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Suppression of a New Allele of the Yeast *RAD52* Gene by Overexpression of *RAD51*, Mutations in *srs2* and *ccr4*, or Mating-Type Heterozygosity

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ABSTRACT

The *RAD52* gene of *Saccharomyces cerevisiae* is involved both in the recombinational repair of DNA damage and in mitotic and meiotic recombination. A new allele of *rad52* has been isolated that has unusual properties. Unlike other alleles of *rad52*, this allele (*rad52-20*) is partially suppressed by an *srs2* deletion; *srs2* mutations normally act to suppress only *rad6* and *rad18* mutations. In addition, although haploid *rad52-20* strains are very X-ray sensitive, diploids homozygous for this allele are only slightly X-ray sensitive and undergo normal meiosis and meiotic recombination. Because *rad52-20* diploids homozygous for mating type are very X-ray sensitive, mating-type heterozygosity is acting to suppress *rad52-20*. Mating-type heterozygosity suppresses this allele even in haploids, because *sir* mutations, which result in expression of the normally silent mating-type cassettes, were identified among the extragenic revertants of *rad52-20*. A new allele of *srs2* and alleles of the transcriptional regulatory genes *ccr4* and *caf1* were among the other extragenic revertants of *rad52-20*. Because other researchers have shown that the RAD51 and RAD52 proteins interact, *RAD51* on a high copy number plasmid was tested and found to suppress the *rad52-20* allele, but *RAD54, 55* and *57* did not suppress. The *RAD51* plasmid did not suppress *rad52-1*. The *rad52-20* allele may encode a protein that has low affinity binding to the RAD51 protein. To test whether the selected revertants suppressed *rad52-20* by elevating the expression of *RAD51*, an integrated *RAD51-lacZ* fusion was genetically crossed into each revertant. Because none of the revertants increased the level of *RAD51-lacZ*, the revertants must exert their effect by one or more mechanisms that are not mediated by *RAD51*.

THERE is considerable evidence that in the yeast *Saccharomyces cerevisiae*, DNA double-strand breaks are repaired by a recombinational mechanism mediated by the genes in the *RAD50* group (*RAD50–57*), most of which are also involved in meiotic recombination (reviewed in PETES *et al.* 1991; GAME 1993). X-ray sensitivity conferred by mutations in these genes is attributable to defects in the repair of X-ray-induced DNA double-strand breaks (HO 1975; RESNICK and MARTIN 1976). Rapid progress is currently being made in understanding the enzymatic roles of several of the genes in this repair pathway. The RAD51 protein shares homology with the bacterial RecA protein (ABOUSSEKHRA *et al.* 1992; BASILE *et al.* 1992; SHINOHARA *et al.* 1992) and has been shown to bind to double-stranded DNA, forming a helical filament structurally similar to that formed by RecA (OGAWA *et al.* 1993). The sequence of the *RAD54* gene reveals homology to a new family of genes from various organisms, all of which may encode DNA helicases (EMERY *et al.* 1991; SCHILD *et al.* 1992). The RAD57 protein contains a potential nucleotide-binding domain, with homology to that of RAD51 (KANS and MORTIMER 1991). The RAD50 protein contains a purine nucleotide-binding domain, and the isolated protein has ATP-dependent DNA binding activity (RAYMOND and KLECKNER 1993). Perhaps the most extensively studied gene in the *RAD50* group is the *RAD52* gene. However, we still do not have a good understanding of its enzymatic role in recombination and recombinational repair.

The *RAD52* gene has been shown by many investigators to be involved both in DNA repair and in mitotic and meiotic recombination (reviewed by GAME 1993). Mutations in this gene cause extreme X-ray sensitivity (RESNICK 1969), the inability to repair double-strand DNA breaks (HO 1975; RESNICK and MARTIN 1976), decreased spontaneous and induced mitotic recombination (PRAKASH *et al.* 1980), greatly reduced sporulation and spore viability (RESNICK 1969; GAME *et al.* 1980; PRAKASH *et al.* 1980), inability to undergo homothallic interconversion of the mating-type locus (MALONE and ESPOSITO 1980), and increased chromosome loss (MORTIMER *et al.* 1981). This gene was cloned by complementation of the *rad52-1* mutation (SCHILD *et al.* 1983a; ADZUMA *et al.* 1984). The sequence of *RAD52* has been determined (ADZUMA *et al.* 1984) but has yielded little information about the potential enzymatic role played by the encoded protein. Regulation studies on *RAD52*, using *lacZ* fusions, have shown that it is not transcriptionally induced by DNA damage (COLE *et al.* 1987) but is by entry of cells into meiosis (COLE *et al.* 1989). The *ENO1* and *GAL1* promoters have been used

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to study the effect of overexpression and controlled expression of *RAD52* in yeast (DORNFELD and LIVINGSTON 1991). That study revealed that overexpression of *RAD52* does not increase the spontaneous mitotic recombination rate or methyl methanesulfonate (MMS) resistance of wild-type cells and that the RAD52 protein activity has a short half-life in cells. Recently, the RAD51 and RAD52 proteins have been shown to interact (SHINOHARA *et al.* 1992; MILNE and WEAVER 1993; DONOVAN *et al.* 1994; C. BENDIXEN and R. ROTHSTEIN, personal communication) (see DISCUSSION).

Most of the studies on *RAD52* have used the *rad52-1* allele (RESNICK 1969), which has recently been shown to have virtually the same phenotype as a *rad52* deletion (BOUNDY-MILLS and LIVINGSTON 1993) or a *rad52* disruption (D. SCHILD, unpublished result). Additional information about *RAD52* has come from studying the *rad52-2* allele and some molecularly constructed alleles. Unlike *rad52-1*, the *rad52-2* allele does not decrease spontaneous mitotic recombination but rather increases it (RESNICK *et al.* 1986; MALONE *et al.* 1988). The meiotic phenotype of *rad52-2*, on the other hand, is similar to that of *rad52-1* or a *rad52* disruption (RESNICK *et al.* 1986). During the original cloning and subsequent subcloning of *RAD52*, an incomplete subclone containing only the N-terminal two-thirds of this gene was shown to complement *rad52-1* (SCHILD *et al.* 1983a; ADZUMA *et al.* 1984). This truncated allele has been introduced into the yeast genome in place of the *RAD52* gene (BOUNDY-MILLS and LIVINGSTON 1993). Analysis of this allele revealed that it has mitotic recombination and repair properties similar to the *rad52-2* allele but has less of an effect on meiosis. This mitotic difference between *rad52-1* and both *rad52-2* and the truncated allele may be partially or fully explained by leakiness of these latter two alleles, which are less sensitive to both X rays and the radiomimetic drug MMS (MALONE *et al.* 1988; BOUNDY-MILLS and LIVINGSTON 1993; and results presented here). BOUNDY-MILLS and LIVINGSTON (1993) have overexpressed *rad52-2* and the truncated allele in strains with the same allele in the chromosome, and have not found increased MMS resistance. They therefore argue against leakiness as an explanation but instead suggest multiple activity domains, one or more of which are retained in *rad52-2* and the truncated allele. Many new temperature-sensitive and partially defective *rad52* alleles have recently been isolated and characterized (KAYTOR and LIVINGSTON 1994).

This article reports on a novel allele of *rad52*, called *rad52-20*, with properties substantially different from those of previously described alleles. The new allele has a quite different effect in diploids and is suppressed by mutations in various genes and by overexpression of *RAD51*. Results presented here suggest that this mutation may affect the interaction of the RAD52 protein with the RAD51 protein (see DISCUSSION).

MATERIALS AND METHODS

Yeast strains: The major strains of *S. cerevisiae* used in this study are listed in Table 1. All other strains were derived from the listed strains, except that strains with multiple centromere markers and *sec59* strains were obtained from the Yeast Genetic Stock Center (Berkeley, CA). Diploid strain XS1241-1C-$\alpha\alpha$ was made by transforming spheroplasted haploid strain XS1241-1C with a *HIS3* containing replicating plasmid and screening the His$^+$ transformants for ploidy. During transformation of spheroplasts, a certain fraction of the transformants are diploid or have higher ploidy. The appearance of small canavanine-resistant colonies was used to screen for nonhaploid transformants; haploids give rise to canavanine-resistant colonies but diploids give few if any such colonies, because resistance is recessive. One apparent nonhaploid transformant was found and was determined to be a diploid by analyzing crosses to a known haploid and a known diploid (data not shown). A derivative of this strain that had lost the plasmid was isolated and used in subsequent experiments. More traditional methods of constructing a diploid homozygous for mating type were not used because of the propensity of *rad52* diploids to lose chromosomes (MORTIMER *et al.* 1981).

Plasmids and isolation of gene deletions: The following yeast replicating plasmids were used in this study: pJR909 (*SIR1* in pRS316), pJR69 (*SIR2* in YCp50), pJR273 (*SIR3* in SEYC58), pJR368 (*SIR4* in YCp50), pJR156 (*MAT***a** *Hin*dIII fragment in YCp50), pJR157 (*MATα* *Hin*dIII fragment in YCp50) (pJR plasmids from J. RINE and colleagues), YEp13-*RAD51*-23, YEp13-*RAD54*-216A, YEp13-*RAD55*-13C (CALDERON *et al.* 1983), YEp13-*RAD52* (SCHILD *et al.* 1983a), and YEp13-*RAD57*-H (SCHILD *et al.* 1983b). Several gene disruptions were constructed for this study. The one-step gene disruption method (ROTHSTEIN 1991) was used to isolate disruptions in yeast. The *sir4::HIS3* disruption was make by cutting pJR276 with *Pvu*II; *HIS3* potential disruptions were screened for sterility. The *ltel::URA3* deletion was made using plasmid pOΔ6 digested with *Eco*RI before transformation (WICKNER *et al.* 1987); *URA3* potential disruptions were screened for cold-sensitive growth at 8°.

X-ray survival curves and patch plate assays: For X-ray survival curves, cells were grown at 30° to mid-log phase (O.D.$_{660}$ of ~0.4-0.8) in liquid YEPD media, except for strains containing replicating plasmids, which were grown in complete media lacking uracil or leucine to maintain selection for the plasmids (SHERMAN *et al.* 1983). Cells were sonicated to disperse clumps of cells, diluted, and plated in duplicate before irradiation with X ray. After irradiation, plates were incubated at 23, 30 or 36° for 5-7 days and then colonies were counted. Final colony counts for survival curves on homozygous *rad52* diploids were done after 10-12 days, because such diploids have many very small and slow growing colonies after X-ray treatment, presumably due to gross aneuploidy (MORTIMER *et al.* 1981). The X-ray source was a Picker X-ray machine with a Machlett OEG 60 tube with a beryllium window, operated at 50 kV and 20 mA, yielding a dose rate of 188 rad/sec. Patch plate assays were done by growing at 30° overnight a master YEPD plate containing patches of strains to be tested and replica plating to one or more plates that were then irradiated for 4-6 min (45-68 krad). Plates were incubated at 23, 30 or 36° for 2-4 days and then patches were scored as X-ray sensitive or resistant and photographed. Patch plates containing dissections normally also contained the two parents of the sporulated diploid as controls.

Isolation of extragenic suppressors of the *rad52-20* allele: After survival curves on *rad52-20* haploid strains, tests revealed that many of the survivors of large X-ray doses were now X-ray resistant. Because X rays are a mutagenic agent, it was not clear if these revertants were preexisting in the

population irradiated, induced by the X-ray treatment itself, or a mixture of both. If some or all were preexisting in the population, then revertants from a single survival curve might not be independent. Therefore, isolated colonies of strain XS1241–1C (*MATα rad52-20 his3*) were patched onto a master plate, and after the patches had grown, this plate was replica plated to a plate that was treated with 56 or 70 krad of X ray. After rare surviving cells grew up into small colonies in a background of dead cells, these colonies were once again replica plated to a plate treated with X rays. From each patch, one colony that showed resistance was picked from the original once X-ray-treated plate and used for further analysis.

β-Galactosidase assays: Cultures were grown to mid-log phase ($O.D._{600}$ of 0.4–0.8) in YEPD at 30°. For each culture, two 10-ml samples were collected on membrane filters: one was irradiated with 30 krad of X rays and the other was the untreated control. The cells on each filter were resuspended in 10 ml of fresh YEPD and shaken at 30° for 90 min. The β-galactosidase assay used *o*-nitrophenyl-β-galactopyranoside and was performed using a protocol described in ELLEDGE and DAVIS (1989). The units for β-galactosidase activity are as described by MILLER (1972) and are proportional to the increase in *o*-nitrophenol per minute per cell.

RESULTS

Initial characterization of the *rad52-20* allele, including suppression by *srs2*: *Srs2* deletions are lethal in a *rad54* background (PALLADINO and KLEIN 1992; F. FABRE, personal communication; D. SCHILD and M. ZARAGOSA, unpublished result). Using the temperature-conditional *rad54-3* allele, which is much more X-ray sensitive at 36 than at 23°, we were able to grow a *rad54-3 srs2Δ* strain at 23° and isolate revertants of the synthetic lethality at 36°; among the revertants of this lethality were a number of new alleles of *rad52*, including *rad52-20*, as well as alleles of *rad51*, *rad55* and *rad57* (D. SCHILD and M. ZARAGOSA, unpublished results). Unlike the other alleles of *rad52* isolated in this way, the *rad52-20* allele showed unusual properties when it was crossed out of the original *rad54-3 srs2Δ* strain background (XS1179–2D in Table 1) it was isolated in. Although segregants containing only the *rad52-20* allele were very X-ray sensitive at 23, 30 and 36°, they were slightly more sensitive at 36° than at lower temperatures (Figure 1). In addition, and much more apparent, segregants that contained mutations in both *rad52-20* and *srs2* (but not in *RAD54*) were much less sensitive to X rays at 23 and 30° than strains with only *rad52-20* and slightly less sensitive at 36° (Figure 1). This indicated that the *srs2* deletion was acting as an extragenic suppressor of the *rad52-20* allele and that the suppression was weaker at 36°. The *srs2* deletion does not suppress the *rad52-1* allele or a *rad52* disruption (data not shown) nor have any other extragenic suppressors of the X-ray-sensitive phenotype of these other *rad52* mutations been reported.

Because of the unusual properties of this allele of *RAD52* compared with the previously isolated alleles, it was important to unambiguously determine that this really was an allele of *RAD52* rather than some other *RAD* gene that interacted with *RAD52* in some unusual and dominant fashion. First, it was determined that the *rad52-20* allele mapped genetically at or very near to where *RAD52* had previously been mapped on the left arm of chromosome 13 (data not shown). Additional evidence that this mutation is an allele of *rad52* comes from analysis of the dissection of a *rad52-7::LEU2/rad52-20* diploid and the observed complementation of *rad52-20* by a plasmid containing the wild-type *RAD52* gene (both discussed below).

X-ray resistance and normal meiosis in homozygous *rad52-20* diploids: Because of the suppression of *rad52-20* by *srs2Δ* and the temperature sensitivity of both the original allele and the suppression, this allele was further characterized for other properties, including the behavior of homozygous *rad52-20* diploids. Unlike other published alleles of *rad52*, this allele had no effect on sporulation or spore viability. The percentage of cells sporulating in closely related *rad52-20/rad52-20*, *rad52-20*/RAD$^+$ and RAD$^+$/RAD$^+$ diploids was very similar, being over 50% in all three strains. Of 48 asci from diploid strain XS1223 (*rad52-20/rad52-20*) that were dissected, 33 gave four viable spore colonies and 85% of all spores produced viable colonies, all of which were *rad52-20*, as expected. Meiotic recombination on chromosome *III* between *leu2* and *MAT* was also normal in XS1223; data from the 39 tetrads with either three or four viable spores gave a map distance for this interval of 38.5 cM [14 parental ditype (PD), 1 nonparental ditype (NPD), 24 tetratype (T)] comparable with the published average distance of 34.7 cM (MORTIMER and SCHILD 1980). The surprising lack of a meiotic phenotype for the *rad52-20* allele was more easily understood when it was determined that diploids homozygous for this allele are only partially X-ray sensitive (Figure 2). The relative X-ray resistance of these diploids was later found to depend on their heterozygosity for the mating type locus (see below).

To determine whether the *rad52-20* allele was dominant or recessive with regards to a *rad52* disruption, the properties of a diploid (XS1354), with one homologue carrying the *rad52* disruption (*rad52-7::LEU2*) and the other the *rad52-20* allele, were examined and compared to a *RAD52/rad52-20* isogenic diploid (XS1355). X-ray survival data on the heteroallelic strain XS1354 showed that it is much more sensitive than *rad52-20* homozygous diploids (Figure 2) but still less sensitive than a *rad52* disruption homozygous diploid, which has similar sensitivity to *rad52* disruption haploid strains. To look for any meiotic effects, both XS1354 and XS1355 were sporulated, tetrads dissected and genetic markers scored in the meiotic segregants. Sporulation in XS1354 (*rad52-7::LEU2/rad52-20*) was ~24%, compared with ~63% for XS1355 (*RAD52/rad52-20*). Spore viability in XS1354 was also reduced to ~32% (two asci with four viable spores, seven with three, 13 with two, 13 with one, and 13 with no viable spores),

TABLE 1

Yeast strains

Strain	Genotype
FPW300	*MATα hpr5Δ59::HIS3 (srs2Δ) his3-11,15 ade2-1 leu2-3,112 ura3-1 trp1-1 can1-100*
XS1179-2D	*MATα rad54-3 hpr5Δ59::HIS3 (srs2Δ) his3 leu2 ura3 trp1 can1*
XS1179-2D-6-1	*MATα rad52-20 rad54-3 hpr5Δ59::HIS3 (srs2Δ) his3 leu2 ura3 trp1 can1*
XS1214-1D	*MAT***a** *rad52-20 his3 ura3 trp1 can1*
XS1214-2B	*MAT***a** *rad52-20 leu2 his3 ura3 trp1 can1*
XS1214-10D	*MATα rad52-20 hpr5Δ59::HIS3 (srs2Δ) his3 leu2 ura3 trp1 can1*
XS1241-1C	*MATα rad52-20 his3*
XS1241-1C-rev.1-9	*MATα rad52-20 his3,* reverted (-rev. 1 through -rev.9) for *rad52-20*
XS1241-11B	*MAT***a** *rad52-20 ura3 trp1 can1*
XS1241-11C	*MATα rad52-20 ura3 trp1 can1*
XS95-6C	*MAT***a** *rad52-1 his3Δ1 leu2-3,112 ura3-52 trp1-289 cir^0*
XS122-49C	*MATα rad52-1 leu2*
XS955-36B	*MATα leu2 ura3 ade2 trp1 his3 lys2 hom3 his3*
DST7	*rad52-7::LEU2* in XS955-36B
F87-17D	*MAT***a** *arg3 ura3 trp1*
XS1258-21B	*MAT***a** *rad52-20 arg3 trp1 ura3*
XS1344-4C	*MAT***a** *rad52-20 met6*
DK330-2B-*fun30::LEU2*	*MATα fun30::LEU2(EcoRV) leu2-3,112 ura3-1 his3-11,15 can1*
YCD32:8D	*MAT***a** *fun21::LEU2 leu2 ura3 his4 trp1 ade2 ade3*
XS1241-1D-*lte1::URA3*	*MATα lte1::URA3 ura3 trp1 can1*
1009-1a	*MAT***a** *ccr4::HIS3 trp1 his3 ura3 leu2 adh1-11*
EGY191-2	*MATα caf1::LEU2 trp1 his3 ura3 leu2 lexA-LEU2*
XS1349-1D	*MAT***a** *rad52-20 leu2 his3*
XS1223	*MAT***a***/MATα rad52-20/rad52-20 ura3/ura3 trp1/trp1 can1/can1*
XS1354	*MAT***a***/MATα rad52-20/rad52-7::LEU2 leu2/leu2 his3/his3 trp1/+ ura3/+ hom3/+ ade2/+ lys2/+*
XS1355	*MAT***a***/MATα rad52-20/+ leu2/leu2 his3/his3 trp1/+ ura3/+ hom3/+ ade2/+ lys2/+*
XS1241-1C-αα	*MATα/MATα rad52-20/rad52-20 his3/his3*
XS1090	*MAT***a***/MATα rad52-7::LEU2/rad52-7::LEU2 leu2/leu2 his3/his3 ura3/ura3 hom3/hom3 lys2/lys2 arg4/+ ade2/+*

All of these strains were constructed for this and related studies, except the following: FPW300 and F87-17D were supplied by HANNAH KLEIN, DK330-2B-*fun30::LEU2* was supplied by ARNIE BARTON, YCD32-8D by CHRIS DAVIS, and 1009-1a and EGY191-2 by CLYDE DENIS. The *lexA-LEU2* construct in strain EGY191-2 does not express *LEU2*.

compared with ~83% for XS1355 (eight with four viable spores, one with three, two with two, one with one, and none with no viable spores). The decreases in both sporulation and spore viability, although significant, are not nearly as great as are normally seen with strains homozygous for other alleles of *rad52* (PRAKASH *et al.* 1980; RESNICK *et al.* 1986). These results suggest that the *rad52-20* allele is recessive to the wild-type gene but is semidominant with regard to a *rad52* disruption, both for DNA repair and sporulation.

A centromere marker (*TRP1*) was used to determine whether the surviving spores in the asci with two viable spores were sister spores (separated at meiosis II) or nonsister spores (separated at meiosis I). Of 13 such dyads for *rad52-20/rad52-7::LEU2* strain XS1354, five were sister pairs and eight were nonsister pairs. Therefore these asci do not appear to have arisen because of a chromosome loss events in the mitotic cells before sporulation, because monosomes normally segregate to produce two viable spores that are sister spores. The map distance between *URA3* and *HOM3* was also determined in XS1354 to examine whether this strain had reduced recombination in meiosis. In the 18 tetrads in which it was possible to determine or infer the segregation of both markers, five were parental, one nonparental and 12 were tetratypes. Although these data are not large enough to get a significant linkage, these data are very similar to the published data for this interval of 538 parental, 101 nonparental, and 1467 tetratypes (MORTIMER and SCHILD 1980). This implies that recombination in this interval is normal in this diploid.

Among the surviving segregants from XS1354, there was no selective advantage between the two *rad52* alleles (35 were *rad52-7::LEU2* and 33 were *rad52-20*). As expected, all of the segregants from XS1354 were found to be X-ray sensitive, with those segregants with the *rad52* disruption (*i.e.*, *LEU2*) slightly more sensitive at 30° than those with the *rad52-20* allele. There was one segregant that, while still slightly X-ray sensitive, was much more resistant than other segregants from this cross. This segregant was Leu$^-$ and the only segregant that failed to mate. It seems likely that this segregant was a *MAT***a**/*α* chromosome *III* disome and that mating type heterozygosity was suppressing the *rad52-20* allele, as discussed later. The segregation data from XS1354 confirms the allelism of *rad52-20* with *RAD52*.

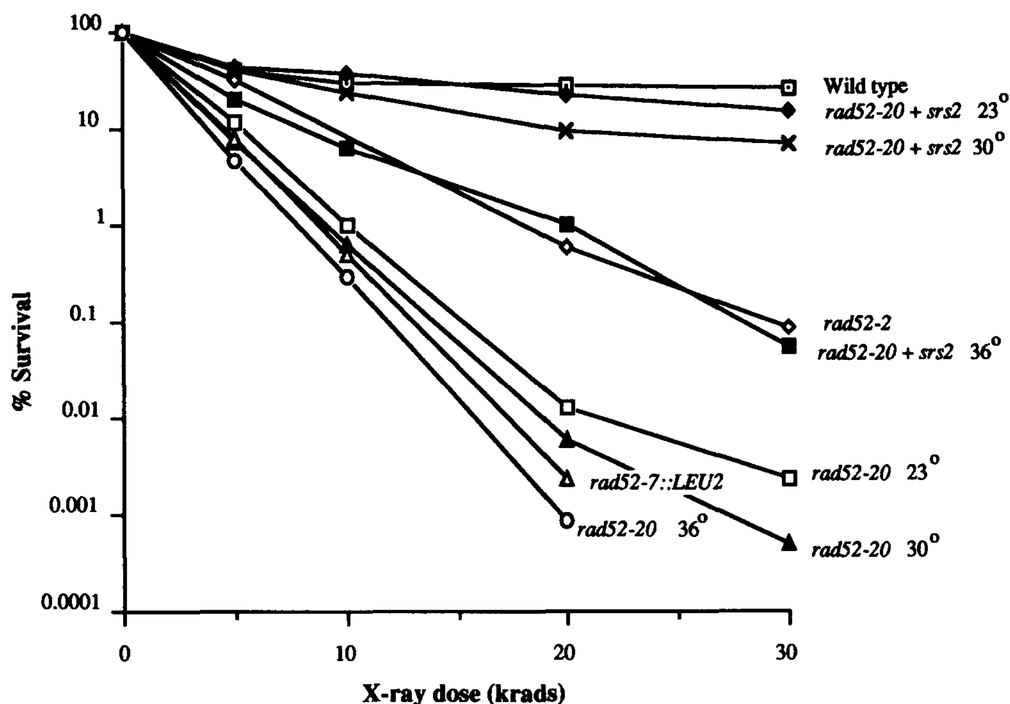

FIGURE 1.—Temperature effects on X-ray survival of *rad52-20* haploids and suppression by *srs2*. Strains XS1214–1D (*rad52-20*) and XS1214–10D (*rad52-20 srs2D*) were incubated at 23, 30 or 36° postirradiation. The wild-type strain XS955–36B, *rad52-2* strain XS1243–5B, and *rad52-7::LEU2* strain DST7 were all incubated at 30° postirradiation; data on these three strains are included for comparison purposes.

Isolation and initial characterization of extragenic suppressors of *rad52-20*: Because the *srs2* deletion acted to suppress the X-ray sensitivity of *rad52-20*, I investigated whether mutations in other genes could also act as extragenic suppressors. The rare survivors of high doses of X rays (75 krad) were tested to determine whether they were X-ray resistant (*i.e.*, reverted for *rad52-20*), and many were (~30%). Nineteen independent revertants were isolated (see MATERIALS AND METHODS), and nine of these strains were extensively characterized (XS1241–1C-rev.1 to -rev.9). The remaining 10 where characterized for mating phenotype only. Plate assays showed that all 19 revertants were less X-ray sensitive than the parent strain they were isolated in (XS1241–1C) at 23 and 30°, and most also at 36°, although none completely reverted the X-ray-sensitive phenotype conferred by *rad52-20*. X-ray survival curves at 30° confirmed the patch plate data for seven revertants and allowed a more quantitative analysis of their survival (Figure 3); survival curves were not obtained for XS1241–1C-rev.3 and -rev.4 because the revertant in XS1241–1C-rev.3 did not segregate as a single gene and XS1241–1C-rev.4 and -rev.8 contain mutations in the same gene (discussed below).

Revertants include mutations in *SIR* genes: To further characterize the original nine revertants, they were outcrossed to a Rad$^+$ parent of the opposite mating type. Unexpectedly, two of the revertant strains (XS1241–1C-rev.5 and -rev.9) mated only very weakly (referred to here as nonmaters). Because these revertants were the survivors of a relatively high X-ray dose, it was possible that the mating defect was unrelated to the suppression of *rad52-20* that was observed in the same strain. The low level of mating did allow diploids to be isolated. Sporulation and dissection of these diploids revealed that the nonmating phenotype segregated two to two and all nonmaters were either strongly Rad$^+$ or moderately Rad$^+$. The strongly Rad$^+$ segregants presumably received the wild-type *RAD52* gene, and the moderately Rad$^+$ segregants had similar resistance to the original revertant in the *rad52-20* background. In addition, in the cross with XS1241–1C-rev.5, all 10 X-ray-sensitive segregants were normal maters, and in the XS1241–1C-rev.9 cross, all 14 were normal maters. These data strongly suggested that the mutation causing nonmating was also suppressing the X-ray sensitivity of *rad52-20*. To test whether the occurrence of two such nonmating revertants out of nine revertants was a chance occurrence, an additional 10 *rad52-20* revertants were isolated and three were found to be nonmaters. Therefore, about a quarter of the *rad52-20* revertants were nonmaters.

In *S. cerevisiae*, many genes have been identified in which mutations lead to a nonmating phenotype. Among these genes are the *SIR1* to *SIR4* loci, whose activities are required for the silencing of the silent mating-type cassettes (reviewed by HERSKOWITZ *et al.* 1992). Because mutations in these genes have previously been shown to suppress the X-ray sensitivity of alleles of *rad55* (LOVETT and MORTIMER 1987), the

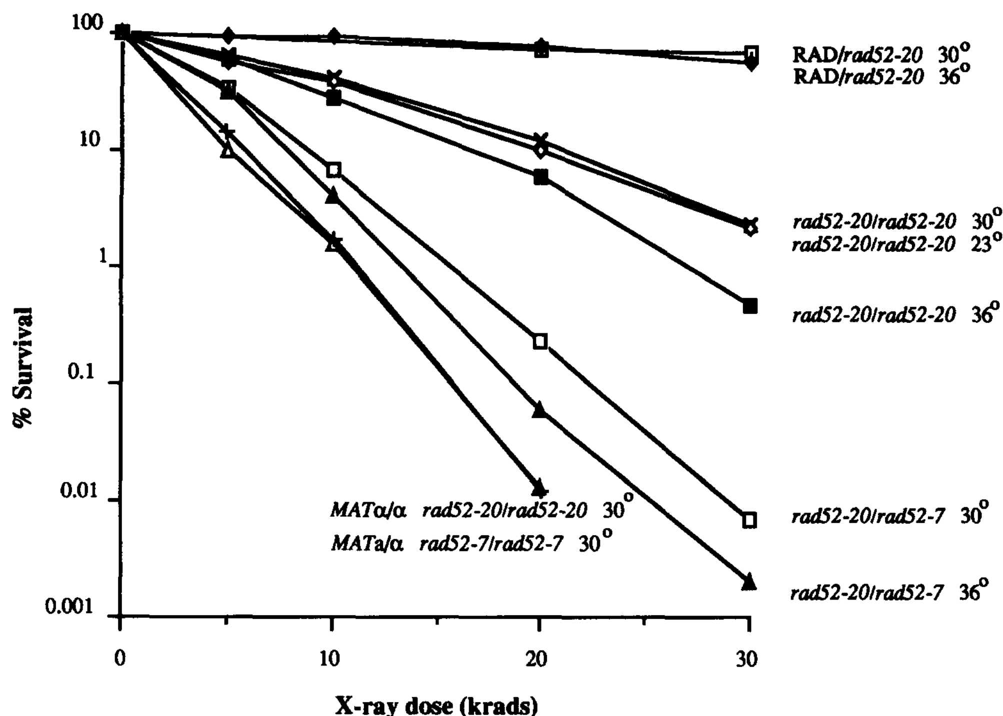

FIGURE 2.—X-ray survival curves of a *rad52-20* diploid and related diploids. Strains XS1223 (*rad52-20/rad52-20*), XS1355 (*RAD52/rad52-20*), XS1354 (*rad52-20/rad52-7::LEU2*) and XS1241-1C-αα (*rad52-20/rad52-20*) were incubated at 23, 30 or 36° postirradiation. XS1223, XS1354 and XS1355 are heterozygous for mating type, whereas XS1241-1C-αα is homozygous for *MATα*. XS1090 is an **a**/α *rad52-7::LEU2/rad52-7::LEU2* diploid (designated by +).

nonmating *rad52-20* revertants were tested for whether they carried mutations in one of these four genes. Because the *sir* mutant phenotype is expressed in haploids, it was not easy to perform genetic complementation experiments, which entail examining the phenotype of diploids. Instead, plasmids containing each of the four *SIR* genes were separately introduced into each of the five nonmating strains and tested for which complemented for mating ability. Each of the nonmating mutations was complemented by one of the four plasmids (data not shown), indicating that each contained a *sir* mutation. The suppressors found in XS1241-1C-rev.5 and -rev.19 were found to be alleles of *SIR4*; the suppressor in XS1241-1C-rev.10 was an allele of *SIR2*, and the suppressor in XS1241-1C-rev.9 and -rev.14 were alleles of *SIR3*. Besides conferring mating ability on each of these revertants, the complementing plasmids did restore some X-ray sensitivity.

As additional evidence that the *sir* mutations are themselves suppressing the *rad52-20* allele, a disruption plasmid was used to disrupt *SIR4* in a *rad52-20* background. A few *sir4* disruptions in a *rad52-20* strain were isolated, and these were suppressed for the X-ray sensitivity of *rad52-20*.

XS1241-1C-rev.1 contains the only intragenic revertant, -rev.3 contains two suppressors, and -rev.7 probably contains an *srs2* allele: Of the nine *rad52-20* revertants examined, only XS1241-1C-rev.1 appears to contain an intragenic revertant. With the exception of XS1241-1C-rev.1, sporulation of diploids constructed by crosses of each of the original revertant strains (in the *rad52-20* background) by a Rad$^+$ strain yielded tetrads with one or two *rad52-20* segregants. Of 10 tetrads with four viable spores each, no *rad52-20* segregants were observed for the cross with XS1241-1C-rev.1, indicating that this is most likely an intragenic suppressor. The other revertants all appear to contain one or more unlinked extragenic suppressors. The revertants that did not affect mating or contain an intragenic suppressor were crossed by a *rad52-20* strain to test for 2:2 segregation of the extragenic suppressor in a homozygous *rad52-20* background. In each case the suppressor segregated 2:2, except XS1241-1C-rev.3, which appeared to contain two weak and unlinked suppressors. The suppressors in XS1241-1C-rev.3 have not been examined further. The suppressor in XS1241-1C-rev.6 was relatively weak and because it did not always segregate as a single mutation in some crosses, it also was not further analyzed.

Because an *srs2* deletion suppressed *rad52-20*, the revertants were tested for whether any might be alleles of *srs2*. A strain carrying both *rad52-20* and *arg3* was constructed and crossed by each revertant strain, because *arg3* is tightly linked to *srs2*. Data from RONG *et al.* (1991) showed no recombinants between *hpr5* (an allele of *srs2*) and *arg3* in 58 tetrads. Only the suppressor in XS1241-1C-rev.7 showed linkage to *arg3* (9 PD, 0 NPD, 0 T). This tight linkage to *arg3*, together with

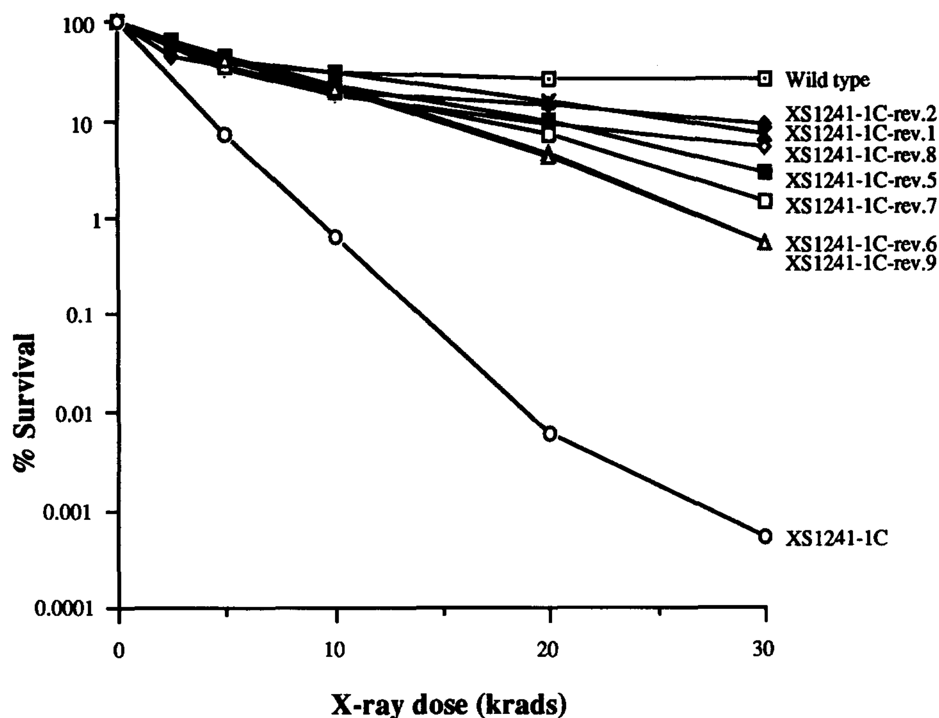

FIGURE 3.—Survival curves of *rad52-20* revertants. XS1241-1C (*rad52-20*) and X-ray-resistant revertants derived from this strain were incubated at 30° postirradiation. Data on the Rad$^+$ (wild type) strain XS955-36B are included for comparison.

the weak suppression observed at 36° for XS1241-1C-rev.7 (data not shown), strongly indicates that the suppressor in this strain is an allele of *srs2*.

XS1241-1C-rev.4 and -rev.8 contain alleles of *ccr4*: Preliminary data indicated that both suppressors in XS1241-1C-rev.4 and -rev.8 were centromere-linked mutations that mapped about the same distance from a centromere. This suggested that these two revertants might be alleles of the same gene. A cross of a *rad52-20* rev.4 strain by a *rad52-20* rev.8 strain resulted in a diploid that when sporulated produced only Rad$^+$ (*i.e.*, suppressed) segregants in eight tetrads, strongly indicating that these two revertants are in the same gene. Extensive crosses to strains containing both *rad52-20* and centromere-linked genes on different chromosomes confirmed the centromere linkage of these suppressors and eliminated most chromosomes (data not shown), except chromosome *I*. A cross, segregating for both the suppressor in XS1241-1C-rev.8 and *ade1* and homozygous for *rad52-20*, showed linkage between this suppressor and *ade1* on chromosome *I* (9 P, 0 NPD, 13 T; 29.5 cM). Additional analysis revealed that the suppressors in both XS1241-1C-rev.4 and -rev.8 map between *fun30* and *fun21* on the left arm. The suppressor in XS1241-1C-rev.8 maps near but not at *lte1* (34 PD, 0 NPD, 4 T; 5.3 cM), which also maps between *fun30* and *fun21*.

CCR4, which maps in this region and encodes a protein involved in transcriptional regulation (MALVAR *et al.* 1992), seemed to be a possible candidate as the suppressor of *rad52-20*, because both *FUN25* and *FUN26*, which also map in this region, encode putative membrane-spanning proteins (OUELLETTE *et al.* 1993). Therefore, a yeast strain with a *ccr4* disruption was obtained from C. DENIS (MALVAR *et al.* 1992) and crossed by a *rad52-20* strain. Results from tetrad analysis of this cross were consistent with *ccr4::HIS3* acting as a suppressor of *rad52-20*; of 10 tetrads with four viable spores, three contained four Rad$^+$ spores, four contained three Rad$^+$ spores and three contained two Rad$^+$ spores, and all spores containing the *ccr4::HIS3* disruption were Rad$^+$. A Rad$^+$ His$^+$ segregant, which was *rad52-20 ccr4::HIS3*, was crossed to a *rad52-20 his3* strain. After sporulation and tetrad analysis, suppression of *rad52-20* segregated with *ccr4::HIS3* in two spores from each of the nine tetrads tested. Additional crosses showed no recombination between the suppressors in either XS1241-1C-rev.4 or -rev.8 and *ccr4::HIS3* (data not shown).

XS1241-1C-rev.2 contains an allele of *caf1*: At the suggestion of C. DENIS, a *caf1* disruption was tested for whether it suppressed *rad52-20*. The *CAF1* gene encodes a *CCR4* associated factor, and *caf1* mutations have a similar phenotype to *ccr4* mutations (M. P. DRAPER, C. SALVADORE and C. L. DENIS, personal communication). A cross of the *caf1::LEU2* disruption strain 191-2 by a *rad52-20 leu2* strain resulted in one tetrad with four Rad$^+$ spores, four with three Rad$^+$ spores and five with two Rad$^+$ spores, and all Leu$^+$ spores were Rad$^+$. A diploid that was homozygous for *rad52-20* and heterozygous for the *caf1::LEU2* disruption resulted in all eight tetrads tested segregating *caf1::LEU2* 2:2 with suppression of *rad52-20*. A cross of a *caf1::LEU2 rad52-20* strain by a *rad52-20* rev.2 strain resulted in 12 tetrads all with four Rad$^+$ spores, indicating that the suppressor in XS1241-1C-rev.2 is an allele of *caf1*.

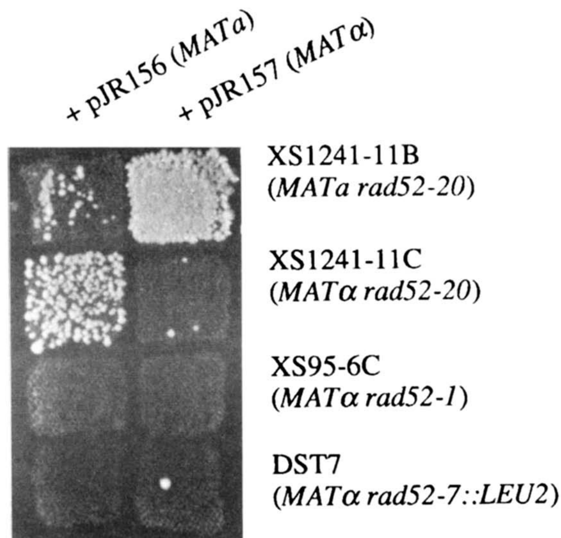

FIGURE 4.—Mating-type heterozygosity in a haploid suppresses *rad52-20*. Strains XS1241–11B (*MAT***a** rad52-20), XS1241–11C (*MATα rad52-20*), XS95–6C (*MATα rad52-1*) and DST7 (*MATα rad52-7::LEU2*) were transformed with *MAT***a** and *MATα* containing plasmids. Transformants were patched on plates lacking uracil and the patches allowed to grow up. These patches were subsequently replica plated to a YEPD plate, which was then irradiated with 60 krad of X ray and incubated for 4 days at 30°.

Mating-type heterozygosity suppresses *rad52-20* in both haploid and diploid strains: Both the resistance of *rad52-20* diploids and *sir* suppression in haploids could be explained by heterozygosity of these strains for mating type. For the *sir* suppression, this hypothesis was tested by introducing *MAT***a** or *MATα* containing plasmids into haploid *rad52-20* strains. Transformants containing a plasmid with the opposite mating-type information from that of the strain transformed were suppressed for *rad52-20* but not when the plasmid contained the same mating-type information as the strain (Figure 4). Heterozygosity for mating type did not suppress the *rad52-1* or *rad52-7::LEU2* alleles (Figure 4). To examine the effect on *rad52-20* of mating-type heterozygosity in diploids, a diploid (XS1241–1C-*αα*) was constructed that was homozygous for both *rad52-20* and *MATα* (see MATERIALS AND METHODS). This diploid was much more sensitive than closely related diploids that were heterozygous for mating type (Figure 2).

Suppression by *RAD51* on a high copy number plasmid: Because mating-type heterozygosity, which affects the transcription of many genes, and mutations in *ccr4*, a transcriptional regulatory gene, both act to suppress *rad52-20*, it seemed possible that increasing the level of one of the *RAD* genes might also act as a suppressor. This was tested by transforming a *rad52-20* strain (XS1214–1B) with the high copy number YEp13 plasmid containing different cloned *RAD* genes and the vector alone as a control. As expected, YEp13-*RAD52* complemented *rad52-20* completely. However, YEp13-*RAD51* also partially complemented (Figure 5). Using the patch plate assay, four independent YEp13-*RAD51* containing transformants were shown to be X-ray resistant and transformants that had lost the YEp13-*RAD51* plasmid were once again as X-ray sensitive as the original strain (data not shown). *RAD54*, *RAD55* and *RAD57* were also tested in this way and none were found to complement (Figure 5 and data not shown). The YEp13-*RAD51* plasmid did not suppress the *rad52-1* allele (Figure 5).

Assaying revertants for the level of *RAD51-lacZ*: To determine whether any of the revertants isolated act to suppress *rad52-20* by increasing the level of RAD51, haploid strains were constructed that contained both an integrated copy of a *RAD51-lacZ* construct (ABOUSSEKHRA *et al.* 1992) and the *rad52-20* mutation and closely related haploid strains that also contained a *rad52-20* revertant. *β*-Galactosidase assays were performed twice on each of these strains with and without treatment of 30 krad of X ray (except the original *RAD51-lacZ* strain, which was only tested once). The results show that the level of *RAD51-lacZ* activity is increased in a *rad52-20* strain compared with the Rad$^+$ control strain (Table 2), presumably due to unrepaired spontaneous damage (see DISCUSSION). None of the revertants tested increased either the constitutive or induced level of *RAD51-lacZ* activity. Actually, all of the revertant strains had lower levels than observed in the unreverted *rad52-20* strain, presumably because the revertants had lower spontaneous damage. These results indicate that these revertants are not suppressing the *rad52-20* mutation by increasing the level of RAD51. In the strain derived from XS1241–1C-rev.8 (containing *ccr4*), the level of the fusion was even slightly lower than in the other strains, possibly indicating that *CCR4* may play some positive role in *RAD51* expression.

DISCUSSION

The *RAD52* gene has been extensively characterized both by genetic and molecular methods, but we still have no understanding of its exact function either in general recombination or in recombinational repair of DNA damage. Biochemical studies have demonstrated that the RAD52 and RAD51 proteins bind to each other (SHINOHARA *et al.* 1992), and this interaction has been confirmed by use of the two-hybrid method (MILNE and WEAVER 1993; DONOVAN *et al.* 1994; C. BENDIXEN and R. ROTHSTEIN, personal communication). MILNE and WEAVER (1993) also found evidence of an interaction between these two proteins in studies examining the effect of overexpression of RAD51 on truncated alleles of *RAD52* and on a *RAD52* homologue from *Kluyveromyces lactis* that functioned in *Saccharomyces cerevisiae* to partially complement *rad52* mutations. As previously discussed (see the introduction), progress in the genetic analysis of *RAD52* has also been made by the char-

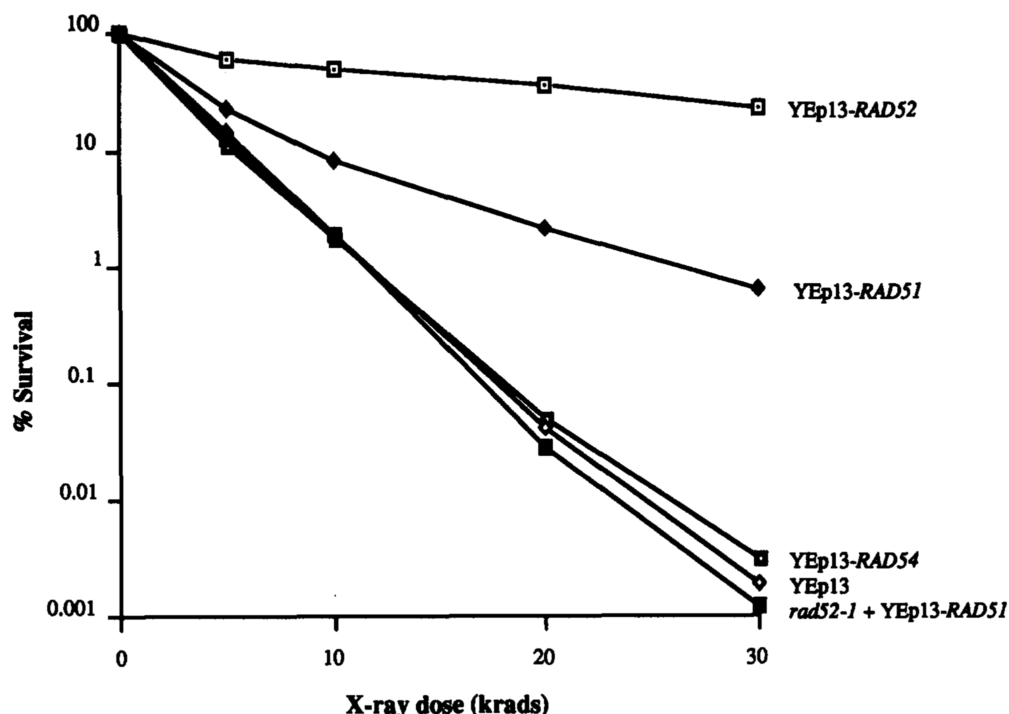

FIGURE 5.—X-ray survival curves of suppression by overexpression of *RAD51*. XS1214–2B (*rad52-20 leu2*) was transformed separately with the YEp13 vector and with YEp13 containing the cloned *RAD51*, *RAD52* and *RAD54* genes. XS122–49C (*rad52-1 leu2*) was transformed with only the YEp13-*RAD51* plasmid. These transformants were grown in media lacking leucine to maintain selection for the plasmids, diluted and plated on YEPD plates, which were then treated with different doses of X rays.

acterization of several different mutant alleles. The new *rad52-20* allele reported here has several unusual properties, such as suppression by a *srs2* deletion and apparently normal meiosis, which add to our knowledge about *RAD52*.

Unlike previously reported *rad52* alleles, the *rad52-20* allele is readily reverted and most of these revertants turn out to be unlinked extragenic suppressors. The isolation and characterization of extragenic suppressors is frequently an excellent method for identifying interacting proteins (JARVIK and BOTSTEIN 1975), but in the current study all suppressors appear to be inactivations of specific genes. Because inactivation of at least six different genes (*caf1*, *ccr4*, *sir2*, *sir3*, *sir4* and *srs2*) can suppress *rad52-20*, it is unlikely that specific alterations in an interacting gene would have been found by the methods used in this study, even if such mutations exist. However, the extragenic suppressors have been enlight-

TABLE 2

Results of *RAD51-lacZ* experiments

Strain	Genotype[a]	Uninduced level of β-gal (no X ray)[b]	Induced level of β-gal (30 krads)[b]	Relative β-gal induction
FF181082	RAD⁺	3.2	15.2	4.8
XS1373-2B	*rad52-20*	12.3	26.8	2.2
		11.8	24.5	2.1
XS1375-3D	*rad52-20* rev.1	3.2	14.6	4.6
		2.4	9.5	4
XS1376-1C	*rad52-20* rev.2 (*caf1*)	2.6	12.3	4.7
		2.4	12	5
XS1377-3A	*rad52-20* rev.5 (*sir4*)	5.2	15	2.9
		4.1	11.6	2.8
XS1379-1B	*rad52-20* rev.7 (*srs2*)	8.2	18.3	2.2
		7.2	12.9	1.8
XS1380-1A	*rad52-20* rev.8 (*ccr4*)	3.2	7.3	2.3
		2.5	5.4	2.2

[a] The radiation genotype only; rev.1. to rev.8 refers here to the revertants present in the original revertant strains, and the mutation in each revertant is in parentheses.

[b] Units of β-galactosidase activity, as described by MILLER (1972).

ening in terms of understanding the properties of this allele of *RAD52*, particularly its weak effect in diploids.

Suppression by overexpression of RAD51: Because the RAD52 and RAD51 proteins have been reported to interact (SHINOHARA *et al.* 1992; MILNE and WEAVER 1993; DONOVAN *et al.* 1994), *RAD51* on a high copy number plasmid was tested and found to suppress *rad52-20* (Figure 5). Yeast strains containing *RAD51* on a high copy number plasmid have greatly increased *RAD51* mRNA levels (G. BASILE, personal communication) and also overexpress the RAD51 protein (SHINOHARA *et al.* 1992). Several other genes in the *RAD52* group did not act as suppressors, except *RAD52* itself, which fully complemented this mutation as expected. Suppression of *rad52-20* by overexpression of RAD51 suggests that this allele of *RAD52* may encode a protein that has a lower affinity for the RAD51 protein. If this is the case, the X-ray sensitivity of *rad52-20* mutants implies that this interaction is biologically crucial for recombinational repair. Although none of the isolated extragenic suppressors of *rad52-20* were in the *RAD51* gene, such mutations would be expected to be quite specific and rare. Therefore, even if they exist, they might not have been found in the limited number of revertants examined to date. Mutagenesis of the cloned *RAD51* gene is planned to look for specific mutations in *RAD51* that might suppress *rad52-20*. In the excision repair pathway, results similar to those reported here have been found: a recently characterized mutation in the *RAD1* excision repair gene can be complemented by overexpression of the RAD10 protein (SIEDE *et al.* 1993), which forms a complex with the RAD1 protein. The authors suggest that their *rad1* mutation results in a protein with a lower affinity for the RAD10 protein, similar to the lower affinity model favored here to explanation the suppression of *rad52-20* by overexpression of *RAD51*. A second model that cannot be ruled out is that the RAD52-20 protein lacks the ability of the normal RAD52 protein to enhance RAD51 activity, but overexpression of RAD51 compensates for this lack of enhancement. If this second model is correct, the RAD52-20 protein may either bind RAD51 normally but not act to enhance its activity or may actually fail to bind RAD51 altogether, even in the presence of increased levels of RAD51. Because *rad52-1* and a disruption allele are not suppressed by *RAD51* overexpression, the implication of this second model is that *RAD52* is at least bifunctional and that *rad52-20* is mutant in only this one function, whereas the other alleles are inactivated for the other or both functions. To explain differences in the effect of *rad51* and *rad52* mutations on a chromosomal inverted repeat, RATTRAY and SYMINGTON (1994) have likewise postulated that *RAD52* may have activities in addition to those of the RAD51-RAD52 protein complex.

Suppression by mating-type heterozygosity: Several lines of evidence demonstrate that *rad52-20* is suppressed by heterozygosity at the mating-type locus. In haploid strains, revertants of *rad52-20* include *sir* mutations (suppressors in XS1241-1C-rev.5 and -rev.9 in Figure 3), which result in the expression of the mating-type information at the silent mating-type cassettes. In addition, plasmids containing mating-type information suppress *rad52-20* in haploid strains with the opposite mating type but not in strains with the same mating-type information as on the plasmids (Figure 4). Similarly, among homozygous *rad52-20* diploids, strains that are heterozygous for mating-type information are only slightly X-ray sensitive, whereas diploids constructed to be homozygous at the mating-type locus are very X-ray sensitive (Figure 2). The apparently normal meiosis in homozygous *rad52-20* diploids is probably also a product of suppression of this allele by mating-type heterozygosity.

Mating-type heterozygosity has long been known to increase the X-ray resistance of diploids (MORTIMER 1958) and also to increase both spontaneous and induced mitotic recombination (reviewed in ESPOSITO and WAGSTAFF 1981). It is assumed that the X-ray resistance is due to the increased capacity for mitotic recombination, because such recombination (including both interhomologue recombination and sister-chromatid exchange) appears to be the only mechanism in yeast of repairing double-strand breaks caused by X rays (reviewed in GAME 1993). The effect on X-ray resistance has been shown to depend on the expression of the *MAT***a***1* and *MAT*α*2* gene products (HEUDE and FABRE 1993). Mating-type heterozygosity has also been shown to increase both the constitutive and damage-induced levels of the *RAD54* transcript, although it had little effect on the level of induction; the *RAD52* transcript, which is uninduced by DNA damage, was unaffected by mating-type heterozygosity (COLE *et al.* 1987). Because *RAD51* is induced by DNA damage in a fashion similar to *RAD54* (ABOUSSEKHRA *et al.* 1992; BASILE *et al.* 1992; SHINOHARA *et al.* 1992) and like *RAD54* has a higher transcript level in *MAT***a**/*MAT*α diploids than haploids (BASILE *et al.* 1992), it seems possible that mating-type heterozygosity was increasing the level of *RAD51*, resulting in the observed suppression of *rad52-20*. Experiments using a *RAD51-lacZ* fusion indicated that at least in a *rad52-20* background a *sir* mutation did not increase the level of *RAD51-lacZ* activity over what was observed in the *rad52-20* strain not expressing both mating types. The uninduced and induced levels of *RAD51-lacZ* expression are higher in the *rad52-20* strain than in a closely related Rad$^+$ strain or any of the strains carrying both *rad52-20* and a suppressor (Table 2). This presumably reflects the presence of spontaneous damage in the *rad52-20* strain that is not repaired; similar results have been observed in *rad52-1* strains for the expression of the DNA damage responsive gene *DDR2* (MAGA *et al.* 1986) and in several *rad50* to *57* strains for the expression of *RAD54* (unpublished observation).

A hypotheses that has not been ruled out is that the suppression is due to a more generalized increase in recombinational-repair capability observed in *MAT*-heterozygous diploids or to the specific increased or decreased level of some other specific component of recombinational repair. Mating-type heterozygosity has been shown to suppress the X-ray sensitivity of *rad55* mutations, but in that case even a deletion of *rad55* was suppressed (LOVETT and MORTIMER 1987), suggesting that that suppression may be different from the allele-specific suppression reported here.

Suppression by *ccr4* and *caf1*: Two of the nine independently isolated suppressors of *rad52-20* have been identified as alleles of *ccr4* (ccr, carbon catabolite repression), and one of the suppressors is an allele of *caf1* (caf, CCR4-associated protein). In addition, deletions and disruptions of *ccr4* and *caf1* act as suppressors of *rad52-20*. The *CCR4* gene was first identified because mutations in this gene suppressed the derepression of the glucose-repressible alcohol dehydrogenase gene *ADH2* and several other glucose-repressible genes (DENIS 1984). The CCR4 protein is postulated to act as a general transcription activator needed for the expression of a number of yeast genes, including several non-glucose-repressible genes (DENIS and MALVAR 1990; DENIS *et al.* 1994). The CCR4 protein contains a leucine-rich tandemly repeated motif that has been indicated in protein–protein interactions (MALVAR *et al.* 1992; DRAPER *et al.* 1994). The *ccr4* mutations have been observed to decrease the expression of several genes. The CCR4 protein is associated with several other proteins, including CAF1 (M. P. DRAPER, C. SALVADORE and C. L. DENIS, personal communication). Recently, *CAF1* has been shown to be allelic with *POP2* (*PGK* promoter directed over production) (SAKAI *et al.* 1992). Because neither the *ccr4* revertant of *rad52-20* nor the *caf1* revertant increased the level of the *RAD51-lacZ* fusion (Table 2), they appear to be suppressing by some other mechanism that is not currently understood. It is possible that *ccr4* and *caf1* suppression is occurring by decreasing the expression of some other yet unidentified gene involved in recombinational repair. It is also possible that these mutations might result in higher levels of the RAD52-20 protein, which might act to increase its activity.

Suppression by *srs2*: Initial characterization of the *rad52-20* allele included the fortuitous finding that a deletion of *SRS2* resulted in suppression of the X-ray sensitivity of *rad52-20*. In addition, one of the *rad52-20* revertants maps very near to where *SRS2* maps and is very likely an allele of this gene. Suppression by *srs2* is somewhat different from the suppression by other suppressors in that even an *srs2* deletion suppresses much stronger at 23 and 30°, as compared with 36°. The *SRS2* gene was first identified because mutations in this gene suppressed the X-ray and UV sensitivity of *rad6* mutations (LAWRENCE and CHRISTENSEN 1979). The *RAD6* gene is involved in the postreplication (sometimes referred to as "error prone") repair pathway and has been shown to encode a ubiquitinating enzyme (JENTSCH *et al.* 1987). Additional alleles of *SRS2* were isolated as suppressors of *rad18* mutations and named *radH* (ABOUSSEKHRA *et al.* 1989) and as mitotic hyperrecombination mutations and named *hpr5* (RONG *et al.* 1991). The *SRS2* gene contains potential DNA helicase domains (ABOUSSEKHRA *et al.* 1989), and the SRS2 protein has recently been shown to have DNA helicase activity (RONG and KLEIN 1993). Suppression of *rad6* and *rad18* mutations by *srs2* mutations or deletions appears to be due to abnormal channeling of some DNA damage into the recombinational-repair pathway (SCHIESTL *et al.* 1990). The interactions between the *SRS2* gene and the recombinational-repair pathway appear to be very complex. Semidominant suppressors of the X-ray and UV sensitivity of homozygous *srs2* diploids have been isolated and found to map in the *RAD51* gene (ABOUSSEKHRA *et al.* 1992). In addition, *srs2* deletions are lethal in a *rad54* background and semilethal in a *rad50* background, resulting in very slow growing colonies (PALLADINO and KLEIN 1992; F. FABRE, personal communication; D. SCHILD and M. ZARAGOSA, unpublished result).

Currently, there is no favored hypothesis to explain the suppression of *rad52-20* by *srs2* mutations. One possibility is that in the absence of the SRS2 protein, the interaction between the RAD52 and RAD51 proteins is stronger and occurs even in the presence of both the RAD52-20 mutant protein and normal levels of the RAD51 protein. If this is the case, it would suggest that the SRS2 protein might be part of a complex of proteins involved in DNA repair. Any explanation for *srs2* suppression must also account for the stronger suppression at temperatures below 36°, even for a deletion of *SRS2*.

Because the revertants of *rad52-20* do not increase the level of *RAD51*, the results of these studies indicate that suppression can occur both by increased RAD51 expression and by one or more other mechanisms. Suppression probably actually occurs by at least three mechanisms, because *srs2* suppression is temperature sensitive. Because both mating-type heterozygosity and the *SRS2* gene have previously been shown to play a role in recombinational repair, further analysis of their role in suppression of this new allele of *rad52* may give us additional clues to the exact role they play.

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Note added in proof: MILNE *et al.* (1995) have indepen-

dently observed that a null allele of *SRS2* suppresses alleles of *RAD52*.

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