Mutations in Recombinational Repair and in Checkpoint Control Genes Suppress the Lethal Combination of \textit{sr}s2\textit{A} With Other DNA Repair Genes in \textit{Saccharomyces cerevisiae}

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ABSTRACT

The \textit{SRS2} gene of \textit{Saccharomyces cerevisiae} encodes a DNA helicase that is active in the postreplication repair pathway and homologous recombination. \textit{sr}s2 mutations are lethal in a \textit{rad5A} background and cause poor growth or lethality in \textit{rad6A}, \textit{rad50A}, \textit{mre11A}, \textit{ssr2A}, \textit{rad27A}, \textit{sgs1A}, and \textit{top3A} backgrounds. Some of these genotypes are known to be defective in double-strand break repair. Many of these lethali
ties or poor growth can be suppressed by mutations in other genes in the DSB repair pathway, namely \textit{rad51}, \textit{rad52}, \textit{rad55}, and \textit{rad57}, suggesting that inhibition of recombination at a prior step prevents formation of a lethal intermediate. Lethality of the \textit{sr}s2A \textit{rad5A} and \textit{sr}s2A \textit{rad54A} double mutants can also be rescued by mutations in the DNA damage checkpoint functions \textit{RAD9}, \textit{RAD17}, \textit{RAD24}, and \textit{MEC3}, indicating that the \textit{sr}s2 \textit{rad54} and \textit{sr}s2 \textit{rad54} mutant combinations lead to an intermediate that is sensed by these checkpoint functions. When the checkpoints are intact the cells never reverse from the arrest, but loss of the checkpoints releases the arrest. However, cells do not achieve wild-type growth rates, suggesting that unrepaired damage is still present and may lead to chromosome loss.

The \textit{SRS2} gene was first identified through dominant mutations that suppressed trimethoprim sensitivity of \textit{rad6} and \textit{rad18} mutants (LAWRENCE and CHRI
tensen 1979). It was again recovered as a suppressor of damage sensitivity of the \textit{rad18} mutant (ABOUSSEKHRA \textit{et al.} 1989) and as a mutant that showed increased spontaneous mitotic gene conversion (RONG \textit{et al.} 1991). These studies placed \textit{SRS2} in the \textit{RAD6} epistasis group for DNA repair, in an error-free postreplication repair pathway. The sequence of the \textit{SRS2} gene revealed high homology to the bacterial repair gene \textit{uvrD}, encoding heli
case II (ABOUSSEKHRA \textit{et al.} 1989). Subsequently, it was demonstrated that the Srs2 protein has DNA helicase activity (RONG and KLEIN 1993). While the biochemical activity of \textit{SRS2} indicates a function in DNA metabolic activity, the precise role of the gene has remained enigmatic.

First, it is not clear why \textit{sr}s2 suppressor alleles are semidominant for the suppressor effect of \textit{rad6} or \textit{rad18} DNA damage sensitivity phenotypes. Second, \textit{SRS2} haploid mutants have a 5- to 10-fold increased sensitivity to UV damage (ABOUSSEKHRA \textit{et al.} 1989; PALLADINO and KLEIN 1992), which is significant but is not of the magni
tude of other UV-sensitive mutants. Haploid \textit{sr}s2 muta
tants are not sensitive to X-rays while diploid mutants are sensitive (ABOUSSEKHRA \textit{et al.} 1989). Third, all the mitotic DNA damage sensitivities are enhanced in mu
tant diploid strains as compared to mutant haploid strains (ABOUSSEKHRA \textit{et al.} 1989). This is unusual and has been interpreted as lethal recombination events occurring between homologous chromosomes in the absence of Srs2 function (ABOUSSEKHRA \textit{et al.} 1989). Thus in the mutant the haploid has a hyperrecombina
tion phenotype while in the diploid some of the inter
homolog recombination events are lethal, while other re
combination events such as intragenic recombina
tion are increased. To make the situation even more compli
cated, the suppression of \textit{rad6} and \textit{rad18} mutants by \textit{sr}s2 mutation is thought to occur by the channeling of repair substrates into the \textit{RAD52} recombination repair pathway (ABOUSSEKHRA \textit{et al.} 1989; RONG \textit{et al.} 1991).

Fourth, in meiosis the gene is required for full spore viability, and in its absence spore viability is \textasciitilde50% and map distances for the \textit{LEU2-HIS4} interval and the \textit{HIS4
dMAT} interval are reduced 2-fold (PALLADINO 1991).

Further clues as to the functional role of \textit{SRS2} have come from identification of double mutant combina
tions with \textit{sr}s2 mutants that are lethal or exhibit extremely poor growth and from suppressors of those \textit{sr}s2 mutant phenotypes. \textit{sr}s2 \textit{rad50} double mutants grow very poorly (RONG \textit{et al.} 1991; PALLADINO and KLEIN 1992). This most likely reflects an essential role for \textit{RAD50} in recombinational repair involving sister chrom
tids (SAEKI \textit{et al.} 1980) and suggests that \textit{SRS2} functions in G2 repair in haploids. The diploid DNA damage sensitivity and lethal recombination of the \textit{sr}s2 mutant is suppressed by semidominant mutations in \textit{RAD51} (ABOUSSEKHRA \textit{et al.} 1992), which encodes a RecA-like

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protein that functions in recombinational repair (Shinohara et al. 1992), again placing SRS2 in a step in homologous recombination that may occur after the RAD51-mediated step, although an alternate interpretation involves negative regulation of homologous recombination pathways by SRS2. ssr2 mutants also suppress nonnull semidominant alleles of RAD51 (Milne et al. 1995; Chant et al. 1996). This has been interpreted as a function for the Srs2 helicase in reversing abortive recombination intermediates.

We have previously reported that ssr2 mutations are lethal in a rad54 null mutant background (Palladino and Klein 1992). This lethality is suppressed by mutations in RAD51, RAD52, RAD55, and RAD57 (Schild 1995). These genes function in the recombinational repair pathway of yeast and are required for homologous recombination (Saeki et al. 1980). These results implicate SRS2 in recombinational repair, although epistasis analysis does not place SRS2 in the RAD52 repair pathway. Nonetheless, there is an interaction between SRS2 and RAD52 as null alleles of SRS2 are able to suppress nonnull alleles of RAD52 (Kaytor et al. 1995; Milne et al. 1995; Schild 1995). Although the mechanism of suppression is not understood, it may reflect a need for the Srs2 protein to stabilize and promote recombination between substrates with limited homology (Paques and Haber 1997). The rad52 alleles may attempt recombination between sequences of reduced homology and this reduced fidelity recombination requires SRS2. In the absence of functional Srs2p, aberrant recombination would be prevented and the rad52 alleles would be suppressed.

Additional evidence for SRS2 functioning in recombinational repair comes from studies of the interaction between SRS2 and SGS1. SGS1 encodes a DNA helicase related to the RecQ family of helicases (Gangloff et al. 1994; Watt et al. 1995; Lu et al. 1996). sgs1 mutant strains are characterized by increased genomic instability (Watt et al. 1996; Yamagata et al. 1998). Although the single null allele mutant strains exhibit normal growth, the double mutant ssr2 sgs1 is inviable or grows extremely poorly (Lee et al. 1999; Gangloff et al. 2000). The growth defect of the ssr2 sgs1 double mutant can be suppressed by mutations in the RAD51, RAD55, or RAD57 genes (Gangloff et al. 2000), indicating that the growth defect is caused by attempted homologous recombination that cannot be completed when both Srs2p and Sgs1p are missing.

To gain additional insight into the biological role of SRS2, we have examined other DNA recombination functions for synthetic interactions with a ssr2 null allele strain. We have also asked what types of mutants suppress ssr2 phenotypes. The results link SRS2 to homologous recombination that is associated with DNA replication and DNA damage that is sensed by DNA damage checkpoints.

MATERIALS AND METHODS

**Yeast strains:** Parental strains used in this study are listed in Table 1. All strains are isogenic and are in the W303 background (W303 genotype: leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-1 RAD5). sgs1 disruption strains were a gift from Rodney Rothstein. The mre11 and exo1 strains were a gift from Lorraine Symington. The rad9, rad17, rad24, and mre11 strains were a gift from Ted Weinert. Strains, if needed, were converted to the RAD5 version of W303 through crosses. The rad54 point mutation strains have been described (Petukhova et al. 1999). All double and triple mutant strains were derived from crosses using the parental single mutant strains listed in Table 1. All recombination and checkpoint mutants are null alleles with the exception of the two indicated rad54 point mutants and the one indicated mre11 point mutant.

**Media and growth conditions:** Standard media were prepared as described (Sherman et al. 1986). All strains were grown on solid media at 30°C.

**RESULTS**

**ssr2 and rad54:** We have previously reported that the ssr2 rad54 double null mutant is lethal (Palladino and Klein 1992). This lethality is suppressed by loss of the homologous recombination functions of RAD51, RAD52, RAD55, or RAD57 (Schild 1995 and this study). However, not all mutations of genes involved in homologous recombination or repair of a double-strand break rescue the ssr2 rad54 lethality. The RAD1 gene encodes an endonuclease that is active in nucleotide excision repair and some recombination reactions (Klein 1988; Schiestl and Prakash 1988; Thomas and Rothstein 1989; Sieve et al. 1993; Tomkinson et al. 1993; Ivanov and Haber 1995; Kirkpatrick and Petes 1997). However, a rad1 null mutation was not able to rescue the ssr2Δ rad54Δ strain.

RAD54 encodes a protein of the SNF2/SWI2 family and contains DNA helicase consensus motifs (Eisen et al. 1995). The protein has demonstrated in vitro ATPase activity and promotes Rad51-mediated strand exchange (Petukhova et al. 1998) via a change in DNA double helix conformation (Petukhova et al. 1999). Two point mutations in the first helicase consensus domain, rad54K341R and rad54K341A, have been shown to abolish ATP hydrolysis and Rad51-promoted homologous DNA pairing as well as mitotic recombination (Petukhova et al. 1999). Both of these mutations in a ssr2Δ background are lethal, showing that the essential function provided by Rad54 protein in a ssr2Δ strain requires the ATPase activity.

Our studies on rad54 vsd54 diploid double mutants had suggested that the double mutant poor growth was due to the accumulation of DNA damage through attempted recombination that was sensed by the DNA damage checkpoint functions. This finding prompted us to speculate that the ssr2 rad54 lethality might be due to the accumulation of DNA damage from attempted recombination that was sensed by the DNA damage checkpoint functions. Therefore the role of the check-
point functions in preventing srs2 rad54 cells from progressing through the cell cycle was assessed by determining the growth phenotype of triple mutants. Triple mutants were segregated in crosses. An example of such a cross is shown in Figure 1. In Figure 1A growth of colonies of the genotype srs2 rad54 rad17, srs2 rad54 rad24, or srs2 rad54 mec3 is compared to a wild-type strain. While the triple mutant strains grow slower than wild type and throw off colonies of varying sizes, these genotypes are viable. In contrast, a rad9 mutation is unable to rescue the srs2 rad54 double mutant (Figure 1B). Figure 1, B and C compares the ability of a rad51 mutation vs. a rad17 mutation to suppress the srs2 rad54 lethality. It can be seen that rad51 is more effective in suppressing the srs2 rad54 lethality, suggesting that the checkpoint mutants suppress the double mutant by release from growth arrest, but DNA damage is still present. Further support for this statement comes from plating efficiencies. One hundred unbudded cells of the srs2 rad54 rad17 and srs2 rad54 rad51 genotypes were micromanipulated to fresh YPD plates. A total of 50/100 srs2 rad54 rad17 cells grew to visible colonies whereas 93/100 srs2 rad54 rad51 cells grew to visible colonies.

**srs2 and rdh54:** The RAD54 and RDH54 genes have related sequences, but the single mutants have unique effects on mitotic recombination and meiotic viability (Klein 1997; Shinojara et al. 1997). We have previously reported that, while in contrast to the srs2 rad54 haploid mutant lethality the srs2 rdh54 haploid mutant shows normal growth, lethality is exhibited in the srs2 rdh54 diploid (Klein 1997). Moreover, this lethality is suppressed by mutation in any one of the recombination repair genes RAD51, RAD52, RAD55, or RAD57 (Klein 1997). Similar to the suppression of srs2 rad54 by DNA damage checkpoint mutations, the srs2 rdh54 diploid lethality is suppressed by mutations in RAD17, RAD24, or MEC3, but not by mutation of the RAD9 gene (Table 2).

**srs2 and rad50/srs2/mre11:** The Rad50, Xrs2, and Mre11 proteins function in a complex to protect DNA ends in end-joining reactions and in meiotic recombination (for a review see Haber 1998). The RAD50, XRS2, and MRE11 genes are not required for mitotic homolog recombination, but are required for recombination between sister chromatids (Säeki et al. 1980). The srs2 null allele mutant has a synthetic growth interaction with null allele mutations in RAD50, XRS2, or MRE11 (see Figure 2A) and this is suppressed by mutations in the

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**TABLE 1**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKY590-1D</td>
<td>MATa srs2::HIS3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY590-6D</td>
<td>MATa srs2::HIS3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY725-6C</td>
<td>MATa srs2::TRP1 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY604-17C</td>
<td>MATa rad50::HIS3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY603-15B</td>
<td>MATa srs2::URA3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY596-1A</td>
<td>MATa rad54::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY596-2B</td>
<td>MATa rad54::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY624</td>
<td>MATa rad54::HIS3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY887</td>
<td>MATa rad54::K314A leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY838</td>
<td>MATa rad54::K341R leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
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<tr>
<td>HKY615-1A</td>
<td>MATa rad1::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
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<td>HKY1039-4D</td>
<td>MATa rad51::HIS3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY614-10B</td>
<td>MATa rad52::TRP1 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY597-2C</td>
<td>MATa rad55::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY598-1B</td>
<td>MATa rad57::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY606-1A</td>
<td>MATa top3::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY606-4D</td>
<td>MATa top3::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY619-3C</td>
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</tr>
<tr>
<td>HKY845-1A</td>
<td>MATa rad9::HIS3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY843-1B</td>
<td>MATa rad17::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY844-11A</td>
<td>MATa rad24::TRP1 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>HKY846-2A</td>
<td>MATa mec3::TRP1 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>1958-10A</td>
<td>MATa sgs1::URA3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>LSY796-3D</td>
<td>MATa sgs1::URA3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>LSY624</td>
<td>MATa exo1::URA3 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>LSY569</td>
<td>MATa mre11::LEU2 leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
<tr>
<td>LSY726</td>
<td>MATa mre11H125N::URA3::mre11D56N leu2-3, 112 his3-11, 15 ade2-1 ura3-1 trp1-1 can1-100</td>
</tr>
</tbody>
</table>

All strains are in the W303 RAD5 background. Strain 1958-10A was received from Rodney Rothstein. Strains LSY796-3D, LSY624, LSY726, and LSY569 were received from Lorraine Symington.
srs2 rad50

Poor growth was not suppressed by mutation because it was of interest to examine the phenotype of the DNA damage checkpoint function outlined in squares. It can be seen that a rad24 mutation, found to be partially defective for an intra-S checkpoint, is a stronger suppressor than the DNA damage checkpoint. The rad51 strain is viable but grows more slowly than other viable genotypes (srs2 outlined in squares. It can be seen that the double mutants srs2 rad17 and rad54 are generated by crossing a rad17 Δ or rad54 Δ strain. Growth is slightly reduced compared to a wild-type control strain. (B) The ability of a rad9 Δ mutation to suppress is compared to the ability of a rad17 Δ mutation to suppress. Srs2 Δ rad54 Δ double mutants are outlined in circles while the triple mutants srs2 Δ rad54 Δ rad9 Δ/ rad17 Δ are outlined in squares. It can be seen that the srs2 Δ rad54 Δ rad17 Δ strain is viable but grows more slowly than other viable genotypes. (C) The ability of a rad51 Δ mutation to suppress srs2 Δ rad54 Δ is shown. Circles indicate the srs2 Δ rad54 Δ double mutants while the triple mutants srs2 Δ rad54 Δ rad51 Δ are outlined in squares. It can be seen that a rad51 Δ mutation is a stronger suppressor than the DNA damage checkpoint.

Recombination repair genes (Table 3). However, the srs2 rad50 poor growth was not suppressed by mutation of the DNA damage checkpoint function RAD17 (Figure 2A). The Mre11 nucleosome activity is not required for viability of srs2 mutants. Since the double null allele growth defect is rescued by recombination repair mutations, this suggests that the poor growth is due to attempted recombination. The fact that the srs2 mre11 Δ-nucleosome-deficient strain is viable indicates that recombinational repair is not impaired in this strain.

RAD50, XRS2, and MRE11 are also required for telomere length and in the mutants telomeres are shortened (Kironmai and Muniyappa 1997; Le et al. 1999). Since double mutants of srs2 combined with either rad50, srs2, or mre11 show poor growth, one possibility is that telomere length is greatly altered in the double mutant. This was excluded by first showing that srs2 strains have normal telomere lengths and then observing that srs2 rad50 strains have telomeres of the rad50 characteristic length (H. Klein and R. Boguslavsky, data not shown).

srs2 and exo1: The EXO1 gene encodes an exonuclease that functions in mitotic recombination (Fiorentini et al. 1997). The exo1 mutant when combined with a mutant in nucleosome involved in DNA replication is lethal (Tishkoff et al. 1997a). This property is shared with genes involved in recombinational repair (Symington 1998). Since the srs2 mutant also has genetic interactions with the same set of genes, the phenotype of the double mutant srs2 exo1 was determined. The double mutant srs2 exo1 was fully viable, showing that neither gene is required for any essential function when the other gene is defective.

srs2 sgs1 and srs2 top3: SGS1 encodes a DNA helicase of the same polarity as the Srs2 DNA helicase (Lu et al. 1996; Bennett et al. 1998). It has been reported that the double mutant srs2 sgs1 is lethal or grows extremely poorly (Lee et al. 1999; Gangloff et al. 2000). This growth phenotype is due to attempted recombination as mutations in the recombinational repair genes RAD51, RAD52, RAD55, or RAD57 rescue the growth defect. We have confirmed these observations. sgs1 cells have been found to be partially defective for an intra-S checkpoint for DNA damage that acts in parallel with the RAD24 checkpoint function (Frei and Gasser 2000). Therefore, it was of interest to examine the phenotype of the srs2 sgs1 rad24 triple mutant. We found that the rad24
Suppression was tested for each mutant individually. A nonnull allele strain. The double mutants, indicated by circles, are fully viable. (B) A rad17 mutation does not suppress the poor growth phenotype of a srs2 rad50Δ double mutant. A cross of srs2Δ rad17Δ by rad50Δ is shown. Circles indicate the srs2Δ rad50Δ double mutants while the triple mutants srs2Δ rad50Δ rad17Δ are outlined in squares.

**DISCUSSION**

All of the double mutant interactions with srs2 and suppression by defects in homologous recombination or the DNA damage checkpoint are summarized in Tables 2 and 3. The findings would indicate that Srs2p acts in recombination after commitment to repair via Top3 protein, which has type I topoisomerase activity. Moreover, the slow growth phenotype of the top3 mutant is suppressed by a sgs1 mutation (Gangloff et al. 1994). Therefore we examined the phenotype of the srs2 top3 double mutant. The double mutant is lethal (Figure 4) and this lethality is also suppressed by loss of the recombination repair pathway through mutations in RAD51, RAD52, RAD55, or RAD57 (Figure 4 and Table 3).

**TABLE 2**

Summary of srs2 interactions with rad54 or rdh54 mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Suppressed by rad51, rad52, rad55, or rad57*</th>
<th>Suppressed by rad17, rad24, or mec1*</th>
<th>Suppressed by rad9</th>
</tr>
</thead>
<tbody>
<tr>
<td>srs2Δ rad54Δ</td>
<td>Lethal</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>srs2Δ rad54K341R</td>
<td>Lethal</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>srs2Δ rad54K341A</td>
<td>Lethal</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>srs2Δ rad5Δ</td>
<td>Haploid is viable</td>
<td>Yes, for diploid</td>
<td>No, for diploid</td>
<td>Yes, for diploid</td>
</tr>
<tr>
<td></td>
<td>Diploid is lethal</td>
<td></td>
<td>No, for diploid</td>
<td></td>
</tr>
</tbody>
</table>

ND, not done.

* Suppression was tested for each mutant individually.
TABLE 3

Summary of srs2 interactions with repair mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Mutant suppression of poor growth/lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>srs2Δ rad50Δ</td>
<td>Very poor growth</td>
<td>Suppressed by rad51, not suppressed by rad17</td>
</tr>
<tr>
<td>srs2Δ xrs2Δ</td>
<td>Very poor growth</td>
<td>Suppressed by rad51</td>
</tr>
<tr>
<td>srs2Δ mre11Δ</td>
<td>Very poor growth</td>
<td>Suppressed by rad51</td>
</tr>
<tr>
<td>srs2Δ mre11H125N-mre11D56N</td>
<td>Normal growth</td>
<td></td>
</tr>
<tr>
<td>srs2Δ exo1Δ</td>
<td>Normal growth</td>
<td></td>
</tr>
<tr>
<td>srs2Δ sgs1Δ</td>
<td>Lethal</td>
<td>Suppressed by rad51, not suppressed by rad1 or rad24</td>
</tr>
<tr>
<td>srs2Δ top3Δ</td>
<td>Lethal</td>
<td>Suppressed by rad51, rad52, rad55, rad57, not suppressed by rad1</td>
</tr>
<tr>
<td>srs2Δ rad27Δ</td>
<td>Lethal</td>
<td>Not suppressed by rad51 or rad17</td>
</tr>
</tbody>
</table>

Not all recombination repair or checkpoint function mutants were tested. The genes tested are indicated.

homologous recombination. If homologous recombination is prevented by inhibiting the initial steps of forming a Rad51 filament on single-stranded DNA, then srs2 rad54 or srs2 rad50/xrs2/mre11 lethality/poor growth can be prevented. This suggests, first, that there exist other pathways in the cell to repair the spontaneous damage that occurs in these double mutants and, second, that once a cell is committed to repair damage through homologous recombination, through action of Rad51p, there is no alternate repair option available. Experiments on in vivo repair of substrates containing a double-strand break indicate an important role for the Srs2 helicase in the repair process (Paques and Haber 1997). The lethality of haploid strains bearing srs2 and rad54 mutations shows that Srs2p can have an important role in repair of spontaneous DNA damage, but in srs2 single mutants there is some substrate specificity as to the action of Srs2p in repair as diploid srs2 mutants show a greatly increased damage sensitivity and it is the attempted homologous recombination between homolog chromosomes, not sister chromatids, that is lethal in the absence of Srs2 helicase (Aboussekhra et al. 1989, 1992).

Studies of srs2 mutants as suppressors of nonnull alleles of RAD51 and RAD52 have provided an alternative interpretation of the role of Srs2p in homologous recombination (Kaytor et al. 1995; Milne et al. 1995; Chanet et al. 1996). These articles have suggested two regulatory functions for SRS2. First, the Srs2 helicase is proposed to antagonize the action of Rad51p and Rad52p in homologous recombination. Through such an activity Srs2p would have an antirecombinase func-

srs2Δ rad24Δ x sgs1Δ

Figure 3.—A rad24Δ mutation does not suppress the poor growth phenotype of srs2Δ sgs1Δ strains. Five tetrads from the cross of a srs2Δ rad24Δ strain to a sgs1Δ strain are shown. The double mutants srs2Δ sgs1Δ are indicated by circles and are inviable or grow very poorly. The triple mutants srs2Δ sgs1Δ rad24Δ are indicated by squares and also are inviable or grow very poorly.

srs2Δ rad51Δ x top3Δ

Figure 4.—Suppression of srs2Δ top3Δ inviability by a rad51Δ mutation. Five tetrads from the cross of a srs2Δ rad51Δ strain to a top3Δ strain are shown. The double mutants srs2Δ top3Δ are indicated by circles and are inviable. The triple mutants srs2Δ top3Δ rad51Δ are indicated by squares and are viable, although they grow slower than other genotypes. The unmarked small colonies carry the top3Δ allele. The reduced growth of the top3Δ strain is not rescued by a rad51Δ mutation, probably accounting for the slower growth of the triple mutant.
tion and could also control use of the homologous recombination pathway to repair a spontaneous damaged substrate. Srs2p would determine which lesions could be processed through homologous recombination. Therefore Srs2p would inhibit homologous recombination prior to the commitment to repair by homologous recombination. In this scheme one would then suggest that, in the absence of Srs2p, substrates are inappropriately targeted for repair through the homologous recombination pathway. When Rad54p is also absent, the processing of substrates through homologous recombination becomes an irreversible lethal action. Rescue through loss of Rad51p, Rad52p, Rad55p, or Rad57p is thought to happen by inhibiting the initial steps of homologous recombination, prior to the Rad54p-mediated step (PETUKHOVA et al. 1998). Rescue through loss of DNA damage checkpoints implies that the cells are arrested due to the accumulation of unrepaired DNA damage that is sensed by these checkpoint genes.

Other studies have suggested that the Srs2 helicase can act at a later step in recombination (RONG et al. 1991; PAQUES and HABER 1997). Whether the helicase acts in concert with Rad54 protein is not known. However, under either model the basic scenario for suppression remains the same, namely, preventing recombination at an early step rescues lethal mutant combinations.

The second regulatory function for Srs2p has been proposed to be repression of a repair pathway that parallels the RAD52 recombination repair pathway (KAYTOR et al. 1995). This remains conjectural, although the recent finding of multiple repair pathways and the RAD59 gene, which is related to RAD52 (RATTRAY and SYMINGTON 1995; BAI and SYMINGTON 1996), provides support for this idea.

The arrest of haploid cells through loss of Srs2 and Rad54 proteins triggers an irreversible arrest that is sensed by DNA damage checkpoint functions. Loss of the checkpoint functions allows cells to grow, but full viability as measured by plating efficiency and colony size is not restored, suggesting that unrepaired damage remains or that chromosome loss is occurring. This cannot be measured in haploid cells for essential chromosomes. The data thus far show that mutations in RAD17, RAD24, and MEC3 can partially suppress the growth arrest, but loss of RAD9 function cannot. Why there is a differential suppression by this group of DNA damage checkpoint genes is not clear, but for some types of DNA damage RAD9 acts additively to RAD17/ RAD24/MEC3 in sensing DNA damage (LYDALL and WEINERT 1995; DE LA TORRE-RUIZ et al. 1998). However, in the DNA damage arrest of rad54 rad54 mutants, no additive effect of a rad9 and a rad24 mutation has been seen in suppression of poor growth (H. KLEIN, unpublished observations).

The Sgs1 helicase is a component of the S-phase checkpoint response acting upstream of the Rad53 kinase (FREI and GASSER 2000). This response has been shown to act in parallel to the checkpoint response that requires RAD24 (FREI and GASSER 2000). The Srs2 helicase has also been shown to be involved in an S-phase checkpoint response that is dependent on RAD17 and RAD24 (LIBERI et al. 2000). The observation that the srs2 sgs1 double mutant poor growth is suppressed by mutations in recombination repair functions, but not by a mutation in the RAD24 checkpoint function, may indicate that the double mutant defect is due to attempted recombination, which can trigger a checkpoint response. Recombination may be an attempt to repair DNA damage that has its origin in a replication defect (FREI and GASSER 2000). Mutation of RAD24 in the srs2 sgs1 double mutant may result in a defect in two parallel checkpoint response pathways, with the result of continued poor growth. Alternatively, loss of the RAD24 checkpoint function may permit the srs2 sgs1 cells to progress through the cell cycle without repairing DNA lesions that have become lethal. Since a rad24 mutation can suppress the lethality of the srs2 rad54 mutant, but not the srs2 sgs1 mutant, this would suggest that the DNA intermediate, presumably related to attempted recombination, that occurs in each of these cell types is different. The difference may be the presence of an unrepaired double-strand break or a structure that can be shuttled into an alternate repair pathway.

It has been suggested that the Sgs1 helicase can act on different substrates, controlled by the action of the topoisomerases I and III and the Srs2 DNA helicase (DUNO et al. 2000). In this regard the srs2 sgs1 poor growth/lethality is proposed to be similar to the srs2 top3 lethality as the alternative helicase/topoisomerase activities of Sgs1/Top3 and Srs2/Top1 are disrupted in these double mutants. What determines the specificity
of the target substrate is not known, but must be linked to recombinational repair.

Srs2 helicase has some interaction with Rad50p/ Xrs2p/Mre11p although whether this is merely genetic or also has a physical basis is not known. We have not detected any interaction with these proteins from two-hybrid screens (H. Klein, unpublished observations). However, the genetic interaction does not involve the Mre11p nuclease function, suggesting that Srs2p may be targeted to open up broken ends by the Rad50p/ Xrs2p/Mre11p complex. The failure of a rad17 mutation to suppress the poor growth phenotype of the srs2 rad50 mutant can be interpreted in two ways. Loss of the RAD17 checkpoint function may permit srs2 rad50 cells to progress through the cell cycle with unrepaired damage, with continued poor growth due to some type of genomic instability. Alternatively, the DNA damage that accumulates in the srs2 rad50 mutant may not be a target for the DNA damage checkpoints that act through RAD17.

We have no information as to when the spontaneous damage is occurring in the srs2 rad54 strain and in the other double mutant combinations that exhibit poor or no growth. SRS2 is induced in expression at the beginning of S phase and is induced in G2 by DNA damage (Heude et al. 1995), suggesting that these phases of the cell cycle may be more prone to DNA damage resulting from loss of Srs2 protein. In the case of the srs2 rad27 lethality, it is likely that the damage occurs during S phase as RAD27 is required for DNA replication (Harrington and Lieber 1994; Reagan et al. 1995; Sommers et al. 1995). If the damage that is targeted for repair by Srs2 helicase, either as a catalytic function in repair or through regulation of which substrate is repaired by the DSB repair pathway, occurs during S phase, this would reinforce the close link between DNA replication and recombination and also bring into play a link between DNA replication and nonrecombination (non-DSB repair) repair modes.

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