# A *Saccharomyces cerevisiae RAD52* Allele Expressing a C-Terminal Truncation Protein: Activities and Intragenic Complementation of Missense Mutations

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Manuscript received June 16, 1992
Accepted for publication September 16, 1992

ABSTRACT

A nonsense allele of the yeast *RAD52* gene, *rad52-327*, which expresses the N-terminal 65% of the protein was compared to two missense alleles, *rad52-1* and *rad52-2*, and to a deletion allele. While the *rad52-1* and the deletion mutants have severe defects in DNA repair, recombination and sporulation, the *rad52-327* and *rad52-2* mutants retain either partial or complete capabilities in repair and recombination. These two mutants behave similarly in most tests of repair and recombination during mitotic growth. One difference between these two alleles is that a homozygous *rad52-2* diploid fails to sporulate, whereas the homozygous *rad52-327* diploid sporulates weakly. The low level of sporulation by the *rad52-327* diploid is accompanied by a low percentage of spore viability. Among these viable spores the frequency of crossing over for markers along chromosome *VII* is the same as that found in wild-type spores. *rad52-327* complements *rad52-2* for repair and sporulation. Weaker intragenic complementation occurs between *rad52-327* and *rad52-1*.

THE central role of the *Saccharomyces cerevisiae RAD52* gene in DNA repair and recombination is recognized by the plethora of mutant phenotypes. Disruption mutations, as well as the *rad52-1* missense mutation, confer extreme X-ray sensitivity (RESNICK 1969; STRIKE 1978), decreased sporulation and spore viability (GAME and MORTIMER 1974; STRIKE 1978; GAME *et al.* 1980; PRAKASH *et al.* 1980; RESNICK *et al.* 1986), decreased mitotic and meiotic recombination (STRIKE 1978; GAME *et al.* 1980; PRAKASH *et al.* 1980; HOEKSTRA, NAUGHTON and MALONE 1986), decreased mating type switching (MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981), and increased chromosome loss (MORTIMER, CONTOPOULOU and SCHILD 1981). Because these phenotypes are the most comprehensive and result from the most drastic genetic alterations, they are considered the null phenotypes. Although all the phenotypes may result from a protein product with a single function, mutational analyses of the gene raise the question whether the phenotypes might result from a protein with multiple activities. Evidence for this possibility comes from the work of MALONE *et al.* (1988) who have shown that although the *rad52-2* mutation produces most of the null phenotypes, mutants bearing this allele have elevated levels of mitotic recombination.

Another complexity arising in the genetic analysis of *RAD52* was revealed in the initial reports on the cloning of *RAD52* (SCHILD *et al.* 1983; ADZUMA, OGAWA and OGAWA 1984). These studies showed that a chromosomal *Bam*HI fragment on a high copy vector complemented a *rad52-1* mutant strain for sensitivity to the radiomimetic alkylating agent methyl methanesulfonate (MMS) and to X-irradiation. Sequencing the cloned gene revealed that the *Bam*HI site is within the *RAD52* open reading frame, allowing expression of only the N-terminal 65% of the protein. The source of the complementation is unclear. The truncated protein may indeed contain DNA repair activity, and its action may have been augmented by its expression from a high copy number plasmid. Another possibility is that the truncated product interacts with the product of the *rad52-1* allele to restore function.

We were interested in examining these possibilities in order to extend the mutational analyses on the multiple roles played by the protein. To do so, we recreated the allele encoding the truncation product by inserting a linker containing stop codons into the *Bam*HI site. This allele was substituted for the wild-type chromosomal copy of the gene to yield a mutant with a single copy of this allele driven by its own promoter. We show that the truncated protein retains activities in repair and recombination. In addition this allele intragenically complements the *rad52-1* and *rad52-2* alleles.

## MATERIALS AND METHODS

**Yeast strains:** A list of the *RAD52* alleles used in this study is given in Table 1, and the genotypes of yeast strains bearing these alleles are shown in Table 2. SSL209, bearing *rad52-1*, is congenic with SSL204. All other haploid strains are isogenic with SSL204, SSL204A or LH330A. The con-

**TABLE 1**

***RAD52* alleles**

| Allele | Type of mutation | Alteration | Source or reference |
|---|---|---|---|
| *rad52-1* | Missense | A90V[a] | RESNICK (1969) |
| *rad52-2* | Missense | P64L[b] | Attributed to R. SNOW; see GAME and MORTIMER (1974) |
| *rad52-169* | Nonsense | Linker insertion causes termination at codon 169 | This study |
| *rad52-327* | Nonsense | Linker insertion causes termination at codon 327 | This study |
| *rad52-ΔHS* | Deletion/disruption | 98% of open reading frame deleted; *LEU2* inserted | DORNFELD and LIVINGSTON (1991) |

[a] Alteration determined by ADZUMA et al. (1984).
[b] Alteration determined in this study.

struction of SSL204 (*RAD52*), SSL209 (*rad52-1*) and SSL212 (*rad52-ΔHS*) has been described previously (DORNFELD and LIVINGSTON 1991). LH330A was provided by Leland Hartwell (MEEKS-WAGNER and HARTWELL 1986).

**Construction of the *rad52-327* and *rad52-169* truncation alleles:** The nucleotide numbering for the various *RAD52* constructions refer to the sequence published by ADZUMA, OGAWA and OGAWA (1984). The clone we used came from DAVID SCHILD and runs from an *Eco*RI site at nucleotide (nt) 78 to a *Sau*3A site at nt 3010 (SCHILD *et al.* 1983). This includes the open reading frame from nt 968 to 2480. An *Xba*I linker containing stop codons in all three reading frames (CTAGTCTAGACTAG, New England BioLabs) was inserted independently into the *Bam*HI (nt 1949) and *Bgl*II (nt 1470) sites of a plasmid-borne copy of *RAD52*. To reflect the position in the 504 amino acid open reading frame, the insertion at the *Bam*HI site was designated *rad52-327*, while the insertion at the *Bgl*II site was designated *rad52-169*.

**Cloning of the *rad52-2* allele:** The *rad52-2* allele was cloned by the polymerase chain reaction (PCR) (SAIKI *et al.* 1988) from both RM137-10D and RM86-13A, two *rad52-2* strains provided by ROBERT MALONE. To confirm that the PCR products contained a *rad52* mutation, the *Bst*EII-*Sph*I fragment (nt 1110–2466) of the wild-type sequence containing most of the open reading frame was replaced with the corresponding fragment of the PCR products. The substituted copies were cloned into the vector YCp50 as a larger

**TABLE 2**

**Strains**

| Strains | Genotype | Source or reference |
|---|---|---|
| RM137-10D | *rad52-2 MAT***a** *ura3-1 ade2-1 lys2-2 met13 can1*$^r$ *tyr1-2* | ROBERT MALONE |
| RM86-13A | *rad52-2 MATα ura3-1 ade2-1* | ROBERT MALONE |
| SSL204 | *RAD52 MATα his3-Δ200 leu2 trp1 ura3-52 ade2-101* | DORNFELD and LIVINGSTON (1991) |
| SSL204A | *RAD52 MAT***a** *his3-Δ200 leu2 trp1 ura3-52 ade2-101* | DORNFELD and LIVINGSTON (1991) |
| SSL212A | *rad52-ΔHS MAT***a** " | DORNFELD and LIVINGSTON (1991) |
| SSL209 | *rad52-1 MATα* " | DORNFELD and LIVINGSTON (1991) |
| SSL209A | *rad52-1 MAT***a** " | Segregant of SSL209 × SSL204A |
| SSL241 | *rad52-327 MAT***a** " | This study |
| SSL242 | *rad52-169 MAT***a** " | This study |
| SSL243[a] | *rad52-2 MATα* " | This study |
| SSL244[b] | *rad52-2 MATα* " | This study |
| SSL245[b] | *rad52-2 MAT***a** " | This study |
| LH330A | *RAD52 MATα ade2 leu2 trp1 hom3 ura3 can1 fcy1 sap3 lys5 cyh2 ade6* | MEEKS-WAGNER and HARTWELL (1986) |
| SSL251 | *rad52-327* " | This study |
| SSL252 | *rad52-169* " | This study |
| SSL253[a] | *rad52-2* " | This study |
| SSL260[c] | *RAD52/RAD52 MAT***a**/*MATα ade2-101/ade2 leu2/leu2 trp1/trp1 ura3-52/ura3 his3-Δ200/+ +/hom3 +/can1 +/fcy1 +/sap3* $\frac{lys5\ cyh2}{+\ \ +} \bullet \frac{ade6}{+}$ | SSL204A × LH330A |
| SSL261 | *rad52-327/rad52-327* " | SSL241 × SSL251 |
| SSL262 | *rad52-169/rad52-169* " | SSL242 × SSL252 |
| SSL263 | *rad52-2/rad52-2* " | SSL245 × SSL253 |
| SSL265 | *rad52-2/rad52-327* " | SSL253 × SSL241 |
| SSL266 | *rad52-1/rad52-327* " | SSL209A × SSL251 |
| SSL267 | *rad52-1/rad52-2* " | SSL209A × SSL253 |
| SSL268 | *rad52-1/rad52-169* " | SSL209A × SSL252 |
| SSL269 | *RAD52/rad52-327* " | SSL241 × LH330A |

[a] *rad52-2* allele derived from RM86-13A.
[b] *rad52-2* allele derived from RM137-10D.
[c] The position of *CEN7* is represented by the closed circle.

*Eco*RI-*Sau*3A fragment (nt 78–3010) containing the entire gene and promoter. These plasmids were transformed into SSL212A cells bearing the deletion-disruption allele, and Ura$^+$ transformants were tested for their sensitivity to MMS. The PCR products from both strains did not provide resistance. Before sequence identification of the *rad52-2* lesion we used two derivative strains, one for each PCR isolate from the two strains provided by ROBERT MALONE. Both behaved identically in the tests made on them. As described under RESULTS, the *rad52-2* mutation disrupts an *Ava*II restriction site at position 1155, and this polymorphism was subsequently used to verify this allele in constructed strains.

**Construction of *rad52-2*, *rad52-327* and *rad52-169* derivatives of SSL204A and LH330A:** The *Eco*RI-*Sau*3A fragment containing each allele was cloned into the *Eco*RI and *Bam*HI sites of YIp5 and targeted into the wild-type strains (SSL204A and LH330A) by *Bst*EII digestion in the *RAD52* coding region before transformation to Ura$^+$. Segregants which had lost the YIp5 *URA3* gene (SCHERER and DAVIS 1979) were tested for MMS sensitivity and analyzed by Southern blotting for definitive restrictions sites (the loss of the 1155 *Ava*II site for *rad52-2* and the presence of an *Xba*I site for *rad52-327* and *rad52-169*). All diploids were constructed by mating the appropriate SSL204A derivative with the appropriate LH330A derivative, as shown in Table 2.

**Expression of *RAD52* alleles from the *ENO1* promoter.** The expression of the wild-type allele from the yeast *ENO1* promoter on the high copy number vector pMAC101 has been described previously (DORNFELD and LIVINGSTON 1991). The *Bst*EII-*Sph*I fragment of each mutant was substituted for the wild-type sequence in this vector.

**MMS survival:** MMS killing was performed as described (PRAKASH and PRAKASH 1977). Briefly, survival of haploid and diploid strains exposed to 0.5% MMS for varying amounts of time was measured by plating appropriate dilutions on YEPD or on selective plates for strains with plasmid borne *RAD52* alleles.

**Mitotic plasmid recombination:** Histidine prototroph formation in SSL204A derivative strains bearing recombination reporter plasmid pBYA819 (shown in Figure 3) was measured by fluctuation analyses using the method of the median (LURIA and DELBRUCK 1943; LEA and COULSON 1949; AHN and LIVINGSTON 1986).

**Chromosome *VII* loss:** Chromosome *VII* loss was measured by fluctuation analyses. Cultures were grown in YEPD medium and plated on YEPD plates containing 10 mg/liter cycloheximide. The median fraction of cells resistant to cycloheximide due to chromosome loss and to recombination was determined for five cultures, and this fraction was used to determine the rate. An account of the method used to distinguish chromosome loss from recombination is provided under RESULTS.

**Sporulation and meiotic crossing over:** Cultures of the diploids were grown in YEPD to late log phase, washed in water and diluted into 0.3% potassium acetate supplemented with required amino acids and nucleic acid bases. The cultures were incubated at 30° for 5–7 days to allow for maximal ascus formation. Only four-spore asci were dissected and scored.

**DNA sequencing:** The DNA sequence of *rad52-2* was determined from the *Bst*EII site at nt 1110 to the *Pst*I site at 2339. Restriction fragments of *rad52-2* were subcloned into the KS+ vector from Stratagene. Double strand sequencing was performed using the T3 and T7 17-mer primers (Stratagene) and Sequenase (USB).

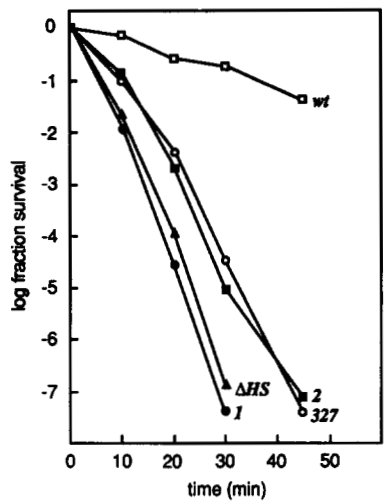


FIGURE 1.—Survival of haploid cells in 0.5% MMS. The numbers identifying the lines represent the *RAD52* alleles present in the strains. □, SSL204A, *RAD52*; ■, SSL243, *rad52-2*; ○, SSL241, *rad52-327*; △, SSL212A, *rad52-ΔHS*; ●, SSL209, *rad52-1*.

## RESULTS

***rad52-327* and *rad52-2* confer partial resistance to MMS:** To determine whether the *rad52-327* mutant is able to repair MMS lesions, we compared its survival to MMS exposure with that of isogenic haploid strains bearing the wild-type allele or other mutant alleles (Figure 1). As expected, the wild-type strain is mostly resistant to MMS exposure, and the deletion strain and the *rad52-1* strain are both extremely sensitive to MMS. Although both the *rad52-327* and the *rad52-2* strains are much more sensitive to MMS than the wild-type strain, exhibiting a drop in survival of five orders of magnitude at 30 min, they retain a low level of resistance to MMS relative to the *rad52-ΔHS* deletion strain, which exhibited a drop in survival of seven orders of magnitude at the same time point.

This partial resistance might result from a limiting amount of a fully active protein or from a partially active protein present in saturating amounts. To distinguish between these possibilities, we expressed the *rad52-327* and *rad52-2* alleles, as well as the wild-type allele, from the constitutive *ENO1* promoter on high copy number plasmids (MATERIALS AND METHODS). Overexpression of the wild-type allele in all mutant strains restored wild-type levels of resistance (data not shown) as expected from a previous study (DORNFELD and LIVINGSTON 1991). Overexpression of either the *rad52-2* or the *rad52-327* allele in strains containing the same chromosomal allele did not increase the MMS resistance of either strain (Figure 2). Indeed, overexpression of *rad52-327* appears to have somewhat of a deleterious affect. These results indicate that the *rad52-327* and *rad52-2* gene products are not rate limiting and, thus, are likely to be partially active proteins.

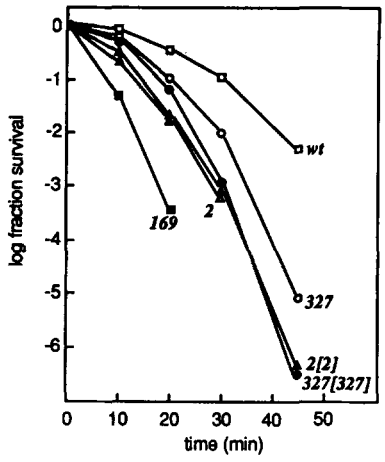


FIGURE 2.—Survival in 0.5% MMS of haploid strains with or without a plasmid bearing a *RAD52* allele under control of the *ENO1* promoter. The numbers identifying the lines represent the *RAD52* alleles present in the strains. The numbers in brackets are the *RAD52* alleles expressed from the plasmid borne *ENO1* promoter. □, SSL204A (*RAD52*) without a plasmid; ○, SSL241 (*rad52-327*) without a plasmid; △, SSL243 (*rad52-2*) without a plasmid; ▲, SSL243 (*rad52-2*) with p*ENO1::rad52-2*; ●, SSL241 (*rad52-327*) with p*ENO1::rad52-327*; ■, SSL242 (*rad52-169*) without a plasmid.

**TABLE 3**

**Survival of cells expressing the *HO* endonuclease**

| Strain | *RAD52* allele | Survival (%)[a] |
|---|---|---|
| SSL204A | *RAD52* | 74.0 |
| SSL212A | *rad52-ΔHS* | 0.4 |
| SSL242 | *rad52-169* | 0.5 |
| SSL241 | *rad52-327* | 9.1 |
| SSL243 | *rad52-2* | 6.7 |

The *HO* gene was under the control of the *GAL1* promoter.
[a] Ratio of colony number on galactose to colony number on glucose times 100%.

***rad52-327* and *rad52-2* are partially able to survive the continual expression of the HO endonuclease:** Because the chromosomal lesions resulting from MMS treatment are numerous and varied, we also characterized the activities of *rad52-327* and *rad52-2* mutants with respect to repair of the single, defined, site-specific, double strand break made by the *HO* endonuclease at *MAT*. This site specific break is the first step in the recombination event leading to mating type switching in yeast. The combination of the wild-type *HO* gene with *rad52-1* is lethal (MALONE and ESPOSITO 1980; WEIFFENBACH and HABER 1981). In our assay for survival to HO endonuclease cutting, we plated cells harboring a plasmid with *HO* under the control of the *GAL1* promoter (HERSKOWITZ and JENSEN 1991) on selective plates containing either glucose or galactose. We found that 74% of wild-type cells formed colonies on the galactose plates, while only 0.5% of null mutant cells (*rad52-ΔHS* or *rad52-169*) were able to form colonies on the galactose plates (Table 3). Although the *rad52-327* and *rad52-2* mutants did not form colonies as efficiently as wild-type cells, they did form colonies more frequently than the null strains (9.1 and 6.7%, respectively). Thus, like the partial resistance to MMS exposure, the *rad52-327* and *rad52-2* mutants are partially capable of overcoming the effect of a double strand break at *MAT*.

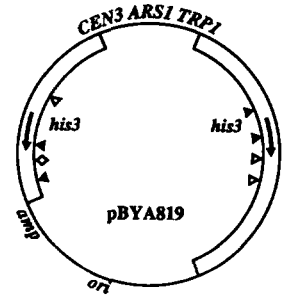


FIGURE 3.—Recombination reporter plasmid pBYA819. Construction of this plasmid and characterization of its recombination in both wild-type and mutant cells have been described elsewhere (AHN and LIVINGSTON 1986; DORNFELD and LIVINGSTON 1992). This plasmid contains *his3* heteroalleles created by insertion of linkers, represented by triangles (open, *Cla*I; closed, *Sac*I) and a diamond (*Sma*I), at two different sites within the *HIS3* coding region and at multiple sites outside the coding region. Plasmid recombination events yield a wild-type *HIS3* allele mostly by gene conversion. Crossing over accompanies some conversion events.

**Both *rad52-2* and *rad52-327* strains are proficient at mitotic plasmid recombination:** *rad52-2* strains are very proficient at spontaneous mitotic recombination, exhibiting rates of recombination of chromosomal markers elevated above the wild-type rate (RESNICK *et al.* 1986; MALONE *et al.* 1988). Since *rad52-327* and *rad52-2* have similar MMS and *HO* endonuclease sensitivities, we tested whether they have a similar effect on mitotic recombination. To measure mitotic recombination, the strains were transformed with a recombination reporter plasmid, pBYA819 (Figure 3), containing *his3* heteroalleles. Previous work from our laboratory (AHN and LIVINGSTON 1986) had shown that this plasmid undergoes recombination, mostly in the form of gene conversion, to yield the wild-type allele. Plasmid recombination occurs 40-fold less frequently in the *rad52-ΔHS* strain (SSL212A) than in the wild-type strain (SSL204) (DORNFELD and LIVINGSTON 1992) and is, therefore, a sensitive assay for mitotic recombination. A comparison of the rate of plasmid recombination (Table 4) shows that the *rad52-ΔHS* deletion-disruption strain and the *rad52-169* truncation strain exhibit a 36- and a 46-fold decrease in the mitotic recombination rate, respectively. In contrast, the *rad52-2* and *rad52-327* strains were recombination proficient, with recombination rates of 46% and 86% of the wild-type rate, respectively. Although we did not observe the hyper-recombination of *rad52-2* previously measured for chromosomal markers in mitosis (RESNICK *et al.* 1986; MALONE *et al.* 1988), we did find that *rad52-327* is like *rad52-2* in retaining recombination functions.

**TABLE 4**

**pBYA819 recombination rates in haploid strains**

| Strain | *RAD52* allele | Rate ($\times 10^{-4}$ events/ cell/generation) |
|---|---|---|
| SSL204A | *RAD52* | 4.3 ± 0.20 |
| SSL212A | *rad52-ΔHS* | 0.12 ± 0.012 |
| SSL242 | *rad52-169* | 0.093 ± 0.007 |
| SSL241 | *rad52-327* | 3.7 ± 0.21 |
| SSL243 | *rad52-2* | 2.0 ± 0.11 |

pBYA819 is depicted in Figure 3. Recombination rates measure the frequency of $His^+$ recombinants.

***rad52-327* and *rad52-2* diploids differ in their amount of chromosome loss:** To further examine recombination and an associated mutant phenotype, chromosome loss, we constructed isogenic, homozygous mutant diploids with three heterozygous markers (*LYS2*, *CYH2* and *ADE6*) along chromosome *VII* on both sides of the centromere. The order of these three markers and their position relative to the centromere is shown in Table 2 for strain SSL260. The heterozygous *CYH2* locus permits selection of $Cyh^r$ cells which arise either by mitotic recombination, *i.e.*, crossing over or gene conversion, or by chromosome loss. In classifying the $Cyh^r$ cells, we used the scheme of MALONE *et al.* (1988) based on the colony color resulting from the heterozygous *ADE6* locus in combination with the homozygous *ade2* locus. $Cyh^r$ colonies which were white were assumed to occur by chromosome loss, whereas red colonies were assumed to occur by recombination. Of the white colonies, those that were $Lys^+$ had an accompanying crossover, while the $Lys^-$ products arose by simple loss. Of the red colonies, those that were $Lys^+$ were classified as resulting from gene conversion and those that were $Lys^-$ were classified as resulting from crossing over in the interval between the centromere and the *CYH2* locus. As shown in Table 5, for all strains the majority of cycloheximide resistant white colonies were $Lys^-$, indicating simple chromosome loss.

The vast majority of $Cyh^r$ colonies arising from the wild-type diploid were red and thus resulted from recombination rather than loss (Table 5). Because at most only one white colony grew on each cycloheximide plate, the rate for chromosome loss given in Table 5 for the wild-type diploid is the value calculated by the method of the median of LEA and COULSON (1949) if one white colony were present on the median plate. Thus, this value is necessarily an overestimate of the true chromosome loss rate.

In contrast, for the null strains, *rad52-1/rad52-169* and *rad52-169/rad52-169*, the majority of $Cyh^r$ colonies were white, and these strains lose a chromosome *VII* homologue more frequently than they recombine the chromosome. Their chromosome loss rates are elevated 67- and 46-fold above the wild-type rate, respectively. Exchange is decreased about 10-fold in both of these null strains. Also, 70% and 63% of events in these strains, respectively, are categorized as gene conversions, as opposed to 5% for the wild-type strain.

Chromosome loss in the *rad52-327/rad52-327* strain is elevated fourfold, which is less than the highly elevated loss rates observed in the null strains. Thus, as seen with other phenotypes, this strain has partial activity for chromosome stability with a phenotype intermediate between the null and wild-type strains. The *rad52-327/rad52-327* strain exhibits an eightfold decrease in recombination. The proportion of conversion-like events increases to 52%. The mutant phenotype with respect to chromosomal recombination is surprising because this allele supports a wild-type rate of plasmid recombination.

The *rad52-2* diploid exhibits elevated chromosome loss, 71-fold greater than wild-type. Unlike the *rad52-327* allele, which exhibits a decrease in mitotic crossing over, the *rad52-2* allele exhibits a 2.6-fold elevation in recombination. Accompanying this increase is an increase in the proportion of events categorized as gene conversions from 5% to 37%. MALONE *et al.* (1988) also observed both the hyperrecombination and the increase in conversion-like events conferred by *rad52-2*. The *rad52-2* mutant's increase in both recombination and chromosome loss is novel in comparison to other mutants which appear to lose chromosomes in response to failed recombination events (MORTIMER, CONTOPOULOU and SCHILD 1981). In addition, the unique behavior of *rad52-2* with respect to chromosome loss and recombination distinguishes it from *rad52-327*.

**A homozygous *rad52-327* diploid sporulates and yields viable spores at a reduced level:** *rad52-1* and *rad52-2* diploids have difficulty in completing sporulation and invariably yield inviable spores (GAME and MORTIMER 1974; STRIKE 1978; GAME *et al.* 1980; PRAKASH *et al.* 1980; RESNICK *et al.* 1986). The inability to sporulate may be the consequence of failed recombination events which leave the chromosomes unable to disjoin properly. Considering that *rad52-327* and *rad52-2* mutants carry out certain mitotic recombination events, homozygous diploids carrying these alleles might be capable of meiotic crossing over, disjunction and viable spore formation. To test this prediction, diploids homozygous for mutant and wild-type alleles were sporulated (Table 6). Unlike all other homozygous mutant diploids including *rad52-2* which fail to yield four spore asci at a level greater than 1.5%, the *rad52-327* diploid strain sporulates and yields viable spores. Neither the proficiency of sporulation (16%) nor the viability of spores (14%) are those of the wild-type diploid (53% and 88%, respectively); indeed, they are significantly below wild-type

**TABLE 5**

**Chromosome *VII* loss and recombination rates**

| Strain | *RAD52* alleles | Loss rate (×10$^{-6}$ events/cell/generation)[a] | Fraction simple loss (Lys$^-$)[b] | Recombination rate (×10$^{-6}$ events/cell/generation)[a] | Fraction gene conversion (Lys$^+$)[c] |
|---|---|---|---|---|---|
| SSL260 | *RAD52/RAD52* | <0.12 (1.0) | 0.6 (4/7) | 28 ± 1.5 (1.0) | 0.05 (5/100) |
| SSL269 | *RAD52/rad52-327* | 0.12 ± 0.01 (1.0) | 1.0 (4/4) | 10 ± 0.62 (0.36) | 0.15 (15/100) |
| SSL265 | *rad52-2/rad52-327* | 0.12 ± 0.01 (1.0) | 1.0 (12/12) | 15 ± 0.88 (0.54) | 0.28 (28/100) |
| SSL261 | *rad52-327/rad52-327* | 0.51 ± 0.05 (4.2) | 0.84 (42/50) | 3.6 ± 0.29 (0.13) | 0.52 (52/100) |
| SSL263 | *rad52-2/rad52-2* | 8.6 ± 0.57 (71) | 0.75 (33/44) | 72 ± 3.9 (2.6) | 0.37 (37/100) |
| SSL266 | *rad52-1/rad52-327* | 2.0 ± 0.14 (15) | 0.96 (24/25) | 7.9 ± 0.49 (0.20) | 0.38 (19/50) |
| SSL268 | *rad52-1/rad52-169* | 8.7 ± 0.59 (67) | 1.0 (25/25) | 3.7 ± 0.28 (0.095) | 0.70 (35/50) |
| SSL262 | *rad52-169/rad52-169* | 5.6 ± 0.38 (46) | 1.0 (50/50) | 2.7 ± 0.20 (0.096) | 0.63 (59/93) |

[a] Value normalized to the *RAD52/RAD52* rate is shown in parentheses.
[b] Ratio of Lys$^-$ colonies to total colonies is shown in parentheses.
[c] Ratio of Lys$^+$ colonies to total colonies is shown in parentheses.

**TABLE 6**

**Four-spore ascus formation and spore viability**

| Strain | *RAD52* alleles | %Four-spore asci[a] | Spore viability (%)[b] |
|---|---|---|---|
| SSL260 | *RAD52/RAD52* | 53 (5) | 88 (112/128) |
| SSL265 | *rad52-2/rad52-327* | 50 (5)[c] | 86 (55/64)[c] |
| SSL266 | *rad52-1/rad52-327* | 44 (2)[d] | 50 (30/60)[d] |
| SSL261 | *rad52-327/rad52-327* | 16 (4)[d] | 14 (23/168)[d] |
| SSL262 | *rad52-169/rad52-169* | 0.6 (3) | ND |
| SSL263 | *rad52-2/rad52-2* | 1.4 (4) | ND |
| SSL267 | *rad52-1/rad52-2* | 0.6 (2) | ND |
| SSL268 | *rad52-1/rad52-169* | 1.4 (2) | ND |

[a] Number of independent sporulations in which four-spore asci were counted is given in parentheses.
[b] Ratio of viable spores to total spores dissected is given in parentheses. ND, not determined.
[c] These values are not significantly different from the wild-type values ($\chi^2 < 1$).
[d] These values are significantly different from the wild-type values ($P < 0.025$).

**TABLE 7**

**Crossing over between heterozygous markers on chromosome *VII***

| Strain | *RAD52* alleles | Percent of spores with recombinant linkage[a] at interval on chromosome *VII*: *LYS5-CYH2* | *CYH2-ADE6* |
|---|---|---|---|
| SSL260 | *RAD52/RAD52* | 37% (40/109) | 60% (65/109) |
| SSL261 | *rad52-327/rad52-327* | 35% (8/23)[b] | 48% (11/23)[b] |
| SSL265 | *rad52-2/rad52-327* | 27% (15/55)[b] | 44% (24/55)[b] |
| SSL266 | *rad52-1/rad52-327* | 40% (12/30)[b] | 50% (15/30)[b] |

[a] Ratio of recombinants to total spores is shown in parentheses.
[b] These values are not significantly different from the wild-type values ($\chi^2 < 1$).

levels. Nevertheless, the results show that this allele confers a low level of function in meiosis. These results also distinguish this allele from *rad52-2* which fails to sporulate to any great extent.

***rad52-327* strains are proficient in meiotic crossing over:** Having viable spores afforded the opportunity to measure whether *rad52-327* can carry out meiotic crossing over between the heterozygous markers on chromosome VII. Because no *rad52-327* tetrads with four viable spores were recovered, map distances based on asci types could not be made. Rather, the data in Table 7 reports the number of spores in which the linkage of heterozygous makers on chromosome *VII* was recombinant. Although spore formation and spore viability are reduced in the *rad52-327* strain, the meiotic crossover frequencies for the two intervals on chromosome *VII* are not statistically different from those of the wild-type strain. The *rad52-327* allele is therefore proficient at both mitotic plasmid recombination and meiotic chromosomal recombination.

***rad52-327* exhibits intragenic complementation with *rad52-1* and *rad52-2*:** The first indication of an interaction between the truncation product encoded by *rad52-327* and the products of the *rad52-1* and *rad52-2* alleles came from the experiments in which *rad52-327* and *rad52-2* were overexpressed from the *ENO1* promoter on high copy plasmids. In addition to being transformed into mutants bearing the same allele, these expression plasmids were transformed into strains with different *rad52* mutations. As shown in Figure 4a, overexpression of *rad52-327* in the *rad52-2* strain increases its MMS resistance to the wild-type level. However, overexpression of *rad52-2* in the *rad52-327* strain does not affect the MMS resistance. Thus, the *rad52-2* and *rad52-327* alleles show intragenic complementation, but the complementation is dosage dependent. Overexpression of *rad52-327* in the *rad52-1* strain also results in an increase in MMS resistance above the level observed when the *rad52-327* allele is overexpressed in its own background (Figure 4b). The intragenic complementation is partial in comparison to the complete restoration of wild-type resistance displayed by the transformed *rad52-2* strain. The specificity of the intragenic complementation is shown by overexpression of the smaller truncation made from *rad52-169*. This truncation, which also covers the single amino acid substitutions in

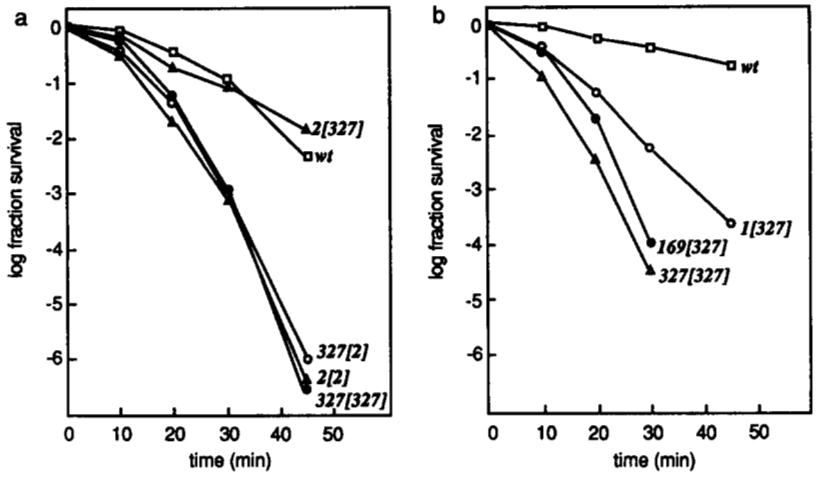


FIGURE 4.—Survival in 0.5% MMS of haploid strains with our without a plasmid bearing a *rad52* allele under control of the *ENO1* promoter. The numbers identifying the lines represent the *RAD52* allele present in the strains. The numbers in brackets are the *RAD52* alleles expressed from the plasmid borne *ENO1* promoter. (a) □, SSL204A (*RAD52*) without a plasmid; ▲, SSL243 (*rad52-2*) with p*ENO1::rad52-2*; ○, SSL241 (*rad52-327*) with p*ENO1::rad52-2*; △, SSL243 (*rad52-2*) with p*ENO1::rad52-327*; ●, SSL241 (*rad52-327*) with p*ENO1::rad52-327*. (b) All strains except SSL204A harbor p*ENO1::rad52-327*. □, SSL204A (*RAD52*); ○, SSL209 (*rad52-1*); ●, SSL242 (*rad52-169*); △, SSL241 (*rad52-327*).

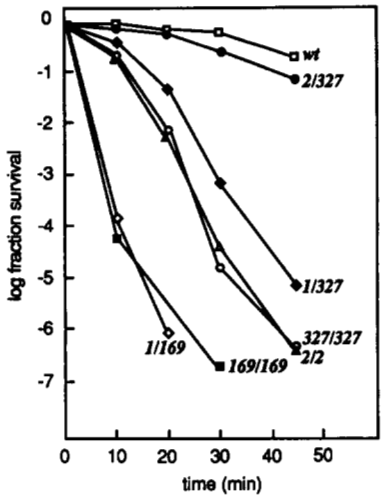


FIGURE 5.—Survival of diploid strains in 0.5% MMS. The numbers identifying the lines represent the *RAD52* alleles present in the diploids. □, SSL260 *RAD52/RAD52*; ●, SSL265, *rad52-2/rad52-327*; ♦, SSL266, *rad52-1/rad52-327*; ○, SSL261, *rad52-327/rad52-327*; △, SSL263, *rad52-2/rad52-2*; ■, SSL262, *rad52-169/rad52-169*; ◇, SSL268, *rad52-1/rad52-169*.

*rad52-1* and *rad52-2* (see below), does not complement either of the mutations (data not shown).

To further explore the intragenic complementation, we constructed diploids heterozygous for the mutant alleles and tested their resistance to MMS exposure. The *rad52-2/rad52-327* heterozygote is significantly more resistant to MMS than either of the two homozygous diploids (Figure 5). The *rad52-1/rad52-327* heterozygote shows a slight increase in MMS resistance over the *rad52-327* homozygous diploid, but, as in the case of the plasmid overexpression (Figure 4b), the intragenic complementation is not as dramatic as in the *rad52-2/rad52-327* diploid.

The heteroallelic diploids were also tested for their ability to sporulate. The *rad52-2/rad52-327* heterozygote sporulates as well as the wild-type strain and significantly above the level of either homozygous diploid (Table 6). In addition, the spore viability (Table 6) and crossing over frequencies (Table 7) are at wild-type levels. The *rad52-1/rad52-327* heterozygote yields an increased, but not wild-type, level of spore formation and spore viability, again showing the less dramatic complementation seen for MMS resistance. The rate of meiotic crossing over (Table 7) is at the wild-type level.

Chromosome loss and recombination were also examined in these strains utilizing the chromosome VII markers described above. As shown in Table 5, the *rad52-2/rad52-327* diploid again shows strong intragenic complementation having two characteristics of the wild-type strain, a high rate of recombination and a low rate of chromosome loss. Interestingly, the *rad52-2/rad52-327* strain, which exhibits a twofold reduction in mitotic recombination relative to wild-type, has neither the 2.6-fold elevation in mitotic recombination of the *rad52-2/rad52-2* strain nor the eightfold decrease in mitotic recombination of the *rad52-327/rad52-327* strain. These alleles thus have a phenotype for mitotic recombination which is intermediate between the individual phenotypes. The *rad52-1/rad52-327* diploid again shows a lesser degree of complementation with a 15-fold increase in loss and a fivefold decrease in recombination.

**Identification of the *rad52-2* mutation:** Because of the similarities between the mitotic phenotypes of *rad52-2* and *rad52-327* mutants, we were interested in learning whether the lesion in *rad52-2* is similar to the truncation-causing nonsense mutation of the *rad52-327* allele. Consequently, the *rad52-2* allele was cloned by PCR independently from two *rad52-2* strains, RM137–10D and RM86–13A (Table 2). Comparing the sequence of this DNA to the wild-type sequence (ADZUMA, OGAWA and OGAWA 1984) revealed a C to T transition at position 1158, changing proline 64 to leucine (data not shown). This mutation also destroys an *Ava*II site at position 1155, resulting in a restriction fragment length polymorphism. By Southern analysis, DNA from a *RAD52* strain contains this site, while DNA from both *rad52-2* strains does not (data not shown).

To confirm that this was the causative mutation in the *rad52-2* allele, we replaced the chromosomal *Bst*EII-*Bgl*II fragment (nt 1110 to 1470) from wild-type and *rad52-2* mutant strains with the corresponding fragment from the other strain. The *rad52-2* fragment confers MMS sensitivity to the wild-type strain, and the *RAD52* fragment confers MMS resistance to the *rad52-2* strain (data not shown). Because *rad52-2* supports mitotic recombination, the wild-type strain receiving the 360-bp *Bst*EII-*Bgl*II fragment from *rad52-2* was transformed with the recombination reporter plasmid pBYA819 and found to carry out plasmid recombination at a rate comparable to the rate determined for the authentic mutant (data not shown). Thus, this single base change results in the MMS sensitivity and recombination proficiency characteristic of the *rad52-2* strain. The *rad52-2* mutation is therefore a missense mutation which is located near the site of the *rad52-1* mutation, another missense mutation which changes alanine 90 to valine (ADZUMA, OGAWA and OGAWA 1984).

## DISCUSSION

**Mutant phenotypes:** In eliminating the C-terminal 35% of the *RAD52* gene product, the *rad52-327* allele changes a 56-kD protein with a calculated isoelectric point near nine into one of 37 kD with an isoelectric point near five. This truncated gene product is partially active in repair of MMS lesions, in recovery from *HO* endonuclease cleavage at *MAT*, in mitotic chromosomal crossing over and in sporulation. The truncated product is substantially active in mitotic plasmid recombination and meiotic crossing over. The *rad52-2* allele is similar to *rad52-327* for many of the mitotic phenotypes measured in this study. It differs from *rad52-327* in that it is hyperrecombinational for chromosomal mitotic recombination and does not support the low level of sporulation found in the truncation strain.

Mutations of *RAD52* fall into at least two classes. Members of one class, which include the *rad52-1* and *rad52-169* alleles, behave like a deletion in that they are deficient in MMS damage repair, mitotic recombination and sporulation. They belong to the null class. Members of the second class, which includes the *rad52-2* and *rad52-327* alleles, maintain some activities while losing others. For example, these two alleles both maintain a low level of MMS damage repair and considerable ability to undergo mitotic plasmid recombination. In their study of *rad52-2*, MALONE *et al.* (1988) were the first to recognize this second class. Their results are slightly different from ours in that they did not observe the low level of MMS resistance which we found. The differences between the two studies might result from strain differences or from differences in experimental protocols such as the extended time period over which we sampled.

There are two explanations for the maintained activities of this second class. (1) These alleles are leaky for *RAD52* function. They express a minimally active mutant protein which has sufficient activity to repair a small percentage of DNA lesions caused by MMS and respond more fully to the relatively infrequent demand for mitotic recombination. (2) The Rad52 protein contains separable activities, one important for DNA repair and another important for recombination.

MALONE *et al.* (1988) also addressed this issue in their study of *rad52-2*. They noted that a simple explanation for the retained activities is that *rad52-2* is a "leaky" mutation. However, they stated that the elevated recombination of chromosomal markers was more consistent with the explanation that "the protein is specifically altered in a way that increases mitotic recombination." We concur that the recombinational proficiency of *rad52-2* and *rad52-327* mutants more likely results from an allele-specific retention of function rather than from leaky mutations. Our concurrence is based on finding that the MMS resistance of mutant strains carrying each of these two alleles was not increased by overexpression of the appropriate allele. Overexpression of leaky alleles would be expected to increase the resistance. Additional support for the hypothesis of multiple activity domains comes from characterization of a class of *rad52* alleles which are temperature sensitive for repair of MMS lesions but competent in mitotic recombination at both permissive and restrictive temperatures (M. D. KAYTOR and D. M. LIVINGSTON, unpublished observation).

**Correlation of mutation types and mitotic phenotypes:** No simple correlation emerges from this study regarding the relationships between the locations or types of the mutations and the resulting phenotypes. Despite the fact that *rad52-1* and *rad52-2* are both missense mutations near the N terminus, they have different effects on repair of lesions caused by the *HO* endonuclease and MMS and on mitotic recombination. By contrast, the *rad52-327* allele, which eliminates the C-terminal 35% of Rad52p, results in similar MMS resistance, *HO* endonuclease survival, and plasmid recombination rates to those of *rad52-2*.

**Correlation of mitotic and meiotic phenotypes:** The difference between the *rad52-327* diploid, which sporulates weakly and undergoes meiotic crossing over, and the *rad52-2* diploid, which is incapable of sporulation, raises questions concerning the role of *RAD52* in meiosis. Mutants harboring each allele have varying degrees of proficiency in tests of mitotic recombination. If mitotic recombination function were a predictor of meiotic function, both might be expected to sporulate at or near the wild-type level. We consider two explanations for this lack of correlation.

First, although both mutants have certain proficiencies in mitotic recombination, neither behaves exactly like the wild type. For example, the *rad52-2* mutant is hyperrecombinational for mitotic chromosomal recombination and exhibits an "altered spectrum" in the location of exchange events (MALONE *et al.* 1988). The *rad52-327* allele supports wild-type levels of plasmid recombination but is deficient for chromosomal recombination. Thus, neither allele may be able to support the quantity or quality of recombination necessary for successful meiosis.

Second, as suggested above, *RAD52* plays multiple roles in repair, recombination and sporulation, and tasks such as sporulation require more of *RAD52*'s activities than do other functions such as mitotic plasmid recombination. This explanation may account for the seemingly wild-type level of crossing over in the few viable spores emerging from the *rad52-327* diploid. Such cells may indeed be capable of meiotic crossing over but fail to carry out fully a second *RAD52* mediated meiotic event. One such event could be meiotic gene conversion which has been suggested to be necessary for chromosome synapsis and to be mechanistically separable from meiotic crossing over (ENGEBRECHT, HIRSCH and ROEDER 1990). One caveat to the "multiple role" conclusion is that its premise–*rad52-327* supports a wild-type level of crossing over–could be faulty. If viable spores survive because they undergo a wild-type level of crossing over and inviable spores die because they carry out much less crossing over, then crossing over is the limiting process and no other activity need be postulated.

We note that separable activities could occur at two levels. The more obvious of the two is that *RAD52* might carry out two or more enzymatic functions. An alternate possibility is that the separable functions occur at the level of substrate recognition. For example, *RAD52* could have only one enzymatic role to play but must recognize different forms of interrupted DNA molecules in order to play that role. Thus, different DNA lesions might be encountered during the various types of mitotic and meiotic recombination, and only a gene product able to recognize all such substrates would be capable of the most demanding task of sporulation. This hypothesis would also account for the *rad52-2* mutant's novel hyperrecombination phenotype coupled with its propensity to lose chromosomes. The mutant protein might take otherwise benign DNA lesions and transform them into ones which lead to chromosome loss or recombination.

Curiously, proficiency in repairing MMS lesions is a better predictor of sporulation function than is mitotic recombination. Comparison of the results on diploid MMS sensitivity (Figure 5) and sporulation (Table 6) indicates that the higher the level of MMS resistance, the greater the percentage of four-spore ascus formation and spore viability. For example, the MMS resistance of the *rad52-1/rad52-327* diploid is slightly greater than the resistance of the *rad52-327/rad52-327* diploid, and the sporulation and spore viability are also improved. However, both MMS resistance and spore viability of the *rad52-1/rad52-327* diploid are lower than the wild-type levels. In turn, the *rad52-2/rad52-327* diploid has wild-type levels both of MMS resistance and of spore formation and viability. The only strain which does not conform to this correlation is the *rad52-2/rad52-2* diploid, which exhibits the same partial MMS resistance as the *rad52-327/rad52-327* diploid but has virtually no sporulation ability.

**Intragenic complementation:** Although neither the *rad52-2* nor the *rad52-327* mutation confer a high level of MMS resistance when they are the only allele present, together they confer a wild-type level of resistance. This dramatic example of intragenic complementation was found both in a haploid *rad52-2* mutant by overexpression of *rad52-327* and in a heteroallelic diploid. In addition to the MMS resistance, the heteroallelic diploid also has a wild-type level of chromosome stability, sporulation and spore viability, and is proficient in recombination of chromosomal markers. A *rad52-1/rad52-327* diploid exhibits a lesser degree of intragenic complementation with respect to MMS resistance and sporulation.

Other examples of mutant protein pairs that restore activity by intragenic complementation fall into two general classes (reviewed by ZABIN and VILLAREJO 1975): (1) mutant proteins that have maintained different activities, and (2) mutant proteins that complement by promoting correct folding of each other. An example of the first class is exhibited by anthranilate synthetase (reviewed in ZALKIN 1973). Component II of anthranilate synthetase contains two separate enzymatic activities: glutamine amido-transferase and anthranilate-5-phosphoribosylpyrophosphate phosphoribosyl transferase. The first activity can be catalyzed by the N-terminal third of the protein (ITO and YANOFSKY 1969; YANOFSKY *et al.* 1971), while the C-terminal two-thirds will catalyze the second reaction (JACKSON and YANOFSKY 1974). An example of the second class is exhibited by glutamic dehydrogenase (GDH). Several mutant GDH proteins have been purified (FINCHAM 1966). When two different inactive mutant polypeptides, $am^{19}$ and $am^{1}$, are combined *in vitro*, the enzymatic activity is recovered (CODDINGTON and FINCHAM 1965). Also, the abnormal electrophoretic mobility of $am^{19}$, which is dependent on the net surface charge, is restored (SUNDARAM and FINCHAM 1964). The folding of one mutant subunit is therefore "healed" by another mutant subunit.

Although all of our observations on intragenic com-

plementation between *rad52* alleles can be made consistent with both models, application of Ockham's razor gives the edge to the folding model. First, there is dosage dependence in the complementation within haploid strains. When *rad52-327* was overexpressed in a *rad52-2* strain, the MMS resistance was greatly increased, but when *rad52-2* was overexpressed in a *rad52-327* strain, the MMS resistance was unchanged (Figure 4a). A similar dosage dependence has been observed in the GDH system. In that system the two inactive GDH proteins complement *in vitro* when the $am^1$ form is in excess, but not when $am^{19}$ is in excess (CODDINGTON and FINCHAM 1966). Thus, the dosage dependence supports a model in which Rad52p has a homotypic quaternary structure in which mutant proteins associate to yield a wild-type structure. Second, quaternary interactions between the polypeptide encoded by *rad52-327* and those encoded by the two missense mutations, *rad52-2* and *rad52-1*, could be hypothesized to compensate for the amino acid alterations of the two missense alleles because the wild-type sequence of the truncation includes the sites altered in the other two alleles. This effect is allele specific because the shorter polypeptide encoded by *rad52-169* might be expected to do the same but does not do so. Third, the location of mutational changes in *RAD52* do not make a separate domain model easy to see. The *rad52-1* allele, which alters a single amino acid of Rad52p, eliminates all repair and recombination functions yet its causative mutation is located close to the alteration in *rad52-2*. If the repair and recombination functions reside in specific regions of the protein, these regions must overlap such that a single amino acid change affects both functions. Also, *rad52-2* and *rad52-1* do not complement (data not shown) as might be expected if the proteins had complementary activities.

While the intragenic complementation of *rad52* alleles is best explained by multimer formation, the phenotypes of the individual *rad52* alleles is most easily explained by multiple activities. These two possibilities are not mutually exclusive because Rad52p may be a multifunctional protein that also acts as a multimer. In addition other factors, such as the interaction of Rad52p with other proteins, may affect the activity of mutant forms. For example, recent evidence suggests that Rad52p interacts with Rad51p (SHINOHARA, OGAWA and OGAWA 1992). Such heterotypic interactions could be responsible for the phenotypes which we have measured.

**Recapitulation:** We set out to understand why the original cloning of *RAD52* by SCHILD *et al.* (1983) and ADZUMA, OGAWA and OGAWA (1984) was successful. Each reported that clones terminating at the internal *Bam*HI site restored either MMS or X-ray resistance to a *rad52-1* strain. We now know that the truncated product confers a low level of MMS resistance. Although the truncated protein provides greater resistance than the *rad52-1* gene product, this plasmid-encoded *RAD52* fragment alone probably could not have provided enough MMS resistance for the cells to survive on agar plates containing MMS during cloning. Fortunately, intragenic complementation of the plasmid-encoded protein fragment with the chromosomal *rad52-1* product provided enough MMS resistance for the cell to survive the MMS selection.

We thank DARIN O'BRIEN for cloning the *rad52-2* allele by PCR and the laboratory of PAUL SILICIANO for their DNA sequencing expertise. We also thank ROBERT ROON and MICHAEL KAYTOR for reading the manuscript. We are appreciative to LELAND HARTWELL and ROBERT MALONE for providing yeast strains. This work was supported by American Cancer Society grant MV-373 and by funds from the Graduate School of the University of Minnesota.

## 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.

AHN, B.-Y., and D. M. LIVINGSTON, 1986 Mitotic gene conversion lengths, coconversion patterns, and the incidence of reciprocal recombination in a *Saccharomyces cerevisiae* plasmid system. Mol. Cell. Biol. **6:** 3685–3693.

CODDINGTON, A., and J. R. S. FINCHAM, 1965 Proof of hybrid enzyme formation in a case on inter-allelic complementation in *Neurospora crassa*. J. Mol. Biol. **12:** 152–161.

CODDINGTON, A., and J. R. S. FINCHAM, 1966 Multiple active varieties of *Neurospora* glutamate dehydrogenase formed by hybridization between two inactive mutant proteins *in vivo* and *in vitro*. J. Mol. Biol. **17:** 503–512.

DORNFELD, K. J., and D. M. LIVINGSTON, 1991 Effects of controlled *RAD52* expression on repair and recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:** 2013–2017.

DORNFELD, K. J., and D. M. LIVINGSTON, 1992 Plasmid recombination in a *rad52* mutant of *Saccharomyces cerevisiae*. Genetics **131:** 261–276.

ENGEBRECHT, J., J. HIRSCH and G. S. ROEDER, 1990 Meiotic gene conversion and crossing over: their relationship to each other and to chromosome synapsis and segregation. Cell **62:** 927–937.

FINCHAM, J. R. S., 1966 *Genetic Complementation*. Benjamin, New York.

GAME, J. C., and R. K. MORTIMER, 1974 A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. **24:** 281–292.

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

HERSKOWITZ, I., and R. E. JENSEN, 1991 Putting the *HO* gene to work: Practical uses for mating-type switching. Methods Enzymol. **194:** 132–145.

HOEKSTRA, M. F., T. NAUGHTON and R. E. MALONE, 1986 Properties of spontaneous mitotic recombination occurring in the presence of the *rad52–1* mutation of *Saccharomyces cerevisiae*. Genet. Res. **48:** 9–17.

ITO, J., and C. YANOFSKY, 1969 Anthranilate synthetase, an enzyme specified by the tryptophan operon of *Escherichia coli*: comparative studies on the complex of subunits. J. Bacteriol. **97:** 734–742.

JACKSON, E. N., and C. YANOFSKY, 1974 Localization of two functions of the phosphoribosyl anthranilate transferase of *Escherichia coli* to distinct regions of the polypeptide chain. J. Bacteriol. **117:** 502–508.

LEA, D. E., and C. A. COULSON, 1949 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–285.

LURIA, S. E., and M. DELBRUCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MALONE, R. E., 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. E., B. A. MONTELONE, C. EDWARDS, K. CARNEY and M. F. HOEKSTRA, 1988 A reexamination of the role of the *RAD52* gene in spontaneous mitotic recombination. Curr. Genet. **14:** 211–223.

MEEKS-WAGNER, D., and L. HARTWELL, 1986 Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44:** 43–52.

MORTIMER, R. K., R. CONTOPOULOU and D. SCHILD, 1981 Mitotic chromosome loss in a radiation-sensitive strain of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **78:** 5778–5782.

PRAKASH, S., and L. PRAKASH, 1977 Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. Genetics **87:** 33–55.

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., J. NITISS, C. EDWARDS and R. E. MALONE, 1986 Meiosis can induce recombination in *RAD52* mutants of *Saccharomyces cerevisiae*. Genetics **113:** 531–550.

SAIKI, R. K., D. H. GELFAND, S. STOFFEL, S. J. SCHARF, R. HIGUCHI, G. T. HORN, K. B. MULLIS and H. A. ERLICH, 1988 Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science **239:** 487–491.

SCHERER, S., and R. W. DAVIS, 1979 Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. Proc. Natl. Acad. Sci. USA **76:** 4951–4955.

SCHILD, D., B. KONFORTI, C. PEREZ, W. GISH and R. MORTIMER, 1983 Isolation and characterization of yeast DNA repair genes: I. Cloning of the *RAD52* gene. Curr. Genet. **7:** 85–92.

SHINOHARA, A., H. OGAWA and T. OGAWA, 1992 Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein. Cell **69:** 457–470.

STRIKE, T. L., 1978 Characterization of mutants of yeast sensitive to X-rays. Thesis, University of California, Davis.

SUNDARAM, T. K., and J. R. S. FINCHAM, 1964 A mutant enzyme in *Neurospora crassa* interconvertible between electrophoretically distinct active and inactive forms. J. Mol. Biol. **10:** 423–437.

WEIFFENBACH, B., and J. E. HABER, 1981 Homothallic mating type switching generates lethal chromosome breaks in *rad52* strains of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1:** 522–534.

YANOFSKY, C., V. HORN, M. BONNER and S. STASIOWSKI, 1971 Polarity and enzyme functions in mutants of the first three genes of the tryptophan operon of *Escherichia coli*. Genetics **69:** 409–433.

ZABIN, I., and M. R. VILLAREJO, 1975 Protein complementation. Annu. Rev. Biochem. **44:** 295–313.

ZALKIN, H., 1973 Anthranilate synthetase. Adv. Enzymol. **38:** 1–39.

Communicating editor: G. R. SMITH