The Yeast *HRS1* Gene Encodes a Polyglutamine-Rich Nuclear Protein Required for Spontaneous and *hpr1*-Induced Deletions Between Direct Repeats

Helena Santos-Rosa*, Beate Clever,† Wolf-Dietrich Heyer† and Andrés Aguilera*

**Departamento de Genetica, Facultad de Biología, Universidad de Sevilla, E-41012 Sevilla, Spain and †Institute of General Microbiology, University of Bern, CH-3012 Bern, Switzerland*

Manuscript received September 11, 1995
Accepted for publication November 15, 1995

ABSTRACT

The *hrs1-1* mutation was isolated as an extragenic suppressor of the hyperrecombination phenotype of *hpr1*Δ cells. We have cloned, sequenced and deleted from the genome the *HRS1* gene. The DNA sequence of the *HRS1* gene reveals that it is identical to *PGD1*, a gene with no reported function, and that the Hrs1p protein contains polyglutamine stretches typically found in transcription factors. We have purified a His(6) tagged version of Hrs1p protein from *E. coli* and have obtained specific anti-Hrs1p polyclonal antibodies. We show that Hrs1p is a 49-kD nuclear protein, as determined by indirect immunofluorescence microscopy and Western blot analysis. The *hrs1*Δ null mutation reduces the frequency of deletions in wild-type and *hpr1*Δ backgrounds sevenfold below wild-type and *rad52* levels. Furthermore, *hrs1*Δ cells show reduced induction of the *GAL1,10* promoter relative to wild-type cells. Our results suggest that Hrs1p is required for the formation of deletions between direct repeats and that it may function in gene expression. This suggests a connection between gene expression and direct repeat recombination. In this context, we discuss the possible roles of Hrs1p and Hpr1p in initiation of direct-repeat recombination.

GENETIC analysis has proven essential for elucidating the mechanisms of homologous recombination in bacteria (see SMITH 1988; LLOYD and SHARPLES 1992; KOWALCZYKOWSKI *et al.* 1994) and in yeast (see PETES *et al.* 1991). Recently, the study of recombination between DNA repeats has contributed significantly to our understanding of the mechanisms of homologous recombination in eukaryotes. Recombination between DNA repeats, in particular direct repeats, is a source for deletion of genetic information, which can have deleterious consequences for the cell. Consequently, it is important to know whether there are cell functions involved in preventing high levels of direct repeat recombination. In addition, the genetic analysis of repeat recombination allows studies in haploid cells where screens for recessive mutations are easily possible.

Different types of mechanisms have been proposed for direct repeat recombination in the yeast *Saccharomyces cerevisiae*. They include reciprocal exchange, single-strand annealing and one-ended invasion (OZENBERGER and ROEDER 1991; FISHMAN-LOBELL and HABER 1992; MEZARD and NICOLAS 1994; PRADO and AGUILERA 1995). The recombinational repair gene *RAD52* is required for almost all types of homologous recombination (see PETES *et al.* 1991). Recently, it has been shown that *RAD52* does not define a single recombination pathway. Instead, *RAD52* is required for multiple pathways, as deduced mainly from genetic studies on *rad51* and *rad57* mutants (AGUILERA 1995; RATTRAY and SYMINGTON 1995). In addition, *RAD52*-independent events have also been described between direct repeats (JACKSON and FINK 1981; OZENBERGER and ROEDER 1991; FISHMAN-LOBELL and HABER 1992). Among the genes known to play an important role in direct-repeat recombination is *RAD1*, which codes for an excision-repair endonuclease that is required to remove DNA heterologies from the recombination event between direct repeats (FISHMAN-LOBELL and HABER 1992).

Although mutations in several yeast genes have been shown to induce recombination between repeats (see PETES *et al.* 1991), only the *HPR1* gene seems to function specifically to prevent direct-repeat recombination. *HPR1* is required to maintain low levels of recombination between direct repeats (AGUILERA and KLEIN 1989a, 1990) but has no effect on other types of genetic recombination (SANTOS-ROSA and AGUILERA 1994). The Hpr1p protein does not seem to be involved in recombination itself (AGUILERA and KLEIN 1989a). We have postulated that in the absence of Hpr1p, yeast cells undergo a high incidence of DNA breaks that are repaired through a nonconservative mechanism of recombination responsible for the hyperdeletion phenotype (SANTOS-ROSA and AGUILERA 1994). Recently it has been shown that Hpr1p is a positive regulator of transcription (ZHU *et al.* 1995), suggesting a dual role for the Hpr1p protein in transcription and direct-repeat recombination.

To understand how deletions between repeats are

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

stimulated in *hpr1*Δ strains and to identify genes involved in the mechanisms of deletion formation, we recently identified five *HRS* genes, mutations in which suppress the hyperdeletion phenotype of *hpr1*Δ strains (SANTOS-ROSA and AGUILERA 1995). These suppressor mutations should identify new functions that either interact physically or functionally with the Hpr1p protein or are directly involved in the formation of deletions between DNA repeats. The *hrs1-1* mutation is of particular interest because it completely suppresses the hyperdeletion phenotype but not the lack of activation of the *GAL1,10* promoter activation observed in *hpr1*Δ strains (SANTOS-ROSA and AGUILERA 1995). In this study, we report the molecular analysis of the *HRS1* gene and we present evidence that this gene is required for spontaneous as well as *hpr1*Δ-induced deletions. Strains carrying a deletion of *HRS1* have levels of direct repeat recombination sevenfold lower than those of wild-type and *rad52* strains. However, *hrs1*Δ cells are not affected in DNA repair. The Hrs1p protein contains polyglutamine and polyglutamine-alanine tracts observed in many transcription factors (GERBER *et al.* 1994). The sequence of *HRS1* reveals that is identical to *PGD1*, named for its Polyglutamine Domain (BRÖHL *et al.* 1994) and for which no function or phenotype had been assigned. We show that Hrs1p is a nuclear protein that may act as a positive regulator of gene expression. Our results suggest a possible connection between gene expression and direct repeat recombination.

MATERIALS AND METHODS

Strains: The yeast strains used in this study are listed in Table 1. All strains constructed for this study are congenic to AYW3-3D, with the exception of AF515-2B. BJ and CSH strains are not genetically related to the strains constructed for this study.

Plasmids: Plasmids used in this study are described 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/l and 500 mg/l, respectively. Plates of SC-FOA medium were prepared by using 1 g/l proline as the nitrogen source. All yeast strains were grown at 30° with horizontal shaking for liquid cultures. Yeast strains were transformed using the lithium acetate method (ITO *et al.* 1983) modified according to SCHIESTL and GIETZ (1989).

Cloning of the *HRS1* gene: Strain SS58-2A carrying the duplication system *leu2-k::URA3-ADE2::leu2-k* was used to screen for plasmids carrying inserts able to complement the *hrs1-1* mutation. Leu^+ transformants were selected on SC-leu supplemented with 75 μg FOA/ml and 16 mg adenine/l. The small amounts of FOA were used to favor the growth of the Ura^- Ade^- cells. On this medium, the *hpr1*Δ *hrs1-1* SS58-2A strain forms white colonies as a consequence of the low frequency of excision (5×10^{-6}) of the *URA3-ADE2* sequences from the duplication system. However, *hpr1*Δ strains form red-sectored colonies as a consequence of the high frequency of excision (4–8%) of the *URA3-ADE2* sequences.

Approximately 44,000 colonies were screened after transformation with the pBS32 library. From these, 12 red-sectoring colonies were selected and tested for the frequency of papillation on SC-FOA. Four different transformants were finally selected that consistently gave *hpr1*Δ levels of papillation on SC-FOA for all Leu^+ segregants tested and for which the *hpr1*Δ hyperrecombination phenotype cosegregated with the Leu^+ phenotype. Plasmid DNA was isolated from the four transformants and propagated through *E. coli*. A large-scale plasmid DNA preparation was made, and the restriction map of the inserts was determined by restriction analysis.

Genetic and biochemical analysis: Genetic analysis was performed by published procedures (SHERMAN *et al.* 1986). UV viability experiments were performed as described previously (AGUILERA and KLEIN 1988). The UV exposure used was 0, 30, 50, 70 and 90 J/m^2. Methyl methanesulfonate (MMS) sensitivity experiments were performed as described previously (PRAKASH and PRAKASH 1977). Samples were taken after incubation in liquid 0.5% MMS for 0, 5, 10, 15, 20, 25 and 30 min.

β-galactosidase was assayed according to GUARENTE (1983) in cell extracts obtained from cultures grown overnight in SC-ura medium supplemented with either 2% glucose or 2% galactose.

Determination of recombination frequencies: Median recombination frequencies were determined using six independent colonies for each strain studied. Yeast strains were grown on YEPD or SC-ura, as appropriate. After three days independent colonies were plated on SC-FOA or SC-his to determine the median frequency of either Ura^- or His^+ recombinants. The viable cell number was determined on YEPD.

The frequency of His^+ Trp^+ gene conversion events and His^+ Trp^- deletion events in the *his3-513::TRP1::his3-537* system (AGUILERA and KLEIN 1988) was calculated by multiplying the median frequency of His^+ recombinants by the proportion of Trp^+ and Trp^- events obtained from independent His^+ recombinants. This proportion was obtained by determining the Trp phenotype of independent His^+ recombinants each of which was isolated from a different YEPD-grown colony.

The proportion of His^+ gene conversion events *vs.* His^+ crossover events in the *his3p::INV* system (*his3-k::LEU2-leu2-r::his3h-URA3*) (AGUILERA and KLEIN 1989b) was determined by isolating independent His^+ recombinants. DNAs from these strains were digested with *Sal*I and subjected to Southern analysis to determine the orientation of the sequence located between the inverted repeats. This orientation indicated whether the His^+ recombination events occurred by crossover or gene conversion (AGUILERA and KLEIN 1989b).

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 (BOLIVAR and BACKMAN 1979). 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 VOGELSTEIN 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°.

TABLE 1

Strains

Strain	Genotype	Source
A3Y3A	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3Δ200*	AGUILERA and KLEIN (1990)
A3Y3T3	*Matα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3Δ200 hpr1Δ3::HIS3*	AGUILERA and KLEIN (1990)
AYW3-3D	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3*	SANTOS-ROSA and AGUILERA (1995)
AYW3-3C	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3 can1-100*	SANTOS-ROSA and AGUILERA (1995)
SS58-2A	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3 hrs1-1*	SANTOS-ROSA and AGUILERA (1995)
X260-3A	*MATa ura3-52 rad52-1*	G. FINK
BJ5464	*MATα leu2Δ1 ura3-52 his3Δ200 trp1 pep4:HIS3 prb1Δ1.6R can1-100*	JONES (1991)
BJ5465	*MATa leu2Δ1 ura3-52 his3Δ200 trp1 pep4:HIS3 prb1Δ1.6R can1-100*	JONES (1991)
CSH89L	*MATa leu2 ade1 ura3 his1 trp5 lys7 met3 spo11*	CSHL[a]
CSH90L	*MATα leu2 ade1 ura3 his1 trp5 lys7 met3 spo11*	CSHL[a]
AF515-2B	*MATα leu2 ura3 his3 trp1 rad1-1*	This study
SSYY1-4B	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3*	This study
SSYY4-6D	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 hpr1Δ3::HIS3 hrs1Δ::LEU2*	This study
SSYY4-6C	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3 hrs1Δ::LEU2*	This study
SSYY4-6A	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3*	This study
SSYY4-6B	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hpr1Δ3::HIS3*	This study
SSAA-8B	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 can1-100*	This study
SSAA-12D	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hrs1Δ::LEU2 can1-100*	This study
SSAA-17B	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hrs1Δ::LEU2 can1-100*	This study
SSAA-17C	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hrs1Δ::LEU2 hpr1Δ3::HIS3*	This study
SSAB-2C	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hrs1Δ::LEU2*	This study
SSAB-4B	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 hrs1Δ::LEU2*	This study
SSIN-14A	*MATα leu2 ura3 his3p::INV*	This study
SSIN-18A	*MATα leu2 ura3 his3-k::LEU2-leu2-r::his3-URA3 can1-100 trp1*	This study
SSIN-11B	*MATα leu2 ade2 ura3 his3p::INV trp1 hrs1Δ::LEU2*	This study
SSIN-17B	*MATα leu2 ade2 ura3 his3p::INV hrs1Δ::LEU2*	This study
SSIN-25B	*MATα leu2 ade2 ura3 his3p::INV hrs1Δ::LEU2 can1-100*	This study
SSXY-9A	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 rad52-1*	This study
SSXY-6A	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 rad52-1 hrs1Δ::LEU2*	This study
SSXY-29A	*MATa leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 rad52-1 hrs1Δ::LEU2*	This study
SSXY-25C	*MATα leu2-k::ADE2-URA3::leu2-k ade2 ura3 his3 rad52-1 hpr1Δ3::HIS3 hrs1Δ::LEU2*	This study
SSGW-4C	*MATα leu2 ade2 ura3 his3 -513::TRP1::his3-537 trp1*	This study
SSGW-3D	*MATa leu2 ura3 his3-513::TRP1::his3-537 hrs1Δ::LEU2 trp1*	This study
SSGW-5A	*MATα leu2 ura3 his3-513::TRP1::his3-537 hrs1Δ::LEU2 trp1*	This study
WDS3C-A	*MATα leu2Δ1 ura3 his3 trp1*	This study
WDS3C-B	*MATα leu2Δ1 ade2 ura3 his3 trp1 pep4::HIS3*	This study
WDS-4B	*MATa leu2-k ura3 his3 trp1 hrs1Δ::LEU2 pep4::HIS3*	This study
WDS-5B	*MATα leu2-Δ1 ura3 his3 trp1 hrs1Δ::LEU2 pep4::HIS3*	This study
WDS-6B	*MATa leu2-k ura3 his3 trp1 hrs1Δ::LEU2 pep4::HIS3*	This study

[a] CSHL: Cold Spring Harbor Laboratory.

DNA sequencing: Plasmids p315S and p315SH were used to construct nested deletions with *E. coli* ExoIII (Pharmacia) according to published procedures (HENIKOFF 1984). Plasmid p315SH was digested either with *Apa*I and *Sal*I or *Pst*I and *Hin*dIII before ExoIII treatment, whereas plasmid p315S was digested with either *Apa*I and *Xho*I or *Sac*I and *Xba*I (these restriction endonucleases cut in the KS polylinker of pRS315). Appropriate clones carrying inserts differing in length by 80–280 bp were isolated from *E. coli* and passed through a Sephadex G-50 column before sequencing. Both DNA strands of a 2138-bp piece of DNA containing the entire *HRS1* gene were sequenced by the dideoxy-chain termination method (SANGER *et al.* 1977) with T7 DNA polymerase (Sequenase) (TABOR and RICHARDSON 1987) and 5′-([α-^{35}S]thio)triphosphate (BIGGIN *et al.* 1980).

Preparation of crude extracts: *E. coli* strain BL21(DE3) transformed with plasmid pT7-7-His(6)HRS1 was grown in LB supplemented with 100 μg/ml ampicillin up to an OD_{600} of 0.8. Expression was induced by adding IPTG to a final concentration of 1 mM. Total protein extracts were prepared after 5 hr of induction according to LAEMMLI (1970).

Yeast cells transformed with plasmid pGAL-HRS1 were grown in basic medium (0.17% YNB, 0.5% ammonium sulphate, 2% sodium lactate, 3% glycerol) to a OD_{600} of 1.0. The culture was then split; one half was supplemented with 2% glucose (repressing conditions) and the other half with 2% galactose (inducing conditions). Protein extracts were prepared after 5 hr using glass beads as described (JOHNSON and KOLODNER 1991).

Purification of His(6)Hrs1p from *E. coli*: The His(6)Hrs1p fusion protein was purified from a 1-liter culture of BL21(DE3) cells transformed with plasmid pT7-7-His(6)-

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)
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)
pRS316	YCp vector based on the *URA3* gene	SIKORSKI and HIETER (1989)
pFUS-A1	YEp expression vector carrying the *GAL*1,10 promoter and the *lacZ* gene	S. JOHNSON
pT7-7	*E. coli* expression vector carrying the T7 promoter	TABOR and RICHARDSON (1985)
pT7-7-His(6)	pT7-7 with a His(6) coding sequence downstream of the T7 promoter	B. CLEVER and W.-D. HEYER
YCpH3	A 10.1-kb *HRS1* genomic insert subcloned in pBS32	This study
YCpH5	A 17-kb *HRS1* genomic insert subcloned in pBS32	This study
YCpH33	A 8.5-kb *HRS1* genomic insert subcloned in pBS32	This study
p315HN2	The 4.8-kb *Hin*dIII fragment of YCpH33, which contains the complete *HRS1* gene, subcloned in pRS315	This study
p315HN3	The 4.2-kb *Hin*dIII fragment from the insert of YCpH33 subcloned in pRS315	This study
p315B2	The 3.3-kb *Bam*HI fragment from the insert of YCpH33 subcloned in pRS315	This study
YCpDB1	YCpH33 in which the 3.3 kb *Bam*HI fragment was removed	This study
p315SH	The 2.0-kb *Sal*I-*Hin*dIII fragment from the insert of YCpH33 subcloned in pRS315	This study
p315S	The 2.2-kb *Sal*I fragment from the insert of p315HN2 subcloned in pRS315	This study
YCpD5H3	The 11.7-kb *Hin*dIII fragment from YCpH5	This study
p316-HRS1	The 4.8-kb *Hin*dIII fragment of p315-HN2, which contains the complete *HRS1* gene, subcloned into pRS316	This study
pDIS1	YCpD5H3 in which the 0.75 kb *Bam*HI internal *HRS1* fragment was replaced with the 2.8-kb *Bgl*II yeast *LEU2* fragment	This study
pT7-7-His(6)HRS1	The 2.84-kb *Dra*I-*Hin*dIII *HRS1* coding region inserted into the *Nhe*I-*Hin*dIII site of pT7-7-His(6)[a]	This study
pGAL-HRS1	The 2.8-kb *Dra*I-*Hin*dIII *HRS1* coding region inserted into the *Xho*I-*HindIII* site of pFUS-A1[b]	This study

[a] The *Nhe*I site was previously blunt-ended with Klenow.
[b] The *Xho*I site was previously blunt-ended with Klenow.

HRS1. Cells were lysed in 6 M guanidine-hydrochloride, 0.1 M NaH_2PO_4, 0.01 M Tris pH 8.0. The His(6)Hrs1p fusion protein was partially purified by affinity chromatography on a Ni-NTA column (QIAGEN). Step elution of His(6)Hrs1p was achieved with 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris pH 7.5 containing increasing concentrations of imidazol (1, 10 and 300 mM). These samples were then separated on SDS-polyacrylamide gels and stained with Coomassie blue. His(6)Hrs1p was further purified by gel elution using the BIOTRAP-BT1000 system (HUNKAPILLER and LUJAN 1986).

Gel electrophoresis and Western blotting: Sodium dodecyl sulfate-polyacrylamide gels (10%, 0.7 mm thick) (LAEMMLI 1970) were used for all protein electrophoresis. Proteins were blotted to nitrocellulose filters as described (TOWBIN *et al.* 1979). Nitrocellulose filters were blocked for 1 hr at room temperature either in TBST (20 mM Tris pH 7.6, 137 mM NaCl, 0.1% Tween-20) containing 3% BSA when the alkaline phosphatase-conjugated secondary antibody (Promega) was used, or in TBST containing 5% nonfat dry milk when the HRP-conjugated secondary antibody (BioRad) was used. Filters were incubated for 1 hr with the primary antibody diluted 1:500 in blocking solution, washed three times for 5 min in the same buffers and incubated for 1 hr with the secondary antibody (Promega 1:7500; BioRad 1:3000). Alkaline phosphatase activity was detected with 0.1 M Tris, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$ containing both tetrazolium and 5-bromo-4-chloro-indolyl phosphate. Peroxidase (HRP) activity was detected by chemiluminescence (ECL, Amersham).

Immunization and affinity purification of antibodies: For rabbit immunization the following protocol was used: day 1, 400 μg; day 15, 200 μg; day 21, test bleed; day 29, 100 μg; day 35, test bleed; day 43, 50 μg; day 49, terminal bleed. For rat immunization the following protocol was used: day 1, 100 μg; day 15, 50 μg; day 21, test bleed; day 29, 30 μg; day 36, terminal bleed. All injections were intradermal using gel eluted His(6)Hrs1p mixed 1:1 with complete Freund's adjuvant for the first injection and with incomplete Freund's adjuvant for further injections. Blood from preimmune, test and terminal bleeds was processed as described (HARLOW and LANE 1988). Anti-Hrs1p specific antibodies were affinity purified on nitrocellulose strips containing His(6)Hrs1p (PRINGLE *et al.* 1991).

In situ immunolocalization: For indirect immunofluorescence log phase *S. cerevisiae* cultures were grown in YEPD and fixed for 90 min in 1% (w/v) formaldehyde pH 7.5–8.0, 1 M sorbitol at 30° with shaking, and processed essentially as described (PRINGLE *et al.* 1991). Affinity-purified rabbit anti-Hrs1p polyclonal anti-serum was incubated overnight with the

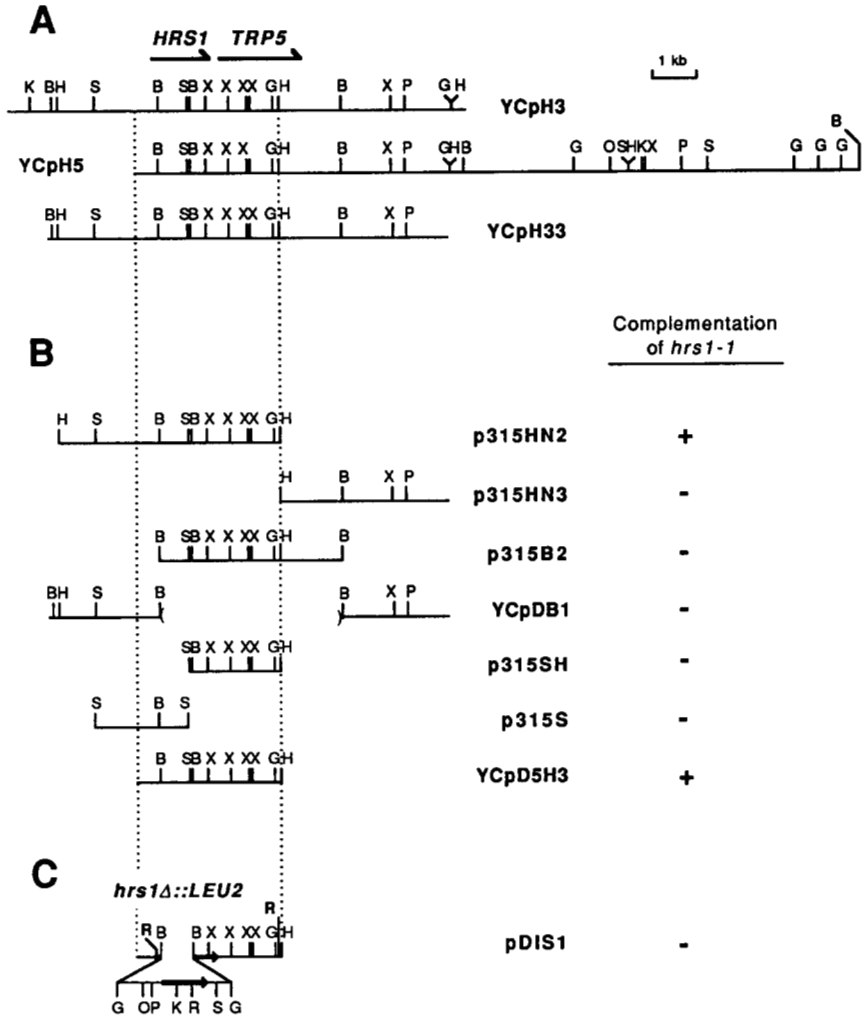

FIGURE 1.—Plasmids. (A) Restriction maps of the three different inserts isolated from plasmids YCpH3, YCpH5 and YCpH33, that complement the *hrs1-1* mutation. (B) Deletion analysis of the region containing the *HRS1* open reading frame showing the ability of each subclone to complement the *hrs1-1* mutation. (C) Plasmid used to delete the *HRS1* gene from the yeast genome. The 0.7-kb *Bam*HI internal fragment of *HRS1* was replaced by the 2.8-kb *Bgl*II fragment containing the entire *LEU2* gene. The *HRS1* and *LEU2* coding sequences are shown as thick arrows. The 4.6-kb *Eco*RI fragment contained between the two *Eco*RI sites marked in bold, **R,** was used to replace the chromosomal *HRS1* gene. Abbreviations of restriction sites are: B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; O, *Xho*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I; and X, *Xba*I. Ony the *Eco*RI sites of the pDIS1 insert are shown.

cells at a 1:10 dilution. As secondary antibody a goat anti-rabbit IgG FITC conjugate (Sigma F-0382) was used in a 1:100 dilution and incubated overnight. Cells were prepared for microscopy in mounting solution [1 mg/ml DABCO (Sigma D-2522) in PBS (16 mM $NaPO_4$, 138 mM NaCl pH 9) containing 90% glycerol and 20 ng/ml DAPI]. Pictures were taken with Kodak TMX-400 film with identical exposure and processing times for wild-type and *hrs1*Δ cells.

RESULTS

Isolation of the *HRS1* gene: The *hpr1*Δ *hrs1-1* haploid strain SS58–2A carrying the duplication system *leu2-k::URA3-ADE2::leu2-k* was transformed with the pBS32 library (F. SPENCER and P. HIETER, unpublished results). More than 40,000 Leu^+ transformants were screened for the red-sectoring phenotype, indicating loss of the *ADE2* marker in the duplication system (see MATERIALS AND METHODS). From these transformants, four clones were identified that restored the high red-sectoring phenotype of *hpr1*Δ cells. Two of them contained the same insert. The maps of the three different DNA inserts able to complement the *hrs1-1* phenotype overlap by 6.8 kb (Figure 1A). The three plasmids were used to retransform to Leu^+ the original *hpr1*Δ *hrs1-1* strain SS58-2A. In all cases the Leu^+ transformants showed *hpr1*Δ levels of Ura^- recombinants, a characteristic that was linked to the Leu^+ phenotype and that was lost when the cells were cured of the plasmid. This result indicates that the 6.8 kb overlapping DNA region complements the *hrs1-1* suppressor phenotype of the *hpr1*Δ hyper-recombination phenotype.

Deletion analysis of this region was performed to define the shortest DNA fragment able to complement the *hrs1-1* mutation. Subcloning experiments were made in centromeric *LEU2*-based plasmids pBS32 or pRS315. The new resulting plasmids (see MATERIALS AND METHODS) were used to transform the *hpr1*Δ *hrs1-1* haploid strain SS58-2A. The results shown in Figure 1B indicated that the *hrs1-1* complementing activity was contained in the 3.1-kb fragment located to the left of the central *Hin*dIII site.

***HRS1* codes for a 431-amino-acid protein with a polyglutamine-rich carboxyterminal region:** The nucleotide sequence of 2138 bp of DNA (EMBL accession number: X81457) was determined (data not shown). The open reading frame predicts a 431 amino acid (46.8 kD) protein. There are several features in the carboxy-terminal half of the putative Hrs1p protein: it contains

```
Hrs1p   256 AKAQAQAQAQAQAQVYAQQSTVQTPITAS-MAAALPNPTPS---------MINSVSPTNVMGTPLTNNMS 315
            A AQAQAQAQAQAQ  AQ    Q P  A      L   TP               S TN   T  TN  S
Gal11p  467 AQAQAQAQAQAQAQAHAQHQPSQQPQQAQQQPTPLHGLTPTAKDVEVIKQLSLDASKTNLRLTDVTNSLS 536

Hrs1p   342 NGSNPNTNTNSNNTPLQSQLN----LNNLTPANILNMSMNNDFQQQQQQQQQQQQPQPQ  396
            N  NP T    NN     Q N    L        L M      QQQQQQQQQQQQ Q Q
Gal11p  637 NNGNPGTTSTGNNNNIATQQNMQQSLQQMQHLQQLKMQQQQQQQQQQQQQQQQQQQQQQQ  695
```

FIGURE 2.—Amino acid homology between the two conserved regions of Hrs1p and Gal11p proteins from *S. cerevisiae*, as obtained with the FASTA algorithm.

almost no charged amino acids, it contains a stretch of alternating alanine and glutamine residues (amino-acid positions 258 to 269), and it contains a continuous stretch of 12 glutamine residues (amino-acid positions 381 to 392). The polyglutamine stretch is surrounded by asparagine rich regions (see Figure 2). The carboxy-terminal portion of the gene had been previously sequenced (ZALKIN and YANOFSKY 1982) as the region located upstream of the *TRP5* gene, which is compatible with our mapping data (see below). Subsequent comparison of *HRS1* with the GenBank release 88.0, EMBL release 42.0 and SWISS-PROT release 30.0 using the FASTA (PEARSON and LIPMAN 1988) and BLAST algorithms (ALTSCHUL *et al.* 1990) showed that the *HRS1* gene was identical to the recently sequenced *PGD1* gene. *PGD1* was identified as a high copy suppressor of the thermosensitive phenotype conferred by a suppressor of a mutation in the *RPO41* gene, which codes for mitochondrial RNA polymerase. No function or phenotype has been assigned to *PGD1* (BRÖHL *et al.* 1994).

Many proteins involved in transcription show short regions of homology with the carboxyterminal region of Hrs1p. These short regions of homologies correspond to the glutamine- or asparagine-rich regions. Among these proteins, the yeast transcriptional regulatory proteins Gal11p/Spt13p (SUZUKI *et al.* 1988) and Ssn6p/Cyc8p (SCHULTZ and CARLSON 1987) contain similarly arranged glutamine-alanine and glutamine stretches in their carboxyterminal regions. The strongest homology is found between Hrs1p and Gal11p (Figure 2).

Deletion of the *HRS1* gene completely suppresses direct-repeat recombination in *hpr1Δ* cells: The genomic *HRS1* gene was replaced by the *hrs1Δ::LEU2* deletion allele in which a 2.8-kb *Bgl*II fragment containing the entire *LEU2* gene was substituted by the 0.7-kb *Bam*HI internal fragment of *HRS1* (see MATERIALS AND METHODS). A red-sectoring *HRS1/hrs1-1 hpr1Δ/hpr1Δ* diploid strain (SS58-2A × AYW3-3C$^-$) containing the *leu2-k::URA3-ADE2::leu2-k* system was transformed (ROTHSTEIN 1983) with a 4.6-kb *Eco*RI fragment containing the *hrs1Δ::LEU2* allele (from plasmid pDIS1; see Figure 1C). Gene replacement of the *HRS1* wild-type copy by the *hrs1Δ::LEU2* allele led to Leu$^+$, white, nonsectoring transformants. Tetrad analysis of one such stable transformant showed that all four spores were viable and the Leu$^+$ phenotype segregated 2$^+$:2$^-$ in 46 tetrads analyzed. Therefore, *HRS1* is not an essential gene. In all cases the Leu$^+$ spores showed suppression of the hyperrecombination phenotype of the *hpr1Δ* mutation, indicating a complete linkage between the *hrs1-1* mutation and the deletion mutation. Southern analysis of two complete tetrads confirmed that two spores in each tetrad carried the expected pattern of bands of the *hrs1Δ::LEU2* allele and the other two carried the wild-type *HRS1* allele (data not shown). These results confirmed that we cloned the *HRS1* gene, and not a suppressor, and that the *HRS1* gene exists in single copy in the yeast haploid genome. Further genetic analysis of different crosses with strains CSH89L and CSH90L revealed that there was a complete cosegregation between the Leu$^+$ phenotype and the hyperrecombination suppressor phenotype of the *hpr1Δ* mutation (14 tetrads analyzed) and that the *hrs1Δ::LEU2* allele was tightly linked to *trp5* (13 PD: 0 NPD: 0 TT tetrads obtained from the *trp5* × *hrs1Δ::LEU2* crosses). This result is consistent with the sequence data indicating that the *HRS1* gene is immediately upstream of the *TRP5* gene on the left arm of chromosome VII.

To quantify the effect of the *hrs1Δ* mutation on *hpr1Δ*-induced recombination we determined the frequency of deletions of the *leu2-k::URA3-ADE2::leu2-k* system in *hpr1Δ hrs1Δ* double mutants by selecting for recombinants on SC-FOA media. Table 3 shows that

TABLE 3

Frequency of deletions (×10^6) in the *leu2-k::URA3-ADE2::leu2-k* direct repeat system in wild-type and mutant strains

Genotype	Strains	Ura$^-$ [a]
Wild type	SSAA-8B	30.0
hpr1Δ	SSYY4-6B	22000
	SSYY1-4B	16000
rad52-1	SSXY-9A	48.0 (0.6)
hrs1Δ	SSAB-2C	7.0 (4.3)
	SSAB-4B	2.6 (11.5)
	SSAA-12D	4.4 (7.0)
hrs1Δ hpr1Δ	SSAA-17C	4.0 (7.5)
	SSYY4-6D	3.6 (8.3)
hrs1Δ rad52-1	SSXY-6A	3.0 (10)
	SSXY-29A	5.0 (6.0)
hrs1Δ hpr1Δ rad52-1	SSXY-25C	3.7 (8.1)

[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. Numbers in parentheses indicate the times decrease below the wild-type value. As a reference, the frequency of deletions previously published for this system in *hrs1-1* strains was 1.5–2.0 × 10^{-5} (SANTOS-ROSA and AGUILERA 1995).

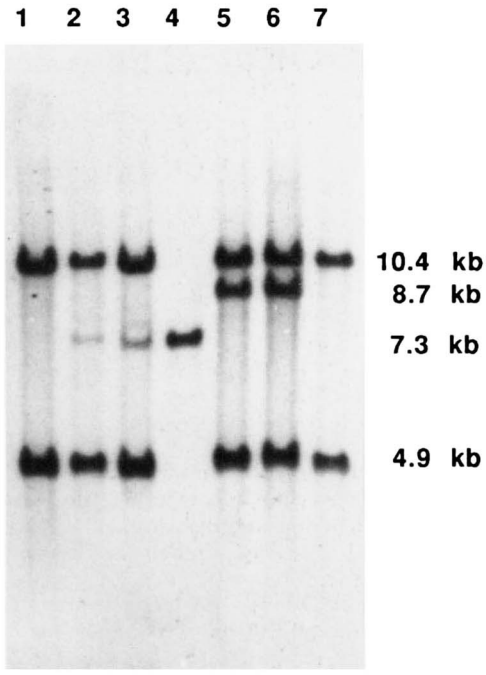

FIGURE 3.—Southern analysis of *Bam*HI-digested genomic DNA from different yeast strains carrying the *leu2-k::ADE2-URA3::leu2-k* direct repeat system. The 1.6-kb *Cla*I-*Sal*I fragment from *LEU2* was used as probe. Strains used were the wild-type strain A3Y3A (lane 1), the *hpr1*Δ strains AYW3-3D (lane 2) and A3Y3T3 (lane 3), the *hpr1*Δ *hrs1*Δ strains SSYY4-6C (lane 5) and SSYY4-6D (lane 6), the *hpr1*Δ *hrs1-1* strain SS58-2A (lane 7), all of them carrying the *leu2-k::ADE2-URA3 leu2-k* direct repeat system, and the A3Y3T3 strain (lane 4) with no duplication system. The 10.4- and 4.9-kb bands correspond to the *leu2* duplicated genes, the 8.7-kb band correspond to the *LEU2* copy used to replace the *HRS1* gene, and the 7.3-kb band correspond to the *leu2* copy remaining in the chromosomal locus after a deletion event took place.

the *hrs1*Δ mutation reduces the frequency of direct-repeat recombination of *hpr1*Δ strains to levels below those conferred by the *hrs1-1* allele (SANTOS-ROSA and AGUILERA 1995), indicating that the original *hrs1-1* mutation was leaky.

We confirmed by genetic reconstruction experiments (data not shown) and by Southern analysis (Figure 3), that the low frequency of colonies formed on SC-FOA in *hrs1*Δ *hpr1*Δ and *hrs1-1 hpr1*Δ strains was a direct consequence of its incapacity to undergo direct-repeat recombination and not a consequence of a possible growth defect of *hrs1* cells on SC-FOA. The Southern analysis (Figure 3) 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-URA::leu2-k* duplication system, corresponds to the single copy of the *LEU2* gene, 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 3, this 7.3-kb band does not appear in *hpr1*Δ *hrs1-1* and *hpr1*Δ *hrs1*Δ double mutants, or in wild-type strains, confirming the complete suppression of the hyperrec phenotype of *hpr1*Δ cells by the *hrs1*Δ mutation.

***HRS1* is required for spontaneous deletions between direct repeats:** The *hrs1*Δ allele reduces the frequency of deletions between direct repeats in *hpr1*Δ cells to seven times below the wild-type level (Table 3). This result suggests that the *hrs1*Δ mutation by itself, might have a hyporec phenotype for deletions in a wild-type background. Indeed, *hrs1*Δ single mutants show a frequency of deletions seven times below the wild-type levels, a phenotype not shown by the original *hrs1-1* mutation. This strong reduction in the frequency of deletions observed in *hrs1*Δ strains is not observed in *rad52-1* strains with the *leu2-k::URA3-ADE2::leu2-k* system, suggesting that *HRS1* is, in contrast to the recombinational repair gene *RAD52*, very important for deletion formation. The double mutant *rad52 hrs1*Δ and the triple mutant *hpr1*Δ *hrs1*Δ *rad52* show the same frequency of deletions as the *hrs1*Δ single mutants, indicating that *hrs1* is epistatic to *rad52*.

The Rec$^-$ phenotype of the *hrs1*Δ mutation was not specific for deletion formation in the *leu2-k::URA3-ADE2::leu2-k* system. The frequency of Ura$^-$ deletions in the 0.75-kb direct repeats of the *his3p::INV* system and the frequency of His$^+$Trp$^-$ deletions in the 6.1-kb direct repeat system *his3-513::TRP1::his3-537* in *hrs1*Δ strains were 10–13 times lower than in wild-type strains (Table 4).

The *hrs1*Δ mutants are not affected in intrachromosomal gene conversion/reciprocal exchange recombination: To test whether *HRS1* is also involved in gene conversion/reciprocal exchange recombination,we determined the effect of the *hrs1*Δ mutation on gene conversion and reciprocal exchange in two different intrachromosomal DNA repeat systems. The frequency of His$^+$ gene conversion/reciprocal exchange recombinants in the 3.0-kb inverted repeats of the *his3p::INV* system in *hrs1*Δ strains was reduced only about threefold (Table 4). It is important to note that in this system the *rad52-1* mutation reduce the frequency of reciprocal exchange/gene conversion events 475-fold (AGUILERA and KLEIN 1989b). Moreover, the frequency of His$^+$Trp$^+$ gene conversion events in the 6.1-kb direct repeat system *his3-513::TRP1::his3-537* was similar to wild-type levels (Table 4). These results indicate that the *HRS1* gene is required for deletion formation between repeats and has only a minor effect on intrachromosomal gene conversion/reciprocal exchange.

The *hrs1*Δ mutants are not affected in UV- and MMS-damage DNA repair: Because mitotic recombination and DNA repair are intimately related, we tested whether the *HRS1* gene was involved in DNA repair. We determined the effect of *hrs1*Δ on the repair of UV and MMS induced damage. We found that the *hrs1*Δ

TABLE 4

Frequency of deletions ($\times 10^6$) and gene conversion/reciprocal exchange of different recombination systems in wild-type and *hrs1*Δ mutant strains

	his3p::INV[a,b]		*his3-513::TRP1::his3-537*[a,b]	
Genotype	His^+ crossovers and gene conversions	Ura^- deletions	His^+ Trp^+ gene conversions	His^+ Trp^- deletions
Wild type	52.0	78.0	31.0	25.0
	30.0	38.5		
*hrs1*Δ	13.0 (3.2)	4.7 (12.4)	48.0 (0.6)	1.9 (13.2)
	13.0 (3.2)	5.5 (10.6)	58.0 (0.5)	1.9 (13.2)

Wild-type strains SSIN-14A and SSIN-18A and the *hrs1*Δ strains SSIN-11B and SSIN-17B were used for the *his3p::INV* system; and the wild-type strain SSGW-4C and the *hrs1*Δ strains SSGW-5A and SSGW-3D were used for the *his3-513::TRP1::his3-537* system.

[a] For each strain six independent colonies were used for a fluctuation test. Median recombination frequencies are given. Ura^- deletion recombinants were scored on SC-FOA medium and His^+ crossover/gene conversion recombinants in SC-His. The frequency of His^+ Trp^+ gene conversion events and His^+ Trp^- deletion events was calculated by multiplying the median frequency of His^+ recombinants determined on SC-his by the ratio of Trp^+:Trp^- events obtained from independent His^+ recombinants, which was 29:24 for the wild-type strain and 206:8 for the *hrs1*Δ strain. The proportion of Trp^- among the total His^+ recombinants was 45.2% for the wild-type strain (53 independent His^+ recombinants analyzed) and 3.9% for the *hrs1*Δ strains (214 His^+ recombinants analyzed). The ratio "His^+ crossovers:His^+ gene conversions" in the *his3p::INV* system was similar for both the wild-type (5:30) and the *hrs1*Δ strains (3:41), as determined by Southern analysis.

[b] Numbers in parentheses indicate the times decrease below the average value of the two median frequencies of the wild-type strains.

strains, as already observed for the *hrs1-1* leaky allele (SANTOS-ROSA and AGUILERA 1995), show the same level of viability after UV irradiation or MMS treatment as wild-type strains, indicating that *HRS1* is not involved in the repair of UV- and MMS-induced damage. Similarly *hrs1*Δ *hpr1*Δ double mutants were neither UV nor MMS sensitive (data not shown).

Meiosis is not affected in *hrs1*Δ strains: Because recombination is essential during meiosis, we asked whether the *HRS1* gene was important for meiosis. We observed that the levels of sporulation of *hrs1*Δ/*hrs1*Δ strains after 3 days at 26° was 10.6–14.5% as compared with 36.7–43.1% for the wild-type versions of the same *hrs1*Δ/*hrs1*Δ strains, obtained by transformation with plasmid p316-HRS1 carrying the complete *HRS1* coding sequence. The levels of germination for the spores of these strains was above 85%. These results indicate that the *hrs1*Δ/*hrs1*Δ strains are proficient in meiosis, although it may occur at reduced efficiency. This suggests that it is unlikely that *HRS1* has a role in meiotic recombination.

***HRS1* is required for activation of gene expression of the *GAL1,10* promoter:** To obtain data on the possible role of Hrs1p on gene expression, as suggested by its deduced amino acid sequence, we determined the levels of activation of the *E. coli LacZ* gene fused to the *GAL1,10* promoter. Table 5 shows that under activation conditions the level of β-galactosidase was 10 times lower in *hrs1*Δ mutants than in wild-type strains, whereas the levels of expression were similar for both genotypes under repressing conditions. This result is consistent with the idea that Hrs1p may function as a positive regulator of gene expression in yeast. Further molecular analysis is required to determine the specific role of *HRS1* on gene expression.

Purification of the Hrs1p protein and anti-Hrs1p antibodies: The Hrs1p protein was expressed as a His(6) fusion protein in *E. coli* under the control of the T7 promoter. Plasmid pT7-7-His(6)HRS1 (see MATERIALS AND METHODS), coding for a Hrs1p fusion protein with the first seven amino-terminal residues (MAPSEIL) replaced by the first 14 amino acids of the pT7-7-His(6) vector (MGGSHHHHHHGMAR), was used to overexpress the His(6)Hrs1p protein in *E. coli*. Denaturing polyacrylamide gels of IPTG-induced *E.coli* extracts revealed the overexpression of a new protein whose molecular weight (52 kD) is in accord with the calculated molecular weight (49 kD) expected for His(6)Hrs1p (Figure 4A). His(6)Hrs1p was purified to apparent homogeneity by affinity-chromatography and gel-elution

TABLE 5

β-galactosidase activity (U) of *HRS1* and *hrs1*Δ strains determined in plasmid pLGSD5, containing the *LacZ* gene under the yeast *GAL1,10* promoter

		β-Galactosidase (U.)[a]	
Genotype	Strains	Glucose	Galactose
HRS1	WDS3C-A	1.05 ± 0.12	1392 ± 42.5
	WDS3C-B	0.78 ± 0.02	1223 ± 138.2
*hrs1*Δ	WDS-4B	1.28 ± 0.21	113.5 ± 15.6
	WDS-5B	0.67 ± 0.003	90.5 ± 8.5
	WDS-6B	0.69 ± 0.16	106.1 ± 20.6

[a] The data shown correspond to the average and the standard deviation of four different determinations for *hrs1*Δ mutants and two for wild-type strains.

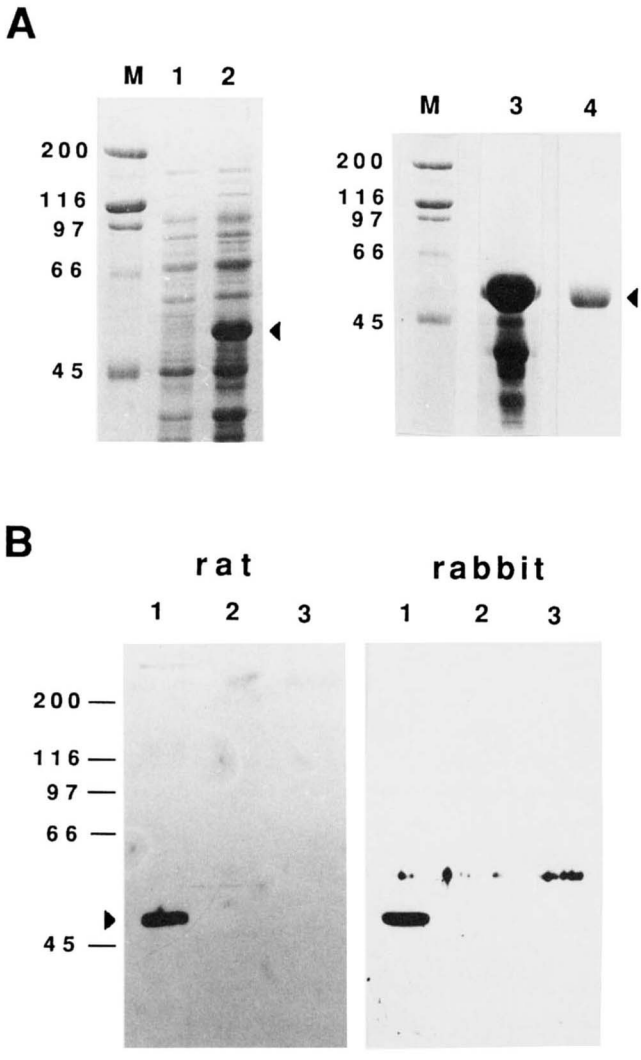

FIGURE 4.—Protein expression. (A) Coomasie blue stained SDS-PAGE (10%) gels of protein extracts from *E. coli* strain BL21 (DE3) transformed with plasmid pT7-7-His(6)HRS1. Shown are 40 μg of total protein extracts from *E. coli* under noninducing (lane 1) and inducing conditions (lane 2), protein fraction resulting after elution of a NTA-Ni column with 0.3 M imidazol (lane 3), and His(6)Hrs1p fusion protein purified to homogeneity by electroelution (lane 4). The position of the His(6)Hrs1p fusion protein is indicated by an arrow. The standard size markers are shown in lanes M. (B) Western blot analysis of yeast protein extracts using antibodies against the His(6)Hrs1p fusion protein obtained from rat (left) and rabbit (right). Forty micrograms of total yeast protein extract were loaded in each lane. The specific primary antibodies were diluted 1:500 and the secondary antibodies (HRP-conjugated goat antirabbit IgG and HRP-conjugated antirat IgG) were diluted 1:3000. Shown are otal protein extracts from strain BJ5464 transformed with plasmid pGAL-HRS1 grown on galactose (lane 1, inducing conditions) and glucose (lane 2, noninducing conditions) and total protein extracts from the wild-type strain SSAA-8B (lane 3).

(Figure 4A). The purified His(6)Hrs1p protein was used to obtain specific anti-Hrs1p antisera from rat and rabbit. The specificity of the purified antisera was shown in Western blots of total yeast protein extracts using either the alkaline-phosphatase (data not shown) or the ECL detection methods (Figure 4B). An ~49-kD protein could be detected when Hrs1p was overexpressed from the pGAL-HRS1 plasmid, which is in excellent accord with the calculated molecular weight of 46,770 for Hrs1p deduced from the DNA sequence (BRÖHL *et al.* 1994). (The 55-kD band observed in the western blot made with rabbit antibodies corresponds to cross-reactivity of the secondary antibody and it is not seen with the affinity-purified primary antibody; data not shown). The endogenous level of Hrs1p in wild-type cells could not be detected despite the use of the sensitive chemiluminiscence system and large amounts of extract (Figure 4B). The ~49 kD protein, expressed by the pGAL-HRS1 plasmid, is a functional Hrs1p protein, as determined by the ability of the pGAL-HRS1 plasmid to complement the *hrs1*Δ mutation (data not shown).

Immunofluorescence localization of the *HRS1* gene product with affinity-purified antibodies: Given the specificity of the affinity-purified antibodies for the *HRS1* gene product, we localized Hrs1p by indirect immunofluorescence. As shown in Figure 5, Hrs1p is highly enriched in the yeast nucleus in cells containing only the chromosomal copy of *HRS1*. Staining with the anti-Hrs1p antibodies was coincident with the DAPI staining of the nuclear DNA. Indirect immunofluorescence of *hrs1*Δ cells showed no signal at all, confirming the specificity of the antiserum. We conclude that Hrs1p is a nuclear protein that is present in the nucleus throughout all stages of the mitotic cell cycle (Figure 5 and data not shown).

DISCUSSION

In this study, we report the molecular analysis of the *HRS1* gene. We have cloned the *HRS1* gene by complementation of the *hrs1-1* mutation, which was identified as an extragenic suppressor of the hyperdeletion phenotype of the *hpr1*Δ mutation (SANTOS-ROSA and AGUILERA 1995). The sequence analysis of the complete *HRS1* gene reveals that it is identical to *PGD1*. No function or phenotype has been previously reported for the *PGD1* gene, that was named for its Polyglutamine Domain. The *PGD1* gene was isolated as an extragenic high-copy suppressor of the *sup1-798* mutation in a search for the *SUP1* gene. The *sup1-798* mutation was identified as an extragenic suppressor of the *rpo41/pet-ts798* mutation altered in the mitochondrial RNA polymerase (BRÖHL *et al.* 1994). The *SUP1* gene remains to be cloned. The *hrs1*Δ null mutation reduces the frequency of deletions in wild-type and *hpr1*Δ backgrounds sevenfold below wild-type and *rad52* levels, and reveals 10 times less activation of gene expression of the *GAL1,10* promoter than wild-type strains. We have purified the His(6)-Hrs1p protein, and we have obtained specific anti-Hrs1p polyclonal antibodies that have allowed us to show that Hrs1p is a ~49-kD nuclear

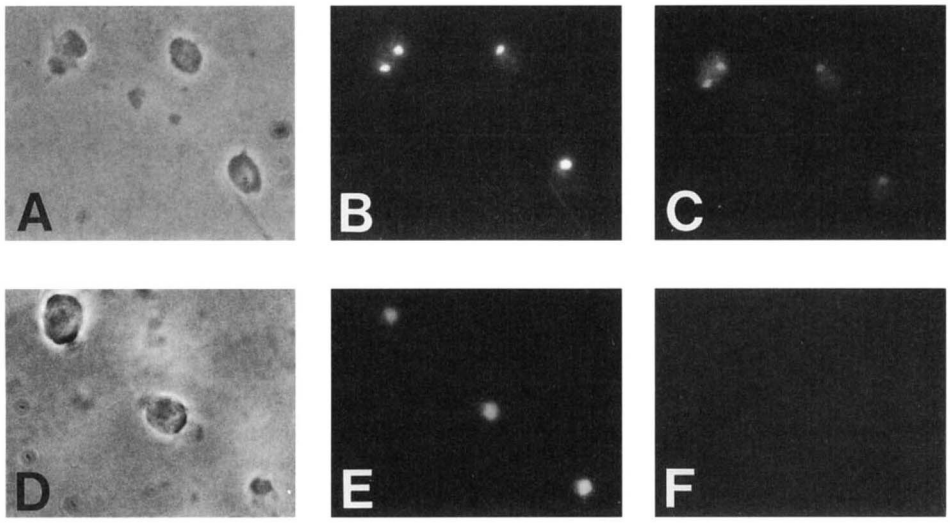

FIGURE 5.—Indirect immunofluorescence of whole vegetative cells. A log-phase culture was fixed and prepared for indirect immunofluorescence as described in the text. (A–C) Wild-type diploid strain made from BJ5464 and BJ5465 haploid strains; (D–F) *hrs1*Δ strain made from SSAA-12D and SSAA-17B haploid strains; (A and D) light microscopy; (B and E) DAPI staining; (C and F) indirect immunofluorescence using an affinity-purified anti-His(6)Hrs1p rabbit polyclonal antibody.

protein. Our results suggest that Hrs1p is a protein required for the formation of spontaneous and *hpr1*Δ-induced deletions between direct repeats and that it may function as a positive regulator of gene expression.

Hrs1p is required for the initiation of spontaneous and *hpr1*-induced deletions between repeats: The observation that spontaneous direct repeat recombination requires the *HRS1* gene is particularly relevant. The levels of recombination of single *hrs1*Δ mutants are sevenfold below the wild-type and *rad52* levels, a phenotype not observed with the original *hrs1-1* mutation. Double mutant analysis showed that the *hrs1* mutation is epistatic to *rad52* and *hpr1* for the repeat-recombination phenotype. These results suggest that *HRS1* is more important and/or may be acting earlier than *RAD52* in direct-repeat recombination. Because *hrs1* mutants are neither affected in the repair of double-strand breaks via direct-repeat recombination nor in the repair of spontaneous (SANTOS-ROSA and AGUILERA 1995), UV- and MMS-induced DNA damage, we believe that Hrs1p is not involved in either DNA repair or recombination itself. Moreover, all recombination events reported to be associated with *hpr1* and *hrs1* mutants are deletions between direct repeats. No effects have been observed on gene conversion and reciprocal exchange, which is consistent with our conclusion that most spontaneous and *hpr1*-induced recombination events occur through a nonconservative recombination mechanism (SANTOS-ROSA and AGUILERA 1994).

Hrs1p may participate in the regulation of gene expression: The amino acid sequence of the Hrs1p protein reveals that it contains a stretch of 12 alternating alanine and glutamine residues and a continuous stretch of 12 glutamine residues at the carboxy-terminal end. This polyglutamine stretch is surrounded by asparagine-rich regions. The homology of the polyglutamine-rich region of Hrs1p with the polyglutamine-rich regions of the yeast transcription activators Gal11p/Spt13p (SUZUKI *et al.* 1988) and Ssn6p/Cyc8p (SCHULTZ and CARLSON 1987), is particularly instructive. The Gal11p protein has been defined as a component of the SRB complex of the RNA polII holoenzyme (KOLESKE and YOUNG 1994). Polyglutamine-rich regions have been shown to correspond to one of the three types of activator domains (acidic, glutamine-rich and proline-rich domains) defined for eukaryotic activators of transcription (MITCHELL and TJIAN 1989). GERBER *et al.* (1994) have shown that homopolymeric stretches of glutamines occur predominantly in transcriptional regulatory proteins and that transcriptional activation is modulated by the number of glutamine repeats. In this study, we show that Hrs1p protein is localized in the nucleus and is required for activation of gene expression of the *GAL1,10* promoter. It has recently been shown that glutamine repeats may form polar zippers, a motif for protein-protein interactions (STOTT *et al.* 1995). This is consistent with the idea that polyglutamine stretches may act as protein-protein multimerization domains (see PASCAL and TJIAN 1991). Although it is still necessary to show at the molecular level that Hrs1p participates in transcription, all these data taken together suggests that this may be the case. Our result is consistent with the recent observation that Hpr1p also participates in transcriptional activation (FAN and KLEIN 1994; ZHU *et al.* 1995).

Role of Hrs1p on the formation of deletions between DNA repeats: The relationship between DNA repeat recombination and gene expression suggested in this study is different from previous observations relating transcription and recombination. First, the increase of recombination levels associated with activation of gene expression in yeast (KEIL and ROEDER 1984; VOELKEL-MEIMAN *et al.* 1987; STEWART and ROEDER 1989; THOMAS and ROTHSTEIN 1989) and other systems (BOURGAUX-RAMOISY *et al.* 1995) is the opposite phenotype observed for *hpr1*Δ strains where the lack of transcription activation is accompanied by a hyper-rec phenotype. Also, the observation that deletions of the *HPR1* and *HRS1*

genes have opposite effects on the stability of direct repeats in yeast clearly suggests that the different recombination levels observed in the mutants are not a consequence of different levels of gene expression. These results suggest that *HRS1* and *HPR1* must have a particular role on the spontaneous initiation of recombination between DNA repeats in yeast.

We propose that the function of Hrs1p on deletion formation may be mediated by a transcription complex. A deletion could occur as a consequence of a retarded, stalled or blocked open transcription initiation complex with negatively supercoiled DNA. The affected DNA region could be more susceptible to DNA breaks, possibly mediated by a nuclease activity, or could be a barrier for DNA replication, which would lead to the deletion of the whole region if flanked by direct repeats. Such a DNA-protein transcription complex would require Hrs1p for its assembly and, eventually, would be the cause for most of spontaneous initiation events leading to deletions between DNA repeats. The Hpr1p protein would be required for the activation of *HPR1*-dependent transcription after the assembly of the open DNA-protein transcription initiation complex. Thus, such a complex would get stalled in *hpr1*Δ cells, leading to the hyper-deletion phenotype.

However, we cannot exclude the possibility that Hrs1p has a more direct role on the initiation of deletion events, which would not be related to its putative role in transcription. It might be possible that Hrs1p participates in deletion formation by contacting recombination or replication factors. In this sense, the acidic activator VP16 has been reported to interact also with replication factor A to induce BVP replication (He *et al.* 1993; Li and Botchan 1993). Also, the components of TFIIH have been shown to participate in both transcription and excision repair (see Drapkin *et al.* 1994).

Further biochemical and molecular studies on the Hrs1p protein will allow us to understand its function, whether or not connected with transcription, in the initiation of spontaneous and *hpr1*Δ-induced deletions.

We thank S. West for supplying the pT7 vectors and strains, R. M. Ríos for technical advice with the protein work, E. Santero and S. Chávez for their critical reading of the manuscript and C. Alaimo for style correction. This work was supported by DGICYT grant PB93-1176-C0201 from the Ministery of Science and Education of Spain and a grant from the Regional Government of Andalucia (Spain) to A.A. Research in W.-D.H.'s laboratory was supported by a career development award (START 31-29254.90) and a grant form the Swiss National Science Foundation. H.S.-R. was the recipient of a PFPI predoctoral fellowship from the Ministery of Science and Education of Spain.

LITERATURE CITED

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, 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, 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.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

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.

Bolivar, F., and K. Backman, 1979 Plasmids of *Escherichia coli* as cloning vectors. Methods Enzymol. **68:** 245–267.

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

Bröhl, S., T. Lisowsky, G. Riemen and G. Michaelis, 1994 A new nuclear suppressor system for a mitochondrial RNA polymerase mutant identifies an unusual zinc-finger protein and a polyglutamine domain protein in *Saccharomyces cerevisiae*. Yeast **10:** 719–731.

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.

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.

Feinbeg, 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.

Gerber, H. -P., K. Seipel, O. Georgiev, M. Höfferer, M. Hug *et al.*, 1994 Transcriptional activation modulated by homopolymeric glutamine and proline stretches. Science **263:** 808–811.

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.

Harlow, E., and D. Lane, 1988 *Antibodies. A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

He, Z., B. T. Brinton, J. Greenblatt, J. A. Hassell and C. J. Ingles, 1993 The transactivator proteins VP16 and GAL4 bind replication factor A. Cell **73:** 1223–1232.

Henikoff, S., 1984 Unidirectional digestion with exonuclease III creates targeted break points for DNA sequencing. Gene **28:** 351–359.

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.

Hunkapiller, M. W., and E. Lujan, 1986 Purification of microgram quantities of proteins by polyacrylamide gel electrophoresis, pp. 89–101 in *Methods of Protein Microcharacterization*, edited by J. Shively. Human Press, Clifton, NJ.

Ito, H., Y. Fukuda, K. Murata and A. Kimura, 1983 Transformation of intact cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

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

Johnson, A. W., and R. D. Kolodner, 1991 Strand exchange protein 1 from *Saccharomyces cerevisiae*. A novel multifuctional protein that contains DNA strand exchange and exonuclease activities. J. Biol. Chem. **266:** 14046–14054.

JONES, E. W., 1991 Tackling the protease problem in *Saccharomyces cerevisiae*. Methods Enzymol. **194:** 428–453.

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.

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

KOWALCZYKOWSKI, S. C., D. A. DIXON, A. K. EGGLESTON, S. D. LAUDER and W. M. REHAUER, 1994 Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:** 401–465.

LAEMMLI, U. K. 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685.

LI, R., and M. R. BOTCHAN, 1993 The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate in vitro BVP-1 DNA replication. Cell **73:** 1207–1221.

LLOYD, R. G., and G. J. SHARPLES, 1992 Genetic analysis of recombination in prokaryotes. Curr. Opin. Genet. Dev. **2:** 683–690.

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.

MITCHELL, P. J., and R. TJIAN 1989 Transcriptional regulation in mammalian cell by sequence-specific DNA binding proteins. Science **245:** 371–378.

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.

PASCAL, E., and R. TJIAN, 1991 Different activation domains of Sp1 govern formation of multimers and mediate transcriptional synergism. Genes Dev. **5:** 1646–1656.

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.

PRINGLE, J. R., A. E. M. ADAMS, D. G. DRUBIN and B. K. HAARER, 1991 Immunofluorescence methods for yeast. Methods Enzymol. **194:** 565–602.

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.

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 hpr1*Δ strains. Mol. Gen. Genet. **16:** 339–346.

SANTOS-ROSA, H., and A. AGUILERA, 1995 Isolation and genetic analysis of extragenic suppressors of the hyper-deletion phenotype of the *Saccharomyces cerevisiae hpr1*Δ mutation. Genetics **139:** 57–66.

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.

SCHULTZ, J., and M. CARLSON, 1987 Molecular analysis of *SNF6*, a gene functionally related to the *SNF1* protein kinase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 3255–3262.

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

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.

STOTT, K., J. M. BLACKBURN, P. J. G. BUTLER and M. PERUTZ, 1995 Incorporation of glutamine repeats makes protein oligomerize: implications for neurodegenerative diseases. Proc. Natl. Acad. Sci. USA **92:** 6509–6513.

SUZUKI, Y., Y. NOGI, A. ABE and T. FUKASAWA, 1988 Gal11 protein, an auxiliary transcription activator for genes encoding galactose-metabolizing enzymes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 4991–4999.

TABOR, S., and C. C. RICHARDSON, 1985 A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:** 1074–1078.

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 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

TOWBIN, H., T. STAEHELIN and J. GORDON, 1979 Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:** 4350–4354.

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.

ZALKIN, H., and C. YANOFSKY, 1982 Yeast gene *TRP5*: structure, function, and regulation. J. Biol. Chem. **257:** 1491–1500.

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