MEIOSIS CAN INDUCE RECOMBINATION IN *RAD52* MUTANTS OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

The *RAD52* and *RAD50* genes have previously been shown to be required for normal meiotic recombination and for various types of recombination occurring in mitotic cells. Recent evidence suggests that *rad52* mutants might be defective in an intermediate recombination step; we therefore examined recombination during meiosis in several *rad52* mutants at several different loci and in genetic backgrounds that yield efficient sporulation and synchronous meiosis. Similar to previous reports, spores from *rad52* diploids are inviable and meiotic recombination is greatly reduced by *rad52* mutations. However, intragenic recombinants were detected when cells were plated on selective media during meiosis; *rad52* mutants experience induction of recombination between homologues under these special conditions. The frequencies of recombination at four loci were considerably greater than the mitotic controls; however, they were still at least 20 times lower than corresponding Rad$^+$ strains. The prototrophs induced by meiosis in *rad52* mutants were not typical meiotic recombinants because incubation in nutrient-rich medium before plating to selective medium resulted in the complete loss of recombinants. We propose that previously observed single-strand breaks that accumulate in *rad52* mutants may be associated with recombinational intermediates that are resolved when cells are returned to selective mitotic media and that the meiosis-induced recombination in *rad52* cells does not involve double-strand breaks.

SEVERAL types of genetic recombination have been identified in the yeast *Saccharomyces cerevisiae*, including homologous chromosome intragenic and intergenic recombination and intrachromosomal, sister chromatid, mating-type switching, plasmid-chromosome, plasmid-plasmid and mitochondrial recombination (ESPOSITO and WAGSTAFF 1981; ESPOSITO and KLAPHOLZ 1981; NASMYTH 1982; ORR-WEAVER and SZOSTAK 1985; DUJON 1981). Many genes have been identified that are involved in the various types of recombination, and some of these genes might be expected to code for products that would have general utility in recombination processes. Mutations in the *RAD52* epistatic

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group of genes (including *RAD50, 51, 52, 54* and *57*) have been shown to affect several types of recombination (GAME 1983). The *RAD52* gene has proved to be particularly interesting because of the pleiotropic consequences that mutations in this gene have on recombination. Originally identified as being X-ray sensitive (RESNICK 1969), *rad52* mutants were shown to be deficient in chromosomal DNA double-strand break repair (HO 1975; RESNICK and MARTIN 1976), and they did not exhibit X-ray- or UV-induced mitotic recombination (RESNICK 1975; PRAKASH *et al* 1980). As might be expected from these effects on chromosomal double-strand break repair, plasmids containing double-strand breaks were much less efficient in recombining with homologous chromosomal regions in *rad52* as compared to Rad^+ strains (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981). In addition to being deficient in mitotic recombination induced by a variety of DNA damaging agents, the *rad52* mutants exhibited low spontaneous rates of intergenic and intragenic mitotic recombination, and they were also defective in the mating-type recombination switching process (MALONE and ESPOSITO 1980).

The *RAD52* epistasis group of genes, and *RAD52* specifically, have also been shown to be required in meiotic recombination. Using a variant of the procedure of SHERMAN and ROMAN (1963) for detecting the induction of genetic recombination during meiotic development, GAME *et al.* (1980) and PRAKASH *et al.* (1980) found that neither *rad52-1* nor *rad50-1* exhibited recombination during meiosis. In addition, the sporulated cultures exhibited reduced viability, as might be expected if recombination were absent (BAKER *et al.* 1976).

Although the *RAD52* gene clearly plays a central role in mitotic and meiotic recombination in yeast, the function of the gene product and the step(s) at which it acts are not known. MALONE and ESPOSITO (1981) suggested that *RAD52* might be involved in an intermediate stage of meiotic recombination based on an analysis using the *spo13* mutation. The *spo13* mutation eliminates the meiosis I reductional division, although other meiotic events including recombination occur normally (KLAPHOLZ and ESPOSITO 1980a,b). Recombination is not required during meiosis in *spo13* mutants, since homologues do not have to pair and segregate away from each other. For example, *rad50 spo13* diploids produce two viable, diploid nonrecombinant spores. On the other hand, *rad52 spo13* mutants produce two inviable spores. Analysis of multiple mutants showed that *rad50* was epistatic to *rad52* (MALONE 1983); that is, *rad50 rad52 spo13* strains produced viable nonrecombinant spores. Based on these observations, it was proposed that *rad50* blocks recombination at an early step, whereas *rad52* blocks recombination at a later step, after recombination intermediates are formed.

A second observation consistent with the hypothesis that *rad52* is blocked at an intermediate step in meiosis was that *rad52* mutants accumulate single-strand interruptions in chromosomal DNA; the number of interruptions corresponds approximately to the number of recombinational events in wild-type cells (RESNICK *et al.* 1981). No single-strand interruptions have been detected in *rad50* cells (RESNICK *et al.* 1984).

If *rad52* mutants were blocked at an intermediate step in recombination,

then it might be possible, under the appropriate conditions, to resolve and detect recombinants. Several factors may have contributed to the inability to detect low levels of recombination during meiosis in previous studies: poor synchrony, inefficient meiosis (*i.e.*, many cells fail to enter meiosis), use of strains that were heterozygous for other genes affecting meiotic recombination, or examination of cultures that happened to have relatively high frequencies of recombination in the starting mitotic population.

We have therefore reexamined meiotic recombination in *rad52* and *rad50* mutants. Using essentially the procedures of SHERMAN and ROMAN (1963), strains were exposed to meiosis-inducing (*i.e.*, sporulation) medium and were returned at various times to selective mitotic medium (return to mitotic medium experiments, RMM). Strains have been utilized that generally exhibit greater levels of sporulation and more synchronous meiosis and have several diagnostic loci to monitor recombination. As previously reported, both *rad50* and *rad52* mutants give rise to completely inviable asci and greatly diminished meiotic recombination; however, we found that *rad52* mutants exhibit a low but significant induction of recombination during meiosis. Interestingly, these recombinants in *rad52* mutants are lost when cells are exposed to rich medium before plating to selective media. No loss of recombinants is observed with wild-type cells, and no meiotic induction of recombination is observed in *rad50* mutants. These results confirm that the *RAD52* gene product is required for the completion of normal meiotic recombination and suggest that, in its absence, structures are developed in meiosis that can lead to complete recombinants in mitotic cells.

MATERIALS AND METHODS

Strains: Two groups of strains were used, and the genotypes are described in Table 1. Strains 1 to 4 contained heteroalleles at three loci: *URA3*, *TRP5* and *LEU1*. Each *rad52* mutant was obtained by backcrossing at least three times to good sporulating wild-type (*RAD52*) strains. Diploids were constructed by prototroph selection immediately before use. (We have found that storage of *rad52* diploids generates a variety of phenotypic suppressors.) Strains 5–9 were derived from at least three backcrosses with SK-1 (KANE and ROTH 1974), which is a homothallic strain that exhibits over 95% sporulation in a synchronous and very rapid fashion. Following the backcrosses, subsequent strains were derived by intercrossing. The presence of *rad1* in a heterozygous, or even homozygous, state does not affect meiotic recombination (RESNICK, GAME and STASIEWICZ 1983).

The *rad52::LEU2* insertion mutants were derived by transforming the appropriate SK-1 *leu2 RAD52* strains with a 2-kb fragment of the *RAD52* gene that contained the *LEU2* gene inserted at the *Bgl*II site within the coding sequence (ADZUMA, OGAWA and OGAWA 1984). The cloned DNA was kindly provided by DAVID SCHILD (University of California, Berkeley). Gene disruption was performed by standard techniques involving treatment of spheroplasts with the cloned insertion fragment (ROTHSTEIN 1983). Colonies arising on medium lacking leucine were tested for ionizing radiation sensitivity and lack of complementation by a *rad52-1* tester mutant. The *rad52::LEU2* mutants were crossed to *RAD52* cells and exhibited 2:2 segregation for the radiation sensitivity and radiation sensitivity cosegregated with leucine prototrophy. The *rad52::LEU2* was a single-copy insertion as confirmed by Southern analysis (data not shown).

Growth and sporulation: Growth and sporulation of strains have been described previously (RESNICK, STASIEWICZ and GAME 1983). Growth before sporulation was in a

TABLE 1

Genotypes of strains

Strain	Diploid	Relevant genotype
1	RM104	*MATa ura3-1 CAN1 leu1-c trp5-c his7-1*
		MATα ura3-13 can1^r leu1-12 trp5-2 his7-2
2	RM105	*MATa rad52-1 ura3-13 can1^r leu1-12 trp5-2 HIS7*
		MATα rad52-1 ura3-1 CAN1 leu1-c trp5-c his7
3	RM106	*MATa rad52-2 ura3-1 CAN1 leu1-c trp5-c HIS7*
		MATα rad52-2 ura3-13 can1^r leu1-12 trp5-2 his7-2
4	RM107	*MATa rad52-2 ura3-1 leu1-c trp5-c cyh2^r HIS7*
		MATα rad52-1 ura3-13 leu1-12 trp5-2 CYH2^s his7-2
5	MR48[a]	*MATa leu2 rad52-1 rad1-1 LYS2 TUP7 can1^r hom3-10 his1-7 TRP2 ade2*
		MATα leu2 RAD52 RAD1 lys2 tup7-1 CAN1 HOM3 his1-1 trp2 ADE2
6	MR49[a]	*MATa leu2 rad52-1 rad1-1 LYS2 TUP7 can1^r hom3-10 his1-7 TRP2*
		MATα leu2 rad52-1 RAD1 lys2 tup7-1 CAN1 HOM3 his1-1 trp2
7	G753[a,b]	*MATa rad50-1 can1^r HOM3 his1-1 TRP2 ade2*
		MATα rad50-1 CAN1 hom3-10 his1-7 trp2 ADE2
8	MR63[a]	*MATa leu2 rad52::LEU2 RAD1 CAN1 HOM3 his1-1 trp2 LYS2 ADE2*
		MATα leu2 RAD52 rad1-1 can1^r hom3-10 his1-7 TRP2 lys2 ade2
9	MR64[a]	*MATa leu2 rad52::LEU2 RAD1 can1^r HOM3 his1-1 trp2 ADE2*
		MATα leu2 rad52::LEU2 rad1-1 CAN1 hom3-10 his1-7 TRP2 ade2

[a] Derived from SK-1 background (KANE and ROTH 1974), which results in high levels and rapid sporulation.

[b] Provided by JOHN GAME.

yeast extract, peptone, potassium acetate medium, and sporulation was in 1% potassium acetate supplemented with necessary bases and amino acids. Sporulation was scored using a phase contrast microscope. Recombination and survival at various times during meiosis were measured as previously described (RESNICK, GAME and STASIEWICZ 1983).

RESULTS

Sporulation and viability in *rad52* mutants after meiosis: All four *rad52* mutations tested caused a significant reduction in sporulation capability (as measured by the percentage of cells that form asci), and essentially all spores which were produced were inviable (Table 2). This is identical to the observations of GAME *et al.* (1980) and PRAKASH *et al.* (1980) with *rad52-1* mutants. Analysis of *rad52* meiotic cultures revealed that some cells (2–40%) retained viability after exposure to sporulation medium. These cells were mitotic in appearance and may reflect cells that did not complete meiosis. Direct analysis by micromanipulation of a single culture of *rad52-1* cells demonstrated that 20% of cells that appeared to be vegetative were viable. For Rad$^+$, 96% of the vegetative cells were viable. Most *rad52-1* cells entered meiosis because an almost twofold increase in DNA was observed (RESNICK *et al.* 1981; J. NITISS and M. A. RESNICK, unpublished results). The final frequency of recombination in the survivors at the end of meiosis (as measured by prototroph production at heteroallelic loci) was reduced by a factor of 100 to 1000 by *rad52*, compared to that found in wild-type cells. The surviving *rad52* vegetative cells

TABLE 2

Sporulation and viability of *rad52* strains after meiosis

Genotype	No. of cultures	Final sporulation (av. %)	Spore viability (av. %)	Final survival (av. %)[a]	Relative reduction in recombination[b]
rad52-1[c] / *rad52-1*	4	16[d]	<0.5	13[d]	10^{-2}–10^{-3}
rad52-1[e] / *rad52-1*	2	41[f]	<0.5	4[f]	10^{-2}–10^{-3}
rad52-2[c] / *rad52-2*	4	18[d]	0.6	45[g]	10^{-2}–10^{-3}
rad52-1[c] / *rad52-2*	8	10[d]	1.0	48[g]	10^{-2}–10^{-3}
rad52::LEU2[e] / *rad52::LEU2*	3	20[f]	<1.0	7[f]	10^{-2}–10^{-3}
RAD52[c] / *RAD52*	3	61[d]	96	96[g]	1
RAD52[e] / *rad52-1*	3	95[f]	ND	100[f]	1
RAD52[e] / *rad52-1::LEU2*	2	93[f]	ND	100[f]	1

[a] Colony forming units/ml at the conclusion of the experiment/colony forming units/ml at t=0.
[b] Average for all loci studied from Tables 3 and 4; results are compared with Rad$^+$ strains.
[c] Strains 1 through 4 of Table 1.
[d] Measured after 48 hr. Incubation in liquid sporulation mixture; cells are plated at the time when final survival is determined (same as [f] and [g]).
[e] Strains 5 through 9 of Table 1.
[f] Measured after 24 hr.
[g] Measured after 32 hr.
ND = not determined.

do show some net increase in recombination over mitotic levels in some cases, but the increase is far below that seen in *RAD52* cells. Vegetative cells remaining in wild-type meiotic cultures at the end of the meiosis exhibit essentially meiotic levels of recombination (MALONE and ESPOSITO 1981).

Induction of recombination in cells during meiosis: The response of cells exposed to sporulation medium is very different in *rad52* as compared to *RAD52* cells (Table 2; Figure 1). Wild-type cells show no loss in viability, whereas *rad52* cells die in sporulation medium. Likewise, wild-type cells sporulate more efficiently than *rad52* mutants. Most importantly, wild-type cells exhibit a meiotic induction of recombination that is two to three orders of magnitude greater than initial mitotic levels. Unlike earlier reports (GAME *et al.* 1980; PRAKASH *et al.* 1980), we observed a significant increase in recombinants in *rad52* strains over the low mitotic control levels, although the maximum value was considerably reduced compared to wild type. The kinetics of recombination at *leu1* in a *rad52-1* strain are shown in Figure 1. Note that the frequency of recombinants decreases somewhat after attaining a maximum value at approximately 20 h (see below). We have examined recombination at two other loci in *rad52-1* strains, as well as repeating the experiment with

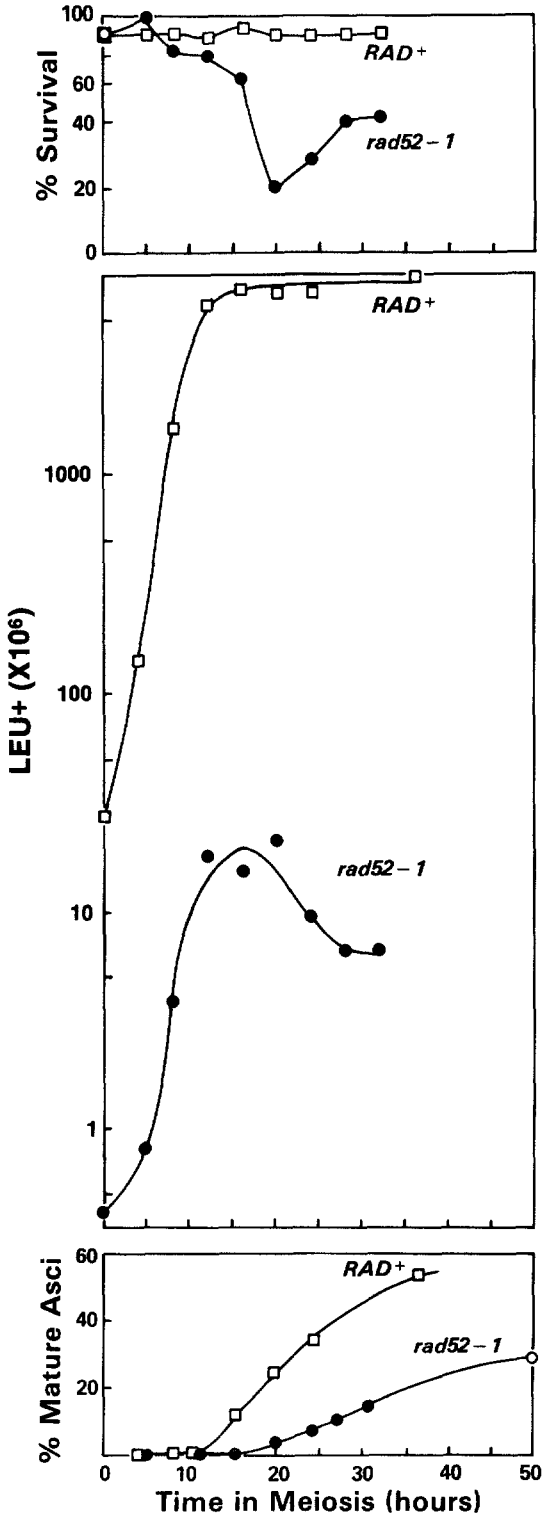

FIGURE 1.—Viability; prototroph frequency and sporulation in RM104, *RAD52/RAD52* (□) and RM105, *rad52-1/rad52-1* (●). *Top panel*: viability of wild-type and *rad52* cells in sporulation medium. *Middle panel*: frequency of leucine prototrophs; both strains are *leu1-C/leu1-12*. *Bottom panel*: sporulation.

rad52-2 and *rad52-1/rad52-2* strains. The extent of induction of recombination is shown in Table 3. In every case there is an increase of recombination after exposure to sporulation medium. In most cases the frequency falls off after the maximum is reached. The relative amount of induction appears greater in *rad52-1* diploids because they show a very low frequency of spontaneous mitotic recombinants.

To support further the observation that recombination was occurring in *rad52* cells in RMM experiments, *rad52* strains exhibiting rapid and synchronous sporulation kinetics were examined. The analysis of recombination induction is shown in Figure 2. There is a large increase in recombinants in *rad52-1* that peaks at about 6 h and follows similar kinetics to the *RAD52* strain. It is interesting to note that in these strains the maximum level attained is higher than the wild-type mitotic value, although it is still 10–20 times less than the wild-type meiotic level (Table 4). The data presented above (Figure 1; Table 3) suggested that the frequency of recombinants falls at later times in meiotic medium. That observation is confirmed by the use of the rapidly sporulating, highly synchronous strains. After 24 h of sporulation, the frequency of recombinants fell to the initial premeiotic level. Both the initial increase and the subsequent fall in recombinant frequency is reflected in the total numbers of recombinants observed, suggesting that recombinants are lost as cells pass through subsequent steps of meiosis.

It is possible that the induction of recombination observed with the *rad52-1* and *rad52-2* strains could be interpreted as being due to leakiness. To address this possibility we constructed a diploid strain containing an insertion in the *RAD52* gene (*rad52::LEU2*) that sporulated synchronously and rapidly. The insert is about 500 base pairs (bp) from the 5′ end of the coding sequence (the *RAD52* coding sequence is approximately 1500 bp [ADZUMA, OGAWA and OGAWA 1984)] and contains all the normal *LEU2* transcriptional and translational controls. The strain is sensitive to γ-irradiation and does not complement *rad52-1* (data not shown). It is likely that no functional *RAD52* gene product is present in the insertion mutant. The induction of recombination in this strain was essentially the same as that observed in the *rad52-1* mutant (Table 4).

Recombination is absent in *rad50* strains: To eliminate the possibility that exposure to sporulation medium followed by a return to mitotic medium results in an induction of recombination in the absence of any meiotic recombination functions, we performed the RMM experiments with *rad50-1* diploids. These diploids do not show increased frequencies of recombinants (Figure 3). This is completely consistent with the proposed role of *RAD50* acting early in meiotic recombination (MALONE 1983).

Exposure of cells to rich growth medium before plating: One interpretation for the induction of recombination in *rad52* diploids is that some initial steps in recombination occur that are then resolved in cells returned to mitotic medium (*i.e.*, medium containing glucose and a nitrogen source). To examine this possibility, we exposed cells to nutrient-rich growth medium (YPD) *after*

TABLE 3

Induction of recombination during meiosis in *RAD52* and *rad52* diploids at various loci

	Recombination frequency											
	ura3 (×10⁶)			*trp5* (×10⁵)			*leu1* (×10⁵)			*rad52* (×10⁵)		
Genotype	Start	Mid	Final	Start	Mid	Final	Start	Mid	Final	Start	Mid	Final
RAD52	4.2	210	280	1.7	1700	1600	2.8	690	790			
RAD52		(12)			(16)			(16)				
rad52-1	0.02	2.5	0.46	0.04	55	30.2	0.04	18	6.6			
rad52-1		(16)			(12)			(12)				
rad52-2	1.6	12	5.5				1.8	21	7.5			
rad52-2		(12)						(12)				
rad52-1	1.3	4.8	7.9	0.70	6.8	6.5	0.56	3.9	3.2	0.5	6.3	1.1
rad52-2		(16)			(16)			(16)			(20)	

The diploids described in Table 1 (numbers 1–4) were exposed to sporulation media for various lengths of time and analyzed for the frequency of prototrophs as described in the text. "Start" values refer to the mitotic values at time 0; "final' values are the frequencies at the last time point measured (usually 32 hr); "mid" values refer to the maximum value obtained, or in the case of *RAD52* cells, the value when the final plateau was initially reached (see Figure 1). The number in parentheses below the mid values indicates the time when the peak or plateau values were reached.

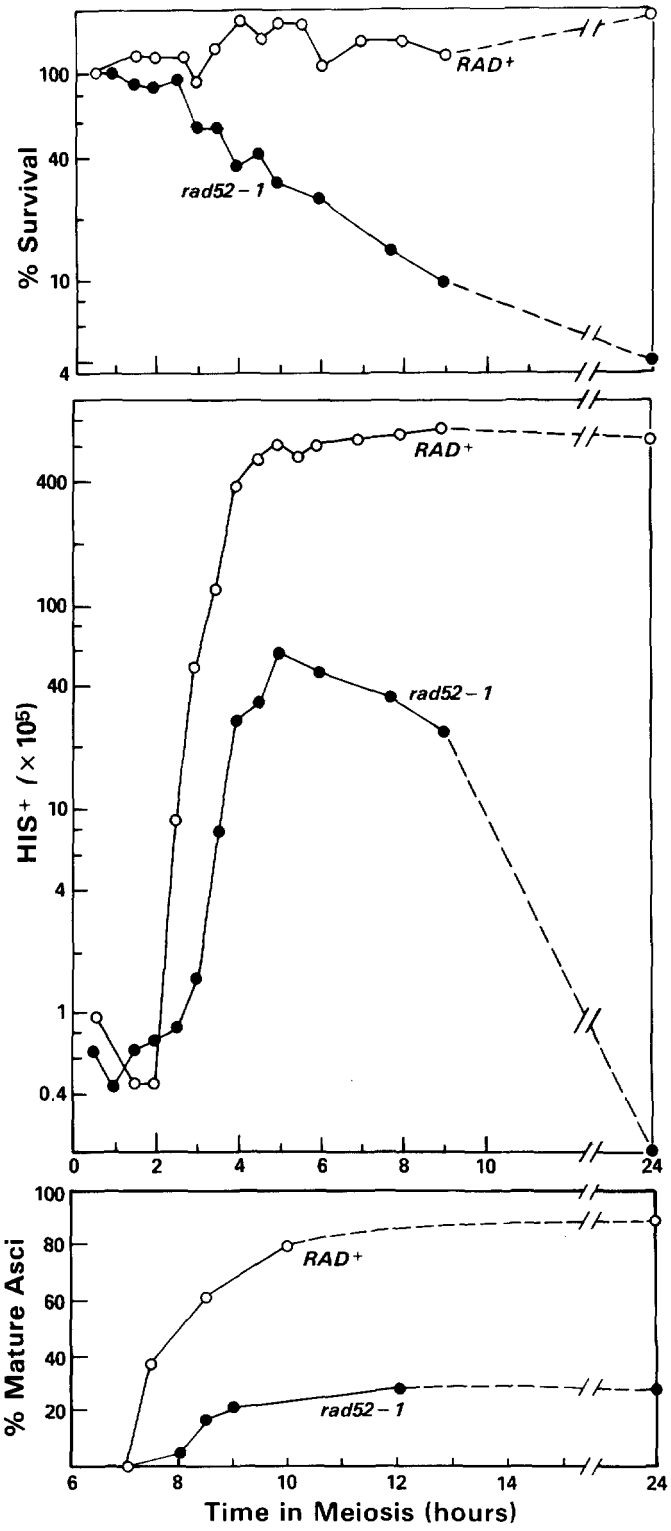

FIGURE 2.—Viability, prototroph frequency and sporulation in SK-1 strains MR48, *RAD52/rad52-1* (○) and MR49, *rad52-1/rad52-1* (●). *Top panel*: viability of wild-type and *rad52* cells in sporulation medium. *Middle panel*: frequency of histidine prototrophs; both strains are *his-1/his1-7*. *Bottom panel*: sporulation.

TABLE 4

Induction of recombination during meiosis at the *HIS1* locus in rapidly sporulating Rad$^+$ and *rad52* diploids

Genotype	Recombination frequency			No. of experiments
	Start ($\times 10^5$)	Mid ($\times 10^5$)	Final ($\times 10^5$)	
RAD52	0.92	770	770	3
rad52-1		(5)		
rad52-1	0.16	45	0.3	3
rad52-1		(9)		
RAD52	1.2	830	830	2
rad52::LEU2				
rad52::LEU2	0.6	31	3	3
rad52::LEU2		(8)		

Recombination frequencies were measured in RMM experiments as described in the text, using strains 5, 6, 8 and 9 of Table 1. The "start" values refer to mitotic values at time 0; "final" values are the frequencies at the last time point measured (usually 24 hr); "mid" values refer to the maximum value obtained, or in the case of Rad$^+$ cells, the frequency when the final plateau was initially reached (see Figure 2). The number in parentheses below the mid values indicates the time when the peak or plateau values were reached.

incubation in meiotic medium and *before* plating to selective medium for prototrophs.

In wild-type *RAD52* cells the level of recombination observed using this protocol is essentially the same as that seen in the original RMM experiments (Figure 4). Exposure to YPD before plating to selective medium does not alter the wild-type frequency of prototrophs. However, there is clearly a drastic reduction in prototroph frequency after exposure to YPD in the *rad52* mutant; in fact, the frequency is reduced to the background mitotic level of cells never exposed to meiosis (Figure 5). Incubation in synthetic complete medium causes a similar loss of prototrophs (data not shown). The loss of recombinants is also seen with heteroalleles in the *LEU1* and the *TRP5* genes and is also seen in *rad52-1* strains that do not have an SK-1 background (data not shown). Thus, the loss of recombinants does not depend on the locus examined or the strain background. To determine if the loss of recombinants is due to this experimental procedure, MR49 cells (*rad52* cells that require histidine and leucine) were held in synthetic media lacking only leucine and histidine. No reduction in recombinants is observed. We conclude from these results that (1) the recombinants formed in *rad52* cells are *not* complete on removal from meiotic medium and that (2) the opportunity for rapid growth somehow reverses or prevents their appearance.

Timing of meiosis-induced recombination in *rad52* strains: Exposure to YPD causes a loss of recombinants in *rad52* cells, but exposure to synthetic complete medium lacking essential nutrients essentially prevents sporulation without causing the loss of recombinants (data not shown). We exploited this to determine the amount of time necessary for the *rad52* recombinants to become stable. The experimental rationale is that by incubating cells in medium that arrests meiosis, but does not allow growth, the recombinants will

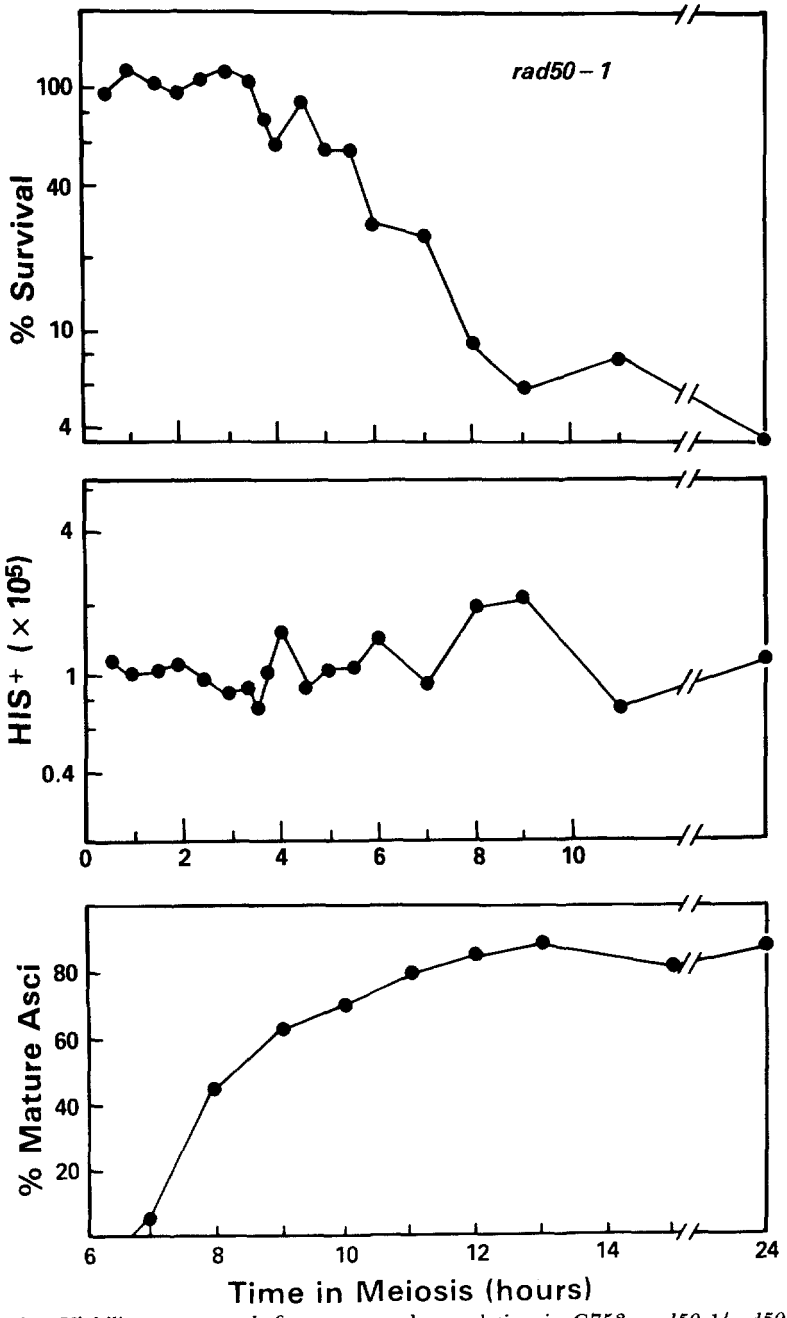

FIGURE 3.—Viability, prototroph frequency and sporulation in G753, *rad50-1/rad50-1*. G753 is an SK-1 strain. *Top panel*: viability of G753 in sporulation medium. *Middle panel*: frequency of histidine prototrophs; G753 is *his1-1/his1-7*. *Bottom panel*: sporulation.

have time to become stable without their loss due to the cells attempting to complete meiosis, which would be a lethal event.

The cells are removed from complete medium lacking histidine and leucine

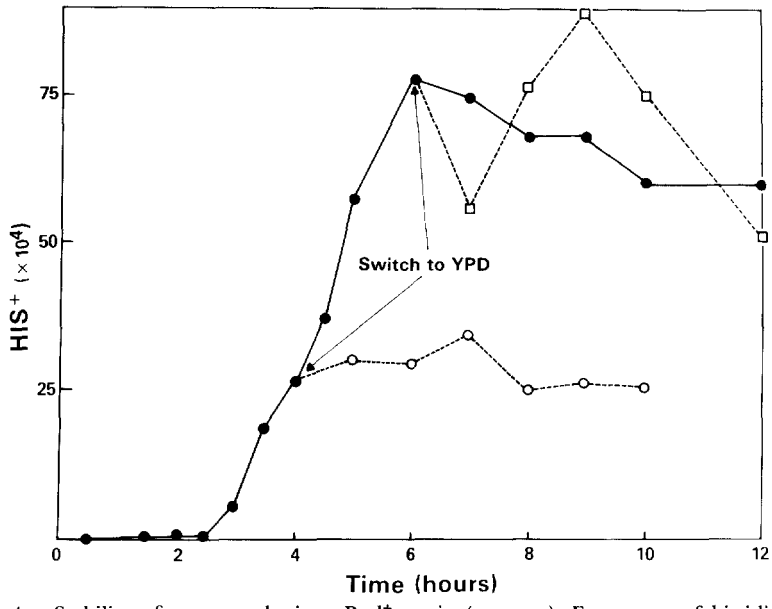

FIGURE 4.—Stability of prototrophs in a Rad$^+$ strain (see text). Frequency of histidine prototrophs in MR48 cells. *RAD52/rad52-1* plated directly to selective (lacking histidine) medium (●) after incubation in sporulation medium; switched to liquid YPD after 4 hr incubation in sporulation medium and then plated at later times to selective medium (○); switched to YPD after 6 hr incubation in sporulation medium and then plated at later times to selective medium (□).

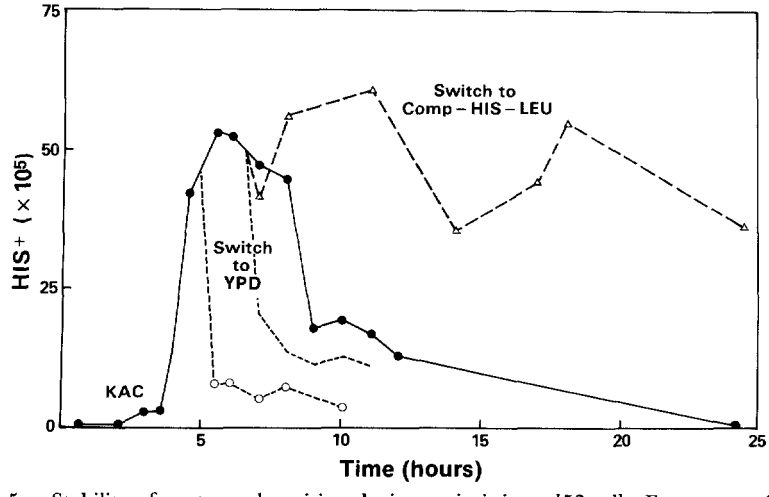

FIGURE 5.—Stability of prototrophs arising during meiosis in *rad52* cells. Frequency of histidine prototrophs in MR49 cells. (1) *rad52-1/rad52-1* cells were plated directly to selective (lacking histidine) medium (●——●) after incubation in meiotic medium; (2) cells were switched to liquid YPD at 5 hr (○– –○) or 6.5 hr (– – –) after incubation in meiotic medium and were plated at later times to selective medium; (3) cells were switched from meiotic medium to synthetic complete medium lacking histidine and leucine and were plated at later times to selective medium lacking only histidine (△— —△).

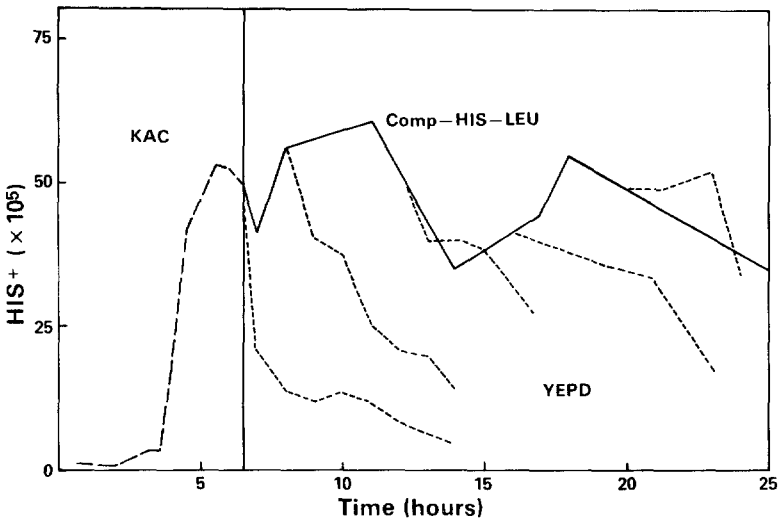

FIGURE 6.—Time required for stabilization of recombinants in *rad52* cells. The MR49 cells, *rad52-1/rad52-1*, from the experiment in Figure 5 were plated directly (— —) to selective (lacking histidine) medium; switched to YPD at 6.5 hr and plated at later times to selective medium (– – –); switched to medium lacking histidine and leucine at 6.5 hr and later plated to selective medium (——). Cells were also transferred from medium lacking histidine and leucine to YPD at 8, 12, 16 and 20 hr (– – –) and subsequently were plated at later times to selective medium.

and are suspended in YPD. The cells are then plated at later times to medium lacking histidine. The results of this experiment are shown in Figure 6. By 13.5 h after the cells were removed from sporulation medium (*i.e.*, $t = 20$ h in Figure 6) and placed in complete medium lacking histidine and leucine, most of the recombinants are stable in YPD. At earlier times, the majority of the recombinants are still lost when the cells are incubated in YPD before plating to selective medium. Thus, the recombination that is seen requires a long time to become stable relative to the amount of time it takes for the cells to complete meiosis.

Examination of recombinants for multiple events: In any specific cell, the increase in recombinants observed during exposure to sporulation medium could reflect a specific event at the diagnostic heteroallelic locus, or it could reflect a more general genome-wide participation in meiotic recombination. We examined a number of recombinants for the presence of other recombination events. As expected from the microscopic appearance of survivors, recombinants almost always seemed to be diploid cells. The data in Table 5 indicate that the selected intragenic recombination event is frequently associated with crossing over. If the prototrophs we observe represent gene conversion (see DISCUSSION), then the association of conversion with crossing over is analogous to the correlation normally found in meiosis or mitotis (FOGEL, MORTIMER and LUSNAK 1983). In addition, we also see evidence for events that could be attributed to nonassociated crossovers, both on the same and on different chromosomes, and these increase with exposure to sporulation medium (Table 5). Of particular significance is the observation that the number

TABLE 5

Evidence for other events occurring in prototrophs arising in *rad52* strains during meiosis

Locus	Chromosome	Crossover frequencies	Time in meiosis (hr)											
			0	5	8	12	16	20	24	28	32	36	46	48
LEU1	VII	A	0.29	0.20	0.48			0.24	0.28	0.28	0.20			0.49
		N	0.15	0.20	0.37			0.44	0.44	0.40	0.36			0.49
		T	0.31	0.24	0.48			0.52	0.60	0.52	0.40			0.56
TRP5	VII	A	0.30	0.40	0.40	0.24	0.40	0.36	0.32	0.36	0.44	0.40	0.48	0.44
		N	0.11	0.28	0.16	0.28	0.20	0.40	0.40	0.36	0.40	0.44	0.52	0.54
		T	0.32	0.40	0.40	0.40	0.52	0.60	0.56	0.56	0.56	0.56	0.60	0.61
URA3[a]	V	A												
		N												
		T	0.31	0.00	0.00			0.24	0.28	0.60	0.48			

$$\text{A} = \text{frequency of associated crossovers} = \frac{\text{no. of colonies showing crossover in interval containing converted locus}}{\text{total no. of colonies}}$$

$$\text{N} = \text{nonassociated crossover} = \frac{\text{no. of colonies showing crossover in any interval other than in A}}{\text{total no. of colonies}}$$

$$\text{T} = \text{total crossovers} = \frac{\text{total no. of colonies showing any crossovers}}{\text{total no. of colonies}}$$

A + N does not necessarily equal T, because some colonies show both associated and nonassociated events.

[a] There were no markers on chromosome *V* that allowed a distinction between associated and nonassociated crossovers. Frequency refers to total discernible crossovers on all chromosomes.

TABLE 6

Spontaneous mitotic recombination in various *rad52* diploids

Genotype	No. of cultures	Relative recombination frequency				
		leu1	*trp5*	*ura3*	*lys2*	*cyh2*
rad52-1 / *rad52-1*	6	0.0040	0.014	0.11	0.13	0.078
rad52-2 / *rad52-2*	8	0.30	1.1	3.0	4.1	
rad52-1 / *rad52-2*	8	0.11	0.21	0.29		0.85
RAD52 / *RAD52*	4	1.0	1.0	1.0	1.0	1.0

Diploids were analyzed for spontaneous mitotic recombination as described in the text. Relative recombination frequency refers to the geometric mean of all cultures of the *rad52* strain divided by geometric mean for all cultures of the wild-type locus.

of cells with nonassociated crossovers tends to increase with time in meiosis up to 20 hr. In some cases the apparent crossover observed in selected prototrophs could not be due to chromosome loss (*e.g.*, the diploid remained heteroallelic at centromere proximal loci or heterozygous for markers on the other side of the centromere).

Mitotic recombination in *rad52* strains: The data in Table 3 indicate that the *rad52-2* allele does not confer a deficiency for mitotic recombination, unlike *rad52-1*. To confirm this observation, multiple independent cultures of *rad52-2* diploids were examined for spontaneous mitotic recombinants at three heteroallelic loci (Table 6). The data indicate that the *rad52-2* allele does not reduce mitotic recombination. It is interesting that, although both *rad52-1* and *rad52-2* have essentially identical repair and homothallic switching defects (data not shown) as well as meiotic deficiencies (see Tables 2 and 3), they differ significantly in their effect on spontaneous mitotic recombination.

DISCUSSION

The *RAD52* gene product is clearly required for genetic recombination to occur at normal levels in either mitotic or meiotic cells. The evidence presented here further supports previous reports that *RAD52* is important in recombination because meiotic spore products are inviable and cells at intermediate stages of meiosis do not exhibit Rad$^+$ levels of recombination. It has been possible, using highly synchronous strains, to demonstrate that meiosis induces significant levels of genetic recombination at several loci in *rad52* mutants, both in terms of frequency and numbers of recombinants, and to subsequently characterize the recombination process. Transient increases in the frequency of intragenic recombinants have also been reported for *rad51* (MORRISON and HASTINGS 1979) and *rad57* (GAME *et al.* 1980) mutants.

We have assumed that the production of prototrophs at heteroallelic loci primarily represents gene conversion events. Although this has been amply

demonstrated both in wild-type meiotic and mitotic cells, prototrophs could also be generated by the occurrence of crossovers between the alleles. Whether the prototrophs we observe in *rad52* cells in RMM experiments are generated by conversion or crossing over, they still represent meiotic recombination in a predominantly recombination-defective cell. The transient recombination events described in these experiments provide insight into the nature of the meiotic defect in *rad52* mutants and, possibly, the events occurring in wild-type cells.

Previously reported properties of *rad52* cells undergoing meiosis, in conjunction with the present observations, suggest that recombination structure(s) are initiated during meiosis in *rad52* mutants. In this study, data are presented which suggest that the structures are resolved when cells are switched out of meiotic medium. Four recent observations support this hypothesis. First, multiple mutant studies have indicated that *rad50* and *spo11* mutations are epistatic to *rad52* in meiosis (MALONE 1983; R. MALONE, unpublished results; S. KLAPHOLZ and R. ESPOSITO, personal communication). One interpretation of this observation is that genetic recombination in meiosis occurs in a series of dependent steps, similar to a biochemical pathway that leads to a final product. An early block in the pathway (*e.g.*, *rad50* or *spo11*) would be epistatic to a later block (*rad52*). Second, *rad52* mutants accumulate single-strand breaks in DNA (RESNICK *et al.* 1981) after exposure to sporulation medium, and *rad50* mutants do not (RESNICK *et al.* 1984; J. NITISS and M. A. RESNICK, unpublished results). The frequency and time of appearance of these breaks strongly suggest they are involved in recombination. It may also be relevant that a nuclease that could possibly be involved in processing the intermediate appears to be lacking in mitotically growing (CHOW and RESNICK 1983) and meiotically developing *rad52* cells (T. Y.-K. CHOW and M. A. RESNICK, unpublished results). Third, *rad52* cells exhibit a limited amount of physically recombined molecules in the region investigated (BORTS *et al.* 1986). Fourth, B. BYERS (personal communication) has observed that the lack of *RAD52* meiotic function does not prevent the appearance of synaptonemal complexes, whereas the absence of *RAD50* function does. All of these observations are consistent with the *rad52* mutation blocking meiotic recombination after it has been initiated.

Further support for the idea that events are initiated in meiosis and are resolved after a return to mitotic medium comes from the observation that cells incubated in YPD or synthetic complete medium immediately before selective mitotic medium do not exhibit recombination induction. If the recombinants were complete, we find it hard to understand why incubation in the rich medium should eliminate them. If, however, the cells contained recombination intermediates, growth might preclude their completion. Specifically, intermediates might be resolved in a nonrecombinogenic fashion when DNA replication and/or chromosome segregation proceeds rapidly. The appearance of recombinants in the cells that are plated directly on selective mitotic medium could be due to the considerably greater time available to resolve recombination intermediates before DNA replication or chromosome segregation occurs, because cells cannot grow on selective medium until the recombinant proto-

troph is formed. No growth was detected microscopically when *his1* heteroallelic cells were exposed to sporulation medium and were subsequently plated on medium lacking histidine (data not shown).

Another explanation for the recombination is that it reflects leakiness of the *rad52* mutant alleles. Two factors suggest that this is not the case. First, quantitatively similar observations have been made with *rad52-1*, *rad52-2* and *rad52::LEU2*. All three of these alleles have comparable deficiencies in sporulation, spore viability and radiation sensitivity. The *rad52::LEU2* allele consists of the entire *LEU2* gene plus surrounding DNA inserted approximately one-third of the way into the *RAD52* coding sequence; therefore, these *rad52* mutants are likely to lack any functional protein. Furthermore, if leakiness were the cause of recombination, the recombination would be expected to be complete before incubation in YPD, as was found for wild-type strains.

We suggest that the intermediate recombination structures induced during meiosis include the single-strand breaks previously described (RESNICK *et al.* 1981) in meiotic *rad52* cells, although it is possible to argue that they are not part of the normal recombination pathway. That intermediate recombination structures include single-strand breaks is consistent with recent observations of HØGSETT and ØYEN (1984). They do not observe single-strand breaks in the ribosomal RNA genes of *rad52-1* cells during meiosis. The ribosomal RNA genes are recombinationally inactive during meiosis even in Rad^+ strains (PETES and BOTSTEIN 1977). Therefore, there is a correlation between lack of strand breaks in a large segment of DNA in *rad52* cells and lack of meiotic recombination in this DNA in Rad^+ cells.

According to most models that have been proposed to explain meiotic recombination, DNA strand breaks are an essential feature (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983). The observation that *rad52* mutants are defective in normal recombination and that the *RAD52* gene product is required for repair of chromosomal DNA double-strand breaks in mitosis might suggest, instead, that the defect in meiosis is related in some way to double-strand breaks. We would argue that the intermediate at which recombination is blocked in *rad52* cells in RMM experiments does not include a double-strand break. Double-strand breaks occurring in *rad52* strains during RMM experiments would be expected to be lethal, just as in *rad52* mitotic cells. Furthermore, double-strand breaks have not been detected in *rad52* meiotic cells (RESNICK *et al.* 1984). It is possible that, in normal meiosis, the *RAD52* gene product or function is required at more than one step; it might be required both *before* and after the putative double-strand break. If this were so, and double-strand breaks were actually involved in meiotic recombination (RESNICK 1976; SZOSTAK *et al.* 1983), *rad52* mutants might be blocked at a step before the development of double-strand breaks.

Related to the problem of the role of *RAD52* in spontaneous recombination, we noted that the mitotic ($t = 0$) values for recombination in *rad52-2* did not appear depressed. We analyzed spontaneous mitotic recombination in the *rad52-2* mutants and found that it occurs at frequencies no lower than in wild-type cells (Table 6). One explanation is that *rad52-2* is a leaky mutation, and

relatively little *RAD52* product is required for spontaneous mitotic recombination. (Even so, the *rad52-2* mutant exhibits deficiencies in meiotic recombination, repair and homothallic switching comparable to *rad52-1*.) An alternative explanation is that one of the two mutations, *rad52-1* or *rad52-2*, has an allele-specific effect on mitotic recombination. We are in the process of testing these possibilities by analyzing the effect of interruptions and deletions of *RAD52* on spontaneous mitotic recombination.

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LITERATURE CITED

ADZUMA, K., T. OGAWA and H. OGAWA, 1984 Primary structure of the *RAD52* gene in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:** 2735–2744.

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

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

CHOW, T. Y.-K. and M. A. RESNICK, 1983 The identification of a deoxyribonuclease controlled by the *RAD52* gene of *Saccharomyces cerevisiae*. pp. 497–455. In: *Cellular Responses to DNA Damage*, Edited by E. C. FRIEDBERG and B. A. BRIDGES. Alan R. Liss, New York.

DUJON, B., 1981 Mitochondrial genetics and functions. pp. 505–635. In: *Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance*, Edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

ESPOSITO, R. E. and S. KLAPHOLZ, 1981 Meiosis and ascospore development. pp. 211–287. In: *Molecular Biology of the Yeast Saccharomyces, Life Cycle and Inheritance*, Edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

ESPOSITO, M. S. and J. E. WAGSTAFF, 1981 Mechanisms of mitotic recombination. pp. 341–370. In: *Molecular Biology of the Yeast Sacchromyces, Life Cycle and Inheritance*, Edited by J. N. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

FOGEL, S., R. K. MORTIMER and K. J. LUSNAK, 1983 Meiotic gene conversion in yeast. pp. 65–107. In: *Yeast Genetics*, Edited by J. F. T. SPENCER *et al.* Springer-Verlag, New York.

GAME, J. C., 1983 Radiation sensitive mutants and repair in yeast. pp. 109–137. In: *Yeast Genetics*, Edited by J. F. T. SPENCER *et al.* Springer-Verlag, New York.

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

HO, K. S. Y., 1975 Induction of DNA double strand breaks by X-rays in a radiation sensitive strain of the yeast *Saccharomyces cerevisiae*. Mutat. Res. **30:** 327–334.

HØGSET, A. and T. ØYEN, 1984 Correlation between suppressed meiotic recombination and the lack of DNA strand breaks in the rDNA genes of *Saccharomyces cerevisiae*. Nucleic Acids Res. **12:** 7199–7213.

KANE, S. and R. ROTH, 1974 Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118:** 8–14.

KLAPHOLZ, S. and R. E. ESPOSITO, 1980a Isolation of *spo12-1* and *spo13-1* from a natural variant of yeast that undergoes a single meiotic division. Genetics **96:** 567–588.

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

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

MALONE, R. and R. E. ESPOSITO, 1980 The *RAD52* gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in yeast. Proc. Natl. Acad. Sci. USA **77:** 503–507.

MALONE, R. and R. ESPOSITO, 1981 Recombinationless meiosis in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **1:** 891–901.

MESELSON, M. and C. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **75:** 4434–4439.

MORRISON, D. P. and P. J. HASTINGS, 1979 Characterization of the mutator mutation *mut5-1.* Mol. Gen. Genet. **175:** 57–65.

NASMYTH, K., 1982 Molecular genetics of yeast mating type. Annu. Rev. Genet. **16:** 439–500.

ORR-WEAVER, T. L. and J. W. SZOSTAK, 1985 Fungal recombination. Microbiol. Rev. **49:** 33–58.

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

PETES, T. D. and D. BOTSTEIN, 1977 Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc. Natl. Acad. Sci. USA **74:** 5091–5095.

PRAKASH, S., L. PRAKASH, W. BURKE and B. A. MONTELONE, 1980 Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae.* Genetics **94:** 31–50.

RESNICK, M. A., 1969 Genetic control of radiation sensitivity in *Saccharomyces cerevisiae.* Genetics **62:** 519–531.

RESNICK, M. A., 1975 The repair of double-strand breaks in chromosomal DNA of yeast. pp. 549–556. In: *Molecular Mechanisms for Repair of DNA,* Part B, Edited by P. C. HANAWALT and R. B. SETLOW. Plenum Press, New York.

RESNICK, M. A., 1976 The repair of double-strand breaks in DNA: a model involving recombination. J. Theor. Biol. **59:** 97–106.

RESNICK, M. A., T. CHOW, J. L. NITISS and J. C. GAME, 1984 Changes in chromosomal DNA of yeast during meiosis in repair mutants and the possible role of a deoxyribonuclease. Cold Spring Harbor Symp. Quant. Biol. **49:** 639–650.

RESNICK, M. A., J. C. GAME and S. STASIEWICZ, 1983 Genetic effect of UV irradiation on excision-proficient and -deficient yeast during meiosis. Genetics **104:** 603–618.

RESNICK, M. A., J. N. KASIMOS, J. C. GAME, R. J. BRAUN and R. M. ROTH, 1981 Changes in DNA during meiosis in a repair-deficient (*rad52*) of yeast. Science **212:** 543–545.

RESNICK, M. A. and P. MARTIN, 1976 The repair of double-stranded breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol. Gen. Genet. **143:** 119–129.

RESNICK, M. A., S. STASIEWICZ and J. C. GAME, 1983 Meiotic DNA metabolism in wild-type and excision deficient yeast following UV exposure. Genetics **104:** 583–602.

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

SHERMAN, F. and H. ROMAN, 1963 Evidence for two types of allelic recombination in yeast. Genetics **48:** 255–261.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. W. STAHL, 1983 The double-strand break repair model for recombination. Cell **33:** 25–35.

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