Volume 11 Number 22 1983 Nucleic Acids Research

Influence of DNA synthesis inhibition on the coordinate expression of core human histone genes during S phase

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Received 18 July 1983; Revised and Accepted 20 October 1983

ABSTRACT

Core histone mRNA metabolism has been examined in S phase HeLa cells recovering from DNA synthesis inhibition by 1 mM hydroxyurea. Using cloned human histone genes as probes for histone mRNA quantitation, the response to and recovery from DNA synthesis inhibition is shown to depend on the position of the cell with respect to the initiation of DNA replication. The incorporation of ^{3}H-uridine into multiple histone mRNAs in recovering cells does not exceed preinhibition levels, and as this incorporation is maximal in early S phase, the synthesis of core histone mRNA is apparently related to the ordered replication of the genome. The total histone mRNA present in interrupted S phase cells after recovery is not significantly different from that present in control cells, and a temporal and functional coupling between histone mRNA levels and the relative rate of DNA synthesis is maintained in perturbed cells.

INTRODUCTION

In several continuously dividing cell lines, both the rate of histone protein synthesis (1-9) and the cellular abundance of core histone mRNA (10-13) increase and decrease in parallel to the rate of DNA synthesis during S phase. Approximately 90% of the total cellular histone protein is synthesized during S phase of the cell cycle, and is highly sensitive to DNA synthesis inhibition (6-9). The remaining 10% of the histone protein synthesis is characterized by its insensitivity to DNA synthesis inhibition and by the differential synthesis of variant histone proteins (7-9). S phase cellular histone mRNA levels are rapidly destabilized ($T_{1/2} \simeq 8$ min) after DNA synthesis inhibition by hydroxyurea (10-16), aphidicolin (11,16) or cytosine arabinoside (16-18), implying that histone protein synthesis in S phase is regulated in part by the availability of histone mRNA, although some exceptions have been documented (14,15).

The further analysis of human histone gene expression is complicated by the polymorphism observed in this middle repetitive family of genomic

sequences (19). At least nine distinct human histone gene clusters have been identified (20-22), but no obvious tandem repeat organization is apparent, and sequence divergence in both coding and noncoding regions has been reported (23). This genetic microheterogeneity gives rise to multiple histone mRNA subspecies (10,24). In the case of at least seven HeLa histone H4 mRNAs, these encode the same amino acid sequence despite significant nucleotide sequence differences (20,24). The analysis of over fifteen core histone mRNAs in synchronized HeLa cells revealed a coordinate regulation with respect to the rate of DNA synthesis (10). Although histone mRNA synthesis and turnover rates have not been directly measured in synchronized human cells, the kinetics of accumulation of histone mRNAs during S phase in HeLa cells suggests that both transcriptional and post-transcriptional mechanisms are operative (10,11). We have recently shown that the apparent rate of histone mRNA synthesis is maximal during early S phase and precedes the maximal accumulation of histone mRNA in mid-S phase (10).

The observed changes in the apparent rate of histone mRNA synthesis during the HeLa S phase prompted further investigation to distinguish whether this synthesis is determined by the rate of DNA synthesis, or whether it is periodic and thus related to the ordered progression through S phase after cells make the G1- to S-phase transition. Hydroxyurea, which rapidly inhibits DNA synthesis and causes the rapid turnover of S phase histone mRNAs (10,15,16) was used to interrupt HeLa cells in early or late S phase, and three metabolic parameters were monitored upon reversal of the inhibition: a) the relative rate of DNA synthesis; b) the accumulation of histone mRNA; and c) the incorporation of ^{3}H-uridine into multiple core histone mRNA subspecies.

The length of S phase is extended when DNA synthesis is inhibited and then allowed to recover. This extension corresponds to the inhibition interval in addition to a 1 hour recovery period, irrespective of whether the DNA synthesis inhibition occurred in early or late S phase. The cellular abundance of histone mRNAs was always proportional to the relative rate of DNA synthesis, and the total cellular histone mRNA present in control S phase cells and in S phase cells which have recovered from DNA synthesis inhibition remained relatively constant. The apparent rate of histone mRNA synthesis (^{3}H-uridine incorporation in a 1 hour labelling interval) in cells recovering from DNA synthesis inhibition rapidly approached but did not exceed preinhibition levels.

As cells recovering from DNA synthesis inhibition in late S phase do not synthesize histone mRNAs at the maximal rates observed in early S phase, but synthesize mRNAs at the lower preinhibition level noted in late S phase, we infer that the apparent rate of histone gene transcription is periodic and is quantitatively related to the ordered progression of cells through S phase, and not directly related to the rate of DNA synthesis. Furthermore, the coordinate regulation of over fifteen core histone mRNA subspecies (10) is apparently maintained despite the metabolic perturbations.

MATERIALS AND METHODS

Materials

Uridine [5,6-^{3}H] (40.5 Ci/mmol), [α-^{32}P]-dCTP (~3,000 Ci/mmol) and En^{3}Hance were purchased from New England Nuclear; [Methyl-^{14}C] thymidine (60 mCi/mmol) was from Amersham; X-ray films were from Eastman Kodak, Co.; hydroxyurea was from Sigma Chemical Co. and restriction endonucleases were from Bethesda Research Laboratories, Inc. or New England Biolabs.

Cell Culture, Synchronization and Pulse Labelling

HeLa S3 cells were grown in suspension culture and were synchronized by two cycles of 2 mM thymidine administration (25).

Relative rates of DNA synthesis were monitored by measuring the incorporation of ^{14}C-thymidine (0.1 µCi/ml, 5 x 10^{5} cells/ml) into acid-precipitable material in a 20 min pulse (10,26).

Total cellular RNA was radiolabelled *in vivo* by incubating cells (2.5 x 10^{6}/ml) in the presence of ^{3}H-uridine (0.1 mCi/ml) for 60 or 90 min at 37°C.

Northern Blot Analysis of Total Cellular RNA

Total cellular RNA was isolated (10), and 50 µg resolved electrophoretically in 1.5% (w/v) agarose, 6% (w/v) formaldehyde gels (10), using 6% (w/v) formaldehyde, 20 mM MOPS (pH 7.0), 5 mM sodium acetate, 1 mM EDTA, as an electrolyte (27). RNA was transferred to nitrocellulose filters, which were hybridized to [^{32}P]-labelled cloned human histone DNA, and washed as described (10). Filters were exposed to preflashed XAR5 or Cronex X-ray film at -70°C, and hybridization quantitated by liquid scintillation spectrometry (10,26). Quenching was corrected by external standardization.

Purification of Cloned Human Histone Gene Sequences

The isolation and characterization of the λCh4A human histone gene recombinants, and the construction of pBR322 subclones [pF0108A (H4), pFF435B (H2A + H2B), and pF0535 (H3)] has been detailed elsewhere (10,20,23). All recombinant DNA manipulations were carried out in accordance with the guidelines established by the National Institutes of Health.

Plasmid DNA was isolated by the sarkosyl lysis procedure (28) and purified further by Biogel A15M (30 x 1.5 cm) gel filtration. Plasmid DNA was either labelled with [^{32}P]-dCTP by nick translation as recommended by Maniatis et al. (29), or immobilized on nitrocellulose filters (50 µg of linearized plasmid DNA/Millipore GSWP010300 filter) as described (10).

Hybrid Selection of Total Cellular ^{3}H-Labelled RNA

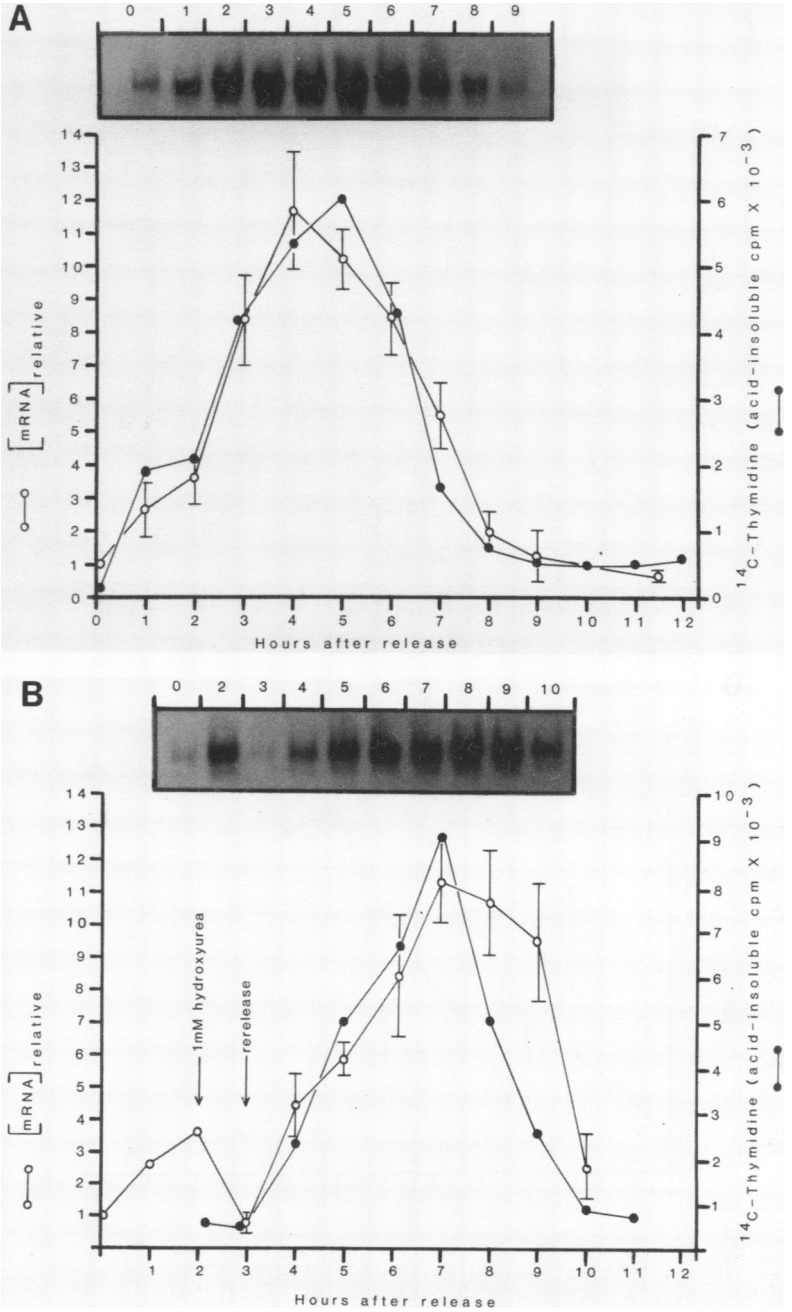
Total cellular RNA (150-300 µg) was hybridized to filter-immobilized plasmid DNA at 43°C or 45°C for 20 hr in 200 µl of a solution containing 50% (w/v) formamide, 0.5% (w/v) SDS, 0.5 M NaCl, 1 mM EDTA, 25 mM Hepes (pH 7.0). Filters were washed, eluted, and the hybridized RNAs resolved by denaturing 6% (w/v) polyacrylamide, 50% (w/v) urea gel electrophoresis as detailed by Plumb et al. (10).

Gels were soaked in En^{3}Hance for 1 hr, then in water for 1 hr, and dried for fluorography with preflashed XAR5 X-ray film at -70°C for 3-14 days. Quantitation was achieved by densitometry and by liquid scintillation spectrometry of gel slices in 10 ml of triton/toluene scintillation cocktail. Quenching was corrected by external standardization.

RESULTS

The Effects of DNA Synthesis Inhibition on Histone mRNA Levels in S Phase HeLa Cells

The kinetics of accumulation of core histone mRNAs during S phase in synchronized HeLa cells (10,11), and the incorporation of ^{3}H-uridine into multiple core histone mRNA subspecies in 1 hr labelling intervals during the cell cycle (10), has indicated that both transcriptional and post-transcriptional events mediate the temporal coupling observed between the rate of DNA synthesis and the intracellular concentration of histone mRNAs. As the maximal apparent rate of synthesis of multiple histone mRNAs in early S phase precedes maximal accumulation of total histone mRNA (10), it suggested that a cell's response to, and recovery


A
0 1 2 3 4 5 6 7 8 9
[mRNA]relative
14C-Thymidine (acid-insoluble cpm X 10-3)
Hours after release
B
0 2 3 4 5 6 7 8 9 10
1mM hydroxyurea
rerelease
[mRNA]relative
14C-Thymidine (acid-insoluble cpm X 10-3)
Hours after release

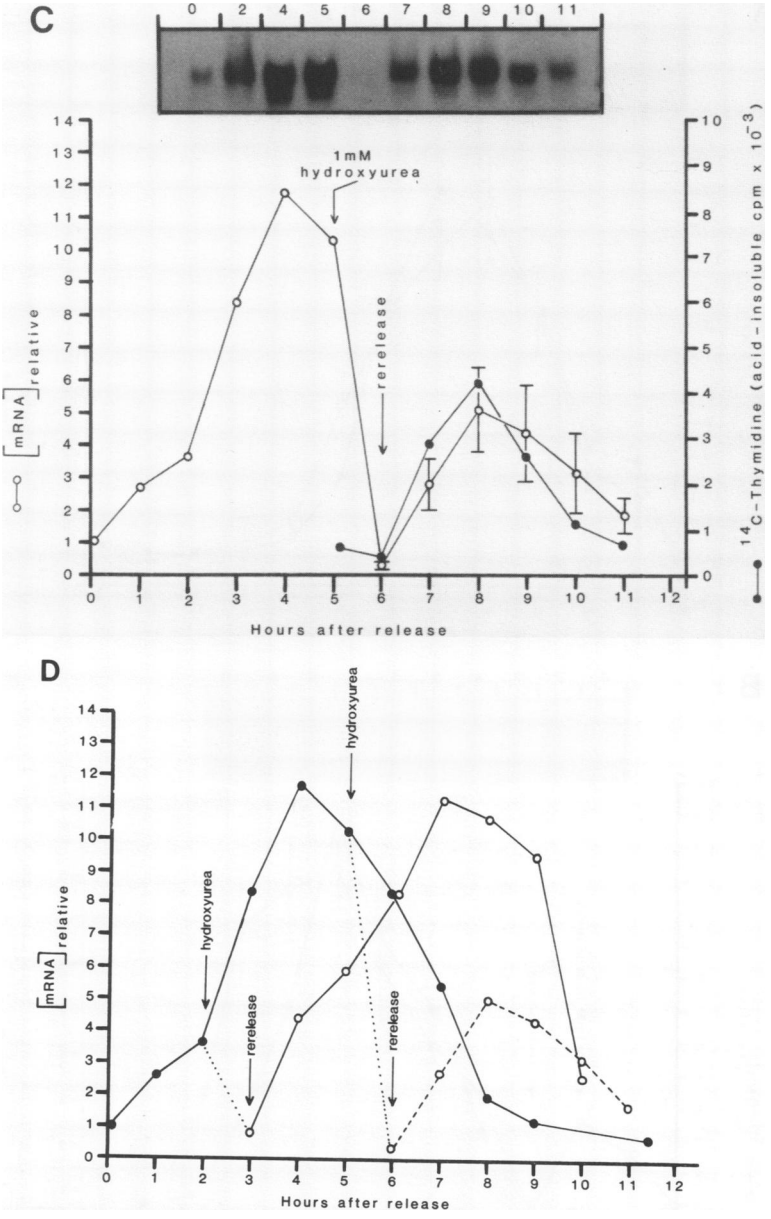


Figure 1: The kinetics of accumulation and turnover of core histone mRNAs in control and interrupted S phase cells. Total cellular RNA was isolated at the times indicated, and 50 µg samples were resolved electrophoretically and analyzed for specific core histone mRNAs by Northern blot hybridization using combinations of ^{32}P-labelled histone gene probes. Autoradiograms [probed with pF0108A (H4) and pFF435B (H2A+ H2B)]

are shown in each panel. Quantitation of hybridization is plotted (o—o) as mean values for at least 6 hybridizations (±S.E.M.) determined by liquid scintillation spectrometry. DNA synthesis rates (●—●) were monitored in parallel by measuring the incorporation of ^{14}C-thymidine (0.1 μCi/ml, 5 x 10^5 cells/ml) into 5% (w/v) trichloroacetic acid insoluble material in a 20 min pulse. A) Control cells progressing through S phase after double thymidine block synchronization. B) Cells in early S phase (2 hr after release from thymidine) were incubated in the presence of 1 mM hydroxyurea for 1 hr, and then released into fresh medium. C) Cells in late S phase (5 hr after release from thymidine) were incubated for 1 hr in the presence of 1 mM hydroxyurea and then released into fresh medium. D) Superimposition of the relative core histone mRNA levels found in control (●—●) and interrupted (o—o early S, and o--o late S) S phase HeLa cells.

from, DNA synthesis inhibition might depend on where in S phase the inhibitor was administered. Cells synchronized at the G1/S boundary were therefore released into S phase, and DNA replication was inhibited with 1 mM hydroxyurea in early or late S phase. Hydroxyurea was then removed and cellular histone mRNA levels were monitored in control and recovering cells by probing electrophoretically resolved, filter-immobilized total cellular RNA with combinations of ^{32}P-labelled cloned human core histone gene DNAs (10,20,23).

The temporal coupling between rates of DNA synthesis and histone mRNA levels in control S phase cells is shown in Fig. 1a, and confirms previous reports (10,11). Administration of 1 mM hydroxyurea to cells in early (2-3 hr into S phase, Fig. 1b) or late S phase (5-6 hr into S phase, Fig. 1c) rapidly inhibited DNA synthesis (over 90% inhibition within 10 min) and, after a 60 min incubation, reduced cellular histone mRNAs to an inhibitor-resistant level corresponding to 85% and 30%, respectively, of levels found in control cells at the G1/S boundary (22% and 2.5% of preinhibition levels respectively, see Fig. 1d). The different levels of inhibitor-insensitive histone mRNAs found in early and late S phase cells are highly reproducible, and are observed whether cells are inhibited with hydroxyurea for 30, 60 or 90 min (data not shown). Although this may reflect the different levels of DNA synthesis inhibition observed in early and late S phase cells treated with 1 mM hydroxyurea (see Fig. 1b-c), differences in the degree of DNA synthesis inhibition were not detectable by pulse-labelling with ^{14}C-thymidine.

The reversal of hydroxyurea inhibition is characterized by the recovery of DNA synthesis and an increase in cellular histone mRNA levels (Fig. 1b-c). The apparent rates of DNA synthesis in control (Fig. 1a)

and hydroxyurea treated cells (Fig. 1b-c), as measured by ^{14}C-thymidine incorporation, are not directly comparable as the pool effects incurred during synchronization with thymidine are reduced by hydroxyurea (data not shown). Nevertheless, the apparent rates of DNA synthesis are proportional to the histone mRNA abundance after release from DNA synthesis inhibition (Fig. 1b-c).

The length of the interrupted S phase is expanded by 1 hr (2 hr including the hydroxyurea treatment interval) as indicated by the decay of histone mRNA levels at hour 11 in treated cells to that level observed at hour 9 in control cells (Fig. 1d). This 1 hr recovery interval is emphasized by the observation that maximal histone mRNA levels in released cells reach those levels found in control cells 1-2 hr earlier. Furthermore, as summarized in Fig. 1d, the total amounts of histone mRNA present in control and perturbed S phase cells after recovery are not significantly different. This suggests that a relatively invariant amount of histone mRNA is required to complete S phase, and the abundance is related to the relative rate of DNA synthesis determined by the ordered progression of cells through S phase.

The Analysis of De Novo Synthesized Multiple Histone mRNAs

As cellular histone mRNA levels are regulated both with respect to the rate of DNA synthesis and temporally with respect to the G1/S boundary, it was of interest to determine the relative rate of histone mRNA synthesis in relation to these metabolic and temporal events. We therefore monitored the incorporation of ^{3}H-uridine into multiple histone mRNA subspecies over 1 hr labelling intervals in S phase HeLa cells during RNA synthesis inhibition and after reversal. This labelling interval does not measure transcription rates *per se*, but the relative incorporation of ^{3}H-uridine into mRNAs reflects, in part, ongoing transcription *in vivo*. Furthermore, this permits the analysis of the regulation of the heterogenous population of core histone gene transcripts, whereas much shorter labelling intervals reduce the specific activities of multiple mRNA subspecies to below the limits of detection by fluorography.

The coordinate regulation of over 15 histone mRNAs in S phase has been described (10), but it is well documented that variant histone proteins are differentially synthesized in the absence of DNA synthesis (7-9). We therefore examined the stability of prelabelled multiple histone mRNAs after a 30 min treatment with hydroxyurea. Synchronized

cells at the G1/S boundary (prerelease), in early S phase (1 hr into S phase), in late S phase (5 hr into S phase) or in G1 (10 hr after release from thymidine block) were labeled with ^{3}H-uridine for 1 hr, and then exposed to 1 mM hydroxyurea for 30 min. Total cellular RNA was hybridized to filter-immobilized cloned human histone DNA and the hybridized RNAs resolved as shown in Fig. 2a. Comparison of the detected mRNA subspecies in control and hydroxyurea treated cells at any one point in the cell cycle reveals that all the subspecies are equally sensitive to DNA synthesis inhibition. However, the total radiolabel present in the hydroxyurea treated mRNA subspecies is significantly higher in early S phase cells compared with late S/G1 phase cells (Fig. 2a).

The synthesis and stability of de novo synthesized histone mRNA subspecies in the presence of hydroxyurea was examined by pretreating synchronized cells for 30 min with 1 mM hydroxyurea, and then for a further 60 min with both hydroxyurea and ^{3}H-uridine. The hybrid-selected mRNAs shown in Fig. 2b indicate that although a low level of incorporation of ^{3}H-uridine does occur, the mRNAs are qualitatively similar to those in control samples, and the relative incorporation is not apparently cell cycle specific. These data suggest that the cell cycle dependent, hydroxyurea-resistant total cellular histone mRNA levels are due to changes in the stability of both total and de novo synthesized histone mRNAs, and this reflects both the post-transcriptional and transcriptional activities of the cell prior to and during hydroxyurea administration (Figs. 2a, 2b and 4a).

Changes in the rate of transcription of the histone genes during the cell cycle are implied by the observed changes in the incorporation of ^{3}H-uridine into histone mRNAs (10). As shown in Figs. 3a and 4a, the incorporation of ^{3}H-uridine into multiple histone mRNAs in 1 hr in vivo labelling intervals is highest in early S phase and precedes maximal total histone mRNA accumulation. In order to determine whether this in vivo incorporation was temporally related to the G1/S boundary, synchronized cells in early (2 hr into S phase) or late S phase (5 hr into S phase) were treated for 1 hr with 1 mM hydroxyurea, and then released from DNA synthesis inhibition. Cells were labelled with ^{3}H-uridine for 1 hr, every hour, and total cellular RNA preparations were hybrid selected for histone mRNAs which were then resolved as shown in Fig. 3. The results are quantitatively summarized in Fig. 4.

The maximal incorporation of ^{3}H-uridine into histone mRNAs also

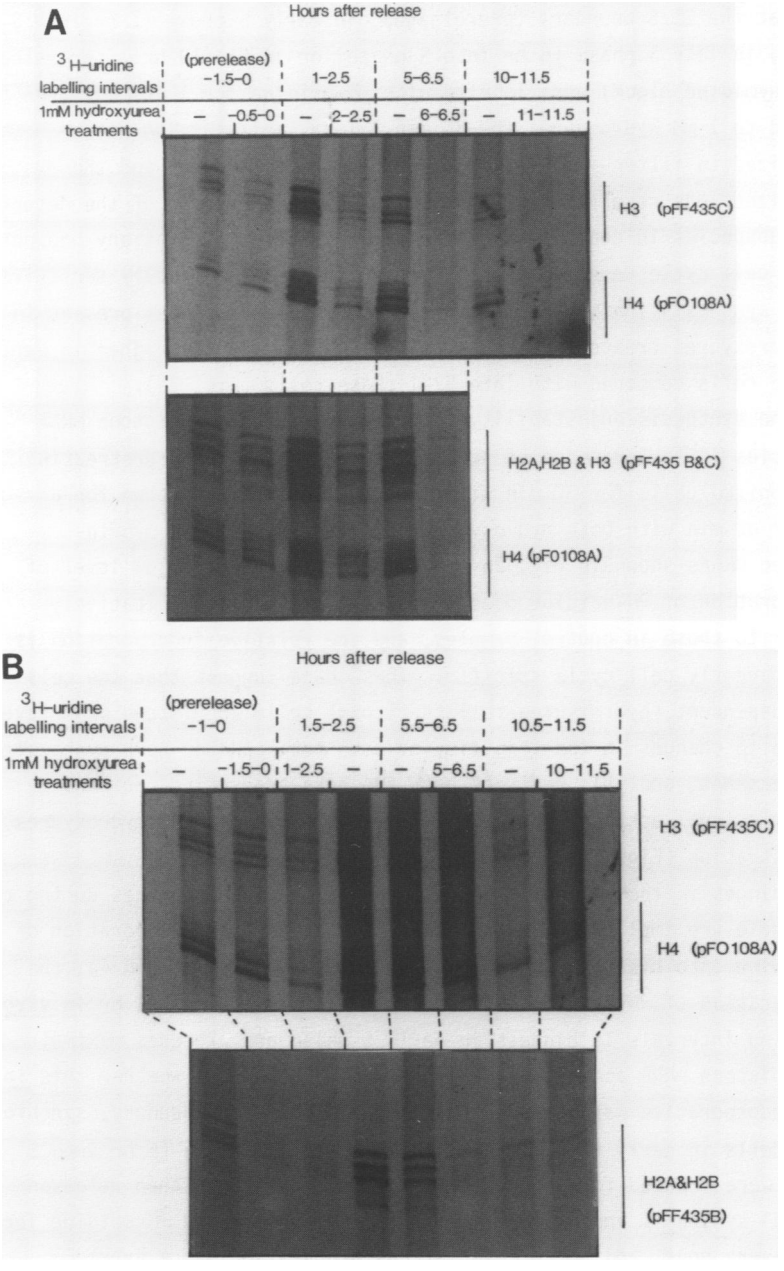
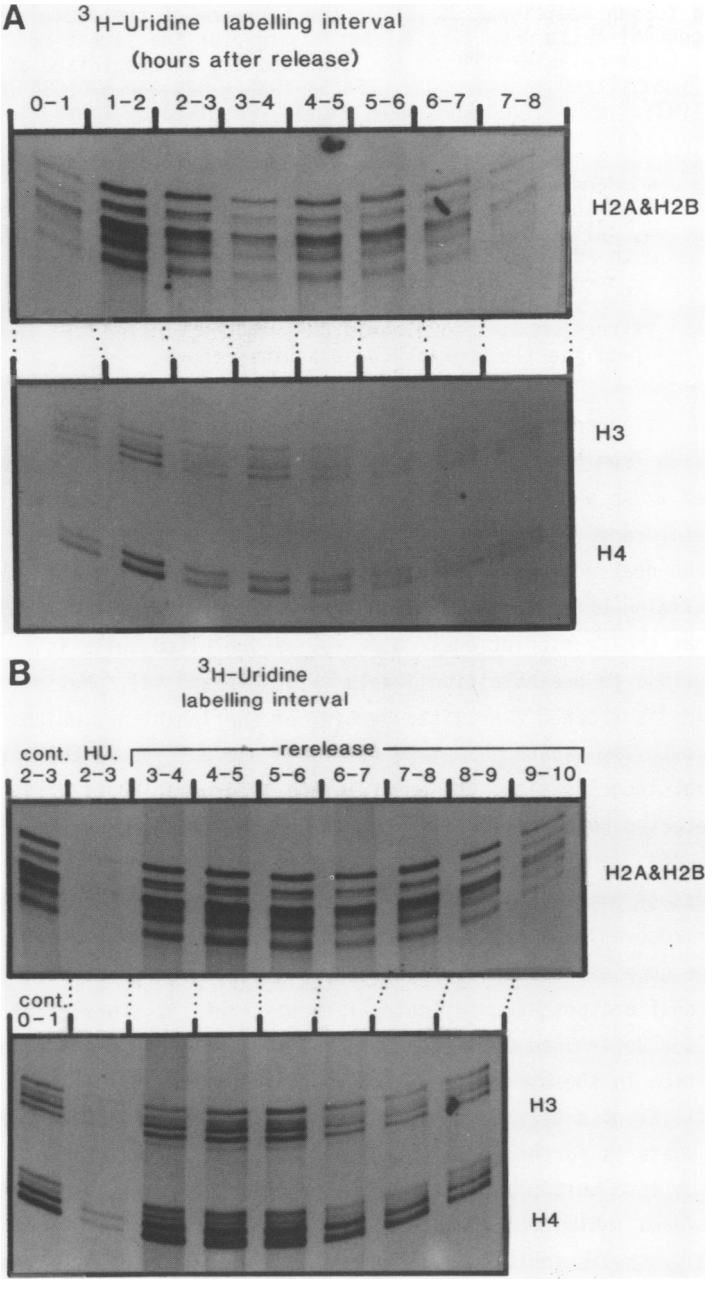


Figure 2: A) The effect of DNA synthesis inhibition on newly synthesized HeLa S phase multiple histone mRNAs. At -1.5 (prerelease), 1, 5 and 10 hr after release from double thymidine block synchronization, cells were labelled with ^{3}H-uridine for 1 hr and then exposed to 1 mM

hydroxyurea for an additional 30 min in the presence of the radiolabel. Untreated control cells were labeled for 90 min over the same time intervals. Total cellular RNA was isolated and 300 µg hybridized at 43°C to filter- immobilized plasmid DNA; pFF435B (H2A + H2B) or pF0108A (H4) and pF0535 (H3). Hybridized RNAs were eluted, resolved by denaturing 6% (w/v) polyacrylamide, 50% (w/v) urea gel electrophoresis and visualized by fluorography. B) The incorporation of ^{3}H-uridine into multiple HeLa S phase histone mRNAs after DNA synthesis inhibition. At -1.5 (prerelease), 1, 5 and 10 hr after release from double thymidine block synchronization, cells were treated with 1 mM hydroxyurea for 30 min, and then labelled with ^{3}H-uridine for an additional hour in the presence of the inhibitor. Untreated control cells were labelled for the last 60 min of the 90 min treatment intervals. Total cellular RNA (300 µg) was hybridized to filter-immobilized plasmid DNA, and hybridized RNAs were eluted and resolved for fluorography as described above.

precedes maximal accumulation of total histone mRNA in early S phase cells released from hydroxyurea administration (Figs. 2b and 4b), and all the detected mRNAs are similarly regulated (Fig. 3b). It is apparent that the total radiolabel incorporated into histone mRNAs rapidly recovers, but does not exceed preinhibition levels, thus indicating that the destabilizing effects of hydroxyurea are readily reversible. This suggests that the transcriptional potential of inhibited cells is maintained close to preinhibition levels (Figs. 4b and 4d). Maximal incorporation is reached 2 hr after removal of the inhibitor, which is consistent with the expanded S phase described above (Figs. 1 and 4). After maximal incorporation, the decay in the incorporation of radiolabel into the detected mRNAs, as cells progress through the interrupted S phase (Fig. 4b), is similar to that in control cells (Fig. 4d). As the total amounts of histone mRNA present in control and interrupted S phase cells after recovery are approximately the same (Fig. 1d), we conclude that histone gene expression is regulated by the modulation of both transcriptional and post-transcriptional events, but the transcriptional parameters are determined by the position of the cell in S phase despite an interruption in the ordered replication of the genome.

The ability of a cell to recover from, and compensate for an extended S phase is further illustrated by the levels of ^{3}H-uridine incorporation into multiple histone mRNA subspecies in cells recovering from hydroxyurea inhibition in late S phase (Figs. 3c and 4c). As in the case of DNA synthesis inhibition and reversal in early S phase, maximal incorporation into histone mRNAs occurs 2 hr after release from inhibition, the incorporation does not fully recover to preinhibition levels, and the subsequent decay in the histone mRNA specific activity is


A
^{3}H-Uridine labelling interval
(hours after release)
0-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
H2A&H2B
H3
H4
B
^{3}H-Uridine
labelling interval
cont.
2-3
HU.
2-3
rerelease
3-4
4-5
5-6
6-7
7-8
8-9
9-10
H2A&H2B
cont.
0-1
H3
H4

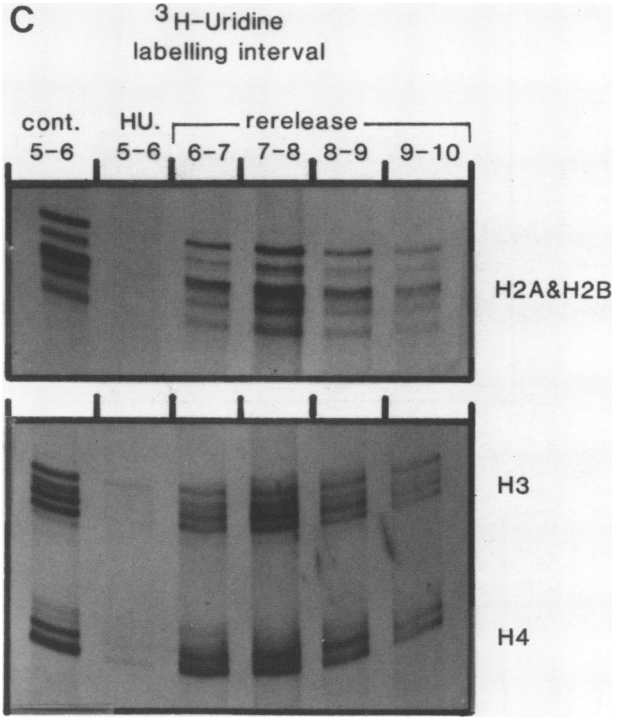
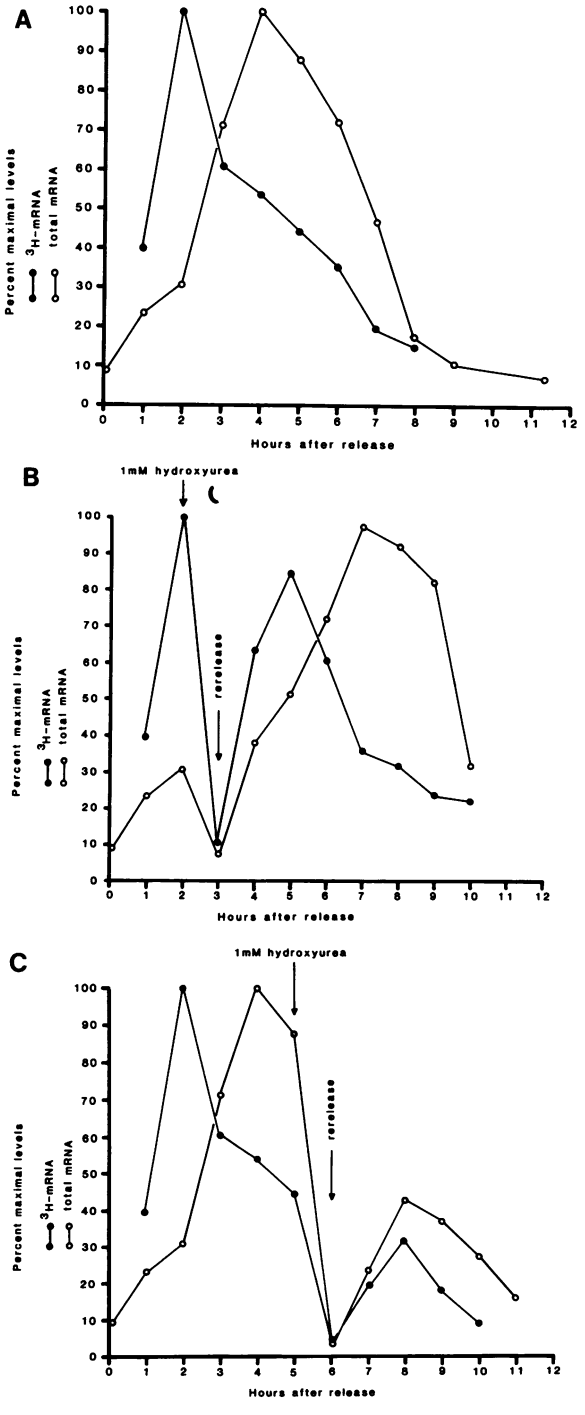


Figure 3: The incorporation of ^{3}H-uridine into multiple core histone mRNAs in control cells and S phase cells recovering from DNA synthesis inhibition. HeLa cells were synchronized by double thymidine blocks, and labelled with ^{3}H-uridine for 1 hr every hour for the intervals indicated. Cells in early (2 hr into S phase) or late (5 hr in to S phase) S phase were treated with 1 mM hydroxyurea for 1 hr, and then released into fresh medium. Total cellular ^{3}H-RNA (150 µg) was hybridized at 45°C to filter-immobilized plasmid DNA as described in methods [pFF435B (H2A + H2B) or pF0108A (H4) and pF0535 (H3)]. Eluted RNAs were resolved by denaturing polyacrylamide gel electrophoresis and visualized by fluorography. A) Control S phase mRNAs. B) mRNA from early S phase cells exposed to hydroxyurea (Hu) and then released. C) mRNAs from late S phase cells exposed to hydroxyurea and then released. Control S phase RNA (cont.) was hybridized and resolved in parallel in fluorographs B and C.

similar to that decay observed in control late S phase cells (Fig. 4d). However, maximal incorporation does not precede maximal histone mRNA accumulation (Fig. 4c). The apparent rate of histone mRNA synthesis appears to be temporally related to the initiation of DNA replication, but total histone mRNA levels remain stoichiometrically coupled to the rate of DNA synthesis (Fig. 4). We conclude that the histone mRNA levels


A
Percent maximal levels
^{3}H-mRNA
total mRNA
Hours after release
B
1mM hydroxyurea
rerelease
Percent maximal levels
^{3}H-mRNA
total mRNA
Hours after release
C
1mM hydroxyurea
rerelease
Percent maximal levels
^{3}H-mRNA
total mRNA
Hours after release

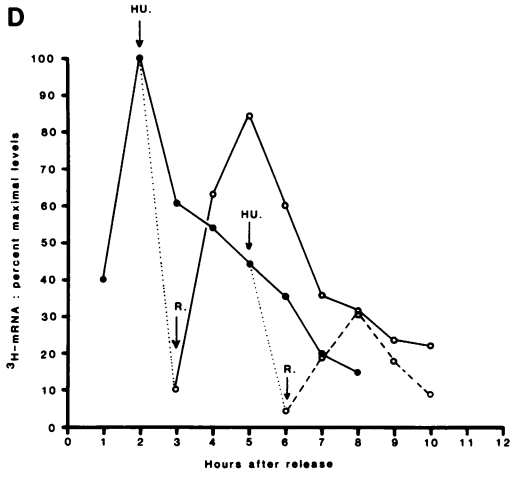


Figure 4: The kinetics of accumulation and turnover of total histone mRNA (o—o) (see Fig. 2), and the relative incorporation of ^{3}H-uridine into 1 hr pulse labelled multiple histone mRNAs (●—●), in control cells and S phase cells recovering from DNA synthesis inhibition. The relative ^{3}H-uridine incorporation into histone mRNAs was determined both by densitometry of fluorograms (see Fig. 4) and liquid scintillation spectrometry of gel slices. Data points are plotted at the end of each labelling interval and represent mean values for at least two hybridizations with filter-immobilized DNA (see Fig. 4). A) Control S phase histone mRNAs. B) Histone mRNAs from cells recovering from DNA synthesis inhibition in early S phase. C) Histone mRNAs from cells recovering from DNA synthesis inhibition in late S phase. D) Superimposition of the relative incorporation of ^{3}H-uridine into core histone mRNAs in control (●—●) and interrupted (o—o) early and (o--o) late S phase HeLa cells.

are coupled to the rate of DNA replication by a post-transcriptional mechanism(s) which can be modulated to compensate for changes in the rate of DNA synthesis in the presence of a rate of histone mRNA synthesis which is quantitatively determined by the cell's position in S phase.

DISCUSSION

Histone gene expression is predominantly coupled to the rate of DNA synthesis in S phase as indicated by the temporal relationship between the rate of DNA synthesis, the rate of histone protein synthesis (1-9) and the cellular abundance of histone mRNAs (10-13). Both transcriptional and post-transcriptional regulatory events have been suggested to modulate the availability of histone mRNA for translation (10-13,15,16).

Studies with yeast (13) and HeLa cells (10) suggest that histone gene transcription is periodic and occurs primarily in early S phase. This periodic synthesis has been examined to determine whether histone mRNA synthesis is related to the rate of DNA synthesis, or whether that synthesis is related to the progression of cells through S phase.

Although the rate of DNA synthesis and the ordered replication of the genome are indistinguishable in continuously dividing cells, the two may be uncoupled in a model system by inhibiting DNA synthesis in synchronized S phase HeLa cells, and then reversing the inhibition. By determining whether or not the apparent rate of histone mRNA synthesis in cells released from DNA synthesis inhibition recovers to levels which exceed preinhibition levels, the periodicity of histone mRNA synthesis may be examined. Synchronized early or late S phase HeLa cells were treated with 1 mM hydroxyurea for 1 hr, and the kinetics of mRNA accumulation and apparent rate of synthesis monitored after release from the inhibition.

The reversal of 1 hr DNA synthesis inhibition during S phase is characterized by the recovery of DNA synthesis and the accumulation of histone mRNA. Histone mRNA levels recover to that level which is found in control S phase cells 1-2 hr earlier, and the interrupted S phase is completed 2 hr after the completion of S phase in control cells. The 2 hr extension of the perturbed S phase includes the 1 hr inhibition interval, indicating that a 1 hr recovery period is required, whether the DNA synthesis inhibition occurred in early or late S phase. Heintz et al. (11) have shown that HeLa H2A mRNA recovers to preinhibition levels within 2-3 hr after release from a 3 hr DNA synthesis inhibition with aphidocolin in early S phase, results which are comparable to those found in cells recovering after a 1 hr inhibition with hydroxyurea (Fig. 1d). In conjunction with the observation that the total histone mRNA levels present in control or interrupted S phase cells are not significantly different, these data imply that the progression of cells through S phase requires histone mRNA in proportion to that DNA yet to be replicated at any given point in S phase, i.e., a temporal and functional relationship.

Although DNA synthesis inhibition induces rapid decay ($T_{1/2}$ = 8 min) (11,14-16) of S phase histone mRNAs, we have observed that the inhibitor-resistant mRNA levels are twice as high in early S phase as in late S phase (7% and 3% of maximal S phase levels respectively). There

is no significant quantitative or qualitative difference in the low level of ^{3}H-uridine incorporation into multiple histone mRNA species after early and late S/G1 cells are pretreated with hydroxyurea (Fig. 2b). On the other hand, the destabilization of de novo synthesized histone mRNAs by hydroxyurea revealed a significantly higher radiolabel content in early S phase, a reflection of the inhibitor-insensitive total histone mRNA levels (Fig. 2a). As the higher radiolabel content cannot be due to synthesis during inhibition (Fig. 2b), we conclude that the difference in inhibitor resistant mRNA levels in early and late S/G1 phase are due in part to changes in the stability of both total and de novo synthesized histone mRNAs during S phase. Histone protein variants are differentially synthesized in the absence of DNA synthesis (7-9), but we have been unable to detect the differential stability and/or synthesis of over fifteen multiple core histone mRNA subspecies labeled in 1 hr pulses. Although the differential translation of histone mRNA subspecies in the absence of DNA synthesis cannot be excluded, a low turnover rate of a second population of histone mRNA subspecies outside cell cycle regulation, and which contributes to the detected inhibitor-sensitive mRNA levels, is suggested. Alternatively, those mRNAs may not be sufficiently complementary to the available genomic histone gene probes for hybridization under the conditions used.

The incorporation of ^{3}H-uridine into multiple histone mRNA subspecies in cells recovering from DNA synthesis inhibition approaches 90% of preinhibition levels after a 1 hr lag, and is consistent with the observed 1 hr extension of S phase. The radiolabel incorporation decays thereafter is parallel with that observed in control S phase cells. As this occurs in cells inhibited and released in early or late S phase, these data suggest that the rate of histone mRNA synthesis is inversely proportional to the ordered replication of the genome, with maximal synthesis being induced upon entry into S phase. This apparent rate of synthesis is not exceeded if DNA synthesis is inhibited and then allowed to resume, and yet histone mRNA does accumulate even in late S phase when the apparent synthesis is 30-40% of maximal. We conclude that cells recover from inhibition and compensate for an extended S phase by modulating the turnover rate of de novo synthesized histone mRNAs. We propose, therefore, that the cellular abundance of histone mRNA in synchronized HeLa cells is regulated by modulating the stability of histone mRNA in the presence of a rate of synthesis which is related to the ordered

replication of the genome initiated at the G1/S boundary (30,31).

ACKNOWLEDGEMENTS

We thank Drs. A. Pardee and F. Marashi for their helpful suggestions. These studies were supported by grants from the National Science Foundation (PCM-80-18075 and PCM-81-18951) and the National Institutes of Health (GM-32010).

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