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Characterization of *HIR1* and *HIR2*, Two Genes Required for Regulation of Histone Gene Transcription in *Saccharomyces cerevisiae*

PETER W. SHERWOOD,[1,2] SHIRLEY V.-M. TSANG,[1] AND MARY ANN OSLEY[1,2]*

Program in Molecular Biology, Sloan Kettering Cancer Center,[1] and Cornell University Graduate School of Medical Sciences,[2] New York, New York 10021

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The products of the *HIR1* and *HIR2* genes have been defined genetically as repressors of histone gene transcription in *S. cerevisiae*. A mutation in either gene affects cell cycle regulation of three of the four histone gene loci; transcription of these loci occurs throughout the cell cycle and is no longer repressed in response to the inhibition of DNA replication. The same mutations also eliminate autogenous regulation of the *HTA1-HTB1* locus by histones H2A and H2B. The *HIR1* and *HIR2* genes have been isolated, and their roles in the transcriptional regulation of the *HTA1-HTB1* locus have been characterized. Neither gene encodes an essential protein, and null alleles derepress *HTA1-HTB1* transcription. Both *HIR* genes are expressed constitutively under conditions that lead to repression or derepression of the *HTA1* gene, and neither gene regulates the expression of the other. The sequence of the *HIR1* gene predicts an 88-kDa protein with three repeats of a motif found in the G_β subunit of retinal transducin and in a yeast transcriptional repressor, Tup1. The sequence of the *HIR2* gene predicts a protein of 98 kDa. Both gene products contain nuclear targeting signals, and the Hir2 protein is localized in the nucleus.

The core histone genes in *Saccharomyces cerevisiae* are under two forms of transcriptional control. Temporal regulation occurs during the cell cycle, where transcription of all four histone gene loci is restricted to late G_1-early S phase (5, 15, 17, 38). Autogenous regulation is a unique response of the *HTA1-HTB1* locus to altered dosage of the genes encoding H2A and H2B. Transcription of this locus is repressed when extra copies of *HTA-HTB* genes are present and derepressed when the *HTA-HTB* copy number is reduced by half (22). Both temporal and autogenous regulation depend on common elements. One element is a *cis*-acting negative site in the *HTA1-HTB1* promoter. When the negative site is deleted from this locus, *HTA1-HTB1* transcription is derepressed throughout the cell cycle (21, 25) and not repressed by elevated levels of H2A and H2B (22). The second element is a set of *trans*-acting *HIR* (histone regulatory) gene products. Mutations in the *HIR1*, *HIR2*, and *HIR3* genes confer phenotypes similar to deletion of the *HTA1-HTB1* negative site; the *HTA1-HTB1* locus becomes constitutively transcribed during the cell cycle (26), and it loses feedback repression by H2A and H2B (22). The concordance of these phenotypes is provided by the observation that the three *HIR* gene products have been characterized genetically as repressors that act through the negative site in the *HTA1-HTB1* promoter (22, 26).

In this study, we have isolated the *HIR1* and *HIR2* genes to determine what roles their gene products play in transcriptional regulation. Neither gene is essential, and null alleles confer the same regulatory defects as the original *hir1* and *hir2* mutations. Both genes encode large proteins that have not been identified previously. The predicted Hir1 protein contains three copies of a motif first noted in the G_β subunit of bovine retinal transducin (11) and also found in a several other yeast proteins, including the general transcriptional repressor, Tup1 (40). Both Hir1 and Hir2 contain a bipartite nuclear localization signal present in nucleoplasmin, Swi5, Cdc25, and a large number of other nuclear proteins (7, 28), and we have demonstrated that Hir2 is a nuclear protein by indirect immunofluorescence.

We have found that the Hir1 and Hir2 proteins are not limiting for their regulatory functions in the cell. Both *HIR* genes are constitutively expressed under a variety of conditions in which transcription of the *HTA1-HTB1* locus is differentially regulated, and neither gene represses *HTA1* transcription when overexpressed. Since the *HIR1* and *HIR2* genes do not appear to encode site-specific DNA binding proteins, the Hir proteins may function as corepressors to assist a repressor that interacts with sequences at the *HTA1-HTB1* negative site.

MATERIALS AND METHODS

Strains and genetic methods. Standard yeast genetic procedures and media were used (33). The yeast strains used in this study are listed in Table 1. The *his4-912δ* and *lys2-128δ* alleles cause histidine and lysine auxotrophies and have been characterized previously (3, 4, 8, 9, 36, 43). The Spt phenotype of strains was determined by replica plating onto SC medium lacking histidine or lysine and then by incubation at 23°C for 3 to 5 days (43). Transformation of yeast cells was by the lithium acetate method (19).

Plasmids. pHIR1 and pHIR2 were isolated from a library of yeast genomic DNA cloned into YCp50 (29) and contain 10-kb (*HIR1*) or 12-kb (*HIR2*) DNA inserts. YCp50-HIR1 is a 3.4-kb *Cla*I-*Sal*I fragment from pHIR1 cloned into the *Cla*I-*Sal*I sites of YCp50. YCp50-HIR2 is a 3.8-kb subclone of pHIR2 constructed by deletion of an 8.2-kb *Cla*I fragment from pHIR2. YIpHIR1 is a 3.5-kb *Eco*RI-*Sal*I fragment from pHIR1 cloned into the *Eco*RI-*Sal*I sites of plasmid YIp5, and YIpHIR2 is a 3.9-kb *Sal*I-*Cla*I fragment from pHIR2 cloned into the *Sal*I-*Cla*I sites of YIp5. YEpHIR1 is a 3.5-kb

* Corresponding author.

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source
PS2138-16B	*MATa hir1-1 his4-912δ lys2-128δ HTA1-HTB1::HTA1-lacZ::LEU2 ura3-52*	34
PS2-7A	*MATa hir2-1 his4-912δ lys2-128δ HTA1-HTB1::HTA1-lacZ::LEU2ura3-52*	34
PS-I18	*MATα HIR1::YIp5-HIR1 his4-912δ lys2-128δ ura3-52*	This study
PS-I27	*MATa hir2::YIp5-HIR2 his4-912δ lys2-128δ HTA1-HTB1::HTA1-lacZ::LEU2 ura3-52*	This study
PSFWΔ1-A	*MATa Δhir1::URA3 his4-912δ lys2-128δ ura3-52*	This study
PSFWΔ2-A	*MATα Δhir2::URA3 his4-912δ lys2-128δ ura3-52*	This study
W303Δ1	*MATa Δhir1::LEU2 ade2-1 ura3-52 trp1 can1 his3 leu2-3,-112*	This study
W303-D	*MATa/MATα leu2-3,-112/leu2-3,-112 ade2-1/ade2-1 ura3-52/ura52 trp1/trp1 can1/can1 his3/his3*	This study
PSD100	*MATa/MATα HIR1::YIp5-HIR1/hir1 ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*	This study
PSD200	*MATa/MATα hir2::YIp5-HIR2/HIR2 ura3-52/ura3-52 his4-912δ/his4-912δ lys2-128δ/lys2-128δ*	This study
2C37-2A	*MATα hir3-1 his4-912δ lys2-128δ ura3-52*	34
MSS12-2B	*MATα HIR4-1 his4-912δ lys2-128δ ade2-1 ura3-52*	M. Spector
FW1238	*MATα his4-912δ lys2-128δ ura3-52*	F. Winston
L210	*MATa spt10-11 his4-917 lys2-128δ trp1Δleu2-3,112*	F. Winston
FW1619	*MATα spt21-1 his4-917 lys2-128δ leu2 ura3-52*	F. Winston
DN105	*MATα Δhta2-htb2::URA3 his3 ura3-52*	D. Norris
DN106	*MATα Δhta1-htb1::URA3 his3 ura3-52*	D. Norris
M90-11D	*MATa cdc28 leu2-3,112 thr1 met2 his3*	M. Whiteway
1078-2B	*MATa cdc4-3 leu2-3,112 trp1 ura3-52 lys2 lys5 his7 ade2 ade6*	B. Beyers
RM14-4B	*MATa cdc7-1 bar1 his6 trp1 ura3 leu2-3,112 ade1*	L. Hartwell
2754-7-4	*MATa cdc8 his7 leu2-3,112*	L. Hartwell
MSS21-5A	*MATα cdc15 ura3-52 trp1*	M. Spector

*Eco*RI-*Sal*I fragment from pHIR1 cloned into the *Eco*RI-*Sal*I polylinker sites of plasmid YEp352. YEpHIR2 was constructed by ligation of a 2.3-kb *Aat*II-*Cla*I fragment from YEp24 (containing the 2 μm circle replication determinant) to an 8.0-kb *Aat*II-*Cla*I fragment from YIpHIR2.

A 9-amino-acid epitope derived from the influenza virus hemagglutinin protein (ha [42]) was inserted into both *HIR1* and *HIR2* by oligonucleotide-directed mutagenesis. Plasmid YEpHIR1-ha was constructed by inserting a double-stranded oligonucleotide (5′-CTAGTTAGCCATACGACGT CCCAGACTACGCTA-3′) into a unique *Xba*I site within the putative open reading frame of *HIR1* in plasmid YEpHIR1. Plasmid YEpHIR2-ha was constructed in two steps. A double-stranded oligonucleotide (5′-CTACCCATACGACG TCCCAGACTACGCTGG-3′) was first inserted into a unique *Pvu*II site within the putative coding region of pHIR2. A 3.9-kb *Sal*I-*Cla*I fragment containing the HIR2 coding region with the ha epitope was then substituted for the same *Sal*I-*Cla*I fragment in YEpHIR2.

RNA analysis. Total RNA was isolated as described previously (23), and 20 μg was subjected to formaldehyde-agarose gel electrophoresis and Northern (RNA) blot analysis (30). A 2.4-kb *Sac*I fragment from the *HTA1-HTB1* locus (16) was used to detected *HTA1* transcripts. This fragment contains the entire *HTA1* gene, as well as a region of the constitutively transcribed *AKY2* (*PRT1*) gene (15, 24). A 1.7-kb *Bam*HI-*Sal*I fragment from YCp50-HIR1 was used to detect *HIR1* transcripts, and a 1.5-kb *Eco*RI-*Sal*I fragment from YCp50-HIR2 was used to detect *HIR2* transcripts.

The 5′ ends of *HIR1* and *HIR2* mRNA were estimated by S1 nuclease protection analysis with total RNA prepared from cells transformed with YEpHIR1 or YEpHIR2. A 1.6-kb *Cla*I-*Acc*I fragment from YCp50-HIR1 was labelled at the *Acc*I site and a 2.6-kb *Eco*RI-*Eco*RV fragment from YIpHIR2 was labelled at the *Eco*RI site with [γ-^{32}P]ATP, by using T4 polynucleotide kinase (22, 25). These fragments were hybridized overnight at 48°C to 20 μg of RNA and analyzed as previously described on a 4% acrylamide–8 M urea gel (25).

DNA analysis. Genomic and plasmid DNAs from *S. cerevisiae* and plasmid DNAs from *Escherichia coli* were prepared as described previously (2, 33). Standard procedures were followed for subcloning DNA fragments and for identifying recombinant clones in bacteria (1, 30). Southern blot analysis (37) utilized radiolabelled DNA hybridization probes prepared by the method of random priming (10), with [α-^{32}P]dATP (NEN). The nucleotide sequence of the *HIR1* and *HIR2* genes was determined by the dideoxy chain termination method (31), with restriction enzyme fragments subcloned into bacteriophage M13mp18 or M13mp19 as templates. The complete nucleotide sequence of both strands of DNA was obtained for each gene, and the sequences spanned all restriction fragment junctions used to create M13 subclones, with the exception of the most extreme 3′ end of the *HIR2 Sal*I-*Cla*I subclone.

Histone gene regulation assay. The Hir phenotype of strains was determined as previously described by analyzing transcription of the *HTA1* gene in cells treated for 30 min with hydroxyurea (26, 34). The levels of *HTA1* RNA were determined by Northern blot analysis; a Hir$^-$ phenotype is manifested as the failure to repress *HTA1* transcription following the inhibition of DNA replication.

Construction of null alleles and genetic analysis of *HIR1* and *HIR2* clones. Plasmid pHIR1::URA3, which contains a deletion-disruption allele of *HIR1*, was constructed by replacing a 1.4-kb *Bgl*II-*Bgl*II fragment within the putative open reading frame of *HIR1* (nucleotides 1000 to 2410) in plasmid YIpHIR1 with a 1.1-kb *Bam*HI fragment containing the *URA3* gene (Fig. 1). A 1.4-kb *Bam*HI-*Sal*I fragment was isolated from pHIR1::URA3, transformed into diploid strain W303-D, and Ura$^+$ prototrophs were selected. Replacement of the *HIR1*$^+$ allele on one homolog was confirmed by Southern blot analysis. A null allele of *HIR2* was constructed in two steps. First, plasmid pBS-HIR2 was constructed by ligating a 2.9-kb *Sal*I-*Eco*RV fragment from pBluescript II KS+ (Stratagene) with a 2.3-kb *Sal*I-*Ssp*I fragment from pHIR2. Second, a 1.2-kb *Hin*dIII fragment within the putative open reading frame of HIR2 (nucleotides 472 to 1738) in plasmid pBS-HIR2 was replaced with a 1.1-kb *Hin*dIII fragment containing the *URA3* gene to construct

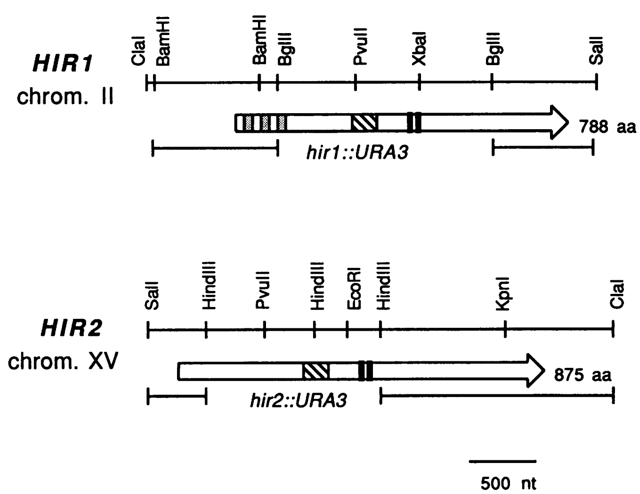

FIG. 1. Restriction enzyme maps of the *HIR1* and *HIR2* genes. The 3.4-kb *Cla*I-*Sal*I fragment containing the *HIR1* gene and the 3.8-kb *Sal*I-*Cla*I fragment containing the *HIR2* gene are shown. Arrows indicate the open reading frames for each gene. Amino acid (aa) motifs in the predicted *HIR1* and *HIR2* gene products are also indicated. Stippled bars represent three β-transducin repeats found in Hir1. Striped bars represent the region of homology between Hir1 and Hir2. Solid bars represent the putative bipartite nuclear localization signals found in Hir1 and Hir2. Regions of the *HIR1* and *HIR2* open reading frames that were deleted and replaced with *URA3* sequences are indicated below the open reading frame maps. The yeast chromosomes (chrom.) on which the two genes reside are also indicated (34). nt, nucleotides.

plasmid pHIR2::URA3 (Fig. 1). A 2.3-kb *Xba*I-*Xho*I fragment was isolated from pHIR2::URA3 and used to transform diploid strain W303-D to uracil prototrophy. Southern blot analysis showed that the $HIR2^+$ gene on one homolog had been replaced with the deletion-disruption allele.

To confirm that the authentic *HIR1* and *HIR2* genes had been isolated, we tested whether the cloned DNA directed integration of a marker gene (*URA3*) to the *HIR1* or *HIR2* locus. Plasmid YIpHIR1 was digested with *Xba*I, which cuts once within the *HIR1* insert, and transformed into strain FW1238 ($HIR1^+$ *his4-912*δ). Ura^+ transformants were selected, and Southern blot analysis was used to identify a strain in which the plasmid had integrated at the putative $HIR1^+$ locus. This strain (PS-I18) was then crossed to strain PS2138-16B (*hir1-1 his4-912*δ) to create heterozygous diploid strain PSD100 (Table 1). Plasmid YIpHIR2 was partially digested with *Pvu*II, which cuts once within the *HIR2* insert, and transformed into strain PS2-7A (*his2-1 his4-912*δ). Following selection for Ura^+ transformants, a strain containing the plasmid integrated at the putative *hir2* locus was identified by Southern blot analysis. This strain (PS-I27) was then crossed with strain FW1238 ($HIR2^+$ *his4-912*δ) to create heterozygous diploid strain PSD200 (Table 1). Following sporulation of both diploids, tetrads were analyzed for their Spt (His) phenotype.

Immunofluorescence. Strains W303Δ1 (Δ*hir1*) and PS2-7A (*hir2*) were transformed to uracil prototrophy with plasmids YEpHIR1 and YEpHIR1-ha or YEpHIR2 and YEpHIR2-ha. Indirect immunofluorescence of fixed cells was performed as previously described (27), with monoclonal antibody 12CA5 (I. Wilson, Scripps Institute), which recognizes the influenza virus hemagglutinin epitope as the primary antibody. DNA was stained with 4′,6′-diamidino-2-phenylindole dihydrochloride. Fluorescence microscopy was carried out by using a Zeiss Axioplan microscope and a Zeiss Neofluar 100× objective. Samples were photographed with TMAX-400 film. The exposure time for fluorescein micrographs was 40 s for both experimental and control strains.

Western blot (immunoblot) analysis. Total yeast proteins were extracted from cells that had been transformed with plasmids YEpHIR1, YEpHIR1-ha, YEpHIR2, or YEpHIR2-ha. Cells (10^7) were collected by centrifugation, resuspended in 110 μl of 33% glycerol–7% sodium dodecyl sulfate (SDS)–0.3 M dithiothreitol–1× protease inhibitor mix ([in micrograms per milliliter] chymostatin, 0.1; aprotinin, 2; pepstatin A, 1; E-64, 7.2; leupeptin, 0.5), and then subjected to two cycles of vortexing with glass beads for 1 min and heating at 95°C for 5 min. After the second cycle of vortexing and heating, samples were centrifuged for 15 s to remove debris, and 15 μl of the supernatant fraction was loaded directly onto a 7.5% polyacrylamide–SDS gel. Following electrophoresis, proteins were transferred to polyvinyl difluoride membranes (Immobilon-P, Millipore) by electroblotting. The filters were blocked (30) and incubated for 12 h at room temperature with a 1:250 dilution of primary antibody (12CA5 monoclonal antibody). Antibody binding was visualized by subsequent incubation with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (30) purchased from Organon Teknika. Western blots were photographed by using Polaroid type 55 film.

Nucleotide sequence accession numbers. The sequences for *HIR1* and *HIR2* have been assigned GenBank numbers LO3838 and LO3839, respectively.

RESULTS

Isolation of *HIR1* and *HIR2* genes. Mutations in either the *HIR1* or the *HIR2* gene prevent transcription of the *HTA1-HTB1* locus from being turned off when DNA replication is inhibited and thus confer a Hir^- (histone regulatory) phenotype (26, 34). We took advantage of our observation that the same mutations also suppress the transcriptional effects of the *his4-912*δ and *lys2-128*δ mutations (34) to isolate DNA clones that contained the *HIR1* or *HIR2* gene. *hir1* and *hir2* mutant strains containing the two δ insertion alleles are phenotypically His^+ Lys^+ (or Spt^-), while HIR^+ strains are His^- Lys^- (or Spt^+). Using a library of yeast genomic DNA cloned into centromere plasmid YCp50 (28), we screened for the wild-type *HIR1* and *HIR2* genes by their ability to complement the Spt^- phenotype of a *hir1* or *hir2* mutant. One transformant with a stable Spt^+ (His^- Lys^-) phenotype was identified for each *hir* mutant, and in each case the Spt^+ phenotype was associated with the presence of a unique plasmid. When the pHIR1 or pHIR2 plasmid was lost by growth of the transformants in nonselective medium, the *hir1* or *hir2* mutant strain recovered its Spt^- (His^+ Lys^+) phenotype. In addition, when the pHIR1 or pHIR2 plasmid was isolated from transformants and retransformed into *hir1* or *hir2* mutant strains, each reconferred an Spt^+ (His^- Lys^-) phenotype.

The minimal DNA sequences encoding *HIR1* or *HIR2* were identified by subcloning fragments of pHIR1 or pHIR2 into YCp50 (Fig. 1). A 3.4-kb *Cla*I-*Sal*I fragment from pHIR1 and a 3.9-kb *Sal*I-*Cla*I fragment from pHIR2 were found to complement the Spt^- phenotype of a *hir1* or *hir2* mutant (Fig. 2A). More importantly, the appropriate *HIR1* or *HIR2* subclone also corrected the Hir^- phenotype of the same *hir* mutants (Fig. 2B). Each subclone specifically complemented the two mutant phenotypes of *hir1* or *hir2*

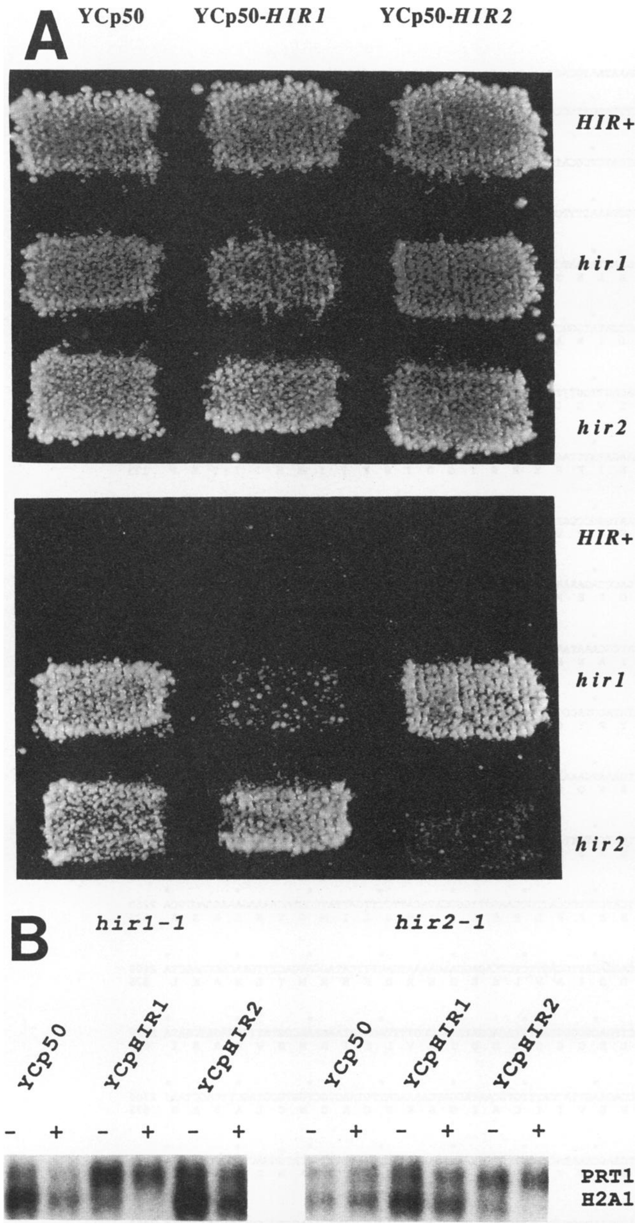

FIG. 2. Complementation of Spt⁻ and Hir⁻ phenotypes by the cloned *HIR1* and *HIR2* genes. Strains FW1238 (*HIR*$^+$), PS2138-16B (*hir1-1*), and PS2-7A (*hir2-1*) were transformed with plasmids YCp50, YCp50-HIR1, or YCp50-HIR2. (A) Spt phenotypes. Top panel, growth on SD plus His medium; bottom panel, growth on SD without His medium. (B) Hir phenotypes. Northern blot analysis of H2A1 and PRT1 (internal control) mRNAs from cells grown in the absence (−) or presence (+) of hydroxyurea for 30 min. A Spt$^-$ Hir$^-$ phenotype is manifested as growth on medium lacking histidine, and the continued production of *HTA1* mRNA in the presence of hydroxyurea.

mutants and had no effect on either the Spt$^+$ (Fig. 2A) or the Hir$^+$ phenotype of *HIR*$^+$ strains (see Fig. 7).

We confirmed that the authentic *HIR1* or *HIR2* gene had been isolated by testing whether the cloned DNA directed integration of a marker gene (*URA3*) to the *HIR1* or *hir2* locus as described in Materials and Methods. Diploid strains PSD100 and PSD200 (Table 1) were sporulated, and the segregation of both the Ura and Spt phenotypes was followed in tetrads. The Spt phenotype segregated 2$^+$:2$^-$ in 20 tetrads resulting from the *HIR1* cross, and every Spt$^+$ segregant was Ura$^+$. No Spt$^-$ segregants were observed among 21 tetrads analyzed from the *HIR2* cross. These results demonstrated tight linkage of YIpHIR1 to the *HIR1* locus and YIpHIR2 to the *hir2* locus. Hybridization of restriction enzyme fragments to yeast chromosomal DNA blots localized *HIR1* to chromosome II and *HIR2* to chromosome XV (Fig. 1) (34). We have shown previously that *HIR2* is linked to the centromere of chromosome XV (34).

Sequence analysis of *HIR1* and *HIR2*. The nucleotide sequences of the *HIR1* and *HIR2* genes revealed that these genes have not been identified previously. The *HIR1* gene (Fig. 3A) contains a single long open reading frame (2,364 nucleotides) that is sufficient to encode a 788-amino-acid protein of 87,923 Da. We mapped the 5′ end of *HIR1* RNA by an S1 nuclease protection assay and identified a single major site of transcription initiation approximately 80 nucleotides downstream of a sequence that could serve as a TATA box (nucleotides 441 to 447) and approximately 150 nucleotides upstream of the putative initiator ATG codon at nucleotides 682 to 684. An epitope-tagged Hir1 protein migrates with an apparent M_r of ~100,000 (see Fig. 8), larger than the protein predicted from the nucleotide sequence. Since translation initiated at a more TATA-proximal ATG (nucleotides 579 to 581) is terminated by a stop codon at nucleotides 662 to 664, this difference may reflect anomolous mobility of the Hir1 gene product or modification of the Hir1 protein.

The predicted Hir1 protein has three distinguishing characteristics (Fig. 4). First (Fig. 4A), it contains a region of 62 amino acids (residues 282 to 343) that is similar to a region in the predicted Hir2 protein (residues 300 to 361 [12]). In this region, 22 residues (35%) are identical, and 31 residues (50%) are conserved between Hir1 and Hir2. Second (Fig. 4B), residues 426 to 443 have the characteristics of the bipartite nuclear localization signal found in nucleoplasmin, Swi5, Cdc25, and a large number of other predominantly nuclear proteins (7, 28). Third (Fig. 4C), the amino terminus contains three copies of an amino acid repeat that was first identified in the β subunit of bovine transducin, a heterotrimeric G protein (11), and subsequently identified in a number of yeast proteins, including Ste4, Cdc4, Cdc20, Tup1, Prp4, and Mak11 (6, 11, 13, 18, 39, 40), as well as in the *Drosophila* enhancer of split gene product (14).

The sequence of the *HIR2* gene (Fig. 3B) contains a single long open reading frame (2,625 nucleotides) sufficient to encode an 875-amino-acid protein of 98,517 Da. Two sites of transcription initiation have been mapped by an S1 nuclease protection assay to approximately nucleotides 50 and 100. There is no recognizable TATA box sequence upstream of the longer protected transcript; however, the latter initiation site is approximately 30 nucleotides downstream of a potential TATA box sequence (nucleotides 64 to 70) and 40 nucleotides upstream of the putative translation initiation codon. This spacing is typical of many yeast genes and makes it likely that the ATG at nucleotides 139 to 141 represents the authentic translation initiation codon. This conclusion is strengthened by Western blot analysis of an epitope-tagged Hir2 protein, which has identified a protein of ~98,000 Da (see Fig. 8).

The putative Hir2 protein contains two features of interest (Fig. 4). The first is the region of homology to the Hir1 protein described above (residues 300 to 361). The second is the presence of a similar bipartite nuclear localization signal

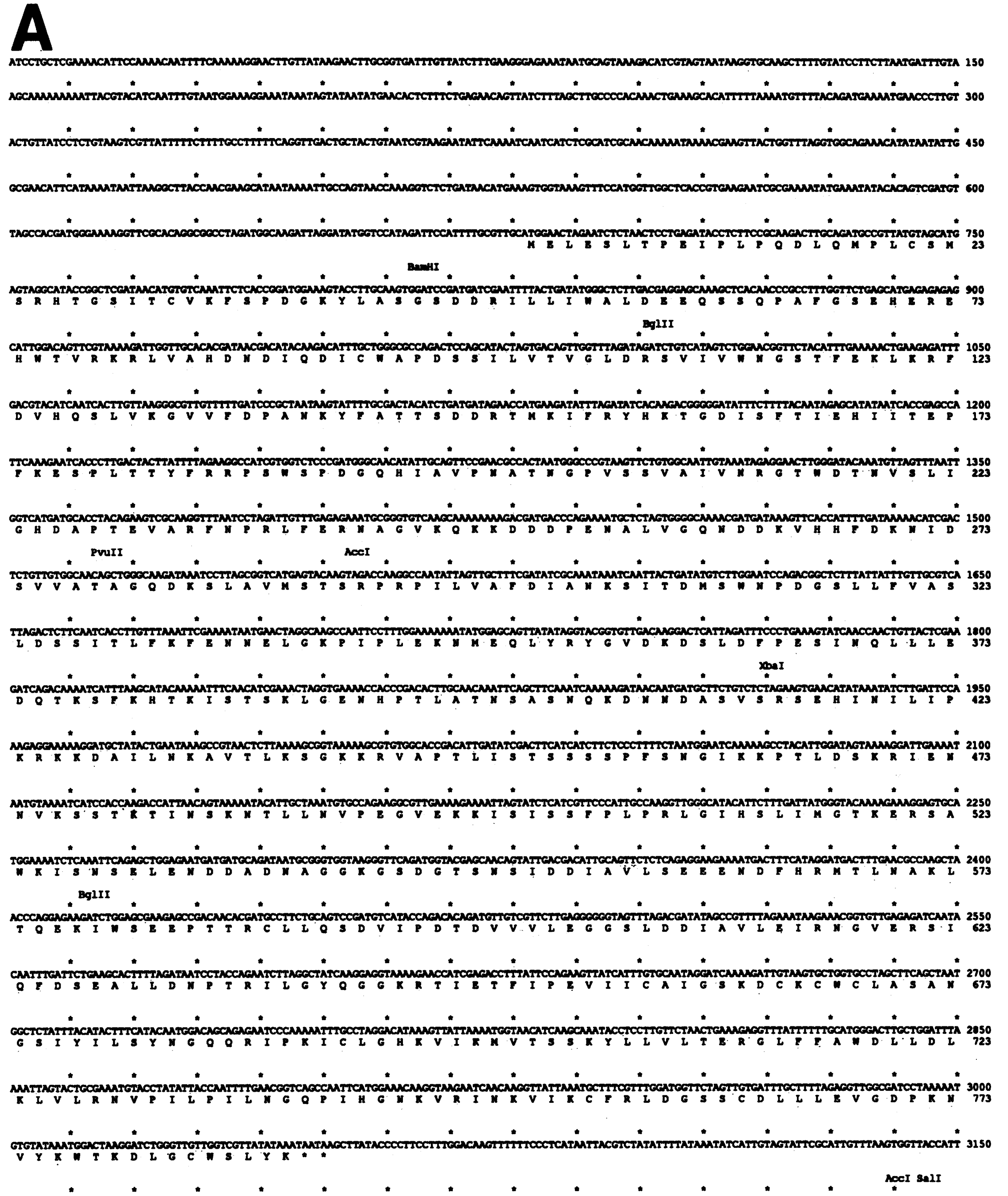

FIG. 3. Nucleotide sequences of the *HIR1* (A) and *HIR2* (B) genes and predicted amino acid residues in the Hir1 and Hir2 proteins.

between residues 454 and 472. Neither Hir1 nor Hir2 contain any of the typical motifs that have been associated with DNA binding proteins.

Phenotypes of *HIR1* and *HIR2* null mutants. Null alleles of *HIR1* and *HIR2* were constructed in vitro (Fig. 1) and integrated into diploid strain W303-D as described in Materials and Methods. Diploid strains heterozygous for either the *HIR1* or the *HIR2* null allele produced four viable spores, indicating that neither *HIR1* nor *HIR2* is an essential gene. A Δ*hir1* Δ*hir2* double mutant is also viable. We have noted no obvious phenotypic differences among isogenic wild-type, Δ*hir1*, Δ*hir2*, and congenic Δ*hir1* Δ*hir2* strains. Each strain grows at the same rate on YPD plates incubated at temperatures ranging from 16 to 37°C and has an identical generation time in liquid SD medium at 30°C. Finally, microscopic examination has revealed no morphological differences among the wild type and single- or double-deletion mutants.

Although null alleles of *HIR1* or *HIR2* do not affect cell viability, both alleles confer Hir$^-$ (see Fig. 6) and Spt$^-$ (data not shown) phenotypes, suggesting that the original *hir1-1* and *hir2-1* mutations represent loss of function alleles.

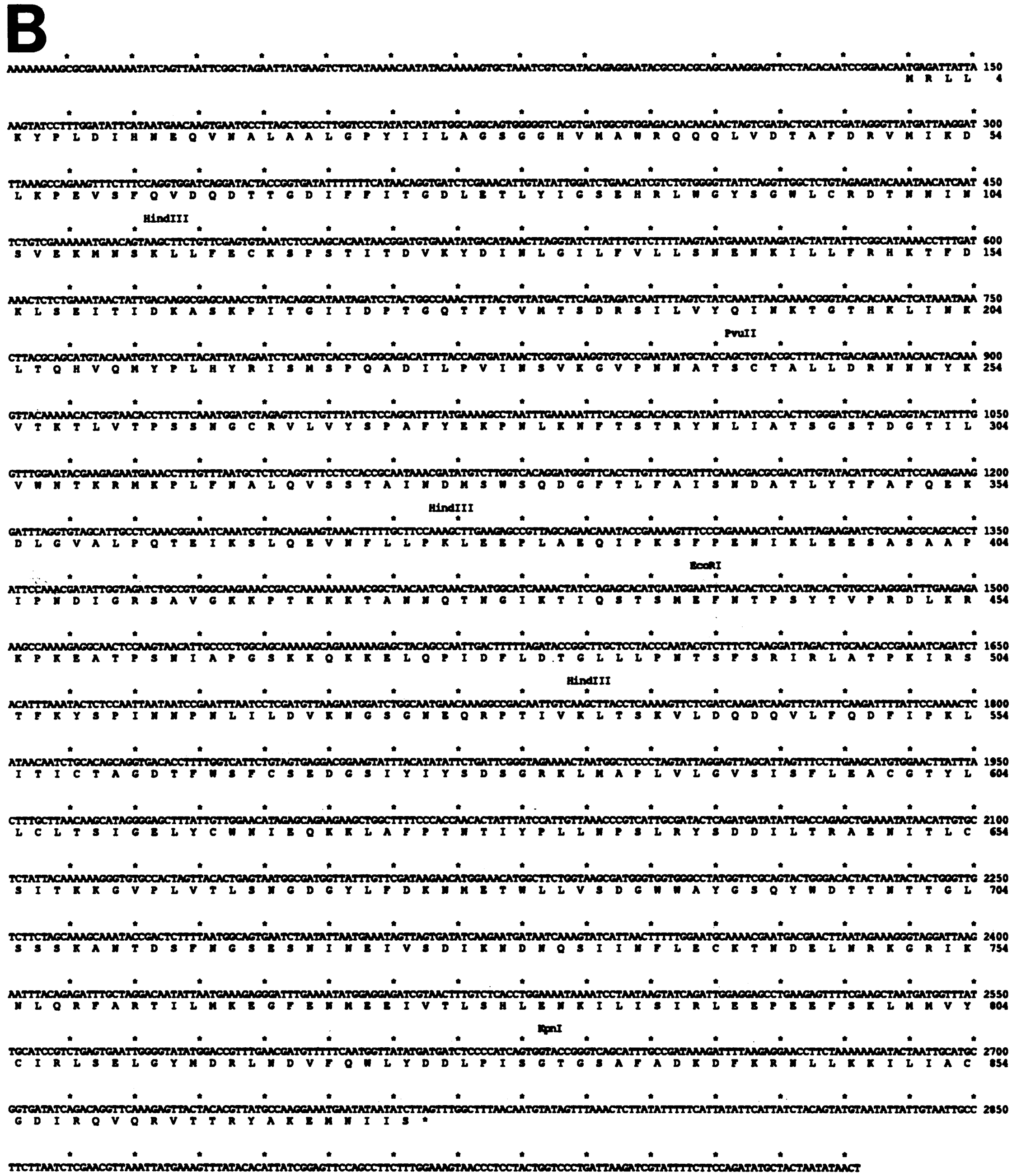

FIG. 3—*Continued.*

Regulation of *HIR1* and *HIR2*. The *HIR1* and *HIR2* gene products have been identified genetically as repressors of yeast histone gene transcription. They are required for the periodic repression of three of the four histone gene loci during the cell cycle (26), as well as for autogenous regulation of the *HTA1-HTB1* locus by H2A and H2B (22). We asked whether the function of Hir1 or Hir2 in these two processes is determined by the differential transcription of the *HIR1* or *HIR2* gene. We therefore measured the levels of *HIR1* or *HIR2* mRNA, each of which is an approximately 2.6- to 2.7-kb-long polyadenylated RNA species, under conditions that lead to either repression or derepression of the *HTA1-HTB1* locus.

We first examined whether the cell cycle-dependent transcription of the *HTA1* gene was correlated with changes in the steady-state levels of *HIR1* or *HIR2* mRNA (Fig. 5A). During the mitotic cell cycle, transcription of the *HTA1-HTB1* locus is repressed until late G_1, when it is activated by completion of the *CDC4*-dependent step (15, 38). Transcription is repressed in S-phase cells by two pathways. In normally cycling cells, it is repressed in early S phase shortly after the execution of the *CDC7*-dependent step; in the absence of *CDC7* function, the histone genes are constitutively transcribed (15). Interruption of S phase with a drug such as hydroxyurea leads to premature repression of *HTA1-HTB1* transcription (21). Finally, the histone genes are not

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A.

         282
HIR1     DKSLAVMSTSRPRPILVAFDIANKSITDMSWNPDGSLLFVA
         | .. |  | | .|.  | .     | ||||  ||  ||
HIR2     DGTILVWNTKRMKPLFNALQVSSTAINDMSWSQDGFTLFAI
         300
                             343
HIR1     SLDSSITLFKFENNELGKPIP
         | |..   | |   .||  .|
HIR2     SNDATLYTFAFQEKDLGVALP
                             361

B.

NUCLEOPLASMIN   155 KRPAatKKAgqAKKKKl
SWI5            636 KKyenvvikrspRKRgR
HIR1            426 KKdAilnKAvtlKsgKKR
HIR2            454 RKPkeatpsniApgsKKqK

C.

HIR1             23 MSRHTGSITCVKFSPDGKYLASGSDDRILLIWA
                 81 LVAHDNDIQDICWAPDSSILVTVGLDRSVIVWN
                123 FDVHQSLVKGVVFDPANKYFATTSDDRTMKIFR

TUP1            441 SPSSDLYIRSVCFSPDGKFLATGAEDRLIRIWD
                481 LQGHEQDIYSLDYFPSGDKLVSGSGDRTVRIWD
                526 EDGVTTVAVS---PGDGKYIAAGSLDRAVRVWD
                572 GTGHKDSVYSVVFTRDGQSVVSGSLDRSVKLWN
                626 YIGHKDFVLSVATTQNDEYILSGSKDRGVLFWD

ß-TRANSDUCIN        FTGHTGDVMSLSLAPDTRLFVSGACDASAKLWD

CONSENSUS           ..GH...V.Sα....D...ααSGS.D..αRαWD
                           I T          T      K
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FIG. 4. Motifs present in the predicted Hirl and Hir2 proteins. (A) Homology between amino acid residues 282 to 343 of Hirl and 300 to 361 of Hir2. Identical residues are indicated by vertical lines, and conserved residues are indicated by dots. (B) Comparison of the putative nuclear localization signal in Hirl and Hir2 to that identified in nucleoplasmin and Swi5. Numbers represent the position of the signal within the four proteins. (C) Comparison of the β-transducin repeats in the Hirl protein to those in Tupl. Residues in Hirl identical to the consensus sequence derived by Fong et al. (11) are indicated by bold letters. Numbers represent the position of the repeats in the two proteins.

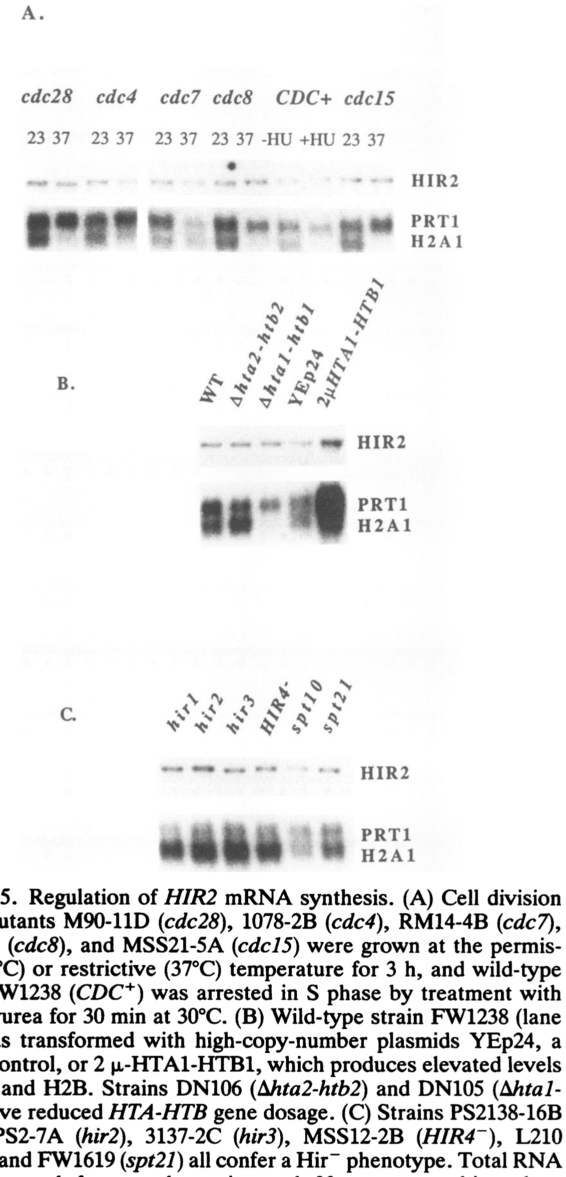

FIG. 5. Regulation of *HIR2* mRNA synthesis. (A) Cell division cycle mutants M90-11D (*cdc28*), 1078-2B (*cdc4*), RM14-4B (*cdc7*), 2754-7-4 (*cdc8*), and MSS21-5A (*cdc15*) were grown at the permissive (23°C) or restrictive (37°C) temperature for 3 h, and wild-type strain FW1238 (CDC^+) was arrested in S phase by treatment with hydroxyurea for 30 min at 30°C. (B) Wild-type strain FW1238 (lane WT) was transformed with high-copy-number plasmids YEp24, a vector control, or 2 μ-HTA1-HTB1, which produces elevated levels of H2A and H2B. Strains DN106 (*Δhta2-htb2*) and DN105 (*Δhta1-htb1*) have reduced *HTA-HTB* gene dosage. (C) Strains PS2138-16B (*hirl*), PS2-7A (*hir2*), 3137-2C (*hir3*), MSS12-2B (*HIR4*$^-$), L210 (*spt10*), and FW1619 (*spt21*) all confer a Hir$^-$ phenotype. Total RNA was extracted from each strain, and 20 μg was subjected to Northern blot analysis, with hybridization probes that detect *HTA1*, *PRT1*, and *HIR2* transcripts. The same blots were then stripped and rehybridized to a probe that detected *HIR1* mRNA, and identical results were obtained (data not shown).

expressed in G_2-phase cells. We measured the levels of *HTA1*, *HIR1*, and *HIR2* mRNAs in *cdc* mutants grown at the permissive temperature or blocked at various points in G_1, S, and G_2 by growth at the restrictive temperature. The *HTA1* gene was regulated as expected at each *CDC* control point, but neither *HIR1* nor *HIR2* mRNA synthesis was regulated in a cell cycle-dependent manner.

We next examined the levels of *HIR1* and *HIR2* mRNAs under conditions in which transcription of the *HTA1-HTB1* locus is autogenously regulated (Fig. 5B). We measured the levels of *HTA1*, *HIR1*, and *HIR2* mRNAs in strains in which *HTA1-HTB1* transcription was either repressed by increased levels of H2A and H2B (2μ*HTA1-HTB1* [22]) or derepressed by reduced levels of the same histones (*Δhta2-htb2* and *Δhtal-htbl* [22]). We again found that the two *HIR* transcripts were present at invariant levels, independent of the effects of the levels of H2A and H2B on the expression of *HTA1*.

We finally asked whether any gene that is known to regulate the *HTA1-HTB1* locus affects the expression of *HIR1* or *HIR2* (Fig. 5C). Mutations in at least eight genes cause a Hir$^-$ phenotype (33, 37a). Some of these genes may confer a Hir$^-$ phenotype because they regulate other *HIR* genes that are primary transcriptional regulators. We examined the levels of *HIR1* and *HIR2* mRNAs in *spt10*, *spt21*, *hirl*, *hir2*, *hir3*, or *HIR4*$^-$ mutants, each of which shows a Hir$^-$ phenotype (33, 37a). None of these mutations affected transcription of either *HIR* gene. Thus, the *HIR1* and *HIR2* genes do not appear to be targets in a sequential cascade of transcriptional regulation.

We also inserted the *HIR1* and *HIR2* genes into a high-copy-number plasmid to assess the effects of overproduction of their gene products. Approximately 30 times more RNA was made from each high-copy-number *HIR* gene relative to that of a single copy *HIR1* or *HIR2* gene (Fig. 6, compare panels 1 and 3 to panels 5 and 7), and it resulted in elevated levels of Hir proteins (see Fig. 8) (33a).

We asked whether overexpression of either gene product could suppress the mutant phenotypes of three strains that were derepressed for transcription of *HTA1*. We initially found that a mutant allele of one *HIR* gene could not be

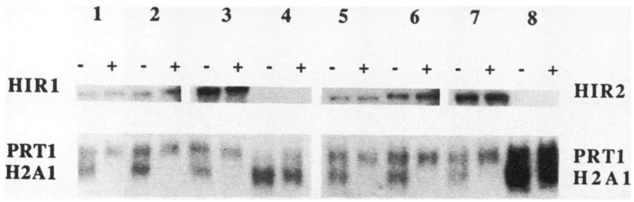

FIG. 6. Northern blot analysis of *HIR1* (lanes 1 to 4) and *HIR2* (lanes 5 to 8) transcripts in strains grown in the absence (−) or presence (+) of hydroxyurea for 30 min. Lanes 1 to 3 and 5 to 7, wild-type strain FW1238 without plasmid (lanes 1 and 5) or transformed with plasmid YCp50-HIR1 (lanes 2), YEpHIR1 (lanes 3), YCp50-HIR2 (lanes 6), or YEpHIR2 (lanes 7); lanes 4, strain PSFWΔ1-A (Δ*hir1*); lanes 8, strain PSFWΔ2-A (Δ*hir2*). Twenty micrograms of total RNA extracted from each strain was subjected to Northern blot analysis. Hybridization probes that detect *HIR1*, *HIR2*, *HTA1*, and *PRT1* transcripts were used. The *HIR1* and *HIR2* blots in lanes 3 and 7 were exposed for 1/25 the time of the remaining lanes to account for the presence of high-copy-number *HIR1* and *HIR2* genes.

suppressed by the overproduction of the wild-type product of the second *HIR* gene (data not shown). We then determined that the derepressed transcription of an *HTA1-lacZ* reporter gene in a Δ*hta1-htb1* strain (22) was not repressed by the overproduction of either Hir1 or Hir2 (data not shown). Finally, we observed that the derepressed transcription of the *HTA1* gene in a *cdc7* mutant could not be corrected by the presence of high-copy-number *HIR1* or *HIR2* genes (data not shown).

The presence of high-copy-number *HIR1* or *HIR2* genes also produced no discernible phenotypes in wild-type cells: neither gene product conferred a Hir^- (Fig. 6, panels 3 and 7) or Spt^- (data not shown) phenotype or repressed transcription of the *HTA1* gene when overproduced (33a). Together, the data suggest that neither Hir1 nor Hir2 is limiting for its regulatory function in the cell.

Intracellular localization and expression of the Hir1 and Hir2 proteins. To detect the Hir proteins, we tagged them with an epitope derived from the hemagglutinin (ha) protein of influenza virus (42) as described in Materials and Methods. The tagged Hir proteins were shown to be functional since a *HIR1*-ha or *HIR2*-ha gene carried on a high-copy-number plasmid complemented both the Hir^- and the Spt^- phenotypes of a *hir1* or *hir2* mutant (data not shown). Indirect immunofluorescence was used to determine the localization of the Hir proteins in cells transformed with a high-copy-number *HIR1*-ha or *HIR2*-ha gene. The presence of a nuclear targeting signal in both Hir proteins would be predicted to confer nuclear localization. No detectable immunofluorescence above background was observed for the epitope-tagged Hir1 protein (data not shown). This may reflect the position of the epitope in the native Hir1 protein, a masking of the epitope by the association of Hir1 with another protein, or low abundance of the Hir1 protein. Results for localization of the Hir2-ha protein are shown in Fig. 7. Although fluorescence was faint, the epitope-tagged protein was only detected in the nucleus (Fig. 7A and C), while a strain expressing a high-copy-number untagged *HIR2* gene showed no nuclear staining (Fig. 7C and D).

To determine the size of the tagged Hir proteins, we performed Western blot analysis of strains carrying the *HIR1*-ha or *HIR2*-ha gene (Fig. 8). In each strain, the primary antibody recognized a single major antigen (lanes 2 and 4) that was not present in strains carrying an untagged *HIR1* or *HIR2* gene (lanes 1 and 3). The apparent molecular mass of the tagged Hir2 protein agreed exactly with the size predicted (98 kDa) from the *HIR2* open reading frame, but the size of the Hir1 protein (~100 kDa) was larger than expected. As discussed above, this apparent discrepancy may result from the electrophoretic behavior of the Hir1 gene product.

We also utilized the epitope-tagged *HIR2* gene to ask whether the levels of Hir2 protein were regulated during the cell cycle. We visualized the tagged Hir2 protein by Western blot analysis of wild-type cells arrested with alpha factor or hydroxyurea and of *cdc28*, *cdc4*, *cdc7*, and *cdc15* mutants grown at the permissive temperature or shifted for one cell generation to the restrictive temperature. We found that the levels of Hir2 protein were unchanged in cells blocked at these points in G_1, S, or G_2 phase (data not shown). In addition, the levels of Hir2-ha protein were unchanged in strains that contained a mutation in the *HIR1* or *HIR3* gene (data not shown). The results suggest that the *HIR2* gene is not regulated at the level of either mRNA or protein synthesis.

DISCUSSION

The yeast histone genes show complex transcriptional regulation in response to distinct intracellular signals. In the cell cycle, transcription of these genes is dependent upon three different signals that are generated as cells progress from G_1 through S phase. Transcription is activated in G_1 following the completion of the *CDC4*-dependent step (15, 38), and repressed in early S phase subsequent to the execution of the late G_1, *CDC7*-dependent step (15). Transcription is prematurely repressed in S phase in response to a third cell cycle signal that is generated by the interruption of DNA replication (21). In addition to these temporal signals, the intracellular levels of H2A and H2B compose a fourth signal; transcription of the *HTA1-HTB1* locus is repressed when H2A and H2B are in excess, and derepressed when these histones are limiting (22). We have identified four *HIR* genes whose products are involved in transmitting these signals (26, 37a). Mutations in these genes cause transcription of the *HTA1-HTB1* locus to be independent of each signal, suggesting that the *HIR* gene products function downstream in a pathway that links both cell cycle progression and intracellular levels of H2A and H2B to the regulation of the *HTA1-HTB1* locus.

In this study, we have isolated and characterized two *HIR* genes to learn how their gene products mediate such diverse regulatory signals. We found that the *HIR1* and *HIR2* genes encode nonessential proteins that are nonetheless required to repress transcription of the *HTA1-HTB1* locus; when either *HIR* gene is deleted, transcription of the histone gene pair is not repressed in response to appropriate regulatory signals.

The differential transcription of the *HTA1-HTB1* locus cannot be accounted for by the regulated transcription of either *HIR* gene. Both *HIR* genes are constitutively transcribed under a wide variety of conditions in which the *HTA1-HTB1* locus is either repressed or derepressed, and the Hir2 protein is present during the G_1, S, and G_2 phases of the cell cycle. Moreover, the pattern of *HIR1* and *HIR2* mRNA synthesis is not altered by mutations in the *SPT10*, *SPT21*, *HIR3*, or *HIR4* gene, each of which confers a Hir^- phenotype (33, 37a). Consistent with the view that the levels of the *HIR1* and *HIR2* gene products are not limiting for their regulatory functions, transcription of the *HTA1* gene is not

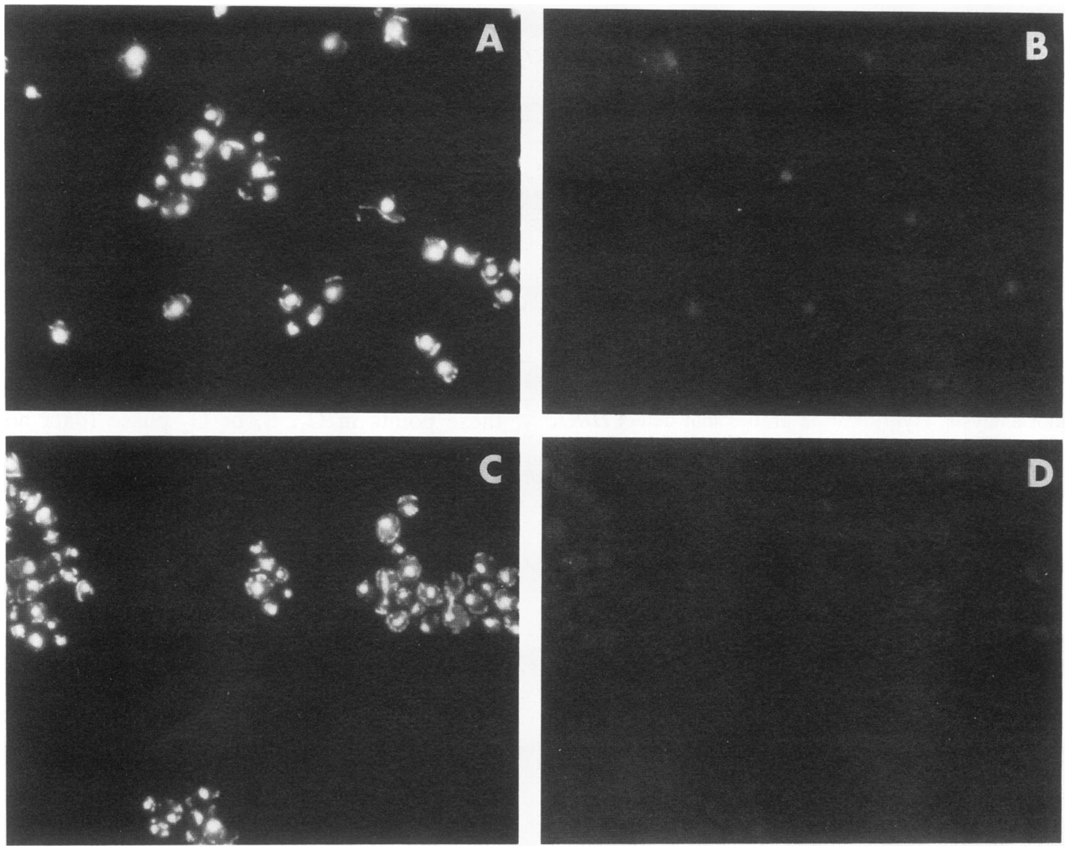

FIG. 7. Nuclear localization of epitope-tagged Hir2 protein. Strain PS2-7A (*hir2-1*) was transformed with plasmid YEpHIR2-ha (A and B) or YEpHIR2 (C and D). (A and C) Cells stained with 4′,6′-diamidino-2-phenylindole dihydrochloride to visualize DNA; (B and D) fluorescein isothiocyanate fluorescence after incubation of cells with monoclonal antibody 12CA5 and fluorescein isothiocyanate-conjugated secondary antibody as described in Materials and Methods.

repressed by overproduction of either the Hirl or the Hir2 protein. These results suggest that the functions of the Hir proteins may be regulated by posttranslational modification and/or by their association with other proteins. Two potential regulators of Hirl or Hir2 function could be the cell cycle proteins, Cdc4 (a β-transducin class protein [see below]), which is required for activation of *HTA1-HTB1* transcription, and Cdc7 (a protein-serine/threonine kinase), which is required for *HTA1-HTB1* repression.

Since mutations in the *HIR1* or *HIR2* gene confer identical regulatory phenotypes, it is formally possible that the function of one *HIR* gene product is to regulate the expression of the second *HIR* gene. We eliminated this possibility by showing that both *HIR1* and *HIR2* mRNAs are produced at invariant levels in wild-type, *hir1*, and *hir2* strains. We have also noted that deletion of both the *HIR1* and *HIR2* genes does not confer additive effects on the derepressed transcription of HTA1 (33a). These data indicate that the Hirl and Hir2 proteins may act in a common regulatory pathway.

The *HIR1* and *HIR2* gene products have been characterized genetically as repressors that act through a negative site in the *HTA1-HTB1* promoter (26). The notion that both proteins might function directly in transcription is supported by the presence of a nuclear localization signal in Hirl and Hir2 and the observation that a Hir2 protein tagged with the influenza virus hemagglutinin epitope is localized in the nucleus. However, the amino acid sequences of Hirl and Hir2 do not contain any of the common motifs associated

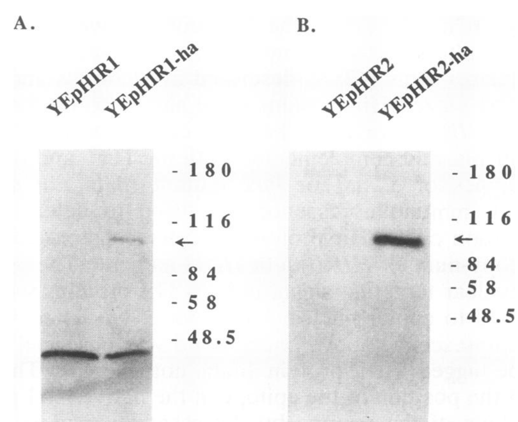

FIG. 8. Western blot analysis of epitope-tagged Hirl and Hir2 proteins. Strain W303Δ1 (Δ*hir1*) was transformed with YEpHIR1 or YEpHIR1-ha (A), and strain PS2-7A (*hir2*) was transformed with YEpHIR2 or YEpHIR2-ha (B). Extracts were prepared, and total proteins were separated on SDS-polyacrylamide gels and transferred to a filter. The filter was incubated with monoclonal antibody 12CA5 and developed as described in Materials and Methods. The *hir1* strain contained an antigen that reacted with the monoclonal antibody even in the absence of the *HIR1*-ha gene. Molecular mass markers α_2-macroglobulin (180 kDa), β-galactosidase (116 kDa), fructose-6-phosphate kinase (84 kDa), pyruvate kinase (58 kDa), and fumarase (48.5 kDa) were included as size standards.

with DNA binding, and *HIR1* or *HIR2* do not encode one of several proteins that bind to specific sequences in the *HTA1-HTB1* negative site or upstream activating sequence element (22a). These observations raise the possibility that Hir1 or Hir2 do not confer repression by directly contacting the *HTA1-HTB1* promoter and suggest that they may function as corepressors to assist a repressor that binds to sequences in the negative site. In this regard, it is intriguing that the predicted Hir1 protein contains three copies of the β-transducin repeat, a motif found in another transcriptional repressor, Tup1 (40). Tup1 also does not bind directly to DNA but has been shown to act with another protein, Ssn6 (20, 23, 32), to represses specific groups of genes in response to signals which determine either the mating type of the cell or the utilization of carbon sources (20, 40). The Tup1/Ssn6 repressor is apparently targeted to particular promoters by its association with additional, site-specific DNA binding proteins.

The Ssn6 protein is characterized by another motif, the TPR snap helix (13, 20, 35). Goebl and Yanagida have noted that many genes encoding TPR proteins are functionally related to those encoding β-transducin proteins and have suggested that particular pairs of TPR and β-transducin proteins interact to perform a common function (13). In the case of Tup1 and Ssn6, whose biochemical (41) and genetic (20) interactions have been demonstrated, one such function is to act in conjunction with site-specific DNA binding proteins such as α2 and Mcm1 to repress transcription of **a**-specific genes (20). It is possible that the Hir1 protein also acts with a TPR class protein to repress transcription of the *HTA1-HTB1* locus by interacting with site-specific DNA binding proteins at the negative site of this locus. While the TPR motif is not found in Hir2, it may occur in another Hir protein (e.g., Hir3 or Hir4), since a mutation in such a TPR class protein would be predicted to confer a Hir^- phenotype. Alternatively, one of the known TPR proteins of yeasts (e.g., Cdc16, Cdc23, Ski3, Prp6, or Ssn6) may act together with Hir1 to regulate *HTA1-HTB1* transcription; however, mutations in none of the genes that encode such proteins confer a Hir^- phenotype (33a).

Mutations in *HIR1* and *HIR2* produce pleiotropic effects on transcription. In addition to derepressing transcription of the *HTA1-HTB1*, *HHT1-HHF1*, and *HHT2-HHF2* loci, *hir* mutations affect the transcription of δ insertion alleles of *HIS4* and *LYS2* by altering the site of transcription initiation (34). It is possible that Hir1 and Hir2 represent another class of general transcriptional repressors that act at diverse yeast promoters. However, we have proposed that the effects of mutant *HIR* gene products on transcription of nonhistone gene loci are indirect and are mediated by altered chromatin structure that results from nonstoichiometric expression of the histone gene loci (34). Thus, some mutations that alter transcription pleiotropically may not define general transcriptional regulators but may act primarily by affecting the expression of chromatin components such as histones.

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REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1989. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
3. **Chaleff, D. T., and G. R. Fink.** 1980. Genetic events associated with an insertion mutation in yeast. Cell **21:**227–237.
4. **Clark-Adams, C. D., and F. Winston.** 1987. The *SPT6* gene is essential for growth and is required for δ-mediated transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**679–686.
5. **Cross, S. L., and M. M. Smith.** 1988. Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3-H4 loci in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**945–954.
6. **Dalrymple, M. A., S. Peterson-Bjorn, J. D. Friesen, and J. D. Beggs.** 1989. The product of the *PRP4* gene of *S. cerevisiae* shows homology to β subunits of G proteins. Cell **58:**811–812.
7. **Dingwall, C., and R. A. Laskey.** 1991. Nuclear targeting sequences—a consensus? Trends Biochem. Sci. **16:**478–481.
8. **Farabaugh, P. J., and G. R. Fink.** 1980. Insertion of the eukaryotic transposable element Ty1 creates a 5-base pair duplication. Nature (London) **286:**352–356.
9. **Fassler, J. S., and F. Winston.** 1988. Isolation and analysis of a novel class of suppressor of Ty insertion mutations in *Saccharomyces cerevisiae*. Genetics **118:**203–212.
10. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
11. **Fong, H. K. W., J. B. Hurley, R. S. Hopkins, R. Miake-Lye, M. S. Johnson, R. F. Doolittle, and M. I. Simon.** 1986. Repetitive segmental structure of the transducin β subunit: homology with the CDC4 gene and identification of related mRNAs. Proc. Natl. Acad. Sci. USA **83:**2162–2166.
12. **Goebl, M.** Personal communication.
13. **Goebl, M., and M. Yanagida.** 1991. The TPR snap helix: a novel protein repeat motif from mitosis to transcription. Trends Genet. **7:**173–177.
14. **Hartley, D. A., A. Preiss, and S. Artavanis-Tsakonas.** 1988. A deduced gene product from the Drosophila neurogenic locus, *Enhancer of split*, shows homology to mammalian G-protein β subunit. Cell **55:**785–795.
15. **Hereford, L. M., S. Bromley, and M. A. Osley.** 1982. Periodic transcription of yeast histone genes. Cell **30:**305–310.
16. **Hereford, L. M., K. Fahrner, J. Woolford, Jr., M. Rosbash, and D. B. Kaback.** 1979. Isolation of yeast histone genes H2A and H2B. Cell **18:**1261–1271.
17. **Hereford, L. M., M. A. Osley, J. R. Ludwig II, and C. S. McLaughlin.** 1981. Cell cycle regulation of yeast histone mRNA. Cell **24:**367–375.
18. **Icho, T., and R. B. Wickner.** 1988. The *MAK11* protein is essential for cell growth and replication of M double stranded RNA and is apparently a membrane associated protein. J. Biol. Chem. **263:**1467–1475.
19. **Ito, H., Y. Fukuda, K. Marata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
20. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1991. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
21. **Lycan, D. E., M. A. Osley, and L. M. Hereford.** 1987. Role of transcriptional and posttranscriptional regulation in expression of histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**614–621.
22. **Moran, L., D. Norris, and M. A. Osley.** 1990. A yeast H2A-H2B promoter can be regulated by changes in histone gene copy number. Genes Dev. **4:**752–763.

22a. **Moran, L., and P. Sherwood.** Unpublished data.

23. **Neigeborn, L., and M. Carlson.** 1987. Mutations causing consti-

tutive invertase synthesis in yeast: genetic interactions with *snf* mutations. Genetics **115**:247–253.

24. **Oechsner, U., V. Magdolen, C. Zoglowek, U. Hacker, and W. Bandlow.** 1988. Yeast adenylate kinase is transcribed constitutively from a promoter in the short intergenic region to the H2A-1 gene. FEBS Lett. **242**:187–193.
25. **Osley, M. A., J. Gould, S. Kim, M. Kane, and L. M. Hereford.** 1986. Identification of sequences in a yeast histone promoter involved in periodic transcription. Cell **45**:537–544.
26. **Osley, M. A., and D. E. Lycan.** 1987. *trans*-acting mutations that alter transcription of *Saccharomyces cerevisiae* histone genes. Mol. Cell. Biol. **7**:4202–4210.
27. **Pringle, J. R., A. E. M. Adams, D. G. Drubin, and B. K. Haarer.** 1991. Immunofluorescence methods for yeast. Methods Enzymol. **194**:565–602.
28. **Robbins, J., S. M. Dilworth, R. A. Laskey, and C. Dingwall.** 1991. Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell **64**:615–623.
29. **Rose, M. D., P. Novick, J. H. Thomas, D. Botstein, and G. R. Fink.** 1987. A Saccharomyces cerevisiae genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60**:237–243.
30. **Sambrook, J., T. Maniatis, and E. F. Fritsch.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
32. **Schultz, J., and M. Carlson.** 1987. Molecular analysis of *SSN6*, a gene functionally related to the *SNF1* protein kinase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:3637–3645.
33. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

33a. **Sherwood, P.** Unpublished data.

34. **Sherwood, P. W., and M. A. Osley.** 1991. Histone regulatory (*hir*) mutations suppress δ insertion alleles in *Saccharomyces cerevisiae*. Genetics **128**:729–738.
35. **Sikorski, R. S., M. S. Boguski, M. Goebl, and P. Hieter.** 1990. A repeating amino acid motif in *CDC23* defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell **60**:307–317.
36. **Simchen, G., F. Winston, C. A. Styles, and G. R. Fink.** 1984. Ty-mediated expression of the *LYS2* and *HIS4* genes of *Saccharomyces cerevisiae* is controlled by the same *SPT* genes. Proc. Natl. Acad. Sci. USA **81**:2431–2434.
37. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.

37a. **Spector, M. S.** Unpublished data.

38. **White, J., S. Green, D. Barker, L. Dumas, and L. Johnston.** 1987. The *CDC8* transcript is cell cycle regulated in yeast and is expressed co-ordinately with *CDC9* and *CDC21* at a point preceding histone transcription. Exp. Cell Res. **171**:223–231.
39. **Whiteway, M., L. Hougan, D. Dignard, D. Y. Thomas, L. Bell, G. C. Saari, F. J. Grant, P. O'Hara, and V. L. MacKay.** 1989. The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. Cell **56**:467–477.
40. **Williams, F. E., and R. J. Trumbly.** 1990. Characterization of *TUP1*, a mediator of glucose repression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:6500–6511.
41. **Williams, F. E., U. Varanasi, and R. J. Trumbly.** 1991. The CYC8 and TUP1 proteins involved in glucose repression in *Saccharomyces cerevisiae* are associated in a protein complex. Mol. Cell. Biol. **11**:3307–3316.
42. **Wilson, I., H. Niman, R. Houghten, A. Cherenson, M. Connolly, and R. Lerner.** 1984. The structure of an antigenic determinant in a protein. Cell **37**:767–778.
43. **Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink.** 1984. Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. Genetics **107**:179–197.