Isolation of Mutants Defective in Early Steps of Meiotic Recombination in the Yeast *Saccharomyces cerevisiae*

Robert E. Malone,[1] Steve Bullard, Michelle Hermiston, Rosemary Rieger, Marc Cool and Anne Galbraith

Department of Biology, University of Iowa, Iowa City, Iowa 52242

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ABSTRACT

Using a selection based upon the ability of early Rec⁻ mutations (*e.g., rad50*) to rescue the meiotic lethality of a *rad52 spo13* strain, we have isolated 177 mutants. Analysis of 56 of these has generated alleles of the known Rec genes *SPO11, MEI4* and *MER1,* as well as defining five new genes: *REC102, REC104, REC107, REC113* and *REC114.* Mutations in all of the new genes appear to specifically affect meiosis; they do not have any detectable mitotic phenotype. Mutations in *REC102, REC104* and *REC107* reduce meiotic recombination several hundred fold. No alleles of *RED1* or *HOP1* were isolated, consistent with the proposal that these genes may be primarily involved with chromosome pairing and not exchange.

GENETIC recombination plays a central role in meiosis; it is required not only to create new combinations of alleles within a chromosome, but also for proper chromosome pairing and the reductional segregation of homologs during meiosis I (BAKER *et al.* 1976). This central role has made the isolation of mutations in meiotic recombination functions difficult; Rec⁻ mutants usually produce aneuploid and inviable meiotic products. Over the last several years, this problem has been at least partially overcome in the yeast, *Saccharomyces cerevisiae.* Part of the reason for progress has been the increasing sophistication of the molecular and genetic techniques available in yeast, and part has been due to a better understanding of the first genes defined that affect meiosis and recombination in yeast.

One of the key steps allowing the isolation and study of Rec⁻ mutations was the discovery of the *SPO13* gene by KLAPHOLZ and ESPOSITO (1980a,b). Mutations in this gene eliminate the reductional division without any major effect on the other processes which occur in meiosis. Genetic recombination occurs at apparently normal levels and is usually followed by an equational division. *A priori,* there should be no particular requirement for recombination in the absence of meiosis I, and several investigators have found that double mutants containing both a *spo13* mutation and a Rec⁻ mutation can generate viable spores that display little or no evidence of recombination (*e.g.,* MALONE and ESPOSITO 1981; KLAPHOLZ, WADDELL and ESPOSITO 1985). That is, the *spo13* mutation "rescues" the Rec⁻ mutation. In the absence of recombination, only equational divisions are observed in *spo13* strains. Using this approach it has been directly demonstrated in meiotic products (spores) that mutations in the *RAD50* gene (MALONE and ESPOSITO 1981), *SPO11* gene (KLAPHOLZ, WADDELL and ESPOSITO 1985), *RED1* gene (ROCKMILL and ROEDER 1988), *HOP1* gene (HOLLINGSWORTH and BYERS 1989), *MER1* gene (ENGEBRECHT and ROEDER 1989), and *MEI4* gene (MENEES and ROEDER 1989) reduce meiotic recombination. In several cases (*e.g.,* the isolation of *HOP1*) the presence of the *spo13* mutation was essential for the original isolation of the mutation which defined the gene.

The use of the *spo13* mutation also allowed meiotic Rec⁻ mutations to be divided into two groups, those that were rescued by a *spo13* mutation (such as those listed above), and those that were not rescued by a *spo13* mutation (such as *rad52* and *rad57*) (MALONE and ESPOSITO 1981; GAME 1983). For example, a *rad52 spo13* strain produces only inviable spores after meiosis. Evidence that the latter group were really Rec⁻ mutations was provided by the examination of triple mutants of *spo13* and one mutant of each class (such as *spo13 rad50 rad52*) (MALONE 1983). Such triple mutants produced viable spores, with, as expected, no evidence of recombination. If the *rad52* class of Rec⁻ mutations were required for some essential meiotic process other than recombination, the triple mutant would not have generated viable spores. If each step in the process of meiotic recombination occurs in a linear dependent pathway, then the two classes of mutations can be interpreted in terms of time of function. MALONE (1983) proposed that the genes defined by mutations that are rescued by *spo13* (*e.g., RAD50*) act "early" in the recombination process,

[1] To whom correspondence should be addressed.

whereas the genes defined by mutations that are not rescued by *spo13* mutations (*e.g., RAD52*) act "later," after recombination has initiated. *S. cerevisiae* has a high frequency of meiotic recombination, and chromosomes undergo multiple exchange events per meiosis. It seems reasonable to suppose that chromosomes tied together by exchanged, but unresolved, strands would not be able to divide equationally.

In an attempt to define all the functions required for the initiation of meiotic recombination, we utilized the information known about *spo13,* early Rec$^-$ mutations, and late Rec$^-$ mutations to *select* for mutations which conferred a defect for the initiation of genetic recombination in meiosis. We anticipated that some of the mutants isolated might confer a defect in the pairing of homologous chromosomes, if that were a prerequisite for initiation of exchange. The ability to apply a selection provides the potential to saturate the system and to define all the genes involved in the initiation of meiotic recombination. Using the selective technique, we isolated 177 Rec$^-$ mutants, and here describe the initial characterization of 56 of them. We have named the new genes defined by these mutations *REC,* since they affect meiotic *rec*ombination, and since the first mutations described in yeast that affected all recombination [not just conversion (ROTH and FOGEL 1971)] were named *REC* by RODARTE-RAMON and MORTIMER (1972). A large number of mutations have been isolated by ESPOSITO *et al.* (1984) that affect mitotic recombination; the genes defined by these mutations have also been named *REC*. The majority of these mutations also affect DNA repair, and some of the genes appear to be required for meiotic recombination. Since none of our mutations affect mitotic recombination (or repair), the genes defined in the experiments described in this paper appear to be in a different class.

MATERIALS AND METHODS

Yeast and bacterial strains and plasmids: The yeast strains used in this paper are shown in Table 1. Many of the strains shown also contain other auxotrophic markers. All diploid *rec* strains used to measure recombination contained the heteroalleles *leu1–12/leu1-c* and a heterozygous recessive canavanine resistance marker (*CAN1/can1*). All bacterial transformations were done with the strain RK1448 as described (HOEKSTRA and MALONE 1985). The plasmid pRM160 containing the *SIR4* gene is shown in Figure 1; its construction is described in the legend to that figure.

Description of media: All media used in these experiments have been described in detail (MALONE and HOEKSTRA 1984). Briefly, YPD is rich medium containing glucose. YPA is identical except that potassium acetate is substituted for glucose and is used to prepare cells for sporulation. Sporulation medium contains 1% potassium acetate with required amino acids and bases added at low levels.

Isolation of Rec$^-$ mutants: The strain RMT14A-1B-39 was grown to 8.2×10^6 cells/ml in 50 ml of YPA medium. Cells were washed once in 10 ml of 0.2 M sodium phosphate buffer (pH = 7.0) and resuspended in an equal volume of phosphate buffer. The cells were mutagenized by exposure to 39 J/m^2 of 254 nm UV light. This resulted in an average of 40% survival. The mutagenized cells were pelleted, resuspended in 50 ml of YPA, and grown for 11.7 hr at 30°. When they reached a concentration of 3×10^7 cells/ml, they were pelleted, washed once in distilled water and once in sporulation medium, and resuspended in 10 ml of sporulation medium. After shaking at 200 rpm at 24° for 48 hr, the cells were examined for the degree of sporulation and pelleted. The pellet was resuspended in 1 ml of SED (1 M sorbitol, 25 mM EDTA, 50 mM dithiothreitol), and placed at 30° for 10 min. Cells were then pelleted, resuspended in 1 ml of 0.1 M Na-citrate (pH = 5.8), 10 mM EDTA containing 10% glusulase (Du Pont), and incubated for 1.5 hr at 30°. The suspension was centrifuged at 3320 rpm for 5 min, and the pellet resuspended in 10 ml H_2O. After incubation at room temperature for 10 min, the suspension was sonicated for two 10 sec periods at a setting of 50 using an Artek model 150 sonicator with microtip. Cells were then washed in phosphate buffer and resuspended in 25% ethanol. Immediately after resuspension the cells were pelleted and washed in phosphate buffer. This final cell suspension was serially diluted and fractions plated on YPG plates to isolate surviving cells and spores. Approximately 10% of the total number of surviving cells were examined further. [Because it is possible that mutants isolated might not be independent (primarily due to the growth period after mutagenesis), the experiment was repeated five times. Mutants isolated in the same experiment may be independent, but mutants isolated in different experiments must be independent. We note which experiment the various mutants came from in RESULTS.] Since mutations in meiotic specific recombination functions were desired, only normal sized colonies (*i.e.,* similar to the size of the starting strain RMT14A-1B-39) were picked for further study. The rationale for this step was that *smaller* colonies might reflect mutants that had an effect on mitotic growth. For example, most *rad50* mutations rescue *rad52 spo13,* but also confer deficiencies on repair and cause slow mitotic growth resulting in small colonies (MALONE *et al.* 1990). We did not observe any colonies that appeared to be significantly *larger* than those made by the starting strain.

Analysis of mutants to find single gene mutations: intercrosses: In order to determine whether a mutant defined a single gene, each mutant was transformed by pRM160 and mated with RM13–22C. The resulting diploid was sporulated and dissected, and at least ten random pairwise intercrosses between *SPO13 RAD52 SIR4* segregants were performed. (These segregants were identified by complementation tests with the appropriate testers except for *SIR4*; these were detected by their ability to mate.) The intercrosses were tested for the ability to sporulate, produce viable spores, and, where appropriate markers were present, undergo meiotic recombination. If the original mutant isolated contained a single recessive mutation (unlinked to *SPO13, RAD52* or *SIR4*), the mutation should segregate 2:2 and ¼ of the intercrosses should display a Rec$^-$ phenotype. [This was monitored by the degree of sporulation, the viability of spores produced, and by comparing the amount of recombination before and after sporulation (by replica plating to appropriate diagnostic media).] Any other pattern was interpreted to indicate that the mutation was complex. For example, if the original mutant contained 2 recessive mutations, both of which were necessary to eliminate meiotic recombination, then only 1/16 of the intercrosses would display a Rec$^-$ phenotype. Only those mutants for which ap-

TABLE 1

Yeast strains

Strain	Relevant genotype	Source
MEI57-14A	*MATα lys2 sir4::HIS3 can1 ura3-52 ade5 spo13-1 ade2 his4-3′Δ::LEU2::his4-260*	MERL HOEKSTRA
RMT14A-1B-39	*MATα lys2 sir4::HIS3 can1 ura3-52 ade5 spo13-1 ade2 his4-3′Δ::LEU2::his4-260 rad52::URA3*	This paper
RM13-22C	*MATα ura3-13 hom3 tyr1-1 cyh2 met13-c*	This paper
	trp5-d leu1-c ade6 ade2-1	This paper
JE102-13Da	*MATα mer1::LEU2 met2*	SHIRLEEN ROEDER
JE102-2Dα	*MATα mer1::LEU2 met2*	SHIRLEEN ROEDER
Y52	*MATa mei4::ADE2 trp1*	SHIRLEEN ROEDER
Y51	*MATα mei4::ADE2 trp1*	SHIRLEEN ROEDER
BR1373-6Da::B84	*MATa red1::ADE2 trp1-1*	SHIRLEEN ROEDER
BR919-8Cα::B84	*MATα red1::ADE2 trp1-289*	SHIRLEEN ROEDER
D1-13-4	*MATa hop1-4::URA3 lys5*	BRECK BYERS
J1-11-1	*MATα hop1-4::URA3 lys5*	BRECK BYERS
A1S11A	*MATa spo11-1 ade1*	This paper
A1S11α	*MATα spo11-1 ade1*	This paper
U1F4A	*MATa rad50-4 ade3*	This paper
U1F4α	*MATα rad50-4 ade3*	This paper
K65-3D	*HO MATa* / *HO MATα*	This paper
RM147	*MATa rec102-1* / *MATα rec102-1*	This paper
RM148	*MATa rec102-3* / *MATα rec102-3*	This paper
RM149	*MATa rec102-4* / *MATα rec102-4*	This paper
RM150	*MATa rec102-5* / *MATα rec102-5*	This paper
RM151	*MATa rec104-1* / *MATα rec104-1*	This paper
RM153	*MATa rec104-3* / *MATα rec104-3*	This paper
RM154	*MATa rec107-1* / *MATα rec107-1*	This paper
RM155	*MATa rec107-2* / *MATα rec107-2*	This paper
RM159	*MATa rec113-1* / *MATα rec113-1*	This paper
RM160	*MATa rec114-1* / *MATα rec114-1*	This paper
RM161	*MATa rec104-1 spo13-1 CAN1 leu1-c* / *MATα rec104-1 spo13-1 can1 leu1-12*	This paper
RM162	*MATa rec107-1 spo13-1 CAN1 leu1-c* / *MATα rec107-1 spo13-1 can1 leu1-12*	This paper
RM163	*MATa rec104-1* / *MATα rec104-1*	This paper
C2-1	*MATa rec107-1* / *MATα rec107-1*	This paper
C3-16	*MATa spo13-1 CAN1 leu1-c* / *MATα spo13-1 can1 leu1-12*	This paper
C3-13	*MATa rec102-1 spo13-1 CAN1 leu1-c* / *MATα rec102-1 spo13-1 can1 leu1-12*	This paper
C3-14	*MATa rec102-1 spo13-1 can1 leu1-c* / *MATα rec102-1 spo13-1 CAN1 leu1-12*	This paper
C4-1	*MATa rec102-1* / *MATα rec102-1*	This paper

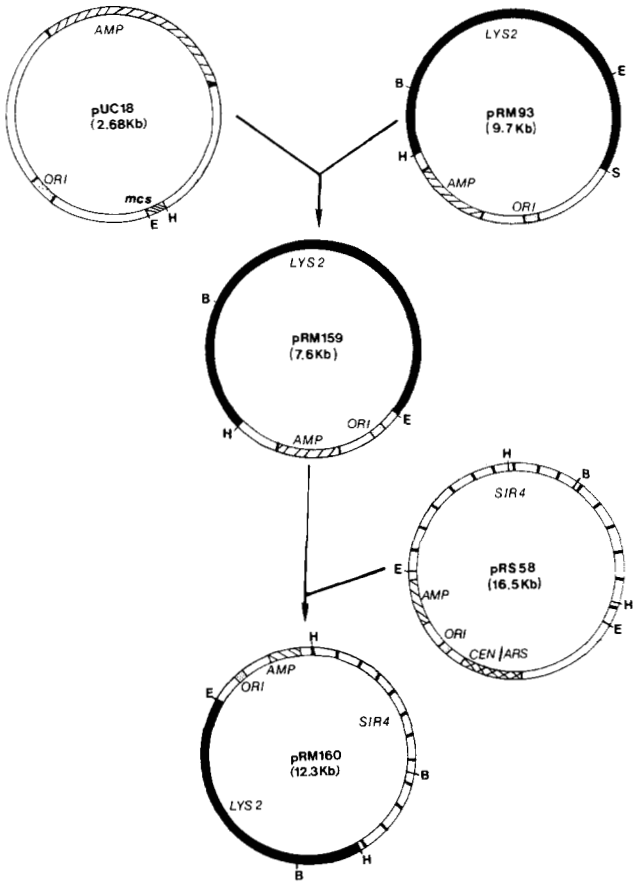

FIGURE 1.—Construction of plasmid to complement the *sir4* mutation. pRM159 was constructed from ligation of the *Hin*dIII-*Eco*RI fragment of pRM93 (this laboratory) containing *LYS2* into the multiple cloning site of pUC18 (YANISCH-PERRON, VIEIRA and MESSING 1985). The *Eco*RI fragment containing the *SIR4* gene was isolated from pRS68 (JASPER RINE, Berkeley) and then inserted into the *Eco*RI site of pRM159 to form pRM160. Although this plasmid was not designed to contain a replication origin, it appears to have a weak *ars* site (our unpublished results).

proximately ¼ of the intercrosses were Rec⁻ were studied further.

Complementation tests: Complementation tests were done with known Rec⁻ mutations (*spo11, red1,* etc.) by mating the "tester" strains (Table 1) with the Rec⁻ segregants defined by the intercrosses. Any Rec⁻ segregants initially found by the intercross procedure were tested for complementation with all other Rec⁻ segregants from all other intercrosses. Subsequently, segregants from all tetrads from intercrosses were tested for complementation with the first allele isolated of the new genes (*i.e., rec102–1, rec104–1, rec107–1, rec113–1, rec114–1*). Complementation tests were done by selecting for diploids, sporulating the diploids, and determining the degree of sporulation and spore viability. Where appropriate markers were present (in most cases), induction of meiotic recombination was measured by replica plating.

Cloning of *REC102, REC104* and *REC107* genes: To clone the wild-type allele of the *rec* mutations, we took advantage of the phenotype conferred upon diploid strains containing the mutation. That is, a Rec⁻ diploid produces inviable spores. Thus a Rec⁻ diploid containing a copy of the wild-type gene on a plasmid should have spore viability restored. The starting diploids were transformed (HINNEN, HICKS and FINK 1978) with a YCp50 yeast library (ROSE *et al.* 1987) obtained from JAN FASSLER (University of Iowa), and Ura⁺ transformants were isolated. The transformants were pooled, spread on sporulation plates, and incubated for 6–7 days at 30°. They were then scraped from the sporulation plate and treated exactly as described above for the original isolation of mutants (spheroplasting, sonication, etc.). Surviving cells were then plated on synthetic complete medium containing the drugs canavanine and cycloheximide. The starting diploid strains were heterozygous for the recessive drug resistance allele; this step selects for cells that were capable of undergoing a reductional division. All colonies obtained were tested for mating type; only those displaying a haploid mating type (**a** or α) were retained. Finally, at least one interval was checked for recombination. Only cells displaying exchange were examined further. DNA was made from isolates passing all tests and used to transform *E. coli.*

Plasmid DNA was isolated from the *Escherichia coli* transformants (MANIATIS, FRITSCH and SAMBROOK 1982) and used to retransform the Rec⁻ diploid. Only those plasmids which restored sporulation, viability and recombination were examined further. Although we obtained several different fragments of DNA for *REC102,* all contained a common region; similar results were obtained for *REC104* and *REC107.* To prove that the cloned region was indeed the gene and not a suppressor, a fragment of the cloned DNA was inserted into the Ura⁺ integrating vector pRS306 (SIKORSKI and HIETER 1989), and transformed into a Rec⁺ strain. Integrants were crossed with the appropriate *rec* mutant, to determine if the integrated *URA3* gene cosegregated with the *REC102, REC104* or *REC107* locus, respectively.

RESULTS

Selection for mutations: To select for mutations conferring a specific defect in the initiation of meiotic recombination, we constructed a strain containing a nonrevertible allele of *rad52* and the *spo13* mutation. It also contained a heteroallelic duplication of the *HIS4* locus that allowed intragenic recombination to be monitored. In order to detect recessive mutations, we used a haploid strain; in *spo13* strains, haploids can go through meiosis and sporulate if both mating types are provided (WAGSTAFF, KLAPHOLZ and ESPOSITO 1982; MALONE 1983). To express both mating types in a haploid cell, we used a nonrevertable mutant allele of the *SIR4* gene that allows expression of the silent mating type copies (MARSHALL *et al.* 1987). The starting strain is shown in Figure 2. Mutagenized cells were exposed to sporulation medium for 7 days, and remaining vegetative cells were removed by a variety of procedures (Figure 2). The experiment was repeated independently five times, and from the five experiments 1475 surviving spore colonies were picked and then examined for the ability to sporulate successfully. After this screen, 208 mutants remained which fell into two classes: (1) the desired class (177) which gave no evidence of intragenic recombination at the *his4* duplication, and (2) a class (31) that was proficient for intragenic recombination at the *his4* duplication. All of the latter mutants were still sensi-

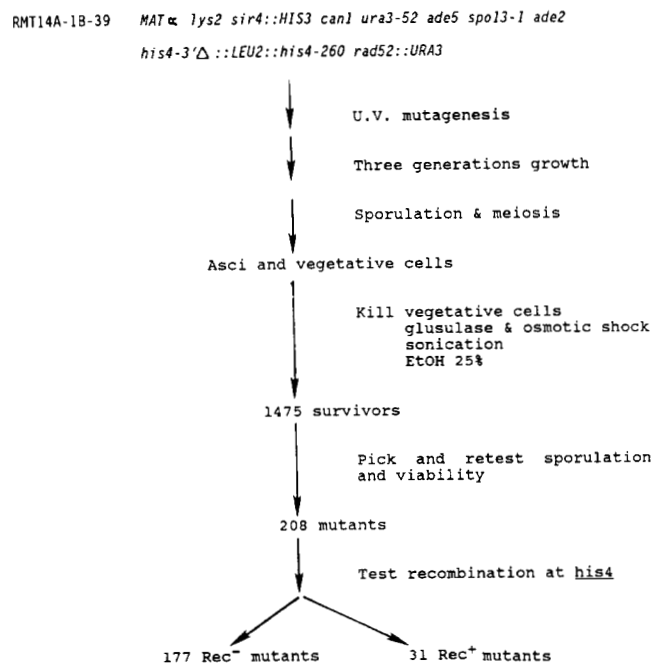

FIGURE 2.—Selection scheme for meiotic early Rec⁻ mutants. The experiment was repeated independently five times and from the five trials a total of 1475 colonies were picked and analyzed. For details, see MATERIALS AND METHODS.

tive to 0.01% methyl methane sulfonate, were Ura⁺, and were unable to complement a *rad52* mutation, indicating that they still contained the *rad52::URA3* mutation. This class of mutations, which appears to bypass the *rad52* defect, will be described elsewhere. This paper focuses on the Rec⁻ class of mutations.

Analysis of mutations: Fifty-six of the Rec⁻ class of mutants were selected for further analysis; they were picked to represent a variety of degrees of sporulation and viability, and, of course, all were Rec⁻ for intrachromosomal exchange as measured by the *his4* duplication. Upon retesting, 55 mutants produced viable spores, indicating that the new mutation present was epistatic to the *rad52::URA3* mutation (data not shown). To begin genetic analysis, all the mutants were transformed with a plasmid (pRM160) containing the *SIR4* gene. The resulting transformants were mated with a Rec⁺ strain (RM13–22C) containing useful markers for genetic analysis. Complementation tests for the known mutations *rad50, spo11, red1, hop1, mer1* and *mei4* were done on all tetrads from the outcrosses. A mutation was considered to complement if sporuation, spore viability and recombination were at wild-type levels. In all cases these three phenotypes segregated together. The outcross of the mutants with RM13–22C also allowed us to determine whether the original mutant isolated contained a single recessive *rec* mutation that rescued a *rad52 spo13* strain (see MATERIALS AND METHODS). Of the 56 mutants analyzed, 23 proved to contain a known Rec⁻ mutation (Table 2); this substantiates the logic of the selection

TABLE 2

List of genes defined by isolated mutations

Genes	No. of isolates	From experiment
Known genes		
SPO11	6	2, 3, 5, 6
MEI4	11	2, 3, 5
MER1	6	1, 3, 5
RAD50	0	
HOP1	0	
RED1	0	
New genes		
REC102	4	5, 6
REC104	3	3, 6
REC107	5	3, 5, 6
REC113	1	5
REC114	1	3

Isolates were determined to represent a mutation in a gene if they did not complement a known mutation in the gene for sporulation ability, ascus viability, and meiotic recombination (see MATERIALS AND METHODS).

used. The failure to isolate *rad50* mutations was expected since we picked only normal sized colonies (see MATERIALS AND METHODS). Likewise, no alleles of *HOP1* or *RED1* were isolated (see DISCUSSION).

Of the remaining 33 that complemented all the known Rec⁻ mutations, 14 appeared to segregate as a single recessive mutation with the phenotype expected for an early Rec⁻ mutation (Tables 2 and 3). These 14 mutations were analyzed further. To determine how many different genes the new single mutations defined, we crossed the first mutation representing each new gene with all other mutants. The resulting diploids were analyzed for ability to sporulate, spore viability, and meiotic recombination. Failure to complement for these three phenotypes allowed us to place the mutation in the same gene. Five new genes were defined by this procedure (Tables 2 and 3). The fact that only one allele of *REC113* and *REC114* was isolated raises the possibility that mutations in other new genes may be present in the 121 mutants that have yet to be examined.

Initial genetic analysis of new Rec mutations: While the restoration of meiotic viability to the *spo13 sir4:HIS3 rad52::URA3* haploid is a strong indication that the mutations isolated were early Rec⁻ mutations, we examined them in diploid strains to verify that they conferred the typical phenotypes of Rec⁻ mutations. We first analyzed the effect that the mutations had on sporulation and viability in normal (*SPO13*) diploids (Table 4). As expected, all of the Rec⁻ mutations reduced sporulation and viability in the presence of the wild type *SPO13* gene. Likewise, none of the new mutations reduced spore viability in the presence of the *spo13–1* mutation (Table 4). We have begun to analyze meiotic recombination in *spo13* diploids (Table 5). For the mutations that have been

TABLE 3

Properties of new mutants isolated in selection

Original isolate No.	Experiment No.	Mutant	Sporulation (%)	Ascus viability
51–32	5	*rec102-1*	38	80
51–39	5	*rec102-2*	41	70
60–38	6	*rec102-3*	48	70
59–33	5	*rec102-4*	36	100
60–46	6	*rec104-1*	31	80
33–44	3	*rec104-2*	40	70
60–32	6	*rec104-3*	44	80
60–14	6	*rec107-1*	3	80
31–47	3	*rec107-2*	57	54
61–28	6	*rec107-3*	51	80
33–47	3	*rec107-4*	59	90
54–58	5	*rec107-5*	58	70
57–38	5	*rec113-1*	31	40
33–55	3	*rec114-1*	50	90
RMT14A-1B-39			3	<1
MEI57-14A			61	89

Mutants were given numbers as isolated. At least two colonies were isolated from each mutant and tested for the properties shown. Experiment number refers to the number of the trial in which the mutant was isolated (see MATERIALS AND METHODS). There were five independent experiments, numbered 1–3 and 5–6; experiment 4 was lost. Sporulation percent was calculated by counting at least 200 cells; values are the averages from at least two colonies. Ascus viability was determined by placing at least 10 asci on YPD and checking for growth after 3 days. RMT14A-1B-39 is the starting strain for the mutagenesis (see Table 1) and was the negative control. MEI57-14A is the parent of RMT14A-1B-39 (see Table 1) and is the positive control. Mutants were placed in complementation groups by tests with the first isolate of each new gene (*e.g.*, *rec102-1*) (see text).

TABLE 4

Sporulation and viability in Rec⁻ diploids

Diploid name	Genotype	*SPO13* diploids: Sporulation (%)	Viable Asci	Calculated spore viability (%)[a]
A. *SPO13* diploids				
K65-3D	*REC*	69	20/20	>53[b]
RM147	*rec102-1*	12	0/37	<0.7
RM149	*rec102-4*	1.5	0/8	<3.1
RM150	*rec102-5*	11	0/9	<2.8
RM151	*rec104-1*	4	0/24	<1.0
RM153	*rec104-3*	0.9	0/35	<0.7
RM154	*rec107-1*	7	0/63	<0.39
RM155	*rec107-2*	6	0/8	<3.1
RM159	*rec113-1*	7	0/10	<2.5
RM160	*rec114-1*	3.9	0/11	<2.3

Diploid name	Genotype	*spo13* diploids: Sporulation (%)	Spore viability[c] (%)
B. *spo13* diploids			
C3-16	*REC spo13-1*	54	255/399 (64)
C3-13	*rec102-1 spo13-1*	52	369/396 (93)
RM161	*rec104-1 spo13-1*	54	62/80 (78)
RM162	*rec107-1 spo13-1*	61	387/440 (88)

[a] For *SPO13* diploids, spore viability is calculated as $1/n$, where n equals 4 times the total number of asci examined.
[b] Actual spore viability determined by dissection of asci was 97%.
[c] For *spo13* diploids, spore viability was measured directly by dissecting asci.

TABLE 5

Meiotic recombination in Rec⁻ strains

Diploid name	Genotype	Frequency of Can^r ($\times 10^4$)		Frequency of Leu$^+$ ($\times 10^4$)	
C3-16	*REC spo13-1*	7900	(1.0)	620	(1.0)
C3-13	*rec102-1 spo13-1*	20	(390)	0.14	(4430)
RM161	*rec104-1 spo13-1*	23	(340)	0.33	(1880)
RM162	*rec107-1 spo13-1*	26.5	(300)	0.344	(1800)

All strains contained a heterozygous canavanine resistance allele and heteroalleles for the *LEU1* gene (see Table 1). Values shown are the geometric mean of at least three cultures. Values in parentheses indicate the reduction caused by the *rec* mutant relative to the wild type. Aberrant meiotic segregation can contribute to the frequency of Can^r colonies in addition to meiotic recombination.

quantitated thus far, the results clearly indicate that they greatly reduce recombination between homologous chromosomes. In fact, mutations in *REC102, REC104* or *REC107* eliminate almost all induction of meiotic recombination at the loci measured. A modest (four- to eight-fold) increase in the frequency of canavanine resistant colonies was observed in *rec102* and *rec107* mutants after meiosis; this is still 300-fold less than the increase observed in wild type strains. Although the exact frequency of recombination in *rec113* and *rec114* strains has not yet been measured, both mutations do reduce meiotic recombination when measured in a replica plating assay (data not shown).

Mitotic effects of the mutations: Mutations in genes affecting meiotic recombination and DNA repair (*e.g.*, *rad50*) can significantly affect the mitotic growth rate of strains containing the mutation (MALONE 1983). None of the mutations isolated so far appear to affect mitotic growth rate; a typical growth curve is shown in Figure 3. Likewise, mutations in the new genes do not confer sensitivity to UV, MMS, or to γ-rays (data not shown). Finally, mitotic recombination frequencies in the mutants are not significantly different from those in wild type strains (Table 6). Thus far, none of the mutants tested confer any observable phenotype upon mitotic cells.

Cloning *REC102, REC104* and *REC107*: In order to clone the *REC* genes we have used a variation of the protocol used by Giroux to clone the *SPO11* gene (GIROUX *et al.* 1986). The scheme is outlined in Figure 4. To demonstrate that the protocol works, we began by cloning *REC102, REC104* and *REC107*. We examined only plasmids which were present in transformants that were recombinant for the diagnostic markers as well as displaying evidence of having undergone a reductional division (see Figure 4). Using this method we have isolated clones that complement *rec102–1 rec104–1* and *rec107–1*. We have inserted

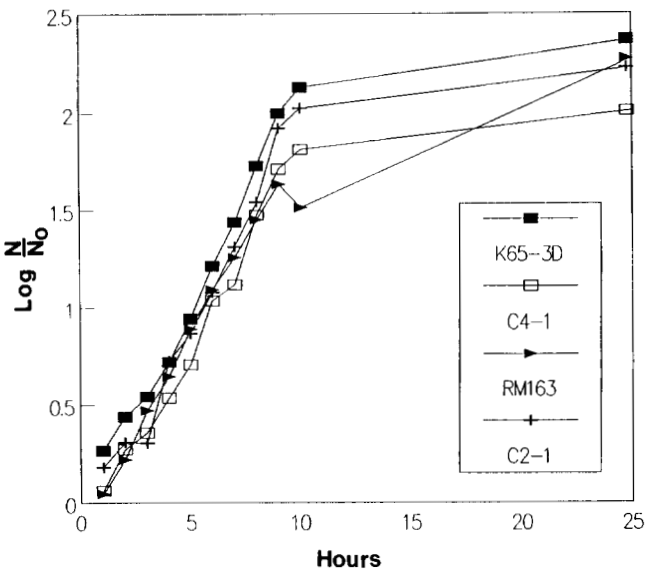

FIGURE 3.—Growth curves of various Rec⁻ diploids. The K65–3D strain was a wild type control (generation time = 86 min). C4–1, a *rec102–1/rec102–1* diploid (92 min). C2–1, a *rec107–1/rec107–1* diploid (88 min). RM163, a *rec104–1/rec104–1* diploid (88 min). Cells were diluted to a concentration of approximately 10^6 cells/ml and grown until they reached saturation. N = number of cells at given time, N_0 = number of cells at start of growth curve. The number of cells were determined by microscope count.

TABLE 6

Mitotic recombination in Rec⁻ strains

Diploid name	Genotype	No. of cultures	Frequency of Can^r ($\times 10^4$)	Frequency of Leu$^+$ ($\times 10^4$)
C3-16	*REC spo13-1*	5	4.7 ± 0.7	0.43 ± 0.4
C3-14	*rec102-1 spo13-1*	5	9.0 ± 3.4	0.47 ± 0.26
RM161	*rec104-1 spo13-1*	5	2.7 ± 0.98	0.41 ± 0.49
RM162	*rec107-1 spo13-1*	5	6.3 ± 4.6	0.54 ± 0.82

Cultures (5 ml) were grown from 100 cells/ml to a concentration of 2×10^7 cells per ml and then analyzed for the frequency of recombinants present in the culture. Values shown are the geometric mean of the recombinant frequencies and the standard deviation is shown below each value. None of the *rec* mutants display frequencies significantly different from those of the wild type.

the selectable marker, *URA3*, into the yeast chromosomal DNA using the cloned DNA to target its integration (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1983). The data in Table 7 indicate that the cloned complementing fragments of *REC102*, *REC104* and *REC107* lead to integration of *URA3* at the gene. We conclude that the clones contain the *REC* genes and not extragenic suppressors.

DISCUSSION

The mechanism whereby homologous chromosomes find each other, pair, and recombine during meiosis is not known but seems likely to involve a relatively large number of gene products. One way to decipher the events that occur during recombination and pairing is to determine which genes are required

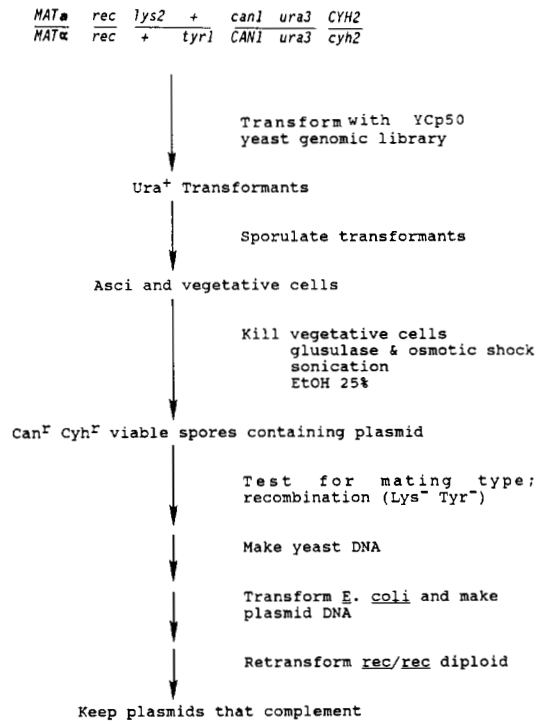

FIGURE 4.—Cloning scheme to isolate the wild-type *REC* genes.

TABLE 7

Segregation of cloned fragments with *REC* genes

Diploid genotype	P	N	T
REC102::URA3 / *rec102-1*	14	0	0
REC107::URA3 / *rec107-1*	15	0	0
REC104::URA3 / *rec104-1*	24	0	0

The *URA3* gene was integrated into the genome using fragments from the cloned complementing DNAs. These strains were crossed with *rec* haploids, sporulated, and dissected. The segregation of the *REC* locus was followed by complementation tests; the segregation of the *URA3* locus was followed by replica plating on plates without uracil. P = parental type tetrad, N = nonparental ditype type tetrad, T = tetra type tetrad.

for the process to occur and then to analyze what their gene products do in meiotic prophase. Currently, several genes are known which are required for meiotic recombination and, in some cases, chromosome pairing and synapsis. Mutations in genes like *RAD52* reduce meiotic recombination, produce inviable spores even in the absence of a reductional division (*i.e.*, *spo13*), and are rescued in a triple mutant (*e.g.*, *rad50 rad52 spo13*). This collection of phenotypes is most easily understood if the gene product is required after recombination has been initiated (MALONE 1983). Consistent with the hypothesis that the *rad52* mutation blocks meiotic exchange at an intermediate point, RESNICK *et al.* (1986) provided evidence that some induction of meiotic recombination can be genetically detected in *rad52* mutants if cells

are returned to mitotic conditions at the appropriate time. However, even the maximal level is less than 5% of the level observed in *RAD52* cells (RESNICK *et al.* 1986). Also in support of the hypothesis is the observation by BORTS, LICHTEN and HABER (1986) that a considerable amount of strand exchange can be physically detected in a *rad52* mutant.

The class of mutants that reduces meiotic recombination but not spore viability in the presence of *spo13* (*e.g.*, *rad50*) is most easily understood if mutations in these genes prevent the initiation of strand exchange, or at least the formation of covalently attached intermediates. Thus, homologs would be free to segregate equationally during meiosis II. Recently, this class (which originally consisted of *rad50* and *spo11*) has been expanded with the discovery of *hop1* mutations by HOLLINGSWORTH and BYERS (1989), and the *red1*, *mer1* and *mei4* mutations by ROEDER and colleagues (ROCKMILL and ROEDER 1988; ENGEBRECHT and ROEDER 1989; MENEES and ROEDER 1989). Mutations in the *HOP1*, *RED1* and *MER1* loci reduce meiotic recombination only to about 10% of the level normally observed. Mutations in the *MEI4* gene appear to completely eliminate meiotic recombination between homologs, as do mutations in *SPO11* and *RAD50* (MALONE and ESPOSITO 1981; KLAPHOLZ, WADDELL and ESPOSITO 1985). Both *hop1* and *red1* mutations prevent the appearance of the synaptonemal complex; this observation, taken together with the relatively high level of recombination (10% of normal) in the mutants, suggests that the wild type products of these genes may act primarily in the process of homolog pairing (ROCKMILL and ROEDER 1990). Recently, HOLLINGSWORTH, GOETSCH and BYERS (1990) have shown that the *HOP1* gene product contains amino acid sequences that would be predicted to bind to DNA, and antibodies to the *HOP1* protein bind to paired meiotic chromosomes. Mutations in *MER1*, however, accumulate axial elements of the synaptonemal complex (ROCKMILL and ROEDER 1990) and, although they only reduce recombination to about the same level as *hop1* and *red1*, may affect recombination directly. Supporting evidence for their hypothesis that *HOP1* and *MER1* affect different steps during meiosis I prophase is the observation that the double mutant *hop1 mer1* has a synergistic effect on meiotic recombination (ENGEBRECT and ROEDER 1989), reducing it almost to the background mitotic level.

We felt that, in order to understand how the processes of chromosome pairing and recombination began, it would be helpful to define as many of the genes involved in the initiation of recombination as possible. The ability of "early" Rec$^-$ mutants to rescue *rad52 spo13* strains allowed a selection to be applied, thus giving us the potential to do a saturation mutagenesis.

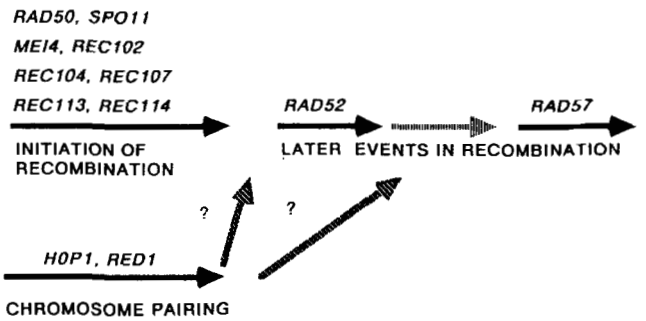

FIGURE 5.—Proposed pathway for recombination and chromosome pairing in meiosis. Because *hop1* and *red1* mutations appear to be required primarily for chromosome pairing, we have listed them on a parallel pathway to recombination (after ENGEBRECHT and ROEDER 1990). However, since they do reduce meiotic recombination to 10% of the normal levels, it seems likely that the pairing pathway and the recombination pathway interact. Note that *mer1* mutants are not placed on either pathway because, although they do rescue a *rad52 spo13* strain (ENGEBRECHT and ROEDER 1989) and were isolated in this mutant search, they only reduce recombination to 10% of the normal meiotic level. This unusual phenotype makes it unclear where *MER1* should be placed.

The selection should obviously generate Rec$^-$ mutants, and we initially felt it might also generate mutations defective in chromosome pairing, if that process were required for exchange. The method clearly worked; mutations were obtained in three known genes (*SPO11, MER1* and *MEI4*), as well as in five new genes (*REC102, REC104, REC107, REC113, REC114*). The data presented indicates that mutations in all of these genes rescue *rad52 spo13* cells. None of our mutants appear to have a phenotype suggesting that the wild type gene is primarily involved in pairing; that is, they appear to be different from the *HOP1, RED1* class. We obtained no alleles of either *RED1* or *HOP1* suggesting that, at least in a haploid strain, they might not efficiently rescue a *rad52* mutation (see below). ENGEBRECHT and ROEDER (1989) have shown that *red1* mutations *can* rescue *rad52 spo13* strains in diploids, even though *red1* only reduces meitoic recombination to 10% of the normal meiotic level. We *did* isolate alleles of *MER1*, which also only reduces meiotic recombination to 10% of the normal level; this experimental observation is consistent with the proposal of ENGEBRECHT and ROEDER (1990) that the defect of *mer1* mutants is primarily in the recombination pathway.

If, indeed, there turn out to be no *hop1* or *red1* mutations in the collection of 177 mutants isolated, it may be because the selection was done with a haploid strain. We propose that the meiotic lethality observed in *rad52 spo13* haploids (MALONE 1983) is primarily due to blocked sister strand exchange. Although both ZAMB and PETES (1981) and PRAKASH and TAILLON-MILLER (1981) have shown that *rad52* mutations do not affect spontaneous mitotic sister strand exchange in ribosomal DNA, the effect on sister strand ex-

change in nonribosomal DNA in meiosis has not been examined. Unresolved exchange events between sister chromatids would obviously inhibit the equational division that occurs in the presence of a *spo13* mutation. Perhaps *mer1* mutations block the initiation of sister strand exchange, but *hop1* and *red1* mutations do not. It has been demonstrated that neither *hop1* nor *red1* mutations eliminate *intra*chromosomal recombination (HOLLINGSWORTH and BYERS 1989; ROCKMILL and ROEDER 1990); These authors have suggested that this observation provides support for the hypothesis that their primary role is in homolog pairing rather than in recombination, *per se*. It is possible that meiotic sister chromatid exchange is also not dependent on gene products whose primary role is in chromosome pairing.

Mutations in all of the five *REC* genes isolated so far appear to confer similar phenotypes; meiotic recombination is greatly reduced and mitotic recombination and repair are unaffected. This suggests to us that the genes are specifically required for the high level of recombination observed in meiotic cells. A number of investigators have proposed pathways for recombination and chromosome pairing during meiosis (MALONE 1983; KLAPHOLZ, WADDELL and ESPOSITO 1985; ENGEBRECHT and ROEDER 1990). We summarize those proposals and include the five new *REC* genes in Figure 5. We propose that the almost complete elimination of meiotic exchange at the two loci examined suggests that these *REC* gene products are required at a point analogous to *RAD50* and *SPO11*. To confirm the magnitude of the recombination deficiency, we are examining more loci on several chromosomes. We are also searching for synaptonemal complexes in the Rec$^-$ diploids, and constructing the appropriate double mutants to examine epistatic interactions. These experiments should help to determine the points in the meiotic recombination pathway where the *REC* gene products act.

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