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A Novel Allele of *RAD52* That Causes Severe DNA Repair and Recombination Deficiencies Only in the Absence of *RAD51* or *RAD59*

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ABSTRACT

With the use of an intrachromosomal inverted repeat as a recombination reporter, we have shown that mitotic recombination is dependent on the *RAD52* gene, but reduced only fivefold by mutation of *RAD51*. *RAD59*, a component of the *RAD51*-independent pathway, was identified previously by screening for mutations that reduced inverted-repeat recombination in a *rad51* strain. Here we describe a *rad52* mutation, *rad52R70K*, that also reduced recombination synergistically in a *rad51* background. The phenotype of the *rad52R70K* strain, which includes weak γ-ray sensitivity, a fourfold reduction in the rate of inverted-repeat recombination, elevated allelic recombination, sporulation proficiency, and a reduction in the efficiency of mating-type switching and single-strand annealing, was similar to that observed for deletion of the *RAD59* gene. However, *rad52R70K rad59* double mutants showed synergistic defects in ionizing radiation resistance, sporulation, and mating-type switching. These results suggest that Rad52 and Rad59 have partially overlapping functions and that Rad59 can substitute for this function of Rad52 in a *RAD51 rad52R70K* strain.

HOMOLOGOUS recombination in *Saccharomyces cerevisiae* requires genes of the *RAD52* epistasis group, including *RAD50–59*, *MRE11*, and *XRS2* (Game and Mortimer 1974; Ivanov *et al.* 1992; Ajimura *et al.* 1993; Bai and Symington 1996). In general, these genes are required for mitotic recombination, meiosis, and resistance to DNA-damaging agents that make double-strand breaks, such as ionizing radiation and radiomimetic chemicals. However, the severity of DNA repair and recombination defects is quite variable between different mutants within the *rad52* group. The *rad52* null mutation results in the most severe phenotype, as measured by various recombination and repair assays (Resnick 1969; Ho 1975; Resnick and Martin 1976; Game *et al.* 1980; Prakash *et al.* 1980; Mortimer *et al.* 1981). The rate of spontaneous mitotic recombination between inverted repeats of the *ade2* gene is reduced by 3000-fold in a *rad52* null mutant strain. On the other hand, individual mutations in any other genes of the *RAD52* epistasis group reduce recombination by no more than 30-fold (Rattray and Symington 1994, 1995). Mating-type (*MAT*) switching is a specialized mitotic recombination event induced by the *HO* endonuclease. During *MAT* switching DNA sequences at the *MAT* locus are replaced by homologous sequences from a distant, unexpressed donor (*HML* or *HMR*) located on the same chromosome. Natural *MAT* switching is dependent on *RAD51–57*. However, only *RAD52* is required in a modified system where the donor is simultaneously not silenced and located on a plasmid (Sugawara *et al.* 1995). When an HO-induced double-strand break (DSB) is made in one homologue in a diploid strain, repair is dependent on *RAD52*. However, in *rad51*, *rad54*, *rad55*, and *rad57* mutants an aberrant repair, termed break-induced replication, occurs, which results in restoration of the broken chromosome arm (Malkova *et al.* 1996).

Recombination between direct repeats can occur by a variety of mechanisms (Klein 1995). When a DSB is made between direct repeats, the ends are processed by a 5′-3′ nuclease to reveal complementary single-stranded regions corresponding to the direct repeats. These sequences can then be annealed, resulting in a deletion product. This mechanism for deletion formation, termed single-strand annealing (SSA), is dependent on *RAD52*, but independent of *RAD51*, *RAD54*, *RAD55*, and *RAD57* (Sugawara and Haber 1992; Ivanov *et al.* 1996). The defect in SSA observed in *rad52* mutants can be partially suppressed by the *rfa1-D228Y* allele, which decreases the affinity of replication factor A (RPA) for single-stranded DNA (Smith and Rothstein 1995, 1999). The requirement for *RAD52* in SSA also depends on the number of repeats, as a DSB in the rDNA locus can be repaired in the absence of *RAD52* (Ozenberger and Roeder 1991). The ubiquitous requirement for *RAD52* in DNA recombination and repair suggests a pivotal role of this gene.

Biochemical and two-hybrid studies demonstrate that Rad52 binds to the Rad51 protein, a homologue of bacterial RecA proteins (Shinohara *et al.* 1992; Milne

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and Weaver 1993; Ogawa *et al.* 1993; Donovan *et al.* 1994; Hays *et al.* 1995; Sung 1997). The observation that high-level expression of *RAD51* suppresses certain *rad52* mutations, but not the null mutation of *RAD52*, is consistent with this direct interaction (Milne and Weaver 1993; Schild 1995; Kaytor and Livingston 1996). Genetic and two-hybrid studies have also identified an interaction between Rad52 and RPA (Firmenich *et al.* 1995; Smith and Rothstein 1995; Park *et al.* 1996; Hays *et al.* 1998). The Rad51 protein mediates a DNA strand exchange reaction, which is stimulated by the single-stranded DNA binding factor RPA (Sung 1994; Sung and Robberson 1995). *In vitro*, efficient strand exchange requires the addition of RPA after Rad51 has nucleated onto the single-stranded DNA. If the single-stranded DNA is incubated with Rad51 and RPA simultaneously, the efficiency of strand exchange decreases dramatically, indicating that RPA can be both a stimulator and an inhibitor. The inclusion of the Rad52 protein can relieve the inhibitory effect of RPA and restores the efficiency of strand exchange to that observed by the sequential action of Rad51 and RPA (Sung 1997; Benson *et al.* 1998; New *et al.* 1998; Shinohara and Ogawa 1998). Thus, Rad52 acts as a cofactor for the Rad51 recombinase in overcoming the competition by RPA for binding to single-stranded DNA. *In vitro* assays reveal that purified Rad52 is capable of binding to both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) and catalyzing annealing reactions between complementary ssDNAs (Mortensen *et al.* 1996). Furthermore, the inhibitory effect imposed by RPA on single-strand annealing is overcome by Rad52 (Shinohara *et al.* 1998; Sugiyama *et al.* 1998).

Homologues of the yeast Rad52 protein have been found in other organisms and in *S. cerevisiae* itself (Bezzubova *et al.* 1993; Milne and Weaver 1993; Ostermann *et al.* 1993; Bendixen *et al.* 1994; Muris *et al.* 1994; Bai and Symington 1996). The conserved amino acids mostly reside at the N-terminal portion of this family of proteins. The N-terminal region has been suggested to be responsible for binding to DNA, while the C-terminal region is thought to be involved in the interaction with the Rad51 protein (Milne and Weaver 1993; Donovan *et al.* 1994; Mortensen *et al.* 1996; Shinohara and Ogawa 1998). Since the discovery of *RAD52*, many mutant alleles have been isolated and characterized (Resnick 1969; Malone *et al.* 1988; Boundy-Mills and Livingston 1993; Kaytor and Livingston 1994, 1996; Schild 1995). Mutations have been identified in both conserved and nonconserved residues of Rad52. The first *RAD52* allele isolated, *rad52-1*, is a missense mutation resulting in an A to V change at position 90, which is conserved among all of the Rad52 family members (Resnick 1969; Adzuma *et al.* 1984). *rad52-1* is indistinguishable from a deletion mutation in almost every aspect examined, including the severe sensitivity to DNA-damaging agents, the deficiency in mating-type switching and other types of mitotic recombination, and the inability to complete meiosis. However, most other *rad52* alleles retain some Rad52 function. For example, the *rad52-2* mutation is caused by a single amino acid change from P to L at position 64, which is conserved across the whole family of Rad52-like proteins except in the yeast Rad59 protein. Despite the fact that the *rad52-2* mutant fails to sporulate successfully and is severely sensitive to methyl methanesulfonate (MMS), the mutant retains significant levels of mitotic recombination and even exhibits a hyper-recombinational phenotype for interchromosomal recombination (Malone *et al.* 1988; Boundy-Mills and Livingston 1993).

In this study we were interested in identifying factors responsible for *RAD51*-independent mitotic recombination. We carried out a screen for recombination-defective mutants in a *rad51* strain containing an inverted-repeat recombination substrate. From this screen a non-null allele of *RAD52* that caused weak γ-ray sensitivity, reduced mitotic recombination, but was not sporulation defective, was identified. This mutation displayed synergistic effects with the *rad51* mutation for inverted-repeat recombination and with the *rad59* mutation for γ-ray sensitivity, mating-type switching, and sporulation.

MATERIALS AND METHODS

Yeast strains: The relevant genotypes of the yeast strains used in this study are given in Table 1. All strains are derivatives of strains W303-1A or W303-1B (Thomas and Rothstein 1989). Strains containing the *ade2-5′Δ-TRP1-ade2-n* construct and a deletion of the *RAD51* gene were described previously (Bai and Symington 1996). A strain containing a deletion-disruption allele of the *RAD59* gene (*rad59::LEU2*), B368-1A, was constructed by PCR using the following primers: 5′ GAGG GAGTCTGTGGCAGTTTAGCACATGCTTTGGACCATT ctcgaggagaacttctagta3′; 5′ ATATGCGTGCCTTTAGCATCC CTCCAATTTGATAAAAGTCGtcgactacgtcgtaaggccg 3′. Bases in uppercase represent *RAD59* sequences and those in lowercase represent *LEU2* sequences. Disruption of the *RAD59* gene was confirmed by PCR. To construct strain B420, strain W1479-11C was first transformed with plasmid pRS414:*MAT***a** to allow mating to B413-8C. Haploid progeny derived from this cross were grown nonselectively and then screened on SC-Trp to identify plasmid-free segregants. All other strains were made by mating the appropriate strains, sporulating and dissecting tetrads from the resulting diploids, and screening haploid progeny for those with the required genotypes.

Plasmids: pRS416:*Erad52* was constructed by inserting a 2.4-kb *Eco*RI-digested, *rad52R70K*-containing fragment at the *Eco*RI site of pRS416. The fragment was made by a PCR reaction performed on the genomic DNA of a *rad52R70K* strain with the use of the following primers: 5′ gataaGAATTCgccta gaatgaaagtaagtgaattagcg 3′; 5′ gatgGAATTCaatgaacctaaggattc cgctg 3′. pRS416:*ERAD52* was made in a similar way except that the inserted fragment was amplified by using wild-type genomic DNA as the PCR template. pRS426:*Erad52* was made by cloning the 2.4-kb *rad52R70K* fragment purified from *Eco*RI-digested pRS416:*Erad52* into the *Eco*RI site of pRS426. YEp24:*RAD51* contains a 3.7-kb *RAD51* fragment inserted at the *Bam*HI site of YEp24. YEp24:*RAD59* contains a *RAD59*

TABLE 1

Yeast strains

Strain	Genotype or description	Source
W303-1A	MATa	Thomas and Rothstein (1989)
W303-1B	MATα	Thomas and Rothstein (1989)
LSY255	MATα rad52::TRP1	Huang and Symington (1994)
B354-1A	MATa rad51::HIS3	Bai and Symington (1996)
B368-1A	MATa rad59::LEU2	This study
B355-6D	MATa ade2::hisG-URA3-his Ghis3::ade2-5′Δ-TRP1-ade2-n	Bai and Symington (1996)
B356-7C	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n	Bai and Symington (1996)
B356-13D	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad51::HIS3	Bai and Symington (1996)
B356-11A	MATα ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad51::HIS3	Bai and Symington (1996)
No. 17	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad51::HIS3 rad52R70K	This study
B400-1A	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad52R70K	This study
B361-4C	MATα ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad59::LEU2	Bai and Symington (1996)
B361-7D	MATα ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad51::HIS3 rad59::LEU2	Bai and Symington (1996)
B365-11C	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad52::TRP1	Bai and Symington (1996)
B413-5C	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad51::HIS3 rad52R70K	This study
B413-8C	MATα ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad52R70K rad59::LEU2	This study
B413-9C	MATa ade2::hisG his3::ade2-5′Δ-TRP1-ade2-n rad51::HIS3 rad52R70K rad59::LEU2	This study
B413-13B	MATα rad52R70K	This study
B384-1A	MATa chromIII::Tn903 (Tn903=IS903-kan^r-IS903)	Bai and Symington (1996)
B385-10C	MATα chromIII::Tn903 (Tn903=IS903-kan^r-IS903) rad51::HIS3	Bai and Symington (1996)
B385-4C	MATa chromIII::Tn903 (Tn903=IS903-kan^r-IS903) rad59-1	Bai and Symington (1996)
B385-1B	MATα chromIII::Tn903 (Tn903=IS903-kan^r-IS903) rad51::HIS3 rad59-1	Bai and Symington (1996)
B386-9B	MATα chromIII::Tn903 (Tn903=IS903-kan^r-IS903) rad52R70K	This study
B386-11C	MATα chromIII::Tn903(Tn903=IS903-kan^r-IS903) rad51::HIS3 rad52R70K	This study
B366-6A	MATa ade2-n	Bai and Symington (1996)
B366-7A	MATa ade2-a	Bai and Symington (1996)
B372	MATa ade2-a MATα ade2-n	Bai and Symington 1996)
B374	MATa ade2-a rad59::LEU2 MATα ade2-n rad59::LEU2	Bai and Symington (1996)
B377	MATa ade2-a rad51::HIS3 MATα ade2-n rad51::HIS3	Bai and Symington (1996)
B380	MATa ade2-a rad51::HIS3 rad59::LEU2 MATα ade2-n rad51::HIS3 rad59::LEU2	Bai and Symington (1996)
B416	MATa ade2-a rad52R70K MATα ade2-n rad52R70K	This study
B417	MATa ade2-a rad52R70K rad59::LEU2 MATα ade2-n rad52R70K rad59::LEU2	This study
B418	MATa ade2-a rad51::HIS3 rad52R70K MATα ade2-n rad51::HIS3 rad52R70K	This study
B419	MATa ade2-a rad51::HIS3 rad52R70K rad59::LEU2 MATα ade2-n rad51::HIS3 rad52R70K rad59::LEU2	This study
W1479-11C	MAT::HIS3 leu2ΔEcoRI::URA3-HOcs::leu2ΔBstEII	Smith and Rothstein (1999)
B420-9D	MAT::HIS3 leu2ΔEcoRI::URA3-HOcs::leu2ΔBstEII	This study
B420-1B	MAT::HIS3 leu2ΔEcoRI::URA3-HOcs::leu2ΔBstEII rad52R70K	This study
B420-3A	MAT::HIS3 leu2ΔEcoRI::URA3-HOcs::leu2ΔBstEII rad59::LEU2	This study
B420-6C	MAT::HIS3 leu2ΔEcoRI::URA3-HOcs::leu2ΔBstEII rad52R70K rad59::LEU2	This study

All strains are in the W303 background (*his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1 can1-100*); only differences from this genotype are noted.

fragment inserted at the *Bam*HI site of YEp24. pRS416-SU that carries the *leu2* inverted repeats and plasmids pFH800 and pBM272-HO used for induction of the HO endonuclease have been described previously (Nickoloff *et al.* 1986; Bai and Symington 1996; Moreau *et al.* 1999).

Cell growth and genetic methods: Cells were grown on either YEPD or synthetic complete (SC) media for most procedures (Sherman *et al.* 1986). Selection for Ura^- cells was performed on SC medium containing 5-fluoroorotic acid (5-FOA) at 1 mg/ml. Selection for G418-resistant cells was on YEPD me-

dium containing 0.5 mg/ml of G418. To induce expression of the HO endonuclease, cultures were grown in synthetic medium containing 2% raffinose as a carbon source prior to the addition of galactose. Yeast mating, sporulation, and tetrad dissection were performed as described (Sherman *et al.* 1986). Cells were grown at 30° unless otherwise indicated. The measurement of recombination rates was as described previously (Bai and Symington 1996). Results for strains not containing the *rad52R70K* mutation have been presented previously (Bai and Symington 1996) and were included here for comparison. Rates for the *ade2* and *leu2* inverted repeats and diploid heteroallelic recombination were determined with three independent isolates and the mean values are presented. Rates were determined only once for the Tn903 substrate and the median values are presented.

Mutagenesis: Mutagenesis was performed as described in Bai and Symington (1996).

Determination of γ-ray sensitivity: Cells were grown in liquid medium at 30° to mid-log phase. Synthetic medium lacking uracil was used to maintain selection for plasmids. Serial dilutions were made from each culture and aliquots of each dilution were spotted onto solid medium YEPD medium or medium lacking uracil. Cells were radiated in a Gammacell-220 ^{60}Co irradiator (Atomic Energy of Canada) with various doses of γ-rays. Percentage survival was measured after 3–4 days of incubation at 30°. Each experiment was repeated three times on independent transformants and the mean value was presented.

Physical analysis of mating-type switching and single-strand annealing: Strains to be tested for mating-type switching (W303-1B, B361-4C, B413-13B, and B413-8C) were transformed to Ura$^+$ with plasmid pBM272-HO. Ura$^+$ plasmid-containing transformants were grown in 5 ml SC-Ura medium for 24 hr. Cells were harvested, washed with water, and used to innoculate 250 ml SC (raffinose)-Ura. Cultures were grown to an OD_{600} of 0.4–0.5. A total of 50 ml of culture was removed for the 0 hr timepoint and then 22.5 ml of 20% galactose was added. One hour after addition of galactose, the cultures were harvested and resuspended in 250 ml of YEPD. Fifty-milliliter samples were removed at 1-hr intervals after induction for DNA analysis. Cells were harvested by centrifugation and washed with water, and the cell pellets were frozen in liquid nitrogen. DNA was extracted from the thawed cell pellets and digested with *Sty*I, and DNA fragments were separated by electrophoresis through 1% agarose gels. DNA fragments were transferred to nylon membranes and hybridized with a 300-bp PCR fragment generated by amplification of *MAT* sequences distal to the HO cut site.

The strains to be used for physical analysis of HO-induced deletion formation (B420-9D, B420-1B, B420-3A, and B420-6C) were transformed to Trp$^+$ using pFH800. Induction of HO was as described above except cells were grown in medium lacking tryptophan. DNA samples were digested with *Spe*I and DNA fragments separated by electrophoresis through 0.8% agarose gels. DNA fragments were transferred to nylon membranes and hybridized with a 400-bp PCR fragment generated by amplification of sequences from the YCL017 ORF, which is adjacent to *LEU2*.

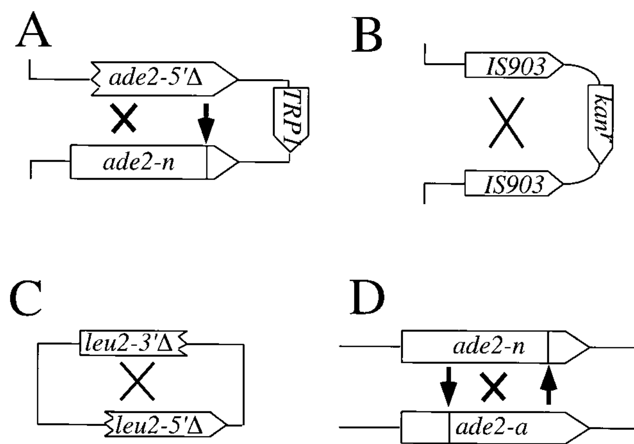

Figure 1.—(A) *ade2* inverted-repeat substrate. The substrate contains an intrachromosomal inverted duplication of heteroalleles of the *ade2* gene integrated at the *HIS3* locus on chromosome *XV*. The heteroalleles are separated by an active *TRP1* gene. One of the *ade2* alleles contains a deletion on the 5′ side (*ade2-5′*Δ), and the other one has a frameshift mutation at a *Nde*I site on the 3′ side (*ade2-n*). Initial strains with nonrecombined substrates (Ade$^-$) form red colonies on nonselective media due to accumulation of a red pigment in the adenine biosynthetic pathway. If recombination between the two alleles produces a wild-type *ADE2* gene, a white sector will be formed within the red colony. Thus, the recombination level of a strain can be visually assessed by colony sectoring and quantitatively determined by measuring the frequency of Ade$^+$ prototrophs within a population of cells. Ade$^+$ recombinants can arise by gene conversion and/or events that invert the *TRP1* gene. (B) The *IS903* inverted-repeat substrate. A fragment of the bacterial transposon *Tn903* was integrated into yeast chromosome *III* between *HIS4* and *HML* loci. The fragment contains the kanamycin resistance gene (*kan^r*) of *Tn903*, flanked by two inverted copies of the *IS903* sequences. The inverted *IS903* repeats can undergo an intrachromosomal reciprocal exchange resulting in inversion of the *kan^r* gene. A yeast strain containing a single copy of the *kan^r* gene in its original orientation is sensitive to 0.5 mg/ml of G418. After inversion the *kan^r* gene confers resistance to G418, possibly because the *kan^r* gene is then expressed more efficiently (Willis and Klein 1987). (C) The *leu2* inverted-repeat substrate. The substrate is carried on a *CEN* plasmid and contains two alleles of the yeast *LEU2* gene that have been truncated for the 5′ and 3′ coding regions, respectively. A functional *LEU2* gene can be generated by recombination between the inverted *leu2* repeats (Prado and Aguilera 1995). (D) Interchromosomal recombination substrate. One of the *ade2* alleles of the diploid contains a fill-in mutation at the 3′ *Nde*I site, and the other one contains a fill-in mutation at the 5′ *Aat*II site (Huang and Symington 1994; Bai and Symington 1996). A functional *ADE2* gene can be generated by interchromosomal recombination.

RESULTS

Isolation of a novel *rad52* allele: To measure the level of recombination *in vivo*, a previously described recombination substrate was utilized (Rattray and Symington 1994) (Figure 1). The substrate is located on chromosome *XV* and consists of inverted heteroalleles of the *ADE2* gene. Both alleles are inactive, but the substrate can be rearranged by recombination events to form a functional *ADE2* gene. Using this substrate, the average rate of recombination was determined to be 1.8×10^{-4} events/cell/generation in wild-type strains and 3.5×10^{-5} in *rad51*Δ mutants. A screen for recombination mutants was performed in the *rad51*Δ background. A *rad51*Δ strain (B356-13D) was mutagenized by *N*-methyl-

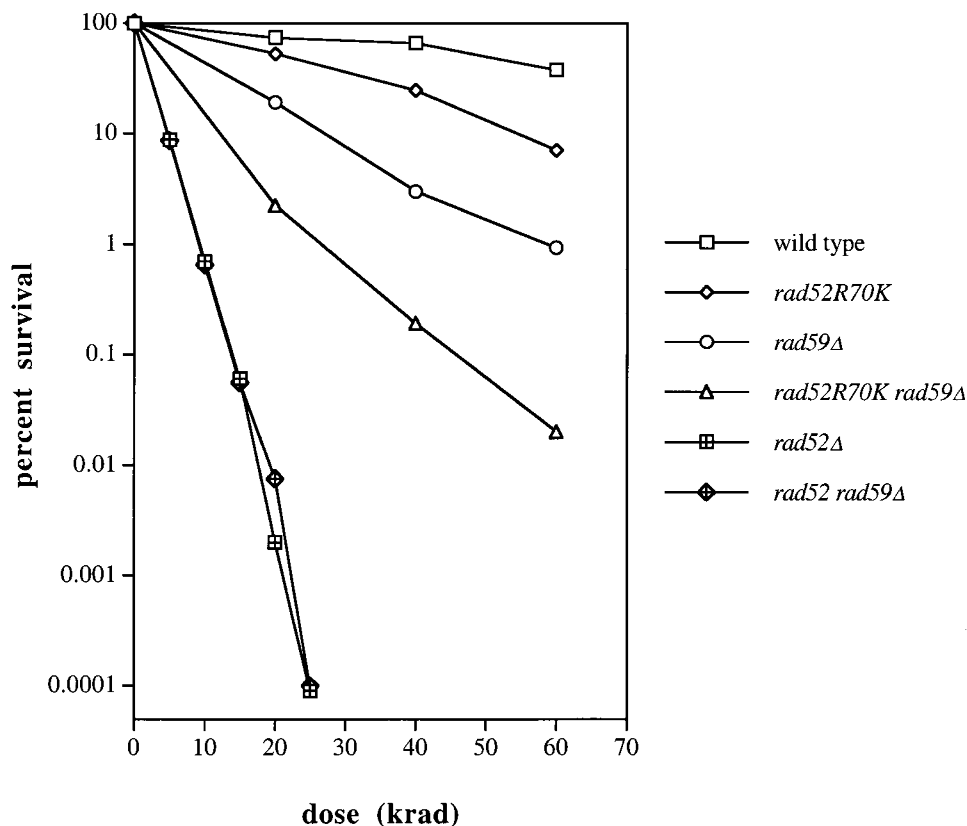

Figure 2.—The *rad52R70K* and *rad59* mutations synergistically increase sensitivity to γ-irradiation.

N′-nitro-*N*-nitrosoguanidine (MNNG) and mutants displaying reduced sectoring were isolated. Approximately 15,000 colonies were screened. Since a *rad52* null mutation was known to dramatically reduce recombination of the *ade2* substrate (>3000-fold), isolated mutants were tested for complementation of γ-ray sensitivity by a *rad52*Δ strain (LSY255). Three mutants completely failed to complement the *rad52*Δ strain in that diploids from the crosses were as sensitive as a *rad52*Δ homozygous diploid strain to γ-irradiation, indicating that these mutants were likely to have acquired *rad52* null mutations. One other mutant, no. 17, partially complemented the *rad52* strain in the γ-ray complementation test, suggesting a partial loss-of-function mutation of *RAD52.* The reduced-sectoring phenotype of no. 17 was not due to loss or mutation of the inverted-repeat recombination substrate, because diploids from the cross between no. 17 and LSY255 sectored at close to the wild-type level, and no. 17 was the only source for the inverted-repeat substrate. Mutant 17 was then backcrossed to an isogenic *rad51* strain (B356-11A). Because the resulting diploids were homozygous for the *rad51*Δ mutation and were defective in meiosis, a *RAD51*-containing plasmid, YEp24:*RAD51*, was introduced into the diploids to complement the defect. The diploids were sporulated and after tetrad dissection plasmid-free haploid progeny were obtained by counterselecting against the plasmid marker gene *URA3* on 5-FOA-containing medium. Haploid progeny were streaked out on YEPD plates and examined for sectoring. The low-sectoring phenotype segregated 2:2 in this backcross, indicating that the unidentified mutation in mutant 17 was a single gene trait. Mutant 17 was derived from a *rad51*Δ strain and was thus extremely sensitive to γ-irradiation. To test whether the unidentified mutation in no. 17 would confer γ-ray sensitivity by itself, a strain that carried the unidentified mutation but had a wild-type *RAD51* gene (B400-1A) was made. B400-1A displayed a mild γ-ray sensitivity, at a level between that of the *rad52*Δ mutant and wild type (see Figure 2). To confirm that the unidentified mutation in no. 17 was allelic to the *RAD52* locus, B400-1A was crossed to LSY255 (*rad52*Δ). Diploids from this cross were capable of sporulation, but at a reduced level. Following tetrad dissection, the haploid progeny were tested for γ-ray sensitivity. The intermediate-sensitivity phenotype representative of the unidentified mutation always segregated away from the severe-sensitivity phenotype rendered by the *rad52*Δ mutation. This result confirmed that the unidentified mutation in mutant 17 was allelic to *RAD52*, or very closely linked.

Cloning and identification of the novel *rad52* allele: DNA fragments containing the unidentified *rad52* allele and the wild-type *RAD52* sequence were amplified by PCR from the genomic DNA of mutant 17 and W303-1A, respectively. On the basis of the sequence of the *RAD52* locus, the wild-type fragment expected was 2.4 kb in length, covering a region extending from 462 bp upstream of the first ATG of the *RAD52 ORF* to 395

TABLE 2

Recombination rates of the *ade2* inverted-repeat substrate

Strain	Relevant genotype	Ade^{+} rate ($\times 10^{-6}$)	Relative rate
B356-7C	*RAD*	180 ± 26	100
B356-13D	*rad51Δ*	35 ± 4	19
B400-1A	*rad52R70K*	33 ± 1	18
B361-4C	*rad59Δ*	43 ± 14	24
B413-8C	*rad52R70K rad59Δ*	10 ± 1	5.6
B413-5C	*rad51Δ rad52R70K*	0.093 ± 0.03	0.051
B361-7D	*rad51Δ rad59Δ*	0.16 ± 0.04	0.089
B413-9C	*rad51Δ rad52R70K rad59Δ*	0.084 ± 0.03	0.047
B365-11C	*rad52Δ*	0.039 ± 0.02	0.022

bp downstream of the stop codon. The amplified PCR fragment carrying the *rad52* allele was of the same size as the corresponding wild-type fragment, indicating that the unidentified mutation in mutant 17 did not involve a large deletion or insertion. The 2.4-kb PCR fragment was cloned into the *Eco*RI site of *pRS416* to create recombinant plasmids containing the mutant allele and the wild-type *RAD52*, respectively. The mutant plasmid (pRS416:*Erad52*), upon transformation into LSY255 (*rad52* null), partially restored the strain's resistance to γ-ray radiation to a level similar to that of B400-1A, whereas the wild-type plasmid (pRS416:*ERAD52*) fully complemented LSY255. By replacing DNA segments from the wild-type sequence with those from the mutant, the location of the unidentified *rad52* mutation was narrowed down to the region 5′ of the single *Bgl*II site in the cloned fragment. DNA sequence analysis revealed a single nucleotide change from G to A at position 209 of the *RAD52 ORF*, resulting in a R to K missense mutation at position 70 of the Rad52 protein. The observed mutation was identified from independently generated PCR fragments ruling out the trivial possibility of artifact from a PCR-derived nucleotide misincorporation. The *rad52* allele in mutant 17 was designated *rad52R70K.*

***rad52R70K* and *rad51Δ* synergistically reduce recombination:** Mitotic recombination, assayed on the *ade2* inverted-repeat substrate, was reduced 5-fold in a *rad51Δ* mutant and colonies sectored at a level slightly lower than the wild-type strain (Rattray and Symington 1994). Strain B400-1A (*rad52R70K*), like a *rad51Δ* mutant, displayed only slightly reduced colony sectoring compared with wild type. However, mutant 17 (*rad51Δ rad52R70K*) was isolated from a *rad51Δ* background by the strong reduction in colony sectoring. Thus, the *rad52R70K* mutation displayed a synergistic effect with the *rad51Δ* mutation. The synergistic reduction in recombination was verified by determining the rates of recombination for each strain (Table 2). Compared with wild-type strains, recombination rates in *rad51Δ* or *rad52R70K* single mutants were reduced only 5-fold, whereas the rates in *rad51Δ rad52R70K* double mutants were reduced 1900-fold.

RAD59, a recombination gene encoding a Rad52 homologue, was identified in the same genetic screen in which mutant 17 was isolated (Bai and Symington 1996). When tested on the *ade2* inverted-repeat substrate, a *rad59Δ* mutation resembled the *rad52R70K* mutation in that *rad59Δ* single mutants showed a 4- to 5-fold reduction in recombination rates, whereas *rad51Δ rad59Δ* double mutants were reduced 1100-fold. The similarity in phenotype between *rad59Δ* and *rad52R70K* strains suggested that both might be defective in the same recombination pathway, in which case a double mutant would be expected to show the same recombination rate as the single mutants. The *rad59Δ rad52R70K* double mutant showed a 3- to 4-fold reduction in the recombination rate compared with strains containing either mutation alone, indicative of additive effects (Table 2). However, a *rad51Δ rad52R70K rad59Δ* triple mu-

TABLE 3

Recombination rates of the Tn903 inverted-repeat substrate

Strain	Relevant genotype	Rate of G418^{r} cells	Relative rate
B384-1A	*RAD*	2.3×10^{-7}	100
B385-10C	*rad51Δ*	5.3×10^{-8}	23
B386-9B	*rad52R70K*	2.4×10^{-8}	10
B385-4C	*rad59-1*	5.1×10^{-8}	22
B386-11C	*rad51Δ rad52R70K*	$<1.0 \times 10^{-8}$	<4
B385-1B	*rad51Δ rad59-1*	$<1.0 \times 10^{-8}$	<4

TABLE 4

Recombination rates of the *leu2* inverted-repeat substrate

Strain	Relevant genotype	Rate of Leu$^+$ cells	Relative rate
B384-1A	*RAD*	$3.3 \pm 1.4 \times 10^{-6}$	100
B385-10C	*rad51Δ*	$5.5 \pm 0.5 \times 10^{-6}$	169
B386-9B	*rad52R70K*	$9.4 \pm 0.9 \times 10^{-7}$	28
B385-4C	*rad59-1*	$5.1 \pm 2.0 \times 10^{-7}$	16
B386-11C	*rad51Δ rad52R70K*	$9.1 \pm 5.3 \times 10^{-7}$	28
B385-1B	*rad51Δ rad59-1*	$4.7 \pm 1.3 \times 10^{-8}$	14

tant showed a rate of recombination similar to those of *rad51Δ rad52R70K* and *rad51Δ rad59Δ* strains, suggesting that the additive effect conferred by the *rad52R70K* and *rad59Δ* mutations is dependent on *RAD51.*

To determine if the *rad52R70K* mutation has a general effect on recombination, rates were determined using several other recombination substrates (Figure 1). Assayed on a substrate consisting of intrachromosomal inverted *IS903* repeats, a *rad52R70K* or *rad51Δ* single mutation reduced recombination slightly, whereas when combined together these two mutations synergistically reduced recombination (Table 3). Similar results were observed for the *rad59-1* mutation, a *rad59* allele indistinguishable from the null mutation in recombination and repair assays (Bai and Symington 1996). Previous studies showed a reduction >100-fold in the rate of recombination of this reporter in a *rad52-1* strain (Willis and Klein 1987). The synergism seen on the *IS903* substrate was consistent with that on the *ade2* substrate. However, a different result was obtained using a substrate where recombination occurs between inverted *leu2* heteroalleles located on a plasmid (Prado and Aguilera 1995). On this substrate a *rad52R70K* mutation reduced recombination 3–4-fold, compared with a 100-fold reduction conferred by the *rad52-1* allele (Prado and Aguilera 1995). A *rad51Δ* mutation had little or no effect and no synergistic effect was found between *rad51Δ* and *rad52R70K* mutations since a double mutant was not significantly different from a *rad52R70K* single mutant strain (Table 4). The effect of *rad52R70K* on interchromosomal recombination was tested by determining the rate of Ade$^+$ prototroph formation between *ade2* heteroalleles located on homologous chromosomes in diploids. In this assay a *rad52R70K* or *rad59Δ* mutation actually elevated the rate of recombination (Table 5). This contrasts with *rad52* null strains, which show a large decrease in the rate of heteroallelic recombination (Hoekstra *et al.* 1986; Resnick *et al.* 1986). The rate in the *rad51Δ* strain was below the measurable range of this assay, as were rates in *rad51Δ rad52R70K*, *rad51Δ rad59Δ*, and *rad51Δ rad52R70K rad59Δ* strains (data not shown). Thus, interchromosomal recombination is dependent on *RAD51*, but not the functions disabled by *rad52R70K* or *rad59Δ* mutations. The *rad52R70K* and *rad59* mutations exhibited similar defects in mitotic recombination using all of these substrates.

***rad52R70K* and *rad59Δ* synergistically increase γ-ray sensitivity:** Mutations in genes required for homologous recombination often result in increased sensitivity to DNA damage agents such as ionizing-radiation and radiomimetic chemicals. Among genes of the *RAD52* epistasis group in *S. cerevisiae*, a mutation in *RAD59* causes an intermediate sensitivity to γ-ray radiation, while null mutations in other genes of the group lead to severe sensitivity. Upon exposure to γ-irradiation, a *rad52R70K* mutant strain displayed a survival level between that of a *rad59Δ* mutant and that of a wild-type strain (Figure 2). The *rad52R70K rad59Δ* double mutant was more sensitive to ionizing radiation than either of the single mutants, indicating a synergistic effect between *rad52R70K* and *rad59Δ* mutations in DNA repair. The *rad52Δ rad59Δ* double mutant was as sensitive to γ-irradiation as the *rad52Δ* single mutant.

***rad52R70K* is suppressed by overexpression of the mutant allele:** In a *rad52R70K* single mutant strain, the introduction of a high-copy-number plasmid carrying the *rad52R70K* allele (pRS426:*Erad52*) partially restored the strain's resistance to γ-irradiation (Figure 3). The sensitivity of the *rad52R70K rad59Δ* double mutant was also partially suppressed by the same plasmid. In neither

TABLE 5

Rates of heteroallelic recombination in diploids

Strain	Relevant genotype	Rate of Ade$^+$ cells	Relative rate
B372	*RAD* / *RAD*	$5.1 \pm 1.5 \times 10^{-7}$	100
B377	*rad51Δ* / *rad51Δ*	$<1.0 \times 10^{-8}$	<2
B416	*rad52R70K* / *rad52R70K*	$2.7 \pm 1.7 \times 10^{-8}$	529
B374	*rad59Δ* / *rad59Δ*	$3.3 \pm 1.7 \times 10^{-8}$	647
B417	*rad52R70K rad59Δ* / *rad52R70K rad59Δ*	$4.8 \pm 1.6 \times 10^{-8}$	941

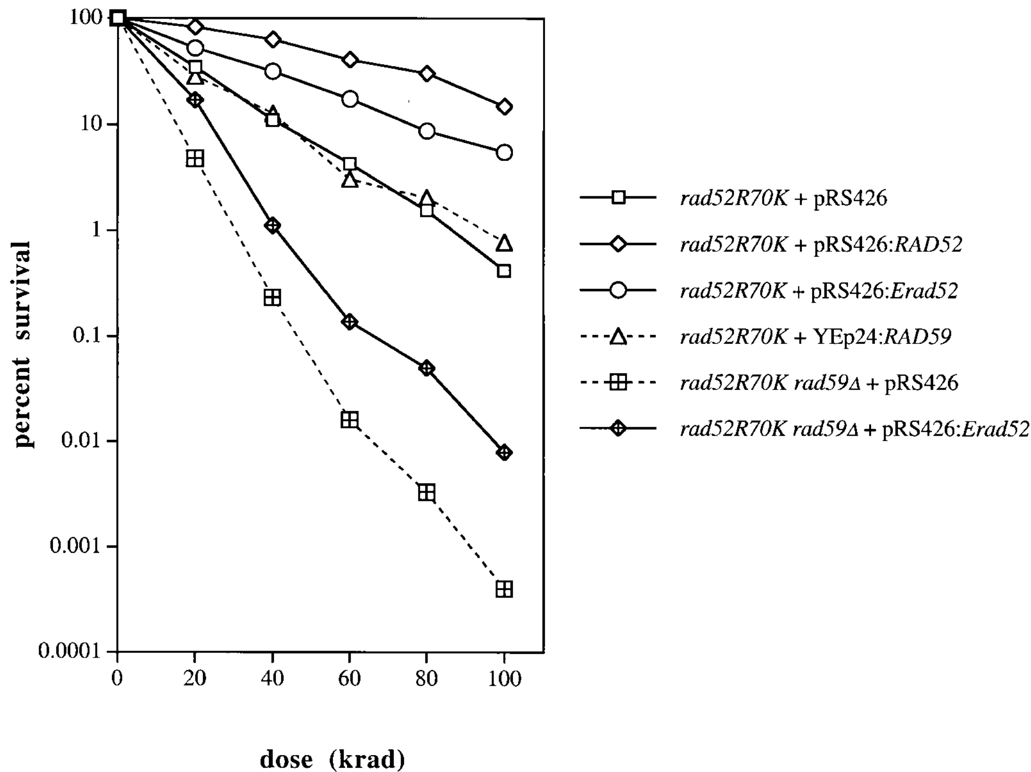

Figure 3.—Suppression of the γ-ray sensitivity of *rad52R70K* strains by increased copy number of the *rad52R70K* allele.

case was the suppression complete. The *rad52R70K* mutation could not be suppressed by YEp24:*RAD59*, a high-copy-number plasmid carrying *RAD59*, and the *rad59Δ* mutation could not be suppressed by pRS426:*Erad52.*

Defects caused by the *rad52R70K* mutation could have arisen from a weakened Rad52 activity and/or a reduced level of Rad52 expression. In either case the mutant phenotype could be suppressed by the overexpression of the mutant allele. However, by Western-blotting analysis using anti-Rad52 antibodies we were unable to detect significant differences in the level of Rad52 between *rad59Δ*, *rad52R70K*, *rad59Δ rad52R70K*, and wild-type strains (data not shown). Thus, it is reasonable to suggest that the *rad52R70K* mutant phenotype is due to impaired activity of the Rad52 mutant protein.

The repair of HO-induced DSBs is defective in *rad52R70K* and *rad59Δ* mutants: γ-Irradiation creates a variety of DNA lesions in addition to double-strand breaks. To determine whether the *rad52R70K*, *rad59Δ*, and *rad52R70K rad59Δ* strains are defective in the repair of a single double-strand break, a mating-type switching assay was performed. The repair of an HO endonuclease-induced double-strand break (DSB) was monitored at the DNA level after induction of HO endonuclease for 1 hr. To measure the formation of switched products, the DNA samples were digested with *Sty*I, which cuts within Y**a** but not Yα sequences. The appearance of a 0.9-kb *Sty*I fragment is indicative of repair of the DSB from the *HMR***a** locus (Figure 4). In the wild-type strain, switching was efficient and completed 3 hr after induction of HO. In both *rad52R70K* and *rad59Δ* mutants switching was delayed and most of the cut DNA was not converted to *MAT***a** product. This defect was even more severe in the double mutant. Although cut fragment was produced and disappeared with normal kinetics, there was a greater reduction in the formation of switched product compared with the single mutants. The disappearance of the 0.7-kb cut fragment suggests that exonuclease degradation from the DSB occurs normally, but subsequent events are defective in these mutants. To ensure that the mutants were proficient in formation of the single-stranded tail at the break site, DNA samples digested with *Sty*I and *Bam*HI were analyzed by alkaline gel electrophoresis (White and Haber 1990; Moreau *et al.* 1999). Single-stranded tails were formed in all of the mutants with the same kinetics indicating no defect in the nuclease-processing step of the reaction (data not shown). At the 5 hr timepoint, cells from all strains were plated on SC-Ura (to select for those containing the HO plasmid) for phenotypic analysis; 63% of wild type, 6.3% of *rad52R70K*, 26.7% of *rad59Δ*, and 1.1 % of the *rad52R70K rad59Δ* colonies were *MAT***a** maters, consistent with the physical analysis.

The repair of DSBs can occur by a variety of mechanisms. When a DSB is made between direct repeats, repair can occur by single-strand annealing. This nonconservative reaction occurs by 5′-3′ degradation from the DSB site to reveal complementary single-stranded regions that can anneal, resulting in deletion of DNA between the repeats (Haber 1995). This reaction is dependent on *RAD52*, but is independent of other tested genes in the *RAD52* epistasis group (Ivanov *et*

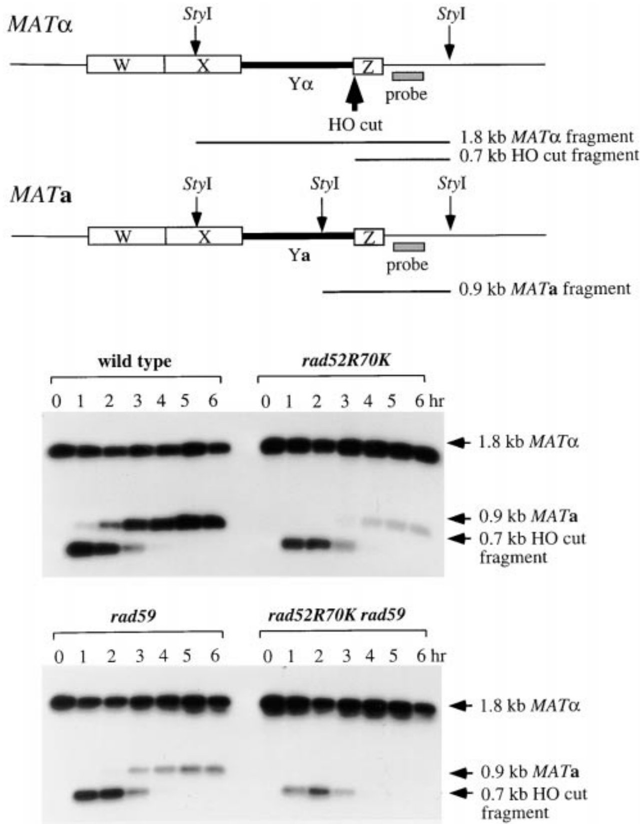

Figure 4.—Kinetics of mating-type switching. A schematic representation of the *MAT***a** and *MAT*α loci indicating the locations of the *Sty*I sites and the hybridization probe (top). HO endonuclease produces a 0.7-kb fragment from the 1.8-kb *MAT*α *Sty*I fragment. A 0.9-kb *Sty*I fragment is produced when the mating type switches from *MAT*α to *MAT***a.** DNA was isolated from cultures prior to galactose induction (0-hr timepoint) and at 1-hr intervals after HO induction.

al. 1996). As the DNA binding domain of Rad52 is in the N-terminal half of the protein, it seemed possible that the *rad52R70K* mutation might affect the DNA binding and annealing properties of the mutant protein. Also, the N-terminal region of Rad52 is conserved with Rad59, suggesting that Rad59 might also participate in strand annealing. Strains containing direct repeats of the *leu2* gene, separated by a copy of the *URA3* gene and vector sequences containing the HO cut site (HO cs), were used to monitor the efficiency of DSBR by single-strand annealing (Figure 5; Smith and Rothstein 1999). HO endonuclease was induced for 1 hr and repair by SSA was monitored at the DNA level by the appearance of a 5.7-kb *Spe*I fragment. In the wild-type strain, cleavage by HO was efficient and all of the cut fragments were converted to deletion products (Figure 5B). For all of the mutants, there was efficient cleavage by HO, but there was a delay in the appearance of deletions and only half of the cut fragments were converted to the deletion products. The *rad52R70K rad59*Δ double mutant showed a decrease in the formation of deletions (27.7% deletion product at 5 hr)

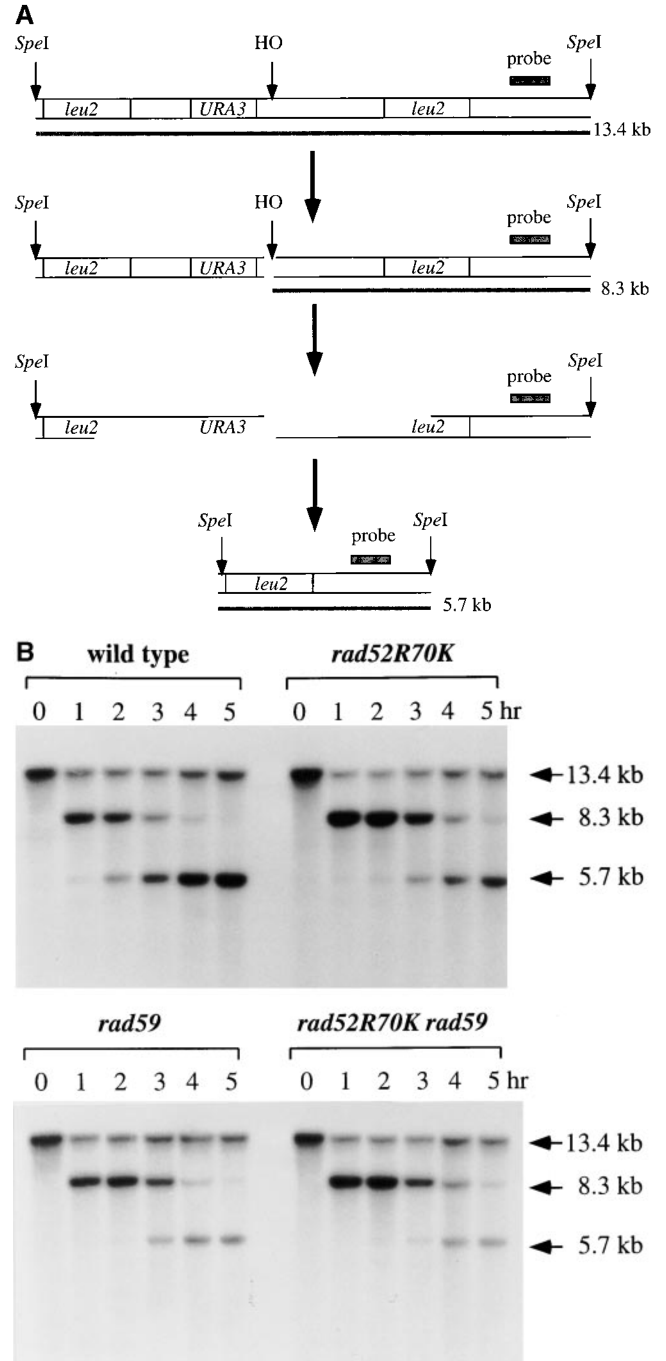

Figure 5.—Kinetics of HO-induced deletion formation. (A) Schematic representation of the *leu2* direct repeat substrate showing the locations of the *Spe*I sites, the HO cleavage site, and hybridization probe. After cleavage by HO, an 8.3-kb fragment is produced from the 13.4-kb *Spe*I fragment. The single-stranded tails formed after resection from the DSB site can anneal to form a deletion product that is detected as a 5.7-kb *Spe*I fragment. (B) DNA was extracted at the times shown and digested with *Spe*I, the position of parental, HO cut fragment, and deletion products are shown to the right of the autoradiogram.

TABLE 6

Sporulation efficiency and spore viability

Strain	Genotype	Number of tetrads with 0–4 viable spores						Spore viability (%)	Sporulation efficiency (%)
		Total	4 viable	3 viable	2 viable	1 viable	0 viable		
B372	*RAD* / *RAD*	100	92	6	2	0	0	98	78
B374	*rad59Δ* / *rad59Δ*	100	53	33	12	2	0	84	65
B416	*rad52R70K* / *rad52R70K*	140	48	58	26	4	4	75	61
B417	*rad52R70K rad59Δ* / *rad52R70K rad59Δ*	47	0	0	2	8	37	6	5

slightly greater than that of the single mutants (36.3% for *rad52R70K* and 35.9% for *rad59Δ*).

***rad52R70K* and *rad59Δ* cause synergistic meiotic defects:** Recombination gene mutations frequently lead to meiotic defects. A diploid strain homozygous for the *rad52R70K* mutation showed a sporulation efficiency of 61%, slightly lower than that of a wild-type diploid strain (78%; Table 6). The spore viability of the *rad52R70K* strain (75%) was also slightly reduced from the wild-type level (98%). A *rad59Δ* homozygous diploid strain sporulated at 65% efficiency, with a spore viability of 84%. However, a diploid strain homozygous for both *rad52R70K* and *rad59Δ* mutations displayed only 5% sporulation efficiency and 6% spore viability, which were much lower than either of the single mutant diploid strains. This result strongly suggests that the biological activities abolished by the *rad52R70K* and the *rad59Δ* mutations are required for yeast meiosis.

DISCUSSION

The isolation of the *rad52R70K* allele in a genetic screen for recombination deficiency is direct evidence that the arginine residue at position 70 of the Rad52 protein is important for function. The *rad52R70K* missense mutation causes defects in mitotic recombination and DNA repair. More interestingly, this mutation shows synergistic effects with the *rad51Δ* mutation for inverted-repeat recombination and with the *rad59Δ* mutation for γ-ray sensitivity, mating-type switching, and sporulation. However, the *rad52R70K* allele retains substantial Rad52 function, because in all of the recombination and repair assays the *rad52R70K* mutation displayed a much less severe defect than a *rad52* null mutation.

***RAD51*-independent recombination:** A single mutation of *rad52R70K*, like a *rad59Δ* mutation, reduces the rate of mitotic recombination only four- to fivefold using inverted-repeat substrates. The synergistic effects of a *rad52R70K* or *rad59Δ* mutation with the *rad51Δ* mutation indicate that *RAD59* and the *rad52R70K* disabled activity are involved in a *RAD51*-independent recombination mechanism. The *RAD51*-independent mechanism cannot completely substitute for the *RAD51*-dependent mechanism and vice versa, since the elimination of either one of them decreases recombination rates. Yet the decrease is not substantial, indicating that each type of mechanism is potent by itself. The *rad51Δ rad52R70K rad59Δ* triple mutant showed a similar rate of recombination as did the *rad51Δ rad52R70K* and *rad51Δ rad59Δ* double mutants, indicating that *RAD59* and the disabled function of *rad52R70K* are likely to function in the same pathway for mitotic recombination. However, the *rad52R70K rad59Δ* double mutant showed a rate of recombination three- to fourfold lower than those of both of the single mutants indicating additive effects. This suggests that *RAD59* and the *RAD52* activity disabled by the *rad52R70K* mutation possess biological functions largely overlapping, yet also with slight differences, in recombination events on the *ade2* inverted-repeat substrate. The synergism displayed by *rad51Δ* with *rad52R70K* or *rad59Δ* for inverted-repeat recombination suggests that there are multiple pathways for recombination of this substrate that are differentially affected by these mutations. Alternatively, Rad51 might have an overlapping function with Rad52 and Rad59. The synergistic defects could also be caused by destabilization of a multiprotein complex. Rad51 and Rad52 are known to interact and we have shown an interaction between Rad52 and Rad59 (A. Davis and L. Symington, unpublished observations).

Most genes of the *RAD52* epistasis group were identified by their requirement for the repair of ionizing-radiation-induced DNA damage (Game and Mortimer 1974), and subsequent studies revealed defects in homologous recombination. However, defects in DNA repair do not always fully correlate with recombination defects. For example, a *rad51Δ* mutant is extremely sensitive to ionizing radiation whereas the mutant exhibits heterogeneous phenotypes for recombination. A *rad51Δ* mutant is largely proficient in mitotic recombination in assays using certain types of inverted-repeat substrates, including both spontaneous and double-

strand break-induced events (Rattray and Symington 1994; Ivanov *et al.* 1996). Neither is *RAD51* required in a recombination assay using two copies of the yeast *MAT* sequences as substrates when the donor sequence is simultaneously not silenced and located on a plasmid (Sugawara *et al.* 1995). *RAD51* is also not required for the formation of Holliday junction intermediates in the rDNA or for sister chromatid joint molecules in meiosis (Schwacha and Kleckner 1997; Zou and Rothstein 1997). However, *RAD51* is required for many recombination events. *RAD51* is essential for normal mating-type switching and is important for heteroallelic recombination in diploids. *rad51*Δ diploids are unable to complete meiosis, but by both physical and genetic assays there is only a 5- to 10-fold reduction in the level of meiotic recombination (Shinohara *et al.* 1992). Mutations in several other genes of the *RAD52* epistasis group (*RAD54*, *RAD55*, and *RAD57*) are similar to *rad51* with respect to heterogeneous phenotypes in recombination. Only the *rad52* mutation is nearly homogeneous in recombination and repair phenotypes, in that a *rad52*Δ mutant is both sensitive to γ-irradiation and defective in mitotic recombination of a variety of substrates. These results suggest that cellular controls for DNA repair and the controls for recombination on specific types of substrates, although partially overlapping, involve functions distinct from each other.

***RAD59* is required for efficient double-strand break repair:** In this study, *RAD59* and the *RAD52* function disabled by the *rad52R70K* mutation were shown to be required for efficient mating-type switching and single-strand annealing, two different DSB-initiated recombination events. By physical analysis, the repair reaction was delayed in both mutants, and there was a 2- to 10-fold reduction in the level of recombination products compared with that of the wild-type strain. There are several possible explanations for the reduction in recombination products. First, as unsynchronized cultures were used for the HO induction experiments, repair could have occurred from a sister chromatid instead of intrachromosomally. The use of the sister chromatid as a donor for repair would not be detected by the physical assay. Although we cannot rule out this possibility, it seems unlikely because both mutants are γ-ray sensitive and repair of lesions in haploid cells is thought to occur by sister chromatid recombination. Furthermore, *rad59*Δ is lethal in combination with *rad27*Δ, suggesting that the recombinational repair of lesions during S-phase or G2 is defective in *rad59*Δ mutants (Symington 1998). Second, the mutants could be defective in the strand invasion or strand-annealing steps of these reactions; this is the model we favor. The displacement of RPA from single-stranded DNA by Rad52 is required for both strand invasion and SSA. A defect in the Rad52-RPA interaction, Rad52 self-interaction, or Rad52-DNA binding could cause the defects observed in the *rad52R70K* strain. Although the biochemical function of Rad59 is currently unknown, the homology between Rad59 and the N-terminal region of Rad52 and the preponderance of basic residues in Rad59 suggest that it is a DNA binding protein. Rad59 could potentially play a similar role to Rad52 in strand annealing, or the complex of Rad52 and Rad59 may be more efficient in these processes than Rad52 alone. The more severe defect of the *rad59 rad52R70K* double mutant is consistent with the proteins having overlapping biochemical activities.

***RAD52* and *RAD59* have partially overlapping functions in meiosis:** As stated earlier, the single *rad52R70K* and *rad59* mutations have little effect on sporulation or spore viability, however, the double mutant is extremely deficient in both spore formation and subsequent viability. This finding suggests that *RAD59* and the function of *RAD52* disabled by the *rad52R70K* mutation have partially redundant functions in meiosis. This is the first evidence of a role for *RAD59* in meiosis and suggests that *RAD59* can carry out a meiotic function of *RAD52* normally mediated by the N-terminal region of the Rad52 protein. *RAD51* and *DMC1*, which encodes a meiosis-specific RecA homologue, also have redundant functions in meiosis. Meiotic recombination products are reduced 5- to 10-fold by mutation of either *RAD51* or *DMC1*, but recombination is severely reduced in the *rad51 dmc1* double mutant (Shinohara *et al.* 1997a). Similarly, *RAD54* and *RDH54*, which encodes a Rad54 homologue, have overlapping functions during meiosis (Klein 1997; Shinohara *et al.* 1997b).

Comparison of *rad52* alleles: The *rad52R70K* allele results from a single amino acid change from arginine to lysine at position 70 that resides in the conserved N-terminal region of the Rad52 protein. Arginine 70 is conserved among all of the Rad52 homologues from various organisms except in the yeast Rad59 protein, where the corresponding residue happens to be lysine. The N-terminal part of Rad52 has been suggested to contain a DNA-binding domain (Milne and Weaver 1993; Mortensen *et al.* 1996; Shinohara and Ogawa 1998). It is possible that the R70K mutation alters the DNA binding capability of Rad52, even though they are similarly charged residues. Alternatively, this position might be important for interaction with other proteins. The N-terminal region of Rad52 is thought to be required for self-association as well as interaction with RPA (Park *et al.* 1996; Hays *et al.* 1998). Previously, some other mutations in the N-terminal region of Rad52 have been characterized and found to display diverse phenotypes (Figure 6). The *rad52-1* (A90V) allele is indistinguishable from a deletion mutation in nearly every aspect tested, including the γ-ray sensitivity and meiosis defects (Resnick 1969; Adzuma *et al.* 1984). The *rad52-2* (P64L) allele causes sporulation deficiency and sensitivity to γ-irradiation and MMS, but the mutant exhibits a hyperrecombinational phenotype for interchromosomal recombination (Malone *et al.* 1988;

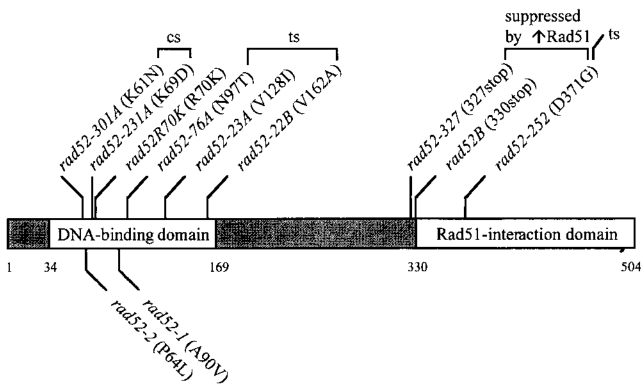

Figure 6.—*RAD52* functional domains and mutant alleles. The locations of the DNA-binding domain and Rad51-interaction domain are from published studies. ts, temperature-sensitive alleles; cs, cold-sensitive alleles. The C-terminal truncation alleles and a C-terminal missense ts allele are suppressed by overexpression of *RAD51.*

Boundy-Mills and Livingston 1993). *rad52-76A* (N97T), *rad52-23A* (V128I), and *rad52-22B* (V162A) alleles give rise to temperature-sensitive phenotypes for cell survival after exposure to MMS or γ-rays, but unconditionally retain the ability to undergo mitotic and meiotic recombination (Kaytor and Livingston 1994). *rad52-301A* (K61N) and *rad52-231A* (K69D) were characterized as cold sensitive with regard to growth on MMS-containing media, but this phenotype was found only using plates containing a low concentration of MMS (Nguyen and Livingston 1997). There are several similarities between strains with *rad52* mutations in this region of the protein. For example, *rad52-2* (P64L) and *rad52R70K* both increase the rate of interchromosomal recombination, but show slight reductions in the rate of inverted-repeat recombination. Strains containing either the *rad52-231A* or *rad52R70K* alleles show similarity in spore viability of homozygous diploids (70% *vs.* 75% at 25°) and survival to HO-induced breaks. Whether the residual activity of the *rad52-2*, *rad52-231A*, and *rad52-301A* strains depends on *RAD59* has yet to be determined. In addition to mutations in the N-terminal portion of Rad52, mutations in the C-terminal portion have also been found to affect Rad52 activity. Among them are a C-terminal truncation allele that exerts dominant negative effects on MMS resistance (Milne and Weaver 1993), another C-terminal truncation allele that retains partial ability to repair DNA damage and to undergo recombination (Boundy-Mills and Livingston 1993), and a temperature-sensitive missense allele that is proficient in mitotic and meiotic recombination (Kaytor and Livingston 1994). Attempts to isolate suppressors of the *rad52* deletion allele have been unsuccessful, indicating that the Rad52 protein possesses an activity not easily bypassed. Suppressors of non-deletion *rad52* alleles have been isolated. The three N-terminal ts alleles are suppressed by any one of a group of suppressors. These suppressors are recessive and involve mutations of different complementation groups (Kaytor and Livingston 1996). The C-terminal truncation alleles, C-terminal missense ts allele, and an allele of undetermined nature (*rad52-20*) are suppressible by the overexpression of Rad51, by mating-type heterozygosity, and in some cases by deletion of *SRS2* (Milne and Weaver 1993; Kaytor and Livingston 1994; Kaytor *et al.* 1995; Schild 1995). The suppression of C-terminal mutations by the overexpression of Rad51 is consistent with the finding that the Rad52 C-terminal is responsible for the protein–protein interaction with Rad51. *rad52-1* or *rad52-2* alleles are not suppressed by any of the above suppressors. Investigation of mutant alleles has improved our understanding of the biological significance of *RAD52* and biochemical analysis of these mutants is likely to shed light on the diverse functions of this protein.

In summary, we identified an unusual allele of *RAD52* that confers DNA repair and recombination defects similar to those caused by mutation of *RAD59.* The similarity between the mutant phenotypes, in combination with the synergistic defects of the double mutant strain, suggest that the two proteins function together and/or have overlapping activities. These results provide further support for the idea that vertebrates have other Rad52-like activities that can compensate for the loss of RAD52 function (Rijkers *et al.* 1998; Yamaguchi-Iwai *et al.* 1998).

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