MOLECULAR AND CELLULAR BIOLOGY, Feb. 1997, p. 545–552 Vol. 17, No. 2
0270-7306/97/$04.00+0

Hir1p and Hir2p Function as Transcriptional Corepressors To Regulate Histone Gene Transcription in the *Saccharomyces cerevisiae* Cell Cycle

MONA S. SPECTOR,[1,2] AMANDA RAFF,[1] HESHANI DESILVA,[1] KENNETH LEE,[1] AND MARY ANN OSLEY[1]*

Program in Molecular Biology, Sloan Kettering Cancer Center, New York, New York 10021,[1] *and Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*[2]

Received 22 August 1996/Returned for modification 4 October 1996/Accepted 4 November 1996

The *HIR*/*HPC* (histone regulation/histone periodic control) negative regulators play important roles in the transcription of six of the eight core histone genes during the *Saccharomyces cerevisiae* cell cycle. The phenotypes of *hir1* and *hir2* mutants suggested that the wild-type *HIR1* and *HIR2* genes encode transcriptional repressors that function in the absence of direct DNA binding. When Hir1p and Hir2p were artificially tethered to yeast promoters, each protein repressed transcription, suggesting that they represent a new class of transcriptional corepressors. The two proteins might function as a complex in vivo: Hir2p required both Hir1p and another Hir protein, Hir3p, to repress transcription when it was tethered to an *HTA1-lacZ* reporter gene, and Hir1p and Hir2p could be coimmunoprecipitated from yeast cell extracts. Tethered Hir1p also directed the periodic transcription of the *HTA1* gene and repressed *HTA1* transcription in response to two cell cycle regulatory signals. Thus, it represents the first example of a transcriptional corepressor with a direct role in cell cycle-regulated transcription.

Histone synthesis is tightly regulated in eukaryotes to ensure that the components for nucleosome assembly are present both at the correct time during the cell cycle and in the proper stoichiometric levels (39). Regulation at the level of transcription plays an important role in the synthesis of the core histones in *Saccharomyces cerevisiae*, and negative regulation has emerged as a significant mechanism in the transcription of six of the eight histone genes (*HTA1-HTB1*, *HHT1-HHF1*, and *HHT2-HHF2*) in this organism (39). A negative site has been identified in the promoters of each of the negatively regulated histone genes in close proximity to upstream activation sequence (UAS) elements (14, 41). The *HTA1-HTB1* negative site has been shown to play a key role in cell cycle-regulated transcription: deletion of this site derepresses *HTA1* and *HTB1* transcription early in G_1 phase, late in S phase, and in G_2/M phase, points in the cell cycle when the two genes are usually not expressed (41), and prevents the same genes from being repressed in S-phase cells when DNA replication has been interrupted (29). The same site is also required for a feedback repression pathway that is triggered by the overproduction of histones H2A and H2B (32). In its absence, *HTA1* and *HTB1* transcription is insensitive to the intracellular levels of these two histone proteins.

Two related genetic screens have identified seven *HIR* and *HPC* (histone regulation and histone periodic control) genes whose products are postulated to encode repressors of *HTA1-HTB1*, *HHT1-HHF1*, and *HHT2-HHF2* transcription (43, 64). Four of these genes (*HIR1*, *HIR2*, *HIR3*, and *HPC2*) have been isolated and extensively characterized (51, 54, 64). None of the genes is essential, and each appears to encode a novel polypeptide. Interestingly, the *HIR1* and *HIR2* genes have been evolutionarily conserved; the human homolog, *HIRA*, encodes a protein that encompasses structural features from both yeast proteins as a single polypeptide (26). These features include seven copies of a WD (β-transducin) repeat (35) that is present at the N termini of both *HIR1* and *HIRA* (26, 51).

Consistent with the idea that some of the Hir and Hpc proteins will directly participate in transcriptional repression, the *HIR1*, *HIR2*, and *HIR3* gene products have been shown genetically to function through the negative site in the *HTA1-HTB1* promoter (43). However, although they are apparently nuclear proteins (51, 54), none of the three contains a motif typically associated with DNA binding, and Hir1p and Hir2p are apparently not present in a factor that binds to the *HTA1-HTB1* negative site (49). This raised the possibility that the Hir1, Hir2, and Hir3 proteins function as transcriptional corepressors, mediating repression through a DNA binding protein that contacts the negative site at each of the negatively regulated histone gene promoters. This class of transcriptional repressors is best exemplified by the Tup1 and Cyc8 (Ssn6) proteins (22, 63), which form a complex that recognizes several different DNA binding proteins at a diverse array of yeast genes, including the α2-Mcm1 repressor at **a**-cell-specific genes (22, 25) and the Mig1 repressor at glucose-repressed genes (59, 61).

To understand the role of the Hir and Hpc proteins in the repression of the core histone genes, we have undertaken a functional analysis of Hir1p and Hir2p. We found that both proteins repressed transcription when artificially recruited to DNA binding sites in yeast promoters, supporting the idea that they represent a new class of transcriptional corepressors. Additional data suggest that Hir1p and Hir2p perform this function as part of a multiprotein complex in vivo: tethered Hir2p depends on the presence of both Hir1p and Hir3p to repress *HTA1* transcription, and the Hir1 and Hir2 proteins can be coimmunoprecipitated from yeast cell extracts. We also found that tethered Hir1p could substitute for the *HTA1-HTB1* negative site to direct transcription of the *HTA1* gene in response to several cell cycle regulatory signals. Thus, Hir1p and Hir2p

* Corresponding author. Mailing address: Program in Molecular Biology, Sloan Kettering Cancer Center, 1275 York Ave., New York, NY 10021. Phone: (212) 639-8156. Fax: (212) 639-2861. E-mail: m-osley@ski.mskcc.org.

TABLE 1. *S. cerevisiae* strains used

Strain	Genotype	Source
W303-1A	*MAT***a** *ura3-1 leu2-3,112 ade2-1 trp1 his3 ssd1*	R. Rothstein
W303Δ1	*MAT***a** *hir1*::*HIS3 ura3-1 leu2-3,-112 ade2-1 trp1 his3 ssd1*	H. DeSilva
W303Δ2	*MAT***a** *hir2*::*URA3 ura3-1 leu2-3,-112 ade2-1 trp1 his3 ssd1*	H. DeSilva
W303Δ3	*MAT***a** *hir3*::*HIS3 ura3-1 leu2-3,-112 ade2-1 trp1 his3 ssd1*	H. DeSilva
MSS27-1D	*MAT***a** *HIR4-1 ura3-1 leu2-3,-112 ade2-1 trp1 his3 ssd1*	M. S. Spector
YEL106	*MAT***a** *bar1*::*LEU2 ura3-1 leu2-3,-112 ade2-1 trp1 his3 ssd1*	F. Cross
BJ5465	*MAT***a** *ura3-52 trp1 leu2Δ1 his3Δ200 pep4*::*HIS3 prbΔ1.6R can1*	E. Jones
K3391	*MAT***a** *clb1Δ clb2^{ts} ura3-1 leu2-3,-112 ade2-1 trp1 his3*	K. Nasmyth
1078-2B	*MAT***a** *cdc4-3 ura3-52 leu2-3,-112 trp1 lys2 lys5 his7 ade2 ade6*	B. Byers

represent the first examples of transcriptional corepressors whose activity directly contributes to cell cycle-regulated transcription.

MATERIALS AND METHODS

Yeast strains and growth conditions. The *S. cerevisiae* strains used in this study are listed in Table 1. Strains with *HIR* mutations are isogenic derivatives of W303-1A. Yeast transformations were performed by the lithium acetate method (20), with selection for the prototrophic marker present on each plasmid. For most studies, strains were grown in SD selective medium (21) at 30°C except for strains K3391 and 1078-2B, which were grown at 24°C.

For cell synchronization, K3391 cells were grown in SD selective medium and arrested in G_1 at Start by treatment with 5 μg α mating factor (Sigma) per ml for 2.5 h; following removal of the mating pheromone by centrifugation and washing, the cells were resuspended in SD selective medium. Strain YEL106 was synchronized following a 2.5-h treatment with 0.05 μg of α mating factor per ml. Samples were taken at 10- to 15-min intervals after release from G_1 arrest for analysis of RNA levels during the cell cycle.

Strain 1078-2B (*cdc4^{ts}*) was grown in SD selective medium at 24°C and also shifted to 37°C for 2.5 h to inactivate the *CDC4* gene product. To inhibit DNA replication, cells were pregrown in SD selective medium, transferred to YPD medium, grown to a density of ~5×10^6 cells/ml, and then treated with 0.2 M hydroxyurea for 30 min (43).

Plasmids. (i) *lacZ* reporter genes. Plasmids with *HTA1-lacZ* reporter genes were all derived from plasmid pCALA1, a *CEN3-LEU2* vector that carries an *HTA1-lacZ* reporter gene under control of the wild-type *HTA1* promoter (41). Plasmid pHTA1Δneg-lacZ contains a 50-bp deletion of sequences from the *HTA1-HTB1* negative site (41). Plasmid pHTA1Δneg-lacZ + lex op contains a 42-bp oligonucleotide with two *lexA* dimer binding sites inserted in place of the missing negative site sequences in plasmid pHTA1Δneg-lacZ (46). *CYC1-lacZ* reporter genes are present on plasmids pAJ1, CK26, and pJK1621, 2μm *URA3* plasmids that carry zero, one, and four *lexA* dimer binding sites inserted 5′ to the *CYC1* UAS elements, respectively, and they were generously provided by A. Johnson (22).

(ii) *lexA* fusion genes. All *lexA* fusion genes were constructed in plasmid pBTM116, a 2μm *TRP1* vector that contains the entire *lexA* coding sequence under control of the *ADH1* promoter (generously provided by Stan Fields). A *lexA-HIR1* fusion gene was constructed by creating a *Sma*I site in the *HIR1* gene immediately before the initiation codon by PCR mutagenesis (3), using the primer 5′AAGGTCTCCCCGGGCATGAAAGTG3′, and inserting the *HIR1* gene as a *Sma*I-*Sal*I fragment into pBTM116. The *lexA-HIR2* fusion gene was constructed by a similar strategy, using the oligonucleotides 5′ACACAATCCC CGGGGATGAGATTA3′ and 5′AGAATCAAAGGGCCCAAATTGTTA3′ to insert *Sma*I sites at both the initiation and termination codons of *HIR2*. All constructs were confirmed by DNA sequence analysis using double-stranded templates (3).

(iii) HA epitope-tagged *HIR* genes. The *HIR1* gene was tagged with a single copy of the hemagglutinin (HA) epitope at a unique *Xba*I site in the *HIR1* open reading frame as previously described (51), and it was present on plasmid YEp352 (2μm *URA3*). The *HIR2* gene was tagged with the same HA epitope at a unique *Pvu*II site, and it was present on the *URA3* 2μm plasmid YEp24 (51). Untagged *HIR* genes were present on the same high-copy-number plasmids as the HA-tagged genes (51).

RNA analysis. Total RNA was extracted from ~1.2×10^8 to 2.5×10^8 exponentially growing cells as previously described (32). *HTA1-lacZ*, *HTB2*, and *RP51A* mRNA levels were measured by a quantitative S1 nuclease digestion assay, using a 1.4-kb *Sma*I-*Cla*I fragment (*lacZ*), a 0.7-kb *Ava*II-*Sal*I fragment (*HTB2*), and a 0.6-kb *Ava*II-*Sal*I fragment (*RP51A*) that had been end labelled with [γ-^{32}P]ATP in the presence of T4 polynucleotide kinase (32). Following electrophoresis using an 8 M urea–4% polyacrylamide gel, the dried gels were subjected to autoradiography. Quantitations were performed on a Fuji phosphoimager, using MACBAS software.

Immunological detection methods. For the immunoprecipitation of Hir proteins, strain BJ5465 (Table 1) was cotransformed with appropriate pairs of HA-tagged or untagged *HIR1* or *HIR2* genes and *lexA-HIR1* or *lexA-HIR2* genes. Immunoprecipitations were performed with ~60 to 450 μg of protein from whole-cell extracts prepared by 10 to 15 cycles of glass bead breakage in a lysis buffer that contained 20 mM HEPES (pH 7.4), 100 mM potassium acetate, 2 mM magnesium acetate, 2 mM phenylmethyl sulfonate (57), and a mix of protease inhibitors (100× mix consists of 10 μg of chymostatin, 200 μg of aprotinin, 100 μg of pepstatin A, 50 μg of leupeptin, and 720 μg of E64 per ml in 10 mM Tris [pH 7.4]). Extracts containing HA-tagged proteins were incubated with 2.5 to 5.0 μl of monoclonal antibody 12C5A (Babco) (36) for 2 h at 4°C in immunoprecipitation buffer (57) (50 mM Tris [pH 7.5], 1% Triton X-100, 150 mM sodium chloride, 500 mM ammonium acetate, 0.5 mg of bovine serum albumin [BSA] per ml) and subsequently incubated with 100 μl of protein G-Sepharose beads (Pharmacia) for an additional 1 to 2 h at 4°C. The Sepharose beads containing immune complexes were collected by low-speed centrifugation and washed three times with immunoprecipitation buffer containing BSA (0.5 mg/ml) and two times with immunoprecipitation buffer without BSA. Approximately 5 to 10% of total HA epitope-tagged proteins were precipitated by these procedures. Following the last wash, the Sepharose beads were resuspended in 20 μl of sodium dodecyl sulfate (SDS) sample buffer (3) and heated at 100°C for 5 min. The eluate was then divided into two parts before loading onto an SDS–7.5% polyacrylamide gel; 5 μl was analyzed for the presence of the HA-tagged protein, and 15 μl was analyzed for the presence of the coimmunoprecipitated LexA fusion protein. Following electrophoresis, the proteins were transferred to Immobilon filters (Dupont-NEN), and Western blot analysis was performed as previously described (51), using polyclonal antibodies against LexA (a generous gift of Roger Brent) at a dilution of 1:2,000 or monoclonal antibody 12C5A (Babco) against the HA epitope at a dilution of 1:1,000. Enhanced chemiluminescence was used for detection (ECL kit from Amersham or Dupont-NEN).

RESULTS

Hir1p and Hir2p function as direct transcriptional repressors. If Hir1p and Hir2p repress *HTA1* and *HTB1* transcription through their recognition of a factor that binds to the *HTA1-HTB1* negative site, we predicted that they would function as direct transcriptional repressors if recruited to the *HTA1-HTB1* promoter. To test this hypothesis, we constructed gene fusions between *HIR1* or *HIR2* and *lexA*, which encodes a bacterial DNA binding protein (7). Both fusion genes were functional as they complemented the Hir$^-$ (43) or Spt$^-$ (50) phenotypes of null mutations in the respective chromosomal genes (data not shown). A *lexA* fusion gene, or *lexA* by itself, was introduced into two wild-type (*HIR*$^+$) yeast strains that carried different *lacZ* reporter genes. The first strain carried a *lacZ* gene controlled by an *HTA1* promoter with its three UAS elements, but without the negative site through which native Hir1p and Hir2p function (43). The second strain carried a similar *lacZ* reporter gene except that two *lexA* dimer binding sites were substituted for the negative site, which naturally occurs between two UAS elements (41).

Both LexA-Hir fusion proteins repressed *HTA1-lacZ* transcription when they were bound to *lexA* DNA binding sites (Fig. 1, *HIR*$^+$). LexA-Hir2p was a stronger repressor than LexA-Hir1p (~5- to 10-fold versus 3- to 5-fold repression), and neither fusion protein repressed transcription in the absence of *lexA* operator sites. Because tethered LexA by itself exerted only a modest effect on transcription (<20% repression), the repression conferred by LexA-Hir1p and LexA-Hir2p resulted from the presence of the Hir1 or Hir2 moiety on the LexA fusion proteins.

To determine whether the Hir proteins were specific transcriptional repressors, we examined whether tethered Hir1p or Hir2p could inhibit transcription in another promoter context.

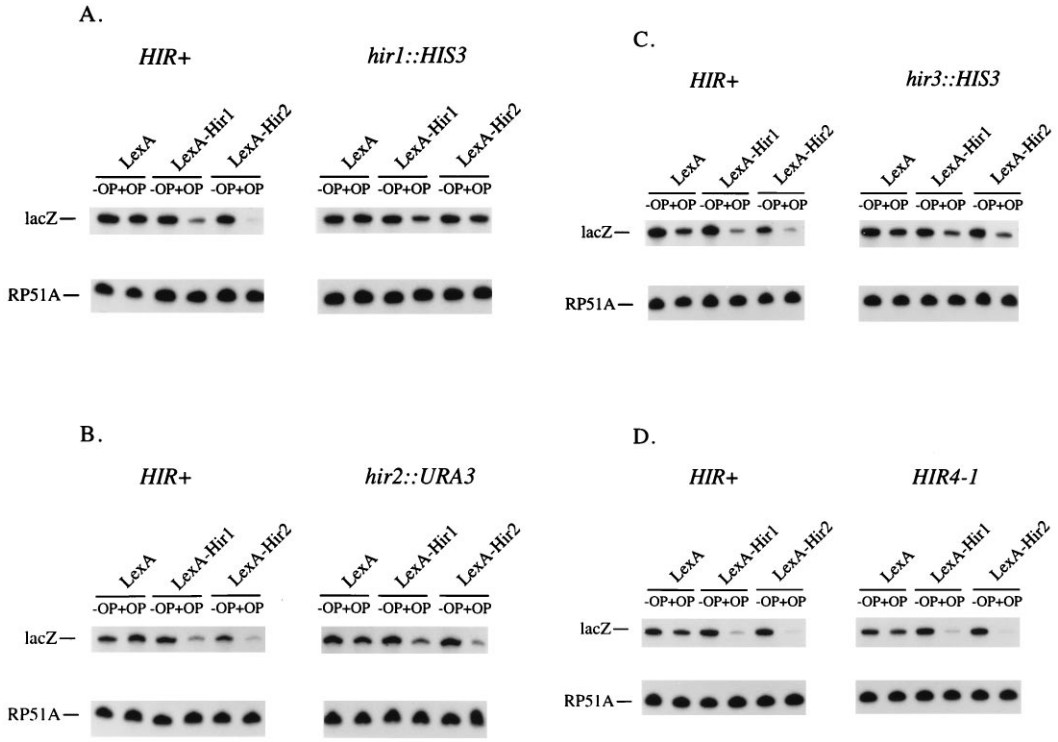

FIG. 1. Tethered Hir1p and Hir2p repress *HTA1-lacZ* transcription. Plasmids carrying a full-length *lexA* gene and either a *lexA-HIR1* or *lexA-HIR2* fusion gene were transformed into a wild-type (HIR^+) strain, three isogenic *HIR* deletion strains, *hir1*::*HIS3* (A), *hir2*::*URA3* (B), and *hir3*::*HIS3* (C), and a strain harboring a dominant mutation in the *HIR4* gene (*HIR4-1*) (D). Each strain was cotransformed with one of two related *HTA1-lacZ* reporter genes; the first contained the *HTA1* promoter without the *HTA1-HTB1* negative site (−OP), and the second contained the same promoter with two *lexA* dimer binding sites substituted for the negative site (+OP). The levels of *lacZ* mRNA produced in the presence or absence of the *lexA* operator sites were measured in each strain background by a quantitative S1 nuclease protection assay and compared to the levels of *RP51A* mRNA, which served as an internal control. In each panel, the HIR^+ controls represent independent experiments.

Each *lexA-HIR* gene was introduced into wild-type (HIR^+) yeast strains that carried two different *CYC1-lacZ* reporter genes, the first with zero and the second with four *lexA* operator sites inserted upstream of the two *CYC1* UAS elements (Fig. 2). The *CYC1* UAS elements bind an activator that is regulated by heme (15), while the *HTA1* UAS elements bind an activator whose function is cell cycle regulated (31). Hir1p and Hir2p also repressed transcription when they were tethered at the *CYC1* promoter. In this promoter context, however, both fusion proteins now functioned as strong transcriptional repressors (>10- to 20-fold repression), perhaps due to the presence of two additional *lexA* dimer binding sites in the *CYC1-lacZ* reporter gene compared with the *HTA1-lacZ* reporter gene.

The Hir proteins repressed transcription when tethered to two different promoters, which suggests that they inhibit transcription by an activator-independent mechanism. Consistent with this notion, we have observed that the Hir1 protein inhibits basal transcription threefold when tethered to multiple *lexA* operator sites in a UAS-less *CYC1-lacZ* reporter gene (40). In addition, because the two Hir proteins repressed transcription when tethered to *lexA* binding sites placed upstream of UAS elements, we conclude that Hir1p and Hir2p inhibit transcription by an active rather than a passive mechanism.

Hir1p and Hir2p functional interactions. Genetic analysis of *hir1*, *hir2*, and *hir3* mutants suggested that the Hir1, Hir2, and Hir3 proteins function together to regulate the same process (48, 51, 53). For example, the three Hir proteins might function in a complex, and tethered Hir1p and Hir2p could repress transcription by assembling other members of the complex. To test this hypothesis, we examined whether tethered Hir1p or Hir2p inhibited *HTA1-lacZ* transcription in different *HIR* mutant backgrounds (Fig. 1). The two fusion proteins showed different functional requirements in this assay. Both LexA-Hir1p and LexA-Hir2p could repress *HTA1-lacZ* transcription in *hir2*Δ (Fig. 1B) and *HIR4-1* (Fig. 1D) strains, but tethered Hir2p was completely unable to repress transcription in a *hir1*Δ strain (Fig. 1A) and showed compromised repression (three-

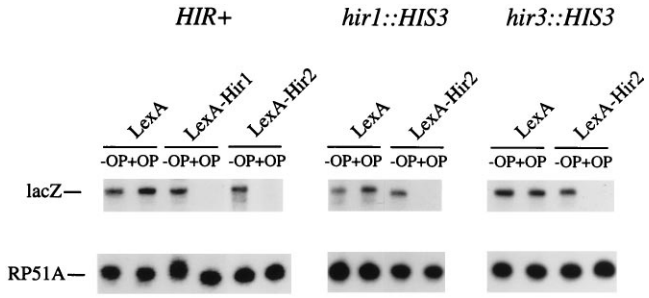

FIG. 2. Tethered Hir1p and Hir2p repress *CYC1-lacZ* transcription. Plasmids carrying a full-length *lexA* gene and either a *lexA-HIR1* or *lexA-HIR2* fusion gene were cotransformed into a wild-type (HIR^+) strain with two different *CYC1-lacZ* reporter genes; the first contained the wild-type *CYC1* promoter (−OP), and the second contained the same promoter with four *lexA* dimer binding sites inserted upstream of the *CYC1* UAS elements (+OP). The same plasmids were also cotransformed into *hir1*::*HIS3* and *hir3*::*HIS3* deletion mutants. The levels of *CYC1-lacZ* mRNA produced in the presence or absence of *lexA* operator sites in each strain were measured by a quantitative S1 nuclease protection assay and compared to the levels of *RP51A* mRNA.

fold reduction) in a *hir3*Δ strain (Fig. 1C). In contrast, tethered Hir1p repressed *HTA1-lacZ* transcription in a *hir3*Δ strain (Fig. 1C) and exhibited a modest but reproducible reduction in its ability to repress transcription a *hir1*Δ background (Fig. 1A). Because the two fusion proteins were made at equivalent levels in both wild-type and *hir* mutant strains (data not shown), we conclude that the *HIR* mutations alter the activity of the Hir proteins rather than their synthesis or stability. Together, the results suggest that the native Hir proteins might function in a complex at the *HTA1* promoter, where Hir1p might carry out the repression function of the complex.

We next examined whether the deletion of *HIR1* or *HIR3* altered the ability of Hir2p to repress transcription when it was tethered at the *CYC1-lacZ* promoter (Fig. 2). In this promoter context, tethered Hir2p repressed transcription in both a *hir1*Δ and a *hir3*Δ mutant, indicating that it requires Hir1p and Hir3p only when it is bound to the the *HTA1* promoter. We tested the possibility that the presence of four *lexA* dimer binding sites at the *CYC1* promoter, which leads to significantly stronger repression by tethered Hir2p, might bypass the normal requirement of LexA-Hir2p for Hir1p or Hir3p. A *CYC1-lacZ* reporter gene with a single *lexA* operator site inserted upstream of the *CYC1* UAS elements was introduced into *HIR*$^+$, *hir1*Δ, and *hir3*Δ strains along with a plasmid carrying a *lexA* or *lexA-HIR2* gene. Tethered Hir2p was able to repress transcription in the absence of either Hir1p or Hir3p in this promoter context as well (data not shown). This finding suggests that Hir2p and Hir1p perform overlapping functions as transcriptional corepressors but that Hir1p has another function that may be specific to its role at the *HTA1* promoter.

Hir1p and Hir2p in vivo associations. We directly tested the hypothesis that Hir1p and Hir2p function as a complex by determining whether the two proteins could be coimmunoprecipitated from yeast cell extracts. A wild-type yeast strain was cotransformed with two high-copy-number plasmids that carried either a *HIR1-HA* gene (51) or a *lexA-HIR2* gene. We used high-copy-number genes because the immunological signal from a single-copy *HIR1-HA* gene was not detectable, and we had previously established that neither fusion protein alters histone gene transcription when overexpressed (45, 51). Antibodies directed against the HA epitope were used to immunoprecipitate Hir1p-HA from cell lysates, and Western blot analysis was then performed with anti-LexA antibodies to determine whether LexA-Hir2p was also present in the immune complexes (Fig. 3A). We found that Hir1p-HA and LexA-Hir2p were specifically associated in these complexes (Fig. 3A, lane 3); no Hir1p-Hir2p interactions were detected if an untagged Hir1 protein was present (Fig. 3A, lane 2) or if an antibody that does not recognize the HA epitope was present during immunoprecipitation (27). In addition, LexA alone was not immunoprecipitated with Hir1p-HA (54). We performed the same analysis after reversing the epitope tags on Hir1p and Hir2p and found that LexA-Hir1p was present in Hir2p-HA immunoprecipitates (Fig. 3B, lane 2). These in vitro associations between Hir1p and Hir2p support the notion that the two proteins might function in a complex in vivo. We have been unable to detect in vivo interactions between Hir1p and Hir2p by using double-hybrid analysis (45), which is perhaps not surprising given the ability of tethered Hir1p and Hir2p to repress basal transcription.

We also tested whether Hir1p self-associates, which might explain the dependence of Hir1p on the presence of the *HIR1* gene product when it is tethered at the *HTA1* promoter (Fig. 1A). High-copy-number plasmids carrying a *HIR1-HA* gene and a *lexA-HIR1* gene were cotransformed into a wild-type strain, and the Hir1p-HA protein was immunoprecipitated from cell extracts with monoclonal antibody 12C5A (Fig. 3C). LexA-Hir1p was present in Hir1p-HA immunoprecipitates (Fig. 3C, lane 6). This association was specific, as it was not observed if a nonspecific antibody was used to precipitate Hir1p-HA (Fig. 3C, lane 5) or if an untagged *HIR1* gene was present instead of a *HIR1-HA* gene (Fig. 3C, lane 3). Thus, Hir1p can associate both with Hir2p and with itself.

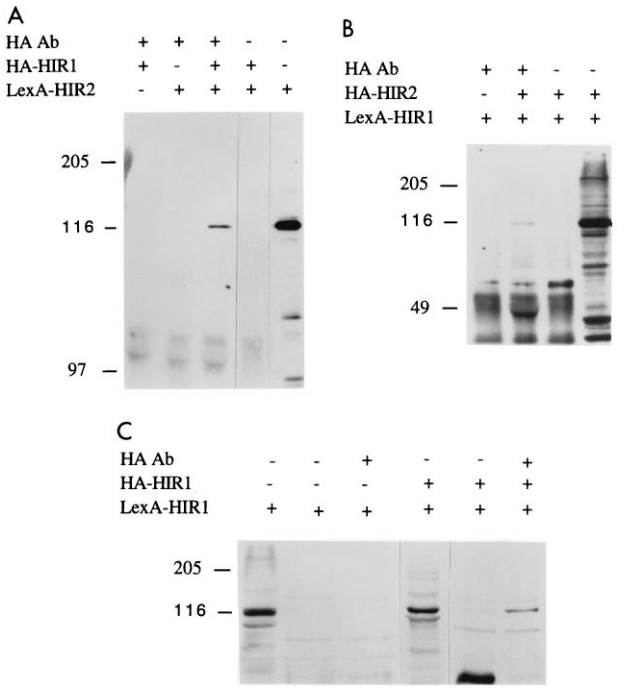

FIG. 3. Hir1p can be coimmunoprecipitated with Hir2p and Hir1p from yeast cell extracts. Strain BJ5465 was cotransformed with plasmids carrying the *HIR1* or *HIR2* gene with or without the HA epitope and plasmids carrying a *lexA-HIR2* or *lexA-HIR1* gene. Whole-cell extracts were incubated with monoclonal antibody (Ab) 12C5A, and the presence of LexA-Hir2p or LexA-Hir1p in immune complexes was monitored by SDS–7.5% polyacrylamide gel electrophoresis and Western blot analysis using polyclonal antibodies against LexA. LexA-Hir1p migrates at a mass of approximately 116 kDa, and LexA-Hir2p migrates at approximately 120 kDa. (Molecular masses are shown in kilodaltons in the left of each panel.) (A) Hir1p-HA and LexA-Hir2p. Lane 1, 440 μg of lysate from cells containing only the *HIR1-HA* gene; lane 2, 220 μg of lysate from cells containing an untagged *HIR1* gene and a *lexA-HIR2* gene; lane 3, 440 μg of lysate from cells containing *HIR1-HA* and *lexA-HIR2* genes; lane 4, 440 μg of lysate from cells containing *HIR1-HA* and *lexA-HIR2* genes incubated without monoclonal antibody 12C5A; lane 5, 6 μg of input lysate from cells containing *HIR1-HA* and *lexA-HIR2* genes. (B) Hir2p-HA and LexA-Hir1p. Lane 1, 60 μg of lysate from cells containing an untagged *HIR2* gene and a *lexA-HIR1* gene; lane 2, 60 μg of lysate from cells containing *HIR2-HA* and *lexA-HIR1* genes; lane 3, 60 μg of lysate from cells containing *HIR2-HA* and *lexA-HIR1* genes incubated without monoclonal antibody 12C5A; lane 4, 6 μg of input cell lysate from cells containing *HIR2-HA* and *lexA-HIR1* genes. (C) Hir1p-HA and LexA-Hir1p. Lane 1, 15 μg of input cell lysate from cells containing an untagged *HIR1* gene and a *lexA-HIR1* gene; lane 2, 150 μg of cell lysate from cells containing an untagged *HIR1* gene and a *lexA-HIR1* gene incubated with a nonspecific antibody (rabbit anti-mouse); lane 3, 150 μg of cell lysate from cells containing an untagged *HIR1* gene and a *lexA-HIR1* gene incubated with monoclonal antibody 12C5A; lane 4, 15 μg of input lysate from cells containing a *HIR1-HA* tagged gene and a *lexA-HIR1* gene; lane 5, 150 μg of cell lysate from cells containing *HIR1-HA* and *lexA-HIR1* genes incubated with a nonspecific antibody (rabbit anti-mouse); lane 6, 150 μg of cell lysate from cells containing *HIR1-HA* and *lexA-HIR1* genes incubated with monoclonal antibody 12C5A. The dark band at the bottom of lane 5 is rabbit anti-mouse antibody, which was used to control for nonspecific immunoprecipitation.

Tethered Hir1p represses transcription in the cell cycle. The *HTA1-HTB1* negative site keeps the *HTA1* and *HTB1* genes repressed for most of the cell cycle, restricting their transcrip-

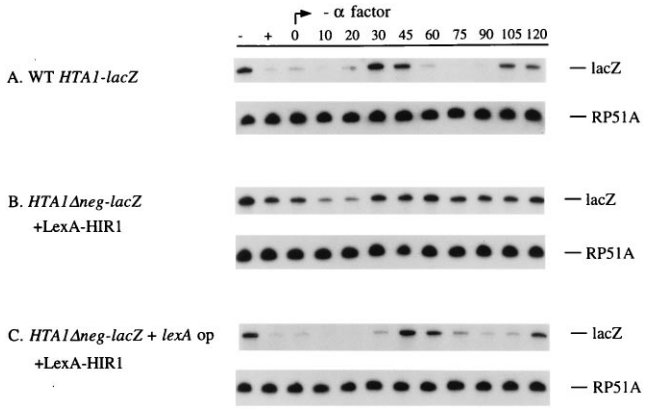

FIG. 4. Tethered Hir1p represses *HTA1-lacZ* transcription during the cell cycle. Strain K3391 (Table 1) was transformed with a *CEN3-LEU2* plasmid that carried one of three different *HTA1-lacZ* reporter genes: an *HTA1-lacZ* gene regulated by the wild-type *HTA1* promoter (WT *HTA1-lacZ*) (A); an *HTA1-lacZ* reporter gene regulated by an *HTA1* promoter from which the *HTA1-HTB1* negative site has been deleted (*HTA1Δneg-lacZ*) (B); and an *HTA1-lacZ* reporter gene regulated by an *HTA1* promoter in which two *lexA* operator sites have been inserted in place of the negative site (*HTA1Δneg-lacZ* + *lexA* op) (C). A plasmid carrying a *lexA-HIR1* fusion gene was also present in panels B and C. Each strain was arrested at Start by treatment with α mating factor at 24°C, and following release from G_1 arrest at 24°C, the levels of *HTA1-lacZ* and *RP51A* mRNAs were measured by a quantitative S1 nuclease protection assay at the indicated times.

tion to the G_1/S phase boundary (29, 41). To test whether the activity of Hir1p and Hir2p as transcriptional repressors is responsible for the periodic accumulation of *HTA1* mRNA, we examined whether tethered Hir1p could substitute for the *HTA1-HTB1* negative site to drive *HTA1-lacZ* transcription in synchronized cells (Fig. 4). We used three strains, each of which carried a different *lacZ* reporter gene. The first strain carried a *lacZ* reporter gene regulated by a chromosomal copy of the *HTA1* promoter, and it served as a control for the effects of the native Hir proteins at the *HTA1-HTB1* negative site (Fig. 4A, WT (wild-type) *HTA1-lacZ*). The other two strains carried either an *HTA1-lacZ* reporter gene with a deletion of the negative site (Fig. 4B, *HTA1Δneg-lacZ*) or the same reporter gene with two *lexA* operators substituted for the negative site (Fig. 4C, *HTA1Δneg-lacZ* + *lexA* op) and LexA-Hir1p.

Each strain was arrested in G_1 at Start by treatment with α mating factor, and after release from the block, the accumulation of *lacZ* mRNA was monitored over an interval of two cell cycles. As we have observed previously (41), transcription of the *lacZ* reporter gene regulated by the wild-type *HTA1* promoter was sharply periodic (Fig. 4A): *lacZ* mRNA was present at very reduced amounts in α-factor-arrested cells and accumulated to peak levels 30 min after release from the G_1 block. When the negative site was deleted from the *HTA1* promoter (Fig. 4B), the periodic pattern of *HTA1-lacZ* transcription was perturbed and *lacZ* transcripts accumulated at all points in the cell cycle, regardless of the presence of LexA-Hir1p. However, when Hir1p was tethered to *lexA* binding sites in place of the *HTA1-HTB1* negative site, periodic transcription was completely restored (Fig. 4C): *lacZ* transcripts were present at very low levels in α-factor-blocked cells, and they accumulated to peak levels by 45 min after release from the G_1 block. The 15-min delay in *lacZ* transcript accumulation relative to wild-type *HTA1-lacZ* mRNA is not significant, as it was also observed for *HTB2* mRNA (data not shown). Restoration of periodic transcription was entirely dependent on the presence of the Hir1p moiety on the tethered fusion protein, as LexA itself was unable to reverse the derepressed pattern of *HTA1Δneg-lacZ* transcription when it was bound to the same *lexA* operator sites (data not shown).

The constitutively transcribed *CYC1* gene becomes cell cycle regulated when the *HTA1-HTB1* negative site is present at its promoter (41, 43). We therefore examined whether Hir1p could confer periodic repression on a *CYC1-lacZ* reporter gene when it was tethered to the *CYC1* promoter (Fig. 5). Tethered Hir1p caused the periodic transcription of the normally constitutive reporter gene, although it skewed somewhat the period of *CYC1-lacZ* transcription relative to that of the chromosomal *HTB2* gene. *CYC1-lacZ* transcription was repressed in early-G_1-phase cells when Hir1p was tethered to the promoter, just like *HTB2* transcription, but its transcription was derepressed almost 15 min earlier than for the *HTB2* gene (Fig. 5B). This difference, which was also observed when the *HTA1-HTB1* negative site was present at the *CYC1* promoter (41), presumably reflects the contribution of histone gene-specific activators to periodic transcription (55). Nonetheless, because LexA is likely to be bound constitutively to its cognate site at both the *HTA1* and *CYC1* promoters, the combined data suggest that it is the regulated activity of Hir1p, or a protein associated with Hir1p, that determines when the *HTA1* and *HTB1* genes will be transcribed during the cell cycle.

Tethered Hir1p responds to two cell cycle regulatory signals. In a second approach, we determined whether tethered Hir1p could respond to specific cell cycle regulatory signals to repress *HTA1-lacZ* transcription. The first signal occurs late in G_1 phase at the *CDC4*-dependent step. Cells blocked at this step fail to derepress *HTA1* transcription or to initiate DNA replication (16, 62). The second signal is produced in S phase when DNA replication is blocked, which results in the premature repression of *HTA1* transcription (18, 29). In a *HIR1* deletion strain, *HTA1* transcription is derepressed when cells are blocked at either of these steps, suggesting that the Hir1 protein is absolutely required at both points of the cell cycle to keep transcription off (43, 54).

When Hir1p was tethered to the *HTA1* promoter, it executed appropriate responses to both cell cycle regulatory signals (Fig. 6). In *cdc4*^{ts} cells arrested at the G_1/S boundary by growth at 36°C (Fig. 6A), tethered Hir1p repressed *HTA1-lacZ* transcription almost to the same extent as if the *HTA1-HTB1* negative site were present in the reporter gene. In S-phase cells arrested by hydroxyurea treatment (Fig. 6B), *lacZ* transcripts regulated either by tethered Hir1p (Fig. 6B, lane 8) or by the

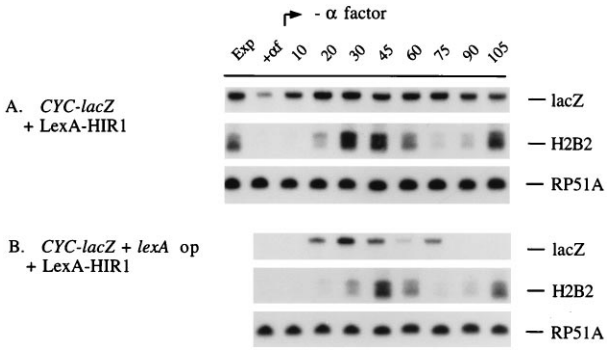

FIG. 5. Tethered Hir1p represses *CYC1-lacZ* transcription during the cell cycle. Strain YEL106 (Table 1) was cotransformed with a plasmid that carried a *lexA-HIR1* fusion gene and either plasmid pAJ1 (*CYC1-lacZ*) (A) or plasmid pJK1621 (*CYC1-lacZ* + *lexA* op) (B). Each strain was arrested at Start by treatment with α mating factor at 30°C, and following release from G_1 arrest, the levels of *CYC1-lacZ*, *HTB2*, and *RP51A* mRNAs were measured by a quantitative S1 nuclease protection assay at the indicated times.

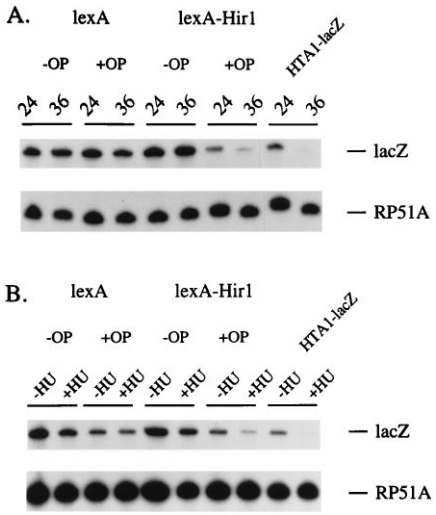

FIG. 6. Response of tethered Hir1p to two cell cycle regulatory signals. Plasmids carrying a *lexA* gene or a *lexA-HIR1* fusion gene were transformed into yeast strains 1078-2B (*cdc4*^{ts}) and W303-1A (*HIR*$^{+}$) (Table 1), each of which carried either an *HTA1-lacZ* reporter gene without the *HTA1-HTB1* negative site (−OP) or an *HTA1-lacZ* reporter gene with two *lexA* operator sites inserted in place of the *HTA1-HTB1* negative site (+OP). A plasmid carrying an *HTA1-lacZ* reporter gene regulated by the wild-type *HTA1* promoter was independently transformed into the same two strains, and it served as a control for the effects of the native Hir proteins at the *HTA1-HTB1* negative site. (A) The *cdc4*^{ts} mutant was grown at 24°C or shifted to 37°C for 2.5 h to inactivate the *CDC4* gene product prior to RNA isolation. (B) The wild-type (*HIR*$^{+}$) strain was grown in the presence or absence of hydroxyurea (+HU or −HU) for 30 min to inhibit DNA replication. The levels of *HTA1-lacZ* and *RP51A* mRNAs were determined by a quantitative S1 nuclease protection assay.

native *HTA1-HTB1* negative site (*HTA1-lacZ*) dropped almost fivefold in abundance.

We next examined whether tethered Hir1p inhibited *HTA1-lacZ* transcription in hydroxyurea-treated cells in the absence of the *HIR1*, *HIR2*, or *HIR3* gene product (Fig. 7). Although all three Hir proteins are required for *HTA1* repression upon replication arrest (43, 64) (Fig. 7A), deletion of *HIR2* or *HIR3* did not alter the ability of tethered Hir1p to inhibit *HTA1-lacZ* transcription in hydroxyurea-treated cells (Fig. 7B). The *hir1*Δ mutation partially eliminated the ability of tethered Hir1p to respond to the replication block, again suggesting that the DNA-bound Hir1 fusion protein requires the presence of wild-type Hir1p to execute its regulatory effect.

These last results prompted us to examine whether tethered Hir2p could repress *HTA1-lacZ* transcription upon replication arrest and whether the *HIR1* or *HIR3* gene products might play a role in its response (Fig. 7C). Tethered Hir2p also caused transcription to be repressed in *HIR*$^{+}$ cells that had been treated with hydroxyurea. However, although Hir1p and Hir3p are required for the function of tethered Hir2p at the *HTA1* promoter in exponential cells, they were dispensable for its role in replication-arrested cells. This finding suggests that the two proteins perform overlapping functions in certain aspects of *HTA1* transcriptional repression.

DISCUSSION

In this study, we show that Hir1p and Hir2p, two yeast proteins that negatively regulate six of the eight core histone genes in the apparent absence of DNA binding, function as transcriptional corepressors. Each protein behaved as a direct transcriptional repressor when artificially recruited to yeast promoters, suggesting that both Hir1p and Hir2p normally function at histone gene promoters through their recognition of a site-specific DNA binding protein. The two Hir proteins may act at these promoters as part of a multiprotein complex: Hir2p required Hir1p and a second protein, Hir3p, to repress transcription when it was directed to the *HTA1* promoter, and Hir1p and Hir2p are physically associated in vivo. The regulated activity of proteins in this complex may account for the periodic expression of the histone genes under its control. Tethered Hir1p faithfully repressed transcription at the *HTA1* promoter in response to a variety of cell cycle regulatory signals, and it could make a normally constitutive gene become periodically transcribed. Hir1p and Hir2p thus represent the first examples in which transcriptional corepressors dictate key aspects of temporally regulated transcription.

Hir1p and Hir2p function as transcriptional corepressors. Although Hir1p and Hir2p act at a negative regulatory site in the *HTA1* promoter (29), the notion that they function without directly binding to this site was initially suggested by the absence of typical DNA binding motifs in either protein (51). This view is strengthened by the observation that a factor that binds in vitro at the *HTA1-HTB1* negative site is still present in extracts prepared from a *hir1*Δ *hir2*Δ double mutant (49). We therefore interpret the results of the tethering experiments to mean that native Hir1p and Hir2p are recruited to histone genes by their recognition of a site-specific DNA binding protein that is present at the promoter of each negatively regulated gene. Thus, the two proteins may be functionally equivalent to transcriptional corepressors such as Tup1p and Ssn6p (11, 19, 22, 60, 63) and Sir1p, Sir2p, Sir3p, and Sir4p (2, 8, 17, 33). In both cases, proteins in the two complexes are targeted

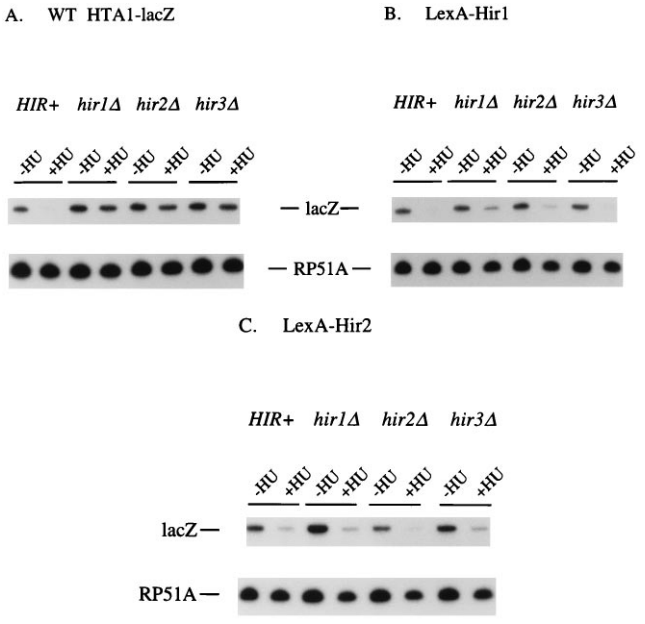

FIG. 7. Tethered Hir1p and Hir2p repress transcription after a replication block in *hir* mutants. *HIR*$^{+}$, *hir1*Δ, *hir2*Δ, and *hir3*Δ strains (Table 1) were transformed with a plasmid carrying an *HTA1-lacZ* gene regulated by the wild-type *HTA1* promoter (WT HTA1-lacZ) (A), a plasmid carrying a *lexA-HIR1* gene and a plasmid carrying an *HTA1-lacZ* reporter gene with two *lexA* operator sites inserted in place of the *HTA1-HTB1* negative site (B), and a plasmid carrying a *lexA-Hir2* gene and the same *HTA1-lacZ* reporter plasmid as in panel B (C). Each strain was grown in the presence or absence of hydroxyurea (+HU or −HU) for 30 min to block DNA replication, and the levels of *lacZ* and *RP51A* transcripts were measured in a quantitative S1 nuclease protection assay. None of the *hir* mutations or hydroxyurea treatment affected the level of *lacZ* mRNA when LexA alone was tethered to the *HTA1-lacZ* promoter.

to particular genes or chromosomal locations through their interactions with site-specific DNA binding proteins. The factor that binds in vitro at the *HTA1-HTB1* negative site may be the site-specific DNA binding factor recognized by Hir1p and Hir2p at this histone gene promoter. Negative sites also occur in the two histone H3-H4 promoters that are regulated by Hir1p and Hir2p (14, 43), but it is not known whether these sites are bound by the same factor. If Hir1p and Hir2p recognize different DNA binding proteins at each of the histone gene promoters, then the two Hir proteins could represent general factors that are differentially recruited to histone gene promoters.

The finding that Hir1p and Hir2p can be coimmunoprecipitated suggests that the two proteins act as a complex in vivo. The Hir tethering assays, however, showed that both proteins are also able to function independently, Hir1p in the absence of Hir2p at the *HTA1* promoter and Hir2p in the absence of Hir1p at the *CYC1* promoter. One interpretation of these results is that Hir1p and Hir2p perform similar or overlapping roles as transcriptional repressors, but at the *HTA1* promoter, native Hir1p provides a second and unique function. Thus, at this promoter, repression might normally occur as the result of the activity of a Hir1p-Hir2p complex. Because the products of five additional *HIR* and *HPC* genes are also required to repress *HTA1* and *HTB1* transcription (43, 64), it is possible that a multiprotein Hir-Hpc complex represses transcription at the *HTA1-HTB1* promoter. We speculate that the *HIR3* gene product may be a third member of such a complex because of its functional interactions with Hir2p. The finding that Hir1p self associates additionally suggests that Hir1p may be present in multiple copies in the putative Hir-Hpc complex.

Because both Hir1p and Hir2p repress transcription in two different promoter contexts, it is unlikely that they function by targeting specific transactivators for inhibition. Several other mechanisms could account for their action. For example, one or both Hir proteins might directly inhibit a member of the RNA polymerase II preinitiation complex. This mechanism has been proposed to account for repression by the Tup1p-Ssn6p corepressor complex (19, 60). Alternatively, the proteins may repress transcription through their effects on chromatin structure, a second mechanism associated with Tup1p-Ssn6p-mediated repression (11, 13) and the likely way by which the Sir3 and Sir4 proteins cause repression at *HM* loci and at telomeres (17, 20a, 58).

We favor the interpretation that Hir1p and Hir2p act on chromatin structure to repress transcription. First, the *HTA1-HTB1* locus is organized into a highly ordered array of nucleosomes (37), and deletion of *HIR1* profoundly disrupts this array (10). Second, this same histone gene locus is also subject to feedback repression by the overproduction of histones H2A and H2B, a response that is dependent on the *HIR1*, *HIR2*, and *HIR3* gene products (32, 51, 54) and also likely to be mediated through changes in chromatin structure. These results suggest that Hir1p and/or Hir2p might interact directly with chromatin constituents to repress transcription. These constituents could be the core histones themselves or other proteins that interact with chromatin. Candidates for such chromatin-associated proteins are the *SPT4*, *SPT5*, and *SPT6* gene products (9, 30, 56, 57), which are required for Hir1p to repress transcription when it is tethered at the *HTA1* promoter (42).

Role of Hir repressors in cell cycle-regulated transcription. Transcriptional activation is the most widely used mechanism to control gene expression during the yeast cell cycle (4, 23), and the regulated binding or activity of transcriptional activators appears to account for the periodic transcription of genes such as *HO*, *CLN1*, and *CLN2* (1, 5, 6, 24, 28, 34, 38, 52). The results of this study represent the first example in which transcriptional corepressors dictate key aspects of periodic control. When Hir1p is brought to two different yeast promoters, it can execute all of the functions provided by the *HTA1-HTB1* negative site to keep transcription repressed at appropriate points in the cell cycle. Tethered Hir2p is also able to direct the periodic transcription of an *HTA1-lacZ* reporter gene in synchronized cells (44), but the results of our functional studies suggest that Hir2p acts with Hir1p at the *HTA1* promoter throughout most of the cell cycle. We speculate that Hir1p, through its activity in a Hir1p-Hir2p complex, might therefore be a major target of regulatory signals in normally cycling cells. However, because Hir1p and Hir2p are able to function independently during replication arrest, both proteins might be separately targeted by regulatory signals triggered by this cell cycle block.

Hir1p and/or Hir2p must be transiently inactivated at the G_1/S phase boundary for *HTA1* transcription to be derepressed. A strong clue as to the nature of the G_1/S-phase regulatory signal comes from the observation that tethered Hir1p represses *HTA1* transcription in *cdc4*^{ts} arrested cells. The *CDC4* gene product is involved in the inactivation of a subset of the Clb-Cdc28 kinases (47), and recently we have found that an active Clb2-Cdc28 complex is required to derepress *HTA1* transcription through the *HIR1* gene product (12).

ACKNOWLEDGMENTS

Stan Fields, Kim Nasmyth, and Fred Cross are thanked for providing yeast strains or plasmids, and Roger Brent is gratefully acknowledged for the gift of anti-LexA antibodies. Peter Sherwood is thanked for initiating studies on the function of Hir1p and Hir2p, and Art Lustig is thanked for helpful discussions.

This study was supported by NIH grant GM40118 to M.A.O.

REFERENCES

1. **Andrews, B. J., and I. Herskowitz.** 1989. Identification of a DNA binding factor involved in cell-cycle control of the yeast *HO* gene. Cell **57:**21–29.
2. **Aparicio, O. M., B. L. Billington, and D. E. Gottschling.** 1991. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. Cell. **66:**1279–1287.
3. **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. Wiley Interscience and Green Publishing Associates, New York, N.Y.
4. **Breeden, L.** 1996. Start-specific transcription in yeast. Curr. Top. Microbiol. Immunol. **208:**95–127.
5. **Breeden, L., and G. Mikesell.** 1994. Three independent forms of regulation affect expression of *HO*, *CLN1*, and *CLN2* during the cell cycle of *Saccharomyces cerevisiae*. Genetics **138:**1015–1024.
6. **Breeden, L., and K. Nasmyth.** 1987. Cell cycle control of the yeast *HO* gene: *cis*- and *trans*-acting regulators. Cell **48:**389–397.
7. **Brent, R., and M. Ptashne.** 1984. A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene. Nature **312:**612–615.
8. **Chien, C. T., S. Buck, R. Sternglanz, and D. Shore.** 1993. Targeting of SIR1 protein establishes transcriptional silencing at *HM* loci and telomeres in yeast. Cell **75:**531–541.
9. **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.
10. **Compagnone-Post, P.** Unpublished data.
11. **Cooper, J. P., S. Y. Roth, and R. T. Simpson.** 1994. The global transcriptional regulators, SSN6 and TUP1, play distinct roles in the establishment of a repressive chromatin structure. Genes Dev. **8:**1400–1410.
12. **Dimova, D., A. Raff, and M. A. Osley.** Unpublished data.
13. **Edmondson, D. G., M. M. Smith, and S. Y. Roth.** 1996. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev. **10:**1247–1259.
14. **Freeman, K. B., L. R. Karns, K. A. Lutz, and M. M. Smith.** 1992. Histone H3 transcription in *Saccharomyces cerevisiae* is controlled by multiple cell cycle activation sites and a constitutive negative regulatory element. Mol. Cell. Biol. **12:**5455–5463.
15. **Guarente, L.** 1992. Mechanism and regulation of transcriptional activation in eukaryotes: conserved features from yeasts to humans, p. 1007–1036. *In* S. L. McKnight and K. Yamamoto (ed.), Transcriptional regulation. Cold Spring

Harbor Laboratory Press, Cold Spring Harbor, N.Y.
16. **Hartwell, L. H.** 1976. Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. J. Mol. Biol. **104:**803–817.
17. **Hecht, A., T. Laroche, S. Strahl-Bolsinger, S. Gasser, and M. Grunstein.** 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell **80:**583–592.
18. **Hereford, L., S. Bromley, and M. A. Osley.** 1982. Periodic transcription of yeast histone genes. Cell **30:**305–310.
19. **Herschbach, B. M., M. B. Arnaud, and A. D. Johnson.** 1994. Transcriptional repression directed by the yeast α2 protein *in vitro*. Nature **370:**309–311.
20. **Ito, H., Y. Fukuda, K. Marata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
20a. **Johnson, L., P. S. Kayne, E. S. Kahn, and M. Grunstein.** 1990. Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating type loci in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **87:**6286–6290.
21. **Kaiser, C., S. Michaelis, and A. Mitchell.** 1994. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
23. **Koch, C., and K. Nasmyth.** 1994. Cell cycle regulated transcription in yeast. Curr. Opin. Cell Biol. **6:**451–459.
24. **Koch, C., A. Schleiffer, G. Ammerer, and K. Nasmyth.** 1996. Switching transcription on and off during the yeast cell cycle: Cln/Cdc28 kinases activate bound transcription factor SBF (Swi4/Swi6) at Start, whereas Clb/Cdc28 kinases displace it from the promoter in G2. Genes Dev. **10:**129–141.
25. **Komachi, K., M. J. Redd, and A. D. Johnson.** 1994. The WD repeats of Tup1 interact with the homeo domain protein α2. Genes Dev. **8:**2857–2867.
26. **Lamour, V., Y. Lecluse, C. Desmaze, M. Spector, M. Bodescot, A. Aurias, M. A. Osley, and M. Lipinski.** 1995. A human homolog of the *S. cerevisiae HIR1* and *HIR2* transcriptional repressors cloned from the DiGeorge syndrome critical region. Human Mol. Genet. **4:**791–799.
27. **Lee, K.** Unpublished data.
28. **Lowndes, N. F., A. L. Johnson, L. Breeden, and L. H. Johnston.** 1992. *SWI6* protein is required for transcription of the periodically expressed DNA synthesis genes in budding yeast. Nature **357:**505–508.
29. **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.
30. **Malone, E. A., J. S. Fassler, and F. Winston.** 1993. Molecular and genetic characterization of *SPT4*, a gene important for transcription initiation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **237:**449–459.
31. **Moran, L.** 1994. Ph.D. thesis. Cornell University, Ithaca, N.Y.
32. **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.
33. **Moretti, P., K. Freeman, L. Coodly, and D. Shore.** 1994. Evidence that a complex of SIR proteins interacts with the silencer and telomere binding protein RAP1. Genes Dev. **8:**2257–2269.
34. **Nasmyth, K., and L. Dirick.** 1991. The role of SWI4 and SWI6 in the activity of G1 cyclins in yeast. Cell **66:**995–1013.
35. **Neer, E. J., C. J. Schmidt, R. Nambudripad, and T. F. Smith.** 1994. The ancient regulatory-protein family of WD-repeat proteins. Nature **371:**297–300.
36. **Niman, H. L., R. A. Houghtern, L. E. Walker, R. A. Reisfeld, I. A. Wilson, J. M. Hogle, and R. A. Lerner.** 1983. Generation of protein-reactive antibodies by short peptides is an event of high frequency: implication for the structural basis of immune recognition. Proc. Natl. Acad. Sci. USA **87:**5373–5377.
37. **Norris, D., B. L. Dunn, and M. A. Osley.** 1988. The effect of histone gene deletions on chromatin structure in *Saccharomyces cerevisiae*. Science **242:**759–761.
38. **Ogas, J., B. J. Andrews, and I. Herskowitz.** 1991. Transcriptional activation of *CLN1*, *CLN2*, and a putative new G1 cyclin (*HCS26*) by SWI4, a positive regulator of G1-specific transcription. Cell **66:**1015–1026.
39. **Osley, M. A.** 1991. The regulation of histone synthesis in the cell cycle. Annu. Rev. Biochem. **60:**827–861.
40. **Osley, M. A.** Unpublished data.
41. **Osley, M. A., J. Gould, S. Kim, M. Kane, and L. Hereford.** 1986. Identification of sequences in a yeast histone promoter involved in periodic transcription. Cell **45:**537–544.
42. **Osley, M. A., and K. Lee.** Unpublished data.
43. **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.
44. **Osley, M. A., and A. Raff.** Unpublished data.
45. **Raff, A.** Unpublished data.
46. **Recht, J., B. Dunn, A. Raff, and M. A. Osley.** 1996. Functional analysis of histones H2A and H2B in transcriptional repression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**2545–2553.
47. **Schwob, E., T. Bohm, M. D. Mendenhall, and K. Nasmyth.** 1994. The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S phase transition in *S. cerevisiae*. Cell **79:**233–244.
48. **Sherwood, P. W.** 1993. Ph.D. thesis. Cornell University, Ithaca, N.Y.
49. **Sherwood, P. W.** Unpublished data.
50. **Sherwood, P. W., and M. A. Osley.** 1991. Histone regulatory (*hir*) mutations suppress δ insertion alleles in *Saccharomyces cerevisiae*. Genetics **128:**729–738.
51. **Sherwood, P. W., S. Tsang, and M. A. Osley.** 1993. Characterization of *HIR1* and *HIR2*, two genes required for regulation of histone gene transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**28–38.
52. **Sidorova, J., and L. Breeden.** 1993. Analysis of the SWI4/SWI6 protein complex, which directs G_1/S-specific transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**1069–1077.
53. **Spector, M. S.** 1994. Ph.D. thesis. Cornell University, Ithaca, N.Y.
54. **Spector, M. S.** Unpublished data.
55. **Spector, M. S., and M. A. Osley.** 1993. The *HIR4-1* mutation defines a new class of histone regulatory genes in *Saccharomyces cerevisiae*. Genetics **135:**25–34.
56. **Swanson, M. S., E. A. Malone, and F. Winston.** 1991. *SPT5*, an essential gene important for normal transcription in *Saccharomyces cerevisiae*, encodes an acidic nuclear protein with a carboxy-terminal repeat. Mol. Cell. Biol. **11:**3009–3019.
57. **Swanson, M. S., and F. Winston.** 1992. *SPT4*, *SPT5*, and *SPT6* interactions: effects on transcription and viability in *Saccharomyces cerevisiae*. Genetics **132:**325–336.
58. **Thompson, J. S., X. Ling, and M. Grunstein.** 1994. The histone H3 amino terminus is required for both telomeric and silent mating locus repression in yeast. Nature **369:**245–247.
59. **Treitel, M., and M. Carlson.** 1995. Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein. Proc. Natl. Acad. Sci. USA **92:**3132–3136.
60. **Tzamarias, D., and K. Struhl.** 1994. Functional dissection of the yeast Cyc8-Tup1 transcriptional co-repressor complex. Nature **369:**758–761.
61. **Tzamarias, D., and K. Struhl.** 1995. Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. **9:**821–831.
62. **White, J. H. M., S. R. Green, D. G. Barker, L. B. Dumas, and L. H. Johnston.** 1987. The *CDC8* transcript is cell cycle regulated in yeast and is expressed coordinately with *CDC9* and *CDC21* at a point preceding histone transcription. Exp. Cell Res. **171:**223–231.
63. **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.
64. **Xu, H., U.-J. Kim, T. Schuster, and M. Grunstein.** 1992. Identification of a new set of cell cycle-regulatory genes that regulate S-phase transcription of histone genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**5249–5259.