Modulation of *Saccharomyces cerevisiae* DNA Double-Strand Break Repair by SRS2 and RAD51

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ABSTRACT

RAD52 function is required for virtually all DNA double-strand break repair and recombination events in *Saccharomyces cerevisiae*. To gain greater insight into the mechanism of RAD52-mediated repair, we screened for genes that suppress partially active alleles of RAD52 when mutant or overexpressed. Described here is the isolation of a phenotypic null allele of SRS2 that suppressed multiple alleles of RAD52 (*rad52B*, *rad52D*, *rad52-1* and *KIRAD52*) and RAD51 (*KIRAD51*) but failed to suppress either a *rad52Δ* or a *rad51Δ*. These results indicate that SRS2 antagonizes RAD51 and RAD52 function in recombinational repair. The mechanism of suppression of RAD52 alleles by *srs2* is distinct from that which has been previously described for RAD51 overexpression, as both conditions were shown to act additively with respect to the *rad52B* allele. Furthermore, overexpression of either RAD52 or RAD51 enhanced the recombination-dependent sensitivity of an *srs2Δ* RAD52 strain, suggesting that RAD52 and RAD51 positively influence recombinational repair mechanisms. Thus, RAD52-dependent recombinational repair is controlled both negatively and positively.

LIVING cells contend with DNA damage as a consequence of normal cellular metabolism as well as external insults such as chemical mutagens and ultraviolet (UV) or ionizing radiation. Failure to accurately repair DNA lesions can lead to mutational loss of factors important for cell growth and viability. In *Saccharomyces cerevisiae*, nearly 100 genetic loci have been shown to be required for the repair of DNA damage (reviewed by GAME 1983; FRIEDBERG *et al.* 1991). Genetic analysis has allowed the assignment of most of these genes into one of three groups, reflecting an underlying organization of pathways for excision repair (RAD3 epistasis group), postreplication repair (PRR; RAD6 epistasis group) and recombinational/double-strand break (DSB) repair (RAD52 epistasis group).

Despite differences in the DNA lesions thought to initiate these pathways, genetic data indicates partial redundancy of function between repair mechanisms. Double mutants between two different epistasis groups often exhibit synergistic (rather than additive) radiation sensitivity (NAKAI and MATSUMOTO 1967; BRENDEN and HAYNES 1973; GAME and COX 1973; SCHIESTL and PRAKASH 1989; GLASSNER and MORTIMER 1994). Thus, the phenotype of a given mutant is influenced by the action of repair proteins in other pathways that may partially substitute for the lost function.

In yeast, the repair of DSBs is mediated almost exclu-

sively by RAD52-dependent recombination. RAD52 epistasis group mutants exhibit pleiotropic defects in mitotic and meiotic recombination and are characterized by extreme sensitivity to agents that cause DSBs such as X-rays and restriction endonucleases (GAME 1983; BARNES and RINE 1985). Within this group, mutations in RAD51, RAD52 and RAD54 result in the most severe repair and recombination defects suggesting that these genes may encode the central components critical for recombinational repair (GAME 1983). Interestingly, RAD51 protein has significant structural similarity with the *Escherichia coli* recombination protein, recA (ABOUSEKHRA *et al.* 1992; SHINOHARA *et al.* 1992). Like recA, RAD51 forms helical filaments on double-stranded DNA and catalyzes DNA-strand exchange *in vitro* (OGAWA *et al.* 1993; SUNG 1994). Recently, we demonstrated the biological relevance of RAD51 self-association *in vivo* with the use of dominant-negative alleles and GAL4 two-hybrid analysis (DONOVAN *et al.* 1994). RAD51 also associates with RAD52 and the characterization of dominant-negative alleles of RAD52 suggests that RAD52 and RAD51 may function as members of a larger DNA repair/recombination complex (MILNE and WEAVER 1993; DONOVAN *et al.* 1994). RAD51 and RAD52 also interact genetically in that overexpression of RAD51 suppresses partially active alleles of RAD52 (MILNE and WEAVER 1993). The combined biochemical and genetic data suggest that RAD51 may function as a positive activator of recombination.

In addition to elements that positively control recombination, negative regulatory elements are also likely

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to exist. Numerous mutations have been isolated that exhibit elevated levels of homologous recombination (e.g., AGUILERA and KLEIN 1988). Although in many cases the hyperrecombination phenotype may be caused by the accumulation of recombinogenic DNA lesions, some hyperrecombination mutants exhibit altered utilization of repair pathways. For instance, mutations in *SRS2* result in an elevated frequency of gene conversion as well as suppression of mutations in *RAD6* or *RAD18* (PRR; LAWRENCE and CHRISTENSEN 1979; ABOUSSEKHRA *et al.* 1989; SCHIESTL *et al.* 1990; RONG *et al.* 1991). Both the hyperrecombination and *rad6Δ* suppression phenotypes of *srs2* mutants are dependent upon *RAD52* and recombinational repair. This suggests that in the absence of the *SRS2* protein, lesions ordinarily repaired by PRR are instead channeled into a recombinational repair pathway (ABOUSSEKHRA *et al.* 1989; SCHIESTL *et al.* 1990; RONG *et al.* 1991). However, although redirecting lesions from PRR to recombinational repair obviates the requirement for PRR function, elevated levels of recombination are not without consequence. *srs2* mutants are characterized by a moderate sensitivity to both UV and methyl methanesulfonate (MMS) damage that can be suppressed in diploids by semidominant mutations in *RAD51*. Therefore, the radiation sensitivity of *srs2* mutants is proposed to result from the inappropriate use of recombinational repair rather than from a defect in repair *per se* (ABOUSSEKHRA *et al.* 1992).

Here we describe the isolation of a phenotypic null allele of *SRS2* that suppressed partially active alleles of *RAD52* and clearly demonstrates an antagonistic interaction between *RAD52* and *SRS2*. Suppression was not limited to alleles of *RAD52* as deletion of *SRS2* also suppressed an allele of *RAD51*. Despite the striking parallels between *RAD51*- and *srs2*-dependent suppression, their mechanisms of action are distinct because they interacted additively with respect to suppression of the *rad52B* allele. Furthermore, we demonstrate that overexpression of either *RAD51* or *RAD52* enhances the MMS sensitivity of an *srs2Δ* strain implicating both *RAD51* and *RAD52* as positive activators of recombination. These data support a model where *RAD52* and *RAD51* function at or before commitment to recombinational repair and where recombination function is governed by both positive (*RAD51* and *RAD52*) and negative (*SRS2*) genetic elements.

MATERIALS AND METHODS

Yeast strains, media and genetic methods: The strains used in this study are displayed in Table 1. Unless otherwise indicated, all strains are derivatives of DWY83 or DWY171 and are isogenic except for the indicated markers. DWY133 is a derivative of DWY97 bearing a spontaneous *srs2-200* mutation. DWY174 (*srs2Δ*) was constructed by a single-step gene disruption (ROTHSTEIN 1991) using an *SRS2* genomic *Acd*

fragment (ABOUSSEKHRA *et al.* 1989) in which the coding sequences between the *Pst*I (bp 1022) and *Cl*aI (bp 4447) sites had been replaced with *TRP1* coding sequences. DWY191, DWY193, DWY198 and DWY199 were derived from the sporulation of a diploid formed by the mating between DWY133 and DWY174. DWY203 is a haploid product from the mating of DWY193 and the nonisogenic DWY129 (generously provided by R. ROTHSTEIN).

All strains were propagated at 30° in either YPD or SC media lacking only those amino acids required for plasmid selection (SHERMAN *et al.* 1986). For solid media, agar was added to 2%. Transformation was performed according to the method of GEITZ *et al.* (1992) with the modification that DMSO was added to 10% immediately before heat shock (HILL *et al.* 1992).

Multicopy suppressor analysis: DWY97 (*rad52Δ*) was cotransformed with plasmids expressing either the *rad52B* truncation (pTM25L) or the *Kluyveromyces lactis* *RAD52* allele (pDBL-(KIRAD52)) (MILNE and WEAVER 1993) and an *S. cerevisiae* YEp213 library (gift of F. WINSTON; CARLSON and BOTSTEIN 1982) or a *kluyveromyces lactis* multicopy library (described below) to obtain 250,000 or 13,000 transformants, respectively that were then pooled in 20% glycerol and frozen in aliquots. Aliquots were thawed and used to inoculate SC-Ura, Leu overnight cultures. Cultures with an OD₆₀₀ of approximately 0.6 were irradiated at 30 kR (*ADH:rad52B*) or 50 or 60 kR [pDBL (KIRAD52)] using a ⁶⁰Co source with a dose rate of 12.9 R/s. One milliliter of irradiated culture was plated on SC-Ura, Leu plates and incubated at 30° for 3 days. Approximately 300 *ADH:rad52B*/YEp213 and 350 pDBL-(KIRAD52)/*K. lactis* library transformants were obtained in the primary screens. In control experiments where the library plasmid had been replaced by an empty vector, a background of ~60 clones was found. Three hundred surviving clones from each selection were rescreened for either X-ray or MMS resistance to yield 9 and 91 resistant clones, respectively. Plasmids were isolated from yeast and introduced into *E. coli* (HOFFMAN and WINSTON 1987) from which hybridization and restriction digestion analysis was performed to identify the genes.

Cloning of *SRS2*: DWY133 was cotransformed with *ADH:rad52B* (pTM25L) and a YCp50 library (ROSE *et al.* 1987). Transformants (3420) were individually resuspended in 20% glycerol and plated on SC-Ura, Leu plates in the presence and absence of 0.0025% MMS. A single MMS-sensitive clone (YCpAFT1) was isolated which contained ~9.5 kb of genomic sequence. Transposon mutagenesis using a mini-γδ transposon (BERG *et al.* 1992) was used to derive clones in which the transposon had interrupted complementing sequences. By sequence analysis and restriction enzyme digestion, all of the insertions were found to have inserted into an ORF encoding *SRS2* (ABOUSSEKHRA *et al.* 1989).

Plasmids and libraries: Construction of *ADH:RAD52* (pTM22), *ADH:KIRAD52* (pTM24), *ADH:rad52B* (pTM25) and *ADH:rad52C* (pTM27) has been described previously (MILNE and WEAVER 1993). To accommodate the *URA3* marker of both libraries used, *LEU2* derivatives of pTM25 (*ADH:rad52B*) and pDBL (KIRAD52) were constructed using by replacing pDB20 vector sequences with those of pDBL (MILNE and WEAVER 1993) to generate pTM25L and pDBL (KIRAD52), respectively. For low-copy number expression of *KIRAD52*, YCpKIRAD52 was constructed by cloning the *K. lactis* *RAD52 Xba*I (filled-in) genomic fragment (MILNE and WEAVER 1993) into the backbone of YCpGal:RIa (BARNES and RINE 1985) digested with *Eco*RI and *Bam*HI (filled-in). The low-copy number *KIRAD51* construct (pRS (KIRAD51)) was similarly constructed (DONOVAN *et al.* 1994). The *S. cere-*

TABLE 1
Yeast strains used in this study

Strain	Genotype	Source or Reference ^a
DWY83	<i>MATα arg4-RV leu2-3,112 cyh^R ura3-52 trp1-289</i>	R. KOLODNER, Harvard Medical School (RKY1734)
DWY97	<i>MATα rad52Δ::ura3 arg4-RV leu2-3,112 cyh^R ura3-52 trp1-289</i>	A. NICOLAS, Université Paris-Sud (ORT118-2) MILNE and WEAVER (1993)
DWY129	<i>MATα rad51::LEU2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>	R. ROTHSTEIN, Columbia University (U687-)
DWY133	<i>MATα aft1-1 rad52Δ::ura3 arg4-RV leu2-3,112 cyh^R ura3-52 trp1-289</i>	This study
DWY171	<i>MATα his3Δ1 cyh^S ade2 ura3-52 trp1-289</i>	R. KOLODNER, Harvard Medical School (RKY1730) A. NICOLAS, Université Paris-Sud (ORT-104-5a)
DWY174	<i>MATα srs2Δ::TRP1 his3Δ1 cyh^S ade2 ura3-52 trp1-289</i>	This study
DWY191	<i>MATα srs2Δ::TRP1 ade2 leu2-3,112 trp1-289 ura3-52</i>	This study
DWY193	<i>MATα srs2Δ::TRP1 his3Δ1 leu2-3,112 trp1-289 ura3-52</i>	This study
DWY198	<i>MATα rad52Δ::ura3 srs2Δ::TRP1 ade2 his3Δ1 leu2-3,112 trp1-289 ura3-52</i>	This study
DWY199	<i>MATα rad52Δ::ura3 srs2Δ::TRP1 ade2 his3Δ1 arg4-RV trp1-289 ura3-52</i>	This study
DWY203	<i>MATα rad51Δ::LEU2 srs2Δ::TRP1 his3 leu2-3,112 trp1 ura3</i>	This study

^a Previous strain designations are displayed in parentheses.

visiae rad52D allele was constructed first in pSKII+ by cloning a *Bst*EII (bp 1114)-*Nla*III (bp 1634) fragment (ADZUMA *et al.* 1984) into pTM10 digested with *Bst*EII and *Sph*I to generate pTM10D. For expression in yeast, *ADH:rad52D* (pTM22D) was created by ligating *Sma*I-*Hind*III (filled-in) fragment into the filled-in *Hind*III site of pDB20 (BECKER *et al.* 1991). Similarly, the *rad52-1* allele (ADZUMA *et al.* 1984) was first constructed in pSKII+. *RAD52* sequences were amplified by PCR utilizing the primers 521-A (5'-GTAATTAATCTTGTTAATCAAATCTTTGG-3') containing the *rad52-1* mutation and 52-1B (5'-CCGCATGCTTTGCTTAGCTACAACGGG-3') introducing an *Sph*I site for subsequent cloning. This PCR product was first ligated into pTM10 digested with *Ase*I and *Sph*I to add sequences 5' to the mutation [pSK(52-1)] and then, 3' sequences were added by cloning the *Bgl*III-*Xmn*I fragment of pTM10 into pSK(52-1) to generate pTM10-1. A yeast expression derivative was created by cloning *rad52-1* coding sequences (*Sma*I-*Hind*III-filled-in) into the filled-in *Hind*III site of pDB20. pGAL-HO was generously provided by R. SCHIESTL (HERSKOWITZ and JENSEN 1991).

For construction of the *K. lactis* genomic library, pDB20 was digested with *Bam*HI to remove the *ADH1* promoter-terminator sequences and create pTM60. Genomic DNA was prepared from *K. lactis* (ATCC No. 8585) by glass-bead disruption (HOFFMAN and WINSTON 1987), partially digested with *Sau*3AI, and fractionated on a sucrose gradient. Fractions containing 7- to 15-kb fragments were ligated into *Bam*HI digested pTM60 and transformed into *E. coli* by electroporation. Approximately 150,000 independent transformants were obtained and DNA prepared from the pooled clones. Analysis of individual clones revealed ~80% recombinants.

MMS and UV sensitivity: Analysis of MMS sensitivity has been described elsewhere (MILNE and WEAVER 1993). Briefly, three independent transformants of each strain were picked into sterile water and titrated in six fivefold serial dilutions. Ten microliter aliquots of each dilution were plated in duplicate on SC plates (selecting for the appropriate plasmids) in the presence and absence of MMS (0.0025% unless otherwise

indicated). Plates were incubated at 30° and colonies were counted daily for 5 days. Data is displayed as the fraction of colonies visible in the presence of MMS relative to the non-MMS control platings and averaged among the three transformants.

Similarly, UV-sensitivity assays were performed by serial dilution of three independent transformants/strain and plating in duplicate on one plate per dose. Plates were irradiated using a Stratalinker UV Cross-linker 2400 for the desired dose. Plates were immediately wrapped in foil to prevent photo-reactivation and incubated at 30° for 2 days before scoring surviving colonies.

RESULTS

Multicopy suppression of *rad52B* and *KIRAD52*: To define the molecular basis for recombinational repair, conditions resulting in increased DSB repair by partially active *RAD52* alleles were sought. Multicopy suppressor analysis was used to isolate genomic sequences that suppress the *rad52B* allele. A *rad52Δ* strain (DWY97) was cotransformed with the partially active *ADH:rad52B* allele and a multicopy *S. cerevisiae* genomic library and subsequently selected for X-ray resistance (30 kR; MATERIALS AND METHODS). Interaction suppressors that could augment *rad52B* activity when overexpressed (like *RAD51*) were expected (MILNE and WEAVER 1993). Although bypass suppressors that could substitute for *RAD52* function were also possible, the dominant-negative effects of *rad52B* might preclude isolation of such suppressors (or even wild-type *RAD52*; MILNE and WEAVER 1993). Eight clones were isolated that exhibited increased X-ray resistance dependent upon expression of both the library plasmid and *rad52B*. On

FIGURE 1.—Suppression of *rad52B* by *aft1-1*. MMS sensitivity was determined by plating serial dilutions of transformants onto selective plates with and without 0.0025% MMS (MATERIALS AND METHODS). Data are displayed as the ratio of colony forming units (CFUs) on MMS plates to the total number on control plates without MMS. DWY133 (*rad52Δ aft1-1*) was transformed with vector controls (pDB20/pDBL; ●) or *ADH:rad52B* (pTM25L, pDBL; ■) and assayed for MMS sensitivity on SC-Ura, Leu plates as described. For comparison, DWY97 (*rad52Δ*) was also transformed with vector controls (pDB20/pDBL; ○), *ADH:RAD52* (pTM22/pDBL; △) and *ADH:rad52B* (pTM25L/pDB20; □) to illustrate no, full and partial levels of complementation of MMS sensitivity of the *rad52Δ* parental strain.

the basis of hybridization and restriction enzyme analysis, each of these clones contained the *RAD51* gene, confirming our previous findings that *RAD51* overexpression suppresses the *rad52B* allele (MILNE and WEAVER 1993). However, the failure to isolate other clones suggests that *RAD51* is the strongest multicopy suppressor of *RAD52*.

We also examined suppression of the partially complementing *KIRAD52* allele in a parallel screen. In this case, a *K. lactis* library was used so that any species differences would be obviated. Plasmids (89) were isolated that consistently conferred radiation resistance and each was shown to contain *KIRAD51* by either hybridization or restriction enzyme analysis. Therefore, the biological basis of *RAD52* activation by *RAD51* is conserved between *K. lactis* and *S. cerevisiae* despite the sequence divergence between these species.

Suppression of *rad52B* and *KIRAD52* by mutation: Insights into the function of *RAD52* can also be gained by the identification and characterization of chromosomal mutations that modulate or bypass *RAD52* activity. A spontaneous mutation (*aft1-1*, Activator of Fifty-Two) was isolated on the basis of suppression of the X-ray sensitivity of a *rad52Δ* strain transformed with the *ADH:rad52B* allele (data not shown). This clone also exhibited increased resistance to the X-ray mimetic agent, MMS (Figure 1). The MMS sensitivity of the *rad52Δ* and *rad52Δ aft1-1* strains were very similar and thus the increase in MMS resistance was dependent upon the presence of the *rad52B* allele (Figure 1). On the basis of mating to a related *rad52Δ* strain, *aft1-1* was determined to be recessive. Dissection of a diploid strain heterozygous for *aft1-1* yielded spores in which the *Aft1* phenotype segregated 2+:2- in 10/10 tetrads examined and thus mapped to a single locus. Western blot analysis of a *rad52Δaft1-1* strain (DWY133) revealed wild-type levels of *RAD51* protein, indicating that the *aft1-1* mutation does not act by merely altering regulation of *RAD51* (data not shown).

Although sensitivity to MMS and X-rays correlates in yeast with defects in DSB repair, damage caused by each of these lesions is heterogeneous and is not limited to DSBs. Therefore, to demonstrate that *aft1-1* activates the *rad52B* allele specifically with regard to the repair of DSBs, we examined the sensitivity of *rad52B aft1-1* strains to a single chromosomal DSB generated at the *MAT* locus by the HO endonuclease (HERSKOWITZ and JENSEN 1991). Repair of this DSB is effected by recombination with silent mating-type cassettes (*HML* and *HMR*) located elsewhere on chromosome III. DWY133 (*rad52Δ aft1-1*) was cotransformed with *ADH:rad52B* and pGAL-HO (containing the gene encoding the HO endonuclease under the control of the *GAL10* promoter) and assayed for DSB sensitivity relative to DWY97 (*rad52Δ*) transformed with either *ADH:rad52B* or fully-complemented with *ADH:RAD52*. Sensitivity was defined as the plating efficiency on SC-Ura, Leu sucrose/galactose plates (HO expression induced) relative to SC-Ura, Leu glucose plates (HO expression repressed). The *aft1-1* mutation increased HO resistance conferred by *rad52B* 500- to 1000-fold relative to the parental *rad52Δ* strain (plating efficiency 57% for *rad52B aft1-1* vs. 0.077% for *rad52B AFT1*). DWY97 complemented with *ADH:RAD52* yielded a wild-type plating efficiency of 79%. As was observed for MMS sensitivity, *aft1-1* had relatively little effect on the DSB sensitivity of a *rad52Δ* strain (plating efficiency 0.14% for *rad52Δ aft1-1* vs. 0.049% for *rad52Δ AFT1*). Thus, the *aft1-1* mutation suppressed the *rad52B* allele to near wild-type levels of repair of HO-induced DSBs.

The *AFT1* gene was cloned by complementation of the *ADH:rad52B* suppression phenotype using a low copy YCp50 library (ROSE *et al.* 1987). A single complementing clone, YCpAFT1, containing ~9 kb of genomic sequences was isolated (MATERIALS AND METHODS). Reintroduction of this plasmid into DWY133 (*rad52Δ aft1-1*) expressing *ADH:rad52B* reduced MMS resistance to a level identical to that of the parental DWY97 strain

(*rad52Δ*) expressing *ADH:rad52B* (data not shown). Sequences required for the complementation of the *Aft1*⁻ phenotype were identified by transposon mutagenesis of YCpAFT1 (MATERIALS AND METHODS; BERG *et al.* 1992). Sequencing of transposon insertion sites and subsequent restriction mapping localized complementing sequences to an ORF encoding SRS2 (ABOUSSEKHRA *et al.* 1989). To unambiguously confirm that *aft1-1* is an allele of *SRS2*, an *aft1-1/srs2Δ::TRP1* diploid was sporulated and dissected. All 10 tetrads examined revealed a segregation pattern of 2 *aft1-1*:2 *srs2Δ* indicating that *AFT1* is indeed allelic with *SRS2*. To further map the *aft1-1* mutation, the *SRS2* gene was isolated from an *aft1-1* strain by gap repair (ROTHSTEIN 1991) of an *SRS2* plasmid deleted for ~80% of the *SRS2* coding sequence (*PstI-PstI*). The inability of this plasmid to complement an *srs2Δ* indicates that the mutation lies between amino acids 33 and 981, whereas an apparently normal restriction map reveals that the defect is likely the result of a point mutation or small deletion (data not shown). To indicate allelism with *SRS2*, the *aft1-1* mutation will be designated as *srs2-200*.

Numerous *srs2* alleles have been identified both as recessive hyperrecombination mutants and as dominant suppressors of the radiation sensitivity of *rad6* and *rad18* mutants (LAWRENCE and CHRISTENSEN 1979; ABOUSSEKHRA *et al.* 1989; SCHIESTL *et al.* 1990; RONG *et al.* 1991; PALLADINO and KLEIN 1992). In general, these mutations are thought to result in the loss of SRS2 function. Not only do most *srs2* mutants phenotypically resemble an *srs2Δ* strain, but also many mutations map to residues thought to be critical for the biochemically determined helicase activity of SRS2 (PALLADINO and KLEIN 1992; RONG and KLEIN 1993). Mechanistically, *srs2* mutations are thought to suppress both *rad6* and *rad18* mutations by channeling DNA lesions into a recombinational repair pathway (ABOUSSEKHRA *et al.* 1989; SCHIESTL *et al.* 1990; RONG *et al.* 1991). To examine whether the *srs2-200* allele suppressed *rad52B* defects through a loss of function or an acquisition of a new activity, an *srs2Δ* allele was constructed by replacing *SRS2* coding sequences with *TRP1*. The *srs2Δ* allele was indistinguishable from *srs2-200* with regard to activation of *rad52* alleles suggesting that the *srs2-200* mutation disrupts the activity of SRS2 (Figure 1 and see below, Figure 3). Furthermore, *srs2-200* suppressed the UV and X-ray sensitivity of a *rad6Δ* strain to a level comparable with that of *rad6Δ srs2Δ* strain (data not shown). Although the following experiments have been performed with both *srs2-200* and *srs2Δ* alleles to yield nearly identical results, data will be presented only for the *srs2Δ* allele.

***srs2* mutations suppress multiple *RAD52* alleles:** Loss of SRS2 function failed to suppress the X-ray and MMS sensitivity of *rad52Δ* strains (Figure 1). Therefore loss of SRS2 activity did not obviate the requirement for

RAD52 function but rather altered the activity of the *RAD52B* protein. To elucidate the mechanism of *srs2Δ* suppression, we examined the effect on a variety of *rad52* mutants including the weakly complementing *rad52B* and *KIRAD52* alleles as well as the inactive *rad52C* allele (Figure 2; MILNE and WEAVER 1993). In addition, we constructed an additional C-terminal truncation allele, *rad52D*, that exhibited minimal complementation of a *rad52Δ* strain (Figure 3A) and was suppressed by overexpression of *RAD51* (data not shown). Finally, the *rad52-1* mutant allele (A90V; ADZUMA *et al.* 1984) was reconstructed and cloned downstream of the *ADH1* promoter, which resulted in the production of an inactive *RAD52* protein that could not be suppressed by *RAD51* overexpression (Figure 3A, data not shown). Each of these alleles was transformed into a *rad52Δ srs2Δ* strain (DWY199) and analyzed for sensitivity to MMS. *srs2Δ* dramatically suppressed both the *rad52B* and *rad52D* C-terminal truncations while having no effect on the N-terminal truncation, *rad52C* (Figure 3B). These findings were qualitatively similar to the pattern of activation that was observed for *RAD51* although the level of suppression by *srs2Δ* was significantly greater (Figure 2; MILNE and WEAVER 1993). Surprisingly, partial suppression was also observed for the *ADH:rad52-1* allele that was dependent upon its overexpression because no significant suppression was observed when an *srs2Δ* was crossed into a *rad52-1* background (data not shown).

Deletion of *SRS2* also activated *KIRAD52* alleles although the results are more complex (Figure 3C). The greatest activation of *KIRAD52* was obtained when using a lower level expression construct (YCpKIRAD52) in which *KIRAD52* is expressed from its endogenous promoter on a low-copy number plasmid. Significantly less activation was observed when higher levels of *KIRAD52* protein were produced from the constitutive *ADH* promoter on a multicopy plasmid (*ADH:KIRAD52*). This is in contrast to activation by *RAD51* in which the highest levels of complementation are observed upon *KIRAD52* overexpression (data not shown). As will be discussed in greater detail, the apparent enhanced sensitivity of an *srs2Δ* strain overexpressing *KIRAD52* likely reflects the inherent MMS sensitivity of *Rad*⁺ *Srs2*⁻ strains.

Deletion of *SRS2* also suppresses an allele of *RAD51*: The finding that a wide variety of *RAD52* alleles were suppressed by *SRS2* mutations prompted us to examine the ability of these mutations to suppress defects in other proteins involved in recombinational repair. For this experiment, the *RAD51* homolog from *K. lactis*, *KIRAD51*, was selected because of its partial activity in *S. cerevisiae* (DONOVAN *et al.* 1994). As was observed with *rad52Δ* strains, the MMS sensitivity of a *rad51Δ srs2Δ* strain (DWY203) is nearly identical to that of a *rad51Δ* strain (DWY203 + YCpAFT1) indicating that deletion

Allele	Suppression		Negative Dominance
	<i>srs2Δ</i>	<i>ADH:RAD51</i>	
<i>RAD52</i>	N/A	N/A	—
<i>rad52B</i>	+++	++	++
<i>rad52C</i>	—	—	++++
<i>rad52D</i>	+++	++	+++
<i>rad52-1</i>	+	—	++
<i>KIRAD52</i>	++++	++++	++++

FIGURE 2.—Summary of *RAD52* alleles. Alleles of *RAD52* are displayed graphically with conserved regions (I, II and III) between *KIRAD52* and *ScRAD52* indicated by overlines (MILNE and WEAVER 1993). Suppression of each *RAD52* allele by deletion of *SRS2* (*srs2Δ*) or overexpression of *RAD51* (*ADH:RAD51*) is indicated by the number of (+) symbols with (—) indicating no effect. The ability of each *RAD52* allele to dominantly interfere with wild-type DSB repair is similarly displayed where the degree of inhibition (X-ray^S) is proportional to the number of (+) symbols. N/A, not applicable.

of *SRS2* does not bypass the requirement for *RAD51* function (Figure 4). However, *rad51Δ* strains transformed with *ADH:KIRAD51* exhibited significantly greater MMS resistance in an *Srs2*[−] genetic background (Figure 4). As isogenic strains were not available, the control for this experiment is DWY203 (*rad51Δ srs2Δ*) complemented with YCpAFT1. Thus, loss of *SRS2* function suppressed defects in at least two proteins required for recombinational repair in *S. cerevisiae*.

Alteration of *srs2Δ* radiation sensitivity by overexpression of *RAD52* alleles: As we have described here, mutations in *SRS2* increase the DNA damage resistance of mutants impaired for recombinational repair. However, in the presence of wild-type recombinational functions (*i.e.*, *Rad52*⁺), *srs2* mutants exhibit moderate sensitivity to both UV and X-irradiation (ABOUSSEKHRA *et al.* 1989, 1992; SCHIESTL *et al.* 1990; PALLADINO and KLEIN 1992). For homozygous *srs2/srs2* diploid strains, both the UV and X-ray sensitivity can be suppressed by semidominant mutations in *RAD51* (ABOUSSEKHRA *et al.* 1992). This implies that the radiation sensitivity of *srs2* mutants is at least in part synthetic—that is, the product of the inappropriate use of recombinational repair mechanisms rather than the result of a defect in DNA repair *per se* (ABOUSSEKHRA *et al.* 1989, 1992).

To define the relationship between *srs2* radiation sensitivity and the relative use of recombinational mechanisms in haploid strains, we transformed a *srs2Δ* strain (DWY191) with a series of *RAD52* alleles that are dominant negatives and inhibit recombinational repair (MILNE and WEAVER 1993). If the UV and MMS sensitivity of *srs2* strains is a consequence of the misuse of recombinational repair, then dominant-negative alleles inhibiting recombination would be predicted to suppress the radiation sensitivity of an *srs2Δ* strain (DWY191). Each of these alleles was confirmed to have a dominant-negative phenotype for MMS repair in a wild-type background (DWY83, Figure 5A). We found that expression of dominant-negative *RAD52* alleles sig-

nificantly suppressed the UV sensitivity of an *srs2* mutant strain (Figure 5B). This suppression was most dramatic at a dose of 30 J/m² although it was also evident at both higher and lower doses (data not shown). The degree of suppression (*KIRAD52* > *rad52C* > *rad52D* > *rad52B*) correlated with the relative strength of the dominant-negative allele, consistent with a model that suppression is due to inhibition of recombinational repair. Overexpression of wild-type *RAD52* and *RAD51* alleles together or separately had little or no effect on the UV sensitivity of *srs2Δ* strains (Figure 5B).

Examining the effects of these alleles on MMS sensitivity of the *srs2Δ* strain was somewhat more complicated, as the repair of MMS-induced lesions requires recombinational repair inhibited by the dominant-negative *RAD52* alleles. Relatively weak dominant-negative *RAD52* alleles, such as *rad52B*, suppressed the MMS sensitivity of an *srs2Δ* strain (Figure 5C). Transformation of stronger alleles, such as *KIRAD52*, enhanced MMS sensitivity, although this was likely the result of inhibition of both inappropriate and appropriate recombinational repair (data not shown). Importantly, overexpression of wild-type *RAD51* and/or *RAD52* resulted in increased sensitization of an *srs2Δ* strain to MMS (Figure 5C) although overexpression of these genes in wild-type strains had no effect even at much higher concentrations of MMS (Figure 5A; data not shown). Effects of overexpression of *RAD51* and *RAD52* were not additive, as overexpression of each protein singly or together resulted in essentially the same degree of sensitivity. By analogy to the moderate sensitivity of an *srs2Δ* strain with wild-type levels of *RAD51* and *RAD52* protein, the observed increased MMS sensitivity of an *srs2Δ* strain overexpressing *RAD51* and/or *RAD52* may reflect an enhanced use of recombinational repair. Thus, based on both the UV and MMS sensitivity data presented here, DNA damage sensitivity of *srs2* mutants appear to accurately reflect the propensity a given strain to perform recombination.

FIGURE 3.—Loss of SRS2 function leads to the suppression of multiple *RAD52* alleles. (A) Partial- and non-complementation of a *rad52Δ* strain transformed with a variety of *RAD52* alleles. DWY97 (*rad52Δ*) was transformed with a vector control (pDB20; ○), *ADH:RAD52* (pTM22; △), *ADH:rad52B* (pTM25; □), *ADH:rad52C* (pTM27; ■), *ADH:rad52D* (pTM22D; ●), *ADH:rad52-1* (pTM28; ▽), *ADH:KIRAD52* (pTM24; ▲) or *KIRAD52* (YCpKIRAD52; ▼) and assayed for MMS sensitivity on SC-Ura plates ($\pm 0.0025\%$ MMS) as described in MATERIALS AND METHODS and Figure 1. (B) Many alleles exhibit increased complementation of the MMS sensitivity of a *rad52Δ* in the absence of SRS2. DWY199 (*rad52Δ srs2Δ*) was transformed with a vector control (pDB20; ○), *ADH:rad52B* (pTM25; □), *ADH:rad52C* (pTM27; ■), *ADH:rad52D* (pTM22D; ●) or *ADH:rad52-1* (pTM28; ▽) and MMS sensitivity assayed as above. (C) In the absence of SRS2, *KIRAD52* exhibits highest levels of apparent complementation when not overexpressed. DWY199 was transformed with either a low-copy number plasmid containing *KIRAD52* genomic sequences (YCpKIRAD52; ▼) or a high-copy plasmid in which *KIRAD52* expression is mediated by the constitutive *ADH1* promoter, *ADH:KIRAD52* (pTM24; ▲) and assayed for sensitivity to MMS as above.

***srs2* and *RAD51* suppress *rad52* mutants by distinct mechanisms:** The *srs2*- and *RAD51*-mediated suppression of *rad52* mutants is particularly unusual due to the wide variety of alleles that were suppressed. Therefore, both suppressors may share a common mechanism of action. To directly address this issue, we examined the combinatorial effects of *SRS2* deletion and *RAD51* overexpression

on the suppression of *rad52* alleles. We selected the *rad52B* allele, as wild-type levels of MMS^R were not attained with this allele in conjunction with either suppressor alone. DWY97 (*rad52Δ SRS2*) and DWY198 (*rad52Δ srs2Δ*) were transformed with *ADH:rad52B* in the presence and absence of *ADH:RAD51* and assayed for MMS resistance. For these experiments, the level of MMS was

FIGURE 4.—Deletion of *SRS2* suppresses a partially active allele of *RAD51*. DWY203 (*rad51Δ srs2Δ*) was transformed with a *KIRAD51* expression construct [pRS(KL51)] along with either vector (pDB20; □) or YCpAFT1 (▲) to complement the deletion of *SRS2*. MMS sensitivity was performed as described in MATERIALS AND METHODS and Figure 1 except that MMS was added to SC-Ura, His plates at a final concentration of 0.00375%. Also shown for comparison is the MMS sensitivity of DWY203 transformed with vector controls (pRS313/pDB20; □) or fully complemented (pRS(SC51)/YCpAFT1; ■). The effective genotype of each strain is shown to the right of the corresponding curve.

increased to 0.005% to accentuate differences between wild-type and mutant strains. Mutation of *SRS2* and over-expression of *RAD51* acted in an additive fashion to further enhance the MMS resistance of an *ADH:rad52B* strain over the levels of either suppressor alone (Figure 6). Therefore, despite the observed similarities between the two suppressors, the molecular basis for their action must be distinct.

DISCUSSION

Interactions between *SRS2* and recombinational repair mechanisms: Both the *srs2-200* and *srs2Δ* alleles functionally suppressed multiple alleles of *RAD52* and *RAD51* (Figures 1–4). The unifying characteristic of each of the suppressible alleles was their partial biological activity. Comparing these alleles, the level of complementation conferred by each to a *rad52Δ* strain correlated with the magnitude of activation resulting from the deletion of *SRS2*. Even the very weak suppression of the *rad52-1* allele can be explained by residual *RAD52* protein activity otherwise below the levels of detection. These findings provide genetic evidence for an antagonistic interaction between *SRS2* and proteins involved in recombinational repair. In a wild-type strain, *SRS2* functions to inhibit the recombinational repair of a subset of DNA lesions thereby allowing PRR. As is demonstrated by the *srs2* suppression of the radiation sensitivity of a *rad6* mutant, removal of *SRS2* allows recombinational repair of lesions that would otherwise be processed by the PRR pathway (ABOUSSEKHRA *et al.* 1989; SCHIESTL *et al.* 1990; RONG *et al.* 1991). Therefore, *SRS2* must inhibit recombinational repair prior to the point where DNA forms are committed to recombination. The genetically observed antagonism between *SRS2* and *RAD52/RAD51* indicate that *RAD52* and *RAD51* also function before commitment to recombina-

tional repair and may represent the direct targets for inhibition by *SRS2*.

We have been unable to observe physical interactions between *SRS2* and either *RAD51* or *RAD52* using two-hybrid fusion analysis (CHIEN *et al.* 1991) suggesting that *SRS2* does not form a stable complex with *RAD52* or *RAD51* (data not shown). Although models in which *SRS2* interacts directly with recombination proteins cannot be excluded, it is possible that *SRS2* inhibits recombination by interacting directly with DNA structures and thereby preventing recombination proteins from binding. Alternatively, *SRS2* could act by physically disrupting protein:DNA complexes that have already formed. This is a particularly attractive model as *SRS2* has a 3' to 5' helicase activity and thus, can translocate along DNA and alter DNA structure (RONG and KLEIN 1993). As we have described here, *SRS2* and *RAD52* interact antagonistically even when the DNA damage is a defined DSB at the *MAT* locus whose repair requires *RAD52*. Therefore, if *SRS2* inhibits the recombinational repair of a subset of DNA lesions, these lesions are likely to differ not by the physical structure of the break but perhaps by the phase of the cell cycle in which the damage was sustained or the availability of homologous sequences.

Synthetic sensitivity of haploid *srs2* mutants to DNA damage: Our observations show that dominant-negative alleles of *RAD52* similarly suppressed the UV and MMS sensitivity of haploid *srs2Δ* strains proportional to the ability of each allele to inhibit recombination (Figure 5). Presumably, inhibiting recombination by dominant-negative alleles of *RAD52* allows the processing of potentially recombinogenic DNA structures by alternate DNA repair mechanisms such as PRR. Consistent with this, inhibition of recombination by semi-dominant mutations in *RAD51* has been shown to restore UV-induced mutagenesis to *srs2* mutants (ABOUS-

FIGURE 5.—Effects of overexpression of *RAD52* alleles on the radiation resistance of wild-type and *srs2Δ* strains. (A) Multiple *rad52* mutant alleles can dominantly interfere with recombinational repair whereas wild-type alleles have no effect. DWY83 (*RAD52 SRS2*) was transformed with vector controls (pDB20/pDBL; ▽), *ADH:RAD52* (pTM22/pDBL; ■), *ADH:RAD51* [pDB20/pDBL(*RAD51*); ●], *ADH:RAD51* and *ADH:RAD52* [pTM22/pDBL(*RAD51*); △], *ADH:rad52B* (pTM25/pDBL; □), *ADH:rad52C* (pTM27/pDBL; ○), *ADH:rad52D* (pTM22D/pDBL; ◇) and *ADH:KIRAD52* (pTM24/pDBL; ▲) and assayed on SC-Ura, Leu plates for sensitivity to 0.0025% MMS as described in Figure 1 and MATERIALS AND METHODS. (B) Dominant-negative alleles of *RAD52* suppress the UV sensitivity of an *srs2Δ* strain. DWY191 (*RAD52 srs2Δ*) was transformed with vector controls (pDB20/pDBL), *ADH:RAD51* [pDB20/pDBL(*RAD51*)], *ADH:RAD52* (pTM22/pDBL), *ADH:RAD51* and *ADH:RAD52* [pTM22/pDBL(*RAD51*)], *ADH:rad52D* (pTM22D/pDBL), *ADH:KIRAD52* (pTM24/pDBL), *ADH:rad52B* (pTM25/pDBL) and *ADH:rad52C* (pTM27/pDBL) and assayed for sensitivity to UV irradiation. As described in MATERIALS AND METHODS, cells were serially diluted on duplicate SC-Ura, Leu plates and one plate of each set irradiated at 30 J/m². UV sensitivity is displayed as the fraction of cells present on the irradiated plate relative to the unirradiated control. (C) Dominant-negative alleles of *RAD52* suppress the MMS sensitivity of an *srs2Δ* strain whereas overexpression of wild-type alleles enhances sensitivity. DWY191 (*RAD52 srs2Δ*) was transformed with vector controls (pDB20/pDBL; ▽), *ADH:rad52B* (pTM25/pDBL; □), *ADH:RAD52* (pTM22/pDBL; ■), *ADH:RAD51* [pDB20/pDBL(*RAD51*); ●] and *ADH:RAD52* and *ADH:RAD51* [pTM22/pDBL(*RAD51*); △] and assayed on SC-Ura, Leu plates for sensitivity to 0.0025% MMS as described in Figure 1 and MATERIALS AND METHODS.

SEKHRA *et al.* 1992). These data also provide additional support for the function of *RAD51* and *RAD52* before or at the point of commitment to recombination. In contrast, mutations in *RAD54* exhibit a synthetic lethal defect in combination with *srs2* alleles implicating

RAD54 in a later step in the recombination process (PALLADINO and KLEIN 1992).

Cell-cycle analysis of UV and X-ray sensitivity reveals that diploid *srs2/srs2* mutants are particularly sensitive in G1 (ABOUSSEKHRA *et al.* 1989). Thus, the damage

FIGURE 6.—Deletion of *SRS2* and overexpression of *RAD51* have additive effects with regard to suppression of *rad52B*. DWY97 (*rad52Δ SRS2*; ○ and ●) and DWY198 (*rad52Δ srs2Δ*; ■ and □) were cotransformed with *ADH:rad52B* (pTM25) and either a vector control (pDBL; ○ and □) or *ADH:RAD51* [pDBL (*RAD51*); ● and ■] and assayed on SC-Ura, Leu plates for sensitivity to 0.005% MMS as described in Figure 1 and MATERIALS AND METHODS. Also included for comparison is the MMS sensitivity of DWY97 (*rad52Δ*) transformed with *ADH:RAD52* (pTM22/pDBL; Δ) to fully complement the MMS sensitivity to wild-type levels.

sensitivity of *srs2* mutants was interpreted to be a consequence of the inappropriate use of homologous chromosomes present in G1 as templates for recombinational repair instead of sister chromatids available in G2 (ABOUSSEKHRA *et al.* 1992). Although this is an attractive model for diploid cells, it fails to explain the recombination-dependent UV and MMS sensitivity of haploid *srs2* strains described here (Figure 5). Instead, haploid *srs2* radiation sensitivity may result from recombination events between DNA lesions and small regions of ectopic sequence similarity leading to genomic rearrangements and chromosomal aberrations. Alternatively, *SRS2* may be required to prevent recombination during specific phases of the cell cycle such as S where newly replicated regions may contain potentially recombinogenic DNA breaks.

We have also characterized the effects of *RAD51* and *RAD52* overexpression on the DNA damage sensitivity of *srs2* mutants. Overproduction of *RAD51* or *RAD52* enhanced the MMS sensitivity of an *srs2Δ* strain without affecting UV^s (Figure 5). This may reflect an increased use of recombinational repair above that observed in an *srs2Δ* with normal levels of *RAD52* and *RAD51*. One interpretation of these results is that *RAD51* or *RAD52* overexpression specifically recruits MMS lesions into recombinational repair systems while having no effect on UV-induced lesions that are generally not repaired by recombination. This would implicate *RAD51* and *RAD52* as positive recombinational activators with important roles in recognition and processing of DNA lesions.

Activation of recombination by *RAD51*: Overexpression of *RAD51* suppressed a wide variety of *RAD52* alleles (summarized in Figure 2). Although *RAD52* and *RAD51* have been shown to physically interact (MILNE and WEAVER 1993; DONOVAN *et al.* 1994), the relative allele nonspecificity of suppression suggests that the mechanism of suppression may be the result of *RAD51* biochemical activity, rather than direct physical interaction with *RAD52*. In addition to significant primary sequence similarity with *recA*, *RAD51* has also been shown to form helical filaments on double-stranded DNA *in vitro* that structurally resemble those formed by *recA* (ABOUSSEKHRA *et al.* 1992; SHINOHARA *et al.* 1992; OGAWA *et al.* 1993). Recently, *RAD51* has also been shown to catalyze strand-annealing and -exchange reactions like *recA* (SUNG 1994). Thus, the compelling structural and functional similarities between *recA* and *RAD51* are consistent with *RAD51* playing a direct role in the formation of critical recombination intermediates which may in turn represent substrates for *RAD52*. These observations are consistent with a proposed role for *RAD51* early in recombination.

Although *RAD51*- and *srs2*-dependent suppression of *rad52* mutants are qualitatively similar, the molecular basis for suppression must be distinct. Overexpression of *RAD51* and deletion of *SRS2* had additive effects with regard to suppression of *rad52B* (Figure 6). This is in contrast to the result of epistasis that would be predicted if *RAD51* overexpression and *SRS2* deletion were mechanistically redundant. Also, we found that *RAD51* overexpression further differed from mutations in *SRS2* by a failure to suppress the UV and X-ray sensitivity of a *rad6Δ* strain (data not shown). In contrast to the antagonistic role of *SRS2*, *RAD51* is likely to function as a direct stimulator of recombinational repair. A positive role for *RAD51* in promoting recombination is consistent with both the genetic observation that *RAD51* overexpression enhances the sensitivity of an *srs2Δ* strain (Figure 5) and the biochemical properties of *RAD51* protein (OGAWA *et al.* 1993; SUNG 1994).

Our data demonstrate that *S. cerevisiae* recombinational repair can be modulated both positively and negatively by altering the levels of antagonistic (*SRS2*) and stimulatory (*RAD51*) effectors. In yeast, this balance is particularly apparent under conditions in which homologous recombination processes are impaired. However, antagonistic effects on homologous recombination may be relevant to other eukaryotic systems in which homologous recombination is normally disfavored relative to nonhomologous/illegitimate recombination events. Therefore, these results have important implications toward the ability to increase the frequency of homologous recombination events in mammalian cells by altering the levels of proteins homologous or analogous to *RAD51* and *SRS2*. Similarly, dysfunctions of the *srs2*-type may explain the elevated levels of recombination

and increased chromosome instability associated with DNA repair deficiencies, such as ataxia telangiectasia and Werners or Bloom syndrome.

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