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trans-Acting Regulatory Mutations That Alter Transcription of *Saccharomyces cerevisiae* Histone Genes

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Using a *Saccharomyces cerevisiae* strain containing an integrated copy of an *H2A-lacZ* fusion gene, we screened for mutants which overexpressed beta-galactosidase as a way to identify genes which regulate transcription of the histone genes. Five recessive mutants with this phenotype were shown to contain altered regulatory genes because they had lost repression of *HTA1* transcription which occurs upon inhibition of chromosome replication (D. E. Lycan, M. A. Osley, and L. Hereford, Mol. Cell. Biol. 7:614–621, 1987). Periodic transcription was affected in the mutants as well, since the *HTA1* gene was transcribed during the G1 and G2 phases of the cell cycle, periods in the cell cycle when this gene is normally not expressed. A similar loss of cell cycle-dependent transcription was noted for two of the three remaining histone loci, while the *HO* and *CDC9* genes continued to be expressed periodically. Using isolated promoter elements inserted into a heterologous *cyc1-lacZ* fusion gene, we demonstrated that the mutations fell in genes which acted through a negative site in the *TRT1 H2A-H2B* promoter.

For reasons that are still not well understood, the accumulation of histone RNAs during the cell cycle of eucaryotic organisms is timed to correspond with the period of chromosome replication (1, 3, 6–8, 17). In the budding yeast *Saccharomyces cerevisiae*, transcriptional controls account primarily for the periodic pattern of histone RNA accumulation. In this organism, transcription of the four core histone genes is restricted to the late G1-early S phase, and both the turn-on of transcription in G1 and the turn-off of transcription in S are separately linked to the completion of specific steps that are required for the initiation of chromosome replication (7). We have recently shown that transcriptional regulation is also responsible for the control of histone RNA levels which occurs during the S phase: when ongoing chromosome replication is arrested with the drug hydroxyurea (HU) or by a temperature-sensitive mutation, transcription of the histone genes is prematurely turned off (10).

Transcription of the divergently transcribed *H2A-H2B* gene pair at the *TRT1* locus depends on repeated activating sequences (UAS) that are located approximately 200 to 300 base pairs (bp) upstream of the transcriptional start sites of both genes (14). These sequences function as cell cycle-dependent activating sequences because in isolation they can activate the transcription of a heterologous gene with correct cell cycle periodicity. A second, unique sequence in the divergent promoter has no intrinsic activating function and acts as a negative regulatory element (14). The presence of this negative element appears to be important for proper transcriptional control in the cell cycle. When it is deleted from the *TRT1* promoter, both forms of cell cycle control are lost: transcription occurs inappropriately in the G1 and G2 phases of the cell cycle (10, 14), and histone RNA continues to accumulate when cells are blocked in the S phase (10).

It is unclear why transcription of the histone genes should depend on the presence of two such different kinds of regulatory elements or how these elements interact. We initiated a study into the roles of the two elements by isolating mutations in regulatory genes which affect cell cycle-dependent transcription. We identified five regulatory mutations that both eliminate S-phase-dependent repression and result in constitutive transcription during the cell cycle. These mutations are in genes that act through the negative site in the *TRT1* promoter and are specific to the yeast histone loci.

MATERIALS AND METHODS

Strains and culture conditions. *S. cerevisiae* MO10 (a *ura3-52 leu2-3,112 trp1-289 his3Δ-1 TRT1::H2A-lacZ::LEU2*) was used to generate regulatory mutations. It contains a 13-amino-acid *H2A-lacZ* fusion gene with a wild-type *TRT1* promoter integrated in single copy at the *TRT1* locus. Complementation tests were performed with yeast strain MW3025-12A (α *ura3 his7 leu2-3, 112 ade2 trp1*). Strains were grown in YEPD (7) or YNB (15) medium supplemented with 100 μg of the appropriate amino acids or bases per ml, except for analysis of HU repression, in which YM-1 (5) medium was used. For the measurement of HU-mediated repression of histone gene expression, exponentially growing cells at a density of 3×10^6 to 5×10^6 cells per ml were incubated in 0.2 M HU (Sigma Chemical Co., St. Louis, Mo.) for 30 min at 30°C. Sodium azide was added to 0.1%, and the cells were then pelleted and frozen at −80°C before isolation of RNA. Cells were synchronized with alpha-factor as previously described (14). Beta-galactosidase assays were performed on cells grown in selective medium (14).

Plasmids. Yeast 2μm plasmids containing *cyc1-lacZ* fusion genes under control of isolated histone promoter elements have been previously described (14). These plasmids were transformed into yeast cells by the lithium chloride procedure (9), using selection for the *URA3*$^+$ gene on the plasmid.

RNA analysis. Total yeast RNA was isolated from 25 to 30 ml of cells at a density of 3×10^6 to 5×10^6 cells per ml. RNA was extracted as previously described (7) and used in either Northern gel (RNA blot) (21) or quantitative S1 nuclease (14) analysis. Total RNA (10 μg) was used in Northern gel analysis, and 20 μg of RNA was used in the S1

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assay. Northern gel blots were hybridized with probes specific to genes from the four histone loci or to the HO (12) and *CDC9* (16) genes. *HTA1* RNA was detected with a 500-bp *Sac*I-*Hin*dIII fragment containing 5′ *HTA1* sequences; *HTB2* RNA was analyzed with either a 1.1-kilobase (kb) *Hin*dIII fragment containing the 3′ end of the *HTB2* gene and the nonhistone protein 2 gene or a 600-bp *Acc*I-*Hin*dIII fragment containing the 5′ portion of the *HTB2* gene. *H3* RNAs from the copy I and copy II loci were detected with a 3.5-kb *Hin*dIII-*Sma*I fragment and a 2.5-kb *Hin*dIII fragment, respectively, both of which were homologous to the entire *H3* coding sequences (19). *HO* RNA was identified by hybridization to an 870-bp *Hin*dIII-*Bam*HI fragment, and *CDC9* RNA was detected by hybridization to a 1.5-kb *Pst*I-*Sac*I fragment. For S1 analysis, a 1.4-kb *Cla*I-*Xho*I fragment was used to identify *cyc1-lacZ* RNA and a 700-bp *Sac*I-*Hin*dIII fragment was used to detect *HTB1* RNA, as previously described (14).

Isolation and characterization of regulatory mutants. Stationary-phase cells of *S. cerevisiae* MO10 were treated with ethyl methanesulfonate (18) under conditions that resulted in 30 to 50% survival, and the mutagenized cells were spread onto YEPD plates and incubated at 23°C. The cells were then replica printed to YNB minus leucine plates containing 100 μg of 5-bromo-4-chloro-3-indoyl-β-galactoside (X-gal; Boehringer-Mannheim Biochemicals, Indianapolis, Ind.) per ml and incubated at both 23 and 36°C. The MO10 cells were pale blue on X-gal plates under both of these conditions. We screened for colonies that were dark blue after overnight incubation and purified clones with this phenotype for more extensive analysis.

Of a total of 5,000 to 6,000 mutagenized cells, we isolated 40 independent, dark blue strains by this screen. Approximately 10 of these strains were pale blue at 23°C and dark blue at 36°C; the remaining mutants were dark blue at both temperatures. We eliminated dark blue strains containing mutations in the *lacZ* gene by a second, functional screen utilizing HU (10). HU was added to a sample of each cell culture as described above, and *H2A-lacZ* RNA levels were measured by the quantitative S1 nuclease assay. Mutants which had lost repression (*hir*) continued to make *H2A-lacZ* RNA in the presence of the replication block, while mutants with altered *lacZ* genes still turned off transcription of the fusion gene when HU was added.

Complementation tests were performed by crossing each mutant strain to MW3025-12A (*HIR*$^+$) and selecting for adenine and histidine prototrophs. HU repression was then measured in each diploid strain to test whether the regulatory mutation was recessive by the criterion of restoration of HU repression. After sporulation of the diploids, segregants containing the regulatory mutation were identified first as dark blue patches on X-gal plates and then by the loss of HU repression. Appropriately marked mutant segregants from each cross were mated in pairwise combinations for further complementation analysis. Complementation was first judged on X-gal plates if both strains contained an *H2A-lacZ* gene; pale blue cells represented complementation and dark blue cells represented a failure to complement. The presence or absence of complementation was then confirmed in the HU repression assay, utilizing the quantitative S1 nuclease assay.

With the exception of the regulatory mutant *hir24*, which had a generation time 1.75 times longer than wild-type cells, the parental and mutant strains grew at the same rate. Sporulation of both *hir24/HIR24* and *hir21/HIR21* diploids, however, revealed slow-growing segregants in almost all tetrads. The cells in such segregants were often large and abnormally shaped, while in the original *hir* haploid strains, cell size and shape were normal. The slow-growth phenotype did not segregate exclusively with the *hir* mutations, suggesting that another mutation(s) was present in such strains. These putative mutations, however, did not affect the 2:2 segregation of *hir* mutations with respect to their relief of HU repression. Instead, they reduced *HTA1* RNA levels even in the absence of the replication block.

RESULTS

Identification of mutants with effects on periodic transcription. In screening for mutants with altered expression of the histone genes, two observations were taken into account. The first is that mutations in individual histone genes do not give phenotypes which can be easily identified, and the second is that as a group these genes are essential (4). We provided a phenotype by utilizing a yeast strain containing an *H2A-lacZ* fusion gene. When such a fusion gene contains the entire histone upstream promoter sequences, it is correctly regulated during the cell cycle, and cells which contain the fusion gene form pale blue colonies on X-gal indicator plates (14). Since the histone genes are essential, we were not able to screen for mutations abolishing their transcription, but we reasoned that we could screen for mutations altering the pattern of periodic transcription. Previous work with the *TRT1* histone genes had shown that removal of a negative site from the promoter of an *H2A-lacZ* fusion gene results in continuous, unregulated transcription during the cell cycle (14). As a consequence of such nonperiodic expression, beta-galactosidase is overexpressed and dark blue colony color develops (14). We utilized this observation as part of an initial phenotypic screen to isolate yeast mutants which had lost control of periodic transcription. A strain containing a single copy of a wild-type *H2A-lacZ* fusion gene integrated at the *TRT1 H2A-H2B* locus was mutagenized with ethyl methanesulfonate, and dark blue colonies were identified on X-gal indicator plates against a large background of pale blue wild-type cells. Although a few of the dark blue clones were dark blue at 36°C and pale blue at 23°C, the majority of dark blue strains overexpressed beta-galactosidase at both 23 and 36°C. The former clones were of interest because they could have represented temperature-sensitive regulatory mutations.

We used a second, functional screen to identify which of the dark blue clones showed altered transcription of the histone genes. This screen was based on our observation that inhibition of ongoing DNA replication with the drug HU represses transcription of the yeast histone genes (Fig. 1A) (10). This repression is dependent on the same negative promoter sequences that affect periodic transcription in the uninterrupted cell cycle (10, 14), and we therefore expected that mutants which had generally lost cell cycle-dependent transcription would be insensitive to the effects of the replication block. All of the dark blue strains were therefore grown into the exponential phase and treated briefly with HU. The levels of RNA produced in the presence and absence of the inhibitor were then measured in an S1 nuclease protection assay (Fig. 1A). Five of the dark blue strains continued to make *H2A-lacZ* RNA in the presence of HU (*hir* = histone regulation); none of the strains were those which had shown a temperature-sensitive dark blue phenotype. With the exception of mutant *hir24*, which had a generation time almost twice as long as that of the parental strain, none of the mutants showed any kind of growth effects (see Materials and Methods).

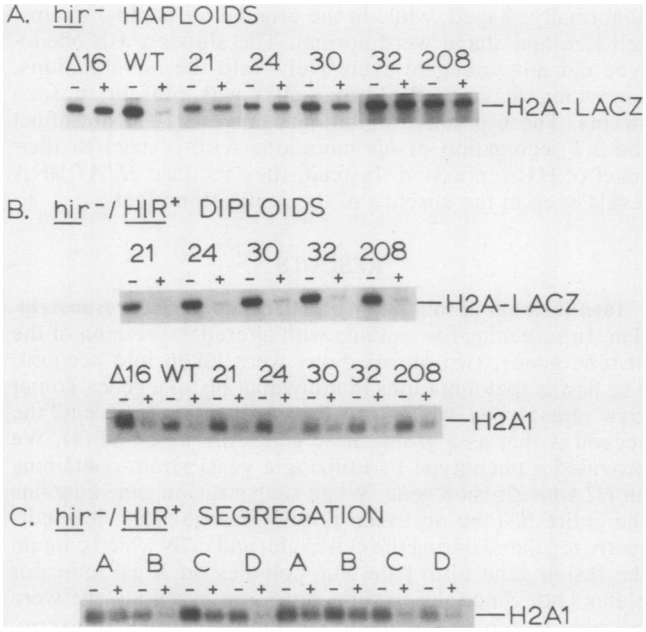

FIG. 1. Characterization of the response of regulatory mutants to HU repression. The levels of *H2A-lacZ* or *H2A1* RNA were measured before (−) or after (+) a 30-min incubation of cells in 0.2 M HU, using either an S1 nuclease protection assay (*H2A-lacZ*) or Northern gel analysis (*H2A1*). (A) *hir*⁻ haploids. Five dark blue mutant strains (*hir21*, *hir24*, *hir30*, *hir32*, *hir208*) isolated after ethyl methanesulfonate-induced mutagenesis of a strain containing an *H2A-lacZ* gene continued to make fusion RNA when DNA replication was blocked with HU. Wild-type cells (WT) turned off transcription under these same conditions, while a fusion gene with a deletion of *TRT1* negative promoter sequences (Δ16) (14) also did not respond to the replication block. (B) *hir*⁻/*HIR*⁺ diploids. Each *hir* mutant strain was crossed to wild-type (*HIR*⁺) cells of *S. cerevisiae* MW3025-12A, and the effect of HU on the levels of *H2A-lacZ* or *H2A1* RNA was followed in the resulting diploids. In each strain, a copy of the wild-type gene (*HIR*⁺) resulted in the restoration of HU repression. *H2A1* RNA levels were also repressed by HU in a diploid formed between a strain containing a deletion of negative promoter sequences in an *H2A-lacZ* fusion gene (Δ16). (C) *hir*⁻/*HIR*⁺ segregation. The effect of HU on the levels of *H2A1* RNA is shown for the meiotic progeny (A to D) of two tetrads that resulted from the sporulation of diploid strain *hir30*/MW3025-12A (*HIR*⁺). In both tetrads, two spore colonies continued to make RNA in the presence of HU (Hir⁻) and two spore colonies turned off (Hir⁺) the production of *H2A1* RNA.

Genetic analysis of regulatory mutants. To define the regulatory mutations in greater detail, we performed a number of genetic tests. Each mutant strain was first crossed to a wild-type strain, and the resulting diploids (*hir*⁻/*HIR*⁺) were analyzed to determine whether transcription of the *H2A-lacZ* gene continued in the presence of HU (Fig. 1B). Since HU repression was restored in every diploid strain, these complementation tests demonstrated that each *hir* mutation was recessive and thereby represented an alteration in a gene encoding a *trans*-acting regulatory protein.

To determine whether the regulatory effects were the result of mutations in single genes, we sporulated each heterozygous diploid and first followed the segregation of spore colony color in 10 of the resulting tetrads. Since the wild-type haploid strain that went into these crosses did not carry an *H2A-lacZ* gene, two spore colonies in each tetrad were blue and two were white. Moreover, the blue spore colonies were almost equally divided between those which were dark blue (Hir⁻) and those which were pale blue (Hir⁺), a segregation pattern which suggested that none of the regulatory mutations were tightly linked to the *H2A-lacZ* gene at the *TRT1* locus. Three tetrads from each of the three complementation groups subsequently identified (see below) were also examined for the segregation of HU-mediated repression, as measured by whether the transcription of the *TRT1 HTA* gene was turned off in the presence of the replication block. The segregation pattern in two such tetrads is shown in Fig. 1C. In each tetrad, two of the spore colonies had lost repression by HU, the expected outcome for mutations in single nuclear genes. Moreover, these data also showed that the regulatory mutations abolished repression not only of an *H2A-lacZ* fusion gene, but of the endogenous *HTA1* gene as well; similarly, the presence of a wild-type regulatory gene in diploid cells eliminated the effects of the regulatory mutations on both an *H2A-lacZ* gene and the *HTA1* gene (Fig. 1B). Thus, the regulatory mutations, which were identified on the basis of their effects on the expression of an *H2A-lacZ* gene, were not fusion-gene specific.

To measure the number of complementation groups represented by the five regulatory mutations, we crossed the mutants in pairwise combinations and assayed for the presence or absence of repression by HU in the resulting diploids (Fig. 2). The five mutants fell into three complementation groups: one complementation group contained *hir30* and *hir32*; the second contained *hir24* and *hir208*; and the third had a single member, *hir21*.

Effect of regulatory mutations on periodic transcription during the cell cycle. Previous work with the *TRT1 H2A-H2B* gene pair has suggested that a common system of transcriptional repression is used both in the normal cell cycle during G1 and G2 and after the inhibition of chromosome replication in S (10). To determine whether the loss of S-phase-associated repression was accompanied by the loss of periodic transcription in the uninterrupted cell cycle, we compared the expression of the *TRT1 HTA* gene during the synchronous growth of a wild-type (*HIR*⁺) strain and two

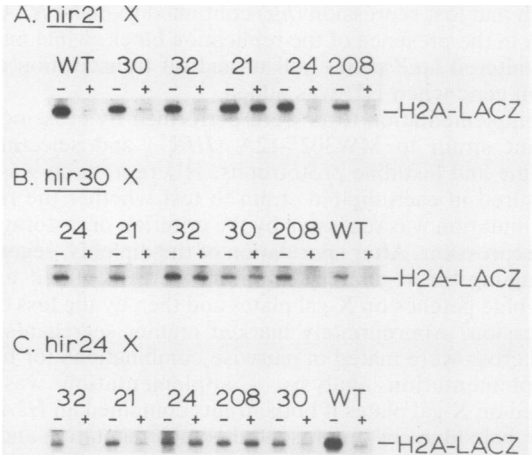

FIG. 2. Estimation of the number of *hir*⁻ complementation groups. *hir*⁻/*hir*⁻ diploid strains were formed by crossing *hir*⁻ haploids in pairwise combinations. Diploids formed by mating *hir*⁻ strains to wild-type cells (WT; *HIR*⁺) or to themselves were included as controls. *H2A-lacZ* RNA levels were measured in each diploid strain before (−) and after (+) HU addition. Complementation occurred whenever HU repression was seen. Failure to complement was indicated by continued production of *H2A-lacZ* RNA in the presence of the replication block.

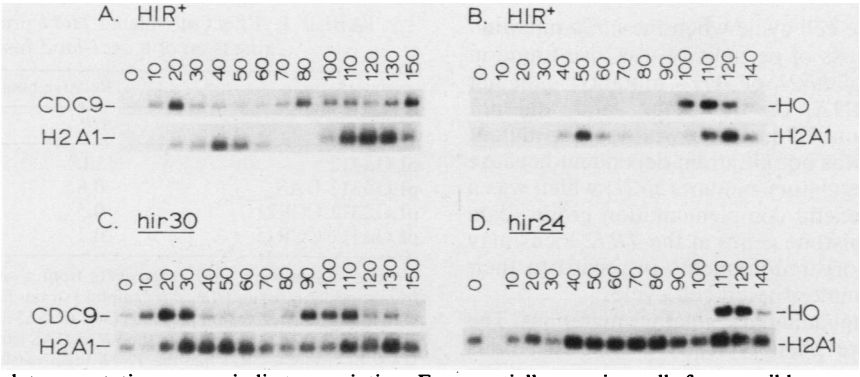

FIG. 3. Effect of regulatory mutations on periodic transcription. Exponentially growing cells from a wild-type strain (*HIR*$^+$; A and B) and two regulatory mutants (*hir30*, C; *hir24*, D) were synchronized in G1 at start with alpha-factor. After release from the block, samples were removed during two subsequent cycles of synchronous growth. The levels of *TRT1 H2A* (*H2A1*), *HO*, and *CDC9* RNAs were measured in these samples by Northern gel analysis. Small buds appeared 20 to 30 min after release from the G1 block, indicating the onset of DNA replication.

regulatory (*hir*) mutants (Fig. 3). In wild-type cells (Fig. 3A and B), the *HTA1* gene was periodically expressed, leading to RNA accumulation only during the S phase (20 to 60 min in the first cell cycle and 100 to 150 min in the second cell cycle). In contrast, significant accumulation of *HTA1* RNA was observed during both the G1 and G2 phases of the cell cycle in each mutant strain (Fig. 3C and D), suggesting that this histone gene was now constitutively transcribed. A regulatory mutant from the third complementation group (*hir21*) also showed a similar loss of periodic transcription (unpublished observations). Therefore, the regulatory genes identified in our screen have a role in both periodic transcription during the cell cycle and DNA replication-associated repression.

Some periodicity in *HTA1* RNA levels could still be observed in the *hir30* mutant strain in particular. This periodicity could reflect either an incomplete loss of repression (e.g., a leaky mutation) or the existence of a second kind of cell cycle-dependent regulatory mechanism. We have previously noted a similar pattern of residual periodicity upon the removal of the negative site from the *TRT1* promoter (10). In that case, we attributed the response to periodic posttranscriptional fluctuations in the stability of full-length histone transcripts (10). The fluctuations in *HTA1* RNA levels seen in the *hir* mutant strain may have the same basis, since in both situations the levels of *H2A-lacZ* fusion RNA, which are not subject to posttranscriptional controls (10), showed little variation throughout the cell cycle (10; unpublished observations).

Besides the genes encoding the four core histones, several other yeast genes are also periodically transcribed during the cell cycle. We therefore asked whether the expression of two such cell cycle-dependent genes, HO (12) and *CDC9* (16), was affected by the presence of the *hir* mutations. RNA from both *CDC9* and *HO* continued to accumulate periodically in the cell cycle, despite the constitutive expression of the *TRT1 HTA* gene (Fig. 3). Thus, the regulatory mutations did not generally eliminate periodic transcription during the cell cycle.

Effect of regulatory mutations on expression of the four histone gene pairs. The four unlinked histone gene loci are coordinately regulated, such that histone RNAs accumulate only during the S phase of the cell cycle (8). Although the *hir* mutations did not generally abolish periodic transcription (see above), it was of interest to determine whether any of them affected this coordinate regulation. We therefore first asked whether the presence of the *hir* mutations resulted in the continued transcription of one gene from each of the four histone gene pairs upon blocking chromosome replication with HU (Fig. 4). In wild-type cells, transcription at each locus was repressed upon incubation with HU. The degree of repression, however, appeared to be somewhat locus specific. For example, the levels of both *TRT1 H2A* RNA and copy I *H3* RNA were strongly repressed (at least 10-fold) upon imposition of the replication block; the levels of *TRT2 H2B* and copy II *H3* RNA, on the other hand, were reduced only three- to fourfold under the same conditions. However, after the addition of HU to the mutant strains, none of the genes were significantly repressed. Thus, the regulatory mutations, which were originally identified because they abolished S-phase-dependent repression of the *TRT1 HTA* gene, were not locus specific and appeared to generally eliminate HU-mediated repression at the four histone loci.

We next asked whether the mutations also affected the periodic transcription of each histone locus by examining the pattern of RNA accumulation during a synchronous cell cycle. Data from the synchronous growth of a wild-type strain (*HIR*$^+$) and one regulatory mutant (*hir30*) are shown in Fig. 5. As we had found for the *TRT1* locus, the copy I and copy II loci showed significant accumulation of RNA in the

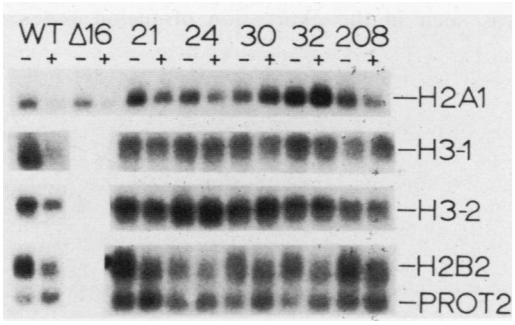

FIG. 4. Effect of *hir* mutations on HU repression of other histone genes. RNA was extracted from wild-type (WT) and mutant (*hir21*, *hir24*, *hir30*, *hir32*, *hir208*) cells before (−) or after (+) incubation in HU. Strain Δ16 contained wild-type *HIR*$^+$ genes and an *H2A-lacZ* fusion gene with a deletion of negative promoter sequences. RNA levels were measured by Northern gel analysis with probes homologous to one gene from each of the four divergently transcribed histone gene pairs: H2A1 refers to RNA from the *TRT1 H2A-H2B* locus; H3-1 and H3-2 refer to *H3* RNA from the copy I and copy II *H3-H4* loci, respectively; H2B2 RNA is from the *TRT2 H2A-H2B* locus, as is PROT2 RNA, which is from a nonhistone gene located 3′ to the *HTB2* gene.

G1 and G2 phases of the cell cycle when the *hir30* mutation was present (a similar loss of periodicity was also found in synchronous cultures of *hir21* or *hir24* mutants; data not shown). *TRT2 H2B* RNA, on the other hand, did not accumulate during G1 and G2 in either wild-type or mutant cells. This latter result was not *hir* strain dependent because it was also noted in a regulatory mutant (*hir21*) which was a member of a second genetic complementation group (data not shown). Thus, the histone genes at the *TRT2* locus may have additional regulatory requirements in regard to their expression during the uninterrupted cell cycle.

Promoter elements responding to regulatory mutations. The regulatory mutants were phenotypically similar to strains that contained a *TRT1* promoter with a deletion of the negative element (CCR; 14). In both cases, S-phase-dependent repression was abolished and transcription occured at inappropriate points (i.e., during G1 and G2) in the cell cycle (10, 14). We therefore asked whether the negative element was the target of the regulatory genes identified in our screen. We obtained *hir*$^-$ *lacZ*$^-$ segregants from each genetic complementation group and transformed these strains with *cyc1-lacZ* fusion genes containing either the *TRT1* activating elements (UAS) substituted for the *CYC1* UAS or the *TRT1* negative element (CCR) inserted adjacent to the constitutive *CYC1* UAS (Fig. 6) (14). We have demonstrated that when the latter plasmid is transformed into wild-type yeast cells, the presence of the *TRT1* negative element repressed *cyc1-lacZ* expression by 2- to 10-fold, as measured either by beta-galactosidase activity on X-gal plates or in liquid medium (14). Using the X-gal plate assay on the mutant strains, it appeared that the negative site no longer repressed *cyc1-lacZ* expression. We therefore measured the actual amount of beta-galactosidase produced by the heterologous fusion genes in wild-type cells and in one of the mutant strains (Table 1). In wild-type cells, approximately equivalent amounts of enzyme were made when the *cyc1-lacZ* fusion gene was driven by either the *CYC1* (pLGΔ312) or *TRT1* (pLGΔ312-UAS) UAS. The presence of the *TRT1* negative site repressed *cyc1-lacZ* gene expression 2-fold when it was inserted upstream of the *CYC1* UAS (pLGΔ312-CCR21) or 10-fold when it was positioned between the *CYC1* UAS and the TATA box (pLGΔ321-CCR15). In an *hir30* mutant background, a dramatic difference was seen in the expression of fusion genes which

TABLE 1. Effect of isolated *TRT1* promoter elements on expression of a *cyc1-lacZ* fusion gene[a]

Plasmid	Relative beta-galactosidase levels	
	HIR$^+$	*hir30*
pLGΔ312	1.0[b]	1.0[c]
pLGΔ312-UAS	0.8	1.4
pLGΔ312-CCR21	0.5	1.5
pLGΔ312-CCR15	0.1	0.6

[a] The strains were *lacZ*$^-$ segregants from a single tetrad which resulted from sporulation of an *HIR*$^+$/*hir30* diploid strain. Each strain was transformed with four *cyc1-lacZ* fusion plasmids (14): pLGΔ312 contained the *CYC1* UAS sites; pLGΔ312-UAS contained the *TRT1* UAS sites substituted for the *CYC1* UAS; pLGΔ312-CCR21 had the *TRT1* negative site inserted upstream of the *CYC1* UAS, and pLGΔ312-CCR15 had the same negative site inserted between the *CYC1* UAS and the TATA box. The values represent the average of four separate assays.

[b] 57 Miller units.

[c] 27 Miller units.

contained the *TRT1* negative element: in each case, beta-galactosidase levels were elevated three- to sixfold compared with those of wild-type cells. A smaller increase (1.7-fold) was also noted for the expression of a *cyc1-lacZ* gene which was driven by the *TRT1* UAS. These data suggested that the negative site in the *TRT1* promoter was the primary target of the *HIR* genes.

We have previously shown that the presence of the *TRT1* negative element in a *cyc1-lacZ* fusion gene can cause this gene to be periodically transcribed, albeit with a different period of expression from the histone genes (14). We therefore asked whether the increased levels of beta-galactosidase were due in part to the abolition of cell cycle-dependent transcription. We found that transcription of a *cyc1-lacZ* fusion gene containing the *CYC1* UAS and *TRT1* negative element continued throughout the cell cycle of an *hir30* strain (Fig. 6B), appearing indistinguishable from the transcription of a fusion gene which contained only the *CYC1* UAS (Fig. 6C). In contrast, the *cyc1-lacZ* fusion gene driven by the *TRT1* UAS still showed periodic transcription in the same mutant background (Fig. 6A). Thus, the *hir30* mutation (and probably the other regulatory mutations as well) eliminated two prominent transcriptional effects attributed to the *TRT1* negative site, suggesting that the *HIR* regulatory system acts primarily through this promoter element.

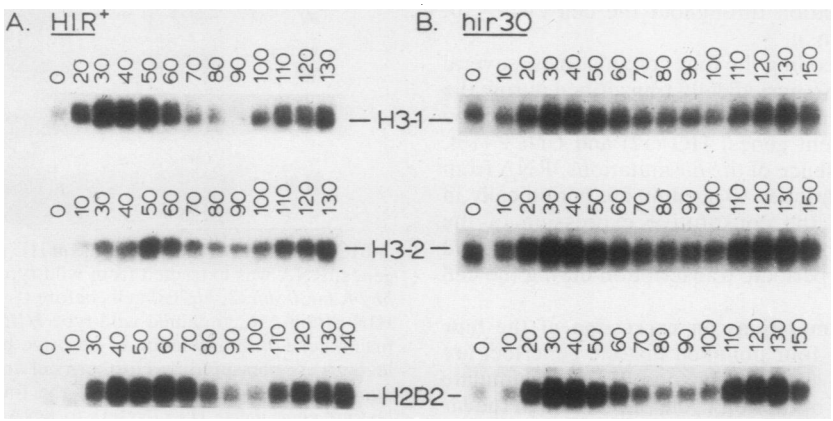

FIG. 5. Effect of a regulatory mutation on the periodic transcription of other histone genes. Cells from a wild-type strain (*HIR*$^+$, A) or the *hir30* regulatory mutant (B) were synchronized with alpha-factor. At intervals after release from the G1 block, the levels of RNA from the *H3* genes at the copy I and copy II loci (H3-1 and H3-2, respectively) and the *HTB* gene at the *TRT2* locus (H2B2) were measured by Northern gel analysis. The *hir30* RNA samples were from the synchrony shown in Fig. 3C.

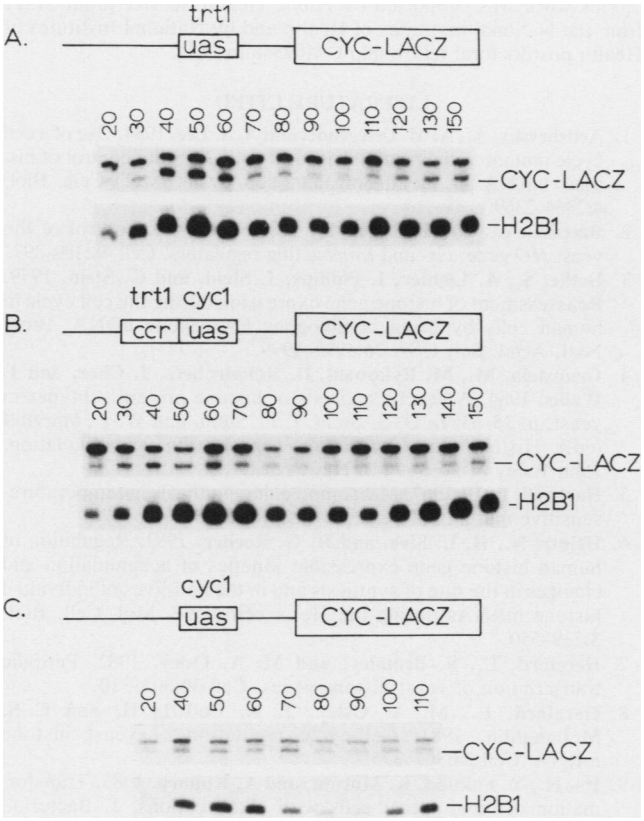

FIG. 6. Effect of a regulatory mutation on isolated histone promoter elements during a synchronous cell cycle. A meiotic segregant carrying an *hir30* mutation was transformed with yeast 2μm plasmids carrying a *cyc1-lacZ* fusion gene with histone promoter elements substituted in the *CYC1* promoter. (A) *cyc1-lacZ* fusion gene containing three copies of the *TRT1* UAS sequences in place of the *CYC1* UAS (14). (B) *cyc1-lacZ* fusion gene containing both the *CYC1* UAS and a restriction fragment with the *TRT1* negative promoter site (CCR; 14). (C) *cyc1-lacZ* fusion gene containing the two *CYC1* UAS elements transformed into a wild-type strain (*HIR*$^+$). The transformants were synchronized in G1 with alpha-factor, and at various times after release from the G1 block, *cyc1-lacZ* and *H2B1* RNA levels were measured in an S1 nuclease protection assay. The band immediately above the *cyc1-lacZ* protected fragment represented reannealed probe. The lower levels of *cyc1-lacZ* RNA seen during the second cell cycle compared with those in the first cycle result from a plasmid-specific effect, since they have also been noted in an identical plasmid in which the *cyc1-lacZ* gene is driven by *HO*-specific promoter elements (2). Probes of different specific activity were used in each panel, so the RNA levels cannot be directly compared.

DISCUSSION

In this paper, we described a method to identify genes involved in the transcriptional regulation of a group of essential yeast genes, the genes encoding the core histones. We took advantage of the observation that an *H2A-lacZ* fusion gene, which is subject to the same regulation as an intact *HTA* gene during the cell cycle, provided a phenotype (dark blue colony color) that could be used to screen for mutants. Using a yeast strain containing such a fusion gene, we isolated mutants which overexpressed beta-galactosidase, reasoning that some of these strains might have altered patterns of histone gene expression. We identified five overexpressors which had lost repression of the *TRT1 HTA* gene which usually occurs upon the arrest of chromosome replication in the S phase (10). The mutations in these strains affected periodic transcription as well, since the *HTA1* gene was also inappropriately expressed during the G1 and G2 phases of the cell cycle. The five mutants fell into three complementation groups, and they identified genes encoding *trans*-acting regulatory proteins.

The histone promoters appear to be complex. The bifunctional *TRT1* promoter contains both positive and negative *cis*-acting elements which individually have effects on transcription (Fig. 7). By promoter deletion analysis, we have previously demonstrated that a repetitive positive element (UAS) is necessary to activate transcription and can on its own activate the transcription of a heterologous gene with correct cell cycle periodicity. These same studies showed that a separate negative element also has a role in regulating transcription. The presence of this site in the *TRT1* promoter is necessary to repress transcription in the S phase after the inhibition of DNA replication (10) as well as in the G1 and G2 phases of the normal cell cycle (10, 14). The genes identified in the present study acted primarily through the negative element in the *TRT1* promoter and eliminated both aspects of its regulation. This result confirmed the importance of the negative site as a promoter regulatory element and suggested that it is a sequence which binds a transcriptional repressor.

Besides the genes encoding the four core histones, only a small number of other yeast genes are periodically transcribed during the cell cycle (7, 13, 16, 20). Two of these periodic genes, *CDC9* and *HO*, continued to show proper cell cycle regulation in the presence of the *hir* mutations. This was not so surprising because the histone genes are transcribed slightly later in G1 than the other periodic genes. Whereas the *HO*, *CDC8*, *CDC9*, and *CDC21* genes are transcribed between the two sequential G1 steps, start and *CDC4* (12; L. Johnston, personal communication), the histone genes are not transcribed until after the completion of the *CDC4*-dependent step (D. Lycan and M. A. Osley, unpublished observations). Thus, it was not unexpected that the histone genes might utilize a separate regulatory system from the remaining periodic genes.

In contrast, the effects of the *hir* mutations on the coordinately expressed histone genes were surprising for two reasons. First, although S-phase-dependent repression was abolished for each of the four histone loci, only the *TRT1*, copy I, and copy II loci showed significant transcription in the G1 and G2 phases of the cell cycle. This result suggests that the repression of transcription triggered in S-phase-

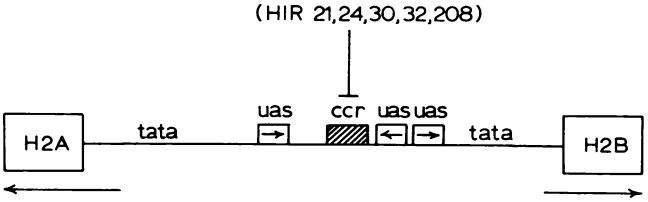

FIG. 7. Some features of the regulation of the *TRT1 H2A-H2B* promoter. A diagram of the divergent *TRT1* promoter is shown. The open boxes represent three 16-bp UAS elements which are found either in whole or in part in the copy I and copy II *H3-H4* promoters (14). The hatched box indicates the negative site (CCR) through which the *HIR* regulatory genes act to repress transcription. This site contains a sequence of dyad symmetry (CGTTAACTATGGT TAGACG) that is the probable target of regulation (14).

arrested cells and the absence of transcription in the G1 and G2 phases of the normal cell cycle may not always utilize the same regulatory pathway, as our previous results had suggested (10). Second, the copy I and copy II promoters (19) do not appear to contain a sequence with extensive homology to the *TRT1* negative element (14) (Fig. 7), even though transcription of the two *H3* genes is affected by the *hir* mutations. Thus, the target(s) of the *HIR* regulatory system in these histone promoters is unknown.

Although the present study demonstrated that the *TRT1* negative site is recognized by a regulatory system which contains at least three genes, the precise relationship of this system to cell cycle-dependent transcription is still puzzling. There must be another regulatory system for periodic transcription which acts on the *TRT1* UAS elements since the isolated UAS can periodically activate transcription of a *cyc1-lacZ* gene in both wild-type and *hir* mutant cells. This would suggest that the negative regulatory system is totally dispensable for cell cycle-dependent transcription. Yet, the deletion of the negative site from the *TRT1* promoter (or the mutation of genes which act through this site) overrides the controls which operate on the UAS elements and results in transcription being activated inappropriately. It is therefore formally possible that the system of negative regulation has a primary role in some other aspect of histone gene regulation and that its effect on periodic expression is only secondary. One aspect of the negative site which was revealed by our analysis of the *TRT1* promoter was its effects on the absolute levels of transcription: when it was deleted from the promoter of an *H2A-lacZ* fusion gene, fusion RNA levels rose not only during G1 and G2, but during the S phase as well (14). In addition, in isolation it moderately or severely repressed the activity of the heterologous *CYC1* UAS elements depending on its position, effects which were relieved by the *hir* mutations. Thus, the negative regulatory system could have evolved initially as a way to modulate the amount of histone RNA which is made. Several recent studies have suggested that correct histone stoichiometry is required for the execution of a number of different intracellular processes (11; D. Norris and M. A. Osley, Mol. Cell. Biol., in press). We have recently found that this stoichiometric balance can be achieved in part by the regulation of histone gene expression. To compensate for the absence of the *TRT2* locus, the *TRT1* locus can increase its level of expression; the *TRT2* locus, however, is expressed at a constant level regardless of the presence or absence of the *TRT1* genes (Norris and Osley, in press). If the regulation shown by the *TRT1* locus is at the level of transcription, then the negative regulatory system might have a role in this response: derepression of the genes at the *TRT1* locus would ensure that enough H2A and H2B are made to maintain correct stoichiometry with respect to H3 and H4. Since the copy I and copy II loci were shown to utilize the regulatory genes identified in this study, they might also respond to a system of stoichiometry control. Moreover, the failure of the *TRT2* locus to respond to changes in gene dosage might be accounted for as well, since this is the only histone locus which was not derepressed in the cell cycle when *hir* mutations were present. We are currently testing this model for the function of the negative regulatory system.

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