Mutations in the Yeast SRB2 General Transcription Factor Suppress hpr1-Induced Recombination and Show Defects in DNA Repair

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ABSTRACT

We have obtained genetic and molecular evidence that the hrs2-1 mutation, isolated as a suppressor of the hyperrecombination phenotype of hpr1Δ, is in the SRB2 gene, which encodes a component of the RNA polIII holoenzyme. A newly constructed srb2Δ allele restores the wild-type levels of deletions in hpr1Δ cells, indicating that the lack of a functional SRB2 transcription factor suppresses recombination between direct repeats. These results suggest a direct connection between transcription and recombination between DNA repeats. On the other hand, the hrs2-1 mutation (renamed srb2-101), in which Gly150 has been changed to Asp, makes cells sensitive to long MMS treatments, a phenotype observed for the srb2Δ null allele only in a hpr1Δ background. This indicates that mutations in the basal transcription factor SRB2 impair DNA repair of MMS-induced damage, which adds a new connection between transcription and DNA repair. We discuss the possibility that hpr1-induced deletions occurred as a consequence of a SRB2-dependent stalled or blocked transcription complex.

RECOMBINATION between direct DNA repeats is a source for deletion of genetic information, which can have deleterious consequences for the cell. This is under genetic control, as shown by the existence of Escherichia coli (see Clark and Low 1988; Smith 1988) and yeast mutations (Petes et al. 1991), as well as human genetic disorders (Fukuchi et al. 1989; Mehn 1993) associated with instability of DNA repeats caused by recombination. To determine the importance of DNA repeats as a source of DNA deletions and other chromosomal aberrations in eukaryotes, it is important to know the mechanisms and the genes that participate in DNA repeat recombination.

It is believed that recombination occurring through reciprocal exchange is not the only mechanism responsible for deletions between repeats. In yeast, this conclusion is supported by different data. Thus, mitotic reciprocal exchange results from recombination pathways depending on the double-strand break (DSB)-repair gene RAD52, whereas deletions result from RAD52-dependent and RAD52-independent pathways (Jackson and Fink 1981; Klein 1988; Schiestl and Prakash 1988). Also, the frequency of deletions do not depend on the length of homology of the recombinant DNA molecules (Yuan and Keil 1990), as is the case for reciprocal exchange (see Petes et al. 1991). In addition, and very importantly, a DSB in nonhomologous DNA sequences flanked by repeats stimulate deletions (Rudin and Haber 1988; Nickoloff et al. 1989) but not reciprocal exchange events (Prado and Aguilera 1995). These results are better explained if deletions between long DNA repeats can occur, in addition to reciprocal exchange, through nonconservative mechanisms such as, for instance, single-strand annealing (SSA) (Ozenberg-erg and Roeder 1991; Fishman-Lobell and Haber 1992) or one-ended invasion crossover (Mezard and Nicolas 1994; Prado and Aguilera 1995).

The genetic analysis of mutations affecting DNA repeat recombination is contributing to identify differences between direct repeat recombination and gene conversion-reciprocal exchange observed between homologous chromosomes or inverted repeats. Thus, the rad1 or rad10 mutants, affected in the Rad1p/Rad10p excision repair endonuclease, have no effect on the frequency of recombination between inverted repeats or homologous chromosomes (Aguilera and Klein 1989b; Aguilera 1995). However, they show a significant reduction in the frequency of spontaneous and DSB-induced deletions between direct repeats (Klein 1988; Schiestl and Prakash 1988, 1990; Fishman-Lobell and Haber 1992; Ivanov and Haber 1995; Prado and Aguilera 1995). It has been suggested that the Rad1p endonuclease removes the single-strand DNA tails generated after exonuclease digestion of a DSB during single-strand annealing (Fishman-Lobell and Haber 1992). Recently, it has also been shown that mutations in the recombinational repair genes RAD51, RAD54, RAD55 and RAD57 are required, at different degrees, for gene conversion/reciprocal exchange recombination. However, they all lead to a hyperrecombination phenotype between direct repeats (McDonald and Rothstein 1994; Aguilera 1995; Liefshtiz et al. 1995; Rattray and Symington 1995). There are also mutations that induce recombination between repeats,
but they have no important effect on reciprocal exchange/gene conversion recombination. Some of these mutations are in the DNA topoisomerase I, II and III genes, TOP1, TOP2 and TOP3, in the silencing gene SIR2, or in the uncharacterized gene RRM3, all of which lead to the genetic instability of the rDNA region (CHRISTMAN et al. 1988; GOTTLIEB et al. 1989; KEIL and MCWILLIAMS 1993).

Mutations in the HPR1 gene strongly stimulate deletions between direct repeats (AGUILERA and KLEIN 1989a, 1990) with no effect on gene conversion/reciprocal exchange recombination (AGUILERA and KLEIN 1989a; SANTOS-ROSA and AGUILERA 1994). To explain how deletions between direct repeats initiate in Hpr1Δ cells, we have postulated that in the absence of Hpr1p, there is a high incidence of recombination-initiation events that are processed through a nonconservative mechanism of recombination (SANTOS-ROSA and AGUILERA 1994). The Hpr1p protein has a nuclear transport motif (SANTOS-ROSA and AGUILERA 1994) and acts as a positive regulator of transcription (FAN and KLEIN 1994; ZHU et al. 1995).

To gain some insight into the mechanism of formation of deletions, we conducted a search for extragenic suppressors of the hyperrecombination phenotype hpr1Δ that identified five genes, HRS1–HRS5 (SANTOS-ROSA and AGUILERA 1995). Among them, the hrs2-1 mutation completely suppressed the hyperdeletion phenotype of hpr1Δ. However, it was the only hrs mutation conferring a MMS-sensitive phenotype and a partial suppression of the hpr1Δ phenotype of lack of gene expression activation (SANTOS-ROSA and AGUILERA 1995). In this study, we show that HRS2 is identical to SBB2, a component of the SRB complex of the RNA polIII holoenzyme (KOLESKE et al. 1992). We also show that a srb2Δ null mutation completely suppresses the hyperrecombination phenotype of hpr1Δ cells, as the hrs2-1 allele did. However, it does not show the same DNA repair- and gene expression-associated phenotypes reported for the hrs2-1 mutation (SANTOS-ROSA and AGUILERA 1995). Our results provide genetic evidence that the hyperdeletion phenotype of hpr1Δ cells is mediated by transcription factors.

### MATERIALS AND METHODS

#### Strains

The yeast strains used in this study are listed in Table 1. Strains constructed for this study are congenic to AYW3-3D.

#### Plasmids

Plasmids used in this study are listed in Table 2.

#### Media and growth conditions

Standard media such as rich medium YEPD, synthetic complete medium (SC) with bases and amino acids omitted as specified, and sporulation medium were prepared as described previously (SHERMAN et al. 1986). L-Canavanine sulfate and 5-fluoro-orotic acid (5-FOA) were added to synthetic medium at concentrations of 60 mg and 750 mg/l, respectively. MMS was added to YEPD or SC plates to the final concentration of 0.02% or 0.017%, respectively. All yeast strains were grown at 30°C with horizontal shaking for liquid cultures. Yeast strains were transformed using

### TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5Y3A</td>
<td>MATα ura3 ade2 his3 leu2-k::URA3-ADE2::leu2-k</td>
<td>AGUILERA and KLEIN (1990)</td>
</tr>
<tr>
<td>AYW3-3D</td>
<td>MATα ura3 ade2 his3 leu2-k::URA3-ADE2::hpr1Δ::HIS3</td>
<td>SANTOS-ROSA and AGUILERA (1995)</td>
</tr>
<tr>
<td>AYW3-1B</td>
<td>MATα ura3 ade2 his3 ade1::URA3-ADE2::ure2-k hpr1Δ::HIS3</td>
<td>SANTOS-ROSA and AGUILERA (1995)</td>
</tr>
<tr>
<td>SS713B-1A</td>
<td>MATα ura3 ade2 his3 leu2-k::URA3-ADE2::leu2-k hpr1Δ::HIS3 sb2-101</td>
<td>SANTOS-ROSA and AGUILERA (1995)</td>
</tr>
<tr>
<td>X260-3A</td>
<td>MATα ura3 ade2 his3 ade1::URA3-ADE2::leu2-k sb2-101</td>
<td>This study</td>
</tr>
<tr>
<td>W19Y1B</td>
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<td>This study</td>
</tr>
<tr>
<td>SB71-5A</td>
<td>MATα ura3 ade2 his3 ade1::URA3-ADE2::leu2-k sb2-101</td>
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<tr>
<td>SB71-8A</td>
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<td>HDY8-7D</td>
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</tr>
<tr>
<td>HDSB-2A</td>
<td>MATα ura3 ade2 his3 ade1::URA3-ADE2::leu2-k sb2-101</td>
<td>This study</td>
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<td>WS1971-3D</td>
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</table>
were grown on YEPD or SC-leu, as appropriate. After 3 days, independent colonies were prepared as described (FEINBERG and VOLGELSTEIN 1983) using digoxigenine-dUTP. Detection of digoxigenine-labeled DNA probes was performed as described (MEAD et al. 1986).

**TABLE 2**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLGSD5</td>
<td>YEp plasmid containing the URA3 gene and the E. coli lacZ gene under the yeast CYC-GAL1,10 promoter</td>
<td>GUARENTE et al. (1982)</td>
</tr>
<tr>
<td>pTZ18U</td>
<td>Vector derived from pUC18</td>
<td></td>
</tr>
<tr>
<td>YEp1</td>
<td>The 10.1-kb EcoRI fragment containing the HIS3 gene subcloned into pBR322</td>
<td></td>
</tr>
<tr>
<td>YEp351</td>
<td>YEp vector carrying the LEU2 gene</td>
<td></td>
</tr>
<tr>
<td>pBS32</td>
<td>YEp vector based on the LEU2 gene</td>
<td></td>
</tr>
<tr>
<td>pRS315</td>
<td>YEp vector based on the LEU2 gene</td>
<td></td>
</tr>
<tr>
<td>YEpM1</td>
<td>A 6.5-kb HRS2/SRB2 genomic insert subcloned in YEp351</td>
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</tr>
<tr>
<td>YEpM4</td>
<td>A 8.9-kb HRS2/SRB2 genomic insert subcloned in YEp351</td>
<td></td>
</tr>
<tr>
<td>YCP18</td>
<td>A 10.3-kb HRS2/SRB2 genomic insert subcloned in pBS32</td>
<td></td>
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<tr>
<td>pMPS41</td>
<td>The left 3.86-kb Sac-Pst fragment from the insert of YEpM4 subcloned in YEp351</td>
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<tr>
<td>pMBH12</td>
<td>The 3.25-kb BamHI-HindIII fragment from the insert of YEpM1 subcloned in pRS315</td>
<td>This study</td>
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<tr>
<td>pMBP13</td>
<td>The 8.25-kb PsI fragment from pMBH12</td>
<td>This study</td>
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<tr>
<td>pHRS21</td>
<td>pMBH12 containing the hrs2-1(srb2-101) allele, instead of HRS2/SRB2</td>
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<td>The 3.25-kb BamHI-HindIII fragment of pHRS21 subcloned in YEp351</td>
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<tr>
<td>pRS40</td>
<td>The 3.1-kb SacI-Pst fragment from the insert of pHRS21 subcloned in pRS315</td>
<td>This study</td>
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<tr>
<td>pKS9</td>
<td>The 0.9-kb PsI fragment from the insert of pHRS21 subcloned in pRS315</td>
<td>This study</td>
</tr>
<tr>
<td>pSRB5</td>
<td>The 0.9-kb PsI fragment from the insert of pMBH12 subcloned into pTZ18U</td>
<td>This study</td>
</tr>
<tr>
<td>pSRB35</td>
<td>The 0.63-kb KpnI-SalI fragment of pMBH2 subcloned into pSRB5</td>
<td>This study</td>
</tr>
<tr>
<td>pSRB35H</td>
<td>The 2.84-kb BamHI-HindIII fragment of YEp1 containing the entire HIS3 gene</td>
<td>This study</td>
</tr>
</tbody>
</table>

Plasmid DNA was isolated from E. coli by CaCl2 gradient centrifugation as described (CLEWELL and HELNÖR 1970). Small-scale plasmid DNA preparations were made as previously published (BIGGIN et al. 1980). Yeast genomic DNA was prepared from 5 ml YEPD cultures as described (SHERMAN et al. 1986). Plasmid yeast DNA was prepared according to HOFFMAN and WINSTON (1987) and used directly to transform E. coli.

**DNA manipulation:** Plasmid DNA was isolated from E. coli by CaCl2 gradient centrifugation as described (CLEWELL and HELNÖR 1970). Small-scale plasmid DNA preparations were made as previously published (BIGGIN et al. 1980). Yeast genomic DNA was prepared from 5 ml YEPD cultures as described (SHERMAN et al. 1986). Plasmid yeast DNA was prepared according to HOFFMAN and WINSTON (1987) and used directly to transform E. coli.

Digoxigenin-dUTP (Boehringer)-labeled DNA probes were prepared as described (FISCHER and VOLGELSTEIN 1984). Hybridization was performed in 50% formamide, 5X SSC, 0.01% N-lauroylsarcosine, 0.02% SDS and 2% Baebohringer Mannheim blocking reagent at 42°C for 18 hr when using digoxigenin-dUTP. Detection of digoxigenin-labeled DNA was performed following Boehringer Mannheim recommendations.

Linear DNA fragments were recovered directly from agarose gels and used in DNA labeling experiments or in ligation reactions with T4 DNA ligase overnight at 14°C.

**Cloning of the HRS2 gene:** Strain SS713B-1A carrying the duplication system leu2-k::URA3-DE2::leu2-k was used to screen for plasmids carrying inserts able to complement the MMS sensitivity phenotype of hrs2-1 mutation in the hpr1Δ background. Leu" transformants were selected on SC -leu supplemented with 0.017% MMS.

From ~80,000 transformants with the MW90 library and 26,900 with the pBS32-based library, a total of 31 candidates (16 and 15 from each library, respectively) were selected for their capacity to grow on SC -leu supplemented with 0.017% MMS (MMS-resistant phenotype). From these, six transformants (four and two from each library, respectively) were selected for which the MMS-resistant phenotype cosegregated with the Leu" phenotype.

**Cloning of the hrs2-1(srb2-101) allele:** Plasmid pHMBH12 was cut with EcoRV (partial digestion) to obtain a 8.0-kb linearized plasmid with a gap of 1145 bp (357 bp corresponding to the SRA23 gene) and with NcoI to obtain a 8.6-kb linearized plasmid with a gap of 570 bp (439 bp corresponding to the SRA23 gene). Both gapped plasmids were used to transform the hpr1Δ/hrs2-1 SS713B-1A strain. All of the 173 transformants obtained from the 1145-bp EcoRV gap containing plasmid complemented the hrs2-1 phenotype (they formed white colonies and were MMS-sensitive, indicating that the gap had been repaired with wild-type information). All of the 160 transformants obtained with the 570-bp NcoI gap containing plasmid did not complement the hrs2-1 phenotype (they formed white colonies and were MMS-sensitive, indicating that the gap had been repaired with the hrs2-1 mutant information). Plasmid DNA was isolated from 10 of these transformants. They all showed the same restriction pattern of pHMBH12. One of them (pHRS21), which was confirmed to be unable to complement the hrs2-1 phenotype, was randomly chosen for DNA sequencing.
**DNA sequencing:** Double-chain DNA was sequenced by the dideoxy-chain termination method (SANGER et al. 1977) with T7 DNA polymerase (Sequenase) and 5'-[α-35S]Triphosphate. The DNA inserts of plasmids pRS03, pRS09 and pMPS41 were sequenced using the M13 reverse primer, the T7 primer or the -40 M13 primer, respectively, as appropriate.

**RESULTS**

**Isolation of the HRS2 gene:** The HRS2 gene was cloned by complementation of the MMS-sensitivity phenotype of the hrs2-1 mutation. The hpr1Δ hrs2-1 haploid strain SS713B-1A carrying the duplication system leu2-k::URA3-ADE2::leu2-k was transformed with the MW90 genomic library, constructed in YEp351 (WALDHEIM et al. 1993) and the pBS32 yeast genomic library (F. SPENCER and P. HIETER, unpublished results). From a total of 106,000 Leu+ transformants plated on SC-MMS lacking leucine, we selected six candidates (four from the MW90 library and two from the pBS32-based library) that were able to reestablish the MMS-resistant and red-sectoring phenotypes of hpr1Δ mutants (see MATERIALS AND METHODS). Plasmid isolation of the six different transformants revealed that we had obtained three different plasmids (three of the plasmids obtained from the MW90 library were identical, and the two plasmids obtained with the pBS32-based library were also identical). The maps of the three different DNA inserts able to complement the hrs2-1 phenotype are shown in Figure 1A. The three different inserts overlap by 1.9 kb. The three plasmids were used to retransform to Leu+ the original hpr1Δ hrs2-1 strain SS713B-1A. In all cases the Leu+ transformants were MMS-resistant and showed hpr1Δ levels of Ura+ recombinants. These phenotypes were linked to the Leu+ phenotype and were lost when the cells were cured of the plasmid. This result indicates that the 1.9-kb overlapping DNA region complements the hrs2-1 mutation. This conclusion was confirmed by deletion analysis of the HRS2 region (Figure 1A), indicating that the unique PstI site of the 1.9-kb overlapping region was located inside the HRS2 gene.

**HRS2 is identical to SRB2:** We sequenced 83 bp from the HRS2 internal PstI site to the right. Comparison of this nucleotide sequence with the GenBank release 88.0 and EMBL release 42.0 using the FASTA algorithms (PEARSON and LIPMAN 1988) showed that HRS2 was identical to SRB2 (KOLESKE et al. 1992), a component of the SRB complex of the RNA polymerase II holoenzyme (THOMSON et al. 1993). A comparative analysis of the restriction map of our cloned fragments with that of the SRB2 region confirmed that our clones contained the SRB2 gene.

**The hrs2-1 (srh2-101) allele determines a SRB2 protein with Gly150 changed to Asp:** The hrs2-1 (srh2-101) allele was cloned by gap-repair as explained in MATERIALS AND METHODS. Sequencing of the terminal 490 bp of the 3’-end of the SRB2 coding region, which corresponds to the entire region recovered by gap repair, revealed that the N-methyl-N-nitro-N-nitrosoguanidine-induced hrs2-1 mutation is a C to A transition, which determines a change of Gly to Asp at the amino acid position 150 of SRB2. This result is the definitive confirmation that the hrs2-1 mutation is an allele of the SRB2 gene. Therefore, we renamed the hrs2-1 allele, srh2-101.

**Deletion of the HRS2/SRB2 gene completely suppresses the hyperrecombination phenotype of hpr1Δ strains:** The genomic SRB2 gene was deleted by gene replacement. The deletion srh2Δ102::HIS3 replaces the 0.3-kb SacII-PstI internal fragment of SRB2 by a 1.32-kb SacII-PstI fragment containing the entire HIS3 gene (Figure 1C). The hpr1Δ strain W19Y-1B carrying the leu2-k::URA3-ADE2::leu2-k system was used to delete the SRB2 gene by omega transformation (ROTHSTEIN 1983) with the 2.7-kb EcoRI fragment obtained from plasmid pSRBR35H. Gene replacement of the SRB2 wild-type copy by the srh2Δ102::HIS3 allele led to His+ stable transformants with poor growth, consistent with previously reported observations (NONE et al. 1989). Southern analysis of several transformants confirmed that they contained the pattern of bands expected for the srh2Δ102::HIS3 allele (data not shown). Tetrads analysis of a cross of one such stable transformant with the hrs2-1(srb2-l01) strain WS1971-3D showed that the His+ phenotype segregated 2+:2- in two tetrads analyzed and that all spores had low frequency of deletions (data not shown), as expected if the deletion of the SRB2 gene suppressed the hpr1 hyper-rec phenotype.

To quantify the effect of the srh2Δ mutation on hpr1Δ-induced recombination, we determined the frequency of deletions of the leu2-k::URA3-ADE2::leu2-k system in hpr1Δ srh2Δ102 double mutants by selecting recombinants on SC-FOA media. Table 3 shows that the srh2Δ102 mutation reduces the frequency of direct-repeat recombination of hpr1Δ strains to wild-type levels. We confirmed that the low frequency of colonies formed on SC-FOA in hpr1Δ srh2Δ102 strains was a direct consequence of its incapacity to undergo direct-repeat recombination and not a consequence of its poor growth phenotype. First, srh2Δ cells have similar efficiency of forming colonies in SC and YEPD as wild-type cells (data not shown). Second, Southern analysis (Figure 2) shows that in hpr1Δ strains carrying the leu2-k::ADE2-URA3::leu2-k system in hpr1Δ srh2Δ102 double mutants by selecting recombinants on SC-FOA media. Table 3 shows that the srh2Δ102 mutation reduces the frequency of direct-repeat recombination of hpr1Δ strains to wild-type levels. We confirmed that the low frequency of colonies formed on SC-FOA in hpr1Δ srh2Δ102 strains was a direct consequence of its incapacity to undergo direct-repeat recombination and not a consequence of its poor growth phenotype. First, srh2Δ cells have similar efficiency of forming colonies in SC and YEPD as wild-type cells (data not shown). Second, Southern analysis (Figure 2) shows that in hpr1Δ strains carrying the leu2-k::ADE2-URA3::leu2-k system, the frequency of deletions of the leu2 duplication system, corresponds to the single copy of leu2-k, and it appears in hpr1Δ strains as a result of the high frequency of deletions of the leu2 duplication system (the relative intensity at which this 7.3-kb band appears with respect to the rest of the bands corresponds to the expected frequency of deletions of the leu2 direct-repeat system). As can be observed in Figure 2, this 7.3-
**TABLE 3**

Frequency of deletions (×10⁶) in the *leu2-K::URA3-ADE2::leu2k* direct repeat system in wild-type and different *hrs2* mutant combinations

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Strains</th>
<th>Deletions (Ura-)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>AYW3-1B</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>A3Y3A</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>W19Y-1B [HPR1]</td>
<td>28</td>
</tr>
<tr>
<td><em>hpr1Δ</em></td>
<td>W19Y-1B</td>
<td>79000</td>
</tr>
<tr>
<td></td>
<td>HDY3-7D [SRB2]</td>
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</tr>
<tr>
<td></td>
<td>HDSB-2B</td>
<td>130000</td>
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<tr>
<td><em>hrs2-1(srb2-101)</em></td>
<td>SB71-5A</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>SB71-8A</td>
<td>70</td>
</tr>
<tr>
<td><em>srb2Δ</em></td>
<td>HDY3-7D [HPR1]</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>HDSB-2A</td>
<td>150</td>
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<tr>
<td><em>hpr1Δ</em> <em>hrs2-1(srb2-101)</em></td>
<td>HDSB-2A</td>
<td>78</td>
</tr>
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<td>HDSB-2C</td>
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<td>HDY3-7D</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>HDSB-2B</td>
<td>190</td>
</tr>
</tbody>
</table>

* For each strain six independent colonies were used for a fluctuation test. Median recombination frequencies are given. Ura- recombinants were scored on SC-FOA medium with or without leucine. Those strains containing a plasmid are indicated with the relevant gene carried by the plasmid between brackets. The plasmids used were YCpA13 for *HPR1* and pMBH12 for *SRB2*. The 310-bp *SacII* internal fragment of *HRS2/SRB2* was replaced by the 1.32-kb *SacII*-fragment containing the entire *HIS3* gene. The *HRS2* and *LEU2* coding sequences are shown as thick arrows. The 2.7-kb *EcoRI* fragment contained between the *EcoRI* site shown and the *EcoRI* site of the polylinker located on the left side (not shown) was used to replace the *SRB2* gene from the chromosomal locus. Abbreviations of restriction sites are as follows: B, *BamHI*; C, *ClaI*; G, *BglII*; H, *HindIII*; K, *KpnI*; N, *NdeI*; P, *PstI*; R, *EcoRI*; S, *SacII*; V, *EcoRV*; X, *XhoI*.

**FIGURE 1.**—Structure of *HRS2*. (A) Restriction maps of the three different DNA inserts isolated from plasmids YCpH18, YEpM1 and YEpM4 that complement the *hrs2-1* mutation. The insert from plasmid YCpH18 and pM1 were obtained independently from two and three different yeast transformants, respectively. (B) Deletion analysis of the region containing the *HRS2* open reading frame showing the ability of each subclone to complement the *hrs2-1* mutation. (C) Plasmid used to delete the *HRS2/SRB2* gene from the yeast genome.
We found that the *srb2Δ102* and *hrs2-1(srb2-101)* mutants showed wild-type levels of viability after UV irradiation (data not shown). This result was independent of whether or not the mutant strains used carried also the *hpr1Δ* mutation. Therefore, *SRB2* is not involved in the repair of UV-induced damage.

When we determined the resistance to MMS as the capacity of the cells to form colonies on YEPD after different times in 0.5% MMS (short exposures to high MMS) (Figure 3), we found that the null *srb2Δ102* mutants had wild-type levels of MMS resistance, independently of whether or not the mutant strains used carried also the *hpr1Δ* mutation (Figure 3). This is different from the MMS-sensitive phenotype observed for *hpr1Δ hrs2-1(srb2-101)* double mutants (SANTOS-ROSA and AGUILERA 1995; Figure 3). However, since we had observed that our *srb2* mutants grew poorly on YEPD-MMS plates in replica-plating experiments, we decided to determine the resistance to MMS as the capacity of the cells to form colonies on YEPD supplemented with 0.02% MMS (long exposures to low MMS) (Figure 4). We observed that the *hrs2-1(srb2-101)* mutation conferred a strong MMS-sensitivity phenotype in both wild-type and *hpr1Δ* backgrounds. However, the *srb2Δ102* mutation only conferred a relatively strong MMS-sensitivity phenotype in a *hpr1Δ* background and a very weak one, if any, in a wild-type background (Figure 4; note: although *srb2Δ102* single mutants grow poorly in YEPD-MMS, they also grow poorly on YEPD). Therefore, mutations in the *SRB2* transcription factor affect the repair of MMS-induced damage, and the strong MMS-sensitive phenotype conferred by *hrs2-1(srb2-101)* is allele-specific.

We have also observed that the *hrs2-1(srb2-101)* and the *srb2Δ102* mutations reduce the levels of activation of gene expression of the *E. coli* LacZ gene fused to the *GALL1,10* promoter, about three- and sixfold below the wild-type levels, respectively (Table 4). In addition, whereas the *hrs2-1(srb2-101)* mutation partially suppresses the phenotype of lack of activation of gene expression of *hpr1Δ* mutants (SANTOS-ROSA and AGUILERA 1995), the null *srb2Δ102* mutation has no suppressor effect (Figure 4). Therefore, the effect of the *srb2-101* mutation on gene expression is also allele-specific.

Finally, *srb2-101* and *srb2Δ102* are loss of function mutations. The *srb2-101* phenotypes on MMS-resistance and transcription activation are recessive. The *srb2-101* allele is leaky, as shown by the ability of the phenotypes of hyperrecombination suppression, MMS resistance and *GAL-lacZ* transcription activation of *srb2-101* mutants to be partially complemented by a multicopy *srb2-101* allele (Table 5).

**DISCUSSION**

We have obtained genetic and molecular evidence that indicates that *hrs2-1*, a mutation that suppresses the hyperrecombination phenotype of *hpr1Δ*, is in the *SRB2* gene. A newly constructed *srb2Δ* allele shows complete suppression of the hyper-rec phenotype of *hpr1Δ*, indicating that a functional *SRB2* transcription factor is required for *hpr1*-induced recombination events between direct repeats. These results suggest a connection between transcription and recombination between DNA repeats. We have also shown that the *srb2-101* mutation, in which G\textsubscript{1538} has been changed to Asp, makes cells sensitive to low MMS treatments, a phenotype observed for the *srb2Δ* null allele only in a *hpr1Δ* background.

The **SRB2** transcription factor is required for *hpr1*-induced recombination: Recently, it has been suggested that *HPR1*, a gene identified through hyper-rec combination mutations (AGUILERA and KLEIN 1989a), encodes a positive regulator of transcription (ZHU et al. 1995). In this study, we have found that mutations in another transcription factor gene, *SRB2*, restore the wild-type levels of recombination in *hpr1Δ* cells. These results indicate a connection between direct repeat recombination and transcription. We provide genetic evidence that hyperrecombination between DNA repeats is produced via transcription factors. Stimulation of recombination by transcription has been previously reported in yeast (KEIL and ROEDER 1984; VOELKEL-MEILMAN et al. 1987; STEWART and ROEDER 1989; THOMAS

![Figure 2](image-url)
FIGURE 3.—Viability curves of the wild-type strain A3Y3A (○), the hpr1Δ strain AW3-3D (■), the srb2-101 strain SB71-5A (●), the srb2Δ-102 strain HDY3-3D (○), the hpr1Δ srb2-101 strain SS713B1A (▲), the hpr1Δ srb2Δ-102 strain HDY3-7D (△) and the rad52-L strain X260-3A (◇) after different times in 0.5% MMS.

and Rothstein (1989) and other systems (Dul and Drexler 1988a,b; Bourgaux-Ramoisy et al. 1995). However, hpr1-induced recombination occurs in cells defective in activated transcription.

The knowledge of the roles of the HPRI and SRB2 factors on transcription is essential to understand their opposite effects on DNA repeat recombination. SRB2 is a component of the SRB subcomplex of the RNA polII holoenzyme that interacts with the CTD of RNA polII. It is believed to be required as mediator for the activation of transcription by the RNA polII holoenzyme (Kim et al. 1994; Koleske and Young 1994). On the other hand, HPRI does not seem to be a sequence-specific activator that recruits the transcription initiation complex into the DNA promoter. Zhu et al. (1995) have shown that HPRI is required for transcription activation of many unrelated genes but not for basal and constitutive transcription. However, only srb2Δ-101, and not srb2Δ-102, partially suppresses the phenotype of lack of activation of gene expression of the GAL1,10 promoter in hpr1Δ cells. Certainly, this can reflect the different abilities of complex with a molecular mass different to that reported for the RNA polIII holoenzyme. In addition, hpr1 mutants do not show srb phenotypes such as cold sensitivity (A. Aguilera, unpublished results). These observations suggest that HPRI and SRB2 play different roles in transcription. It is likely that HPRI acts at a later step than SRB2 in transcription. This would be consistent with our data suggesting that HRS2(SRB2) acts at an earlier step than HPRI in direct-repeat recombination (Santos-Rosa and Aguilera 1995) and with the observation that srb2Δ does not suppress the lack of gene expression of the GAL1,10 promoter of hpr1 mutants (Table 4).

Both the srb2-101 and srb2Δ-102 mutants have a similar effect on the frequency of deletions of hpr1 cells and the level of activation of the GAL1,10 promoter in HPRI cells. However, only srb2-101, and not srb2Δ-102, partially suppresses the phenotype of lack of activation of gene expression of the GAL1,10 promoter in hpr1Δ cells.

FIGURE 4.—Growth phenotypes on YEPD plates without MMS (−MMS) and supplemented with 0.02% MMS (+MMS) of the hpr1Δ strain A3Y3A-3D, the srb2-101 strain SB71-5A, the srb2Δ-102 strain HDY3-3D, the hpr1Δ srb2-101 strain SS713B1A, the hpr1Δ srb2Δ-102 strain HDY3-7D and the rad52-L strain X260-3A. For each strain and plate, five patches corresponding to 10 µl of different diluted suspensions of an overnight culture are presented. The approximate number of cells plated in each patch were from left to right: 3 × 10^4, 3 × 10^5, 3 × 10^6, 3 × 10^7 and 3 × 10^8 (this latter one is not shown in the rad52 control).
According to our results, we consider the possibility and YOUNG (1994). Indeed, the srb2-1 allele also showed contacts. This would be consistent with the observation of hprl-induced deletions independent of its role in specific phenotypes not shared by the null mutation modified SRB2-101 factor could participate in transcription complexes that could function in a HPR1-independent manner. In this context, the specific phenotype of the srb2-101 allele could be a consequence of a putative involvement of the Gly150 residue in protein-protein contacts. This would be consistent with the observation that SRB2 participates in a protein complex (KOLESKE and YOUNG 1994). Indeed, the srb2-I allele also showed specific phenotypes not shared by the null mutation (NONET and YOUNG 1989).

Whether SRB2 has a direct role in the formation of hprl-induced deletions independent of its role in transcription is an open question. However, we believe that the recombination phenotypes of hprl and srb2 mutants may be related to their effects in transcription. According to our results, we consider the possibility that, in hprl single mutants, transcription complexes could get stalled on HPR1-dependent genes. In hprl srb2 double mutants, the assembly or stability of ternary transcription complexes could be greatly reduced as a consequence of the lack of functional SRB2 factor. Thus, the stalled transcription complex would be the cause of the hyper-rec phenotype of hprl cells. Our recent observation that hrs1 mutants, which completely suppress hprl-induced recombination, are affected in a protein, HRS1, with structural and functional similarities to transcription factors (SANTOS-ROSA et al. 1996) is consistent with this hypothesis. FAN et al. (1996) have recently reported the identification of TFIIB and the RPBl subunit of RNA polII among suppressors of the thermosensitivity phenotype of hprl cells. However, mutations in the respective structural genes of these proteins only suppress 10% of hprl-induced recombination.

Mutations in the SRB2 transcription factor show defects in DNA repair: We have observed that srb2 mutants are affected in the repair of MMS-induced damage. However, this effect is different for the two alleles analyzed. Thus, whereas the srb2-101 confers a clear allele-specific phenotype of sensitivity to long MMS treatments in either HPR1 and hprl genetic backgrounds (Figure 4), the srb2Δ102 confers such phenotype only in a hprlΔ background (Figures 3 and 4). Also, the severe MMS-sensitive phenotype of hrs2-1(srb2-101) is not accompanied by the slow growth phenotype and the lower levels of activation of gene expression observed for srb2Δ102. These results suggest that the SRB2 transcription factor has a function related to DNA repair that is independent of its effect on gene expression. Thus, it is unlikely that the MMS phenotype of srb2 mutants were a consequence of a specific effect of SRB2 on transcription of different RAD genes. This conclusion is corroborated by the recent observation that mutants in the RAD51, RAD54, RAD55 and RAD57 genes lead to a hyperdeletion phenotype between repeats (McDONALD and ROTHSTEIN 1994; AGUILERA 1995; LIEFSHITZ et al. 1995; RATTRAY and SYMINGTON 1995). If the MMS-sensitivity phenotype of srb2 mutants were a consequence of a reduction in the expression of these RAD genes, we would not expect the suppression of the hyperdeletion phenotype of hprl mutants.

### TABLE 4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Glucose (U)</th>
<th>Galactose (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0.29 ± 0.02</td>
<td>1807.5 ± 123.7</td>
</tr>
<tr>
<td>hrs2-1(srb2-101)</td>
<td>0.12 ± 0.02</td>
<td>574.5 ± 67.1</td>
</tr>
<tr>
<td>srb2Δ</td>
<td>0.17 ± 0.01</td>
<td>301.5 ± 112.2</td>
</tr>
<tr>
<td>hprlΔ</td>
<td>0.05 ± 0.01</td>
<td>15.1 ± 2.5</td>
</tr>
<tr>
<td>hprlΔ hrs2-1 (srb2-101)</td>
<td>0.09 ± 0.01</td>
<td>83.1 ± 19.0</td>
</tr>
<tr>
<td>hrs2Δ srb2Δ</td>
<td>0.05 ± 0.01</td>
<td>9.6 ± 10.2</td>
</tr>
</tbody>
</table>

*The data shown correspond to the average and the standard deviation of two different determinations. Two different strains were used for each genotype. In each case, the results were the same for both strains and the data of one strain is shown. Data shown correspond to strains AW3-1B (wild type), SS713B-1A transformed with YCPa13 (hrs2-1), HDY3-7D transformed with YCPa13(srb2Δ), W19Y-1B (hprlΔ), SS713B-1A (hprlΔ hrs2-1) and HDY3-7D (hprlΔ srb2Δ).

The β-galactosidase activity of srb2 mutant combinations determined in plasmid pLGSD5, containing the LacZ gene under the yeast GAL1,10 promoter.

### TABLE 5

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Deletions (×10³)*</th>
<th>MMS*</th>
<th>Glucose (U)*</th>
<th>Galactose (U)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>YEpl[-]</td>
<td>56</td>
<td>S</td>
<td>1.01 ± 0.12</td>
<td>193.6 ± 28.4</td>
</tr>
<tr>
<td>YCP[SBB2]</td>
<td>28000</td>
<td>R</td>
<td>0.78 ± 0.07</td>
<td>19.2 ± 2.6</td>
</tr>
<tr>
<td>YCP[srb2-101]</td>
<td>61</td>
<td>S</td>
<td>1.96 ± 0.49</td>
<td>199.9 ± 76.6</td>
</tr>
<tr>
<td>YEpl[srb2-101]</td>
<td>1200</td>
<td>R</td>
<td>0.86 ± 0.12</td>
<td>40.6 ± 16.7</td>
</tr>
</tbody>
</table>

*Deletion and β-galactosidase data were obtained as explained in Tables 2 and 3. MMS sensitivity data (R, resistant; S, sensitive) were obtained as shown in Figure 5. The strain analyzed was SS713B-1A (hprlΔ srb2-101) transformed with YEpl351 (YEpl[-1]), pMBH12 (YCP[SBB2]), pHRS21 (YCP[srb2-101]) and YEplHRS21 (YEpl[srb2-101]).

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J. I. Piruat and A. Aguilera
The observation that mutations in SRB2 can impair DNA repair adds a new connection between RNA polII transcription and DNA repair. It is known that different polypeptides of the TFIIF transcription factor are excised repair proteins, as is the case for the yeast RAD3 and RAD25/SSL2 genes or their human homologues (for reviews see ABOUSSEKIRA and WOOD 1994; CLEAVER 1994; DRAPKIN et al. 1994). Since MMS is a methylating agent as well as a radiomimetic chemical (PRAKASH and PRAKASH 1977), we do not know the molecular nature of the DNA repair pathway impaired in srb2 mutants, whether excision repair or recombinational repair. However, this raises the question of whether a putative DNA repair-related function of SRB2, required in a hpr1Δ background, might play an active role in the hpr1-induced recombination events.

**How could deletion events be initiated in hpr1 cells?**

The main question raised by our results is whether or not hyperrecombination in hpr1 cells is mediated by transcription. Certainly, this question needs to be answered at the molecular level.

We cannot exclude the possibility that SRB2 could serve to recruit components of the DNA repair and/or recombination machinery, in the absence of which certain types of DNA repair and recombination events would not occur. However, according to our hypothesis that a SRB2-dependent transcription complex may be stalled in hpr1 cells, we consider two possible alternatives to explain the formation of deletions. One alternative is that the replication apparatus may not be able to pass through the stalled DNA-protein complex. This would lead to unreplicated DNA molecules, with free ends at the DNA regions flanking the blocked transcription complex. In *E. coli*, VILETTE et al. (1995) has proposed that deletions in plasmids may result from collisions between converging replication and transcription machineries, and KUZMINOV (1995) has proposed that “collapsed” replication forks can explain the hyper-rec phenotype of the replication terminator. Another alternative is that the stalled SRB2-dependent DNA-protein complex could cause nucleosome hypersensitive sites in the DNA. The resulting free DNA ends or breaks could be repaired through a nonconservative recombination mechanism, such as single strand annealing or one-ended invasion crossover, responsible for the deletion events.

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**LITERATURE CITED**


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