MOLECULAR AND CELLULAR BIOLOGY, Mar. 1995, p. 1698–1708 Vol. 15, No. 3
0270-7306/95/$04.00+0

HPR1 Encodes a Global Positive Regulator of Transcription in *Saccharomyces cerevisiae*

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Received 19 May 1994/Returned for modification 14 July 1994/Accepted 28 December 1994

The Hpr1 protein has an unknown function, although it contains a region of homology to DNA topoisomerase I. We have found that *hpr1* null mutants are defective in the transcription of many physiologically unrelated genes, including *GAL1*, *HO*, *ADH1*, and *SUC2*, by using a combination of Northern (RNA) blot analysis, primer extension, and upstream activation sequence-*lacZ* fusions. Many of the genes positively regulated by *HPR1* also require *SWI1*, *SWI2-SNF2*, *SWI3*, *SNF5*, and *SNF6*. The transcriptional defect at *HO* and the *CCB*::*lacZ* upstream activation sequence in *hpr1* mutants is partially suppressed by a deletion of *SIN1*, which encodes an HMG1p-like protein. Elevated gene dosage of either histones H3 and H4 or H2A and H2B results in a severe growth defect in combination with an *hpr1* null mutation. However, increased gene dosage of all four histones simultaneously restores near-normal growth in *hpr1* mutants. Altered in vivo Dam methylase sensitivity is observed at two *HPR1*-dependent promoters (*GAL1* and *SUC2*). Most of the Hpr1 protein present in the cell is in a large complex (10^6 Da) that is distinct from the *SWI-SNF* protein complex. We propose that *HPR1* affects transcription and recombination by altering chromatin structure.

The *HPR1* gene was identified in a screen for mutations that increase intrachromatid recombination (1). Cells with null mutations in *HPR1* are viable and display a 100-fold elevated rate of mitotic intrachromatid recombination. The DNA sequence of *HPR1* revealed two small regions with limited homology to *TOP1* (2), the gene encoding DNA topoisomerase I, and a separate more extensive homology to the human V(D)J recombination-activating gene *RAG1* (50). The mechanism by which the Hpr1 protein influences recombination is unknown. Recently, mutants that suppress the hyperrecombination phenotype of *hpr1* mutants have been isolated (11).

Mutations in genes affecting recombination could define factors that participate directly in the recombination process or that affect recombination indirectly through alteration of other aspects of DNA metabolism such as transcription, DNA replication, or chromatin structure. For example, many *cdc* mutants that affect DNA synthesis display a mitotic hyperrecombination phenotype (14). In addition, active transcription of both RNA polymerase II (45) and RNA polymerase I (48) promoters has been shown to result in a higher rate of mitotic recombination. Mutations in *TOP1* and *TOP2* (encoding DNA topoisomerase II) cause a higher rate of mitotic recombination in ribosomal DNA (8, 20) even though they are required together for transcription of the ribosomal DNA (6).

Transcription by RNA polymerase II of many inducible genes requires the *SWI1*, *SWI2-SNF2*, *SWI3*, *SNF5*, and *SNF6* genes (51). These five *SWI-SNF* products are part of a large protein complex referred to as the *SWI-SNF* complex (7, 10, 33). Suppression analysis of a *swi1* mutation led to the identification of several *SIN* (Swi-independent) loci (44). Mutations in the *SIN1* gene (26), which encodes an HMG1p-related protein, or in *HHT2* (a.k.a. *SIN2*), which encodes histone H3 (26a), alleviate the requirement for the *SWI1* gene in transcription. In addition, deletion of one of the two gene clusters that encode histones H2A and H2B restores transcription of several genes in the absence of *SWI2*, *SNF5*, or *SNF6* (15). These genetic studies form the basis for the hypothesis that *SWI* products act positively in transcription to antagonize the negative action of the *SIN* products and other chromatin components.

We report here that *HPR1* is required for transcription of many of the same genes that require the *SWI-SNF* products. Cells with null mutations in *HPR1* display a synthetic growth defect in combination with a semidominant allele of the HMG1-related gene *SIN1* (26) or with increased gene dosage of both histones H3 and H4 or histones H2A and H2B. However, increased gene dosage of all four histones simultaneously restores near-normal growth in *hpr1* mutants. Altered in vivo Dam methylase sensitivity is observed at two *HPR1*-dependent promoters (*GAL1* and *SUC2*). Biochemical experiments show that Hpr1p is a member of a large protein complex that is distinct from the *SWI-SNF* protein complex. We present a model in which *HPR1* affects both transcription and recombination through its effect on chromatin structure.

MATERIALS AND METHODS

DNA manipulation and gel electrophoresis. Plasmid DNA was isolated and purified with Qiagen columns (Qiagen Corp., Chatsworth, Calif.). Restriction enzymes, alkaline phosphatase, and DNA ligase and the DNA polymerase large fragment (Klenow) were purchased from New England Biolabs, GIBCO BRL Technologies, Inc., and Boehringer Mannheim Biochemicals, respectively. AmpliTaq DNA polymerase was purchased from Perkin-Elmer Corp., Norwalk, Conn. The boiling method was used for plasmid DNA minipreparation (17). Genomic DNA was isolated from *Saccharomyces cerevisiae* cells as described elsewhere (35). Nu-SIEVE GTG and GTG agarose (genetic technological grade; FMC) was used for isolating DNA fragments for cloning purposes.

Oligonucleotides for PCR primers. The oligonucleotides used to amplify *HPR1-M* (Y322-G550) for cloning into pQE16 for antibody production were 5′-GCGGGGATCCACTGAGTATTACGCG-3′ and 5′-GGCGGGATCCTC CACCGGAAGTT-3′. The oligonucleotides used to amplify *HPR1* for cloning in frame with the GAL4 DNA binding domain were 5′-GCGGGGATCCGTAT GTCTAATACCG-3′ and 5′-GGGCGGATCCTTTCATATCTTGGGT-3′. The PCR primers were synthesized by Operon Technologies Inc., Alameda, Calif.

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TABLE 1. Yeast strains used

Strain	Genotype[a]	Reference or source
CY184	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* rDNA::*ADE2*	This study
CY257	*MAT***a** *swi1*Δ::*LEU2 lys2 ade2 his3 ura3*	I. Herskowitz
CY342	*MAT***a** *leu2-1 his3-200 ura3-99 ade2-101 lys2-801*	C. Peterson
CY407	*MAT***a** *swi2*Δ::*HIS3 leu2-1 his3-200 ura3-55 ade2-101 ade3-52 lys2-801*	C. Peterson
CY527	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* rDNA::*ADE2 hpr1-102*::*HIS3*	This study
CY605	*MAT***a** *trp1*Δ*ura3-52 lys2 ade2 leu2 his3*Δ*200 hpr1-102*::*HIS3 ho*::*HO-lacZ*	I. Herskowitz
IH2563	*MAT***a** *swi3*Δ::*TRP1 lys2-801 ade2-101 his3-*Δ*200 ura3-52 leu2-*Δ*1 trp1-*Δ*1*	I. Herskowitz
GGYI::171	*MAT***a** *his3 leu2 gal4*Δ *gal80*Δ *GAL* UAS::*lacZ ade2 tyr1*	S. Fields
YZY3	*MAT*α*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* rDNA::*ADE2 hpr1-103*::*LEU2*	This study
YZY8	*MAT*α *ade2-1 ura3-1 his3-11,15 rp1-1 leu2-3,112 can1-100* rDNA::*ADE2 URA3*::*DAM*	This study
YZY9	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* rDNA::*ADE2 URA3*::*DAM hpr1-103*::*LEU2*	This study
YZY10	*MAT***a** *trp1*Δ *ura3-52 lys2 ade2 leu2 his3*Δ*200 hpr1-102*::*HIS3 ho*::*HO-lacZ sin1*Δ::*TRP1*	This study
YZY21	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* rDNA::*ADE2 sin1*Δ::*TRP1*	This study
YZY22	*MAT*α *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100* rDNA::*ADE2 hpr1-103*::*HIS3 sin1*Δ::*TRP1*	This study

[a] rDNA, gene for rRNA.

Yeast strains. All yeast strains used are listed with relevant genotypes in Table 1.

Plasmid construction. All plasmids used are listed in Table 2.

(i) pCB93. For pCB93, a 3.7-kb *Bam*HI-*Sal*I DNA fragment containing *HPR1* was cloned into the *Bam*HI-*Sal*I sites in pUC19.

(ii) pBS1. For pBS1, an internal *Bgl*II-*Xba*I fragment from pCB93 was removed and replaced with a 1.8-kb *Xba*I-*Bam*HI fragment containing *HIS3* isolated from YCp407. pBS1 was used to disrupt *HPR1* with *hpr1-102*::*HIS3* following linearization with *Sal*I and *Eco*RI.

(iii) pYZ1. For pYZ1, a 3.7-kb *Bam*HI-*Sal*I fragment containing *HPR1* was isolated from pCB93 and cloned into the *Bam*HI-*Sal*I sites in pRS316 (40).

(iv) pYZ12. For pYZ12, a 2,250-bp *Bam*HI fragment of the *HPR1* open reading frame generated by PCR using pYZ1 as a template was fused to the GAL4 DNA binding domain at the *Bam*HI sites in pMA424.

(v) pYZ29. For pYZ29, a 3.7-kb *Bam*HI-*Sal*I fragment containing *HPR1* from pYZ1 was cloned into the *Bam*HI-*Sal*I sites in pB2440 (12).

(vi) pYZ31. For pYZ31, a 719-bp *Bam*HI fragment of *HPR1* (Y322-G550) was generated by PCR using pYZ1 as a template and was fused in frame to the 6-histidine tag by ligating at the *Bgl*II and *Bam*HI sites of pQE16.

(vii) pYZ33. For pYZ33, a 1.4-kb internal *Bgl*II-*Nru*I fragment was removed from pYZ1 and replaced with a 2.5-kb *Bgl*II-*Sal*I fragment (made blunt with Klenow enzyme) containing *LEU2* obtained by digesting YEp13. pYZ33 was used to disrupt *HPR1* with *hpr1-103*::*LEU2* following linearization with *Bam*HI and *Hin*dIII.

(viii) pYZ69. For pYZ69, a 2.25-kb *Eco*RI fragment from pJC102 (39) and a 1.76-kb *Eco*RI fragment from pTS4 (39) were both cloned into the *Eco*RI site of pRS314.

β-Galactosidase activity assay. Plasmids pRY121Δ10 and pBA161 (34) were transformed into a wild-type strain, CY184, and an *hpr1-102*::*HIS3* mutant, CY527. pRY121Δ10-containing strains were grown in 2% galactose in yeast extract-peptone (YEP) medium, whereas strains containing the pBA161 plasmid or *HO*::*lacZ* integrated were grown in 2% glucose in YEP medium. Cultures were grown to early log phase (A_{600} = 0.5). Crude protein extracts were made by the glass bead method (25). The protein concentration was determined by Bio-Rad assay. Ten micrograms of protein extract was used for β-galactosidase liquid assays (41). Reaction mixtures were incubated at 28°C for 20 min for *GAL1*::*lacZ* and 16 h for *HO*::*lacZ* and *CCB*::*lacZ* fusions.

RNA analysis. Cultures were grown to early log phase in the media indicated in Fig. 1. A standard protocol for isolating total yeast RNA was used (23). For Northern (RNA) blots, 20 μg of total RNA was electrophoresed in a formaldehyde-agarose gel (1.5% agarose) prior to transfer to a nitrocellulose membrane and hybridization with [α-^{32}P]dATP-labeled DNA probes. DNA fragments used as the hybridization probes were as follows: *SUC2*, a 2.1-kb *Hin*dIII-*Sph*I fragment from pB1281, and *ACT1*, a 1.0-kb *Xho*I-*Hin*dIII fragment from pRB149 (30). Ten micrograms of total RNA was analyzed by primer extension (26). DNA primers used for primer extension were as follows: *GAL1*, 5′-GCGCTAGAAT TGAACTCAGG-3′, and *ADH1*, 5′-CTTTGGCTTTGGAACTGG-3′.

Purification of an internal Hpr1 peptide (Hpr1p-M). Two-liter *Escherichia coli* M15/pREP4 cultures containing pYZ31 were grown in 2× yeast extract-tryptone medium containing 100 μg of ampicillin per ml and 25 μg of kanamycin per ml at 37°C. The cultures were induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 2 mM at an A_{600} of 0.8. Cultures were grown continuously for an additional 5 h. The protein purification procedure using Ni-nitrilotriacetic acid resin was as described in the QIAexpressionist manual (Qiagen Inc.) except that in all buffers, the final concentration of β-mercaptoethanol was 10 mM. Protein was eluted from the column in a buffer of 8 M urea–0.1 M sodium phosphate–0.01 M Tris-HCl, pH 8.0 (or pH 6.3), containing 300 mM imidazole. More than 95% of the loaded Hpr1p-M was eluted from the Ni-nitrilotriacetic acid column with the above two buffers. Urea was removed from the concentrated protein by dialysis against phosphate-buffered saline. The protein was concentrated with a Centriprep 10 column (Amicon Inc., Beverly, Mass.).

Preparation of rabbit antiserum to Hpr1p-M. Rabbit polyclonal antiserum against Hpr1p-M was produced by using the purified Hpr1p-M in two rabbits by Berkeley Antibody Co., Richmond, Calif. The rabbits were injected with Hpr1p-M in complete Freund's adjuvant as follows: 500 μg on day 0 and 250 μg on days 21, 45, 67, 89, and 114. Antiserum was prepared from bleedings of the rabbits on days 0 (preimmune), 34, 56, 78, 100, 125, and 135.

Western blotting (immunoblotting). Ten micrograms of crude protein extract made by glass bead lysis (25) was loaded on sodium dodecyl sulfate (SDS)–10% polyacrylamide gels. Proteins were electrotransferred onto a nitrocellulose mem-

TABLE 2. Plasmids used

Plasmid	Genotype or phenotype	Reference or source
pB1281	Ap^r *URA3 SUC2*	G. Fink
pB2440	Ap^r *LEU2* 2μm	R. D. Gietz and A. Sugino
pB2446	Ap^r *URA3* 2μm	R. D. Gietz and A. Sugino
pBA161	*URA3* 2μm *CCB*::*lacZ*	34
pBD7	*URA3* 2μm *SWI3*	32a
pBD13	*URA3* 2μm *SWI1*	32a
pBS1	Ap^r *hpr1*Δ*Bgl*II-*Xba*I::*HIS3*	This study
pCB351	*URA3 SIN1 CEN*	26
pCB352	*URA3 SIN1-2 CEN*	44
pJC102	*URA3 CEN* H2A	39
pMA424	*HIS3 GAL4B* 2μm	11a
pQE16	Ap^r *HIS*×6	Qiagen Corp.
pRB149	*URA3* Ap^r *ACT1*	30
pRD56	*URA3 CEN* p*GAL1,10-GST*	I. Herskowitz
pREP4	Km^r *lacI*	Qiagen Corp.
pRM200	*TRP1 CEN* H3 H4	27
pRS314	*TRP1 CEN*	40
pRS316	*URA3 CEN*	40
pRY131Δ10	*URA3* 2μm p*GAL*::*lacZ*	34
pTS4	*URA3 CEN* H2B	39
pWB51	Ap^r *sin1*Δ::*TRP1*	26
pYZ1	*URA3 CEN HPR1*	This study
pYZ12	HIS3 GAL4B::*HPR1*	This study
pYZ29	*LEU2* 2μm *HPR1*	This study
pYZ31	Ap^r *HIS*×6 HPR1p-M	This study
pYZ33	Ap^r *hpr1*Δ*Bgl*II-*Nru*I::*LEU2*	This study
pYZ69	URA3 CEN H2A H2B	This Study
YCp407	*HIS3 CEN*	G. Fink
YEp13	*LEU2* 2μm	G. Fink
YIpDAM	Ap^r *URA3 DAM*	16

TABLE 3. Transcriptional defect at the *GAL1*, *CCB*, and *HO* UAS elements in an *hpr1* mutant and suppression by a *sin1* deletion[a]

UAS element	TATA	β-Galactosidase activity [U/mg (%)] in:			
		CY184	CY527	YZY22	YZY21
GAL1	*GAL1*	1,311 (100)	30 (2)	17 (1)	985 (75)
CCB	*CYC1*	126 (100)	11 (6)	110 (59)	138 (74)
HO	*HO*	10.1 (100)	0.25 (2)	2.3 (23)	7.7 (76)

[a] A *URA3* 2μm plasmid containing *GAL1*::*lacZ* (pRY121Δ10) was transformed into an *HPR1* wild-type strain (CY184) and an *hpr1-102*::*HIS3* mutant strain (CY527). A *URA3* 2μm plasmid carrying *CCB*::*lacZ* (pBA161) was transformed into CY184, CY527, a *sin1*Δ::*TRP1* strain (YZY21), and a *sin1*Δ::*TRP1 hpr1-102*::*HIS3* double mutant strain (YZY22). For the integrated *HO*::*lacZ* strain, the *SIN1* gene was disrupted in an *hpr1-102*::*HIS3 ho*::*HO-lacZ* strain (CY605) to become *sin1*Δ::*TRP1 hpr1-102*::*HIS3 ho*::*HO-lacZ* (YZY10). p*HPR1.CEN* (pYZ1) was transformed into both CY605 and YZY10 as the *HPR1* wild-type controls. Crude protein extracts were isolated from the above cultures, which were grown in 2% galactose (*GAL1*::*lacZ*) or 2% glucose (*CCB*::*lacZ* and *HO*::*lacZ*) to early log phase. Protein concentration was determined by Bio-Rad assay. Ten micrograms of protein was incubated at 28°C for 20 min for the *GAL1*::*lacZ* strain and 16 h for *HO*::*lacZ*- and *CCB*::*lacZ*-containing strains. β-Galactosidase units were defined as amount of enzyme required to hydrolyze 1 nmol of *o*-nitrophenyl-β-D-galactoside per min per mg of protein at 28°C and pH 7. All results were the averages from three experiments, and the standard error was always less than 7%.

brane. Blots were probed with polyclonal Hpr1p-M antiserum at a 1:20,000 dilution and goat anti-rabbit immunoglobulin G conjugates (Bio-Rad) at a 1:10,000 dilution. The blots were reacted with enhanced chemiluminescence reagents (Amersham) and exposed to X-ray film.

Superose 6 chromatography. Extracts were prepared from 200-ml yeast cultures and fractionated on a fast protein liquid chromatography (FPLC) Superose 6 column as previously described (10, 33). Fractions were trichloroacetic acid precipitated, resuspended in SDS sample buffer, and electrophoresed on 10% Laemmli gels. Western blots were probed with either affinity-purified rabbit α-Swi3p antibodies (34) or crude rabbit α-Hpr1p antibodies, then processed, and developed with a chemiluminescent substrate as previously described (34).

In vivo Dam methylase sensitivity. For the *GAL1-10* promoter region, restriction enzyme-digested purified yeast genomic DNA was resolved by electrophoresis in 1% agarose gels in 1× Tris-acetate-EDTA buffer. DNA was transferred to nylon membranes (43), and the membrane was hybridized with a 690-bp *Nde*I-*Xba*I fragment from the *GAL1-10* promoter region of pRD56. For the *SUC2* promoter region, restriction enzyme digests of the purified DNA were resolved by electrophoresis in a mixture of 1.75% Nu-SIEVE GTG and 0.75% GTG agarose in 1× Tris-borate-EDTA buffer. The probe used for hybridization was an 874-bp *Eco*RI-*Hin*dIII fragment from the *SUC2* promoter region from plasmid B1281. The *HPR1* strain containing an integrated *E. coli dam* gene is YZY8, and the *hpr1-103*::*LEU2* derivative (YZY9) containing the *dam* gene was constructed by disrupting the *hpr1* gene with pYZ33 in YZY8.

RESULTS

***HPR1* is required for efficient transcription of several physiologically unrelated genes. (i) UAS::*lacZ* fusions.** An *HPR1* strain and an otherwise isogenic *hpr1-102*::*HIS3* mutant were compared for transcriptional activity from three upstream activation sequence (UAS)::*lacZ* fusions (Table 3). The *GAL1* promoter used here is a synthetic promoter containing two of the four binding sites for Gal4p and the *GAL1* TATA box (13). The *CCB* (cell cycle box) element contains three copies of a cell cycle-regulated element found in the *HO* upstream regulatory region and the *CYC1* TATA (3, 22, 32). The *GAL1* and *CCB* promoter fusions to *lacZ* were contained on plasmids. For *HO*, an integrated fusion to the wild-type promoter (*HO* TATA) was assayed. All three of these UAS elements are less efficiently utilized (between 16- and 44-fold) in the *hpr1* null mutant than in the *HPR1* parent strain. Since *HPR1* is not required for efficient transcription of all genes (see below) and because each of the TATA elements is different, it is unlikely that *HPR1* is required for TATA function. The copy number of the *GAL*::*lacZ* and *CCB*::*lacZ* plasmids is the same in the *hpr1* and *HPR1* strains (data not shown).

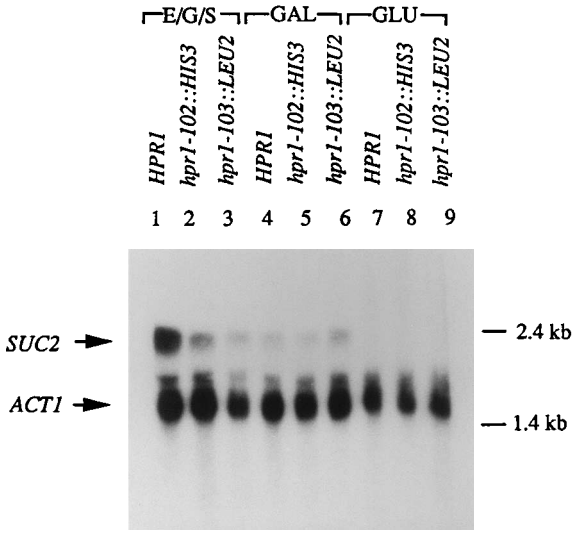

FIG. 1. *SUC2* and *ACT1* RNA levels in *hpr1* mutants. Strains were subcultured in YEP-raffinose medium overnight. These precultures were harvested, and the pellets were washed with double-distilled H_2O three times before being inoculated in YEP medium containing the following: ethanol (2%), glycerol (2%), and sucrose (0.2%) (E/G/S); galactose (2%) (GAL); or glucose (2%) (GLU). Cultures were grown to early log phase (A_{600} = 0.5). Total RNA was isolated and quantified by reading the A_{260}. Twenty micrograms of each sample was applied to a 1.5% formaldehyde–agarose denaturing gel. RNA was then transferred to a nitrocellulose membrane. Hybridization to radiolabeled DNA probes was performed at 42°C. Probes used in this experiment are described in Materials and Methods. Strains: *HPR1*, CY184; *hpr1-102*::*HIS3*, CY527; *hpr1-103*::*LEU2*, YZY3. Arrows denote the positions of *SUC2* and *ACT1* transcripts. RNA molecular size markers were run in a neighboring lane.

(ii) Northern blot analysis. To determine whether *HPR1* is required for transcription from native genes, we have performed Northern blot analysis for the *SUC2* and *ACT1* genes (Fig. 1). *SUC2* transcription is glucose repressed but can be derepressed in ethanol-glycerol-sucrose medium (29), whereas *ACT1* transcription is not affected by the carbon source. The strains were grown in YEP medium containing either ethanol-glycerol-sucrose, galactose, or glucose and were harvested during log-phase growth (A_{600} = 0.5) prior to RNA isolation. Comparing lane 1 with lanes 2 and 3 in Fig. 1, we observed that *HPR1* null mutants display lower steady-state levels of *SUC2* mRNA under derepressing conditions. This reduction was observed for two different deletion alleles of *HPR1*. By contrast, *ACT1* transcription does not require *HPR1* on any of the three carbon sources tested. *HPR1* is also not required for transcription of *TOP1* and *TOP2* (data not shown), indicating that *HPR1* is required for transcription of only a subset of genes. The transcription of rRNAs also does not require *HPR1*, since an equal amount of ethidium bromide-stained rRNA was obtained from an equal number of cells from both *hpr1* and *HPR1* strains (data not shown).

(iii) Primer extension of *GAL1* and *ADH1* mRNA. We performed primer extension analysis of *GAL1* and *ADH1* mRNA isolated from *hpr1* and *HPR1* strains in order to determine whether *HPR1* is required for proper start site selection (Fig. 2). The results show that while transcription of the *GAL1* gene is reduced in *hpr1* deletion strains, start site selection (19) is not altered (Fig. 2A). The RNA samples used in these experiments are identical to those used in the Northern blot analyses shown in Fig. 1. Figure 2B shows primer extension analysis of *ADH1* mRNA in *HPR1* and *hpr1* mutants. *ADH1* is transcribed when cells are grown in either glucose, galactose, or ethanol-

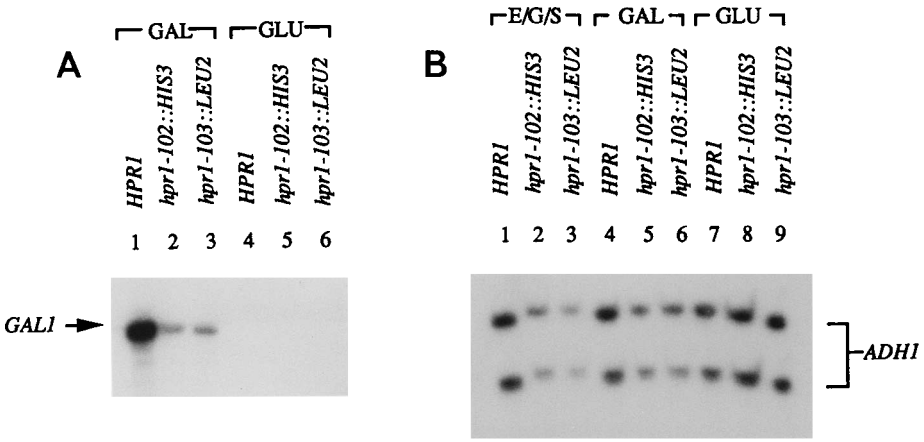

FIG. 2. *HPR1* is required for *GAL1* transcription. The culture conditions, abbreviations, and strains used in these experiments were the same as those described in the legend to Fig. 1. (A) Primer extension analysis using a *GAL1* primer. Total RNA was isolated from cultures which were grown in either YEP-galactose or YEP-glucose. Ten micrograms of total RNA was used for each primer extension reaction. The arrow indicates the *GAL1* primer extension product. (B) Primer extension analysis using an *ADH1* primer. The RNA used in this experiment was the same as that used in panel A. Ten micrograms of total RNA was used for each reaction. The two *ADH1* primer extension products are identified.

glycerol-sucrose medium (4). Two major mRNA start sites have been observed (34, 47). Transcription during growth on glucose medium does not require *HPR1* (compare lane 7 with lanes 8 and 9), but transcription during growth on ethanol-glycerol-sucrose or galactose shows a small but consistent dependency on *HPR1*. These effects also are observed with two different null alleles of *HPR1. SWI1*, *SWI2*, and *SWI3* are also required for efficient *ADH1* transcription on ethanol-glycerol-sucrose medium but not on glucose or galactose medium (34). The mRNA sites are identical to those observed previously (19, 47).

***HPR1* does not contain transcriptional activation activity when fused to the Gal4p DNA binding domain.** Transcriptional activators are often modular in structure, containing distinct DNA binding and transcriptional activation domains. The function of each domain is often maintained in a heterologous context, with the activation domain of one factor functioning when fused to the DNA binding domain of another (5). Many activators of transcription contain acidic domains that are required for function (37). When such a domain is fused to a sequence-specific DNA binding protein that lacks its own activation domain, such as the Gal4 N-terminal peptide, an appropriate reporter gene that contains the *cis*-acting DNA binding elements for Gal4p can be activated by the protein fusion in vivo (5). We have used PCR to fuse precisely the entire *HPR1* open reading frame to DNA encoding the Gal4p N-terminal 147 amino acids (pYZ12). pYZ12 was transferred into GGY::171, which contains a *GAL*-UAS::*lacZ* reporter gene. The resulting fusion proteins are produced in vivo, as evidenced by the fact that they complement the growth defect of an *hpr1* null mutant and can be detected on Western blots (data not shown). β-Galactosidase activity was measured by both filter assay and liquid assay. The fusion protein is not able to activate transcription from its cognate reporter contruct (data not shown). Thus, *HPR1* does not contain a transcriptional activation domain that functions in this assay.

The defect in *HO*::*lacZ* and *CCB*::*lacZ* expression in *hpr1* mutants is suppressed by a *sin1* null mutation. The defect in transcription of the *HO* gene observed in *swi* mutants is alleviated by mutations in *SIN* genes (44), some of which are in known chromatin components, including histone H3 (26, 26a). Therefore, we tested whether the transcriptional defects at *HO* and the *CCB* UAS observed in *hpr1* mutants were also suppressed by a *sin1* mutation. To do this, the *SIN1* gene was disrupted in an *hpr1-102*::*HIS3 ho*::*HO-lacZ* strain (CY605) to generate YZY10. In addition, a *CCB*::*lacZ URA3* 2μm plasmid was transferred into a *sin1*Δ::*TRP1* strain (YZY21) and an *hpr1-102*::*HIS3 sin1*Δ::*TRP1* strain (YZY22). The above strains were assayed quantitatively for β-galactosidase activity. The results of assays for β-galactosidase activity (Table 3) show that the *sin1*Δ::*TRP1* allele partially suppresses the transcription defect at *HO* and at the *CCB* UAS observed in *hpr1* mutants. Whereas the *hpr1-102*::*HIS3* mutant has only 2% of wild-type *HO*::*lacZ* expression, the *hpr1 sin1* double mutant has 23% of the wild-type level. A similar suppression by *sin1*Δ::*TRP1* is seen at the *CCB* UAS where *hpr1-102*::*HIS3* mutants have 6% of wild-type *CCB*::*lacZ* expression and *hpr1 sin1* double mutants have 59% of the wild-type level. In contrast, *GAL1*::*lacZ* expression is not suppressed in *hpr1 sin1* double mutants (Table 3).

***HPR1* is not required for transcription of *SWI1*, *SWI2*, or *SWI3*.** Because the phenotypes of *hpr1* and *swi* mutants are similar, we reasoned that the transcriptional defects observed in *hpr1* mutants might be a secondary consequence of an effect on *SWI* gene transcription. To address this, we examined the steady-state levels of *SWI1*, *SWI2*, and *SWI3* mRNA in *hpr1* and *HPR1* strains. Northern blot analysis indicates that *hpr1* mutants do not alter *SWI1*, *SWI2*, or *SWI3* steady-state mRNA levels (data not shown).

Hpr1 protein is equally abundant in *swi* mutant and *SWI* strains. The Swi3 protein is degraded more rapidly in *swi1* or *swi2* mutants (34), resulting in a lower steady-state level of Swi3p, probably because the *SWI-SNF* protein complex fails to form in *swi1* or *swi2* mutants (33). If the Hpr1 protein were part of the same complex, then it might also be degraded more rapidly in *swi1*, *swi2*, or *swi3* mutants and then be present at a lower steady-state level. Therefore, we determined the steady-state level of Hpr1 protein in crude extracts from *swi1*, *swi2*, and *swi3* mutants by Western analysis using antibodies to Hpr1p (see Materials and Methods). Figure 3A shows a Western blot that demonstrates that the polyclonal antibody is specific to the Hpr1 protein. A major band is observed in wild-type extracts at the predicted molecular mass for Hpr1p (88 kDa; lane 1), whereas no signal is present in an *hpr1-102*::*HIS3*

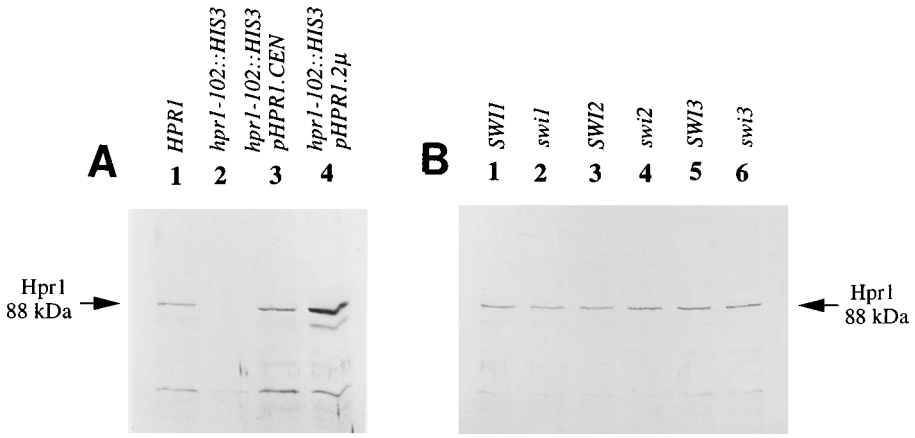

FIG. 3. Western blot analysis of the Hpr1 protein in *swi1*, *swi2*, and *swi3* mutants. (A) Specificity of the Hpr1p antibody. Lane 1, *HPR1* wild-type strain (CY184); lane 2, *hpr1-102*::*HIS3* mutant (CY527); lane 3, *HPR1* on a *CEN.ARS* plasmid (pYZ1) in CY527; lane 4, *HPR1* on 2μm (pYZ29) in CY527. Protein extracts were isolated from cultures at early log phase. (B) Hpr1 protein level in *swi1*, *swi2*, and *swi3* mutants and isogenic wild-type strains. Extracts were prepared from a *swi1* mutant strain (CY257) containing *SWI1* on a 2μm plasmid, pBD13 (lane 1), or a 2μm vector, pB2446 (lane 2); a *SWI2* wild-type strain (CY342) (lane 3); a *swi2* mutant strain (CY407) (lane 4); and a *swi3* mutant strain (IH2563) containing *SWI3* on a 2μm plasmid, pBD7 (lane 5), or a 2μm vector, pB2446 (lane 6). Ten micrograms of crude protein was loaded in each lane. Arrows denote the 88-kDa Hpr1 protein.

mutant extract (lane 2). Expression of *HPR1* from *CEN.ARS* (lane 3) and 2μm (lane 4) plasmids in an *hpr1-102*::*HIS3* mutant results in the appearance of the *HPR1* product. Figure 3B shows that the Hpr1 protein is equally abundant in *swi1*, *swi2*, and *swi3* mutant extracts and corresponding isogenic wild-type extracts.

Hpr1p is a component of a large protein complex distinct from the *SWI-SNF* complex. Given the similar phenotypes of *hpr1* and *swi-snf* mutants, *HPR1* could encode an unidentified subunit of the *SWI-SNF* protein complex or be required for its assembly. The *SWI-SNF* complex contains the products of the *SWI1*, *SWI2*, *SWI3*, *SNF5*, and *SNF6* genes as well as four additional subunits whose genes have not been identified (7, 10, 33). We prepared whole-cell extracts from *HPR1* and *hpr1* deletion strains and fractionated these extracts on an FPLC Superose 6 gel filtration column. The elution of the *SWI-SNF* complex was monitored by Western blot analysis using antibodies to the Swi3p subunit. As observed previously, the peak of Swi3 protein was in fraction 19, which corresponds to an apparent molecular mass of about 2 MDa (Fig. 4C) (33). Elution of Swi3p in fraction 19 requires an intact *SWI-SNF* complex, as fractionation of extracts prepared from *swi* or *snf* mutants shifts the peak of Swi3 protein (33). Figure 4A shows that in an extract made from an *hpr1* deletion strain, Swi3p still elutes from Superose 6 in fraction 19, indicating that *HPR1* is not required for assembly of the *SWI-SNF* complex. Furthermore, in an extract from a wild-type strain the peak of Hpr1 protein over Superose 6 was in fraction 21, which corresponds to an apparent molecular mass of about 1 MDa, smaller than the *SWI-SNF* complex (Fig. 4B). In addition, the Hpr1 protein (88 kDa) is not the same size as any of the unidentified *SWI-SNF* complex proteins (59, 61, 73, and 82 kDa) (7). No additional Hpr1 protein was detected in other fractions that would indicate a species of smaller apparent molecular mass, such as 88-kDa monomeric Hpr1p (32a). These results suggest that Hpr1p is not a subunit of the *SWI-SNF* complex and is not required for its assembly and indicate that Hpr1p may be a component of a distinct, large protein complex.

***hpr1* null mutants have synthetic growth defect in combination with *SIN1-2*, a dominant allele of an *HMG1*-like gene, or with elevated histone gene dosage.** The *SIN1* gene encodes a product with some similarity to HMG1 (26). The semidominant *SIN1-2* allele was isolated as a suppressor of the transcriptional defect at the *HO* promoter in *swi1* mutants (44). Introduction of a *CEN.ARS* plasmid carrying the *SIN1-2* allele into *hpr1* null mutants results in a strain that grows somewhat slowly at 30°C and has a marked growth defect at 37°C (Fig. 5). The *SIN1-2* plasmid does not cause any growth defect at 30 or 37°C in an isogenic *HPR1* strain. In contrast to results obtained with the semidominant *SIN1-2* allele, an *hpr1 sin1* double null mutant does not show a marked growth defect at either 30 or 37°C (data not shown). Thus, the special allele of *SIN1* is not tolerated in the absence of *HPR1* function.

In another example of a genetic interaction with chromatin components, *hpr1* mutants carrying extra copies of the genes for histones H3 and H4 (*HHT* and *HHF* genes) on a *CEN.ARS* plasmid (27) show a pronounced growth defect at 37°C, in contrast to isogenic *HPR1* controls (Fig. 6). Similarly, increased gene dosage of the genes encoding histones H2A and H2B (*HTA* and *HTB*) in *hpr1* mutants causes a marked growth defect at 37°C (Fig. 6). However, simultaneous expression of all four histone encoding genes from *CEN.ARS* plasmids restores growth in *hpr1* mutants to the level seen with vector alone (Fig. 6). Thus, *hpr1* mutants are hypersensitive to histone gene dosage imbalances.

Altered in vivo methylase sensitivity of the *GAL1* and *SUC2* promoters in *hpr1* mutants. Sensitivity of promoter regions to nucleases has been used as a probe to examine chromatin structure (49). A method has been developed to examine the sensitivity of yeast promoters to methylation in vivo from an exogenously introduced *E. coli* Dam methylase (42). It was shown that two *Sau*3AI sites in the *GAL1* gene, one in the promoter and another 310 bp downstream in the coding region, displayed little methylation under conditions of transcriptional repression and much greater methylation under conditions of transcriptional activation (42). This difference was attributed to a change in chromatin structure upon transcriptional activation. Because the phenotypes of *hpr1* mutants observed to date would be consistent with *HPR1* playing a role in altering chromatin structure to facilitate transcription, we have examined the in vivo methylation sensitivity of the *GAL1* and *SUC2* promoter regions in an *hpr1* mutant.

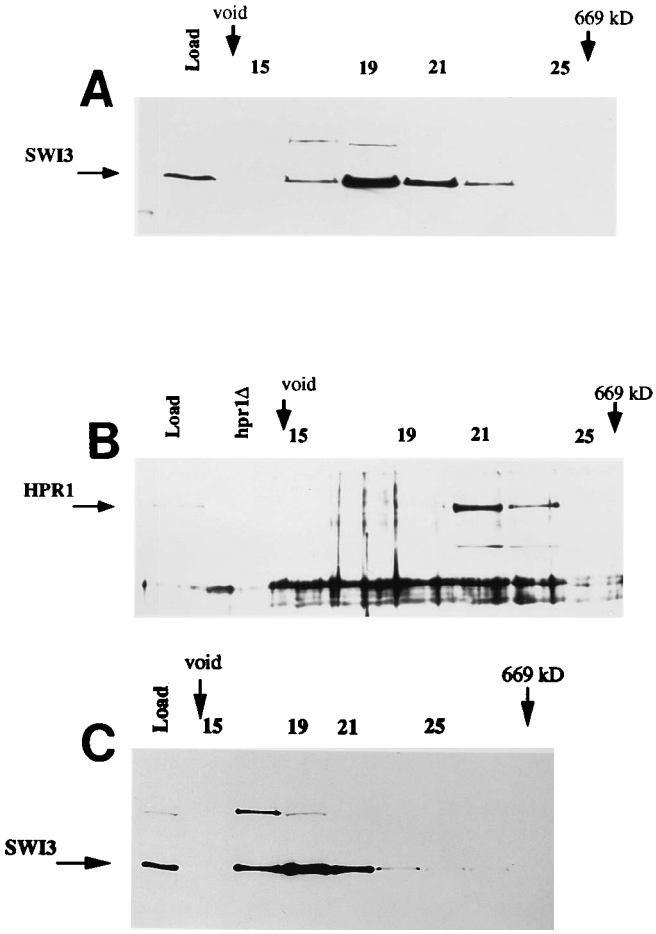

FIG. 4. Hpr1p is not a component of the *SWI-SNF* complex. Arrows denote the Swi3 and Hpr1 proteins. (A) Whole-cell extract was prepared from CY527 (*hpr1-102*::*HIS3*) and fractionated on Superose 6, and fractions were analyzed for Swi3 protein by Western blot analysis. The Superose 6 column was calibrated with marker proteins thyroglobulin (669 kDa; fraction 25), apoferrin (443 kDa; fraction 28), β-amylase (200 kDa; fraction 30), and bovine serum albumin (66 kDa; fraction 33). Void volume was estimated by using high-molecular-mass plasmid DNA. (B) Elution of Hpr1p from gel filtration. Whole-cell extract was prepared from *HPR1* wild-type strain CY184 and fractionated on an FPLC Superose 6 gel filtration column, and elution of Hpr1 protein was followed by Western blot analysis. Crude extract prepared from an *hpr1-102*::*HIS3* strain (CY527) was loaded in lane 2 as a control. (C) Whole-cell extract was prepared from CY184 and fractionated on an FPLC Superose 6 gel filtration column. Fractions were analyzed for Swi3 protein by Western blot analysis.

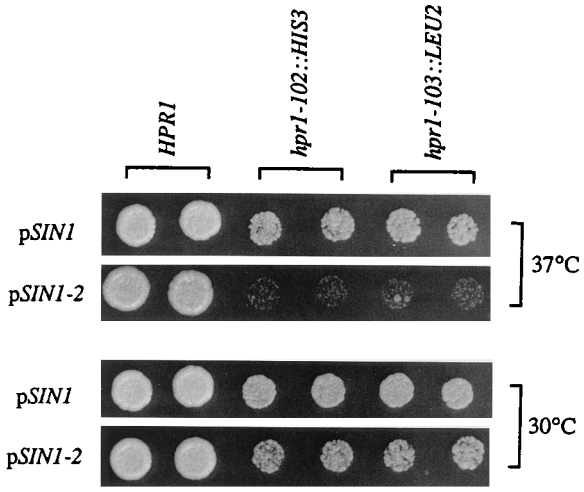

FIG. 5. Synthetic growth defect of *hpr1* mutants with *SIN1-2* at 37°C. The *SIN1-2* dominant mutant (pCB352) and wild-type *SIN1* (pCB351) were harbored by *CEN.ARS* plasmids and were transformed into *HPR1* (CY184), *hpr1-102*::*HIS3* (CY527), and *hpr1-103*::*LEU* (YZY3) strains at 30°C. Purified transformants were grown in liquid medium at 30°C to saturation. The cell density was adjusted by A_{600} reading. Equal numbers of cells were applied on the selective plates and then incubated at 30 and 37°C as indicated.

In the Singh and Klar system, expression of the *E. coli* Dam methylase from an integrated plasmid causes efficient methylation of many *Sau*3AI (GATC) sites in the yeast genome. *S. cerevisiae* contains little or no naturally methylated DNA (36). The degree to which any given *Sau*3AI site is methylated in vivo can be assessed by subsequently digesting purified genomic DNA with *Sau*3AI and two *Sau*3AI isoschizomers that are methylation state-sensitive restriction enzymes. *Sau*3AI cuts GATC sites regardless of the methylation state of the adenine, whereas *Dpn*I cuts only dimethylated GATC sites and *Mbo*I cuts only unmethylated GATC sites.

GAL1. We introduced the *E. coli dam* gene into an *HPR1* strain by integrating plasmid YIpDAM (16) at the *URA3* locus and then disrupted *hpr1* with plasmid pYZ33. These isogenic strains, YZY8 (*HPR1 dam*$^+$) and YZY9 (*hpr1-103*::*LEU2 dam*$^+$), were used in this study. To examine methylation sensitivity in the *GAL1* promoter, cultures were grown separately in galactose, glucose, or raffinose. Figure 7A shows a partial restriction map of the *GAL1-10* divergent promoter region. Genomic DNA was isolated and digested to completion with *Eco*RI and *Ava*I. This generates a 0.85-kb fragment that spans

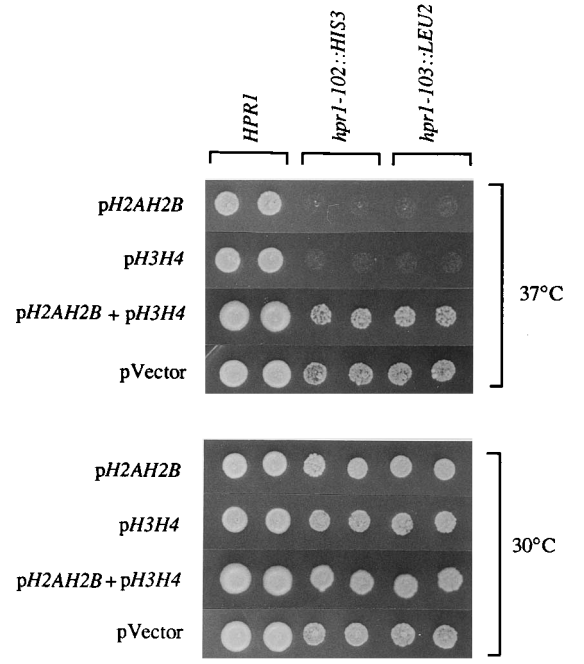

FIG. 6. Synthetic growth defect of *hpr1* mutants with elevated histone gene dosage. pH2AH2B (pYZ69) contains *HTA1* and *HTB2*, which encode histones H2A and H2B, respectively. pH3H4 (pRM200) contains *HHT2* and *HHF2*, which encode histones H3 and H4, respectively. pVector is pRS314. These *CEN.ARS* plasmids were transformed or cotransformed into CY184 (*HPR1*), CY527 (*hpr1-102*::*HIS3*), and YZY3 (*hpr1-103*::*LEU2*) at 30°C. Purified transformants were incubated at both 30 and 37°C. The experiment was performed as described in the legend to Fig. 5.

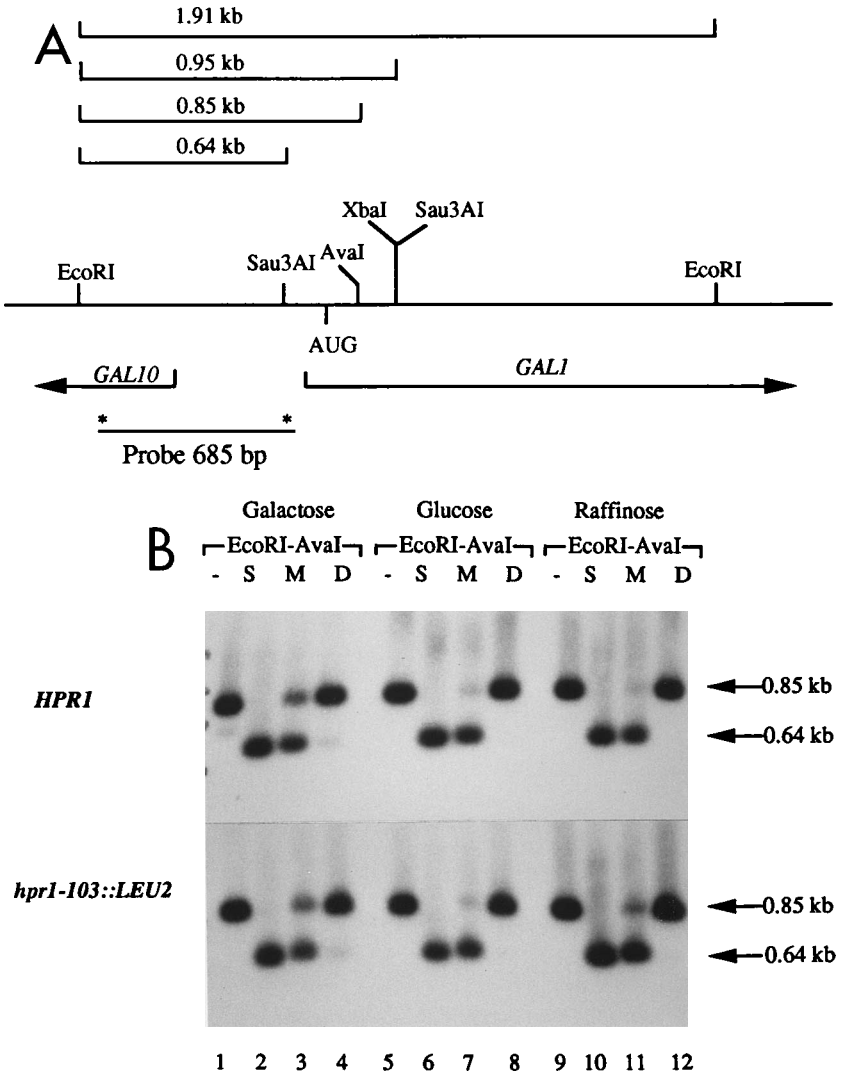

FIG. 7. *hpr1* mutants display altered in vivo methylation sensitivity of the *GAL1-10* divergent promoter region. (A) Partial restriction map of the *GAL1-10* divergent promoter. The position of the 685-bp probe is marked. (B) In vivo methylation sensitivity of the *GAL1* promoter *Sau*3AII site in *HPR1*/*dam*$^+$ (YZY8) and *hpr1-103*::*LEU2*/*dam*$^+$ strains. Yeast chromosomal DNA was isolated and purified from exponential-phase cultures grown in medium containing either galactose, glucose, or raffinose. Purified DNA (5 μg) was digested with *Eco*RI-*Ava*I and then with *Sau*3AII (S), *Mbo*I (M), or *Dpn*I (D). −, DNA was digested with only *Eco*RI-*Ava*I. Digested DNA was loaded on a 1% agarose gel. DNA was transferred to a nylon membrane. The membrane was probed with a radiolabeled 685-bp *Nde-Xba*I fragment isolated from pRD56. Arrows indicate the 0.85- and 0.64-kb DNA fragments.

the leftmost (promoter) *Sau*3AI, site in Fig. 7A. Further digestion of genomic DNA was then performed using *Sau*3AI, *Dpn*I, and *Mbo*I to assess the extent of methylation of the promoter *Sau*3AI site. Digestion with any of these isoschizomers generates a 0.64-kb fragment (Fig. 7B) and a 0.21-kb fragment that runs off the gel under these conditions.

The wild-type strain shows greater sensitivity to methylation when grown in galactose (transcriptionally induced) than in either glucose (transcriptionally repressed) or raffinose (non-induced, nonrepressed) medium. For the *HPR1* strain, digestion with *Eco*RI and *Ava*I generates a 0.85-kb fragment (Fig. 7B, lanes 1, 5, and 9) that is detected with the 685-bp probe shown in Fig. 7A. Further digestion with *Sau*3AI generates the 0.64-kb *Eco*RI-*Sau*3AI fragment (lanes 2, 6, and 10). Digestion with *Sau*3AI is complete because it is insensitive to adenine methylation. Digestion of the *Eco*RI-*Ava*I 0.85-kb fragment with *Mbo*I and *Dpn*I allows one to determine the extent of methylation of the promoter *Sau*3AI site. Digestion with *Mbo*I occurs only in the fraction of *Eco*RI-*Ava*I fragments that are not methylated at the *Sau*3AI site. In lane 3 digestion with *Mbo*I is 64.1% complete (quantitation by phosphorimaging), indicating that 64.1% of the *Sau*3AI sites are unmethylated and that 35.9% are hemi- or dimethylated. Digestion with *Dpn*I occurs only in the fraction of *Eco*RI-*Ava*I fragments that are methylated on both strands (dimethylated). Quantitation of lane 4 indicates that 5.4% of the GATC sites are dimethylated. In contrast, on glucose no dimethylation is detectable (lane 8) and 88.3% of the *Sau*3AI sites are unmethylated while 11.7% are hemi- or dimethylated (lane 7). To simplify the analysis, one can compare the fraction of GATC sites that are resistant to *Mbo*I digestion (i.e., the percent hemi- or dimethylated) in glucose- and galactose-grown wild-type cells. On glucose the proportion of the promoter GATC site that is hemi- or dimethylated is 11.7% (lane 7), and on galactose it is 35.9% (lane 3). Raffinose-grown cells display an intermediate level of

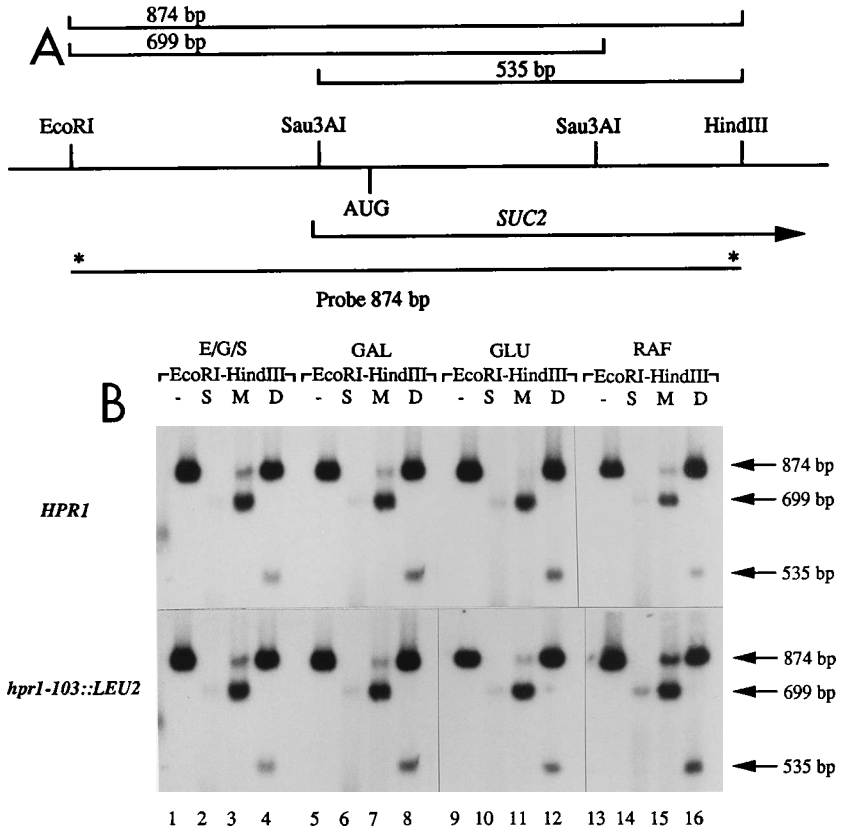

FIG. 8. *hpr1* mutants display altered in vivo methylation sensitivity at the *SUC2* promoter. (A) Partial restriction map of the *SUC2* promoter. The position of the 874-bp probe is marked. The promoter *Sau*3AII site is located 28 bp upstream from the TATA box. (B) In vivo methylation sensitivity of the of *SUC2* promoter *Sau*3AII sites in *HPR1*/*dam*$^+$ (YZY8) and *hpr1-103*::*LEU2*/*dam*$^+$(YZY9) strains. Yeast chromosomal DNA was isolated and purified from exponential-phase cultures. The medium was either YEP–2% ethanol–2% glycerol–2% sucrose (E/G/S), YEP-galactose (GAL), YEP-raffinose (RAF), or YEP-glucose (GLU). Five micrograms of purified DNA was digested with *Eco*RI-*Hin*dIII first and then with *Sau*3AII (S), *Mbo*I (M), and *Dpn*I (D). −, DNA was digested with only *Eco*RI-*Hin*dIII. Digested DNA was loaded on a 1.75% Nu-Sieve GTG and 0.75% GTG agarose gel. DNA was transferred to a nylon membrane. The membrane was probed with a radiolabeled 874-bp *Eco*RI-*Hin*dIII fragment isolated from B1281. Arrows designate the 874-, 699-, and 535-bp fragments.

hemi- or dimethylation, 17.0% (lane 11). This is qualitatively consistent with published results (42).

In an *hpr1-103*::*LEU2* mutant, the levels of hemi- and dimethylation when cells are grown in glucose (14.5 versus 11.7% in *HPR1* strains; Fig. 7B, lane 7) or galactose (38.1 versus 35.9% in *HPR1* strains; lane 3) are similar to those in the wild type. However, the *hpr1-103*::*LEU2* mutant displays considerably greater hemi- and dimethylation (25.8 versus 17.0% in *HPR1* strains; lane 11) of the promoter GATC site when cells are grown in raffinose. This difference has been observed in three independent experiments. The *hpr1-103*::*LEU2* mutant also showed slightly increased sensitivity to methylation of the *Sau*3AI site in the coding region (data not shown). This small difference was the same on all three carbon sources. Thus, the *hpr1* mutant displays altered in vivo methylation sensitivity at the *GAL1* promoter under noninducing conditions.

SUC2. The *SUC2* gene is transcriptionally repressed on glucose medium, partially derepressed on galactose and raffinose medium, and fully derepressed on ethanol-glycerol-sucrose medium (29). Our methylation sensitivity experiments at *SUC2* indicate the following: (i) an *hpr1-103*::*LEU2* mutant shows elevated methylation sensitivity of the promoter *Sau*3AI site and the coding sequence *Sau*3AI site on raffinose medium (partially derepressed) and to a lesser extent on ethanol-glycerol-sucrose medium (fully derepressed), and (ii) on all carbon sources and in both the *hpr1* mutant and wild-type strains, the promoter *Sau*3AI site is more sensitive to methylation than the site within the coding region.

These conclusions were based on the following data. Figure 8A shows a restriction map of the *SUC2* promoter region. Genomic DNAs were purified and digested to completion with *Eco*RI and *Hin*dIII. This generates an 874-bp fragment that can be detected by using the same 874-bp fragment as a hybridization probe. Further digestion at one or both of the *Sau*3AI sites could generate any of the set of fragments shown schematically in Fig. 8A. Digestion at the *Sau*3AI promoter site (located 28 bp upstream from the TATA box) (38) alone generates 535- and 339-bp fragments, whereas digestion of the coding region site alone generates 699- and 175-bp fragments. Digestion at both *Sau*3AI sites would yield an additional 360-bp fragment. Thus, excluding the case where significant digestion occurs at both *Sau*3AI sites, the appearance of the 535-bp fragment is indicative of digestion at the promoter GATC site and the appearance of the 699-bp fragment is indicative of digestion at the coding region GATC site. The three smaller fragments run off the gels under these conditions and are not shown.

Digestion with *Dpn*I, which cuts only dimethylated GATC sites, shows that under all conditions and in both mutant and wild-type strains, the 535-bp fragment is more abundant than the 699-bp fragment, which is not detectable. This indicates that the promoter GATC site is methylated to a greater extent

than the coding region site under all conditions. Furthermore, the *hpr1-103*::*LEU2* mutant shows greater dimethylation of the promoter GATC site than wild-type strains on raffinose (Fig. 8B, lane 16; 36.4% versus 16.6% in *HPR1* strains; quantitation by phosphorimaging). Similar levels of dimethylation of the promoter GATC site are seen on glucose (lane 12; 25.6% for *hpr1* strains versus 25.7% for *HPR1* strains) and galactose (35.8% for *hpr1* strains versus 31.7% for *HPR1* strains). On ethanol-glycerol-sucrose the *hpr1-103*::*LEU2* mutant shows somewhat more dimethylation of the promoter GATC (lane 4; 42.1% for *hpr1* strains versus 33.0% for *HPR1* strains). These results are different from those at *GAL1* in that an *hpr1* mutant shows hypersensitivity to *SUC2* methylation under conditions in which *SUC2* is being expressed, whereas at *GAL1* hypersensitivity is seen under conditions in which *GAL1* is not being expressed.

Digestion with *Mbo*I, which cuts only unmethylated GATC sites, shows that the coding region *Sau*3AI site is frequently unmethylated (because the 699-bp fragment is abundant; for example, Fig. 8B, lane 3, 12.5% hemi- or dimethylated for *HPR1*). To assess methylation at the promoter *Sau*3AI site under conditions in which there is significant digestion at the coding site (such as in lane 3), one must determine the abundance of both the 535-bp fragment and the double digestion product (360 bp) which is not visible in Fig. 8B. Quantitation of these fragments from lane 3 indicates that the promoter *Sau*3AI site is hemi- or dimethylated 58.9% of the time in *HPR1* strains versus 12.5% for coding region site. This is consistent with the *Dpn*I digestion data which indicate that the promoter site is, under all conditions, more sensitive to methylation than the coding region site. *Mbo*I digests also indicate that in the *hpr1-103*::*LEU2* mutant, the coding region *Sau*3AI site is more sensitive to methylation than in *HPR1* on raffinose, as evidenced by the greater relative abundance of the 699-bp fragment (lane 15; 50.5% hemi- or dimethylation versus 15.6% in *HPR1* strains). In addition, the promoter *Sau*3AI site is also more readily methylated in *hpr1-103*::*LEU2* mutants, as evidenced by the greater abundance of the 360-bp double digestion product following *Mbo*I digestion (Fig. 8B, lane 15) in *HPR1* (25.5% hemi- or dimethylation) versus *hpr1* (70.7% hemi- or dimethylation [data not shown]) strains. Methylation of both *Sau*3AI sites is not different between *hpr1* and *HPR1* strains on glucose, galactose, or ethanol-glycerol-sucrose medium, as judged by *Mbo*I digestion. In summary, the *hpr1-103*::*LEU2* mutant displays greater methylation sensitivity of both the *SUC2* promoter and coding region GATC sites than an *HPR1* strain under a partially derepressed condition (raffinose).

DISCUSSION

We present evidence that *HPR1* is required for efficient transcription of a subset of yeast genes. Many of the genes that require *HPR1* for transcription also require an intact *SWI-SNF* complex (51) and the carboxy-terminal tail of the largest subunit of RNA polymerase II (52). We find that Hpr1p is not a component of the *SWI-SNF* complex but may be a component of a distinct 1-MDa complex. Altered in vivo Dam methylase sensitivity is observed at two *HPR1*-dependent promoters (*GAL1* and *SUC2*). The requirement of *HPR1* for efficient *HO* and *CCB*::*lacZ* transcription is partially suppressed by a deletion of *SIN1*, which encodes a protein related to a known chromatin component. Strains with null mutations in *HPR1* display a synthetic growth defect at 37°C in combination with a semidominant allele of *SIN1* (26) or when histone gene dosage is altered and imbalanced by expression of either H3 and H4 or H2A and H2B from *CEN.ARS* plasmids. Simultaneous gene dosage increases for all four histones restore viability to *hpr1* deletion mutants. The sensitivity of *hpr1* mutants to alterations in chromatin components suggests that the transcriptional and recombinational phenotypes are a secondary consequence of aberrant chromatin structure. We propose that *HPR1* influences chromatin structure in a manner required to facilitate transcription of some genes.

***HPR1* and the *SWI* genes.** Although many of the genes that require *HPR1* for transcription are the same as those requiring the *SWI-SNF* products, Hpr1p does not appear to be part of or required for assembly of the large protein complex containing Swi1p, Swi2p, Swi3p, Snf5p, and Snf6p, because the *SWI-SNF* complex migrates normally on Superose 6 when isolated from an *hpr1* deletion mutant. Nearly all of the Hpr1p in the cell is present as part of a separate complex of about 10^6 Da. Presumably the *SWI-SNF* and Hpr1p-containing complexes are required for separate steps in the process of transcriptional induction. One feature distinguishing between *HPR1* and *SWI* gene functions is that *HPR1* is required for transcription of a *CCB* element UAS (Table 3) while the *SWI* products are not (34).

Deletion of the C-terminal tail of the largest subunit of RNA polymerase II, RPB1p, results in transcription defects that are also similar to those in *swi-snf* mutants (31, 52). The RNA polymerase II holoenzyme has been purified and shown to consist of a complex that is somewhat smaller than the *SWI-SNF* complex (24). The complex contains several general transcription factors (24), *SRB* gene products (21, 46), and several unidentified proteins. However, Hpr1p does not appear to be a member of the holoenzyme complex (53).

Like the *SWI* products, Hpr1p does not contain any of the motifs characteristic of DNA binding proteins such as basic/helix-loop-helix, helix-turn-helix, homeodomain, or bZIP domains (18). It does contain two domains of 54 and 130 amino acids with 25% identity and 50% similarity to *S. cerevisiae* DNA topoisomerase I (2). The function of these homology regions is unclear, since they lie in very poorly conserved regions of the *TOP1* product. Highly conserved regions found in the *TOP1* family of type I DNA topoisomerases are not found in the *HPR1* product. Thus, the regions in *TOP1* that are homologous to *HPR1* may be required for an aspect of *TOP1* function that does not involve its DNA-relaxing activity. As has been suggested for the *SWI* products (34), Hpr1p or the Hpr1 complex could facilitate communication between gene-specific activators and general transcription factors or might interact with the carboxy-terminal domain of the largest subunit of RNA polymerase II. Alternatively, *HPR1* could facilitate nucleosome disassembly during transcription. Attempts to determine the molecular mechanism of *HPR1* and *SWI* gene function will be greatly facilitated by the development of an in vitro transcription system in *S. cerevisiae* that is dependent on chromatin structure.

In vivo Dam methylase sensitivity of *HPR1*-dependent promoters. Methylation sensitivity of promoter regions is generally higher under conditions of active transcription and lower under conditions of repression (49). This is presumed to be the result of remodeling of chromatin necessary to facilitate transcription. Expression of the *E. coli* Dam methylase in *S. cerevisiae* (42), which lacks natural DNA methylation (36), allows one to examine any GATC sequence in the genome for its sensitivity to methylation without disrupting the natural state of chromatin structure by extraction of nuclei. The *GAL1*, *GAL7*, *GAL10*, *PHO5*, *HMR***a**, *HML***a**, *STE2*, and *STE3* promoters were shown to undergo a transition to a state of en-

hanced sensitivity to methylation following activation of transcription (42).

Mutations in *HPR1* enhance the in vivo methylation sensitivity at two *HPR1*-dependent promoters, *GAL1* and *SUC2*. At *GAL1*, methylation hypersensitivity is observed on the noninducing carbon source raffinose. This suggests that the chromatin structure at *GAL1* is abnormal in the absence of *HPR1*, even when *GAL1* is not being expressed. This result is more meaningful than a decrease in methylation sensitivity under inducing conditions, a result that could be a consequence of a failure at any point in the signal transduction pathway leading to transcriptional activation of that gene. Surprisingly, there is little or no *HPR1* dependency on methylation sensitivity under inducing conditions for *GAL1*. In contrast to *GAL1*, the greatest difference in *SUC2* methylation sensitivity is observed under a partially derepressing condition. While the signal-to-noise ratio in these experiments is low (wild type changes from 10% methylation on glucose to 30% methylation on galactose), we have consistently observed a significant enhancement of promoter methylation sensitivity in *hpr1* mutants grown on raffinose.

Our results also indicate that upon induction of the SUC2 promoter, an enhanced in vivo methylation sensitivity is observed in a promoter GATC site similar to what was previously observed at *GAL1*, *PHO5*, and *HMR*. Furthermore, the methylation sensitivity of the promoter site in *SUC2* is much greater under all conditions than that of a GATC site in the coding sequence.

Synthetic growth defect of *hpr1* mutants in the presence of *SIN1-2* and elevated histone gene dosage. Whereas a deletion of *SIN1* partially suppresses the defect in *HO* transcription in *hpr1* mutants, the semidominant *SIN1-2* allele, isolated as a suppressor of a *swi1* deletion, is not tolerated at 37°C in the absence of *HPR1*. *SIN1* is distantly related to the HMG1 class of high-mobility group proteins (26), making the *SIN1* product a possible chromatin component. If the *SIN1* product were ordinarily removed from chromatin by Hpr1p or the Hpr1p complex, then the *SIN1-2* mutant could encode a protein that is lethal if bound permanently to chromatin.

Another genetic interaction between an *hpr1* mutation and a chromatin component is the severe growth defect observed in the presence of elevated H3-H4 or H2A-H2B gene dosage. Overexpression of histones has been shown to affect gene expression in yeast cells (9), and an imbalance in the expression of histone subunits H3 and H4 or H2A and H2B can result in chromosome instability and cell death (28). These studies used high-copy-number plasmids, while we see an effect using low-copy-number plasmids. If *HPR1* were involved in assembly or disassembly of histones onto DNA following or preceding DNA replication or transcription, then an *hpr1* mutant might be sensitive to even a slight imbalance in levels of the different subunits. Supporting this argument is the result that expression of histones H2A and H2B from a *CEN.ARS* plasmid suppresses the growth defect observed when H3 and H4 are made from a *CEN.ARS* plasmid in *hpr1* strains.

Hyperrecombination and transcription. Enhanced rates of transcription are associated with enhanced rates of recombination at both RNA polymerase I (48) and RNA polymerase II (45) promoters in *S. cerevisiae*. However, the phenotype of an *hpr1* null mutation is diminished transcription and enhanced recombination. One major class of mitotic hyperrecombination mutants is those that affect DNA synthesis (14). We suggest that these facts can be reconciled as follows. In the absence of *HPR1*, chromatin structure is altered such that DNA replication is slowed, leading to persistent nicks that are recombinogenic (14). This same altered chromatin structure is incompatible with effective transcription of a subset of mRNAs. This is supported by our finding that at least one *HPR1*-regulated promoter (*GAL1*) is hypersensitive to in vivo methylation under a noninducing condition. This indicates that *hpr1* mutants may have altered chromatin structure even in the absence of active transcription.

ACKNOWLEDGMENTS

We thank Cora Coburn for the initial observation that an *hpr1* mutant was defective in *HO* transcription and that this defect can be suppressed by a *SIN1* deletion. We also thank Ira Herskowitz, Gerry Fink, Michael Grunstein, and Robert Malone for many strains and plasmids. We are grateful to Ira Herskowitz, Erin O'Shea, David Pellman, and Nikki Levin for comments on the manuscript.

This work was supported by NIH grant GM46877 to M.F.C. and NIH grant GM49650-01 and March of Dimes Birth Defects Foundation grant II 5-FY93-0922 to C.L.P. C.L.P. is a scholar of the Leukemia Society of America.

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