Mutations in the RNA Polymerase II Transcription Machinery Suppress the Hyperrecombination Mutant *hpr1Δ* of *Saccharomyces cerevisiae*

Hua-Ying Fan, Kenneth K. Cheng and Hannah L. Klein

Department of Biochemistry and Kaplan Cancer Center, New York University Medical Center, New York, New York 10016

Manuscript received September 29, 1995
Accepted for publication December 4, 1995

ABSTRACT

The *soh1, soh2* and *soh4* mutants were isolated as suppressors of the temperature-dependent growth of the hyperrecombination mutant *hpr1* of *Saccharomyces cerevisiae*. Cloning and sequence analysis of these suppressor genes has unexpectedly shown them to code for components of the RNA polymerase II transcription complex. *SOH2* is identical to *RPB2*, which encodes the second largest subunit of RNA polymerase II, and *SOH4* is the same as *SUA7*, encoding the yeast transcription initiation factor TFIIB. *SOH1* encodes a novel 14-kD protein with limited sequence similarity to RNA polymerases. Interestingly, *SOH1* not only interacts with factors involved in DNA repair, but transcription as well. Thus, the Soh1 protein may serve to couple these two processes. The Soh1 protein interacts with a DNA repair protein, Rad5p, in a two-hybrid system assay. Soh1p may functionally interact with components of the RNA polymerase II complex as suggested from the synthetic lethality observed in *soh1 rpb1Δ104, soh1 soh2-1* (*rpb2*), and *soh1 soh4* (*sua7*) double mutants. Because mutations in *SOH1, RPB2* and *SUA7* suppress the hyperrecombination phenotype of *hpr1* mutants, this suggests a link between recombination in direct repeats and transcription.

MITOTIC DNA recombination can occur in response to DNA damage. Mutations in genes involved in DNA metabolism, such as DNA repair or replication, can result in hyperrec phenotypes (for review, see KLEIN 1995), which arise from the accumulation of nicked or gapped single and double-stranded DNA and can be repaired through recombination repair. Mutations in genes that normally repress mitotic recombination in favor of a strict repair pathway can also yield a hyperrec phenotype.

hpr1 was originally isolated as a hyper-recombination mutant that increases the rate of a deletion event between direct repeats by 500–1000-fold over wild type (AGUILERA and KLEIN 1988). The hyperrecombination phenotype of *hpr1* mutants is dependent on the *RAD52* gene (AGUILERA and KLEIN 1988). This suggests that the deletion event observed in *hpr1* mutants is processed by the *RAD52*-dependent recombination repair pathway. The Hpr1p protein shows significant homology to topoisomerase I (encoded by *TOP1*) in yeast (AGUILERA and KLEIN 1990), although no topoisomerase activity has been reported. Overexpression of the *TOP1* gene does not suppress the elevated recombination rate of *hpr1*. In contrast to *top1* mutants, no increase in superhelicity of plasmids, nor increase in the recombination rate at the rDNA locus is observed in *hpr1* mutants (AGUILERA and KLEIN 1990). However, a *hpr1Δ* mutant is lethal in combination with either *top1, top2ts* or *top3* mutations, while each single mutant is viable (AGUILERA and KLEIN 1990). A *hpr1Δ* mutant is also inviable when combined with a deletion of the copy I histone H3-H4 genes (FAN and KLEIN 1994), suggesting that Hpr1p plays a role, directly or indirectly, in modulating chromatin structure. Such a role has also been postulated by ZHU *et al.*, in an independent study (1995). The Hpr1p protein has also been suggested to function as a positive transcription regulator, as the expression of the *SUC2* and *GAL1* genes is reduced in *hpr1* mutants (ZHU *et al.* 1995).

Suppressors of *hpr1* (*soh* mutants) were isolated by the ability to restore viability to a *hpr1Δ* strain at 37° (FAN and KLEIN 1994). The *soh1Δ* mutant suppresses 90% of the increased recombination frequency of a *hpr1Δ* mutant. The Soh1 protein shows limited homology to RNA polymerases. Here, we report the cloning of the *SOH2* and *SOH4* genes and further characterization of the *SOH1* gene. *SOH2* and *SOH4* encode two essential components of the RNA polymerase II transcription complex and *soh1* mutations interact genetically with mutations in genes that encode components of the RNA polymerase II complex. Mutations in these *SOH* genes suppress the hyperrecombination mutant *hpr1*, suggesting a link between transcription and recombination. A precedent for the linking of transcription and recombination has come from several studies in yeast. The *HOT1* DNA fragment, containing an RNA polymerase I (Pol I) promoter sequence, can stimulate the recombination of an adjacent gene. This stimulated recombination can be abolished by inserting a Pol I terminator sequence between the *HOT1* and the neigh-

Corresponding author: Hannah L. Klein, Department of Biochemistry, NYU Medical Center, 550 First Ave., New York, NY 10016.
E-mail: kleinh01@mcrcr.med.nyu.edu

boring gene (VOELKEL-MEIMAN and ROEDER 1990). Repeated DNA sequences that are constitutively transcribed by RNA polymerase II have also been found to have an elevated recombination rate (THOMAS and ROTHSTEIN 1989).

We also show that Soh1p interacts with Rad5p physically. This observation further suggests a transcription and recombination link to a Rad5p-mediated repair pathway. *RAD5* encodes an ATPase and the Rad5p protein is a member of the Snf2p/Swi2p putative DNA helicase family (JOHNSON *et al.* 1994). In addition, the Rad5p protein contains a cysteine-rich region and a leucine-zipper motif (JOHNSON *et al.* 1992). The *RAD5* gene belongs to the *RAD6* epistasis group, which represents the postreplication DNA repair pathway, consisting of both error-prone and error-free components. *RAD5* may not have a direct role in recombination, but is involved in the destabilization of poly(GT) tracts in the genome (JOHNSON *et al.* 1992). Mutations in *RAD5* result in a 10-fold decrease in the rate of poly(GT) tract instability, suggesting that the Rad5p protein may enhance a transient primer-template dissociation reaction during replication.

MATERIALS AND METHODS

Yeast strains: The strains used in this study are listed in Table 1. All strains are isogenic and in the W303 strain background, with the exception of strains HFY954, EGY48, FY84 and N400. Strains used for determining recombination rates were derived from W628-2A, HFY1125-11B, HKY442-1B and HKY442-3C.

Plasmids: Plasmids used in this study are listed in Table 2. pM107 was kindly provided by M. HAMPSEY. pSH18-34, pEG202 and pJG4-5 were kindly provided by R. BRENT. pLexA-Snf2 was kindly provided by M. CARLSON.

Media and growth conditions: Standard media were prepared as described (SHERMAN *et al.* 1986). All strains were grown on solid media or in liquid media at 30°, except for the testing of temperature-dependent growth at 37°.

Cloning of *SOH2* and *SOH4*: Strains with a genotype *soh1-1 soh2-1 hpr1* or *soh1-1 soh4-4* grow extremely slowly at 30°, and complementation of these phenotypes was used to clone the *SOH2* and *SOH4* genes. Strains HFY281-3A and HFY899-1C have the genotypes described above and were used to clone *SOH2* and *SOH4*, respectively, by transformation with a partial *Sau*3A yeast genomic library constructed in the vector pBS32 (F. SPENCER and P. HIETER, unpublished data). pBS32 is a *LEU2* derivative of the vector YCp50 (J. TRUEHEART, unpublished observations). Yeast transformation was carried out according to the lithium acetate procedure of ITO *et al.* (1983), as modified by HILL *et al.* (1991). Leu^+ transformants that exhibited normal growth at 30° were selected and plasmids were recovered into *Escherichia coli*. The bacterial transformants were subjected to colony hybridization using *SOH1* as a probe to detect the *SOH1* gene, thereby eliminating the *SOH1*-containing colonies from further studies.

Cloning and sequencing of the *rad5-535* mutation: Strain HFY218-13C (*rad5-535 soh1-1*) failed to grow in the presence of 0.012% methyl methanesulfonate (MMS). The position of the *rad5-535* mutation was determined by the gap-repair method according to ORR-WEAVER and SZOSTAK (1983). The plasmid pL2-1 was digested with *Nhe*I-*Hpa*I, *Nru*I-*Bgl*II and *Bgl*II-*Hpa*I to generate three different deletions within the *RAD5* gene. Plasmids that have acquired the *rad5-535* allele were identified and the region containing the *rad5-535* mutation was deduced to be within a 250-bp *Nhe*I-*Hpa*I fragment. This fragment was used to substitute the corresponding fragment from the plasmid pL2-1, which contains the wild-type *RAD5* gene, producing plasmid pL94-1. Because pL94-1 failed to complement *rad5-535*, the 250-bp *Nhe*I-*Hpa*I fragment was determined to contain the *rad5-535* mutation. This fragment of pL94-1 was then subcloned into pBluescript-SK (Stratagene), forming plasmid pL100-1. Both strands of pL100-1 were sequenced by the dideoxy-chain termination method (SANGER *et al.* 1977) using the universal primer, T7 DNA polymerase (Sequenase 2.0, US Biochemical) (TABOR and RICHARDSON 1987) and 5′-[$a^{35}S$]thio-triphosphate (BIGGIN *et al.* 1983).

MMS sensitivity: Yeast strains were grown in rich medium (YEPD) to saturation phase at 30°. Cells were pelleted and washed once in sterile water. Cells were pelleted again and resuspended in 0.05 M KH_2PO_4 (pH 7.0) at a concentration of 10^7 cells/ml. These cells were treated with 1% MMS. At 10-min intervals, 0.5 ml MMS-treated cells were added to 0.5 ml 10% $Na_2S_2O_3$ to inactivate the MMS. The cells were then serially diluted, plated on rich medium and incubated at 30°. Survival was determined after 2 days.

UV sensitivity: Yeast strains were grown in rich medium (YEPD) to saturation phase at 30° and then serially diluted onto YEPD plates. Cells were irradiated with UV light, by inverting the plates onto a UV transilluminator (Chromo-VUE model TM36, 300 nm) with the lids removed. Different exposure times were used. These plates were incubated in the dark at 30° for 2 days, and the number of surviving cells was then determined.

β-galactosidase activity assays: Strains containing a reporter plasmid were grown overnight at 30° in complete minimal synthetic media without appropriate amino acids to select for plasmids. Overnight cultures were then diluted into fresh selective medium at a density of 3×10^6 cells/ml and grown at 30° for ~7 hr until reaching a density of $2-4 \times 10^7$ cells/ml. Cells were then harvested and resuspended in breaking buffer (100 mM Tris-Cl, pH 8, 1 mM dithiothreitol, 20% glycerol). The cells were lysed by vortexing with glass beads. Cell debris and glass beads were removed by centrifugation. The crude extracts were assayed as described by MILLER (1972). The specific β-galactosidase activity was calculated using the formula: $1.7 \times (A_{420})/[(0.0045)$ (protein concentration) (assay time) (extract volume)] and expressed as nanomoles *o*-nitrophenyl β-D-galactopyranoside (ONPG) cleaved per minute per milligram protein.

DNA manipulations: Plasmid DNA was recovered from yeast transformants according to HOFFMAN and WINSTON (1987). Plasmid DNA was isolated from *E. coli* by the alkaline lysis procedure (SAMBROOK *et al.* 1989). Plasmid DNA was treated with restriction endonucleases according to the manufacturer's specifications. Genomic DNA was purified from yeast cells according to ROSE *et al.* (1990), then digested with restriction enzymes, and run on agarose gels. Gels were subsequently blotted to nitrocellulose paper in 10× SSC (1.5 M NaCl, 0.15 M Na citrate) overnight. Filters were then UV cross-linked. DNA fragments were labeled with ^{32}P-α-dCTP by random priming according to FEINBERG and VOGELSTEIN (1984). Hybridization was performed in 1× Denhardt's, 6× SSC, 0.25 M NaH_2PO_4 (pH 6.0), 30 mM $Na_4P_2O_7$ and 200 ng single-stranded salmon sperm DNA at 65° for 18 hr. For ligation, DNA fragments were gel purified (QIAEX, gel purification kit from QIAGEN) and ligated using T4 DNA ligase (Boehringer Mannheim) at 16° overnight.

Tetrad analysis: Fresh diploids were plated on sporulation plates and incubated at room temperature for 3 days and then were digested with zymolyase 20T (2 mg/ml) for 5 min at

TABLE 1

Yeast strains

Strain	Genotype	Source
W303-1A	*MATa ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100 rad5-535*	R. ROTHSTEIN
W303-1B	*Matα ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100 rad5-535*	R. ROTHSTEIN
U755	*MATa TRP1* in W303 background	R. ROTHSTEIN
U768-4C	*MATa hpr1Δ3::HIS3* in W303 background	R. ROTHSTEIN
U768-1C	*MATα hpr1Δ3::HIS3* in W303 background	R. ROTHSTEIN
HFY14	*MATα soh1-1 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY5	*MATα soh2-1 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY22	*MATα soh4-1 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY20	*MATα soh4-2 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY19	*MATα soh4-3 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY7	*MATα soh4-4 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY6	*MATα soh4-5 hpr1Δ3::HIS3* in W303 background	FAN and KLEIN (1994)
HFY218-13C	*MATa soh1-1 TRP1* in W303 background	FAN and KLEIN (1994)
HFY219-7C	*MATa soh2-1* in W303 background	FAN and KLEIN (1994)
HFY221-4C	*MATa soh4-1 TRP1* in W303 background	FAN and KLEIN (1994)
HFY204-1A	*MATa soh4-2* in W303 background	FAN and KLEIN (1994)
HFY214-13B	*MATa soh4-3* in W303 background	FAN and KLEIN (1994)
HFY212-7A	*MATa soh4-4 TRP1* in W303 background	FAN and KLEIN (1994)
HFY202-7D	*MATa soh4-5* in W303 background	FAN and KLEIN (1994)
HKY442-1B	*MATa leu2-k::ADE2-URA3::leu2-k* in W303 background	FAN and KLEIN (1994)
HKY442-3C	*MATα leu2-k::ADE2-URA3::leu2-k hpr1::HIS3* in W303 background	FAN and KLEIN (1994)
HKY579-10A	*MATa ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100*	This study
HKY578-2C	*MATa rad5Δ::URA3* in W303 background	This study
HFY281-3A	*MATa soh1-1 soh2-1 hpr1Δ3::HIS3* in W303 background	This study
HFY899-1C	*MATα soh1-1 soh4-4* in W303 background	This study
HFY986-1A	*MATα soh1Δ3::URA3 LEU2* in W303 background	This study
HFY986-1C	*MATa soh1Δ3::URA3 TRP1 RAD5* in W303 background	This study
HFY1125-11B	*MATα gal10ΔKpnI::URA3::gal10Δ3′, gal4::LEU2,* in W303 background	This study
W628-2A	*MATa gal80::LEU2, rad52::TRP1, gal10ΔKpnI::URA3::gal10Δ3′* in W303 background	R. ROTHSTEIN
N400	*Mata ura3-52 his3Δ200 leu2-3,112 rpb1Δ187::HIS3* + (pC6 [*CEN LEU2 rpb1Δ104*])	R. A. YOUNG
FY84	*MATa ura3-52 his3Δ200 leu2Δ1 lys2-128δ*	F. WINSTON
HFY954	*MATa rpb1::HIS3 SOH1 ura3-1 ade2-1 leu2,3-112 his3-11,15* + pC6 [*rpb1Δ104*] *MATα rpb1::HIS3 SOH1 ura3-52 ADE2 leu2,3-112 his3Δ200*	This study
HFY954-2	*MATa rpb1::HIS3 soh1Δ3::URA3 ura3-1 ade2-1 leu2-3,112 his3-11,15* + pC6 [*rpb1 Δ104*] *MATα rpb1::HIS3 SOH1 ura3-52 ADE2 leu2-3,112 his3Δ200*	This study
EGY48	*MATa his3 trp1 ura3-52 leu2 LexAop-LEU2*	R. BRENT

30°. Tetrads were dissected using a micromanipulator under a light microscope.

Determination of recombination rates: Recombination rates were calculated according to the median method of LEA and COULSON (1948) as described (AGUILERA and KLEIN 1988). For the *gal10* duplication system, strains were streaked out on solid YP-sucrose medium (1% yeast extract, 2% peptone, and 2% sucrose), and were incubated at 30° for 4 days. For the *leu2* duplication system, strains were streaked out on solid YEPD medium and incubated at 30° for 3 days. Nine colonies from each strain were used in each fluctuation test. A minimum of three different strains of the same genotype were used for each recombination rate determination.

RESULTS

Isolation of the *soh* mutants: We have previously described the isolation of the *soh* mutants that are suppressors of the hyperrecombination mutant *hpr1Δ* of *S. cerevisiae* (FAN and KLEIN 1994). *SOH1* encodes a putative 14-kD protein that shows slight homology to RNA polymerases. Here, we describe the cloning of two additional *SOH* genes, *SOH2* and *SOH4,* and the further characterization of the *SOH1* gene.

***SOH4* encodes TFIIB:** Five alleles of *soh4* were isolated and both dominant and recessive alleles were recovered (FAN and KLEIN 1994). *soh1-1 soh4* double mutants show extremely slow growth, and complementation of this phenotype was used to clone *SOH4.* The obtained *SOH4* clone was sequenced, and is identical to the *SUA7* gene, which encodes yeast TFIIB and is involved in the selection of transcription initiation sites (PINTO *et al.* 1992). To ascertain that *SUA7* is *SOH4,* the *SUA7* locus was tagged with a marker gene, *URA3,* by transformation and the *soh4-3* mutation was shown to tightly link to the *SUA7* locus (PD:NPD:TT = 100:0:0). To determine whether any of the five *soh4* alleles are involved in transcription start site selection, the pM107 plasmid was introduced into all five *soh4* mutant strains. The pM107 plasmid contains a *cyc1-5000* promoter-*lacZ* fusion construct with an aberrant ATG at the leader

TABLE 2

Plasmids

Name	Description	Source
pEG202	2μ, *HIS3*, LexA DNA binding domain fused to the *ADH1* promoter	R. BRENT
pJG4-5	2μ, *TRP1*, Gal4p activation domain fused to the *GAL1* promoter	R. BRENT
pSH18-34	2μ, *URA3*, 8 *lexA* operator-*lacZ*	R. BRENT
pLexA-Snf2	LexA (1-87)-Snf2 (14-1096)	M. CARLSON
pM107	*cyc1-5000-lacZ*	M. HAMPSEY
pL2-1	p*RAD5*, *CEN*, *ARS*, *URA3*, Amp^R	This study
pL94-1	*Nhe*I, *Hpa*I fragment of *rad*5-535 replaces the corresponding fragment of *RAD5* in pL2-1	This study
pL100-1	*Nhe*I, *Hpa*I fragment of *rad*5-535 in pBluescript	This study
pL47-8	*SOH1* in pJG4-5	This study
pL50-1	*SOH1* in pEG202	This study
pL58-5	Rad5p (1-801) in pEG202	This study
pL103-48	Rad5p (1-1169) in pEG202	This study
pS5S14-1	originally isolated *SOH2* clone from pBS32 library	This study
pS5S14-1*Hin*dIII	Self-ligation of the longest *Hin*dIII fragment of plasmid pS5S14-1	This study
pL14-11	5.6-kb *Eco*RI fragment of pS5S14-1 cloned into pRS314	This study

sequence that is not in frame to the *lacZ* coding sequence (HAMPSEY 1991). *SUA7* strains, with normal transcription start sites, will use the aberrant ATG as the translation start codon and fail to synthesize functional β-galactosidase. On the other hand, *sua7* mutants can alter the transcription start site pattern at the *cyc1* locus and then use the endogenous translation start codon ATG to make functional β-galactosidase. Two original *sua7* mutants, *sua7-1* and *sua7-3*, had an increase of five- and threefold over wild type in β-galactosidase activity, respectively (M. HAMPSEY, personal communication). Based on the results of Table 3, the *soh4* alleles do not seem to have a dramatic role in the transcription start site selection. *soh4-3* and *soh4-5* are the two best alleles for increasing the *cyc1-5000-lacZ* expression, and yield an increase of 2.3-fold over wild type. This suggests that some of these *soh4* mutant alleles, especially *soh4-1*, *soh4-2* and *soh4-4*, may suppress the *hpr1* mutant with no impairment in transcription initiation site selection.

TABLE 3

Expression of the *cyc1-5000-lacZ* construct in *soh4* mutation strains

Genotype	β-Galactosidase Activity[a]	Fold over wild type
SOH4	5.2 ± 2.2	1.0×
Soh4-1	9.0 ± 0.3	1.7×
soh4-2	7.0 ± 1.2	1.3×
soh4-3	12.0 ± 0.3	2.3×
soh4-4	9.0 ± 2.4	1.7×
soh4-5	12.1 ± 2.1	2.3×

[a] The activity is expressed as nanomoles/minute/milligram protein. The number is the average of the activities from four independent transformants, and two determinations were performed for each colony. The activity is expressed as the mean rate ± the standard deviation of the four determinations.

***SOH2* encodes a subunit of RNA polymerase II:** *SOH2* was cloned by complementation of the extremely poor growth phenotype of a *soh2-1 soh1-1 hpr1*Δ triple mutant at 30°. The DNA insert from the *SOH2* clone (pS5S14-1) obtained was used to probe a membrane containing an ordered array of yeast recombinant clones and was found to hybridize to a λ clone containing *PDR5* on chromosome *XV*. Genetic mapping data showed that the *soh2-1* allele is linked to *ADE2* at a distance of 31 cM (PD:NPD:TT = 45:2:49).

To delimit the minimum complementation region of the *SOH2* clone, the pS5S14-1*Hin*dIII and pL14-11 plasmids were constructed from the plasmid pS5S14-1. These two plasmids contain a 300-bp overlapping sequence (Figure 1). Neither of these two plasmids complemented the *soh2-1* phenotype (extremely slow growth in combination with *soh1-1*), indicating that the common insert sequence between pS5S14-1*Hin*dIII and pL14-11 contained an internal fragment of the *SOH2* gene. A *Hin*dIII-*Eco*RI fragment containing part of the potential *SOH2* gene was cloned into pBluescript-SK, sequenced, and found to be identical to the *RPB2* gene in the GenBank database (accession number M15693).

RPB2 is an essential gene and encodes the second largest subunit of RNA polymerase II. The *rpb2* allele we isolated (*soh2-1*) is viable at 30° with a slightly reduced growth rate but does not have the Spt^- phenotype that has been reported for the *rpb2-501~505* alleles (HEKMATPANAH and YOUNG 1991). The assay system we used was the *lys2-128δ* allele, which contains a δ sequence of the *Ty* transposon inserted in the amino-terminal coding region of the *LYS2* gene. In contrast to some mutant alleles of *RPB2* (HEKMATPANAH and YOUNG 1991), *soh2-1* failed to restore *LYS2* expression that had been inactivated by the δ sequence insertion (data not shown).

We further examined whether the *soh2-1* mutant caused another well-characterized phenotype of *rpb2*

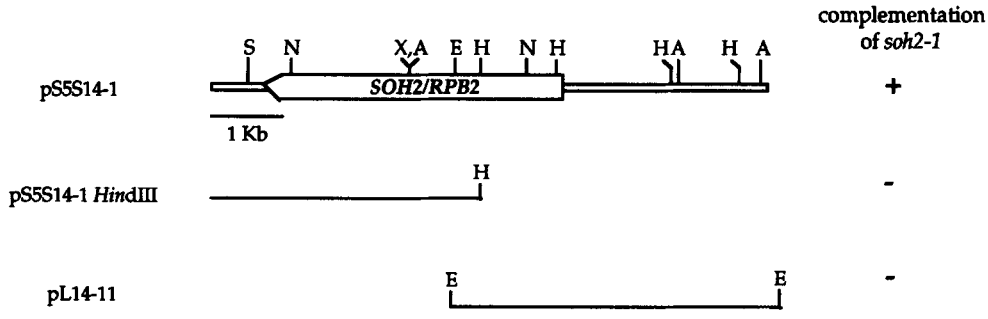


FIGURE 1.—Structure of the *SOH2* gene. The restriction map of the yeast DNA insert in the original clone of *SOH2*, pS5S14-1 is shown. Displayed below this map are subclones and the ability of these subclones to complement the *soh2-1* mutant. Complementation was tested in a diploid strain of the genotype of *soh1-1/soh1-1 soh2-1/SOH2 hprl*Δ*/hprl*Δ by determining the meiotic segregation pattern at 30°. If a plasmid contains the *SOH2* gene, then a 3+:1- or 4+:0 segregation for viability will be observed, and none of the poorly growing spores will contain the *SOH2* plasmid. In contrast, if a plasmid does not contain the *SOH2* gene, a 2+:2 segregation for viability will be observed. Neither the pS5S14-1 *Hin*dIII nor the pL14-11 plasmids complemented the *soh2-1* phenotype. E, *Eco*RI; X, *Xho*I; S, *Spe*I; A: *Ava*I; H, *Hin*dIII; N, *Nru*I.

alleles, Srb⁻. The C-terminal domain (CTD) of the Rpb1 protein (the largest subunit of RNA polymerase II) consists of 26–27 copies of direct heptapeptide repeats, Pro-Thr-Ser-Pro-Ser-Thr-Ser (SCAFE *et al.* 1990; THOMPSON *et al.* 1993). A minimum of 10 copies of heptapeptide repeats is essential for viability, while 10–12 copies result in a conditional lethal phenotype. The *rpb1*Δ*104* allele, containing $11^{3}/_{7}$ copies of the heptapeptide repeats, is cold sensitive, heat sensitive and has an inositol auxotrophy (NONET *et al.* 1987). A screen for suppressors of the *rpb1*Δ*104*^{cs} mutant uncovered a group of mutants termed *srb*. The Srb proteins are believed to interact with the C-terminal domain of the Rpb1 protein (NONET and YOUNG 1989). New mutant alleles of *RPB2* were recovered among the *srb* mutants (R. A. YOUNG, personal communication). To determine whether *soh2-1* acts as a *srb* mutant, we assayed the ability of the *soh2-1* mutant to suppress the cold sensitivity of *rpb1*Δ*104*^{cs}. A *rpb1-104 soh2-1* double mutant was constructed and *soh2-1* did not suppress the cold sensitivity of *rpb1-104* (data not shown), indicating that *soh2-1* is a separation of function mutant, which is different from *spt* or *srb* mutants. This is further strengthened by the observation that three different *srb/rpb2* alleles, *rpb2-551*, *rpb2-553* and *rpb2-554*, that we obtained from R. A. YOUNG do not suppress the *hprl*Δ mutant.

***hprl* and transcription:** Mutations in the RNA polymerase II complex suppress the hyperrecombination phenotype of *hprl* mutants, suggesting that the *hprl*-induced recombination is related to transcription. This hypothesis was tested by assaying recombination rates of a *gal10* duplication in different transcription states (THOMAS and ROTHSTEIN 1989). The recombination system has two heteroalleles of *gal10* that are separated by a selectable marker, *URA3*. Both of these copies are under the control of the native *GAL10* promoter, which can be activated by the Gal4p protein, and repressed by the Gal80p protein. The rate of recombination deletion events (FOA^{r}) was determined for *hprl* mutants. In the *gal80* background, the *GAL10* promoter is highly expressed and in the *gal4* background, the *GAL10* expression is repressed. A 20-fold increase in the recombination rate was observed in *HPR1 gal80* strains compared to *HPR1 gal4* strains, in agreement with published data (THOMAS and ROTHSTEIN 1989). This indicates that recombination occurs more frequently when the region is in a transcriptionally active state than when it is in a transcriptionally repressed state. As shown in Table 4, a 100-fold increase in the rate of deletion events was observed in *hprl* mutants over that in *HPR1* strains, in the transcriptionally active *gal80* background. In addition, only a 10-fold increase in the number of deletion events was detected in *hprl vs. HPR1* in the transcriptionally repressed *gal4* background. These results further support the hypothesis that the hyperrecombination phenotype of *hprl* mutants is related to transcription.

Interaction between *SOH1* and RNA polymerase II: Because the *soh1-1 soh2*(*rpb2*) double mutant showed poor growth and some *rpb2* alleles can suppress *rpb1* mutants, we were interested in examining the effect of

TABLE 4

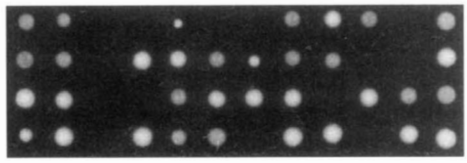
Recombination rates in different transcription states

Genotype	Recombination rate ($\times 10^{-5}$)[a]
HPR1 gal80	29 ± 7.2
hprl gal80	3400 ± 600
HPR1 gal4	1.1 ± 0.1
hprl gal4	17 ± 3.4

[a] Rates were calculated as described in MATERIALS AND METHODS from 5-FOA resistance frequencies of strains carrying the duplication *gal10*Δ*KpnI::URA3::gal10-3'*Δ (THOMAS and ROTHSTEIN 1989). Strains were grown on YP-sucrose plates for 4 days at 30° before assaying recombination rates. Each rate was calculated from three independent fluctuation tests on three strains of the same genotype, and is expressed as the mean rate ± the standard deviation of the three determinations.

A. HFY954

rpb1::HIS3/rpb1::HIS3 SOH1/SOH1 ade2-1/ADE2 ura3-1/ura3-52 + pC6 [*rpb1Δ104*]



B. HFY954-2

rpb1::HIS3/rpb1::HIS3 soh1::URA3/SOH1 ade2-1/ADE2 ura3-1/ura3-52 + pC6 [*rpb1Δ104*]

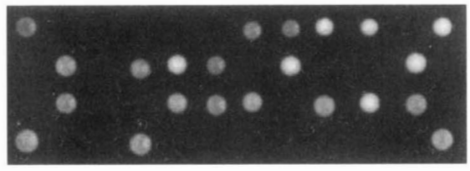


FIGURE 2.—The *soh1Δ rpb1Δ104* mutant is lethal. A *soh1Δ3::URA3 rpb1::HIS3* strain containing a plasmid C6 [*rpb1Δ104*] is dead, as shown by the tetrad analyses of HFY954 and HFY954-2 strains. (A) HFY954 is homozygous for *rpb1::HIS3* and *SOH1*. The *rpb1Δ104* mutation is maintained on an extragenic plasmid. The *rpb1Δ104* mutation rescues the lethal endogenous *rpb1* deletion mutation and results in a cold-sensitive phenotype. All viable spores are His^+, Leu^+, and Ura^-. The inviable spores result from either a loss of the C6 plasmid or general spore inviability. (B) Plasmid pL34-1 contains the *soh1* deletion construct, which is marked with a selectable marker, *URA3*. A 3.7-kb *Not*I and *Xho*I fragment of pL34-1 was used to transform HFY954. Transformants were checked by Southern blot analysis to determine whether the *soh1* deletion construct inserted at the correct locus. All of the viable spores are His^+, Leu^+, and Ura^-, and no viable spores are Ura^+. Together, these data suggest that mutant strains with a genotype of *soh1Δ3::URA3, rpb1::HIS3* + pC6 [*rpb1Δ104*] are dead at 30°.

the *soh1Δ* mutation on a *rpb1* CTD truncation allele (*rpb1Δ104*). The inability to recover a *soh1Δ rpb1Δ* + pC6 [*rpb1Δ104*] double mutant from 50 tetrads from a *soh1Δ* by *rpb1Δ* + pC6 [*rpb1Δ104*] cross suggests a possible lethality of a *soh1Δ rpb1Δ* + pC6 [*rpb1Δ104*] strain. (The *rpb1Δ104* allele is maintained on an extragenic plasmid C6, and the endogenous *RPB1* copy is replaced with the *HIS3* marker). To confirm this possibility, we constructed a heterozygous *soh1Δ* mutation in the homozygous *rpb1Δ* + pC6 [*rpb1Δ104*] diploid background by transforming a DNA fragment containing a *soh1* deletion construct, *soh1Δ3::URA3* into the strain HFY954. Southern blot analysis confirmed that the *soh1Δ3::URA3* DNA fragment had inserted into one chromosomal *SOH1* locus in the diploid strain. Homozygous *rpb1Δ* + pC6 [*rpb1Δ104*] diploids with homozygous *SOH1* or heterozygous *soh1Δ3::URA3*, were sporulated and the tetrad analyses are shown in Figure 2. Spore inviability in the first diploid which is homozygous *SOH1*, resulted either from losing the plasmid C6 (the *rpb1Δ* mutant is inviable at 30°) or general strain spore inviability. However, in the heterozygous *soh1Δ3* diploid, no more than two viable spores were recovered from each tetrad, and no viable Ura^+ spores, carrying the *soh1* deletion, could be obtained. These results indicate that a *soh1Δ3 rpb1Δ* + pC6 [*rpb1Δ104*] double mutant is lethal. The combination of a viable mutant *soh1Δ* and a conditional lethal mutant *rpb1Δ104* results in a synthetic lethality, suggesting an interaction between these two mutants.

TABLE 5

Transactivation activity of the LexA-Soh1p fusion protein

Plasmids	X-Gal Plates	β-Galactosidase activity[a]	Fold increase
pLexA	White	4.1 ± 1.8	1×
pLexA-Soh1p	Blue	57 ± 6.8	14×
pLexA-Snf2p	Blue	250 ± 40	61×

pSH18-34 is the reporter plasmid (Table 2).

[a] The activity is expressed as nanomoles/minute/milligram protein. The number is the average of the activities from three independent transformants ± the standard deviation of the three independent transformants.

Soh1p and transcription: The genetic interactions of *soh1* with *rpb1, rpb2,* and *sua7* strongly suggest a role of the Soh1 protein in transcription. This observed synthetic lethality of *soh1* with transcription mutants is specific, as *soh1* has been tested with many nonspecific null mutants (such as *top1, rad51* and *srs2*) and no synthetic lethality has been found. Therefore, a fusion protein containing the DNA binding domain of the LexA and Soh1p was constructed. As shown in Table 5, this LexA-Soh1p fusion protein is able to bind a *LexA* operator and activate a downstream reporter gene, *lacZ*. An increase of 14-fold in the β-galactosidase activity is observed in a strain containing the plasmid pL50-1 (Table 5). The transactivation activity of a LexA-Snf2p fusion protein has been reported (LAURENT *et al.* 1991) and was used here as a positive control. The β-galactosidase activity induced by the LexA-Soh1p fusion protein is not as high as that induced by the LexA-Snf2p fusion protein, but the transactivation activity is significant and reproducible.

***soh1Δ* enhances the DNA repair defect of a *rad5-535* mutant:** The original *soh1-1* mutant, in the W303 strain background, was found to be sensitive to a DNA-alkylation agent, methyl methane sulfonate (MMS), at a concentration of 0.012% (Figure 3A). In the first attempt to clone *SOH1,* we used this MMS-sensitive phenotype for complementation, and recovered only *RAD5* clones that complemented the *soh1-1* MMS sensitivity. *RAD5* encodes a putative DNA helicase with a demonstrated ATPase activity (JOHNSON *et al.* 1994). The Rad5p protein is involved in DNA repair, and a null mutation yields a defect in DNA repair that results in a MMS sensitive phenotype. However, we noted that the *RAD5* gene did not complement other phenotypes associated with the *soh1-1* mutant such as the suppression of *hpr1*

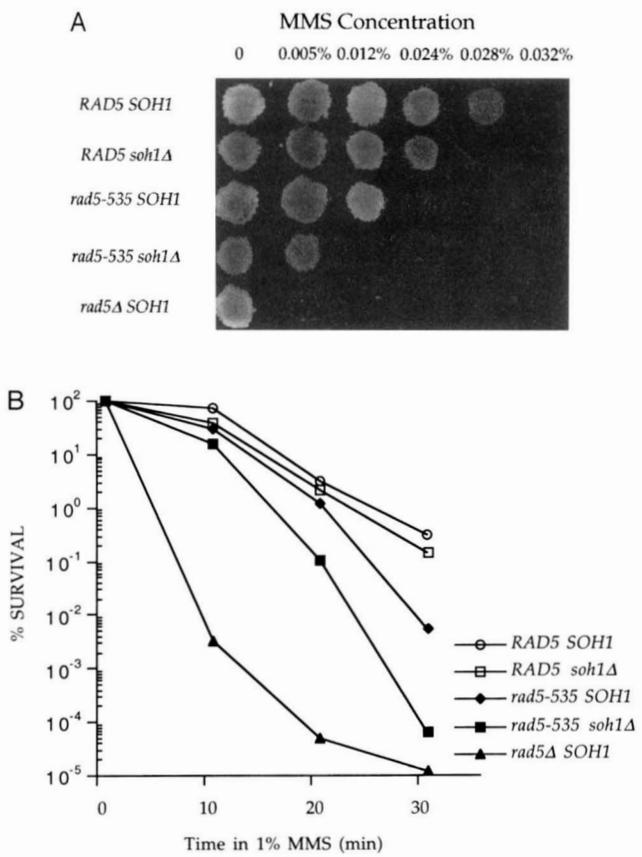


FIGURE 3.—MMS sensitivity of the *rad5-535* mutant is enhanced by a mutation in the *SOH1* gene. (A) Freshly grown patches of cells were replica plated to YEPD plates containing different concentration of MMS and incubated at 30° for 24 hr. These plates were then replica plated to YEPD plates and grew at 30° for 16 hr. Strains used in this study with corresponding genotypes are HFY579-10A: *RAD5 SOH1*, HFY986-1C: *RAD5 soh1*Δ, U755: *rad5-535 SOH1*, HFY986-1A: *rad5-535 soh1*Δ, and HKY578-2C: *rad5*Δ *SOH1*. (B) MMS survival curve of *soh1* and *rad5* mutant strains. Strains were incubated in phosphate buffer (pH 7.0) containing 1% MMS. Cells were collected every 10 min and plated on rich medium. The number of surviving cells was determined after 2 days. The *soh1-1* strain is as MMS sensitive as the *soh1*Δ strain in all strain backgrounds.

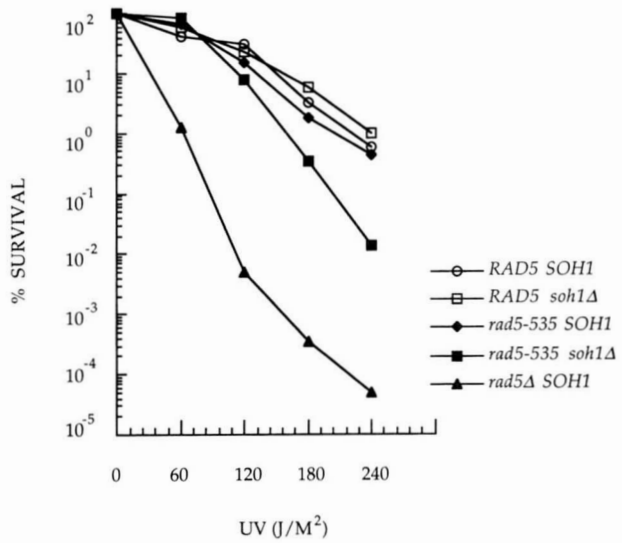


FIGURE 4.—Survival of *soh1* and *rad5* mutants after UV irradiation. Strains used in this study are the same as in Figure 3. Neither *rad5-535* nor *soh1*Δ is sensitive to UV irradiation. The *soh1*Δ and *rad5-535* double mutant is more sensitive than either of the single mutants.

temperature-dependent growth. Moreover, *soh1-1* was not allelic to *RAD5* by genetic tests. This suggested that there was a *rad5* mutation in the W303 strain background. This potential *rad5* mutation was cloned and sequenced. A G-to-A transition change was found when the W303 *rad5* sequence was compared with the published *RAD5* sequence. This change results in a Gly-to-Arg change at position 535, which is within the nucleotide binding consensus sequence, ^{535}GXGKT, of the Rad5p protein. This mutant allele has been named *rad5-535*. A *rad5-535* mutant, which is slightly MMS sensitive and considered to be a weak allele of *rad5*, is present in the W303 strain background, which we have used in the lab. To further confirm this, we replaced the W303 *rad5* allele with the *RAD5* wild-type allele. This single gene change resulted in increased MMS resistance of the W303 strain. Moreover, the *soh1–1* mutant only is slightly MMS sensitive in the *RAD5* background (Figure 3).

The *soh1*Δ *RAD5* strain does not have any obvious defects in DNA repair because it is resistant to DNA damaging agents, such as UV and X-ray irradiation and MMS treatment (data not shown and Figures 3 and 4). *rad5-535* is a weak allele of *RAD5* and shows slight sensitivity to MMS and UV (Figures 3 and 4). Increased sensitivity to both MMS and UV was observed in a *rad5-535 soh1*Δ double mutant (Figures 3 and 4). Therefore, we conclude that a *soh1* mutation enhances the DNA repair defect of a *rad5-535* mutant.

***rad5* does not suppress *hpr1*:** Although a *soh1* mutation enhances the DNA repair defect of a *rad5-535* mutant, this enhancement is not responsible for the initial isolation of *soh1-1* as a *hpr1* suppressor. *soh1* suppresses a *hpr1* mutant in a *RAD5* background and a *rad5* null mutant does not suppress the temperature sensitivity of a *hpr1* mutant (Figure 5).

A further indication that a *rad5* mutant does not suppress *hpr1* is that a *rad5* deletion mutation does not suppress the hyperrecombination phenotype of a *hpr1* mutant (Table 6). The phenotypes of *hpr1* are not influenced by the genotype of *RAD5*. A *hpr1 rad5-535* mutant is as hyperrecombination as a *hpr1 RAD5* mutant and a *hpr1 RAD5* mutant is as temperature sensitive as a *hpr1 rad5-535* mutant (Table 6).

Soh1p interacts with a DNA repair protein: The genetic interaction between *soh1* and *rad5-535* suggested that the Soh1 protein might interact with the Rad5 protein. We used the two-hybrid system to examine this possibility. To construct the LexA DNA binding domain-Rad5 fusion protein, different fragments of the

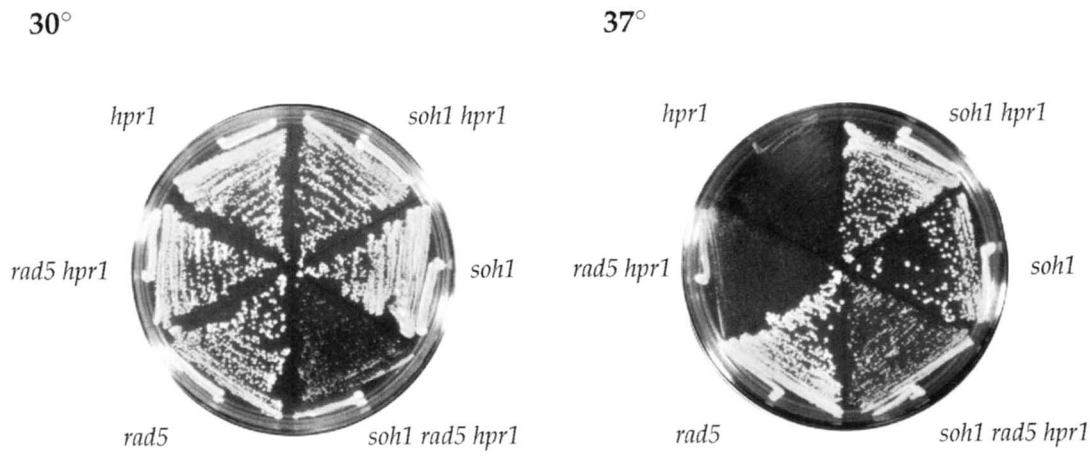


FIGURE 5.—*soh1* suppresses *hpr1* while *rad5* fails to suppress *hpr1*. Strains with different combinations of *soh1*, *rad5*, and *hpr1* mutations were streaked on rich medium, and then incubated at both 30° and 37°.

RAD5 gene were cloned into the pEG202 plasmid (GYURIS *et al.* 1993), which contains the LexA DNA binding domain under the control of the *ADH1* promoter. The Gal4p activation domain-Soh1p fusion protein was produced by cloning the *SOH1* gene into the pJG4-5 plasmid, which contains the Gal4p activation domain under the *GAL1* promoter control. If two fusion proteins interact with each other, and bring the LexA DNA binding domain and the Gal4p activation domain together, this protein complex is able to activate the reporter genes, *LEU2* and *LacZ*.

As shown in Table 7, the Rad5p protein interacts with the Soh1p protein. In addition, the first two-thirds of the Rad5p protein (residues 1-801) is sufficient for this interaction. This result correlates with the genetic interaction described above, and suggests that the Soh1p protein may have a role in DNA repair.

DISCUSSION

Soh1p and DNA repair: We have described the further characterization of the *SOH1* gene. In the process of cloning *SOH1*, we found a genetic interaction between *soh1-1* and *rad5-535*. The Rad5-535 mutant protein contains a mutation at the consensus sequence of the putative helicase domain I, which is responsible for ATP binding. This mutant is slightly MMS sensitive. *soh1* mutants do not have an obvious defect in DNA repair, but *soh1* mutations enhance the DNA repair defect of a *rad5-535* mutant significantly. The Soh1p protein was shown to interact with the Rad5p protein by the two-hybrid system. The first two-thirds of the Rad5p protein, which includes the putative helicase domains IIII, is sufficient for this interaction.

The observation that *soh1* mutants enhance the *rad5-535* defect in DNA repair indicates that the Soh1p protein has a role in DNA repair. Because a *soh1* null mutant does not demonstrate an obvious defect in DNA

TABLE 6

Recombiantion rates of a *hpr1* mutant in various *rad5* strain backgrounds

Genotype	Recombination Rate ($\times 10^{-6}$)[a]	Growth at 37°
RAD5 HPR1	5.4 ± 1.6	+
rad5-535 HPR1	3.8 ± 1.8	+
rad5Δ HPR1	28 ± 8.4	+
RAD5 hpr1Δ	2400 ± 980	−
rad5-535 hpr1Δ	2700 ± 1400	−
rad5Δ hpr1Δ	3000 ± 730	−

[a] Rates were calculated as described in MATERIALS AND METHODS from 5-FOA resistance frequencies of strains carrying the duplication *leu2-k::ADE2-URA3::leu2-k*. Each rate was calculated from three independent fluctuation tests on three strains of the same genotype and is expressed as the mean rate ± the standard deviation of the three determinations.

TABLE 7

Quantitative assay of the interaction between Soh1p and Rad5p

Fusion proteins[a]	β-galactosidase activity[b], fold over control
Gal4-Soh1p + LexA-control insert[c]	1.1 ± 0.9, 1×
Gal4-Soh1p + LexA-Rad5p (1-1169)[d]	27.9 ± 1.6, 25×
Gal4-Soh1p + LexA-Rad5p (1-801)[d]	30.8 ± 4.1, 28×

[a] The fusion plasmids plus the reporter plasmid, pSH18-34, were transformed into the yeast strain EGY48 for a β-galactosidase activity assay.

[b] The activity is expressed as nanomoles/minute/milligram protein. The number is expressed as the average of the activities of three independent transformants ± standard deviation from three determinations.

[c] Plasmid pL111-3 contains the LexA-control insert fusion. The *Nru*I-*Hpa*I fragment of *RAD5* (400 bp) was cloned in the pEG202 plasmid in wrong orientation with respect to the *RAD5* transcript to form pL111-3. The control insert is ~46 amino acids and was used as a negative control in this assay.

[d] LexA-Rad5p (1-1169) is a fusion containing the full-length Rad5p. LexA-Rad5p (1-801) contains the first two-thirds of the Rad5p protein.

repair, the Soh1p protein is an accessory protein, but not an essential component of DNA repair. Two possibilities are suggested for the Soh1p protein. First, the role of Soh1p may be structure oriented. The Rad5-535 mutant protein may bind ATP less efficiently, and this defect may be exacerbated by the absence of the Soh1p protein. In this situation, the conformation of a Rad5p repair complex would be altered and the Rad5-535 protein would bind ATP even less efficiently, resulting in a more severe defect in DNA repair. A second hypothesis is that the Soh1p protein is involved in processing DNA damage to a recombination substrate. In the absence of Soh1p, the recombination machinery is partially suppressed and more DNA lesions are channelled to the Rad5p-mediated repair pathway. The Rad5-535p repair complex may not function as efficiently as the wild-type Rad5p repair complex and thus a *rad5-535* mutant would show a slight sensitivity to DNA damaging agents. In the absence of the Soh1p protein, more DNA lesions are channelled to the Rad5p-mediated repair pathway, rendering a *rad5-535 soh1* double mutant more sensitive to DNA damaging agents.

Soh1p and transcription: The fact that the *soh1* and *soh2-1* (*rpb2*) mutants are viable at 30°, but that the combination of a *soh1 soh2-1* double mutant gives extremely poor growth suggests an interaction between *SOH1* and *RPB2*. A similar interaction exists between *soh1* and *soh4* (*sua7*). A further indication of an involvement of *SOH1* in transcription comes from the interaction between a *soh1* mutation and a mutation in the RNA polymerase II largest subunit gene *RPB1*. A *rpb1Δ104* mutant is viable at 30°, but not in the *soh1Δ* background. In addition, the LexA DNA binding domain-Soh1p fusion protein is able to bind to a *LexA* operator and to activate a downstream reporter gene. Based on these data, it is likely that the Soh1p protein has a role in transcription. Unlike the basal transcription factor genes (like *RPB1*, *RPB2* and *SUA7*), *SOH1* is not an essential gene for growth. Thus, Soh1p may be an accessory protein for transcription.

Precedent for linking transcription and DNA repair comes from the finding that the Rad3p and Rad25p DNA helicases, which are involved in the nucleotide excision DNA repair pathway, are essential for RNA polymerase II transcription in *S. cerevisiae* (Feaver *et al.* 1993; Guzder *et al.* 1994a; Svejstrup *et al.* 1994). *RAD3* is an essential gene for growth and has an indispensable role in transcription. Viable *rad3* point mutants have been identified that are defective in DNA repair, but are proficient in the Rad3p transcription function (Sung *et al.* 1988). Some of these mutants have been shown to be defective in the DNA helicase activity of the Rad3p protein. Therefore, the helicase activity of the Rad3p protein is essential for DNA repair but not for transcription. In contrast, the DNA helicase activity encoded by *RAD25* (*SSL2*) is essential for both transcription and DNA repair (Guzder *et al.* 1994b). On the basis of the phenomenon that the nucleotide excision repair and transcription are coupled and the results we present here, it is reasonable to hypothesize that the Rad5p-mediated DNA repair pathway may be associated with the RNA polymerase II complex and that Soh1p is one of the coupling factors.

Recombination and transcription: We have shown that *SOH2* and *SOH4* are known genes, *RPB2* and *SUA7*, respectively, and they are essential for growth. The recovered *soh2* and *soh4* alleles do not appear to have many of the defects described for the known *rpb2* and *sua7* mutants. *soh2-1* does not have a Spt$^-$ or Srb$^-$ phenotype. Some of the *soh4* alleles are unlikely to be involved in transcription start site selection, and we refer to them as Sua$^+$. Mutations in *RPB2* have been recovered that have a Sit$^-$ phenotype (Arndt *et al.* 1989), which permits transcription of *HIS4* in the absence of three normally required activators Gcn4p, Bas1p and Bas2p. We have not determined whether *soh2-1* has a Sit$^-$ phenotype. However, it is unlikely that *soh2-1* has a Sit$^-$ phenotype, because *sit2* (*rpb2*) mutants grow very slowly and are inositol auxotrophs whereas the *soh2-1* mutant exhibits normal growth and is an inositol prototroph. Therefore, *soh2-1* and *soh4* mutants may represent new classes of *RPB2* and *SUA7* mutants.

The identification of mutations in *RPB2* (the *soh2-1* mutant) and *SUA7* (*soh4-15* mutants) that lead to a suppression of the temperature sensitive growth of a hyperrecombination mutant, *hpr1*, suggests first, that this phenotype is a result of a transcription-related lethality and second, that there is a link between recombination and transcription in *hpr1* mutants. We have found that the *soh2* and *soh4* alleles do not impair transcription start site selection, as they have no Spt$^-$ phenotype or Sua$^-$ phenotype. However, these mutants show a reduced growth rate compared with an isogenic wild-type strain. We believe that the mutations act to slow down RNA polymerase II transcription process, possibly affecting the transition from the initiation phase to the elongation phase. This would suggest that the temperature lethality of *hpr1* mutants results from inefficient transcription. Simply slowing the metabolism rate of cells by using a poor carbon source such as galactose or potassium acetate does not compensate for the absence of the Hpr1 protein at 37° (H. Fan and H. L. Klein, unpublished result).

The *hpr1Δ* mutation is lethal in combination with a mutation in any of the three *TOP* genes, including a *top1* mutation, and also with deletion of copy I of the histone H3-H4 genes. The synthetic lethality can be rescued by mutations in *RPB2* (*soh2-1*) and *SUA7* (*soh4-15*), suggesting that the basis behind these synthetic lethal phenotypes is transcription related and that the synthetic lethality and the temperature-sensitive growth of *hpr1* have a common origin.

Mutations in the components of the Pol II transcription complex, *rpb2* and *sua7*, suppress ~10% of the

elevated recombination rate that results from the *hpr1Δ* mutation (FAN and KLEIN 1994), suggesting that the elevated recombination rate observed in *hpr1Δ* mutants is related to Pol II transcription. Hyperrecombination may not be completely suppressed because only a partial suppression of the *hpr1*-induced lethal event is needed to restore viability (the phenotype used to isolate the *soh* mutants) and hence the *soh* mutants may be leaky alleles for the recombination-suppression phenotype or because a fully suppressing *rpb2* or *sua7* mutant is itself lethal.

To further elucidate the relationship between *hpr1*-induced recombination and recombination, the *gal10* direct repeat recombination system was assayed in *hpr1Δ* mutants. A 10-fold increase in recombination rate was observed in the absence of the Hpr1p protein in the *gal4* strain background, when the recombination assay system is in a repressed transcription state (Table 4). A 100-fold increase in recombination rate was observed when the *hpr1Δ* mutation was introduced into the *gal80* mutant background, which is in a constitutively expressed transcription state. These two observations further indicate that DNA lesions accumulate in the absence of the Hpr1 protein, and these DNA lesions are resolved by recombination events. Furthermore, the level of *hpr1*-induced recombination is higher when the recombination assay system is in the transcriptionally active state (*gal80*) than that in the transcriptionally repressed state (*gal4*), suggesting that either more DNA lesions are produced when DNA is highly expressed in the absence of the Hpr1 protein or more likely transcription activation opens chromatin structure and thereby makes DNA more accessible to recombination machinery.

We propose that in the absence of the Hpr1p protein, DNA lesions accumulate that are recombinogenic. Because no double-strand breaks and no sensitivity to UV radiation, X-ray or MMS have been observed in *hpr1* mutants, the lesions accumulating in *hpr1* mutants must be of a different type. Moreover, *hpr1* mutants do not show increased spontaneous mutation rates (AGUILERA and KLEIN 1990). The lesions may be related to some secondary structure that forms in transcription and is normally removed by the combined action of the Hpr1p protein and the topoisomerases. The secondary structure may impede the transcription complex but can be removed or bypassed by a pathway that involves the Soh1p protein. Normally, this process is not recombinogenic, but when the lesion occurs in direct repeats, it may be removed by an intrachromatid deletion event. Soh1p may not be directly involved in the recombination reaction. Instead, it may function to channel the lesions into the appropriate recombination repair pathway. In the absence of Soh1p, a repair complex associated with the Pol II transcription machinery will take over and repair the lesions. This complex may involve the Rad5p protein. We believe that the same type of lesions that causes the hyperrecombination phenotype also results in the temperature lethality. At 37° the repair process may be less efficient and block transcription. We believe that it is the reduction in transcription of essential genes that results in the *hpr1* lethality at 37°. The *soh2* and *soh4* mutants may act to slow down transcription, possibly the transition from initiation to elongation, and thereby may allow a repair complex to be recruited to the lesion before the transcription event is aborted.

We thank MICHAEL GARABEDIAN for discussion and critical reading of this manuscript. We thank RODNEY ROTHSTEIN, F. WINSTON, RICK YOUNG, MICHAEL HAMPSEY and ROGER BRENT for strains, plasmids and discussion of unpublished results. We also thank MARIAN CARLSON for the pLexA-Snf2 plasmid. This work was supported by National Institutes of Health grant GM-30439. Computing was supported by National Science Foundation grant DIR-8908095.

LITERATURE CITED

AGUILERA, A., and H. L. KLEIN, 1988 Genetic control of intrachromosomal recombination in *Saccharomyces cerevisiae*. I. Isolation and genetic characterization of hyper-recombination mutations. Genetics **119:** 779–790.

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

ARNDT, K. T., C. A. STYLES and G. R. FINK, 1989 A suppressor of a *HIS4* transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell **56:** 527–537.

BIGGIN, M. D., T. J. GIBSON and G. F. HONG, 1983 Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80:** 3963–3965.

FAN, H.-Y., and H. L. KLEIN, 1994 Characterization of mutations that suppress the temperature-sensitive growth of the *hpr1Δ* mutant of *Saccharomyces cerevisiae*. Genetics **137:** 945–956.

FEAVER, W. J., J. Q. SVEJSTRUP, L. BARDWELL, A. J. BARDWELL, S. BURATOWSKI *et al.*, 1993 Dual roles of a multiprotein complex from S. cerevisiae in transcription and DNA repair. Cell **75:** 1379–1387.

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

GUZDER, S. N., H. QIU, C. H. SOMMERS, P. SUNG, L. PRAKASH *et al.*, 1994a DNA repair gene *RAD3* of *S. cerevisiae* is essential for transcription by RNA polymerase II. Nature **367:** 91–94.

GUZDER, S. N., P. SUNG, V. BAILLY, L. PRAKASH and S. PRAKASH, 1994b Rad25 is a DNA helicase required for DNA repair and RNA polymerase II transcription [see comments]. Nature **369:** 578–581.

GYURIS, J., E. GOLEMIS, H. CHERTKOV and R. BRENT, 1993 Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell **75:** 791–803.

HAMPSEY, M., 1991 A tester system for detecting each of the six base-pair substitutions in *Saccharomyces cerevisiae* by selecting for an essential cysteine in iso-1-cytochrome c. Genetics **128:** 59–67.

HEKMATPANAH, D. S., and R. A. YOUNG, 1991 Mutations in a conserved region of RNA polymerase II influence the accuracy of mRNA start site selection. Mol. Cell. Biol. **11:** 5781–5791.

HILL, J., K. A. DONALD, D. E. GRIFFITHS and K. A. DONALD, 1991 DMSO-enhanced whole cell yeast transformation [published erratum appears in Nucleic Acids Res. 1991 Dec 11;19(23):6688]. Nucleic Acids Res. **19:** 5791.

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 yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JOHNSON, R. E., S. T. HENDERSON, T. D. PETES, S. PRAKASH, M. BANKMANN *et al.*, 1992 *Saccharomyces cerevisiae RAD5*-encoded DNA repair protein contains DNA helicase and zinc-binding sequence motifs and affects the stability of simple repetitive sequences in the genome. Mol. Cell. Biol. **12:** 3807–3818.

JOHNSON, R. E., S. PRAKASH and L. PRAKASH, 1994 Yeast DNA repair protein Rad5 that promotes instability of simple repetitive sequences is a DNA-dependent ATPase. J. Biol. Chem. **269:** 28259–28262.

KLEIN, H. L., 1995 Genetic control of intrachromosomal recombination. Bioassays **17:** 147–159.

LAURENT, B. C., M. A. TREITEL and M. CARLSON, 1991 Functional interdependence of the yeast Snf2, Snf5, and Snf6 proteins in transcriptional activation. Proc. Natl. Acad. Sci. USA **88:** 2687–91.

LEA, D. E., and C. A. COULSON, 1948 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–284.

MILLER, J. L., 1972 *Experiments in Molecular Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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.

NONET, M., D. SWEETSER and R. A. YOUNG, 1987 Functional redundancy and structural polymorphism in the large subunit of RNA polymerase II. Cell **50:** 909–915.

ORR-WEAVER, T. L., and J. W. SZOSTAK, 1983 Yeast recombination: the association between double-strand gap repair and crossing-over. Proc. Natl. Acad. Sci. USA **80:** 4417–4421.

PINTO, I., D. E. WARE and M. HAMPSEY, 1992 The yeast *SUA7* gene encodes a homolog of human transcription factor TFIIB and is required for normal start site selection *in vivo.* Cell **68:** 977–988.

ROSE, M. D., F. WINSTON and P. HIETER, 1990 In *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 In *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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

SCAFE, C., D. CHAO, J. LOPES, J. P. HIRSCH, S. HENRY *et al.*, 1990 RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. Nature **347:** 491–494.

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

SUNG, P., D. HIGGINS, L. PRAKASH and S. PRAKASH, 1988 Mutation of lysine-48 to arginine in the yeast Rad3 protein abolishes its ATPase and DNA helicase activities but not the ability to bind ATP. EMBO J. **7:** 3263–3269.

SVEJSTRUP, J. Q., W. J. FEAVER, J. LAPOINT and R. D. KORNBERG, 1994 RNA polymerase transcription factor IIH from yeast. J. Biol. Chem. **269:** 28044–28048.

TABOR, S., and C. C. RICHARDSON, 1987 DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **84:** 4767–4771.

THOMAS, B. J., and R. ROTHSTEIN, 1989 The genetic control of direct-repeat recombination in *Saccharomyces*: the effect of *rad52* and *rad1* on mitotic recombination at *GAL10*, a transcriptionally regulated gene. Genetics **123:** 725–738.

THOMPSON, C. M., A. J. 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.

VOELKEL-MEIMAN, K., and G. S. ROEDER, 1990 A chromosome containing *HOT1* preferentially receives information during mitotic interchromosomal gene conversion. Genetics **124:** 561–572.

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: S. JINKS-ROBERTSON