

Regulation of Histone mRNA in the Unperturbed Cell Cycle: Evidence Suggesting Control at Two Posttranscriptional Steps

MICHAEL E. HARRIS,^{1†} RUTH BÖHNI,^{2‡} MARTIN H. SCHNEIDERMAN,^{3,4§} LAKSHMAN RAMAMURTHY,³
DANIEL SCHÜMPERLI,^{2‡} AND WILLIAM F. MARZLUFF^{1,3*}

Department of Chemistry,¹ Department of Biological Sciences,⁴ and Institute for Molecular Biophysics,³ Florida State University, Tallahassee, Florida 32306, and Institut für Molekularbiologie II der Universität Zürich, 8093 Zürich, Switzerland²

Received 26 November 1990/Accepted 1 February 1991

The levels of histone mRNA increase 35-fold as selectively detached mitotic CHO cells progress from mitosis through G₁ and into S phase. Using an exogenous gene with a histone 3' end which is not sensitive to transcriptional or half-life regulation, we show that 3' processing is regulated as cells progress from G₁ to S phase. The half-life of histone mRNA is similar in G₁- and S-phase cells, as measured after inhibition of transcription by actinomycin D (dactinomycin) or indirectly after stabilization by the protein synthesis inhibitor cycloheximide. Taken together, these results suggest that the change in histone mRNA levels between G₁- and S-phase cells must be due to an increase in the rate of biosynthesis, a combination of changes in transcription rate and processing efficiency. In G₂ phase, there is a rapid 35-fold decrease in the histone mRNA concentration which our results suggest is due primarily to an altered stability of histone mRNA. These results are consistent with a model for cell cycle regulation of histone mRNA levels in which the effects on both RNA 3' processing and transcription, rather than alterations in mRNA stability, are the major mechanisms by which low histone mRNA levels are maintained during G₁.

Histone proteins are synthesized coordinately with DNA, and changes in histone protein synthesis are mediated by rapid changes in histone mRNA concentration (10, 13, 32). There are two major groups of histone genes: (i) those coding for the cell cycle-regulated, replication-dependent histones and (ii) the constitutively expressed replacement variant histones (39, 40). The replication-dependent histone genes lack intervening sequences, and the mRNAs end in a 3' stem-loop structure which is formed by an endonucleolytic cleavage (9, 22). The replacement variant histone H3.3 gene contains intervening sequences, and the mRNA ends in a 3' poly(A) sequence (6, 38).

Detailed studies of the changes in histone mRNA metabolism during the cell cycle have largely been conducted in cells synchronized by using inhibitors of DNA synthesis (13, 26), serum starvation (8), or temperature-sensitive mutants (2, 3, 18, 19, 33). Although in one study centrifugal elutriation (1) was used to avoid the effects of drug-induced synchrony, the lack of complete synchronization did not allow precise measurement of the changes in histone mRNA levels during the cell cycle. These studies all showed that the rate of histone gene transcription varied only three- to fivefold during the cell cycle, indicating that much of the regulation must be posttranscriptional.

Posttranscriptional regulation at two steps, mRNA degradation and 3' processing of pre-mRNA, has been implicated in control of histone mRNA levels in a number of studies. The levels of replication-dependent histone mRNAs decrease rapidly upon inhibition of DNA synthesis as a result

of increased degradation (4, 10, 32). The 3' stem-loop structure is required for their rapid degradation (11, 15, 24), and this process also requires that the mRNA be actively translated (11). Inhibitors of protein synthesis prevent the rapid degradation of histone mRNA (4, 35). The replacement variant histone mRNAs, which lack the 3' stem-loop structure, are stable when DNA synthesis is inhibited (5, 32). The same 3' end structure is required for 3' end formation and is involved in regulating 3' end formation. This was demonstrated in a temperature-sensitive mutant arrested in G₁ (18) and in cells synchronized by serum starvation or G₁ arrest by indomethacin (34). We report here the changes in histone mRNA levels that occur during the CHO cell cycle, using cells synchronized by selective detachment of mitotic cells. Our results indicate that quantitative changes in RNA 3' processing, but not mRNA stability, play an important role during the progression of cells from G₁ to S phase, whereas the rapid decrease in histone mRNA levels during G₂ phase is primarily due to reduced mRNA stability.

MATERIALS AND METHODS

Selection and culture of cells. CHO cells were grown and selected in McCoy's 5a medium supplemented with 5% calf bovine serum (Hyclone). Cells were selected essentially by the method of Terasima and Tolmach (36) as modified by Schneiderman et al. (27, 28), using a semiautomated shaker apparatus. Selected mitotic cells were pooled on ice for no longer than 4 h for each experiment. Cooled cells grew and entered S phase exactly as did cells that were plated immediately after selection. These cells also had identical changes in histone mRNA concentrations. Mitotic cells were plated in temperature- and pH-adjusted medium at a density of 10⁶ cells per 25-cm² flask for RNA analysis. Mitotic indices were determined by scoring a minimum of 300 cells from preparations of pooled cells (27). The mitotic indices were greater than 97% for all experiments, and fewer than 0.5% of the

* Corresponding author.

† Present address: Department of Biochemistry, University of Alabama, Birmingham, AL 35294.

‡ Present address: Division of Developmental Biology, Department of Zoology, University of Bern, 3012 Bern, Switzerland.

§ Present address: Department of Radiology, University of Nebraska Medical Center, Omaha, NE 68198-0145.

cells were synthesizing DNA 1 h after plating. In some experiments, cells were continuously treated with 5 μ g of cycloheximide (CHM) per ml prior to selection.

Mitotic cells used to monitor the position of the synchronous cells in the cell cycle were grown on coverslips at the same density as the cells used for RNA analysis. They were pulse-labeled for 15 min (starting at the times indicated in the figure legends) in medium containing 222 kBq of [¹²⁵I]iododeoxyuridine. After labeling, the cells were washed three times with Hanks balanced salt solution and fixed in methanol. The cells were coated with NTB2 autoradiographic emulsion and exposed up to 10 days. The labeling index was generated after scoring of a minimum of 300 cells at each time.

Analysis of mRNA content. Total cell RNA was prepared, and histone mRNA levels were analyzed by using a quantitative S1 nuclease assay as described previously (12, 32). To assay the H3.2 mRNA levels, the H3-614 gene was labeled at the 3' end of the *SalI* site at codon 58 of the H3 gene. To assay the H3.3 mRNA levels, the H3.3-921 gene was 5' labeled at the *BglII* site at codon 85 of the gene (37). To assay the U₅H mRNA and the endogenous hamster H2a mRNA, the H2a gene was labeled at the 3' end of the *AvaI* site at codon 20 or the 5' end of the *NarI* site at codon 43. The two transcripts from plasmid pgptCX/-230+118 were analyzed by using a riboprobe protection assay (see Fig. 4A). Conditions were as previously described (12). The amount of U1 RNA was also assayed by using a riboprobe protection assay which detects both U1b and U1a small nuclear RNAs (snRNAs) (17).

Introduction of exogenous genes into CHO cells. The pgptCX/-230+118 gene described by Stauber et al. (33, 34) was introduced into CHO cells by transfection, using the Polybrene method of Chaney et al. (7) as described previously (16). Stable transformants were selected for resistance to mycophenolic acid as previously described (23). The intact histone H2a-614 gene and the U₅H gene were introduced into CHO cells by using Polybrene (7) together with the plasmid pSV2neo, which confers resistance to G418. Stable transformants were selected for resistance to G418 as previously described (16). The U₅H gene contains the mouse U1b snRNA promoter (20, 21) and the first 5 nucleotides (nt) of the U1 snRNA joined to the 5' untranslated region of the histone H2a-614 gene (12, 14). This gene encodes a normal histone H2a mRNA and protein and has been described previously (25).

RESULTS

Levels of H3.2 and H3.3 mRNA in G₁ and S phase. Highly synchronous cultures of CHO cells were obtained by selective detachment of mitotic cells. Less than 0.5% of the mitotic cells incorporated [¹²⁵I]iododeoxyuridine within 1 h after selection, and more than 97% of the cells eventually entered S phase. At the time of selection, all cells were between 4 and 22 min before division (29). Note that as the cells progress their level of synchrony decreases. The length of G₁ varied between 3 and 4.5 h among the experiments. It took 3 to 5 h from the time the first cells entered S phase until >95% of the cells were in S phase (see Fig. 2). Desynchronization is such that by 10 to 11 h the cells were distributed from late S through G₁ (31).

To determine the amount of histone H3.2 mRNA, we used an S1 nuclease assay, utilizing the mouse H3-614 gene as a probe. The hamster mRNA protects the coding region of this gene from S1 nuclease digestion as a result of the high homology between hamster and mouse H3.2 mRNAs (16),

resulting in a single protected fragment of 240 bp when the *SalI* site was labeled at the 3' end. To determine the amount of the replacement variant histone H3.3 mRNA, we used the mouse histone H3.3 pseudogene (37) as a probe. This probe completely protects the hamster histone H3.3 mRNA, resulting in a 365-nt protected fragment. Figure 1A shows the levels of H3.2 and H3.3 mRNAs as cells progressed from M to S phase. To accurately quantify the amounts of histone mRNA, RNA from different numbers of cells were assayed on the same gel (gel not shown), and the results are plotted in Fig. 2A. We observed a 35-fold increase in the amount of H3.2 mRNA between G₁ and S phase. The amount of H3.2 mRNA began to increase as early as 1 h after division, before the cells entered S phase (Fig. 2B; see below). By 3.5 h, the amount of H3.2 mRNA had reached half of its maximal level, and about 50% of the cells had entered S phase. After 5 h 90% of the cells were in S phase (Fig. 2A); by 7 h more than 95% of the cells were in S phase, and the histone mRNA had reached its maximal level. In sharp contrast, the levels of H3.3 mRNA remained relatively constant, changing only twofold between M and S phase, as expected for a constitutively expressed gene (Fig. 1). The levels of the H3.2 mRNA were normalized to the amount of H3.3 RNA. Similar results were obtained when the H3.2 mRNA levels were normalized to the amount of U1 snRNA (Fig. 3D).

The initial accumulation of histone H3.2 mRNA precedes the onset of S phase. At 1.0 and 1.5 h after mitosis there was a detectable increase in H3.2 mRNA levels (>3-fold), while the labeling index of these cells was less than 1%. Figure 1B shows an experiment in which H3.3 and H3.2 mRNAs were measured simultaneously. Similar results were observed in eight separate experiments. Because the two probes used had similar specific activities, the amounts of H3.3 and H3.2 mRNA in G₁-phase cells were similar. The data from two separate experiments are plotted in Fig. 2B. There was a threefold increase in histone H3.2 mRNA during G₁ which reached a plateau prior to the onset of S phase. The major accumulation of histone mRNA occurred as cells entered S phase (Fig. 2A).

Regulation of histone mRNA levels between G₁ and S phases. Intact mouse histone genes transfected into CHO cells are regulated qualitatively and quantitatively in parallel with the endogenous hamster genes, allowing us to dissect the contribution of different regulatory steps by using a transfected gene. The H2a-614 gene was introduced into CHO cells, and synchronous cells from pools of stable transformants were analyzed. Expression of the transfected mouse H2a-614 gene paralleled expression of the endogenous hamster H2a genes (Fig. 3). The ratio of expression of the H2a-614 gene to the endogenous hamster histone genes was 27 \pm 2% at each time. Thus, an intact mouse histone gene is regulated properly, both qualitatively and quantitatively, allowing us to estimate the contribution of different aspects of histone mRNA metabolism by using appropriately constructed genes.

The rapid 35-fold increase in histone mRNA concentrations between G₁ and S phases could be due to alterations in the rate of histone gene transcription, the efficiency of processing of histone mRNA, an increase in the half-life of histone mRNA, or a combination of effects on all three of these parameters. We designed experiments to test the contribution of each of these parameters to regulation of histone mRNA levels. A gene, U₅H, was constructed which had a constitutive U1 snRNA promoter followed by the complete coding region and normal 3' end of the H2a-614 gene (Fig. 4B). The U₅H gene produces a transcript with a

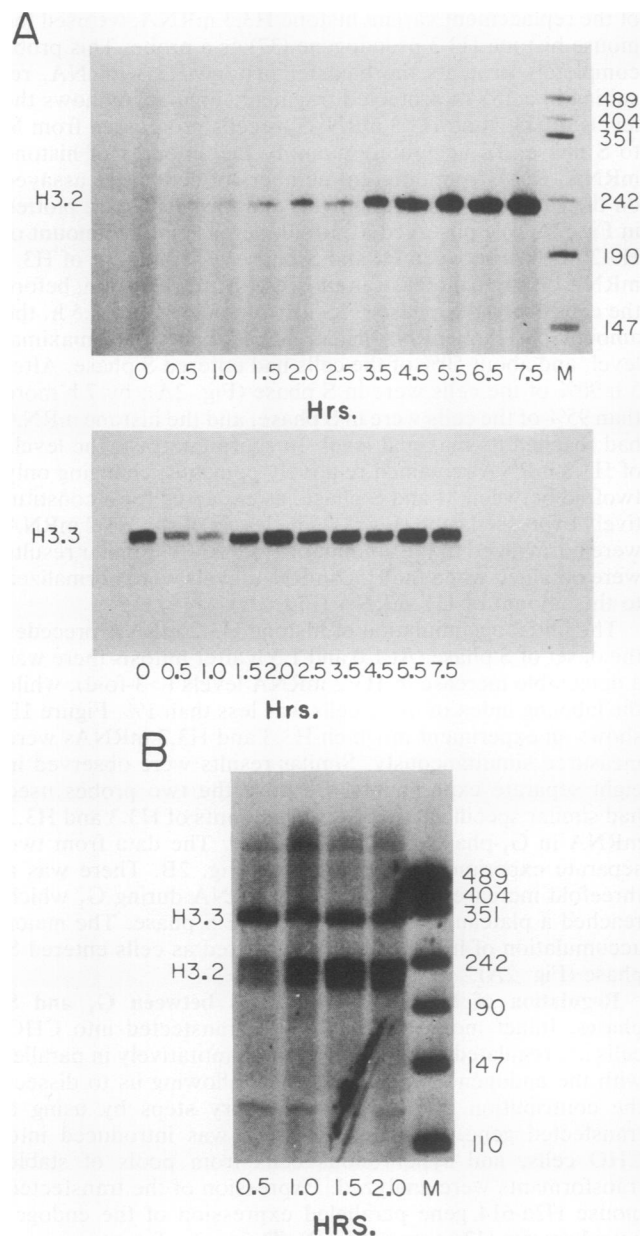


FIG. 1. H3.2 and H3.3 mRNA levels during the cell cycle. Cells were synchronized by mitotic selection, and total cell RNA was prepared; 2×10^6 cells were plated for each time point. Each assay represents RNA from 4.0×10^5 cells except for the 0.5- and 1-h time points, at which fewer cells were recovered. The amounts of histone H3.2 RNA and H3.3 RNA were analyzed by using a quantitative S1 nuclease assay. The S1-resistant DNA fragments were resolved by electrophoresis and detected by autoradiography. The times after selection are indicated below each lane. In the experiment shown in panel A, the H3.2 and H3.3 histone mRNA levels were determined separately. The low levels of H3.3 mRNA at 0.5 and 1.0 h were due to a lower recovery of cells in these samples (see panel B). In panel B, the H3.2 and H3.3 mRNAs from a separate experiment in which equal number of cells were recovered were measured simultaneously, using a mixture of H3.3 and H3.2 probes of similar specific activity. The H3.2 protected fragments were resolved into three bands in this experiment.

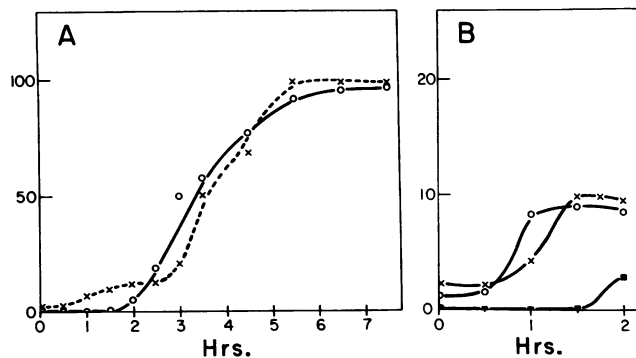


FIG. 2. H3.2 RNA levels during the cell cycle. (A) Amount of H3.2 RNA (as a percentage of the maximal level, after normalization to the amount of H3.3 mRNA; \times) and labeling index of cells (\circ) plotted versus the time after plating mitotic cells. (B) Results of two experiments showing the H3.2 mRNA level during the first 2 h after plating of mitotic cells. The labeling index was $<0.5\%$ until 2 h after plating.

histone 3' end and shows normal regulation of mRNA half-life when DNA synthesis is inhibited (25). This gene allows us to assess the effect of transcription on cell cycle regulation. A second gene, pgptCX/-230+118, contains the simian virus 40 (SV40) early promoter, the coding body of the bacterial xanthine-guanine phosphoribosyltransferase gene (*gpt*), and, at its 3' end, the SV40 small-t intron and early polyadenylation signal. Introduced into the 3' untranslated part of the bacterial sequence is the 3' part of the mouse histone H4 gene, including the histone 3' end signal (Fig. 5A). This gene yields an mRNA with a histone 3' end whose half-life is not regulated because of the long distance from the termination codon to the histone 3' end (11). The contribution of RNA 3' processing to histone gene regulation can be separately assessed with use of this gene, by measuring the relative usage of the histone 3' end and SV40

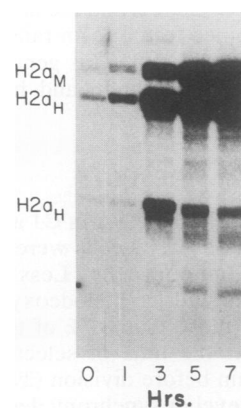


FIG. 3. Expression of the mouse H2a-614 gene in CHO cells. Cells transfected with the intact H2a-614 gene (16) were synchronized by mitotic shake-off. Equal numbers of cells were collected at the indicated times after mitotic shake-off, total cell RNA was prepared, and the amounts of H2a-614 and endogenous hamster H2a mRNAs were measured by S1 nuclease mapping. The probe used was the H2a-614 gene labeled at the 3' end of the *Ava*I site at codon 20. This probe protects the entire mouse H2a-614 mRNA (H2a_M) and the endogenous hamster H2a mRNAs up to the termination codon (H2a_H). Numbers below the lanes indicate the time after plating that the cells were harvested.

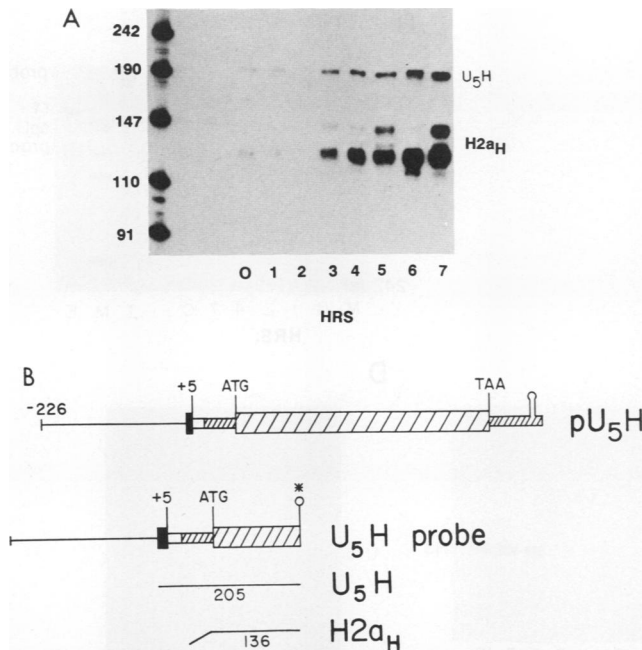


FIG. 4. (A) Expression of the *U₅H* gene in synchronous CHO cells. CHO cells stably transfected with the *U₅H* gene were synchronized by mitotic shake-off, and the amounts of *U₅H* mRNA and endogenous histone *H2a* mRNA were assayed by S1 nuclease assay. (B) Structure of the *U₅H* gene and strategy used for S1 nuclease mapping. The probe was the *U₅H* gene labeled at the 5' end of the *Nar*I site in the *H2a* coding region (codon 43). The protected fragments are *U₅H* (209 nt; protection by the *U₅H* mRNA) and *H2a_H* (130 nt; protection by the endogenous hamster *H2a* mRNA). The minor band at 145 nt is also due to protection by an endogenous hamster *H2a* mRNA. The time after selection is indicated below each lane. The 2-h sample had half as much RNA assayed as in the other lanes, as judged by determination of the amount of U1 snRNA in each sample (not shown).

polyadenylation site, as previously described (12, 32). To measure the relative half-life of histone mRNA in *G₁* and S phases we used actinomycin D (dactinomycin) to block histone mRNA synthesis or CHM to stabilize the histone mRNA.

Figure 4 shows the expression of the *U₅H* gene during the cell cycle. The mRNA produced from this gene has a histone 3' end and shows normal posttranscriptional regulation when cells are treated with inhibitors of DNA synthesis (25). The *U₅H* mRNA increases about 10-fold as cells progress from *G₁* to S phase, compared with the 35-fold increase of the endogenous histone mRNA (Fig. 4). When the intact mouse *H2a-614* gene is transfected into CHO cells, there is a 35-fold increase in histone mRNA between *G₁* and S phases exactly paralleling the increase in the endogenous hamster *H2a* mRNAs (see Fig. 3). The 3.5-fold difference in accumulation we interpret as the contribution of the increase in transcription rate to the increase in histone mRNA levels between *G₁* and S phases. Thus, there remains about a 10-fold effect which is posttranscriptional.

The fusion gene *pgptCX/-230+118* (Fig. 5A) was introduced into the CHO cells to assess the contribution of 3' end formation to the change in histone mRNA levels during the cell cycle. This hybrid transcription unit, after transfection into mammalian cells, gives rise to two major RNA species (33): a short one with the histone *H4* 3' end and a longer

transcript polyadenylated at the SV40 polyadenylation site. The short histonelike RNA is regulated qualitatively in parallel with the endogenous histone genes in a temperature-sensitive cell cycle mutant (33) or in fibroblast cells that are synchronized by serum starvation (34), i.e., conditions under which regulation of histone RNA 3' processing has been demonstrated (18, 34). However, because the histone gene 3' end is located 540 nt 3' to the stop codon of the *gpt* gene, the mRNA remains stable when DNA synthesis is inhibited (34), as predicted from our previous results on the regulation of histone mRNA half-life (11). Proper regulation of histone mRNA degradation requires that the termination codon be within 200 nt of the 3' end of the mRNA (11). Thus, expression of the short histonelike RNA encoded by *pgptCX/-230+118* can be taken as an indicator of regulation at the level of histone RNA 3' processing (34).

Figure 5B shows the results of an experiment using mitotically selected cells transfected with the *pgptCX/-230+118* gene. The short RNA which ends at the normal histone 3' end gives rise to a protected fragment of 401 nt (proc); the long polyadenylated RNA protects a fragment of 521 nt (rt). In addition, there is a minor transcript at 442 nt (spl) which is due to the use of a cryptic 5' splice site 41 nt downstream of the histone 3' end by some polyadenylated transcripts (33, 34). The relative proportions of the proc RNAs reflect the relative efficiency of histone 3' end formation. If the histone 3' end is not formed, then transcription continues and the polyadenylated transcript is formed. In both mitotic and *G₁* cells, the polyadenylated transcript predominates. As cells enter S phase, the proportion of transcripts ending at the histone 3' end increases about eightfold (Fig. 5B; compare the 1-h and 7-h time points; Table 1). This proportion persists throughout S phase. The increase in processing efficiency started prior to S phase in three independent experiments, as did the increase in histone mRNA concentration. The amount of both transcripts decreased between the end of S phase and mitosis (compare the 10- and 0-h points in Fig. 5B), with a greater decrease in the histone 3' end, probably reflecting the down-regulation of histone 3' end formation in *G₂* (see Discussion).

The change in endogenous histone *H3* mRNA levels in the same cells is shown in Fig. 5C. These changes are similar to the changes seen in Fig. 1, with a small increase in *G₁* phase (2-h time point) and a much larger increase as cells enter S phase. The magnitude of the increase in the endogenous histone mRNA is much greater than that of the transfected *pgptCX/-230+118* gene, due both to the additional regulation of transcription and to the relatively high levels of the *pgptCX/-230+118* transcripts present in the mitotic cells. As an internal control, the levels of U1 snRNA in the same cells are shown in Fig. 5D; the amounts of both U1a and U1b snRNA remained constant (as a proportion of total RNA). Essentially identical results were obtained in three separate experiments. These results are summarized in Table 1. The combination of the three- to fourfold effect on transcription and the eightfold effect on 3' end formation account for most if not all of the increase in histone mRNA levels between *G₁* and S phases.

Stability of histone mRNA in *G₁*. The results presented above suggest that the regulation of histone mRNA half-life is not important between *G₁* and S phases but rather that 3' end formation and transcription are regulated. To measure the relative half-lives of histone mRNA in *G₁*- and S-phase cells, we used two approaches. First, we treated cells in *G₁* phase with dactinomycin and monitored the change in histone mRNA levels. Second, we took advantage of the fact

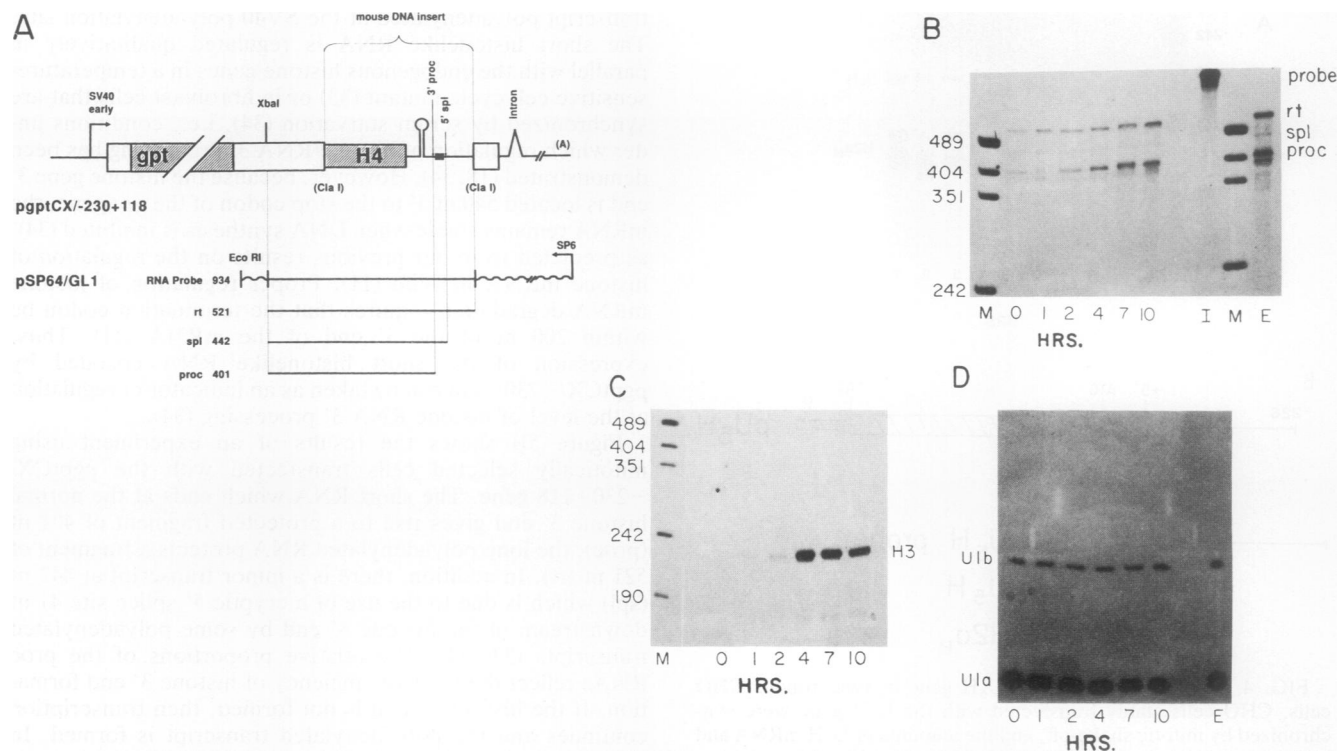


FIG. 5. Structure and expression of the pgptCX/-230+118 gene in synchronous CHO cells. (A) The *gpt* transcription unit of pgptCX/-230/+118 (16) consists of the SV40 early promoter (arrow), a bacterial DNA fragment (thick bar) containing the coding body of the *gpt* gene, the SV40 small-t intron (intron), and early polyadenylation site [(A)]. The mouse DNA insert contains part of the coding region (thinner, hatched bar) of a mouse histone H4 gene, the 3'-terminal hairpin loop and spacer element (black box) containing the RNA 3' processing signal, plus additional 3' spacer sequences. A uniformly labeled RNA probe for RNase mapping was obtained by runoff transcription with SP6 RNA polymerase from plasmid pSP64/GL1 digested with *EcoRI*. Wavy lines represent the part of the probe not present in pgptCX/-230+118. rt, Part of the probe protected by readthrough transcripts traversing the entire histone DNA insert; spl, part of the probe protected by differentially spliced readthrough RNA generated by utilization of a cryptic 5' splice site present in the post-H4 spacer (16); proc, part of probe protected by correctly processed histonelike RNA. The lengths of the relevant fragments are indicated in nucleotides. (B) The pgptCX/-230+118 gene was introduced into CHO cells by stable transfection, and mitotic cells were selected as described for Fig. 1. The polyadenylated and nonpolyadenylated transcripts from this gene were assayed by using the riboprobe as described above. The 401-nt fragment (proc) is the result of protection by the nonpolyadenylated transcript ending at the histone 3' end, and the 521-nt fragment (rt) is the result of protection by the polyadenylated transcript. Lanes M, pUC18 digested with *HpaII*; lane I, the input labeled riboprobe RNA; lane E, RNA from exponentially growing cells. A 1- μ g sample of RNA was analyzed in each time point. The times after selection are indicated below each lane. (C) The levels of hamster H3 mRNA were measured as for Fig. 1 in the same samples, using 1 μ g of RNA and the mouse H2a-614 gene labeled at the *Sall* site as a probe. The lanes are labeled as in panel B. (D) The levels of hamster U1a and U1b snRNAs were measured by using a riboprobe copy of the mouse U1b gene as a probe as described previously (17); 400 ng of RNA from the cells in the experiment shown in panel B was assayed. Lanes are labeled as in panel B.

TABLE 1. Quantitative changes in efficiency of 3' end formation^a

Time (h) after selection	Labeling index	proc RNA	rt RNA	proc/rt
0	<1.0	11.3 (2.9)	15.3 (0.6)	0.74 (4.8)
1	<1.0	4.0 (1.0)	25.5 (1.0)	0.15 (1.0)
2	<1.0	24.3 (6.2)	37.0 (1.4)	0.67 (4.3)
4	52	50.0 (12.7)	40.4 (1.6)	1.23 (8.0)
7	96.5	64.0 (16.2)	49.0 (1.9)	1.30 (8.5)
10	94	55.1 (14.1)	70.5 (2.8)	0.78 (5.1)

^a The gel in Fig. 4B was quantified by densitometry. Numbers represent arbitrary densitometer units. These were normalized relative to the values at 1 h after selection, and the normalized figures are given in parentheses. Note that the rise in processing efficiency starts prior to the entry of cells into S phase. Similar results were obtained in three separate experiments. For definitions of proc and rt RNAs, see the legend to Fig. 5.

that inhibiting protein synthesis stabilizes histone mRNA. Thus, if histone mRNA is being rapidly synthesized but also rapidly degraded in the cytoplasm, inhibiting protein synthesis would result in large increase (four- to fivefold) in histone mRNA levels (10, 35).

Figure 6A shows the effect of treating cells in G₁-phase (1.5 h after shake-off; lanes 1, 3, and 4) or S phase (7 h after shake-off; lanes 5 and 7) with dactinomycin for 30 or 60 min. In G₁-phase cells, histone mRNA levels decreased by about 40% in 30 min (Fig. 6A, lane 2) and 70% in 1 h (lane 4), consistent with a half-life of histone mRNA of about 40 min, similar to the values estimated by Heintz et al. (13) for HeLa cells. A very similar decrease of 40% in 30 min was observed in S-phase cells (lane 7), suggesting that the half-life of histone mRNA does not differ significantly between G₁ and S phases. Although it is difficult to precisely estimate the amount of decrease of histone mRNA in G₁ phase following dactinomycin treatment, the fact that the histone mRNA is

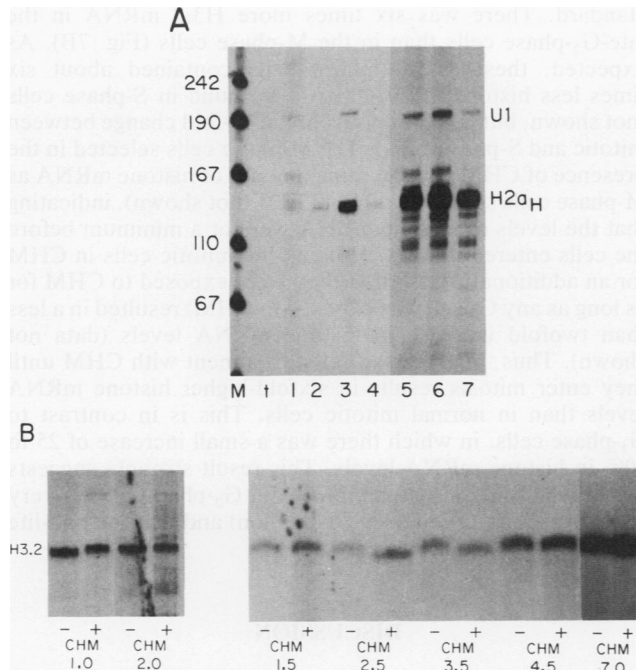


FIG. 6. Effects of dactinomycin D and CHM treatment on G_1 - and early-S-phase cells. (A) Synchronous CHO cells transfected with the U_5H gene were selected by mitotic shake-off. RNA was prepared and assayed by S1 nuclease mapping as for Fig. 3. Lanes 1 and 3 show analysis of RNA from cells incubated for 1.5 and 2.5 h, respectively. After incubation for 1.5 h, cells were treated with dactinomycin (5 μ g/ml) for 30 min (lane 2) or 1 h (lane 4). Lanes 5 and 6 show analysis of RNAs from cells incubated for 7 and 8 h, respectively. After incubation for 7 h, cells were treated with dactinomycin (5 μ g/ml) for 30 min (lane 7). (B) Cells were treated with 0.1 mM CHM for 1 h before the indicated time of harvest, which is the time after plating of mitotic cells. The amounts of H3.2 mRNA were measured by S1 nuclease mapping as described in the legend to Fig. 1. RNA from 4×10^5 cells was used for each time point. The two panels represent separate experiments. In the experiment on the right, the cells entered S phase slightly later than in the experiment in Fig. 1, accounting for the relatively low level of histone mRNA in the cells 4.5 h after plating.

still detectable after treatment with dactinomycin for 1 h (lane 4) indicates that the histone mRNA is not extremely unstable in G_1 -phase cells.

To independently estimate the half-life of histone mRNA in G_1 - and S-phase cells and to investigate the role that degradation of histone mRNA might play in depressing the levels of histone message in G_1 , we treated synchronous cells with CHM at various times during G_1 and S phases and measured the changes in amount of histone mRNA (Fig. 6B). The maximum increase in histone mRNA levels during both G_1 and S phases as a result of CHM treatment for 1 h was 25 to 40%. This value is consistent with a half-life of about 45 min, assuming that CHM stabilizes histone mRNA equally in both phases of the cell cycle. A similar increase in histone mRNA levels was observed in exponentially grown cells treated with CHM for 1 h (not shown). The results were quantified by densitometry (Table 2). Because blocking degradation did not result in a multifold increase in mRNA levels, and because CHM has little effect on histone gene transcription in exponentially growing cells (10, 35), the observed small (25 to 40%) increase in histone mRNA levels

TABLE 2. Changes in histone H3.2 mRNA levels after CHM treatment^a

Time (h) after selection	Labeling index (control)	H3.2 mRNA concn	
		-CHM	+CHM
1.0	0	3	4
2.0	0	10	16
2.5	0.5	10	15
3.0	15	15	23
7.5	95	100	140

^a Synchronous cells were treated with CHM for 1 h at different times during the cell cycle. The times indicate the time after plating at the end of the CHM treatment. The labeling indices of the cells incubated without CHM were determined. Cells treated with CHM were not labeled with [¹²⁵I]thymidine during the treatment time. The amount of histone H3.2 mRNA was determined by densitometry of autoradiographs of S1 nuclease assays similar to those in Fig. 5. Values have been corrected for variable recovery of cells based on the amounts of H3.3 mRNA in each sample. The amount of H3.2 mRNA in the control cells 7.5 h after plating was assigned a value of 100.

is probably due to further stabilization of an already stable mRNA.

Thus, these two independent measurements of histone mRNA half-life in G_1 - and S-phase cells give similar results, suggesting that the half-lives of histone mRNA in G_1 and S phases are similar, about 45 min. These results suggest that the regulation of histone mRNA half-life is not important between G_1 and S phases but rather that 3' end formation and transcription together are regulated at this time. Most importantly, these results are consistent with the interpretation that the changes in histone mRNA levels observed as cells progress from mitosis through G_1 and into S phase are due principally to changes in the rate of histone mRNA biosynthesis (i.e., transcription and 3' processing).

Regulation of histone mRNA levels in G_2 . The 35-fold decrease in histone mRNA levels in the 90 min between the end of S phase and mitosis requires that the half-life of histone mRNA be less than 18 min during this time, i.e., considerably shorter than the half-life in G_1 and S phases. It was impossible to measure the histone mRNA levels directly during G_2 because desynchronization is such that by 10 to 11 h the cells were distributed from late S phase to G_1 phase, making it impossible to obtain a pure population of G_2 cells by plating mitotic cells (31). However, by combining mitotic cell selection with CHM treatment, it is possible to obtain mitotic cells which contain mRNAs stabilized while the cells are in G_2 (29, 30). CHM prevents cells which have not progressed past a transition point located an average of 70 min before division (40 to 50 min into G_2) from reaching mitosis. Cells which have passed this transition point enter mitosis at the normal rate (29). After treatment with CHM, 10 shakes were collected at 10-min intervals and pooled into two groups. The first group contained shakes 1 to 5, and the second group contained shakes 6 to 10. The histone mRNA content in these two groups reflects the amount of histone mRNA in the cells at the time of CHM addition when the cells were in mitosis and G_2 , respectively. The strategy for obtaining these cells is described below and in the legend to Fig. 7.

The G_2 -CHM transition point (TP_{CHM}) is located 70 min before division (28). During selection, after exposure to CHM, only those cells that have progressed past the TP_{CHM} can progress into mitosis and be selected. Cells selected during the 50 min immediately after CHM addition were considered to be in M phase at the time of CHM treatment because mitosis is 42 min long. They are referred to as

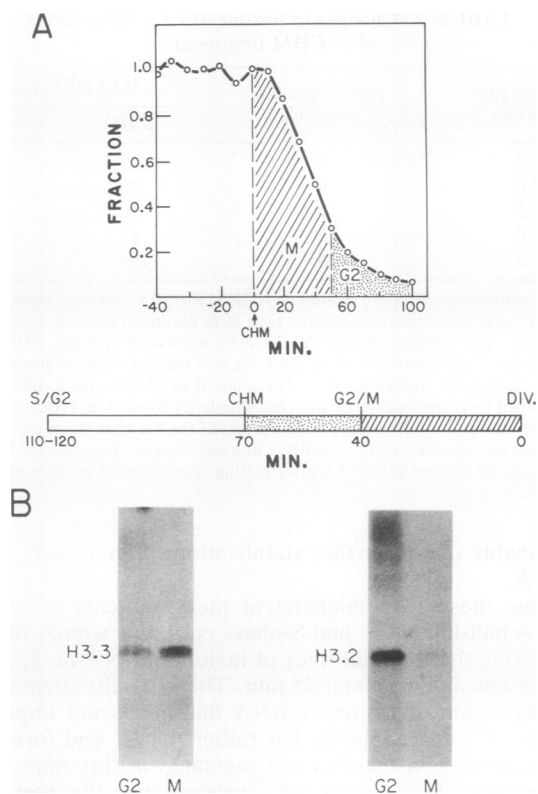


FIG. 7. Histone mRNA levels in M- and G₂-phase cells. (A) Selective detachment of mitotic cells treated in mitosis or G₂ with CHM. To establish a base line for cell progression, mitotic cells were selected for 60 min before addition of CHM (-60 to 0 on the abscissa). These cells were discarded. At 0 min, immediately after harvesting of the selection-shake, CHM (5 μ g/ml) was added to the medium used to refeed the cells so that CHM was continuously present for the remainder of the experiment. From 10 to 50 min after the addition of CHM (selection-shakes 1 to 5 posttreatment), the cells in mitosis at the initiation of treatment were selected and pooled. From 60 to 100 min after the addition of CHM (selection-shakes 6 to 10 posttreatment), the cells in G₂ at the initiation of treatment were selected and pooled. The ordinate is the number of cells selected as a fraction of the controls (1 = 7.5×10^5 cells per flask, nine treatment flasks). The time line represents the cell cycle from the S/G₂ boundary to division and shows the major, relevant events. The cells pooled from the hatched and dotted areas in the graph correspond to the hatched and dotted areas of the time line. (B) RNA from approximately 10^7 G₂- and 3×10^7 M-phase cells was analyzed by S1 nuclease assay for H3.3 and H3.2 mRNA content. The low amount of H3.3 mRNA in the G₂ cells reflects the lower number of cells in this sample.

M-phase cells in Fig. 7B. Cells selected 60 to 100 min after CHM addition were considered to be late-G₂-phase cells at the time of treatment. These cells are referred to as G₂ cells in Fig. 7B. They are cells that have progressed into M phase but contain H3.2 mRNA which was stabilized when the cells were in G₂ phase. Cells which were in S phase or early G₂ phase at the time of treatment were blocked from entering mitosis by CHM (29). Both the mitotic and G₂-phase cells were analyzed for H3.3 and H3.2 mRNA content. The average cell cycle age of these two populations differed by about 25 min.

To maintain uniformity, total cell RNA was extracted from approximately the same number of cells in both groups. In addition, the H3.3 mRNA level was used as an internal

standard. There was six times more H3.2 mRNA in the late-G₂-phase cells than in the M-phase cells (Fig. 7B). As expected, these late-G₂-phase cells contained about six times less histone mRNA than was found in S-phase cells (not shown, but this follows from the 35-fold change between mitotic and S-phase cells). The M-phase cells selected in the presence of CHM had the same amount of histone mRNA as M-phase cells selected without CHM (not shown), indicating that the levels of histone mRNA were at a minimum before the cells entered mitosis. Holding the mitotic cells in CHM for an additional 2 h (so that they were exposed to CHM for as long as any G₂ cell was exposed to CHM) resulted in a less than twofold increase in histone mRNA levels (data not shown). Thus, in G₂-phase cells, treatment with CHM until they enter mitosis results in sixfold-higher histone mRNA levels than in normal mitotic cells. This is in contrast to G₁-phase cells, in which there was a small increase of 25 to 40% in histone mRNA levels. This result strongly suggests that the half-life of histone mRNA in G₂-phase cells is very short (less than 17 min; see Discussion) and that the half-life in G₁ phase is much longer.

DISCUSSION

The steady-state level of an mRNA is a function of the rate of synthesis and the rate of degradation. Synthesis of functional cytoplasmic mRNA includes transcription, processing, and transport. During the cell cycle, histone mRNA concentrations are altered rapidly at two different times. First, as cells enter S phase there is a large (35-fold) increase; second, between S phase and mitosis there is a large (35-fold) decrease in histone mRNA. Several studies using cells synchronized by various methods or cells treated with inhibitors of DNA synthesis have determined that the rate of transcription of histone mRNA is reduced by a factor of only 3 to 5 in cells not synthesizing DNA (3, 8, 13, 19, 32). These include resting fibroblasts (8) and temperature-sensitive cells arrested in G₁ (19), in which histone mRNA content is less than 2% of the S-phase level (8, 19). Similarly, a number of studies have shown that the half-life of histone mRNA is reduced when S-phase cells are treated with inhibitors of DNA synthesis. This reduction in the histone half-life requires that the mRNA is being actively translated (11), and hence degradation is prevented by inhibitors of protein synthesis (4, 35). Schümperli and co-workers have recently shown that histone mRNA 3' end formation is inefficient in a temperature-sensitive mutant arrested in G₁ (18) and in cells synchronized by serum starvation (34). This finding suggests that 3' end formation is a regulatory step which is normally important in cell cycle regulation (18, 34).

The results reported here indicate that all of these different steps in histone mRNA metabolism are important in the cell cycle regulation of histone mRNA in CHO cells: (i) changes in histone mRNA stability, important in regulating the level of histone mRNA between the end of S phase and mitosis; (ii) RNA 3' end formation, important in G₁ phase; and (iii) the relatively small (three- to fourfold) change in transcription rate. We were not able to obtain enough synchronous cells to directly measure transcription rates or 3' processing efficiency.

Change in histone mRNA levels from G₁ to S phase. The results shown in Fig. 3 to 5 strongly suggest that the increase in histone mRNA levels between G₁ and S phases is due to a small increase (three- to fivefold) in the rate of transcription and a larger increase (six- to eightfold) in the efficiency

of histone 3' end formation. There is little or no change in the half-life of histone mRNA in the G₁-to-S-phase transition.

The difference in the amount of increase between the U₅H gene, which has a U1 snRNA promoter, and the H2a-614 histone gene is due to the amount of transcriptional regulation of the histone H2a-614 gene. This difference is three- to fourfold, which we ascribe to the transcriptional component of the cell cycle regulation of histone mRNA.

The relative increase of nonpolyadenylated versus polyadenylated transcripts from the pgptCX/-230+118 gene strongly suggests that 3' end formation is regulated during the G₁-to-S-phase transition in continuously cycling cells. This confirms our previous findings obtained with cells induced to reenter the cell cycle by serum stimulation (34). The relative increase in abundance of the nonpolyadenylated transcripts occurs as cells enter S phase. Transcription of the SV40 promoter on the pgptCX/-230+118 gene is not strongly altered during the cell cycle and is controlled for by measuring the ratio of the polyadenylated and nonpolyadenylated transcripts. Because the half-life of the nonpolyadenylated pgptCX/-230+118 transcript is not affected by inhibitors of DNA synthesis (34), the eightfold change in the relative abundance of this transcript represents the contribution of regulation of 3' end formation. Since it takes a significant amount of time for the relatively stable transcripts from this gene to decay at the end of S phase, maximal differences in the ratio of the polyadenylated and nonpolyadenylated transcripts are observed in G₁-phase cells rather than in mitotic cells (Table 1).

These results are consistent with differences in histone mRNA half-life not being important between G₁ and S phases. We estimated the half-life of histone mRNA in two ways during G₁- and S-phase cells, and the two estimates gave similar results. Blocking transcription with dactinomycin allowed us to estimate the relative half-life of histone mRNA in G₁- and S-phase cells (Fig. 5A), and the half-lives of histone mRNA were similar in G₁ and S phases. We also indirectly measured the half-life of histone mRNA in CHO cells by taking advantage of the fact that CHM stabilizes histone mRNA (35). The extent of increase in histone mRNA content after CHM treatment will depend on the half-life of histone mRNA. For example, if the half-life of histone mRNA is much less than 1 h, treatment with CHM for 1 h results in a large and rapid increase in histone mRNA levels (10, 35). However, if the half-life of histone mRNA is about 1 h, treatment with CHM will result in a much smaller increase in histone mRNA levels. In agreement with these predictions, we have reported (10) that histone mRNA levels were rapidly increased by CHM treatment of cells that had histone mRNA levels reduced by inhibitors of DNA synthesis (as a result of the short half-life of histone mRNA under these conditions). With this approach, it is clear that the stability of H3.2 mRNA is about the same in both G₁- and S-phase CHO cells (Fig. 6B; Table 2). The small increase in histone mRNA after 1 h of CHM treatment is consistent with a histone mRNA half-life of about 1 h.

In conclusion, the 3- to 4-fold change in transcription rates between G₁ and S phases coupled with the 6- to 8-fold change in 3' end formation can account for most, if not all, of the observed 35-fold change in histone mRNA levels. In contrast, the half-life of histone mRNA, which is dramatically reduced during G₂ phase (see below), is apparently the same in G₁ and S phases.

Changes in histone mRNA levels from S phase to mitosis. In the 90 min between the end of S phase and the selection of mitotic cells, the amount of histone mRNA drops 35-fold.

Therefore, the 90 min represents at least five half-lives, giving a maximum calculated half-life in G₂ phase of 17.5 min. This is a maximum because histone mRNA levels could have reached a minimum before the cells were selected in mitosis, and some histone mRNA synthesis could continue for at least part of this time. Cells treated with CHM in late G₂ phase (between the TP_{CHM} and the beginning of mitosis) had histone mRNA levels greater than those of mitotic cells, indicating that the histone mRNA had not all been degraded by this time. The late-G₂-phase cells had at least fourfold more histone mRNA than did the mitotic cells (Fig. 6B), and these populations differed in cell cycle time by an average of only 25 min, indicating that the half-life of histone mRNA in late G₂ was at most 12 min.

In addition, between the end of S phase and mitosis the ratio of the short histonelike transcript to the polyadenylated RNA from the pgptCX/-230+118 gene decreases by eightfold. Because transcription of this gene continues in G₂, this finding suggests that 3' formation is greatly reduced in G₂ cells. The eightfold decrease in the nonpolyadenylated pgptCX/-230+118 transcript between the end of S phase and its lowest value (1 h after selection) indicates that this transcript has a minimal half-life of about 50 min (i.e., similar to that of a normal histone mRNA in G₁ or S phase).

Model for regulation of histone mRNA levels during the cell cycle. In G₁ phase, there is a very low rate of histone RNA biosynthesis, as a result of reduced transcription and inefficient processing. As cells approach S phase, the transcription rate increases and processing becomes efficient. The histone mRNAs have similar half-lives in both G₁- and S-phase cells. At the end of S phase, histone mRNA is rapidly degraded and both the transcription rate and processing efficiency are reduced. By the time cells reach mitosis, the histone mRNA half-life has returned to that in G₁- and S-phase cells. Note that because histone mRNA is equally stable in G₁-phase cells (defined as cells not synthesizing DNA) and S-phase cells, there may be accumulation of histone mRNA (and histone protein) initiating just prior to entry into S phase. This would allow the cell to have a supply of histones to assemble nascent chromatin immediately upon initiation of DNA replication. This result implies that the coupling of histone mRNA stability to DNA replication is only seen in S- and G₂-phase cells and that the system for rapid degradation of histone mRNA may not be present in G₁-phase cells.

ACKNOWLEDGMENTS

We thank Ta-Jen Liu for the CHO cells transfected with the H2a-614 gene and Duane Pilch for the CHO cells transfected with the U₅H gene.

This work was supported by grants GM29832 to W.F.M. and CA41270 to M.H.S. from the National Institutes of Health and by grant 3.036.87 from the Swiss National Science Foundation and subsidiaries of the State of Zürich to D.S. The visit of R.B. to the Marzluff and Schneiderman laboratories was made possible by a travel grant from the Roche Research Foundation.

REFERENCES

- Alterman, R.-B., S. Ganguly, D. H. Schulze, W. F. Marzluff, and A. I. Skoultchi. 1984. Cell cycle regulation of mouse histone H3 mRNA metabolism. *Mol. Cell. Biol.* 4:123-132.
- Artishevsky, A., A. Graftsky, and A. S. Lee. 1985. Isolation of a mammalian sequence capable of conferring cell cycle regulation to a heterologous gene. *Science* 230:1061-1062.
- Artishevsky, A., S. Wooden, A. Sharma, E. Resendez, and A. S. Lee. 1987. Cell-cycle regulatory sequences in a hamster histone promoter and their interactions with cellular factors. *Nature*

- (London) 238:823-827.
4. Baumbach, L. L., F. Marashi, M. Plumb, G. Stein, and J. Stein. 1984. Inhibition of DNA replication coordinately reduces cellular levels of core and H1 histone mRNAs: requirement for protein synthesis. *Biochemistry* 23:1618-1625.
 5. Brown, D. T., S. E. Wellman, and D. B. Sittman. 1985. Changes in the levels of three different classes of histone mRNA during murine erythroleukemia cell differentiation. *Mol. Cell. Biol.* 5:2879-2886.
 6. Brush, D., J. B. Dodgson, O. R. Choi, P. W. Stevens, and J. D. Engel. 1985. Replacement variant histone genes contain intervening sequences. *Mol. Cell. Biol.* 5:1307-1317.
 7. Chaney, W. G., D. R. Howard, J. W. Pollard, S. Sallustio, and P. Stanley. 1986. High frequency transfection of CHO cells using polybrene. *Somatic Cell Mol. Genet.* 12:237-244.
 8. DeLisle, A. J., R. A. Graves, W. F. Marzluff, and L. F. Johnson. 1983. Regulation of histone mRNA production and stability in serum-stimulated mouse fibroblasts. *Mol. Cell. Biol.* 3:1920-1929.
 9. Gick, O., A. Krämer, W. Keller, and M. L. Birnstiel. 1986. Generation of histone mRNA 3' ends by endonucleolytic cleavage of the pre-mRNA in a snRNP-dependent *in vitro* reaction. *EMBO J.* 5:1319-1326.
 10. Graves, R. A., and W. F. Marzluff. 1984. Rapid, reversible alterations in histone gene transcription and histone mRNA levels in mouse myeloma cells. *Mol. Cell. Biol.* 4:351-357.
 11. Graves, R. A., N. B. Pandey, N. Chodchoy, and W. F. Marzluff. 1987. Translation is required for regulation of histone mRNA degradation. *Cell* 48:615-626.
 12. Graves, R. A., S. E. Wellman, I.-M. Chiu, and W. F. Marzluff. 1985. Differential expression of two clusters of mouse histone genes. *J. Mol. Biol.* 183:179-194.
 13. Heintz, N., H. L. Sive, and R. G. Roeder. 1983. Regulation of human histone gene expression: kinetics of accumulation and changes in the rate of synthesis and in the half-lives of individual histone mRNAs during the HeLa cell cycle. *Mol. Cell. Biol.* 3:539-550.
 14. Hurt, M. M., N. Chodchoy, and W. F. Marzluff. 1989. The mouse histone H2a.2 gene from chromosome 3. *Nucleic Acids Res.* 17:8876-8876.
 15. Levine, B. J., N. Chodchoy, W. F. Marzluff, and A. I. Skultchi. 1987. Coupling of replication type histone mRNA levels to DNA synthesis requires the stem-loop sequence at the 3' end of the mRNA. *Proc. Natl. Acad. Sci. USA* 84:6189-6193.
 16. Levine, B. J., T.-J. Liu, W. F. Marzluff, and A. I. Skultchi. 1988. Differential expression of individual members of the histone multigene family due to sequences in the 5' and 3' regions of the genes. *Mol. Cell. Biol.* 8:1887-1895.
 17. Lobo, S. M., W. F. Marzluff, A. C. Seufert, W. L. Dean, G. A. Schultz, C. Simerly, and G. Schatten. 1988. Localization and expression of U1 RNA in early mouse embryo development. *Dev. Biol.* 127:349-361.
 18. Lüscher, B., and D. Schümperli. 1987. RNA 3' processing regulates histone mRNA levels in a mammalian cell mutant. A processing factor becomes limiting in G1-arrested cells. *EMBO J.* 6:1721-1726.
 19. Lüscher, B., C. Stauber, R. Schindler, and D. Schümperli. 1985. Faithful cell-cycle regulation of a recombinant mouse histone H4 gene is controlled by sequences in the 3'-terminal part of the gene. *Proc. Natl. Acad. Sci. USA* 82:4389-4393.
 20. Marzluff, W. F., D. T. Brown, S. Lobo, and S. S. Wang. 1983. Isolation and characterization of two linked mouse U1b small nuclear RNA genes. *Nucleic Acids Res.* 11:6255-6270.
 21. Moussa, N. M., A. S. E. L. Din, S. M. Lobo, and W. F. Marzluff. 1987. A mouse U1b-2 gene with extensive sequence similarity to a rat U1a gene for 670 nucleotides 5' to the gene. *Nucleic Acids Res.* 15:3622.
 22. Mowry, K. L., and J. A. Steitz. 1987. Both conserved signals on mammalian histone pre-mRNAs associate with small nuclear ribonucleoproteins during 3' end formation *in vitro*. *Mol. Cell. Biol.* 7:1663-1672.
 23. Mulligan, R. C., and P. Berg. 1981. Selection of animal cells that express the *Escherichia coli* gene coding for xanthine-guanosine phosphoribosyltransferase. *Proc. Natl. Acad. Sci. USA* 78:2072-2076.
 24. Pandey, N. B., and W. F. Marzluff. 1987. The stem-loop structure at the 3' end of histone mRNA is necessary and sufficient for regulation of histone mRNA stability. *Mol. Cell. Biol.* 7:4557-4559.
 25. Pilch, D. R., and W. F. Marzluff. Gene Expression, *in press*.
 26. Plumb, M., J. Stein, and G. S. Stein. 1983. Coordinate regulation of multiple histone mRNAs during the cell cycle in HeLa cells. *Nucleic Acids Res.* 11:2391-2410.
 27. Schneiderman, M. H. 1981. A rapid membrane filter technique for preparing tissue culture cells for scoring of mitotic and labeling indices. *Stain Technol.* 56:45-47.
 28. Schneiderman, M. H., W. C. Dewey, and D. P. Highfield. 1971. Inhibition of DNA synthesis in synchronous Chinese hamster cells treated in G1 with cycloheximide. *Exp. Cell Res.* 67:147-155.
 29. Schneiderman, M. H., W. C. Dewey, D. B. Leeper, and H. Nagasawa. 1972. Use of the mitotic selection procedure for cell cycle analysis. Comparison between the X-ray and cycloheximide G2 markers. *Exp. Cell Res.* 74:430-438.
 30. Schneiderman, M. H., and K. G. Hofer. 1980. The target for radiation-induced division delay. *Radiat. Res.* 84:462-476.
 31. Schneiderman, M. H., K. G. Hofer, and S. Schneiderman. 1988. Cell progression after selective irradiation of DNA during the cell cycle. *Radiat. Res.* 116:283-291.
 32. Sittman, D. B., R. A. Graves, and W. F. Marzluff. 1983. Histone mRNA concentrations are regulated at the level of transcription and mRNA degradation. *Proc. Natl. Acad. Sci. USA* 80:1849-1853.
 33. Stauber, C., B. Lüscher, R. Eckner, E. Lötscher, and D. Schümperli. 1986. A signal regulating mouse histone H4 mRNA levels in a mammalian cell cycle mutant and sequences controlling RNA 3' processing are both contained within the same 80-bp fragment. *EMBO J.* 5:3297-3303.
 34. Stauber, C., and D. Schümperli. 1988. 3' processing of pre-mRNA plays a major role in proliferation-dependent regulation of histone gene expression. *Nucleic Acids Res.* 16:9399-9413.
 35. Stimac, E., V. E. Groppi, Jr., and P. Coffino. 1984. Inhibition of protein synthesis stabilizes histone mRNA. *Mol. Cell. Biol.* 4:2082-2087.
 36. Terasima, T., and L. J. Tolmach. 1963. Variations in several responses of HeLa cells to X-irradiation during the division cycle. *Exp. Cell Res.* 3:344-351.
 37. Wellman, S. E., P. J. Casano, D. R. Pilch, W. F. Marzluff, and D. B. Sittman. 1987. Characterization of mouse H3.3-like histone genes. *Gene* 59:29-39.
 38. Wells, D., and L. Kedes. 1985. Structure of a human histone cDNA: evidence that basally expressed histone genes have intervening sequences and encode polyadenylated mRNAs. *Proc. Natl. Acad. Sci. USA* 82:2834-2838.
 39. Wu, R. S., and W. M. Bonner. 1981. Separation of basal histone synthesis from S phase histone synthesis in dividing cells. *Cell* 27:321-330.
 40. Zweidler, A. 1984. Core histone variants of the mouse: primary structure and expression, p. 373-395. *In* G. Stein, W. Stein, and W. Marzluff (ed.), *Histone genes: structure, organization and regulation*. John Wiley & Sons, New York.