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Inhibition of DNA Replication Coordinately Reduces Cellular Levels of Core and H1 Histone mRNAs: Requirement for Protein Synthesis†

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ABSTRACT: Cellular levels of H1 and core histone mRNAs have been examined in exponentially growing HeLa S3 cells as a function of DNA synthesis inhibition under varying concentrations of three DNA synthesis inhibitors. Total cellular histone mRNAs were analyzed by Northern blot hybridization, and their relative abundance was shown to be stoichiometrically and temporally coupled to the rate of DNA synthesis. In the presence of cytosine arabinoside, hydroxyurea, or aphidicolin, a rapid, proportionate decrease of histone mRNA levels resulted in an apparent mRNA half-life of less than 10 min. Using inhibitors of transcription and translation, we show that transcription is not necessary for the coordinate decrease of histone mRNA levels that occurs when DNA synthesis is inhibited. When protein synthesis is inhibited by addition of cycloheximide, core and H1 histone mRNAs do not decrease in parallel with reduced rates of DNA synthesis but instead are stabilized and accumulate with time, thus uncoupling histone mRNA levels and DNA replication. These last observations suggest that protein synthesis, either of histones or of some unidentified regulatory molecules, is required for the stoichiometric turnover of H1 and core histone mRNAs coordinate with reduced rates of DNA synthesis.

Histone genes comprise a family of moderately reiterated sequences (Wilson & Melli, 1977; Kedes, 1979) whose products, histone proteins, play key roles in the structural and transcriptional properties of the eukaryotic genome (Isenberg, 1979; McGhee & Felsenfeld, 1980; Felsenfeld & McGhee, 1982; Weisbrod, 1982). It has been well established that histone proteins are required for packaging eukaryotic DNA into chromatin; hence, it is not surprising that histone genes are expressed predominantly during the S phase of the cell cycle. A temporal and functional coupling of histone gene expression and DNA replication in most eukaryotic cells is suggested by the synthesis of histone proteins (Spalding et al., 1966; Robbins & Borun, 1967; Stein & Borun, 1972; Wu & Bonner, 1981; Delegane & Lee, 1972; Marashi et al., 1982) and histone mRNAs (Borun et al., 1967, 1975; Jacobs-Lorena et al., 1973; G. Stein et al., 1975; J. Stein et al., 1975; Tarnowka et al., 1978; Parker & Fitschen, 1980; Hereford et al., 1981, 1982; Heintz et al., 1983; Plumb et al., 1983b; Sittman et al., 1983) in conjunction with DNA synthesis. Although evidence points to control of this relationship by both transcriptional (Borun et al., 1967, 1975; G. Stein et al., 1975; Hereford et al., 1981, 1982; Heintz et al., 1983; Plumb et al., 1983a; Sittman et al., 1983) and posttranscriptional (Butler & Mueller, 1973; Borun et al., 1975; Heintz et al., 1983; Plumb et al., 1983a; Plumb et al., 1983b; Sittman et al., 1983) processes, the mechanisms regulating coordinate DNA replication and histone gene expression remain largely unresolved.

One approach to studying the relationship between histone gene expression and DNA replication has involved the use of metabolic inhibitors to examine the requirements for specific biochemical events. Early studies, which used high concentrations of DNA synthesis inhibitors such as Ara-C,[1] HU, or aminopterin, showed that DNA synthesis, histone protein synthesis (Spalding et al., 1966; Robbins & Borun, 1967; Stein & Borun, 1972; Borun et al., 1975; Marashi et al., 1982), histone mRNA levels (Breindl & Gallwitz, 1973, 1974a,b; Borun et al., 1975; Gallwitz, 1975; G. Stein et al., 1975; Stahl & Gallwitz, 1977; Stein et al., 1977a; Shephard et al., 1982), and radiolabeled precursor incorporation into histone mRNA (Spalding et al., 1966; Borun et al., 1967, 1975; Craig et al., 1971; Adesnik & Darnell, 1972; Schochetman & Perry, 1972; Butler & Mueller, 1973; Jacobs-Lorena et al., 1973; Perry & Kelley, 1973) were all diminished. However, these early studies lacked a direct assay for cellular levels of specific histone mRNAs and did not address the stoichiometry of the relationship between DNA replication and histone gene expression. The availability of cloned human histone genes (Heintz et al., 1981; Sierra et al., 1982) and the development of sensitive methods for detection of mRNAs have permitted us to examine systematically the nature of this relationship.

In this study, we have used a wide range of concentrations of three DNA synthesis inhibitors, Ara-C, HU, or aphidicolin, to examine the relationship between DNA replication and histone gene expression in HeLa S3 cells. Regardless of the

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[1] Abbreviations: Ara-C, cytosine arabinoside; HU, hydroxyurea; Act-D, actinomycin D; TCA, trichloroacetic acid.

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inhibitor or the concentration tested, the steady-state levels of core and H1 histone mRNAs and the rate of DNA synthesis were temporally and stoichiometrically coupled. Additionally, data are presented that suggest that protein synthesis is necessary to mediate the stoichiometric turnover of core and H1 histone mRNAs, coordinately with the decreased rate of DNA synthesis.

Materials and Methods

Materials. [5,6-^{3}H]Uridine (40.5 Ci/mmol), [*methyl*-^{3}H]thymidine (20.0 Ci/mmol), L-[*methyl*-^{3}H]leucine (60 Ci/mmol), and [α-^{32}P]dCTP (~3000 Ci/mmol) were purchased from New England Nuclear; X-ray films were from Eastman Kodak Co; proteinase K was obtained from E. Merck; HU was from Sigma Chemical Co. Ara-C and cycloheximide (Acti-dione) were kindly provided by Upjohn Co., and aphidicolin was provided by Imperial Chemical Industries. Act-D was obtained from either Sigma Chemical Co. or the National Cancer Institute.

Cell Culture. HeLa S3 cells were grown in suspension culture at 37 °C in Joklik-modified minimum essential medium (GIBCO) supplemented with 7% calf serum. Cells were maintained in exponential growth at (2.5–5.0) × 10^5 cells/mL and monitored daily by packed cell volume and/or use of a hemocytometer.

Assessment of DNA, RNA, and Protein Synthesis. Relative rates of DNA, RNA, or protein synthesis, in the presence or absence of metabolic inhibitors, were monitored by incorporation of [^{3}H]thymidine (1 μCi/mL), [^{3}H]uridine (2 μCi/mL), or [^{3}H]leucine (1 μCi/mL), respectively, into TCA-precipitable material [10% (w/v) TCA for RNA and DNA, 20% for protein]. Labeling was initiated at various times following the addition of the inhibitors to 2 mL (5 × 10^5 cells/mL) of exponentially growing HeLa cells and continued for 20 min at 37 °C. TCA-precipitable material was collected on 0.45-μm nitrocellulose filters (Millipore type HA) and washed 4 times with 2 mL of cold 10% TCA. The filters were dissolved in 1 mL of ethylene glycol monoethyl ether (Cellusolve), and radioactivity was measured by liquid scintillation spectrometry in 10 mL of scintillation cocktail—72% (v/v) toluene, 24% (v/v) cellusolve, and 4.2% (v/v) Liquiflor (from Research Products International). Each assay was performed in duplicate.

Stock solutions of Ara-C, HU, Act-D, and cycloheximide were prepared in Spinner Salts (GIBCO), and aphidicolin was dissolved in dimethyl sulfoxide. All samples containing aphidicolin were adjusted to final concentrations of 0.5% (v/v) dimethyl sulfoxide.

Human Histone Gene Probes. The isolation and characterization of the λCh4A recombinant phages containing human histone genes have been described (Sierra et al., 1982). Genomic restriction fragments were subcloned into pBR322 (Stein et al., 1984; Carozzi et al., 1984) and contained the following human histone genes: H1 (pFN C16), H2A + H2B (pFF435B or pFF435D), H3 (pFF435C), and H4 (pF0108A). Plasmid DNA was isolated as described (Clewell & Helenski, 1970). ^{32}P-Labeled probes [(0.8–1.6) × 10^8 dpm/μg] were obtained by "nick translation" (Maniatis et al., 1975) of appropriate plasmid DNAs with [α-^{32}P]dCTP.

Identification of HeLa Histone mRNAs. HeLa total cellular RNA was isolated (Plumb et al., 1983a), electrophoresed under denaturing conditions in 1.5% (w/v) agarose–6% (w/v) formaldehyde gels (Rave et al., 1979), and then transferred to nitrocellulose membranes (Thomas, 1980). For detection of core histone mRNAs, the nitrocellulose blot was prehybridized at 50 °C for 6 h in 20 mL of 50% (v/v) formamide–5X SSC buffer (1X SSC = 0.15M NaCl–0.015 M sodium citrate, pH 7.2)–5X Denhardt solution [1X Denhardt = 0.02% (w/v) polyvinyl pyrollidone–0.02% (w/v) Ficoll 400]–1% (w/v) glycine–100 μg/mL of *Escherichia coli* total nucleic acids. Hybridization with a ^{32}P-labeled histone gene probe was performed in prehybridization buffer without glycine for 40 h at 47 °C. For detection of H1 histone, mRNA prehybridization and hybridization were carried out at 42 °C. After hybridization, filters were washed once in 250 mL of each of the following: 5X SSC buffer–1X Denhardt solution for 20 min at room temperature, 5X SSC buffer–1X Denhardt solution for 35 min at 60 °C, 2X SSC buffer–0.1% (w/v) sodium dodecyl sulfate for 35 min at 60 °C, and 1X SSC buffer–0.1% (w/v) sodium dodecyl sulfate for 35 min at 60 °C. Filters were briefly air dried on Whatman 3MM paper and exposed to preflashed XAR-5 film under Kodak X-OMAT regular or Du Pont Lightning-Plus intensifying screens at –70 °C. Hybridization was quantitated by both densitometric analysis of autoradiograms and liquid scintillation spectrometry of the RNA blots.

Results

Effects of DNA Synthesis Inhibition on Core Histone mRNA Levels. The correlation between core histone mRNA steady-state levels and the rate of DNA synthesis in exponentially growing HeLa S3 cells was examined after a 60-min treatment of HeLa cells with various concentrations of the DNA synthesis inhibitors Ara-C (7 nM to 16 μM), HU (1 μM to 1 mM), or aphidicolin (0.001–2 μg/mL). Total cellular RNA was isolated, fractionated electrophoretically, and transferred to nitrocellulose; core histone mRNAs were assayed by hybridization to ^{32}P-labeled human histone gene clones. In parallel cultures, DNA synthesis was monitored as described under Materials and Methods. Although the three DNA synthesis inhibitors have different mechanisms of action, a time-course analysis revealed that maximal inhibition of DNA synthesis and the reduction of core histone mRNA levels occurred within 30 min of exposure, regardless of the inhibitor or concentration used (data not shown).

The data in Figure 1 represent the results of three independent experiments in which HU was used to inhibit DNA synthesis. Upon treatment with increasing concentrations of HU, the cellular levels of mRNAs for each of the core histones decrease, with little variation among the different mRNAs (see Figure 1A). The levels of each of the individual core histone mRNAs were averaged and are shown in Figure 1B, along with the rate of DNA synthesis at each concentration of HU. The rate of DNA synthesis and the steady-state levels of core histone mRNA are closely coupled, ranging from approximately 3% of control levels at 1 mM HU to 90% of control at 1 μM HU, with 50% inhibition occurring at approximately 50 μM HU. An "inhibitor-insensitive" population of core histone mRNA exists in the presence of 1 mM HU and is estimated to be less than 10% of the level in untreated exponentially growing cells.

The results of a similar analysis carried out with varying concentrations of Ara-C are shown in Figure 2, which represents a quantitative summary from three independent experiments for all core histone mRNAs. Analysis of individual core histone mRNA levels suggests that these RNAs are coordinately controlled in the presence of this inhibitor (data not shown). Any statistical variation reflects the range of values obtained from independent experiments and not differences in the levels of individual histone mRNA species. There is a stoichiometric decrease in both core histone mRNA levels and the rate of DNA synthesis with increasing concentrations

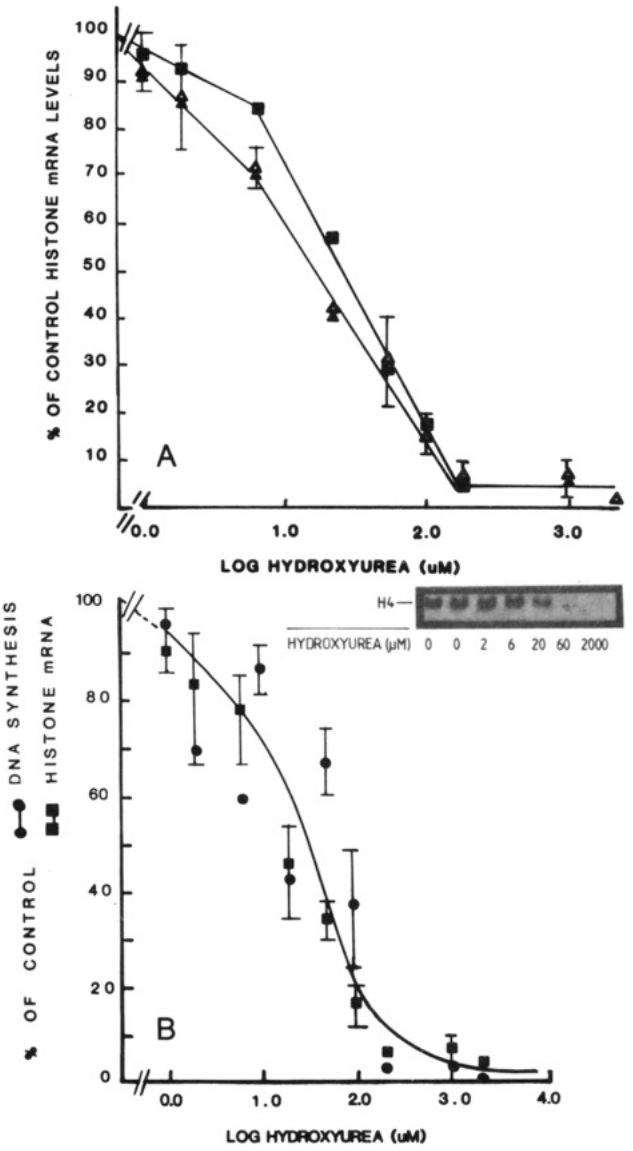

FIGURE 1: Effect of hydroxyurea on HeLa core histone mRNA levels. Total cellular RNA was isolated from cells exposed to the hydroxyurea concentrations indicated, and 50 μg/sample was analyzed by Northern blot hybridization with the following probes: H2A + H2B (pFF435B), H3 (pFF435C), and H4 (pF0108A). Hybridization was quantitated as described under Materials and Methods. (A) Analysis of individual core histone mRNA levels in the presence of HU: H2A + H2B (■), H3 (▲), and H4 (△). Brackets indicate the standard deviations from three independent experiments. (B) Effects of hydroxyurea on core histone mRNA levels (■) and the rate of DNA synthesis (●). At the indicated HU concentrations, the levels of individual core histone mRNAs were analyzed and averaged. The brackets indicate standard deviations calculated from five independent experiments. A representative autoradiogram is presented showing H4 histone mRNA levels in the presence of 2–2000 μM HU.

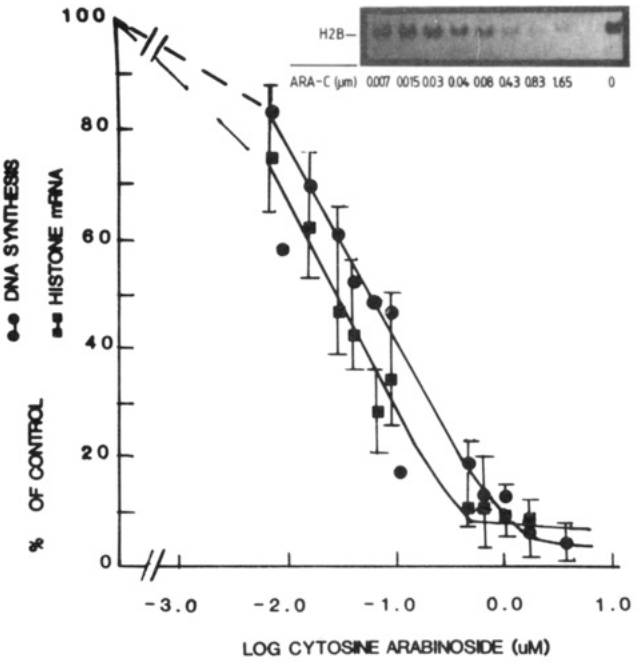

FIGURE 2: Effect of cytosine arabinoside on the cellular abundance of core histone mRNAs. Total cellular RNA was isolated and analyzed for histone mRNA levels by Northern blot hybridizations to ^{32}P-labeled histone gene probes. A representative autoradiogram depicts the decrease in histone H2B mRNA levels in cells treated with increasing concentrations of cytosine arabinoside. The graph, a summary of three independent experiments, represents the mean relative abundance of histone H4, H3, H2A, and H2B mRNAs (■) and the DNA synthesis rates (●), monitored as described under Materials and Methods.

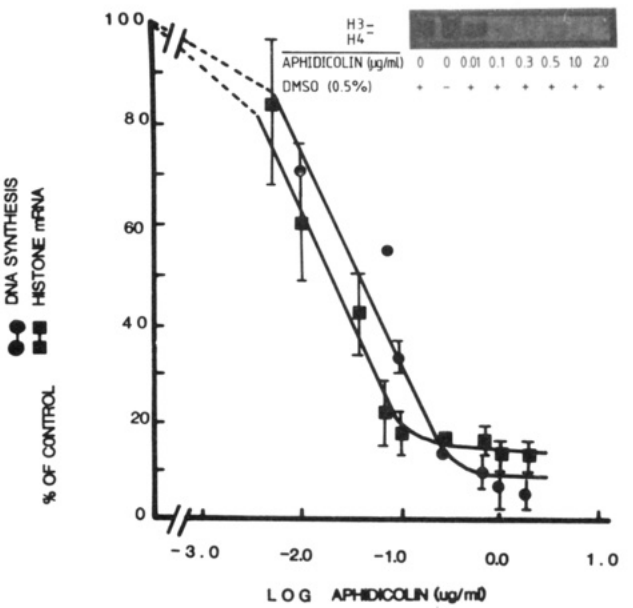

FIGURE 3: Effect of aphidicolin on HeLa core histone mRNA levels. The cellular abundance of core histone mRNAs was assessed with respect to treatment with increasing concentrations (0.001–2.0 μg/mL) of aphidicolin. A representative autoradiogram shows the coordinate decrease of H3 and H4 mRNA levels in the presence of increasing aphidicolin concentrations, as detected by hybridization to ^{32}P-labeled H3 (pFF435C) and H4 (pF0108A) probes. The graph summarizes the results from three independent experiments and shows the mean relative abundance of H2A, H2B, H3, and H4 mRNAs (■) and the average rates of DNA synthesis (●) at varying concentrations of aphidicolin.

of Ara-C (Figure 2). Histone mRNA levels were 90% of control at 7 nM Ara-C and decreased concomitantly with the rate of DNA synthesis to less than 10% of control at 1.6 μM, with 50% levels being reached at 0.03 μM. The inhibitor-insensitive population of core histone mRNAs, estimated to be 8–10% of levels in untreated cells, was detected in the presence of concentrations of Ara-C greater than 1.6 μM.

The results obtained with aphidicolin (0.001–2 μg/mL) to inhibit DNA synthesis are shown in Figure 3, which summarizes the levels of core histone mRNAs and the rates of DNA synthesis from several independent experiments. As observed with HU and Ara-C, there is a stoichiometric decrease in core histone mRNA levels and the rate of DNA synthesis upon treatment with aphidicolin. Although maximal inhibition of the rate of DNA synthesis (>95%) occurs at 1.0 μg/mL aphidicolin, there still exists an inhibitor-insensitive

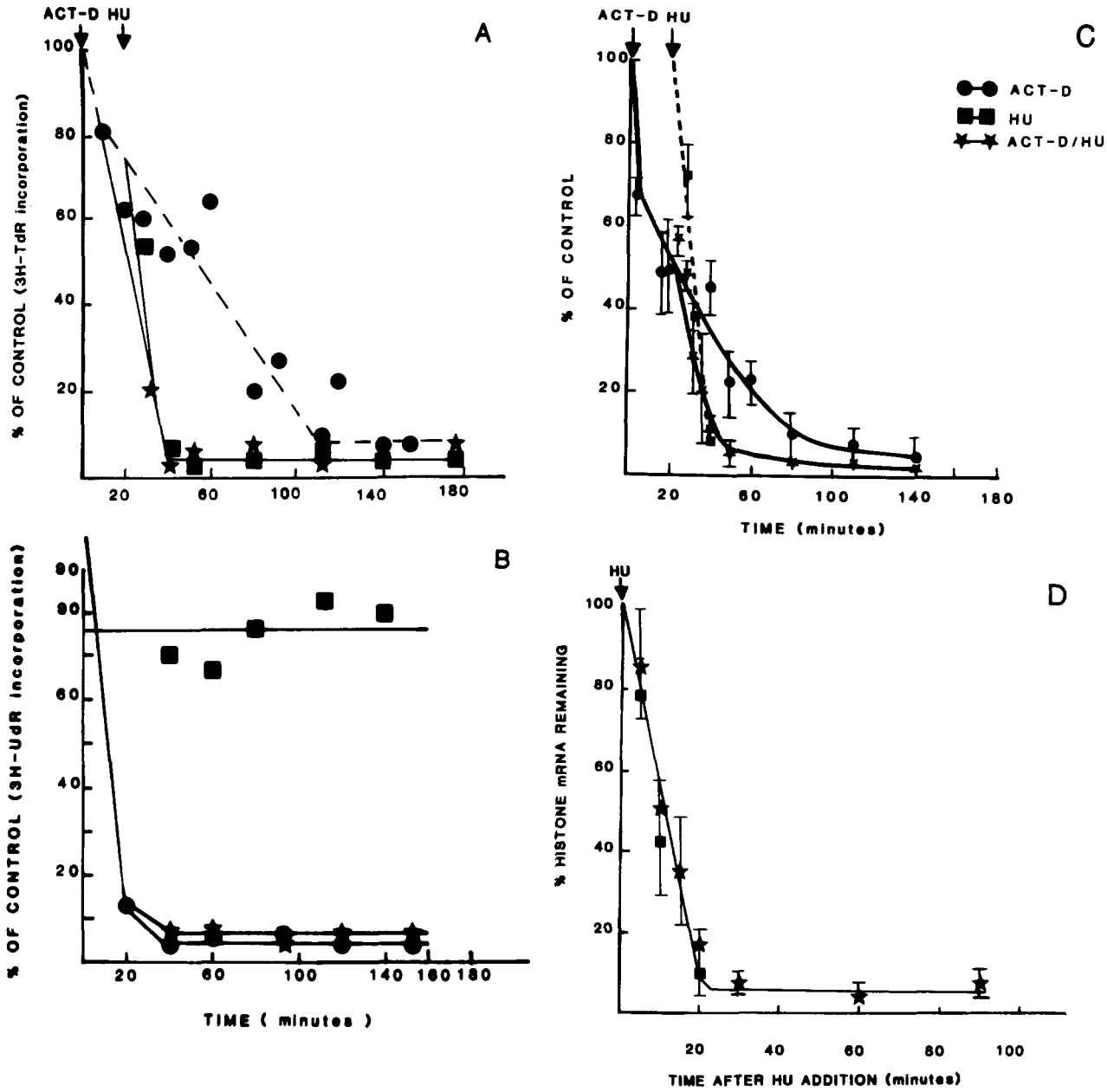

FIGURE 4: Analysis of the relationship between core histone mRNA levels and the rate of DNA synthesis as a function of Act-D concentration. (A and B) The relative rates of DNA synthesis (A) and of RNA synthesis (B) in the presence of Act-D (●), HU (■), or Act-D followed by HU treatment (solid star). (C) Steady-state levels of core histone mRNAs in the presence of Act-D (●), HU (■), or Act-D followed by HU (solid star). The data are plotted as a relative percentage of levels in untreated, exponentially growing cells. (D) Core histone mRNA levels in the presence of HU alone (■) or Act-D/HU (solid star) after addition of HU to cells. Core histone mRNA levels were analyzed as described under Materials and Methods, and the percent of histone mRNA remaining was calculated relative to mRNA levels after 3 min in the presence of HU.

level of core histone mRNA, which is approximately 15% of control levels in exponentially growing HeLa cells. This inhibitor-insensitive level is slightly higher in aphidicolin-treated cells than in cells treated with HU or Ara-C, but the significance of this observation remains to be resolved.

Effect of Inhibition of Transcription on Core Histone mRNA Levels. To determine the involvement of transcription in coupling of histone mRNA steady-state levels to the rate of DNA replication, exponentially growing HeLa cells were exposed to Act-D (5 μg/mL), and DNA synthesis, RNA synthesis, and the relative abundance of each of the core histone mRNAs were measured. In parallel cultures, HU was added 20 min after introduction of Act-D; other cultures contained HU alone. The results of two independent experiments are summarized in Figure 4. In cells treated with Act-D alone, transcription was inhibited to 10% of control levels by 20 min, while DNA synthesis decayed approximately linearly over a 2-h period. However, the levels of core histone mRNA, after an initial more precipitous decrease, paralleled the decrease in the rate of DNA synthesis. Each of the core histone mRNAs appears to be coordinately controlled in the presence of Act-D (not shown).

When RNA synthesis was inhibited by Act-D prior to inhibition of DNA synthesis with 1 mM Hu (Figure 4A–C), the rate of DNA synthesis decreased rapidly to less than 5% of control within 20 min of HU addition, with a parallel decrease in core histone mRNA levels. The rate and extent of decrease of histone mRNA levels and DNA synthesis are indistinguishable from those observed with HU alone. Although HU alone had little effect on net transcription, which remained at approximately 80% of the control for a period of 2 h after inhibition of DNA synthesis, the half-life of core histone

mRNAs was estimated to be between 5 and 10 min whether or not RNA synthesis had previously been blocked (Figure 4D). These results suggest that transcription is not required to initiate the coordinate turnover of cellular histone mRNAs that accompanies inhibition of DNA replication and imply that posttranscriptional regulation is operative.

Requirement of Protein Synthesis for Core Histone mRNA Turnover. Possible posttranscriptional controls governing histone gene expression in relation to DNA synthesis were studied by examining the effect of protein synthesis inhibition on the coupling of cellular histone mRNA levels and the rate of DNA replication. Early studies had indicated that when protein synthesis was blocked, DNA synthesis rapidly decreased to 20% of control levels (Butler & Mueller, 1973; Gallwitz, 1975), while polysomal histone mRNA levels did not decrease in parallel (Butler & Mueller, 1973; Breindl & Gallwitz, 1974b; Gallwitz, 1975). These results suggested that DNA replication and polysomal histone mRNA levels were uncoupled in the absence of protein synthesis. Using cloned human histone genes as hybridization probes, we have directly quantitated cellular levels of core histone mRNAs in exponentially growing HeLa cells after treatment with cycloheximide (10 μg/mL). In parallel cultures, HU (1 mM) was added after 5 min of cycloheximide treatment, and the rates of DNA and protein synthesis and core histone mRNA levels were assayed (Figure 5).

In the presence of cycloheximide alone, net DNA synthesis and protein synthesis decrease rapidly to approximately 20% of control levels by 20 min (Figure 5A,B). The remaining 15–20% incorporation of [^{3}H]leucine is at least in part attributable to charged tRNAs precipitated with the radiolabeled proteins by cold 20% TCA. Cycloheximide treatment of intact cells resulted in stabilization and accumulation of core histone mRNAs, ranging from 100% of control levels at 5 min to approximately 200% at 90 min (Figure 5C). Inhibition of protein synthesis 5 min prior to blocking DNA synthesis with HU completely prevents the decrease in histone mRNA levels observed in the presence of HU alone (Figure 5C). Instead, there is an accumulation of histone mRNA ranging from 100% of control at 5 min to approximately 120% at 90 min. All of the core histone mRNAs are similarly affected by these inhibitors. It appears that in the presence of cycloheximide core histone mRNA levels and the rate of DNA replication are uncoupled, suggesting that protein synthesis is necessary for maintenance of the normal interrelationship between histone gene expression and DNA synthesis.

Coupling of H1 Histone mRNA Levels and DNA Synthesis. Recently, Carozzi et al. (1984) isolated a λCH4A recombinant clone containing a human H1 histone gene. We have used a subclone containing this H1 histone gene (pFNC16) to study H1 gene expression in log-phase HeLa cells incubated with various metabolic inhibitors (Figure 6). Figure 6A shows the coordinate regulation of H1 and core histone mRNAs in the presence of Act-D and HU. The rate of DNA synthesis (see Figure 4A) and the levels of core (Figure 4C) and H1 mRNAs decrease in parallel (Figures 4A and 6A). A small molecular weight RNA species (~7 S in size) also hybridizes to sequences present in pFNC16 (Figure 6A). The cellular levels of this 7S RNA do not change as a function of progression through the cell cycle or after exposure to 1 mM hydroxyurea (Plumb et al., 1983a). Figure 6B indicates that in the presence of cycloheximide H1 histone mRNA levels are stabilized and accumulate with time, in parallel with the increase in core histone mRNA levels, although the level of H1 mRNA accumulation may be somewhat lower. H1 and core histone

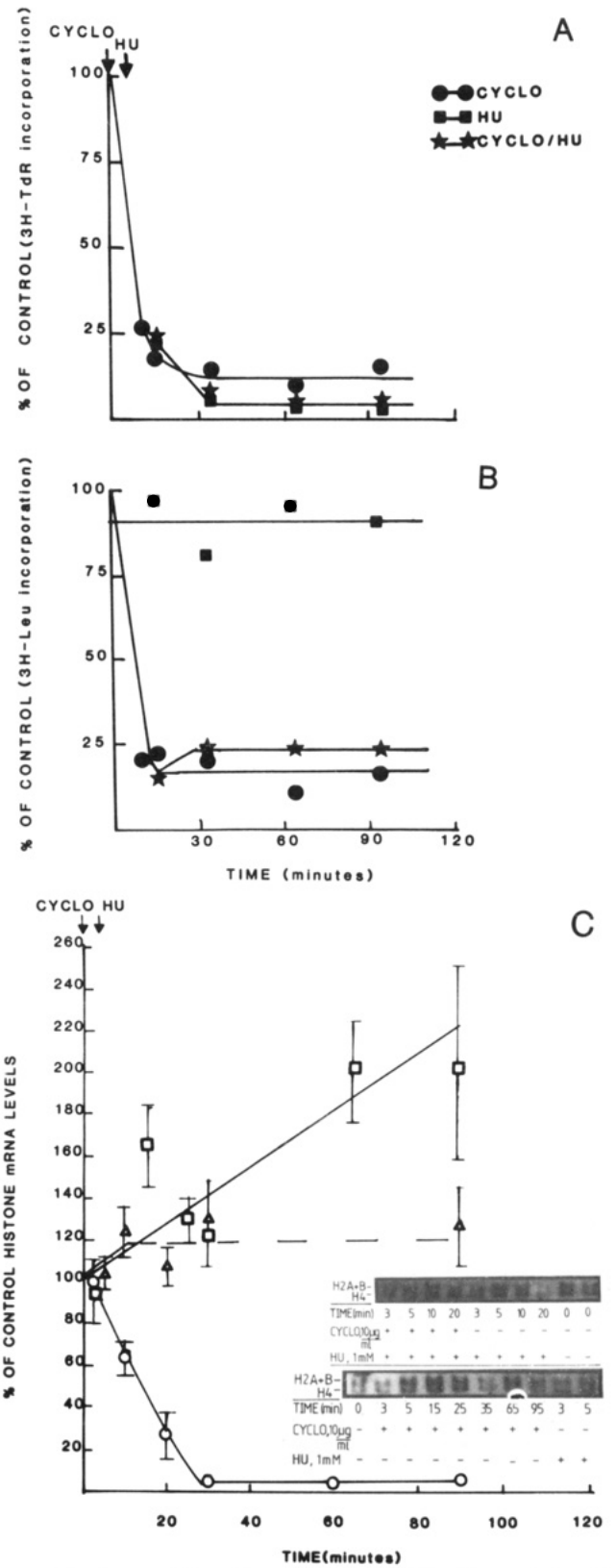

FIGURE 5: Accumulation of core histone mRNAs when protein and DNA synthesis are inhibited. (A and B) The relative rates of DNA (A) and protein (B) synthesis in the presence of cycloheximide (●), HU (■), or cycloheximide followed by addition of HU (solid star). The data are presented as percentages of values obtained with untreated cells. (C) The accumulation of core histone mRNAs in the presence of cycloheximide (□) or cycloheximide/HU (△) as a function of the length of exposure to the inhibitors, as well as the decrease in mRNA levels in the presence of HU (○). Core histone mRNA levels were analyzed and plotted as percentages of values obtained with untreated cells. The autoradiogram is from a representative Northern blot hybridized to pFF435B and pF0108A.

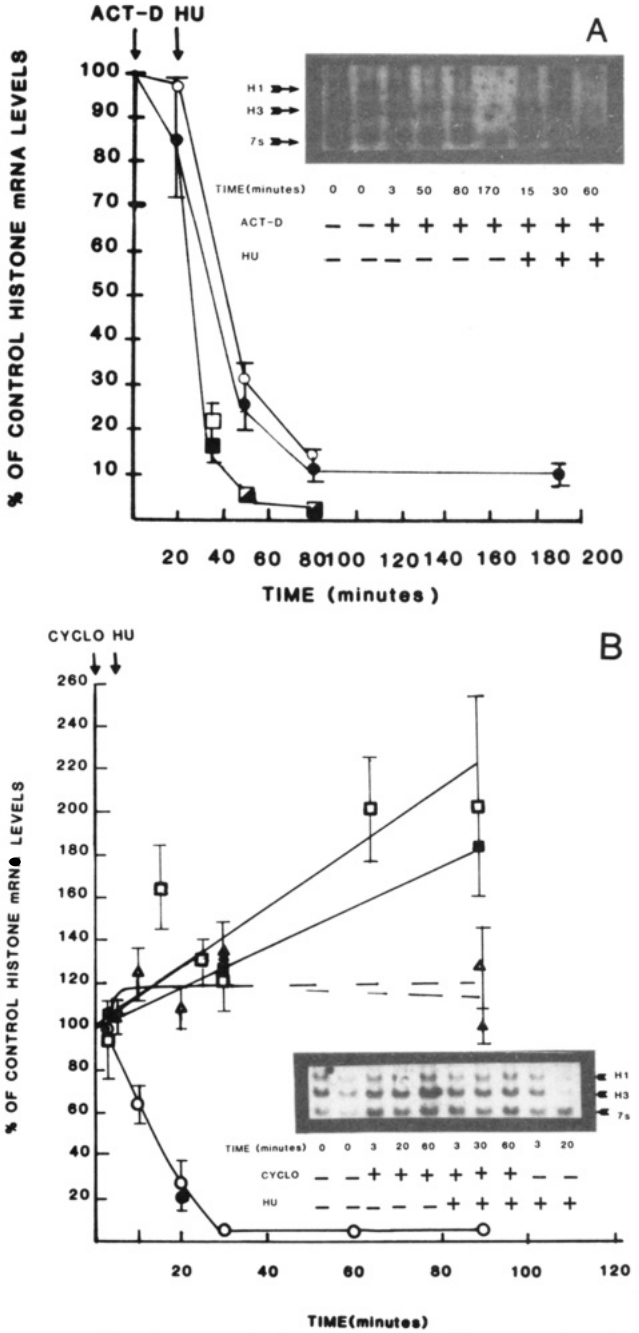

FIGURE 6: Coordinate regulation of H1 and H3 histone mRNAs in the presence of metabolic inhibitors. Total cellular RNA was isolated from cells exposed to Act-D ± hydroxyurea or cycloheximide ± HU at the indicated times. Aliquots of 50 μg/sample were resolved electrophoretically and analyzed by Northern blot hybridization with radiolabeled pFNC16 (H1 and 7S RNA) and pFF435C (H3) histone gene subclones as probes. (A) Analysis of H3 (closed symbols) and H1 (open symbols) mRNA levels in the presence of Act-D (circles) and Act-D and HU (squares) with time. The graph represents a summary of four independent Northern blots, and the mean values for H1 and H3 mRNA levels were calculated as percentages of untreated cells. The autoradiogram is from a Northern blot probed with pFNC16 and pFF435C. (B) Analysis of H3 (open symbols) and H1 (closed symbols) mRNA levels in the presence of cycloheximide (squares), cycloheximide and HU (triangles), and HU (circles) as a function of length of treatment. The graph represents a summary of four Northern blots, and the mean values of H1 and H3 mRNA levels were calculated as a percentage of untreated cells. The autoradiogram is from a representative Northern blot hybridized to pFNC16 and pFF435C.

mRNAs also appear to be coordinately controlled in the presence of several concentrations of each of the DNA synthesis inhibitors (data not shown). These results suggest that similar mechanisms may be operative in the coupling of HeLa H1 and core histone mRNA levels with the rate of DNA synthesis.

Discussion

We have addressed the relationship between histone gene expression and DNA replication by the systematic use of metabolic inhibitors to perturb DNA replication, transcription, and mRNA translation, while analyzing cellular levels of histone mRNAs. Our objectives were 3-fold: first, to assay the cellular representation of individual core and H1 histone mRNAs by hybridization to radiolabeled human histone gene probes, in order to determine the stability of these mRNAs in the presence of reduced rates of DNA synthesis; second, to obtain information concerning the stoichiometry of the relationship between histone mRNA steady-state levels and the rate of DNA synthesis; and third, to preferentially inhibit different events involved in DNA replication, assaying histone mRNA levels under these conditions.

Our laboratory (G. Stein et al., 1975; Stein et al., 1977a; Detke et al., 1979; Plumb et al., 1983a,b) and others (Borun et al., 1967, 1975; Hereford et al., 1982; Heintz et al., 1983) have previously reported that cellular levels of histone mRNAs are regulated both transcriptionally and posttranscriptionally. In this paper we have presented evidence that suggests that the coupling of histone gene expression and DNA synthesis is a temporal, stoichiometric, and functional relationship and that the turnover of histone mRNA is controlled primarily at a posttranscriptional level. We propose that the histone proteins themselves may be involved in determining the stability of histone mRNAs in response to changes in the rate of DNA synthesis. Such a relationship is consistent with the role of histone proteins in packaging newly replicated DNA into chromatin.

Initially, we assessed the stoichiometry of the relationship between histone mRNA levels and the rate of DNA synthesis by using three different inhibitors of DNA replication. Ara-C is a competitive inhibitor for dCTP binding to DNA polymerases α and β (*Cancer Chemotherapy Annuals*, 1980; Fridland, 1977) and inhibits DNA replication. At low concentrations (nanomolar), it inhibits initiation of DNA replication almost exclusively (Fridland, 1977), while at higher concentrations (micromolar), it inhibits both initiation and elongation of newly synthesized DNA (Fridland, 1977). It has been reported that Ara-CTP is incorporated into newly replicated DNA (Momparler, 1969), thus irreversibly inhibiting replication. Ara-C also causes an imbalance in the ribonucleotide and deoxyribonucleotide pools, as it increases the intracellular concentration of dCTP (Momparler, 1969; Cohen, 1966). HU at millimolar concentrations inhibits ribonucleotide reductase, which results in a decrease in the conversion of ribonucleotides to deoxyribonucleotides, thus reversibly inhibiting DNA synthesis (Striefel & Howell, 1981; Radford, 1982). HU also causes an imbalance in intracellular nucleotide precursor pools (Walker, 1977). Aphidicolin is a specific inhibitor of DNA polymerase α (Noy, 1979; Witz, 1979; Huberman, 1981). The postulated mechanism of inhibition involves binding to a site distinct from, but possibly overlapping, the nucleotide binding site on the polymerase (Huberman, 1981).

Our results show that in the presence of a broad concentration range of each of these DNA synthesis inhibitors, there is a stoichiometric relationship between core and H1 histone mRNA levels and the rate of DNA synthesis. Because Ara-C, HU, and aphidicolin each have different mechanisms of action, our data suggest that the key regulatory component controlling histone mRNA availability and stability is proportional to the

rate of DNA synthesis occurring in the cell but is not related to any one specific event in the process of DNA replication itself. The mRNAs for the five classes of histones respond similarly to DNA synthesis inhibition. However, consistent with recent reports by Bonner and co-workers (Wu & Bonner, 1981; Wu et al., 1982), we detect a "basal" level of histone mRNA that is insensitive to DNA synthesis inhibition. In agreement with the results of others (Heintz et al., 1983; Plumb et al., 1983a,b), the basal level of histone mRNAs remains approximately the same (8–15% of untreated log-phase control) regardless of the inhibitor or its concentration.

To address possible mechanisms operative in the coupling of histone mRNA steady-state levels with decreased rates of DNA replication, transcription was inhibited with Act-D. Despite the inhibition of RNA synthesis, histone mRNA levels and DNA synthesis decreased in parallel, but not as rapidly as when cells were treated with an inhibitor of DNA replication alone. The rapid loss of cellular histone mRNAs that occurred in the presence of a DNA synthesis inhibitor was not prevented by prior treatment with Act-D. These results suggest that in the presence of DNA synthesis inhibitors, histone mRNA levels are controlled at a posttranscriptional level. Furthermore, transcription is not necessary for the apparent destabilization of histone mRNAs that occurs in conjunction with DNA synthesis inhibition. Our results are in agreement with earlier Act-D inhibition studies in which pulse-labeled 7–9S polysomal RNAs decreased in parallel with inhibition of DNA synthesis (Borun et al., 1967).

Our results suggest that protein synthesis is necessary to maintain the parallel and functional relationship between cellular levels of core and H1 histone mRNAs and the rate of DNA synthesis. Consistent with earlier observations from in vitro translation studies that inhibition of protein synthesis prevents the loss of histone mRNAs from polysomes when DNA synthesis is inhibited (Butler & Mueller, 1973; Breindl & Gallwitz, 1974b; Gallwitz, 1975; Stahl & Gallwitz, 1977), we have found that total cellular histone mRNAs accumulate when protein synthesis is blocked either alone or prior to inhibition of DNA replication. However, the accumulation of core histone mRNAs is greater in the presence of cycloheximide alone than in cycloheximide plus HU. This result suggests that HU partially inhibits histone gene transcription while histone mRNA turnover is greatly reduced. While this paper was in preparation, similar findings concerning the stabilization of human and murine core histone mRNAs in the presence of protein synthesis inhibitors were reported (Stimac et al., 1983; Sittman et al., 1983). One interpretation of these results is that an unidentified labile regulatory molecule may be involved in the posttranscriptional coupling of histone gene expression and DNA replication. An equally viable interpretation, the one which we favor, is that histone proteins may either directly or indirectly determine cellular levels of histone mRNAs.

The latter interpretation would be consistent with a feedback mechanism by which the amount of histone protein synthesis necessary to package DNA into chromatin is mediated through control of histone mRNA stability. Autoregulation has been proposed as a mechanism operative in the control of other eukaryotic genes. For example, the amount of unpolymerized tubulin (Cleveland et al., 1981) and the amount of secreted tropoelastin (Frisch et al., 1983) may feedback inhibit their own production by controlling stability of their respective mRNAs. There is also evidence in murine tumor cells for translational repression that can limit the synthesis of specific polypeptides through selective feedback on translation of their respective mRNAs (Yenofsky et al., 1983). The specific posttranscriptional events that may be involved in regulating the coupling of histone gene expression and DNA replication remain to be resolved.

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