EFFECTS OF THE RAD52 GENE ON RECOMBINATION IN SACCHAROMYCES CEREVISIAE

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ABSTRACT

Effects of the rad52 mutation in Saccharomyces cerevisiae on meiotic, γ-ray-induced, UV-induced and spontaneous mitotic recombination were studied. The rad52/rad52 diploids undergo premeiotic DNA synthesis; sporulation occurs but inviable spores are produced. Both intra- and intergenic recombination during meiosis were examined in cells transferred from sporulation medium to vegetative medium at different time intervals. No intragenic recombination was observed at the hisl-1/hisl-315 and trp5-2/trp5-48 heteroalleles. Gene-centromere recombination also was not observed in rad52/rad52 diploids. No γ-ray- or UV-induced intragenic mitotic recombination is seen in rad52/rad52 diploids. The rate of spontaneous mitotic recombination is lowered five-fold at the hisl-1/hisl-315 and leu1-c/leu1-12 heteroalleles. Spontaneous reversion rates of both hisl-1 and hisl-315 were elevated 10 to 20 fold in rad52/rad52 diploids.—The RAD52 gene function is required for spontaneous mitotic recombination, UV- and γ-ray-induced mitotic recombination and meiotic recombination.

Major advances have been made in our understanding of the phenomenon of meiotic recombination in eukaryotes from genetic studies on fungi, particularly the yeast Saccharomyces cerevisiae (Fincham and Day 1971; Fogel and Hurst 1967; Fogel and Mortimer 1971). Gene conversion during meiosis of yeast appears to involve the formation of asymmetrical heteroduplexes that may be 1000 nucleotide pairs long (Fogel and Mortimer 1969, 1970). Mismatch repair of heteroduplexes results in gene conversion, whereas the lack of mismatch repair leads to post-meiotic segregation. Gene conversion is associated with a high frequency of crossing over of flanking markers that is sufficient to account for all of the exchange events occurring during meiosis in yeast (Hurst, Fogel and Mortimer 1972).

Mitotic recombination, on the other hand, is not as well understood. From recent work in S. cerevisiae, it appears that spontaneous mitotic recombination may involve formation of very long symmetrical heteroduplexes in G1 that can be resolved by DNA replication (Esposito 1978). X-ray- and UV-induced mitotic recombinants have also been reported to arise during the G1 phase of the cell

cycle in *S. cerevisiae* (Wildenberg 1970; Fabre 1978). In order to understand the role of various gene products in meiotic and mitotic recombination, it is necessary to obtain mutants affecting these processes. Since the proposed models of recombination involve nicking of DNA, formation of heteroduplexes and repair synthesis (Holliday 1964; Meselson and Radding 1975) and since many mutants of *Escherichia coli* defective in DNA repair are also recombination deficient (Clark 1973; Eisenstark 1977; Howard-Flanders 1968; Rothman, Kato and Clark 1975), we have begun studies on mutants of the yeast *S. cerevisiae* that are defective in DNA repair and in sporulation (Game and Mortimer 1974). Prominent among these mutants are the radiation-sensitive mutants rad50, rad51, rad52, rad53, rad54, rad55, rad56, rad57 and rad6. Here we report on studies of mitotic and meiotic recombination in rad52 mutants, demonstrating that the *RAD52* gene is involved in meiotic recombination, γ-ray- and UV-induced mitotic recombination and spontaneous mitotic recombination.

**MATERIALS AND METHODS**

**Strains:** The genotypes of the strains used in this study are given in Table 1. The map distances, in centimorgans, of markers are indicated below and are compiled from Hawthorne and Mortimer (1960), Fogel and Hurst (1967) and Mortimer and Hawthorne (1966, 1973). Chromosomes are designated by Roman numerals.

<table>
<thead>
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The rad 52-1 mutant was obtained from M. Resnick; other strains were obtained from S. Fogel and R. Rothstein.

**Media:** The following media were used: YPD, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, solidified with 2% Bacto-agar; YPA, 2% Bacto-peptone, 1% yeast extract, 1% potassium acetate; sporulation medium (SPM), 2% potassium acetate, pH adjusted to 7.0 with acetic acid and supplements required by the particular strain used added to a final concentration of 75 μg/ml. Synthetic complete and synthetic omission media were used to score for prototrophs and consisted of the following: synthetic complete (SC), 0.67% Difco yeast nitrogen base without amino acids, 2% dextrose, 2% Bacto-agar, adenine sulfate 20 μg/ml, L-arginine-HCl 20 μg/ml, L-histidine-HCl 20 μg/ml, DL-homoserine 100 μg/ml, L-isoleucine 30 μg/ml, L-leucine 30 μg/ml, L-lysine-HCl 30 μg/ml, L-methionine 20 μg/ml, L-phenylalanine 50 μg/ml, L-tyrosine 30 μg/ml, uracil 20 μg/ml and L-valine 150 μg/ml. Synthetic complete minus histidine (SC-his) and synthetic complete minus tryptophan (SC-trp) were used to score for histidine and tryptophan prototrophs, respectively, and consisted of the above constituents lacking histidine and tryptophan, respectively.

**Premeiotic DNA synthesis:** Cells were grown in YPA medium containing 2 μCi/ml 14C-uracil and 50 mg/liter of unlabeled uracil to a density of 1 × 10⁷ cells/ml. At that time, cells were washed and transferred to SPM at a density of 1 × 10⁷ cells/ml. Cells were then incu-
bated in SPM at 30° and 0.5 ml aliquots were withdrawn at various times after transfer to SPM for a 48-hr period. The samples were then added to tubes on ice containing 0.5 ml 2 N NaOH, mixed well and incubated for 18 hr at 37°. Samples were precipitated by the addition of 50 μg of carrier DNA and 0.5 ml 50% TCA per tube. Samples were collected by filtration onto glass fiber filters, washed 5 times with 5% TCA, rinsed once with cold distilled water and once with cold 95% ethanol, dried and counted in LiquiScint-toluene scintillation fluid (New England Nuclear) in a Beckman LS-250 liquid scintillation counter. Increase in 14C counts in DNA during sporulation represents premeiotic DNA synthesis (Simchen, Pirson and Salts 1972).

Meiotic prototrophy: Freshly mated diploids were used for all experiments. After subcloning on appropriate omission media, YPA medium was inoculated with three to four colonies of each strain. Cultures were incubated at 30° for about 16 hours or until the cell density had reached 1–2 x 10^7 cells/ml. At that time, cells were washed and resuspended in SPM at the same density. At various intervals after transfer to SPM, aliquots were withdrawn, sonicated and plated on SC for viability determinations and on SC-his and SC-trp to select for prototrophic intragenic recombinants. Sporulation was monitored by microscopic examination of samples.

Determination of rates of mitotic prototrophy to histidine independence and tryptophan independence: For every strain examined, freshly mated diploids were used because the frequency of mitotic prototrophy for either marker was higher even in diploids stored at 4° for long periods of time than it was in freshly mated diploids. This was true of hisf-1/hisf-35 and trp5-2/trp5-48 diploids, whether they were heterozygous or homozygous for rad52. A group of culture tubes containing 1 ml YPD medium per tube was inoculated from a single clone at 100 cells per tube. The frequencies of histidine-independent and tryptophan-independent cells in the inoculum were determined by plating about 2000 cells on a total of 10 SC-his and 10 SC-trp plates and counting colonies growing on each type of medium. Only those experiments that had an initial frequency of zero HIS+ and TRP+ cells in the inoculum were used in the determinations. Cultures were incubated at 30° for three days, at which time the frequency of HIS+ and TRP+ clones in each culture was determined by plating appropriate dilutions on SC-his and SC-trp media and on SC to determine viability. SC plates were incubated for three days before scoring, whereas SC-his and SC-trp plates were scored after three to five days of incubation at 30°. Twenty-five cultures were used per strain for determining the rate of mitotic prototrophy shown in Table 3. For determining rates of prototrophy, the mean number of histidine-independent or tryptophan-independent cells per culture was determined by the method of the median of Lea and Coulson (1949). Rates were obtained by dividing the mean number of histidine-independent or tryptophan-independent cells by the mean number of cells in the cultures.

Determination of rates of spontaneous mutation: A clone obtained from freshly mated and subcloned diploids was suspended in sterile distilled water and used to inoculate 11 independent cultures at a density of about 1000 cells/ml YPD. Cultures were grown to stationary phase and then plated on the appropriate selective medium for determining the number of prototrophs that had arisen in each culture. Only those experiments that had an initial frequency of zero HIS+ and TRP+ cells in the inoculum were used in the determinations. Viability was determined by plating appropriate dilutions on synthetic complete medium. In order to obtain large enough numbers of colonies growing on SC-his and SC-trp, three-day cultures of 40 ml each were concentrated four-fold, washed, and plated when rates of mutation of hisf-1 and trp5-2 were determined. One ml cultures were used for determining the rate of mutation of trp5-48, while 10 ml cultures were used for hisf-135. Rates were determined by the method of median of Lea and Coulson (1949), as described above.

Determination of UV- and γ-ray-induced mitotic prototrophy: Freshly mated and subcloned diploids were suspended in sterile distilled water at a density of 1 x 10^8 cells per ml. Cells in suspension were distributed into scintillation vials, subjected to γ-ray irradiation, and plated on SC for viability determinations and on SC-his and SC-trp for determination of prototroph frequencies. The irradiation was from a 6000 Curie Cobalt-60 source whose dosimetry is given in McKee and Lawrence (1979). UV irradiation was carried out by irradiating cells on the surface of plates at a fluence of 1 J/m²/sec. The radiation source and its dosimetry are given in Lawrence and Christensen (1976).
RESULTS

Effects in meiosis

Premeiotic DNA Synthesis: Before determining the effect of the rad52 mutation on recombination during meiosis, it was necessary to determine whether premeiotic DNA synthesis occurs in rad52/rad52 diploids, since commitment to recombination is not observed in mutants that do not undergo premeiotic DNA synthesis (BAKER et al. 1976; ROTH and LUSNAK 1970). Figure 1 shows that premeiotic DNA synthesis in the rad52/rad52 diploid is similar to that in the wildtype RAD52/RAD52 diploid. Although results for only one strain of each genotype are given in Figure 1, two more strains of each genotype gave similar results.

Intragenic recombination: Strains with two heteroallelic sites, his1-1/his1-315 on chromosome V and trp5-2/trp5-48 on chromosome VII were constructed in order to determine the effect of the rad52 mutation on meiotic intragenic recombination. In addition, the strains were heterozygous for hom3, arg6, and ilv1, so that intergenic recombination could be monitored (see Table 1). The centromere markers ura3, leu1 and met14, located on chromosomes V, VII and XI, respectively, were also present in the heterozygous state so that meiotic segregation could be monitored. The genotypes of the strains used are given in Table 1. Intragenic recombination at the his1 and trp5 loci was monitored by withdrawing cells from liquid sporulation medium at different time intervals and

![Graph](image-url)

**Figure 1.**—Premeiotic DNA synthesis in RAD+/RAD+ (●) and rad52/rad52 (○) diploids. Aliquots of cells were removed at various times after transfer to sporulation medium. The alkali-stable, acid precipitable counts (DNA) were determined in each sample. The ratio of the amount of counts in DNA at a given time over the amount in DNA at the time of transfer to sporulation medium is plotted in the figure.
### Table 1

#### Genotypes of strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tbody>
<tr>
<td>LP-854</td>
<td>LYS2</td>
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<tr>
<td>LP-855</td>
<td>LYS2</td>
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<td>LP-1416</td>
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**Notes:**
- LP682-1C  
- LP687-7D  
- LP687-3C  
- LP682-3D  
- LP687-10D 
- LP682-1C  
- LP682-3B  
- LP687-7D  
- LP682-1C  
- PR86-2C   
- PR86-2C   

**Genotype Details:**
- **LYS2**: LYS2 allele
- **HOM3**: HOM3 allele
- **his1-315**: his1-315 allele
- **arg6**: arg6 allele
- **ilv1-1**: ilv1-1 allele
- **trp5-48**: trp5-48 allele
- **rad52-1**: rad52-1 allele
- **ura3**: ura3 allele
- **HOM3**: HOM3 allele
- **his1-315**: his1-315 allele
- **arg6**: arg6 allele
- **ilv1-1**: ilv1-1 allele
- **TRP5**: TRP5 allele
- **LEU1**: LEU1 allele
- **rad52-1**: rad52-1 allele
- **URA3**: URA3 allele
- **hom3**: hom3 allele
- **his1-1**: his1-1 allele
- **ARG6**: ARG6 allele
- **ilv1-1**: ilv1-1 allele
- **LEU1**: LEU1 allele
- **rad52-1**: rad52-1 allele
- **PR86-2C**: PR86-2C allele
- **PR86-2G**: PR86-2G allele
- **LP682-3B**: LP682-3B allele
- **LP687-7D**: LP687-7D allele
- **LP682-1C**: LP682-1C allele
- **LP687-3C**: LP687-3C allele
- **LP682-3D**: LP682-3D allele
- **LP687-10D**: LP687-10D allele
- **LP-855**: LP-855 allele
- **LP-1383**: LP-1383 allele
- **LP-1384**: LP-1384 allele
- **LP-1415**: LP-1415 allele
- **LP-1416**: LP-1416 allele
plating them on SC, SC-his and SC-trp media. The frequency of histidine or tryptophan prototrophs arising during meiosis in diploids homozygous for rad52 (LP-854, LP-1383 and LP-1384), heterozygous for rad52 (LP-855, LP-1415) and in a diploid homozygous for RAD52 (LP-1416) is given in Figure 2 and Figure 3, respectively. Prototrophs of both types arise with similar kinetics in rad52/RAD52 and RAD52/RAD52 diploids, where the frequency of histidine and tryptophan prototrophs begins to rise after about five hours in sporulation medium. In rad52 homozygous diploids, on the other hand, there is essentially no increase in either histidine or tryptophan prototrophs during sporulation. The frequencies observed are at the levels found in mitotic cells. By 50 hours in sporulation medium, the frequency of histidine-independent colonies per 10⁶ colony-

![Graph showing frequency of histidine prototrophs as a function of time of incubation in sporulation medium.]
EFFECTS OF RAD52

Figure 3.—Frequency of tryptophan prototrophs as a function of time of incubation in sporulation medium in LP-1416, RAD+/RAD+ (○); LP-855 and LP-1415 RAD+/rad52 (▲ and △, respectively); and in LP-1384 and LP-854, rad52/rad52 (○ and □, respectively). All strains contained trp5-2/trp5-48 heteroalleles.

Forming units has risen to about 6000 in the RAD52/RAD52 and rad52/RAD52 diploids, whereas in the rad52/rad52 diploids, it is less than 10 (Figure 2). A similar pattern of prototroph accumulation is observed for the trp5-2/trp5-48 heteroallelic pair (Figure 3).

In addition to the lack of prototroph accumulation during meiosis, the viability of rad52 homozygous diploids decreases to 10–20% after 50 hours in SPM, whereas the viability of rad52/RAD52 and RAD52/RAD52 diploids remains constant throughout sporulation (Figure 4).

Commitment to meiosis among intragenic recombinants: In order to determine whether the histidine and tryptophan prototrophs recovered from sporulation medium at different times were committed to meiotic chromosome segre-
Figure 4.—Viability as determined by plating appropriate dilutions of cells on SC medium after various times of incubation in sporulation medium in LP-1416, *RAD+/RAD+* (), LP-855 and LP-1415, *RAD+/rad52* ( and , respectively); and LP-1384 and LP-854, *rad52/rad52* ( and , respectively).

Viability, 50–150 histidine and tryptophan prototrophs at each time point were tested for auxotrophy for the centromere-linked marker *leu1* in the *leu1/+* heterozygotes. Figure 5 gives the percent of histidine prototrophs committed to meiotic chromosome segregation and percent of asci as various times during sporulation of a diploid homozygous for *RAD52*. As expected, cells committed to meiotic chromosome segregation begin to appear after eight hours, whereas the intragenic recombinants begin to rise earlier, after five hours in sporulation media (Sherman and Roman 1963; Esposito and Esposito 1974). In the wild-type *RAD52/RAD52* strain, the final percent of cells committed to meiotic chromosome segregation and the percent of ascus formation was 50 to 60%. The tryptophan prototrophs gave similar results for commitment to meiosis.

In the *rad52/rad52* homozygotes, meiotic chromosome segregation was studied in tryptophan prototrophs obtained from sporulation medium at 24 hours. Six subclones from each of the 130 *TRP+* colonies were examined for auxotrophy for the centromere-linked marked *leu1*. Meiotic chromosome segregation was not observed in *rad52/rad52* diploids as determined by the lack of leucine auxotrophs. After 24 hours in sporulation medium, the wild-type homozygotes, on the other hand, have almost reached the plateau for commitment to meiotic segregation.
The rad52/rad52 homozygotes were variable in sporulation in different strains. The percent of asci formed in various rad52/rad52 strains ranged from nearly zero to 30%. The spores from rad52/rad52 strains were found to be inviable.

Gene-centromere recombination: Histidine and tryptophan prototrophs obtained from sporulation medium at various times were examined for gene-centromere recombination. Since the strains were heterozygous for several loci, the appearance of auxotrophs for these heterozygous loci in cells uncommitted to meiotic segregation is due to recombination (Esposito and Esposito 1974; Esposito, Plotkin and Esposito 1974). Histidine and tryptophan prototrophs from RAD52/RADS2 were replica-plated onto media lacking homoserine, arginine, lysine or leucine. Table 2 gives the gene-centromere recombination in tryptophan and histidine prototrophs of wild-type diploids calculated from the fraction of cells uncommitted to meiotic chromosome segregation and taken from sporulation medium at 11 hours. At the arg6 and lys2 loci, we already observe the recombination level characteristic of meiotic cells in the prototrophs of wild-type diploids. The higher-than-expected frequency of recombination involving arg6 and lys2 loci among tryptophan prototrophs is not statistically significant.
**TABLE 2**

Gene-centromere recombination among tryptophan and histidine prototrophs uncommitted to meiosis

<table>
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<th>Strain</th>
<th>Genotype</th>
<th>Total colonies tested</th>
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<th><strong>HIS</strong>&lt;sup&gt;*&lt;/sup&gt;</th>
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<tr>
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<td></td>
<td></td>
<td>hom&lt;sup&gt;3&lt;/sup&gt;</td>
<td>arg&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>LP-1416</td>
<td>RAD52</td>
<td>128&lt;sup&gt;†&lt;/sup&gt;</td>
<td>9.0</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>RAD52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1384</td>
<td>rad52</td>
<td>130&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td></td>
<td>rad52</td>
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* % Gene-centromere recombination was calculated, according to Esposito and Esposito (1974), only in the fraction of cells uncommitted to meiotic chromosome segregation as follows:

\[ \% \text{Gene-centromere recombination} = \frac{c \times mT}{T \times mT} \times 100, \]

where \( c = 2 \times \# \text{ of } -/- \text{ auxotrophs}; mT = 2 \times \# \text{ of leucine auxotrophs}; \) these represent cells committed to meiosis; \( T = \) total colonies.

** Maximum expected gene-centromere recombination is 33.3% since only one-half of the equational divisions of second-division segregation (tetratypes) are recovered as homozygotes in this system.

† Prototrophs from LP-1416 were withdrawn from sporulation medium at 11 hours.
‡ Prototrophs from LP-1384 were withdrawn from sporulation medium at 24 hours. Six subclones from each TRP<sup>+</sup> colony were analyzed.

Gene centromere recombination was examined among tryptophan prototrophs from the rad<sup>52</sup>/rad<sup>52</sup> strain (LP-1384) at 24 hours in SPM. These TRP<sup>+</sup> colonies of rad<sup>52</sup>/rad<sup>52</sup> were subcloned and six subclones of each colony were examined by replica plating onto media lacking homoserine, arginine, lysine or leucine. Since there is no enhancement of intragenic recombination in rad<sup>52</sup>/rad<sup>52</sup> during meiosis, the recovered prototrophs arose during mitosis. Therefore, subclones of prototrophs were examined in order to ensure the recovery of any auxotrophs arising due to intergenic recombination during meiosis. However, in rad<sup>52</sup>/rad<sup>52</sup> homozygotes, no gene-centromere recombination was observed after 24 hours in sporulation medium. Tryptophan prototrophs from the rad<sup>52</sup>/rad<sup>52</sup> strain recovered at 5 and 10 hours from sporulation medium also gave no evidence of intergenic recombination or meiotic chromosome segregation.

In order to examine further intergenic recombination and meiotic chromosome segregation, heterozygous can<sup>+</sup>/+ diploids of RAD52/RAD52, RAD52/rad52 and rad52/rad52 were transferred to sporulation medium, and at various time intervals, samples were withdrawn and plated on SC+can—arg to select for canavanine-resistant colonies and on SC for viability. The appearance of canavanine-resistant colonies during sporulation is due to intergenic recombination or meiotic chromosome segregation. Figure 6 gives the percent of canavanine-resistant colonies and asci appearing during sporulation in RAD52/RAD52, RAD52/rad52 and rad52/rad52 diploids. In the RAD52/RAD52 and RAD52/rad52 diploids, a rise in the frequency of canavanine-resistant colonies is apparent beginning at eight hours and reaching a final level of 60–70%. The
Figure 6.—Percent of canavanine-resistant colonies arising from can+/- diploids (closed symbols) and percent asci (open symbols) as a function of time of incubation in sporulation medium in RAD+/RAD+ (○, □); RAD+/rad52 (▲, △) and rad52/rad52 (■, □) diploids. Canavanine-resistant cells were scored on synthetic complete medium lacking arginine and supplemented with 80 mg/l of L-canavanine sulfate (SC — arg + can).

appearance of canavanine-resistant colonies correlates rather well with the frequency of asci. In the rad52/rad52 diploid, on the other hand, no increase in the frequency of canavanine-resistant colonies occurs even though approximately 25% asci are observed. The rad52/rad52 diploid was also heterozygous for ade6 and ade2. The appearance of red colonies during sporulation of these diploids would be indicative of intergenic recombination or meiotic chromosome segregation. However, no increase in the number of red colonies was observed in the rad52/rad52 diploids during sporulation. In conclusion, no intragenic or intergenic meiotic recombinants were observed in rad52/rad52 diploids at a variety of loci examined.

Giemsa staining of rad52/rad52 diploids: The rad52/rad52 diploids were stained with giemsa after 48 hours in sporulation medium. The percent of cells with one, two and four chromatin bodies was 65%, 21% and 14%, respectively. A total of 400 cells was examined for these determinations.
Effects in mitotic cells

Spontaneous mitotic recombination: Since meiotic recombinants are not seen in rad52/rad52 diploids, we determined whether the rad52 mutation also affected mitotic recombination. Experiments were carried out as described in MATERIALS AND METHODS. Table 3 gives the results for rates of spontaneous mitotic prototrophy at his1-1/his1-315 and trp5-2/trp5-48 heteroalleles in rad52/rad52, rad52/RAD52 and RAD52/RAD52 diploids. In rad52/RAD52 heterozygotes, there is about a two-fold reduction in the rate of histidine prototrophy compared to that in the RAD52/RAD52 homozygous diploid. Similar results are observed for the trp5-2/trp5-48 heteroalleles. In the rad52/rad52 diploids, the rate of histidine prototrophy is only 20-30% of that in the RAD52/RAD52 diploid, whereas the rate of tryptophan prototrophy in rad52/rad52 diploids is increased 6- to 9-fold over that in the RAD52/RAD52 diploid. In order to determine the contribution of spontaneous mutations to these prototrophs, reversion rates were examined in homoallelic diploids and are given in Table 4. The spontaneous mutation rate to histidine prototrophy in his1-1/his1-1 rad52/rad52 diploids is about 10-fold greater than in the wild type diploids. The reversion rate of his1-315 is more than 20 times higher in the rad52/rad52 diploids than in the RAD52/RAD52 diploids. The increase in reversion rates at trp5-2 and trp5-48 are about

TABLE 3

Spontaneous mitotic prototrophy at his1-1/his1-315 and trp5-2/trp5-48 sites

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Mean No. of cells per culture (1)</th>
<th>Median No. HIS+ arising per culture (2)</th>
<th>Mean No. HIS+ rate (3)</th>
<th>Median No. TRP+ arising per culture (4)</th>
<th>Mean No. TRP+ rate (5)</th>
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<tbody>
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<td>LP-1416</td>
<td>RAD52/RAD52</td>
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<td>5050</td>
<td>653</td>
<td>1.92</td>
<td>2475</td>
</tr>
<tr>
<td>LP-855</td>
<td>RAD52/rad52</td>
<td>3.46 × 10^8</td>
<td>1855</td>
<td>271</td>
<td>0.78</td>
<td>1150</td>
</tr>
<tr>
<td>LP-855</td>
<td>RAD52/rad52</td>
<td>1.13 × 10^8</td>
<td>835</td>
<td>136</td>
<td>1.2</td>
<td>235</td>
</tr>
<tr>
<td>LP-1415</td>
<td>RAD52/rad52</td>
<td>2.83 × 10^8</td>
<td>1850</td>
<td>271</td>
<td>0.96</td>
<td>1288</td>
</tr>
<tr>
<td>LP-854</td>
<td>rad52/rad52</td>
<td>3.4 × 10^7</td>
<td>65</td>
<td>16</td>
<td>0.47</td>
<td>1400</td>
</tr>
<tr>
<td>LP-854</td>
<td>rad52/rad52</td>
<td>3.84 × 10^7</td>
<td>40</td>
<td>11.4</td>
<td>0.30</td>
<td>1713</td>
</tr>
<tr>
<td>LP-1384</td>
<td>rad52/rad52</td>
<td>3.02 × 10^7</td>
<td>80</td>
<td>19</td>
<td>0.63</td>
<td>1925</td>
</tr>
</tbody>
</table>
### TABLE 4

Spontaneous homoallelic reversion rates in Diploids at his1 and trp5 loci

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Mean No. of cells per culture (1)</th>
<th>Median No. of prototrophs arising per culture (2)</th>
<th>Reversion rate (3/1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-87</td>
<td>RAD52</td>
<td>5.45 \times 10^9</td>
<td>68</td>
<td>3.1 \times 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>RAD52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1938</td>
<td>rad52/rad52</td>
<td>3.16 \times 10^9</td>
<td>530</td>
<td>29.1 \times 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>rad52/rad52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-77</td>
<td>RAD52</td>
<td>29.52 \times 10^8</td>
<td>70</td>
<td>0.58 \times 10^{-8}</td>
</tr>
<tr>
<td></td>
<td>RAD52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1901</td>
<td>rad52/rad52</td>
<td>14.7 \times 10^8</td>
<td>1310</td>
<td>13.6 \times 10^{-8}</td>
</tr>
<tr>
<td></td>
<td>rad52/rad52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-87</td>
<td>RAD52</td>
<td>5.45 \times 10^9</td>
<td>467</td>
<td>15 \times 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>RAD52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1938</td>
<td>rad52/rad52</td>
<td>3.15 \times 10^9</td>
<td>910</td>
<td>46.3 \times 10^{-9}</td>
</tr>
<tr>
<td></td>
<td>rad52/rad52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1843</td>
<td>RAD52</td>
<td>1.53 \times 10^6</td>
<td>473</td>
<td>5.4 \times 10^{-7}</td>
</tr>
<tr>
<td></td>
<td>RAD52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-77</td>
<td>RAD52</td>
<td>2.95 \times 10^9</td>
<td>9117</td>
<td>3.7 \times 10^{-7}</td>
</tr>
<tr>
<td></td>
<td>RAD52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP-1844</td>
<td>rad52/rad52</td>
<td>5.99 \times 10^7</td>
<td>1428</td>
<td>3.6 \times 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>rad52/rad52</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

three and ten times greater in rad52/rad52 diploids than in RAD52/RAD52 diploids, respectively. The trp5-48 allele is suppressed by all ochre suppressors and the trp5-2 allele is suppressed by class I ochre suppressors (Hawthorne and Leupold 1974); spontaneous mutation rates are known to be elevated at ochre-suppressor loci in rad52 strains (von Borstel, Cain and Steinberg 1971).

We expect the spontaneous mutation rate in his1-1/his1-315 rad52/rad52 heteroallelic diploids to be 8.26 \times 10^{-4}, an average of reversion rates in corresponding homoallelic diploids. The rate of spontaneous intragenic recombination in his1-1/his1-315 rad52/rad52 diploids is about 4.2 \times 10^{-4} after subtracting the mutation rates. The average reversion rate in his1-1/his1-315 RAD52/RAD52 diploids is 4.5 \times 10^{-4}, and the rate of spontaneous intragenic recombination in these diploids is 19.15 \times 10^{-7}. Thus, a five-fold reduction occurs in the rate of spontaneous intragenic mitotic recombination at the his1-1/his1-315 sites in rad52/rad52 diploids as compared with the RAD52/RAD52 diploids. At the trp locus, we are still left with a heteroallelic recombination rate of 3-6 \times 10^{-6}.
in rad52/rad52 diploids after subtracting the mutation rates. It is not clear if this increase is real or an artifact due to high reversion rates. In order to clarify the role of rad52 in spontaneous mitotic recombination, we examined the rate of heteroallelic recombination resulting in prototrophy at leu1-c/leu1-12 in RAD52/RAD52 and rad52/rad52 diploids. Neither of these alleles is suppressed by suppressor loci. The rate of prototrophy in rad52/rad52 diploids is $0.86 \times 10^{-8}$, which is only 20% the rate ($4.14 \times 10^{-8}$) in RAD52/RAD52 diploids. We conclude that the RAD52 gene product plays a role in spontaneous mitotic recombination.

**γ-ray- and UV-induced mitotic recombination:** Figure 7A gives the survival following γ-ray irradiation and Figure 7B gives the survival following UV irradiation, while Figures 8 and 9 present the results of γ-ray- and UV-induced heteroallelic recombination at the his1-1/his1-315 and trp5-2/trp5-48 sites in rad52/rad52, rad52/RAD52 and RAD52/RAD52 diploids. No γ-ray-induced prototrophs were recovered at the his1 or trp5 loci among the rad52/rad52 diploids (Figure 8). The rad52/rad52 diploids gave some UV-induced prototrophs at the his1 heteroallelic sites at UV fluences above 20 J/m². At 40 J/m², the frequency of UV-induced prototrophs at his1 was 350/10⁷ colony forming units (cfu) in rad52/rad52 diploids and over 8000/10⁷ cfu in both RAD52/RAD52 and rad52/RAD52 diploids. At trp5, few UV-induced prototrophs are observed in rad52/rad52 diploids below 20 J/m². However, UV-induced prototrophs do appear above 20 J/m² in rad52/rad52 diploids. At 40 J/m², the frequency of UV-induced prototrophs in rad52/rad52 diploids is 1800/10⁷ cfu, while the frequency in the rad52/RAD52 and RAD52/RAD52 diploids varies from 9200 to 13,600 per 10⁷ cfu. Since some UV-induced prototrophs were observed at both his1 and trp5 loci in rad52 diploids, we examined the frequency of UV-induced reversion in his1-1/his1-1, his1-315/his1-315, trp5-2/trp5-2 and trp5-48/trp5-48 homoallelic rad52 diploids. We found that UV-induced mutations can account for all of the prototrophs observed in his1-1/his1-315 and trp5-2/trp5-48, rad52/rad52 heteroallelic diploids. We conclude that γ-ray- and UV-induced heteroallelic recombination is absent in rad52/rad52 diploids.

**DISCUSSION**

Premeiotic DNA synthesis occurs in rad52/rad52 diploids, but no intragenic or intergenic recombinants during meiosis are recovered at any of the several loci tested. Sporulation occurs to varying degrees in different rad52 strains, producing inviable spores. Game and Mortimer (1974) also reported greatly reduced spore viability in rad52 diploids. Even though first and second meiotic divisions occur during sporulation in rad52 diploids, we have found no auxotrophs for leu1 from leu1/+ diploids, no canavanine-resistant cells from can⁴/+ diploids, and no red colonies from ade6/+ ade2/+ cells resulting from meiotic chromosome segregation. This suggests that the products following the first meiotic division in rad52 diploids are inviable. Our failure to recover meiotic recombinants cannot be due to inviability of meiotic segregants since commitment to recombination occurs before commitment to meiotic chromosome segregation. It also appears unlikely that rad52 strains are defective in DNA
Figure 7.—Survival curves following α-ray irradiation (A) and UV irradiation (B) in RAD+/RAD+ (LP-1416), (●); RAD+/rad52 (LP-1415), (△); and rad52/rad52 (LP-1384), (○), diploids.
replication; one would expect such a defect to prevent nuclear division. All of the cell division cycle mutants of *S. cerevisiae* defective in DNA replication stop before or during nuclear division (Hartwell et al. 1974). Recombination-defective mutants of *Drosophila melanogaster* show elevated frequencies of chromosome nondisjunction at the first meiotic division (Baker et al. 1976). The inviability of spores and the decline in viability of *rad52/rad52* cells in sporulation media may be due to nondisjunction and/or abnormal DNA resulting from a block during recombination.

The effect of *rad52* on γ-ray- and UV-induced mitotic intragenic recombination strengthens the notion that the *RAD52* gene product functions in recombination. In *rad52* diploids, γ-ray-induced heteroallelic recombination is absent at both the *his1* and *trp5* loci. Resnick (1975) also observed a lack of γ-ray-induced heteroallelic recombination at the *arg4* locus. UV-induced intragenic recombina-
EFFECTS OF RAD52

Figure 9.—Dose-response curve for induction of histidine (A) and tryptophan (B) mitotic prototrophs following UV irradiation of heteroallelic RAD+/RAD+ (LP-1416), (○); RAD+/rad52 (LP-1415), (△); and rad52/rad52 (LP-1384), (○), diploids.

The RAD52 gene product seems to be required for meiotic as well as γ-ray- and UV-induced recombination. The degree of its requirement in spontaneous mitotic recombination is less clear, since we observe about one-fifth the wild type level of recombination at his1-1/his1-1315. It may be that the rad52-1 mutant allele affects spontaneous mitotic recombination to a lesser degree, while different mutant alleles of rad52 may turn out to be entirely deficient in spontaneous mitotic recombination. For example, the recA and lexB mutations of the RECA gene differ remarkably in their properties. The recA mutant is deficient in recombination, and the lexB mutant is proficient in recombination (Morand, Goze and Devoret 1977). Or, there may be alternate pathways for spontaneous mitotic recombination.
Mutants specifically defective in meiotic recombination in *S. cerevisiae* have been isolated and characterized; three mutants, con1, con2 and con3 allow pre-meiotic DNA synthesis, but show no prototroph formation for heteroalleles at the *leu2* locus. The *con1* mutation blocks sporulation, while *con2* and *con3* mutants produce inviable ascospores (Roth and Fogel 1971; Fogel and Roth 1974). The *rec1*, *rec2*, *rec3* and *rec4* mutants of *S. cerevisiae* are deficient in X-ray-induced mitotic intragenic recombination, and the *rec1*, *rec3* and *rec4* mutants are also deficient in UV-induced mitotic intragenic recombination (Rodarte-Ramon and Mortimer 1972; Rodarte-Ramon 1972). The *rec4* mutant blocks single-site conversions and increases the frequency of co-conversions that include both mutant sites of the heteroalleles. Intergenic meiotic recombination or sporulation are not affected in *rec4* strains (San Filippo 1976). In contrast to the *rec4* mutation, which affects the length of the conversion segment but not intergenic recombination or sporulation during meiosis, the *rad52* mutation affects both intragenic and intergenic meiotic recombination and inviable spores are formed during meiosis.

In addition to the effects on recombination, we have found that spontaneous mutation rates of *his1-1* and *his1-315*, which are not nonsense alleles, are 10 to 20 fold higher in *rad52* than in the wild-type cells. Spontaneous mutation rates of ochre suppressors are also increased in *rad52* strains (von Borstel, Cain and Steinberg 1971). The effect of *rad52* on spontaneous mutations appears to be a general one.

The *rad52* mutation of yeast resembles the *recA* mutation of *E. coli* in its deficiency in recombination. However, the *RAD52* gene function does not seem to be involved in error-prone repair of UV- or X-ray-induced damage to DNA, whereas the *RECA* gene product is absolutely required for error-prone repair (Kondo et al. 1970; Witkin 1969). The *recA* mutants are also highly UV sensitive, whereas the *rad52* mutants are not very UV sensitive. The *RECA* gene has been implicated in the formation of joint molecules during recombination (Holloman and Radding 1976); the *RECA* protein has also been shown to catalyze renaturation of single-stranded DNA (Weinstock, McEntee and Lehman 1979). The *RAD52* gene product may play a similar role in synopsis.

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**LITERATURE CITED**


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