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Role of Transcriptional and Posttranscriptional Regulation in Expression of Histone Genes in *Saccharomyces cerevisiae*

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We analyzed the role of posttranscriptional mechanisms in the regulation of histone gene expression in *Saccharomyces cerevisiae*. The rapid drop in histone RNA levels associated with the inhibition of ongoing DNA replication was postulated to be due to posttranscriptional degradation of histone transcripts. However, in analyzing the sequences required for this response, we showed that the coupling of histone RNA levels to DNA replication was due mostly, if not entirely, to transcriptional regulatory mechanisms. Furthermore, deletions which removed the negative, cell cycle control sequences from the histone promoter also uncoupled histone transcription from DNA replication. We propose that the arrest of DNA synthesis prematurely activates the regulatory pathway used in the normal cell cycle to repress transcription. Although posttranscriptional regulation did not appear to play a significant role in coupling histone RNA levels to DNA replication, it did affect the levels of histone RNA in the cell cycle. Posttranscriptional regulation could apparently restore much of the periodicity of histone RNA accumulation in cells which constitutively transcribed the histone genes. Unlike transcriptional regulation, periodic posttranscriptional regulation appears to operate on a clock which is independent of events in the mitotic DNA cycle. Posttranscriptional recognition of histone RNA must require either sequences in the 3′ end of the RNA or an intact three-dimensional structure since *H2A*- and *H2B-lacZ* fusion transcripts, containing only 5′ histone sequences, were insensitive to posttranscriptional controls.

In *Saccharomyces cerevisiae*, histone mRNAs accumulate to significant levels only in the S phase of the cell cycle, coincident with the peak in the rate of DNA replication (19). This coupling between histone gene expression and DNA replication is thought to be achieved through a combination of transcriptional and posttranscriptional controls. Transcriptional regulation ensures that histone RNA is synthesized only at the appropriate time in the cell cycle. The rate of histone *H2B* transcription has been shown to increase dramatically late in G1, rising to a peak by early S phase and then falling steadily throughout S to almost nondetectable levels in G2 and M (16). In addition to transcriptional regulation, however, the link between histone RNA accumulation and DNA replication has been postulated to involve posttranscriptional changes in histone RNA stability (16, 19). If ongoing DNA replication is inhibited in yeast cells, there is a rapid drop in histone RNA levels (19). A similar coupling between DNA replication and histone RNA levels has also been observed in mammalian cells (1, 3, 5–7, 15, 22, 29, 30). Although it has been directly demonstrated in higher cells that existing histone mRNAs are specifically destabilized when the cells are treated with inhibitors of DNA replication (1, 3, 5–7, 15, 22, 29, 30), it is not known whether such posttranscriptional mechanisms are activated in yeast cells.

Posttranscriptional modulation of histone RNA levels has been postulated to occur at other stages of the cell cycle as well. For example, the peak in the rate of histone gene transcription precedes the peak in RNA accumulation in the cell cycle (16). If the histone transcripts synthesized late in G1 were less stable than those made in the S phase, this could account for the shift in RNA accumulation toward the S phase and the peak in the rate of DNA replication. In addition, *cdc7* mutants arrested at the G1/S boundary in the cell cycle transcribe the histone genes at maximal rates but accumulate only 30 to 50% of the maximum levels of RNA (16). Again, this observation could be accounted for if the RNA made late in G1 was less stable.

In this study, we investigated the role of posttranscriptional regulation in the control of histone gene expression in *S. cerevisiae*. Using *H2A*- and *H2B-lacZ* fusion genes, we showed that the drop in RNA levels observed when DNA synthesis was inhibited was due mostly, if not entirely, to transcriptional and not posttranscriptional regulatory mechanisms. The sequences required for this response reside in the histone promoter, and we showed that deletions which removed sequences necessary for periodic transcription in the cell cycle (24) also uncoupled histone transcription from DNA replication. To determine whether posttranscriptional mechanisms might play a role in regulating yeast histone RNA levels elsewhere in the cell cycle, we used two mutations which result in constitutive transcription of the histone genes. These mutations allowed us to uncouple transcriptional from posttranscriptional regulation for the first time and therefore to determine directly whether posttranscriptional mechanisms affected the levels of either histone or histone-*lacZ* fusion RNAs at any stage of the cell cycle. We showed that in the absence of transcriptional regulation, full-length *H2B* transcript levels were modulated posttranscriptionally. This posttranscriptional regulation was periodic but was apparently independent of other steps in the mitotic cell cycle such as DNA replication and mitosis. Unlike the full-length transcripts, fusion RNAs, some of which contained significant amounts of the histone 5′ coding sequence, did not appear to respond to posttranscriptional controls. Posttranscriptional recognition of histone RNAs must therefore require either an intact transcript or sequences that reside in the 3′ end of the gene.

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MATERIALS AND METHODS

Plasmids. Construction of *H2A-lacZ* fusion genes has been described (26). The 80-amino-acid *H2B-lacZ* gene was constructed by inserting the 1.1-kilobase *Hin*dIII fragment from *TRT1* (17) into the *lacZ* vector in the opposite orientation to that used for *H2A* fusion genes. *H2B-lacZ* fusion genes containing fewer than 80 amino acids of the *H2B* 5′ coding sequence were derived from this plasmid by Bal 31 deletion and insertion of *Bam*HI linkers to regenerate in-frame fusion genes (gift of R. Moreland). The pFB1-7Cen plasmid was constructed by digesting pBL101 (gift of L. Guarente) with *Bam*HI and *Sma*I and replacing the *CYC1* sequences with the *Bam*HI-*Sma*I fragment from a seven-amino-acid *H2B-lacZ* gene. Similarly, the pFB1-80μ plasmid was constructed by replacing the *CYC1* sequences in pLGΔ312 (8) with the *Bam*HI-*Sma*I fragment from the 80-amino-acid *H2B-lacZ* gene. Construction of pCALA and its promoter deletion derivatives has been described (24). The *GAL10*-regulated *H2B-lacZ* plasmid, pNIRV2b, was constructed by digesting pJT24 (4) with *Sac*I and *Xho*I, isolating the vector fragment, and joining it to the smallest *Sac*I-*Xho*I fragment from the Δ49 deletion derivative of pCALA (24).

Strains and media. Typically, 5 to 10 μg of plasmid DNA was transformed into yeast strain DB747 (**a** *ura3-52 leu2-3 leu2-112 trp1-289 his3*; D. Botstein), 2754-7-4 (**a** *cdc8 his7 leu2*; W. Fangman), or GRF167 (α *ura3 his3*; G. Fink) by the lithium chloride procedure (20). Transformants were selected on minimal medium plates supplemented with 100 μg of the required amino acids per ml minus the amino acid used for selection. The strains were grown in liquid media (minus the amino acid required for selection) supplemented with 2% glucose, except for those transformed with *GAL10*-controlled plasmids, which were grown in a mixture of galactose and glucose to induce moderate expression of the fusion gene. DB747 cells were synchronized by treatment with α-factor for one generation (3 h) to arrest cells in G1 at "START" (13, 18). The α-factor was removed by centrifugation, and the cells were suspended in fresh medium to initiate synchronous growth.

The Δ16′ transplacement strain was constructed by the procedure of Rothstein (28) to replace the wild-type promoter at the genomic *TRT1* (17) locus with the deleted Δ16′ promoter. A *Hin*dIII-*Sac*I fragment from the *TRT1* locus, which includes the 3′ end of the *H2B* gene and the *TRT1 ars* element (26), was cloned into pUC13. The yeast *URA3* gene was inserted downstream of the *H2B* gene at a *Bam*HI site, and then the *Hin*dIII fragment containing the deleted Δ16′ histone promoter (24) was joined to the *Hin*dIII-*Sac*I fragment. A 3.6-kilobase partial *Hin*dIII-*Sac*I fragment containing 13 amino acids of *H2A*, the entire *TRT1* intergene region, an intact *H2B* gene, and the *URA3* gene was gel purified and used to transform yeast strain DB747. Ura$^+$ transformants were selected and screened by Southern blot analysis for the replacement of the chromosomal *TRT1* promoter with the deleted Δ16′ promoter.

Quantitative S1 nuclease analysis. Total RNA was extracted from 20 to 30 ml of cells as previously described (19). Transcript levels were determined by a quantitative S1 nuclease mapping assay (2, 24). The S1 probes used were double stranded but dephosphorylated such that only the strand homologous to the RNA of interest was labeled. Fusion-specific probes were labeled at the *Cla*I site in *lacZ*. The *TRT1 H2B* probe was labeled at the *Hin*dIII site at amino acid 80, and the ribosomal protein 51 (*RP51*) probe was labeled at the *Ava*II site in the second exon (31). The S1 hybridization reactions contained 4 to 10 μg of total RNA, depending on the abundance of the RNA of interest. S1-resistant fragments were analyzed on 4% acrylamide–8 M urea gels and detected by autoradiography. Quantitation was performed with a Helena Laboratories Quick-Scan R&D densitometer.

HU arrest. Cells were grown in selective media and then diluted into YPD or YM-1 medium (11) for approximately 3 h until the cell density reached 4×10^6 to 8×10^6 cells per ml. Solid hydroxyurea (HU) (Sigma Chemical Co.) was then added directly to the cultures to a final concentration of 0.2 M. Arrest in S phase was confirmed by visual observation of HU-treated cells to ensure that >90% of the culture arrested as large budded cells. In addition, a positive control was always included in the S1 hybridization reactions to ensure that HU treatment had repressed endogenous histone RNA levels. Finally, *RP51* RNA levels were also determined as a control for nonspecific inhibitory effects of HU. In some experiments involving extended time in HU (more than 60 min), some depression of *RP51* RNA levels was observed. In such cases, the *RP51* RNA levels were used in quantitation to adjust the histone RNA levels to compensate for the effect of general, and not histone-specific, repression of transcription.

RESULTS

Response of histone-*lacZ* RNA levels to inhibition of DNA synthesis. The inhibition of ongoing DNA replication in *S. cerevisiae* results in a rapid and specific drop in histone RNA levels (19). To elucidate the sequences and regulatory mechanisms involved in coupling histone RNA levels to DNA synthesis, we constructed a number of *H2A*- and *H2B-lacZ* fusion genes. If the levels of a hybrid histone-*lacZ* transcript could be shown to be linked to DNA replication, then the location of the histone sequences required for this response could be determined by analyzing fusion genes containing decreasing amounts of the histone coding sequence. All of the histone-*lacZ* fusion genes contained the entire *TRT1* (17) histone intergene region with the upstream promoter sequences and RNA start sites, as well as varying amounts of either the *H2A* or *H2B* 5′ coding sequence fused in frame to

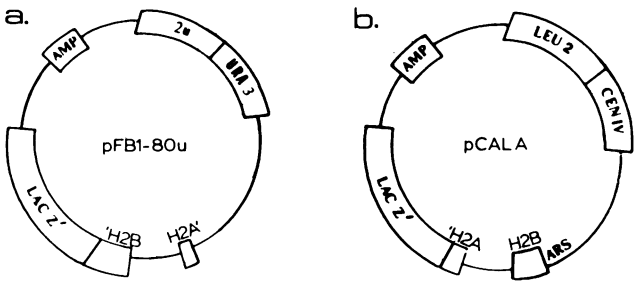

FIG. 1. *H2A* and *H2B* fusion plasmids. (a) The *H2B-lacZ* fusion plasmids contain from 7 to 80 amino acids of the *H2B* coding sequence fused in frame to the *E. coli lacZ* gene, the histone intergene region, and a 13-amino-acid fragment of *H2A*. The pFB1-80μ plasmid shown has 80 amino acids of the coding sequence and contains the yeast *URA3* gene for selection and the 2μ origin of replication for stable maintenance of the plasmid in yeast cells (at approximately 10 copies per cell; unpublished observations). The *H2A* and *H2B* genes are transcribed from different strands of the DNA. (b) The *H2A-lacZ* plasmid pCALA contains 13 amino acids of the *H2A* coding sequence fused in frame with the *lacZ* gene, the entire intergene region, the *H2B* gene, and the *ars* sequence from the *TRT1* locus. The yeast *cenIV* sequence allows stable maintenace of the plasmid at ca. one copy per cell, and the *LEU2* gene permits selection in yeast cells.

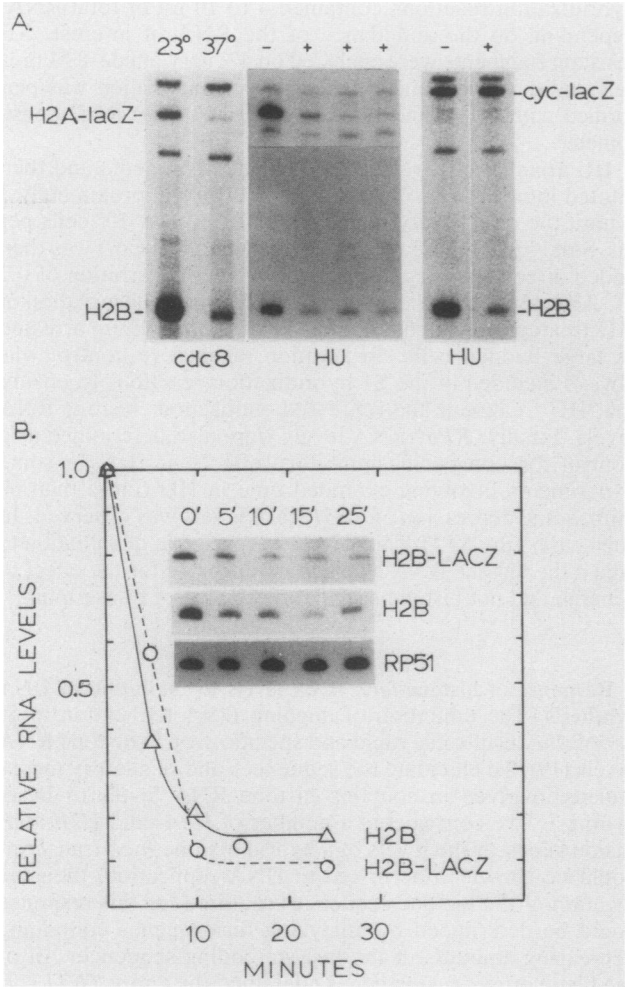

FIG. 2. Histone-*lacZ* RNA levels are coupled to DNA replication. (A) *H2A-lacZ* RNA levels are sensitive to inhibition of DNA synthesis. Left panel: yeast strain 2754-7-4 (*cdc8*) was transformed with the *H2A-lacZ* plasmid pCALA. Transformants were grown in selective media at 23°C, diluted into YPD medium for 3 h, and then shifted to the nonpermissive temperature (37°C). Total RNA was extracted from cells at 23°C and 25 min after the shift to 37°C, and the levels of histone *H2B* and *H2A-lacZ* RNAs were determined by a quantitative S1 nuclease assay. Four micrograms of the total RNA was hybridized in probe excess with double-stranded probes specific for *TRT1 H2B* and histone-*lacZ* fusion transcripts. Center panel: strain DB747 was transformed with the *H2A-lacZ* plasmid pfusA1-3 (26), the integrative form of plasmid pCALA. Transformants with two copies of the *H2A-lacZ* gene integrated into the chromosome were grown at 30°C (−), HU was added (+), and samples were removed 15, 60, and 120 min later. RNA was extracted, and 4 μg of total RNA was then hybridized with probes specific to fusion and *H2B* transcripts. Because the two probes have different specific activities, the levels of *H2B* and *H2A-lacZ* RNAs cannot be compared directly. Right panel: strain DB747 was transformed with the *cyc1-lacZ* plasmid pLGΔ312 (8). Transformants were grown at 30°C in the absence of HU (−) or for 15 min in the presence of HU (+). Ten micrograms of RNA was used in the hybridization reaction with probes specific for the *cyc1-lacZ* and *H2B* transcripts. The additional, unlabeled bands in all panels were due to the reannealed probes. (B) Kinetics of HU response. Strain DB747 was transformed with pFB1-7Cen, a centromere plasmid derivative of pFB1-80μ (Fig. 1A) which contains the seven-amino-acid *H2B-lacZ* fusion gene. Transformants were grown into the exponential phase and treated with HU, and samples were removed every 5 min. RNA levels at each time point were determined by S1 nuclease analysis with 4 μg of total RNA, 10 ng of the *lacZ* and *TRT1 H2B* probes, and 7 ng of the *RP51* probe. The autoradiograms (shown in the inserts) were quantitated by densitometry, and the relative *H2B* and *H2B-lacZ* RNA levels were plotted as a function of time after the addition of HU.

the *Escherichia coli lacZ* gene (Fig. 1). Transcription of these genes has been shown to be cell cycle regulated due to sequences in the intergene region (24).

If synchronized yeast cells carrying the temperature-sensitive *cdc8* mutation are shifted to the nonpermissive temperature in mid-S phase, there is an immediate termination of ongoing DNA chain elongation and a concomitant drop in the levels of *H2B* mRNA (19). To determine whether histone-*lacZ* RNAs show a similar response, *cdc8* cells were transformed with a 13-amino-acid *H2A-lacZ* fusion gene (Fig. 1b), and exponentially growing transformants were shifted to the nonpermissive temperature. At various times after the shift, the levels of both fusion and *H2B* RNAs were determined. Because histone and histone fusion RNAs accumulate only during the S phase, it was unnecessary to synchronize cells since only those cells in S contributed to the results. The levels of both *H2B* and *H2A-lacZ* RNAs dropped after the shift to the nonpermissive temperature (Fig. 2A). This indicates that, like the intact histone transcripts, *H2A-lacZ* fusion RNA levels responded to the inhibition of DNA replication.

To confirm these results, as well as to determine whether *H2B-lacZ* fusion transcripts are also coupled to DNA replication, we used HU, an inhibitor of DNA chain elongation used frequently in mammalian cells. We tested the effects of HU on histone and histone-*lacZ* RNA levels in *S. cerevisiae* by using cells containing either the 13-amino-acid *H2A-lacZ* gene or one of various *H2B-lacZ* genes containing from 7 to 80 amino acids of the *H2B* 5′ coding sequence. Transformants were grown at 30°C, and HU was added to a concentration that inhibits DNA replication in yeast cells (14). The levels of both endogenous *H2B* and *H2A-lacZ* fusion RNAs dropped rapidly after HU addition (within 15 min) to a level which remained constant over the next 2 h. (Fig. 2A, center panel). Furthermore, all of the various-length *H2B-lacZ* RNAs, including that from the seven-amino-acid fusion gene (see below), were also sensitive to the inhibition of DNA synthesis (data not shown). This response was specific to histone and histone fusion RNAs since transcripts from a *cyc1-lacZ* fusion gene (9) were not affected by inhibition of DNA synthesis (Fig. 2A, right panel). Therefore, these results corroborate those obtained by using the *cdc8* mutation to inhibit DNA replication and indicate that the levels of both *H2A-lacZ* and *H2B-lacZ* RNAs are specifically affected by the interruption of ongoing DNA synthesis.

We next compared the kinetics of the disappearance of fusion and endogenous histone RNAs after the inhibition of DNA synthesis. Cells transformed with the seven-amino-acid *H2B-lacZ* fusion gene were treated with HU, and the levels of *H2B* and *H2B-lacZ* fusion RNAs were measured in samples removed at 5-min intervals. The two RNAs appeared to decay with kinetics identical to a similar steady-state level (10 to 15% of exponential levels), although the final level of the full-length *H2B* RNA remained somewhat higher than that of the *H2B-lacZ* fusion RNA (Fig. 2B). (A similar difference between the final levels of intact and fusion RNAs can also be seen in Fig. 2A, center panel.) The concentrations of HU used to inhibit DNA replication in these experiments did not affect gene expression in general

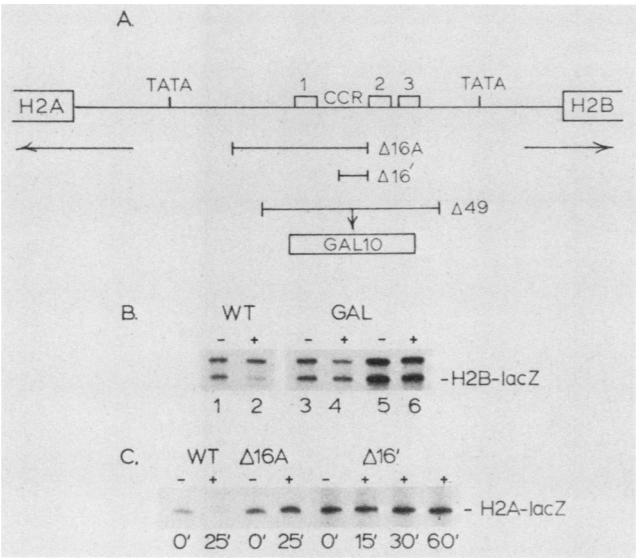

FIG. 3. Sequences required to couple transcription to replication. (A) *TRT1* histone promoter mutations. A schematic diagram of the histone upstream promoter region with the elements required for cell cycle control of transcription is shown. Boxes 1, 2, and 3 represent the reiterated upstream activating site elements, and the negative cell cycle control region (CCR) is indicated (24). The endpoints of the Δ16A and Δ16′ promoter deletions are shown. Deletion Δ49 removes the entire upstream promoter region and was used to construct the *GAL10*-regulated *H2B-lacZ* fusion gene on plasmid pNIRV2b. (B) HU insensitivity of *GAL10* promoter. Strain DB747 was transformed with an 80-amino-acid *H2B-lacZ* fusion gene regulated by the histone promoter (pFB1-80μ; Fig. 1A), and strain GRF167 (wild type for galactose regulation) was transformed with the identical *H2B-lacZ* gene under *GAL10* control (pNIRV2b). Transformants were treated with HU, and samples were taken before (−) and 25 min after (+) HU addition. The levels of *H2B-lacZ* RNA were determined by S1 nuclease analysis with 3 μg of total RNA and 10 ng of fusion-specific probe. Lanes 1 and 2, Levels of fusion RNA in cells transformed with the cell cycle-regulated *H2B-lacZ* gene; lanes 3 through 6, *H2B-lacZ* RNA levels from the *GAL10*-regulated fusion gene in cells grown in 1.2% galactose–0.8% glucose (lanes 3 and 4) or 1.6% galactose–0.4% glucose (lanes 5 and 6). (C) HU insensitivity of histone promoter mutants. DB747 cells were transformed with the 13-amino-acid *H2A-lacZ* gene (Fig. 1B) containing either the wild-type histone promoter or the promoter deletion Δ16A or Δ16′. Transformants were treated with HU as above, and samples were removed before (−) and after (+) addition of the drug at the times indicated. The *H2A-lacZ* RNA levels were determined by the quantitative S1 nuclease assay.

since no change in the levels of RP51 RNA, a species which is not sensitive to the arrest of DNA synthesis, could be detected during the time course of the experiment (Fig. 2B). These results show that fusion and full-length histone transcripts responded almost identically to the inhibition of DNA replication. The fact that hybrid transcripts with as few as 7 amino acids of the *H2B* 5′ coding sequence and 13 amino acids of the *H2A* 5′ coding sequence were sensitive to the inhibition of DNA synthesis implicates sequences in the 5′ regions of these genes in this response.

Coupling of fusion RNA levels to DNA replication is due to transcriptional regulation. Histone mRNA has been shown to be specifically destabilized in mammalian cells treated with inhibitors of DNA replication (1, 3, 5–7, 15, 22, 29, 30). To determine whether the drop in fusion RNA levels observed in *S. cerevisiae* was due to a similar posttranscriptional mechanism or was the result of transcriptional regulation, we replaced histone promoter sequences in an *H2B-lacZ* fusion gene (pFB1-80μ, Fig. 1a) with the galactose-regulated *GAL10* promoter (10). All the upstream promoter sequences required for cell cycle-regulated transcription are deleted in this construction, but the TATA box and the 5′ RNA leader region remain intact (Fig. 3A). Thus, although transcription of this fusion gene is regulated by galactose, the actual transcripts are identical in structure to those from the original cell cycle-regulated *H2B-lacZ* gene (pFB1-80μ).

To determine whether these identical transcripts would respond in the same way to the inhibition of DNA replication, we transformed yeast cells with either the cell cycle-regulated or *GAL10*-regulated *H2B-lacZ* gene. Transformants were grown either in glucose (cell cycle-regulated gene) or in two mixtures of galactose plus glucose to induce low-to-intermediate levels of fusion RNA (*GAL10*-regulated gene) and then treated with HU. As expected, the levels of fusion RNA were repressed by HU in cells containing the cell cycle-regulated gene (Fig. 3B, lanes 1 and 2). In marked contrast, the *H2B-lacZ* RNAs from cells with the *GAL10*-regulated gene were completely insensitive to the inhibition of DNA synthesis (Fig. 3B). This uncoupling could not have been due to overexpression of *H2B-lacZ* RNA because uncoupling was observed when the levels of fusion RNA induced by galactose were identical to those produced from the histone promoter in cells containing the cell cycle-regulated fusion gene (Fig. 3B, cf. lanes 1 and 3). These results indicate that an intact histone promoter is required to couple histone-*lacZ* RNA levels to DNA replication. Thus, in *S. cerevisiae*, the coupling of fusion RNA levels to DNA replication must be achieved by transcriptional and not posttranscriptional mechanisms.

To confirm that transcriptional control was in fact responsible for the drop in RNA levels observed after HU addition, as well as to define more precisely the promoter sequences involved in this response, we tested two mutations in the histone promoter for their sensitivity to the inhibition of DNA synthesis. These mutations, which delete 200 nucleotides (Δ16A) and 54 nucleotides (Δ16′) from the center of the bidirectional histone promoter (Fig. 3A), remove sequences known to be required for the cell cycle control of histone transcription (24). Genes controlled by these mutant promoters are not transcribed periodically but are transcribed constitutively throughout the cell cycle. To determine whether these deletions also removed sequences required to couple RNA levels to DNA replication, we transformed yeast cells with an *H2A-lacZ* fusion gene containing either the wild-type histone promoter or one of the deleted promoters. Transformants were treated with HU during exponential growth, and *H2A-lacZ* RNA levels were determined. Whereas the fusion RNA levels dropped after HU addition in cells transformed with the wild-type gene, no change in RNA levels was detected in cells containing genes with either of the deleted promoters, even after 60 min in HU (Fig. 3C). The same result was observed after the inhibition of DNA synthesis at 36°C in *cdc8* cells transformed with an *H2A-lacZ* gene containing the Δ16A promoter mutation (data not shown). These results confirm that transcriptional control must be responsible for coupling fusion RNA levels to the rate of DNA replication. Furthermore, the data indicate that the smaller 54-nucleotide deletion was sufficient to uncouple transcription from DNA replication. This deletion also eliminates the regulation of histone transcription in the cell cycle (24), which suggests that the same regulatory

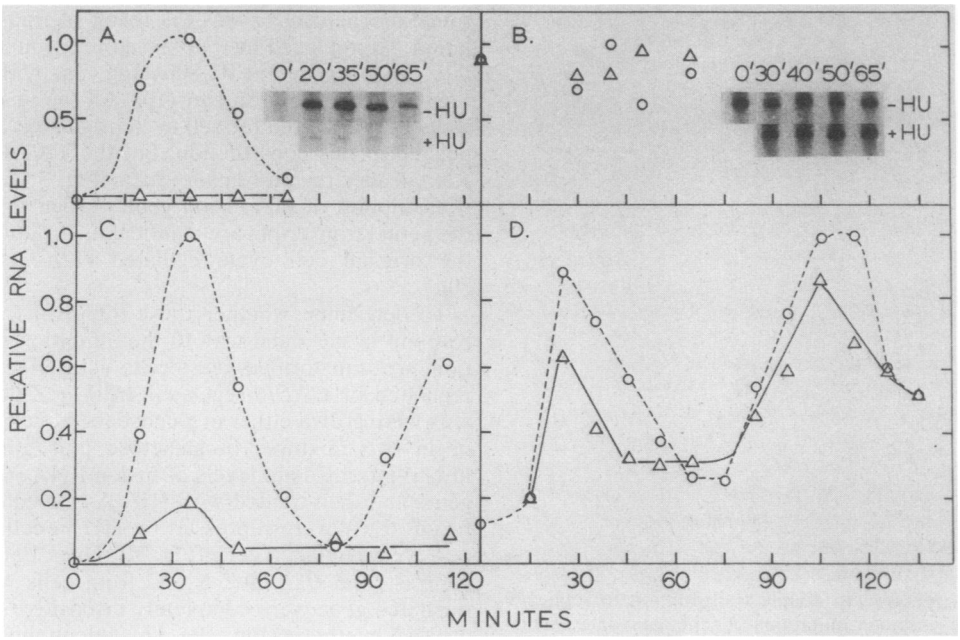

FIG. 4. Posttranscriptional regulation in the cell cycle. (A, B, and C) Strain DB747 was transformed with plasmid pCALA carrying the 13-amino-acid *H2A-lacZ* fusion gene with either the wild-type histone promoter (panels A and C) or the Δ16A promoter deletion (panel B). (D) The Δ16′ transplacement strain, which carries the Δ16′ promoter deletion at the chromosomal *TRT1* locus. Cells were synchronized with α-factor and released into YPD or YM-1 medium plus (△) or minus (○) HU. RNA was extracted from samples taken at 10 to 15-min intervals. (A and B) The levels of *H2A-lacZ* RNA were determined by a quantitative S1 nuclease assay and are shown in the inserts. The autoradiograms were quantitated by densitometry, and the relative levels of fusion RNA were graphed as a function of time after release from α-factor. (C and D) The levels of the endogenous *TRT1 H2B* transcript were determined by quantitative S1 nuclease analysis with 10 to 15 ng of the *TRT1*-specific *H2B* probe and quantitated by densitometry. The levels of *RP51* RNA in each sample were also determined and used in quantitation (panel D) to adjust the +HU RNA levels at the later time points (see Materials and Methods).

molecule(s) used for cell cycle control may be responsible for coupling histone transcription to DNA replication.

Posttranscriptional regulation in the cell cycle requires an intact *H2B* transcript. Although the coupling of fusion RNA levels to DNA replication during S phase is primarily due to transcriptional and not posttranscriptional control, posttranscriptional mechanisms might still affect histone RNA levels elsewhere in the cell cycle. As we have shown, the stability of histone transcripts can be altered in yeast cells (25). However, whether changes in histone RNA stability occur in a regulated, periodic fashion was unknown. Regulation of histone RNA stability might contribute to or perhaps alter the pattern of histone RNA accumulation in the cell cycle imposed by periodic transcription.

To investigate whether posttranscriptional mechanisms regulate levels of histone or histone-*lacZ* RNA or both, we used the histone promoter mutations, Δ16A and Δ16′. Because genes containing these deletions are transcribed constitutively, these mutations allowed us to uncouple transcriptional from posttranscriptional regulation and to assess directly whether posttranscriptional mechanisms could affect the levels of either histone or histone-*lacZ* RNA in the cell cycle. To assay posttranscriptional regulation of the fusion transcripts, we transformed cells with the 13-amino-acid *H2A-lacZ* fusion gene controlled by either the transcriptionally regulated or a constitutive histone promoter. To study regulation of the intact transcripts, we replaced the wild-type histone promoter at the *TRT1* chromosomal locus with a constitutive promoter by using the gene transplacement procedure (28). Cells were synchronized in G1 by using the yeast mating pheromone, α-factor, and the levels of *H2B* and fusion RNAs were measured at various times after release from the block. In addition, part of each synchronized culture was released into medium containing HU so that the effect of HU on the pattern of accumulation of fusion and intact *H2B* RNAs could be determined. The results of this analysis are shown in Fig. 4.

If the stability of the fusion or full-length histone transcripts was regulated in the cell cycle, then cells lacking transcriptional regulation should still have exhibited cell cycle-dependent fluctuations in these RNAs due to posttranscriptional mechanisms. The normal pattern of fusion RNA accumulation is shown in Fig. 4A; when the *H2A-lacZ* gene was transcriptionally regulated, the fusion RNA accumulated in the cell cycle with the same periodicity as did endogenous *H2B* transcripts (cf. Fig. 4A and C). However, when transcription was constitutive (Fig. 4B), elevated levels of fusion RNA were observed in G1 (at 0 min) and throughout the cell cycle into G2. Although there was some scatter in the fusion RNA levels in this experiment, these fluctuations did not correlate with the cell cycle. Thus, in the absence of transcriptional control, there does not appear to be any additional cell cycle regulation of fusion RNA levels through changes in RNA stability.

In contrast to the fusion transcripts, the full-length histone transcripts appeared to be posttranscriptionally regulated. When the levels of intact *H2B* RNA were measured in cells in which *H2B* transcription was constitutive, substantial cell cycle fluctuations (approximately threefold between S phase and G2) in the levels of *H2B* RNA were observed (Fig. 4D). These fluctuations were not so pronounced as those found in cells in which *H2B* transcription was regulated (in which 20-fold changes occurred; Fig. 4C). Nonetheless, in the absence of transcriptional control, the levels of histone RNA

showed periodic changes which were presumably due to posttranscriptional alterations of histone RNA stability during the cell cycle. Since the fusion transcripts did not show this response, the hybrid transcripts either lacked the specific sequences or the proper three-dimensional structure necessary for posttranscriptional recognition and control.

The addition of HU to synchronized cells containing either regulated or constitutively transcribed genes confirmed the conclusions reached from our experiments with exponentially growing cells. The levels of both fusion and *H2B* RNAs were repressed in cells in which these genes were transcriptionally regulated (Fig. 4A and C). (In synchronized cells, a slight accumulation of histone RNA in the presence of HU is expected since histone transcription is activated in late G1, before the point in the cell cycle at which both HU and *cdc8* arrest DNA synthesis. The reason for the lack of fusion RNA accumulation is unclear but may have reflected a greater instability of the hybrid transcript.) In contrast to the effect of HU in cells with the intact histone promoter, fusion RNA levels were not depressed by the inhibition of DNA synthesis at any stage of the cell cycle if the transcriptional regulatory sequences were deleted (Fig. 4B). This confirms our earlier conclusion that the promoter deletions remove sequences required for this level of regulation. Finally, the accumulation of *H2B* RNA also did not seem to be significantly affected by the addition of HU to the promoter mutant (Fig. 4D). However, since the peak of accumulation in the first cell cycle was somewhat reduced, we cannot exclude the possibility of a small posttranscriptional component to the coupling of full-length histone transcripts to DNA replication.

Perhaps the most interesting result of these experiments, however, is illustrated by the pattern of *H2B* RNA accumulation in the presence of HU (Fig. 4D). Synchronized yeast cells treated with HU arrest in S phase as large budded cells, and all further progress through the mitotic cell cycle is blocked; without DNA synthesis, no spindle formation, mitosis, or cell separation occur. Thus, it is not surprising that histone transcription in wild-type cells was not reinitiated after the HU arrest point (Fig. 4C). However, when histone transcription was constitutive, posttranscriptional regulation appeared to occur periodically despite the block in the cell cycle (Fig. 4D). Thus, the *H2B* RNA levels dropped and rose again with normal cell cycle periodicity even though the mitotic cell cycle was blocked in early S phase. This suggests that posttranscriptional regulation may operate on a periodic timer which runs independently of steps in the mitotic cell cycle. Such a clock might be linked to some aspect of cell growth since cells arrested by HU continue to grow in size despite the block in the mitotic DNA cycle.

DISCUSSION

We previously demonstrated the importance of transcriptional regulation in the periodic accumulation of histone RNAs during the *S. cerevisiae* cell cycle (16). However, it was not known whether the regulation of histone gene expression involved posttranscriptional regulation of histone RNA stability as well. We therefore investigated the role of posttranscriptional mechanisms in the regulation of histone RNA levels in two different cell cycle phenomena.

In *S. cerevisiae*, as in higher eucaryotes, the interruption of ongoing DNA replication leads to a rapid drop in the levels of histone RNA. Posttranscriptional turnover of existing histone RNA has been postulated to account for much of this decline in higher eucaryotic cells (1, 3, 5–7, 15, 22, 29, 30), although a transcriptional component is also present in some cells (7, 15, 22, 29). On the basis of the rapid disappearance of histone RNA in temperature-shifted *cdc8* cells, we previously proposed that degradation of existing RNA is the major mechanism in yeast cells for coupling histone RNA levels to DNA replication (19). However, in analyzing the histone sequences required for this response, we demonstrated that sequences in the histone promoter are actually responsible for the effect of DNA synthesis inhibitors on histone RNA levels. Our results suggest that the inhibition of ongoing DNA chain elongation with either the mutation *cdc8* or the drug HU acts primarily to repress histone gene transcription. Although we cannot entirely exclude the possibility that interruption of DNA replication has some minor effect on the stability of full-length histone transcripts, the rapid drop in RNA levels can be accounted for primarily by inhibition of transcription followed by normal decay of histone transcripts. Since the half-life of yeast histone mRNAs is shorter (25) than that of histone transcripts in higher eucaryotic cells (3, 15, 22, 27, 29, 30), posttranscriptional destabilization of histone RNA may not be as important in yeast cells for coupling RNA levels to DNA replication.

We have proposed that the cessation of histone RNA synthesis in the cell cycle is due to repression of transcription (16) and depends on specific negative regulatory (CCR) sequences in the histone promoter (24). We showed here that deletions which eliminated these negative control sequences also made histone transcription insensitive to the inhibition of DNA replication. This suggests that the inhibition of DNA synthesis triggers a repression of transcription (using these same sequences) similar to that which occurs in the normal cell cycle. However, the repression which occurs when DNA synthesis is arrested must occur earlier than that observed in the normal cell cycle since histone RNA accumulation is negligible in cells grown in the presence of DNA synthesis inhibitors. We propose that there is a cell cycle-specific repressor which is activated sometime in S phase in the normal cell cycle and which is prematurely activated when DNA synthesis is arrested immediately after its initiation by inhibitors of DNA chain elongation. Thus, a common regulatory molecule would be activated by two different pathways. Although it is tempting to imagine that the termination of DNA synthesis could act as the signal to activate the repressor in both cases, the pathway must be more complex since during the normal cell cycle, the rate of histone transcription begins to fall before the peak in the rate of DNA replication (16). Interestingly, the signal for coupling histone transcription to DNA replication must depend on the actual arrest of ongoing DNA synthesis and not on the lack of replication per se. Cells which carry the temperature-sensitive *cdc7* mutation (a defect in the initiation of DNA replication) arrest at the G1/S boundary at the nonpermissive temperature, and despite the lack of DNA synthesis at this stage of the cell cycle, no inhibition in the rate of histone transcription is observed (16). This suggests that the regulatory molecule(s) required to repress transcription cannot be activated in the cell cycle until after DNA synthesis has been initiated.

We expanded our search for posttranscriptional regulation in *S. cerevisiae* by assaying for posttranscriptional controls at other points in the cell cycle. By using histone promoter mutations which result in the constitutive transcription of the histone genes (24), we were able to uncouple transcriptional from posttranscriptional regulation and to assess directly whether posttranscriptional controls affect the levels

of either histone or histone-*lacZ* RNA in the cell cycle. We found that in the absence of transcriptional regulation, the levels of full-length *H2B* RNA still showed substantial cell cycle-dependent fluctuations. These fluctuations, which were presumably due to posttranscriptional degradation of histone RNA, were about threefold between S phase and G2. Although these changes were less dramatic than those observed in cells with intact transcriptional controls (20-fold changes between S and G2), they do indicate that periodic posttranscriptional modulation of histone RNA levels can occur. The simplest interpretation of these results is that posttranscriptional mechanisms make a 3-fold contribution to the total 20-fold fluctuation in histone RNA levels in the cell cycle by destabilizing histone transcripts in G1 and G2. However, it is also possible that posttranscriptional destabilization is a response to the inappropriate synthesis of histone RNA in the G1 and G2 phases of the cell cycle and that normally, when transcription is repressed at these stages, the posttranscriptional response is not activated. Interestingly, the periodicity imposed by posttranscriptional mechanisms alone did not displace the peak in *H2B* RNA accumulation. Thus, *H2B* transcripts still acumulated with the same cell cycle periodicity as did the other histone transcripts. The major biological role of posttranscriptional regulation in the cell cycle could therefore be similar to its effect in dosage compensation (25); that is, it might be a mechanism for maintaining an equimolar stoichiometry between the four different histone transcripts.

In contrast to full-length histone transcripts, histone-*lacZ* fusion RNAs did not appear to be posttranscriptionally regulated; in the absence of transcriptional regulation, the levels of fusion RNA did not fluctuate in the cell cycle. In addition, our experiments suggest that fusion transcripts may be insensitive to dosage compensation as well. When the rate of *H2B* transcription is doubled by increasing the gene copy number, the stability of the *H2B* transcript is decreased such that no increase in the levels of stable RNA occur (25). However, when the rate of transcription of the *GAL10*-regulated *H2B-lacZ* fusion gene was increased by raising the galactose concentration, the levels of stable fusion RNA also increased. Since the *GAL10*-regulated fusion gene contained more than half of the 5′ end of the *H2B* gene (the entire untranslated leader region plus 80 of 130 amino acids of the coding sequence), the sequences required for posttranscriptional recognition and destabilization of *H2B* RNAs must either reside in the 3′ end of the gene or else an intact three-dimensional structure is necessary. It is interesting in this context that sequences responsible for posttranscriptional regulation of the mouse *H4* gene have been shown to reside in the 3′ region of the gene (21).

An intriguing aspect of the posttranscriptional regulation we observed is that it appears to be independent of the mitotic cell cycle. In contrast to transcriptional control, which is tightly linked to DNA replication and cell cycle progress, posttranscriptional regulation of histone RNA continued to act periodically even when the cell cycle was blocked in S phase by the addition of HU. This behavior is reminiscent of the control of bud formation in temperature-sensitive *cdc4* yeast cells, which continue to initiate buds once every 100 to 150 min at 36°C (about one generation time) despite the block in the cell cycle (12). Although we cannot eliminate the possibility that the continued cycling in RNA levels was somehow an artifact induced by the conditions of S-phase arrest, these results suggest that there is a regulatory clock which runs independently of steps in the nuclear DNA cycle.

Why are the histone genes so tightly regulated, with controls at both the transcriptional and posttranscriptional levels? Histone RNA levels seem to be linked both to the rate of DNA replication and to each other. Perhaps histones have a role, as yet unidentified, in some cellular assembly process(es) which requires precisely regulated levels. For example, Meeks-Wagner and Hartwell suggested that multiple levels of histone regulation may have evolved to ensure a high fidelity of chromosome transmission; if an equimolar ratio of each of the four histones was a requirement in vivo for correct nucleosome assembly, then precisely controlled levels of histone gene expression might be necessary to ensure stable chromosome transmission and hence cell viability (23).

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