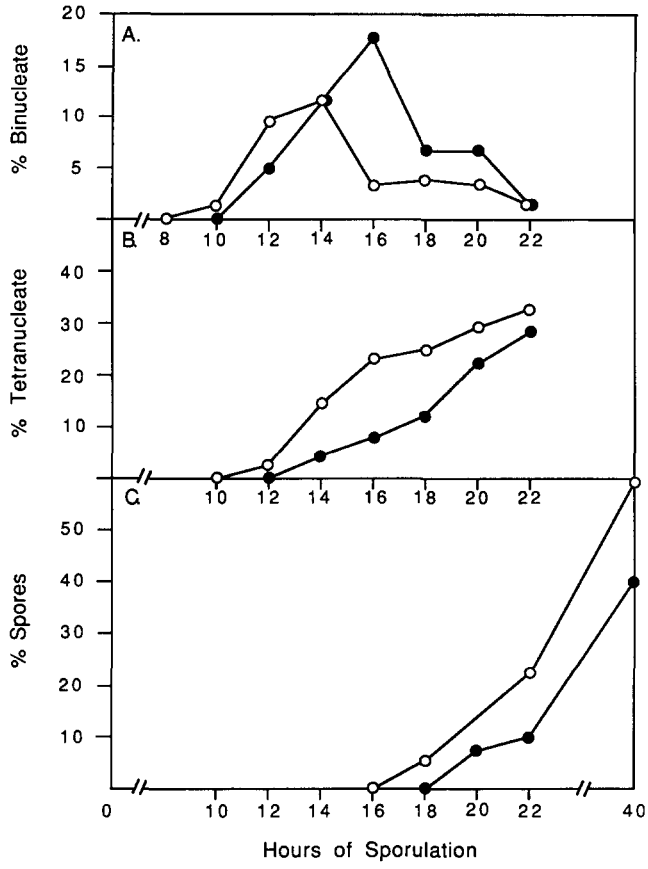

FIGURE 3.—Meiotic time course in wild-type and *rec102* strains. The numbers of binucleate cells, tetranucleate cells and tetrads as a percent of total cells are indicated by the open circles for the wild-type strain, JB128, and by the black circles for the mutant strain, JB247. Cells from each time point were stained with DAPI and examined in the fluorescence microscope. In addition, cells were examined in the light microscope to assess spore formation. JB128 and JB247 are isogenic diploids.

from the *rec102* mutant (data not shown). Thus, axial elements can develop completely in *rec102* strains but they cannot align to form SC.

Meiotic chromosome segregation is delayed in *rec102* strains: The analysis of spreads of meiotic nuclei suggested that meiotic chromosome segregation might be delayed in *rec102* strains. At 15 hr, only 3% of the mutant nuclei were undergoing the meiosis I division and there was no evidence of meiosis II divisions. In contrast, at 15 hr in wild type, 18% of the cells were in meiosis I and 4% in meiosis II. To examine more closely the progression of wild-type and mutant strains through meiosis, cells were harvested at various time points, stained with DAPI and examined in the fluorescence microscope to identify binucleate (meiosis I) and tetranucleate (meiosis II) cells. In addition, cells from each time point were examined in the light microscope for spore formation.

In wild type, binucleate (Figure 3A) and tetranucleate (Figure 3B) cells were first observed at 10 and 12 hr, respectively. In the mutant, binucleate and tetranucleate cells were not detected until 12 and 14 hr, respectively. The maximum number of binucleate cells was observed at 14 hr in wild type. In the mutant, the peak of binucleate cells was at 16 hr, by which time the number of wild-type cells undergoing the first meiotic division had decreased significantly. Spore formation was also delayed by about 2 hr in the mutant (Figure 3C). These results indicate that meiosis I chromosome segregation and subsequent events are delayed in the absence of the *REC102* gene product.

The percent of diploids detectable as binucleate cells was higher in the *rec102* mutant than in wild type (Figure 2A). When the binucleate cells at all time points are summed, it is evident that the number of binucleate cells detected in the mutant is approximately 1.5 times that observed in wild type. The increase in binucleates indicates that mutant cells spend more time than wild type between the completion of meiosis I and the completion of meiosis II. In other words, the second division is delayed even more than the first division.

Mapping of the *REC102* gene: The *REC102* gene was localized to chromosome *XII* by Southern blot analysis of electrophoretically separated yeast chromosomes (CHU, VOLLRATH and DAVIS 1986) (data not shown). A *rec102-12::LEU2* strain (JB262) was crossed to a strain (a spore of PPA187) carrying *URA3* integrated near *CDC25* on chromosome *XII*. Tetrad analysis indicated that the *REC102* gene is 13.3 cM from *CDC25* (58 PD:0 NPD:21 TT). The *rec102-12::LEU2* strain (JB262) was also crossed to a haploid carrying *URA3* integrated near *STE11* (JB294) and tetrad analysis indicated that *REC102* is 22.4 cM from *STE11* (21 PD:0 NPD:17 TT). *CDC25* and *STE11* are 41 cM apart on the right arm of chromosome *XII*; *CDC25* is centromere-proximal (MORTIMER *et al.* 1989). Our analysis places *REC102* in the interval between *CDC25* and *STE11*, closer to *CDC25*.

***REC102* is a previously defined gene:** MALONE *et al.* (1991) have recently described the isolation of yeast mutants defective at early steps in meiotic recombination. A cross was carried out between the *rec102-1* mutant (RMA3R2a) described by MALONE *et al.* (1991) and the mutant characterized in this study (JB262). The resulting diploid produced inviable spores indicating that the two mutations fail to complement. In addition, when a *rec102* homozygous diploid, JB23-11A, was transformed with a plasmid containing the wild-type *REC102* gene (MALONE *et al.* 1991), the resulting transformants produced viable spores. Thus, we have designated the mutants characterized in this study as *rec102* alleles.

DISCUSSION

In this study, we present a detailed phenotypic characterization of mutants at a locus designated

REC102 by MALONE *et al.* (1991). MALONE and coworkers isolated several *rec102* mutants on the basis of their ability to restore spore viability to *rad52 spo13* strains. They demonstrated that *rec102* strains produce inviable spores as *SPO13* diploids and showed that the *rec102-1* mutation eliminates meiotic gene conversion. They also showed that *rec102* does not affect mitotic recombination and suggested that it reduces intergenic meiotic recombination.

Our results confirm and extend those of MALONE *et al.* (1991). We demonstrate that *rec102* mutants are defective, not only in interchromosomal gene conversion, but also in reciprocal crossing over between homologues. Since crossovers are required for chiasma formation (JONES 1987), the defect in reciprocal exchange probably accounts for the observed spore lethality. In addition, we have shown that a *rec102* mutation improves the spore viability of *spo13* diploids and eliminates the reductional and aberrant segregations that normally occur in a *spo13* background. This result is consistent with the hypothesis that interchromosomal crossing over leads to connections between homologues that hinder equational chromosome segregation in a *spo13* background (WAGSTAFF, KLAPHOLZ and ESPOSITO 1982; HOLLINGSWORTH and BYERS 1989).

Our results also demonstrate that the *REC102* gene product is required for recombination between homologous sequences on nonhomologous chromosomes. It has been suggested that ectopic gene conversion reflects an homology search process that precedes and is necessary for chromosome synapsis (CARPENTER 1987; SMITHIES and POWERS 1986). If so, some meiotic mutants might be proficient in ectopic recombination, but deficient in the exchange events that occur after chromosome pairing. However, the *rec102* mutant, like all of the other yeast Rec$^-$ mutants characterized to date, reduces ectopic recombination to the same extent that it reduces recombination between homologues (ROCKMILL and ROEDER 1990; STEELE, MORRIS and JINKS-ROBERTSON 1991).

Class 1 mutants can be classified into a number of groups based on the examination of spreads of meiotic chromosomes. The *red1* mutant fails to assemble SC or any obvious precursors in SC assembly (ROCKMILL and ROEDER 1990). In the *rad50* null mutant, short stretches of incompletely developed axial elements are apparent (ALANI, PADMORE and KLECKNER 1990). The *mer1* mutant (ENGEBRECHT and ROEDER 1989) is similar to the *rec102* mutant described here in that both mutants assemble full-length, but unpaired, axial elements. In wild-type strains, axial element development and synapsis occur simultaneously; therefore, full-length axial elements are not observed in the absence of SC (ALANI, PADMORE and KLECKNER 1990). Thus, the *rec102* and *mer1* mutations must uncouple the development of axial elements from the formation of tripartite SC. The *rad50S* mutation has a similar effect on SC assembly, but it is not a class 1 mutant (*i.e.*, *rad50S* is not *spo13*-rescued (ALANI, PADMORE and KLECKNER 1990)).

rec102 adds to the growing list of yeast mutants that are defective in both meiotic recombination and SC assembly. With the exception of *spo11-1* (which may not be a null mutant) (KLAPHOLZ, WADDELL and ESPOSITO 1985), all mutations that decrease recombination prevent synapsis and *vice versa*. These observations are consistent with the hypothesis that recombination enzymes play a role in the homology search process and consequently are required for the synapsis of homologues. SMITHIES and POWERS (1986) and CARPENTER (1987) have proposed that homologue recognition involves direct DNA-DNA interactions catalyzed by strand transfer enzymes. Recognition may involve strand transfer reactions or the formation of earlier intermediates in recombination, such as three-stranded DNA (HSIEH, CAMERINI-OTERO and CAMERINI-OTERO 1990; RAO, DUTRIEX and RADDING 1991). The observation that the formation of double-strand breaks precedes the assembly of tripartite SC (ALANI, PADMORE and KLECKNER 1990) is consistent with the hypothesis that at least the initiation of recombination is required for synapsis. Furthermore, double-strand breaks do not occur in at least some class 1 mutants (CAO, ALANI and KLECKER 1990). Some or all of the class 1 mutants may be defective in the formation of the early recombination intermediates that are required to align homologous chromosomes as a necessary prerequisite to both recombination and synapsis.

Extensive SC is formed during meiosis in haploid yeast (LOIDL, NAIRZ and KLEIN 1991) and haploid plants (VON WETTSTEIN, RASMUSSEN and HOLM 1984), indicating that synapsis can take place between nonhomologous DNA segments. If recombination enzymes play a role in synapsis, then the SC observed in haploids may depend on interactions between dispersed repeated sequences (such as transposons or tRNA genes). Alternatively, some of the steps involved in homology searching may be required for nonhomologous synapsis, but the search for homology may not have to be successful in order for synapsis to proceed. It should be noted that, in *rec102*, *mer1* and *rad50S* strains, the axial elements that are formed do not fold back on themselves to engage in extensive intrachromosomal pairing and nonhomologous chromosomes do not synapse with each other as they do in wild-type haploids. Thus, the *REC102*, *RAD50* and *MER1* gene products are apparently required to carry out some aspect of synapsis that is required for both

homology-dependent and homology-independent SC formation.

In the *rec102* mutant, meiosis I chromosome segregation (and subsequent events) are significantly delayed. This observation suggests that the transition from prophase to the meiosis I division may depend on a signal that is transmitted either by the *REC102* gene product directly or by some product of *REC102* action. Even in the absence of the *REC102* gene product, the block in Meiosis I chromosome segregation is eventually bypassed and meiosis proceeds, perhaps due to an alternative signaling pathway. The meiosis I division is not delayed in several other class I mutants including *mei4* (T. M. MENEES, P. B. ROSS-MACDONALD and G. S. ROEDER, manuscript submitted for publication), *red1* (ROCKMILL and ROEDER 1990), *mer1* (ENGEBRECHT and ROEDER 1989) and *spo11* (KLAPHOLZ, WADDELL and ESPOSITO 1985; GIROUX, DRESSER and TIANO 1989).

In the *rec102* mutant, the meiosis II division is delayed even more than meiosis I, as indicated by the accumulation of binucleate cells. A delay in meiosis II is observed for *rad50S* (ALANI, PADMORE and KLECKNER 1990) and *mei4* (T. M. MENEES, P. B. ROSS-MACDONALD and G. S. ROEDER, manuscript submitted for publication) strains, even though these mutants undergo the first meiotic division with kinetics similar to wild type. In the case of the *rad50S* mutant, it has been shown that the delay in meiosis II is associated with a marked delay in the duplication and separation of meiosis II spindle pole bodies (ALANI, PADMORE and KLECKNER 1990). This perturbation in the kinetics of the meiosis II division may be a secondary effect of the alterations in recombination and synapsis during meiosis I prophase.

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