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

José I. Piruat and Andrés Aguilera

Departamento de Genética, Facultad de Biología, Universidad de Sevilla, E-41012 Sevilla, Spain

Manuscript received January 23, 1996
Accepted for publication April 25, 1996

ABSTRACT

We have obtained genetic and molecular evidence that the *hrs2-1* mutation, isolated as a suppressor of the hyperrecombination phenotype of *hpr1*Δ, is in the *SRB2* gene, which encodes a component of the RNA polII holoenzyme. A newly constructed *srb2*Δ allele restores the wild-type levels of deletions in *hpr1*Δ 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 Gly_{150} has been changed to Asp, makes cells sensitive to long MMS treatments, a phenotype observed for the *srb2*Δ null allele only in a *hpr1*Δ 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; MEYN 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) (OZENBERGERG 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; LIEFSHITZ *et al.* 1995; RATTRAY and SYMINGTON 1995). There are also mutations that induce recombination between repeats,

Corresponding author: Andrés Aguilera, Departamento de Genetica, Facultad de Biología, Apdo. 1095 de la Universidad, 41080 Sevilla, Spain. E-mail: aguilo@cica.es

TABLE 1

Strains

Strain	Genotype	Source
A3Y3A	*MATα ura3 ade2 his3 leu2-k::URA3-ADE2::leu2-k*	AGUILERA and KLEIN (1990)
AYW3-3D	*MAT***a** *ura3 ade2 his3 leu2-k::URA3-ADE2::leu2-k hpr1Δ3::HIS3*	SANTOS-ROSA and AGUILERA (1995)
AYW3-1B	*MAT***a** *ura3 ade2 his3 trp1 can1-100 leu2-k::URA3-ADE2::leu2-k*	SANTOS-ROSA and AGUILERA (1995)
SS713B-1A	*MAT***a** *ura3 ade2 his3 leu2-k::URA3-ADE2:leu2-k hpr1Δ3::HIS3 srb2-101*	SANTOS-ROSA and AGUILERA (1995)
X260-3A	*MAT***a** *ura3 rad52*	G. FINK
W19Y-1B	*MATα ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 can1-100*	This study
SB71-5A	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::URA3-ADE2:leu2-k srb2-101*	This study
SB71-8A	*MATα ura3 ade2 his3 leu2-k::URA3-ADE2:leu2-k srb2-101*	This study
HDY3-7D	*MATα ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 srb2Δ102::HIS3*	This study
HDY3-7C	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1*	This study
HDY3-3D	*MATα ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k srb2Δ102::HIS3 can1-100*	This study
HDSB-2A	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 srb2-101 can1-100*	This study
HDSB-2B	*MATα ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 srb2Δ102::HIS3 can1-100*	This study
HDSB-2C	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 srb2-101 can1-100*	This study
HDSB-2D	*MATα ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 srb2Δ102::HIS3 can1-100*	This study
WS1971-3D	*MAT***a** *ura3 ade2 his3 trp1 leu2-k::URA3-ADE2::leu2-k hpr1Δ4::TRP1 srb2-101 can1-100*	This study

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 MCWILLIANS 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 *SRB2,* a component of the SRB complex of the RNA polII 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° with horizontal shaking for liquid cultures. Yeast strains were transformed using

TABLE 2

Plasmids

Plasmid	Description	Source
pLGSD5	YEp plasmid containing the *URA3* gene and the *E. coli lacZ* gene under the yeast *CYC-GAL1,10* promoter	GUARENTE *et al.* (1982)
pTZ18U	Vector derived from pUC18	MEAD *et al.* (1986)
YIp1	The 10.1-kb *Eco*RI fragment containing the *HIS3* gene subcloned into pBR322	STRUHL and DAVIS (1980)
YEp351	YEp vector carrying the *LEU2* gene	HILL *et al.* (1986)
pBS32	YCp vector based on the *LEU2* gene	F. SPENCER and P. HIETER
pRS315	YCp vector based on the *LEU2* gene	SIKORSKI and HIETER (1989)
YEpM1	A 6.5-kb *HRS2/SRB2* genomic insert subcloned in YEp351	This study
YEpM4	A 8.9-kb *HRS2/SRB2* genomic insert subcloned in YEp351	This study
YCpH18	A 10.3-kb *HRS2/SRB2* genomic insert subcloned in pBS32	This study
pMPS41	The left 3.86-kb *Sad-Pst*I fragment from the insert of YEpM4 subcloned in YEp351	This study
pMBH12	The 3.25-kb *Bam*HI-*Hin*dIII fragment from the insert of YEpM1 subcloned in pRS315	This study
pMBP13	The 8.25-kb *Pst*I fragment from pMBH12	This study
pHRS21	pMBH12 containing the yeast *hrs2-1(srb2-101)* allele, instead of *HRS2/SRB2*	This study
YEpHRS21	The 3.25-kb *Bam*HI-*Hin*dIII fragment of pHRS21 subcloned in YEp351	This study
pRS-03	The 0.31-kb *Sac*I-*Pst*I fragment from the insert of pHRS21 subcloned in pRS315	This study
pRS-09	The 0.9-kb *Pst*I fragment from the insert of pHRS21 subcloned in pRS315	This study
pSRB5	The 0.9-kb *Pst*I fragment from the insert of pMBH12 subcloned into pTZ18U	This study
pSRB35	The 0.63-kb *Kpn*I-*Sac*I fragment of pMBH2 subcloned into pSRB5	This study
pSRB35H	The 2.84-kb *Bam*HI-*Xho*I fragment of YIp1 containing the entire *HIS3* gene subcloned into pSRB35. It contains the *srb2Δ102::HIS3* allele	This study

the lithium acetate method (ITO *et al.* 1983) modified according to SCHIESTL and GIETZ (1989).

Genetic and biochemical analysis: Genetic analysis was performed as previously published (SHERMAN *et al.* 1986). UV and MMS viability were determined as described previously (AGUILERA and KLEIN 1990).

β-galactosidase was assayed according to GUARENTE (1983). Permeabilized yeast cells were prepared from 8-hr-old log-phase cultures in synthetic medium supplemented with either 2% glucose or 2% galactose, which had been inoculated from overnight cultures in synthetic medium suplemented with 3% lactate and 2% glycerol as the carbon source.

Determination of recombination frequencies: Recombination frequencies were calculated as the median value of six independent colonies for each strain studied. Yeast strains were grown on YEPD or SC −leu, as appropriate. After 3 days, independent colonies were plated on SC-FOA to determine the median frequency of Ura^- recombinants. The viable cell number was determined on SC or SC −leu, as appropriate.

DNA manipulation: Plasmid DNA was isolated from *E. coli* by CsCl gradient centrifugation as described (CLEWELL and HELINSKI 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*.

Digoxigenine-dUTP (Boehringer)-labeled DNA probes were prepared as described (FEINBERG and VOLGELSTEIN 1984). Hybridization was performed in 50% formamide 5× SSC, 0.01% N-laurosylsarcosine, 0.02% SDS and 2% Boehringer Mannheim blocking reagent at 42° for 18 hr when using digoxigenine-dUTP. Detection of digoxigenine-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°.

Cloning of the *HRS2* gene: Strain SS713B-1A carrying the duplication system *leu2-k::URA3-ADE2::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 phentotype). 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 pMBH12 was cut with *Eco*RV (partial digestion) to obtain a 8.0-kb linearized plasmid with a gap of 1145 bp (357 bp corresponding to the *SRB2* 5′-end coding region) and with *Nco*I to obtain a 8.6-kb linearized plasmid with a gap of 570 bp (439 bp corresponding to the *SRB2* 3′-end coding region). Both gapped plasmids were used to transform the *hpr1Δ hrs2-1* SS713B-1A strain. All of the 173 transformants obtained from the 1145-bp *Eco*RV gap containing plasmid complemented the *hrs2-1* phenotype (they formed red-sectoring colonies), indicating that the gap had been repaired with wild-type information. All of the 1600 transformants obtained with the 570-bp *Nco*I-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 pMBH12. 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-^{35}S]thio)triphosphate. The DNA inserts of plasmids pRS-03, pRS-09 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 *hpr1*Δ *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 (WALDHERR *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 *hpr1*Δ 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 *hpr1*Δ *hrs2-1* strain SS713B-1A. In all cases the Leu$^+$ transformants were MMS-resistant and showed *hpr1*Δ 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 *Pst*I 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 *Pst*I 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 (THOMPSON *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(srb2-101)* allele determines a SRB2 protein with Gly$_{150}$ changed to Asp: The *hrs2-1(srb2-101)* 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 G 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, *srb2-101*.

Deletion of the HRS2/SRB2 gene completely suppresses the hyperrecombination phenotype of *hpr1*Δ strains: The genomic *SRB2* gene was deleted by gene-replacement. The deletion *srb2*Δ*102::HIS3* replaces the 0.3-kb *Sac*II-*Pst*I internal fragment of *SRB2* by a 1.32-kb *Sac*II-*Pst*I fragment containing the entire *HIS3* gene (Figure 1C). The *hpr1*Δ 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 *Eco*RI fragment obtained from plasmid pSRB35H. Gene replacement of the *SRB2* wild-type copy by the *srb2*Δ*102::HIS3* allele led to His$^+$ stable transformants with poor growth, consistent with previously reported observations (NONET and YOUNG 1989). Southern analysis of several transformants confirmed that they contained the pattern of bands expected for the *srb2*Δ*102::HIS3* allele (data not shown). Tetrad analysis of a cross of one such stable transformant with the *hrs2-1(srb2-101)* 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 *srb2*Δ mutation on *hpr1*Δ-induced recombination, we determined the frequency of deletions of the *leu2-k::URA3-ADE2::leu2-k* system in *hpr1*Δ *srb2*Δ*102* double mutants by selecting recombinants on SC-FOA media. Table 3 shows that the *srb2*Δ*102* mutation reduces the frequency of direct-repeat recombination of *hpr1*Δ strains to wild-type levels.

We confirmed that the low frequency of colonies formed on SC-FOA in *hpr1*Δ *srb2*Δ*102* strains was a direct consequence of its incapacity to undergo direct-repeat recombination and not a consequence of its poor growth phenotype. First, *srb2*Δ 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 *hpr1*Δ strains carrying the *leu2-k::ADE2-URA3::leu2-k* duplication system there is a weak 7.3-kb *Bam*HI band hybridizing with the *LEU2* probe used. This 7.3-kb band, not observed in wild-type strains carrying the *leu2-k::ADE2-URA3::leu2-k* duplication system, corresponds to the single copy of *leu2-k*, and it appears in *hpr1*Δ 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-

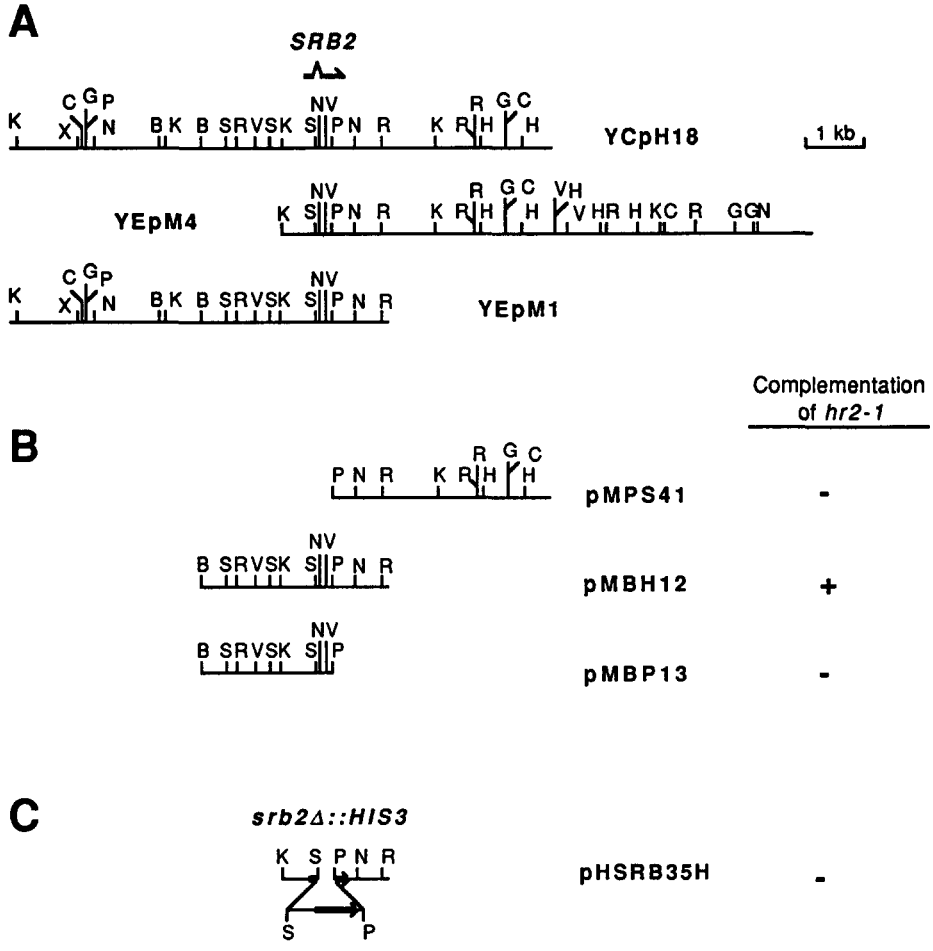

FIGURE 1.—Structure of *HRS2*. (A) Restriction maps of the three different DNA inserts isolated from plasmids YCpH18, YEpM1 and YEpM4 that complement the *hrs2-1* mutation. The insert from plasmid YCpH18 and pM1 were obtained independently from two and three different yeast transformants, respectively. (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. The 310-bp *Sac*I-*Pst*I internal fragment of *HRS2/SRB2* was replaced by the 1.32-kb *Sac*I-*Pst*I fragment containing the entire *HIS3* gene. The *HRS2* and *LEU2* coding sequences are shown as thick arrows. The 2.7-kb *Eco*RI fragment contained between the *Eco*RI site shown and the *Eco*RI 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, *Bam*HI; C, *Cla*I; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; N, *Nco*I; P, *Pst*I; R, *Eco*RI; S, *Sac*I; V, *Eco*RV; X, *Xho*I.

TABLE 3

Frequency of deletions ($\times 10^6$) in the *leu2-K::URA3-ADE2::leu2-k* direct repeat system in wild-type and different *hrs2* mutant combinations

Genotype	Strains	Deletions (Ura−)[a]
Wild type	AYW3-1B	56
	A3Y3A	73
	W19Y-1B [*HPR1*]	28
hpr1Δ	W19Y-1B	79000
	HDY3-7D [*SRB2*]	47000
	HDSB-2B [*SRB2*]	130000
hrs2-1(srb2-101)	SB71-5A	42
	SB71-8A	70
srb2Δ	HDY3-7D [*HPR1*]	49
	HDSB-2B [*HPR1*]	150
hpr1Δ hrs2-1(srb2-101)	HDSB-2A	78
	HDSB-2C	64
hpr1Δ srb2Δ	HDY3-7D	130
	HDSB-2B	190

[a] 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*.

kb band does not appear in *hpr1Δ hrs2-1(srb2-101)* and *hpr1Δ srb2Δ102* double mutants, as well as in wild-type strains, confirming the suppression of the hyper-rec phenotype of *hpr1Δ* cells by the *srb2Δ102* mutation.

Finally we have observed that the frequency of recombination of both *hrs2-1(srb2-101)* and *srb2Δ102* single mutants are similar to the wild-type values, indicating that the *SRB2* gene is required for the formation of *hpr1*-induced deletions between repeats, but not for the formation of spontaneous deletions.

The *hrs2-1(srb2-101)* mutation shows allelic-specific MMS sensitivity and gene expression phenotypes: Although the recombination phenotype conferred by the *hrs2-1(srb2-101)* and *srb2Δ102* alleles were similar, *srb2Δ102* strains grow more slowly than *hrs2-1(srb2-101)* strains. We have determined that the generation time in YEPD medium was 92 min for wild-type strains, 132 min for *hrs2-1(srb2-101)* strains, and 170 min for *srb2Δ102* strains, independently of whether or not the *srb2* strains were also mutated in the *HPR1* gene. This result suggests that *hrs2-1(srb2-101)* and *srb2Δ102* are not functionally equivalent alleles. Thus, since we have previously reported a MMS-sensitive phenotype for the *hpr1Δ hrs2-1(srb2-101)* mutants, we decided to determine whether the *hrs2-1(srb2-101)* and *srb2Δ102* mutations had different effects on the repair of UV- and MMS-induced damage and gene expression.

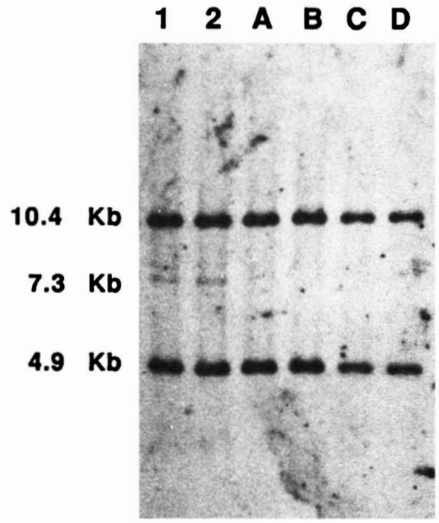

FIGURE 2.—Southern analysis of *Bam*HI-digested genomic DNA from different yeast strains carrying the *leu2-k::ADE2-URA3::leu2-k* direct repeat system. The 1.6-kb *Cla*I-*Sal*I fragment from *LEU2* was used as probe. Strains used were the *hpr1Δ SRB2* strains W19Y-1B (lane 1) and HDY3–7C (lane 2), and four spores from one single tetrad, the *hpr1Δ srb2-101* spores HDSB-2A (lane A) and HDSB-2C (lane C), and the *hpr1Δ srb2Δ102* spores HDSB-2B (lane B) and HDSB-2D (lane D), all of them carrying the *leu2-k::ADE2-URA3::leu2-k* direct repeat system. The 10.4- and 4.9-kb bands correspond to the *leu2* duplicated alleles, and the 7.3-kb band corresponds to the *leu2* copy remaining in the chromosomal *locus* after a deletion event took place.

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 *GAL1,10* 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 allelic-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 Gly_{150} 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.

The SRB2 transcription factor is required for *hpr1*-induced recombination: Recently, it has been suggested that *HPR1,* a gene identified through hyper-recombination 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-MEIMAN *et al.* 1987; STEWART and ROEDER 1989; THOMAS

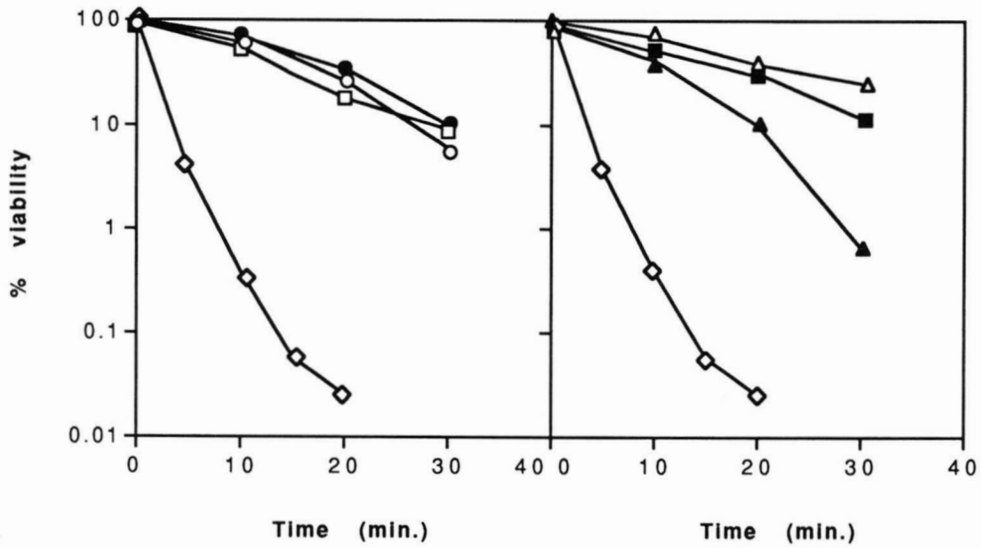

FIGURE 3.—Viability curves of the wild-type strain A3Y3A (□), the *hpr1*Δ strain AYW3-3D (■), the *srb2-101* strain SB71-5A (●), the *srb2*Δ*-102* strain HDY3-3D (○), the *hpr1*Δ *srb2-101* strain SS713B-1A (▲), the *hpr1*Δ *srb2*Δ*102* strain HDY3-7D (△) and the *rad52-1* strain X260-3A (◇) after different 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 HPR1 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, HPR1 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 HPR1 is required for transcription activation of many unrelated genes but not for basal and constitutive transcription, HPR1 does not transactivate transcription when fused to the DNA-binding domain of GAL4, and HPR1 seems to participate in a protein complex with a molecular mass different to that reported for the RNA polII holoenzyme. In addition, *hpr1* mutants do not show *srb* phenotypes such as cold sensitivity (A. AGUILERA, unpublished results). These observations suggest that HPR1 and SRB2 play different roles in transcription. It is likely that HPR1 acts at a later step than SRB2 in transcription. This would be consistent with our data suggesting that HRS2(SRB2) acts at a earlier step than HPR1 in direct-repeat recombination (SANTOS-ROSA and AGUILERA 1995) and with the observation that *srb2*Δ does not suppress the lack of gene expression of the *GAL1,10* promoter of *hpr1* mutants (Table 4).

Both the *srb2-101* and *srb2*Δ*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 *HPR1* cells. However, only *srb2-101,* and not *srb2*Δ*102,* partially suppresses the phenotype of lack of activation of gene expresssion of the *GAL1,10* promoter in *hpr1*Δ cells. Certainly, this can reflect the different abilities of

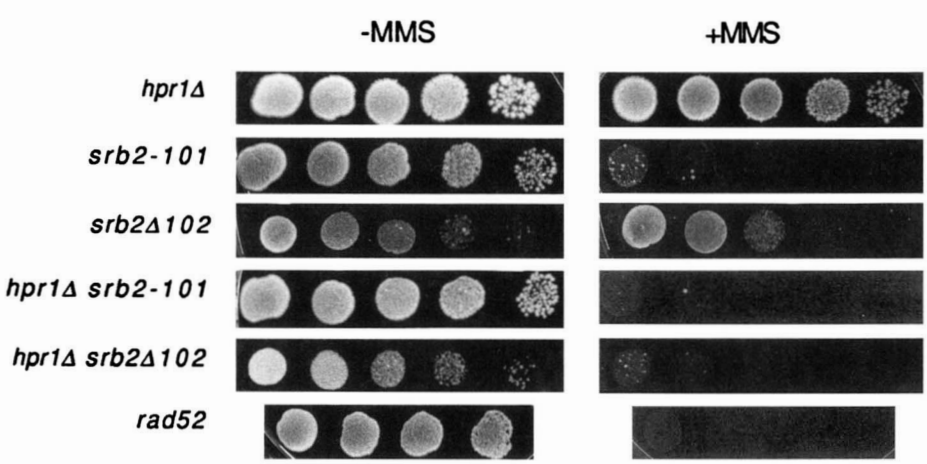

FIGURE 4.—Growth phenotypes on YEPD plates without MMS (−MMS) and supplemented with 0.02% MMS (+MMS) of the *hpr1*Δ strain A3YW3-3D, the *srb2-101* strain SB71-5A, the *srb2*Δ*-102* strain HDY3-3D, the *hpr1*Δ *srb2-101* strain SS713B-1A, the *hpr1*Δ *srb2*Δ*102* strain HDY3-7D and the *rad52-1* strain X260-3A. For each strain and plate, five patches corresponding to 10 μl of differently diluted suspensions of an overnight culture are presented. The approximate number of cells plated in each patch were from left to right: 3×10^6, 3×10^5, 3×10^4, 3×10^3 and 3×10^2 (this latter one is not shown in the *rad52* control).

TABLE 4

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

Genotype	β-galactosidase (U)[a]	
	Glucose	Galactose
Wild type	0.29 ± 0.02	1807.5 ± 123.7
hrs2-1(srb2-101)	0.12 ± 0.02	574.5 ± 67.1
srb2Δ	0.17 ± 0.01	301.5 ± 112.2
hpr1Δ	0.05 ± 0.01	15.1 ± 2.5
hpr1Δ hrs2-1 (srb2-101)	0.09 ± 0.01	83.1 ± 19.0
hpr1Δ srb2Δ	0.05 ± 0.01	9.6 ± 10.2

[a] The data shown correspond to the average and the standard deviation of two different determinations. Two different strains were used for each gentoype. In each case, the results were the same for both strains and the data of only one strain is shown. Data shown correspond to strains AYW3-1B (wild type), SS713B-1A transformed with YCpA13 (*hrs2-1*), HDY3-7D transformed with YCpA13(*srb2Δ*), W19Y-1B (*hpr1Δ*), SS713B-1A (*hpr1Δ hrs2-1*) and HDY3-7D (*hpr1Δ srb2Δ*).

the SRB2 factor to contact other proteins, whether or not in the same complex. It might be possible that the 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 Gly_{150} 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 *srb2-1* 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 *hpr1*-induced deletions independent of its role in transcription is an open question. However, we believe that the recombination phenotypes of *hpr1* and *srb2* mutants may be related to their effects in transcription. According to our results, we consider the possibility that, in *hpr1* single mutants, transcription complexes could get stalled on HPR1-dependent genes. In *hpr1 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 *hpr1* cells. Our recent observation that *hrs1* mutants, which completely suppress *hpr1*-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 RPB1 subunit of RNA polII among suppressors of the thermosensitivity phenotype of *hpr1* cells. However, mutations in the respective structural genes of these proteins only suppress 10% of *hpr1*-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 *hpr1* genetic backgrounds (Figure 4), the *srb2Δ102* confers such phenotype only in a *hpr1Δ* 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 suppresion of the hyperdeletion phenotype of *hpr1* mutants.

TABLE 5

Genetic characterization of different *hpr1Δ srb2-101* transformants

Plasmid	Deletions (×10⁶)[a]	MMS[a]	β-galactosidase (U)[a]	
			Glucose	Galactose
YEp[−]	56	S	1.01 ± 0.12	193.6 ± 28.4
YCp[*SRB2*]	28000	R	0.78 ± 0.07	19.2 ± 2.6
YCp[*srb2-101*]	61	S	1.96 ± 0.49	199.9 ± 76.6
YEp[*srb2-101*]	1200	R	0.86 ± 0.12	40.6 ± 16.7

[a] Deletion and β-galactosidase data were obtained as explained in Tables 2 and 3. MMS sensitivity data (R, resistent; S, sensitive) were obtained as shown in Figure 5. The strain analyzed was SS713B-1A (*hpr1Δ srb2-101*) transformed with YEp351 (YEp[−]), pMBH12 (YCp[*SRB2*), pHRS21 (YCp[*srb2-101*]) and YEpHRS21 (YEp[*srb2-101*]).

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 TFIIH transcription factor are excision repair proteins, as is the case for the yeast *RAD3* and *RAD25/SSL2* genes or their human homologues (for reviews see ABOUSSEKHRA 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 *hpr1*Δ 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 *terminus.* Another alternative is that the stalled SRB2-dependent DNA-protein complex could cause nuclease 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.

We thank H. SANTOS-ROSA for providing strains and technical tips, S. CHÁVEZ, F. PRADO and E. SANTERO for their critical readings of the manuscript and C. ALAIMO for style correction. This work was supported by Dirección General de Investigación Cienfica y Técnica grant PB93-1176-C02-01 from the Ministery of Science and Education of Spain and a grant from the Regional Government of Andalucia (Spain) to A.A. J.-I.P. was the recipient of a predoctoral fellowship from the Ministry of Science and Education of Spain.

LITERATURE CITED

ABOUSSEKHRA, A., and R. D. WOOD, 1994 Repair of UV-damaged DNA by mammalian cells and *Saccharomyces cerevisiae.* Curr. Opin. Genet. Dev. **4:** 212–220.

AGUILERA, A., 1995 Genetic evidence for different *RAD52*-dependent intrachromosomal recombination pathways in *Saccharomyces cerevisiae.* Curr. Genet. **25:** 298–305.

AGUILERA, A., and H. L. KLEIN, 1989a Genetic and molecular analysis of recombination events in *Saccharomyces cerevisiae* occurring in the presence of the hyper-recombination mutation *hpr1.* Genetics **112:** 503–517.

AGUILERA, A., and H. L. KLEIN, 1989b Yeast intrachromosomal recombination: long gene conversion tracts are preferentially associated with reciprocal exchange and require the *RAD1* and *RAD3* gene products. Genetics **123:** 683–694.

AGUILERA, A., and H. L. KLEIN, 1990 *HPR1,* a novel yeast gene that prevents intrachromosomal excision recombination, shows carboxy-terminal homology to the *Saccharomyces cerevisiae TOP1* gene. Mol. Cell. Biol. **10:** 1439–1451.

BIGGIN, M. D., J. GIBSON and G. F. HONG, 1980 Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80:** 3963–3965.

BOURGAUX-RAMOISY, D., D. GENDRON and P. BOURGAUX, 1995 A hotspot for promoter-dependent recombination in polyomavirus DNA. J. Mol. Biol. **248:** 220–224.

CHRISTMAN, M. F., F. S. DIETRICH and G. R. FINK, 1988 Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. Cell **55:** 413–425.

CLARK, A. J., and K. B. LOW, 1988 Pathways and systems of general recombination in *Escherichia coli,* pp.155–215 in *The Recombination of the Genetic Material,* edited by K. B. LOW. Academic Press, New York.

CLEAVER, J. E., 1994 It was a very good year for DNA repair. Cell **76:** 1–4.

CLEWELL, D., and D. HELINSKI, 1970 Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9:** 4428–4440.

DRAPKIN, R., A. SANCAR and D. REINBERG, 1994 Where transcription meets repair. Cell **77:** 9–12.

DUL J. L., and H. DREXLER, 1988a Transcription stimulates recombination. I. Specialized transduction of *Escherichia coli* λ*trp* phages. Virology **162:** 466–470.

DUL J. L., and H. DREXLER, 1988b Transcription stimulates recombination. II. Generalized transduction of *Escherichia coli* by phages T1 and T4. Virology **162:** 471–477.

FAN, H.-Y., and H. L. KLEIN, 1994 Characterization of mutations that suppress the temperature-sensitive growth of the *hpr1*Δ mutant of *Saccharomyces cerevisiae.* Genetics **137:** 1–12.

FAN, H.-Y., K. K. CHENG and H. L. KLEIN, 1996 Mutations in the RNA polymerase II transcription machinery suppress the hyperrecombination mutant *hpr1*Δ of *Saccharmomyces cerevisiae.* Genetics **142:** 749–759.

FEINBERG, A. P., and B. VOLGELSTEIN, 1984 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137:** 266–267.

FISHMAN-LOBELL, J., and J. E. HABER, 1992 Removal of nonhomologous DNA ends in double-strand break recombination: the role of the yeast ultraviolet repair gene *RAD1.* Science **258:** 480–484.

FUKUCHI, K., G. M. MARTIN and R. J. MONNAT, JR., 1989 Mutator phenotype of Werner syndrome is characterized by extensive deletions. Proc. Natl. Acad. Sci. USA **86:** 5893–5897.

GOTTLIEB, S., J. WAGSTAFF and R. E. ESPOSITO, 1989 Evidence for two pathways of meiotic intrachromosomal recombination in yeast. Proc. Natl. Acad. Sci. USA **86:** 7072–7076.

GUARENTE, L. R., 1983 Yeast promoters and *LacZ* fusions designed to study expression of cloned genes in yeast. Methods Enzymol. **101:** 181–191.

GUARENTE, L. R., R. YOCUM and P. GIFFORD. 1982 A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. Proc. Natl. Acad. Sci. USA **79:** 7410–7414.

HILL, J. E., A. M. MYERS, T. J. KOERNER and A. TZAGALOFF, 1986 Yeast/*E. coli* shuttle vectors with multiple unique restriction sites. Yeast **2:** 163–167.

HOFFMAN, C. S., and F. WINSTON, 1987 A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli.* Gene **57:** 267–272.

ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

IVANOV, E. L., and J. E. HABER, 1995 *RAD1* and *RAD10*, but not other excision repair genes, are required for double-strand break-induced recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:** 2245–2251.

JACKSON, J. A., and G. R. FINK, 1981 Gene conversion between duplicated genetics elements in yeast. Nature **292:** 306–311.

KEIL, R. L., and A. D. MCWILLIANS, 1993 A gene with specific and global effects on recombination of sequences from tandemly repeated genes in *Saccharomyces cerevisiae*. Genetics **135:** 711–718.

KEIL, R. L., and G. S. ROEDER, 1984 *cis*-acting, recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell **57:** 377–386.

KIM, Y.-J., S. BJORKLUND, L. Y. LI, M. SAYRE and R. KORNBERG, 1994 A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:** 599–608.

KLEIN, H. L., 1988 Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. Genetics **120:** 367–377.

KOLESKE, A. J., and R. A. YOUNG, 1994 An RNA polymerase II holoenzyme responsive to activators. Nature **368:** 466–469.

KOLESKE, A. J., S. BURATOWSKI, M. NONET and R. A. YOUNG, 1992 A novel transcription factor reveals a functional link betwen the RNA polymerase II CTD and TFIID. Cell **69:** 883–894.

KUZMINOV, A., 1995 Collapse and repair of replication forks in *Escherichia coli*. Mol. Microbiol. **16:** 373–384.

LIEFSHITZ, B., A. PARKET, R. MAYA and M. KUPIEC, 1995 The role of DNA repair genes in recombination between repeated sequences in yeast. Genetics **140:** 1199–1211.

MCDONALD, J. P., and R. ROTHSTEIN, 1994 Unrepaired heteroduplex DNA in *Saccharomyces cerevisiae* is decreased in *RAD1 RAD52*-independent recombination. Genetics **137:** 393–405.

MEAD, D. A., E. SZCZESNA-SKORUPA and B. KEMPER, 1986 Single-stranded DNA 'blue' T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering. Protein Eng. **1:** 67–74.

MEYN, M. S., 1993 High spontaneous intrachromosomal recombination rates in Ataxia-Telangiectasia. Science **260:** 1327–1330.

MEZARD, C., and A. NICOLAS, 1994 Homologous, homeologous, and illegitimate repair of double-strand breaks during transformation of a wild-type strain and *rad52* mutant strain of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:** 1278–1292.

NICKOLOFF, J. A., J. D. SINGER, M. F. HOEKSTRA and F. HEFFRON, 1989 Double-strand breaks stimulate alternative mechanisms of recombination repair. J. Mol. Biol. **27:** 527–541.

NONET, M. L., and R. A. YOUNG, 1989 Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of *Saccharomyces cerevisiae* RNA polymerase II. Genetics **123:** 715–724.

OZENBERGERG, B. A., and G. S. ROEDER, 1991 A unique pathway of double-strand break repair operates in tandemly repeated genes. Mol. Cell. Biol. **11:** 1222–1231.

PEARSON, W. R., and D. J. LIPMAN, 1988 Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:** 2444–2448.

PETES, T. D., R. E. MALONE and L. S. SYMINGTON, 1991 Recombination in yeast, pp. 407–521 in *The Molecular and Celular Biology of the Yeast Saccharomyces: Genome*, Vol. 1, edited by J. BROACH, E. W. JONES and J. R. PRINGLE. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

PRADO, F., and A. AGUILERA, 1995 Role of reciprocal exchange, one-ended invasion crossover and single-strand annealing on inverted and direct repeat recombination in yeast: different requirements for the *RAD1*, *RAD10* and *RAD52* genes. Genetics **139:** 109–123.

PRAKASH, L., and S. PRAKASH, 1977 Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. Genetics **86:** 33–55.

RATTRAY, A. J., and L. S. SYMINGTON, 1995 Multiple pathways for homologous recombination in *Saccharomyces cerevisiae*. Genetics **139:** 45–56.

ROTHSTEIN, R. J., 1983 One step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

RUDIN, N., and J. E. HABER, 1988 Efficient repair of HO-induced chromosomal breaks in *Saccharomyces cerevisiae* by recombination between flanking homologous sequences. Mol. Cell. Biol. **8:** 3918–3928.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-termination inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SANTOS-ROSA, H., and A. AGUILERA, 1994 Increase in incidence of chromosome instability and non-conservative recombination between repeats in *Saccharomces cerevisiae hpr1Δ* strains. Mol. Gen. Genet. **16:** 339–346.

SANTOS-ROSA, H., and A. AGUILERA, 1995 Isolation and genetic analysis of extragenic supressors of the hyper-deletion phenotype of the *Saccharomyces cerevisiae hpr1Δ* mutation. Genetics **139:** 57–66.

SANTOS-ROSA, H., B. CLEVER, W.-D. HEYER and A. AGUILERA, 1996 The yeast *HRS1* gene encodes a polyglutamine-rich nuclear protein required for spontaneous and *hpr1*-induced deletions between direct repeats. Genetics **142:** 705–716.

SCHIESTL, R. S., and R. D. GIETZ, 1989 High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. Curr. Genet. **16:** 339–346.

SCHIESTL, R. H., and S. PRAKASH, 1988 *RAD1*, an excision repair gene of *Saccharomyces cerevisiae*, is also involved in recombination. Mol. Cell. Biol. **8:** 3619–3626.

SCHIESTL, R. H., and S. PRAKASH, 1990 *RAD10*, an excision repair gene of *Saccharomyces cerevisiae*, is involved in the *RAD1* pathway of mitotic recombination. Mol. Cell. Biol. **10:** 2485–2491.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

SMITH, G. R., 1988 Homologous recombination in procaryotes. Microbiol. Rev. **52:** 1–28.

STEWART, S. E., and G. S. ROEDER, 1989 Transcription by RNA polymerase I stimulates mitotic recombination in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:** 3464–3472.

STRUHL, K., and R. W. DAVIS, 1980 A physical, genetic and transcriptional map of the cloned *his3* region of *Saccharomyces cerevisiae*. J. Mol. Biol. **136:** 309–332.

THOMAS, B. J., and R. ROTHSTEIN, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

THOMPSON, C. M., A. KOLESKE, D. M. CHAO and R. A. YOUNG, 1993 A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:** 1361–1375.

VILETTE, D., S. D. ERHRLICH and B. MICHEL, 1995 Transcription-induced deletions in *Escherichia coli* plasmids. Mol. Microbiol. **17:** 493–504.

VOEKEL-MEIMAN, K., R. L. KEIL and G. S. ROEDER, 1987 Recombination-stimulating sequences in yeast ribosomal DNA correspond to sequences regulating transcription by RNA polymerase I. Cell **48:** 1071–1079.

WALDHERR, M., A. RAGNINI, B. JANK, R. TEPLY, G. WIESENBERGERN *et al.*, 1993 A multitude of suppressors of group II intron-splicing defects in yeasts. Curr. Genet. **24:** 301–306.

YUAN, L.-W., and R. L. KEIL, 1990 Distance-independence of mitotic intrachromosomal recombination in *Saccharomyces cerevisiae*. Genetics **124:** 263–273.

ZHU, Y., C. L. PETERSON and M. F. CHRISTMAN, 1995 *HPR1* encodes a global positive regulator of transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15:** 1698–1708.

Communicating editor: F. WINSTON