Coordinate Turnover of Nuclear and Cytoplasmic Histone Messenger RNA following Inhibition of DNA Replication in HeLa S3 Cells†

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ABSTRACT: We have examined the metabolism of human H4 histone mRNA in the nucleus and cytoplasm of HeLa S3 cells following inhibition of DNA synthesis to address the extent to which histone mRNA stability in these cellular compartments is coupled to DNA replication. The nuclear and cytoplasmic levels of histone mRNAs encoded by the pF0108A human H4 histone gene were determined by S1 nuclease analysis using a ^{32}P-labeled probe that could distinguish pF0108A transcripts from those of other members of the H4 histone multigene family. Hydroxyurea treatment resulted within 15 min in a 75% reduction in the level of histone H4 mRNA in the nucleus, which corresponds to the 85% decrease observed for H4 histone mRNA in the cytoplasm. The kinetics of nuclear and cytoplasmic H4 mRNA turnover following hydroxyurea treatment were also similar. Northern blot analysis using a ^{32}P-labeled mitochondrial cytochrome *b* probe indicated that the association of cytoplasmic RNA with the nuclear fraction was less than 0.5%. Treatment of cells with a protein synthesis inhibitor resulted in a 1.3-fold increase in nuclear H4 histone mRNA levels and a 1.5-fold increase of H4 mRNA in the cytoplasm after 45 min. Together, these results indicate that nuclear and cytoplasmic H4 histone mRNAs respond similarly to metabolic perturbations that influence message stability and that mechanisms operative in the turnover of histone mRNAs in the nucleus and cytoplasm may be similar.

It has been well documented that in most eukaryotic cells, histone gene expression is temporally and functionally coupled to DNA replication, occurring predominantly during the S phase of the cell cycle (Prescott, 1966; Allfrey et al., 1963; Robbins & Borun, 1967; Spaulding et al., 1966; Stein & Borun, 1972; Wu & Bonner, 1981; Lennox & Cohen, 1984). Although specific mechanisms operative in the regulation of histone gene expression remain to be determined, it is evident that control resides at both the transcriptional and posttranscriptional levels (Jacobs-Lorena et al., 1972; Stein et al., 1975; Parker & Fitschen, 1980; Heintz et al., 1983; Plumb et al., 1983a; Sittman et al., 1983). In human and murine cells, there is a coordinate and stoichiometric relationship between DNA synthesis, histone protein synthesis, and cellular levels of histone mRNAs, with a 15–50-fold increase during S phase (Heintz et al., 1983; Plumb et al., 1983a; Rickles et al., 1982; Sittman et al., 1983; DeLisle et al., 1983; Alterman et al., 1984). However, while an increase in histone gene transcription is observed during early S phase, this is only 3–7-fold increased over a basal level of transcription that occurs throughout the cell cycle and in nondividing cells (Heintz et al., 1983; Plumb et al., 1983a,b; Sittman et al., 1983; Alterman et al., 1984). It therefore appears that the onset of DNA replication is associated with an enhancement, rather than an activation, of histone gene transcription and is also accompanied by a decrease in the turnover of histone mRNAs. At the natural completion of DNA replication and following inhibition of DNA synthesis, there is a rapid and selective destabilization of histone mRNAs that parallels the decline in DNA and histone protein synthesis (Heintz et al., 1983; Plumb et al., 1983a,b; Baumbach et al., 1984). This turnover of histone mRNAs is posttranscriptionally mediated, occurs in the absence of RNA synthesis, and is dependent on protein synthesis.

Despite the importance of histone mRNA turnover for modulating cellular levels of histone mRNA, our understanding of cellular and molecular parameters that are involved is minimal. Therefore, to further address the rapid and selective destabilization of histone mRNA that occurs in the absence of DNA synthesis, we have examined the extent to which turnover occurs in the nucleus and cytoplasm, the kinetics of nuclear and cytoplasmic histone mRNA turnover, and the requirements for protein synthesis. Results are presented which suggest that these parameters of histone mRNA turnover are similar in both the nucleus and cytoplasm.

Materials and Methods

Cell Culture, Subcellular Fractionation, and Isolation of RNA. HeLa S3 cells were grown in suspension culture at a concentration of (2.5–5) × 10^5 cells/mL at 37 °C in Joklik-modified minimal essential medium (GIBCO) supplemented with 7% calf serum. Exponentially growing cells were used in all experiments, and for each experiments, cells were obtained from the same suspension culture. Control, hydroxyurea-treated (1 mM), or cycloheximide-treated (12 μg/mL) cells were harvested by centrifugation at 1000*g* for 2 min at 4 °C. Subcellular fractionations were carried out essentially following the procedures of van Eekelen et al. (1981) and Mariman et al. (1982). RNAs from nuclear and cytoplasmic fractions were isolated by using the methods described by Clejek et al. (1982) and Long et al. (1979). Details of our applications of these methods have been reported (Bandyopadhyay et al., 1986).

DNA Synthesis Analysis. The rate of DNA synthesis in control or treated cells was monitored as described by Stein and Borun (1972) by pulse labeling cells for 30 min with [^{3}H]thymidine (1 μCi/mL). Trichloroacetic acid (TCA) (10%) precipitable material was collected on Millipore filters

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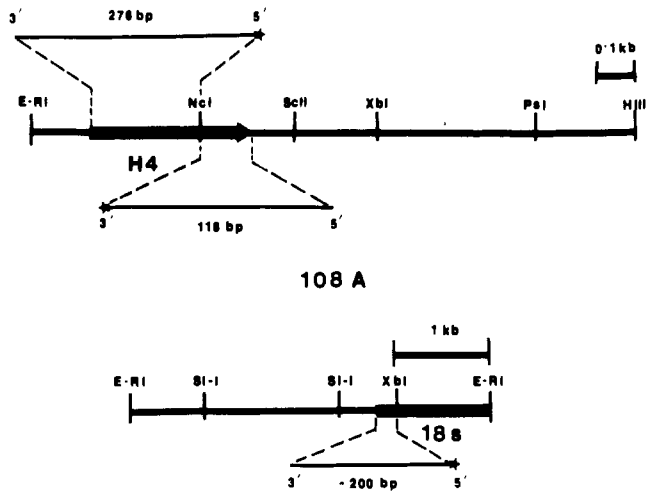

FIGURE 1: Partial restriction maps of a human cell cycle dependent histone H4 gene (pF0108A) and a ribosomal gene (LS-2) cloned in pBR322. Restriction enzymes indicated are *Eco*RI (E-RI), *Nco*I (NCI), *Sac*II (SCII), *Xba*I (XbI), *Pst*I (PsI), *Hin*dIII (HIII), and *Sal*I (SII). The bold arrow in 108A represents the length of H4 mRNA and the direction of transcription. *Nco*I–*Sac*II and *Nco*I–*Eco*RI fragments were used as probes in S1 nuclease analysis. The upper and lower lines indicate the expected sizes of the protected probes after S1 digestion. Stars at the ends of the lines indicate the labeled ends. LS-2 contains part of the 18S ribosomal gene (bold line) and 5′ upstream spacer sequences. The 5′ end-labeled *Xba*I–*Sal*I fragment was used as probe in the analysis.

(0.45 μm, type HA) which were solubilized in 1 mL of ethylene glycol monoethyl ether, and radioactivities were measured in a liquid scintillation spectrometer.

Northern Blot Analysis. Total cytoplasmic, nuclear, or total cellular RNAs (50 μg of each) were electrophoretically separated in 1.5% agarose–formaldehyde gels. RNAs were transferred to nitrocellulose essentially following the method described by Southern (1975). Prehybridization was performed at 38 °C for 6 h in 20 mL of 50% formamide, 5 × SSC [3 M NaCl and 0.3 sodium citrate (pH 7.0)], 1% glycine, 5 × Denhardt's buffer, and 100 μg/mL *Escherichia coli* DNA. Hybridization was at 38 °C for 48 h in 20 mL of solution containing 50% formamide, 5 × SSC, 15 mM potassium phosphate buffer (pH 7.2), 5 × Denhardt's buffer, 100 μg/mL *E. coli* DNA, and thermally denatured, ^{32}P-labeled (nick-translated) mouse cytochrome *b* DNA (1 × 10^6 cpm/mL). Filters were sequentially washed with 100 mL of the following solutions for 45 min at 65 °C: (1) 5 × SSC and 1 × Denhardt's buffer; (2) 2 × SSC and 0.1% sodium dodecyl sulfate (SDS); (3) 1 × SSC and 0.1% SDS; (4) 0.1 × SSC and 0.1% SDS. Filters were air-dried and exposed to preflashed Kodak XAR-5 film with intensifying screens at −70 °C.

S1 Nuclease Probes. (*A*) *H4 Histone Gene Probe.* pF0108A (Figure 1), a cell cycle dependent human H4 histone gene subcloned in pBR322 (Sierra et al., 1982), was linearized by cleavage with *Nco*I. For 3′ end labeling, 5′ overhangs of *Nco*I digests were repaired with deoxynucleoside triphosphates in the presence of [α-^{32}P]dCTP using the Klenow fragment of DNA polymerase, as described by Maniatis et al. (1982). This two-end-labeled, linearized DNA was digested with *Sac*II, and a 238 base pair (bp) fragment was isolated and used as probe.

For 5′ end labeling, the *Nco*I-linearized DNA was dephosphorylated by digestion with bacterial alkaline phosphatase and then phosphorylated with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP as described by Maniatis et al. (1982). The 5′ end-labeled DNA was restricted with *Eco*RI and the 490 bp fragment isolated for use as probe.

Specific activities of these probes were determined by measuring 10% TCA-precipitable radioactivity and the absorbance at 260 nm.

(*B*) *Ribosomal Gene Probe.* A human 18S ribosomal gene cloned in pBR322, LS-2 was linearized with *Xba*I. The DNA was dephosphorylated by bacterial alkaline phosphate treatment, phosphorylated with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP, and digested with *Sal*I. The 800 bp fragment was isolated and used as a probe.

Determination of Saturation Point of Hybridization. Two hundred nanograms of the 238 bp, 3′ end-labeled pF0108A probe described above was serially diluted with equal volumes of H_2O. Aliquots of the probe were hybridized with 50 μg of HeLa cell total cytoplasmic RNA in 25 μL of 80% formamide and 1 × hybridization buffer [0.4 M NaCl, 40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (Pipes) (pH 6.4), and 1 mM ethylenediaminotetraacetic acid (EDTA)] for 3 h at 55 °C. S1 nuclease digestion was carried out in 8 volumes of S1 nuclease buffer [250 mM NaCl, 30 mM NaOAc (pH 4.6), and 1 mM $ZnSO_4$] with 1500 units/mL S1 nuclease at 37 °C for 30 min. Products were fractionated electrophoretically in 6% polyacrylamide–7 M urea gels. The gels were dried and autoradiographed. The extent of hybridization was quantitated by densitometric scanning of the autoradiograms.

S1 Nuclease Analysis. The method of Weaver and Weissman (1979) was used with only minor modifications which have been reported (Bandyopadhyay et al., 1986). All S1 analyses to measure the levels of histone H4 mRNA were performed as described above, using 50 μg of HeLa cell cytoplasmic RNAs and 100 μg of nuclear RNAs. Probe concentrations were 160 ng of the 3′ end-labeled *Nco*I/*Sac*II fragment and 200 ng of the 5′ end-labeled *Nco*I/*Eco*RI fragment of pF0108A. For ribosomal RNA analysis, the hybridization temperature was 61 °C. All other steps of the procedure remained the same as those used for analysis of histone RNAs.

RESULTS

Distribution of Histone H4 RNA in the Cytoplasm and Nucleus. These studies were carried out by using a cell cycle dependent human H4 histone gene designated pF0108A. To examine the metabolism of histone mRNAs in the nucleus and cytoplasm, it was initially necessary to establish the representation in the nuclear and cytoplasmic subcellular fractions of mRNAs encoded by the pF0108A H4 histone gene, and the extent to which cytoplasmic material is associated with isolated nuclei. Cellular levels of pF0108A H4 histone mRNA were determined by S1 nuclease protection analysis, to permit discrimination between mRNAs transcribed from this copy of the H4 histone gene and those RNAs transcribed from other H4 histone genes. Despite the apparent complete conservation of amino acid sequences in human H4 histone proteins, there are significant variations in the nucleotide sequences of the H4 histone genes, both in the protein coding regions (predominantly third position changes) and in the regions of the gene encoding the nontranslated 5′ and 3′ segments of the mRNAs (length and nucleotide substitutions).

Consistent with our previous observations (Bandyopadhyay et al., 1986), S1 nuclease analysis of nuclear, cytoplasmic, and total cellular RNAs from exponentially growing HeLa S3 cells indicated that 98% of the pF0108A H4 histone mRNAs are present in the cytoplasm and 2% are in the nucleus. To address the extent to which cytoplasmic material is associated with the nuclear fraction, the representation of mitochondrial cy-

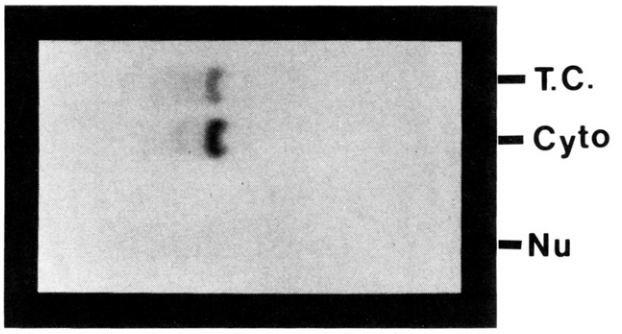

FIGURE 2: Distribution of mitochondrial cytochrome *b* mRNA in two subcellular fractions. Fifty micrograms each of total cytoplasmic and nuclear RNAs was electrophoresed in an agarose gel, transferred to nitrocellulose, and hybridized with nick-translated, mouse cytochrome *b* gene probe as described under Materials and Methods. Radioactive bands were present only in TC and Cyto lanes where total cellular and cytoplasmic RNAs, respectively, were analyzed. The autoradiogram shows no band at the corresponding position in the nuclear (Nu) lane.

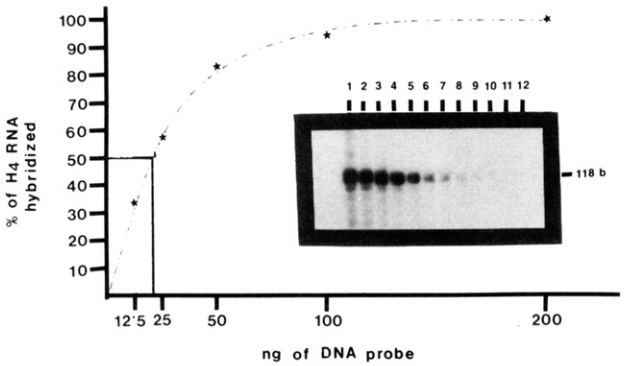

FIGURE 3: Establishment of DNA excess condition for S1 nuclease analysis and determination of saturation point of hybridization. The inset shows an S1 nuclease analysis with 50 μg of total cytoplasmic RNA and 3′ end-labeled *Nco*I–*Sac*II probe (Figure 1) in the indicated amounts. The size of the protected probe was 118 nucleotides. The autoradiograms were scanned, and the densitometric values are represented in graphical form.

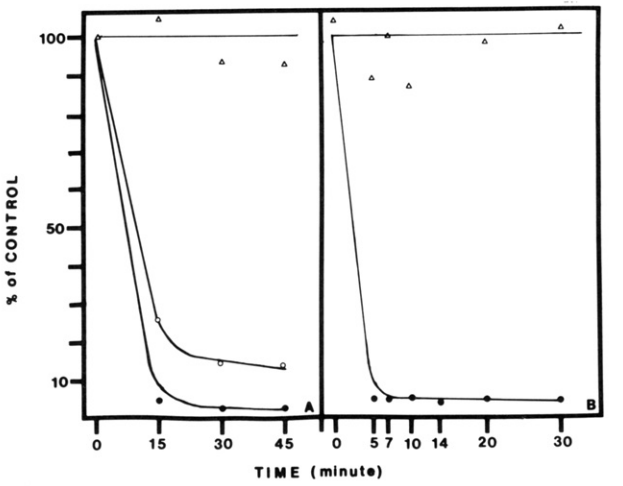

FIGURE 4: Rate of DNA synthesis in cells treated with hydroxyurea (●) or cycloheximide (○) for various lengths of time. Panels A and B represent two different experiments. Incorporation of [^{3}H]thymidine as a function of inhibition was depicted as a percent of control [(△) cells without treatment]. Each point is the average value of two separate observations.

tochrome *b* mRNA was determined. Total cellular, nuclear, and cytoplasmic RNAs (50 μg) were fractionated electrophoretically in a 1.5% agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled (nick-translated) mitochondrial cytochrome *b* DNA probe. As shown in Figure 2, an autoradiogram of such a Northern blot indicates the presence of cytochrome *b* mRNA in total cellular and cytoplasmic RNAs without a detectable amount in the nuclear fraction. In a longer exposure of the blot, a very weak signal in the nucleus became detectable. Densitometry indicated that the level of cytochrome *b* mRNA in the nuclear fraction was less than 0.5% of that in the cytoplasm. It therefore appears that the presence of cytoplasmic material in the nuclear fraction is minimal and insufficient to account for the presence of 2% of the cellular H4 histone mRNAs in the nucleus.

Metabolic Stability of H4 mRNA in the Nucleus and Cytoplasm following Inhibition of DNA or Protein Synthesis. The stability of pF0108A H4 histone mRNA was compared in the nucleus and cytoplasm following inhibition of DNA replication or protein synthesis. The representation of RNAs encoded by the pF0108A H4 histone gene was determined by S1 nuclease analysis. The aim of this study was to determine whether mechanisms operative in histone mRNA turnover are similar in the nucleus and in the cytoplasm.

We established that the S1 nuclease assays used in these studies were carried out in DNA excess by determining the saturation point of hybridization. By carrying out hybridization in DNA excess, the hybridization signal is a reflection of cellular levels of pF0108A H4 histone mRNAs. Figure 3 (insert) shows results from an S1 nuclease analysis carried out with 50 μg of total cytoplasmic RNA and a 3′ end-labeled *Nco*I–*Sac*II fragment of pF0108A (Figure 1) as the probe. The RNA was maintained at the same concentration for each of the reactions, while the DNA probe was serially diluted (e.g., 200 ng in lane 1, 100 ng in lane 2, 50 ng in lane 3, etc.). The 118-nucleotide fragment of protected probe was resolved in a 6% polyacrylamide–7 M urea denaturing gel. When autoradiograms were scanned and densitometric data were plotted as a percent of pF0108A histone H4 mRNA hybridized against fixed amounts of probe (Figure 3), it was established that >50 ng of double-stranded probe was required to saturate the pF0108A H4 histone mRNA present in 50 μg of total cytoplasmic RNA.

H4 histone mRNA levels were monitored at three time points (15, 30, and 45 min) after exposing exponentially growing HeLa S3 cells to a DNA synthesis inhibitor (1 mM hydroxyurea) or to a protein synthesis inhibitor (12 μg/mL cycloheximide). DNA synthesis was monitored in aliquots of cells from the same cultures (Figure 4A), and inhibition of DNA synthesis in hydroxyurea- and cycloheximide-treated cells was expressed as a percent of control. The incorporation of [^{3}H]thymidine decreases to less than 10% of control in hydroxyurea-treated cells and to less than 25% of control in cycloheximide-treated cells within 15 min of treatment. To examine the representation of pF0108A H4 mRNA, S1 nuclease analysis was carried out on RNAs isolated from the nucleus and cytoplasm of untreated control, hydroxyurea-treated, and cycloheximide-treated cells. Total cytoplasmic (50 μg) or total nuclear (100 μg) RNAs were hybridized with 160 ng of the 3′ end-labeled *Nco*I–*Sac*II fragment of pF0108A (Figure 5A,B). After S1 nuclease digestion, a 118-nucleotide, S1-protected DNA fragment was resolved as a single major band in a 6% polyacrylamide–7 M urea gel. The level of cytoplasmic H4 mRNA in hydroxyurea-treated cells decreased to 13% of control within 15 min of treatment (Figures 5A and 6). Longer exposure of the cells to this DNA synthesis inhibitor further decreased the cytoplasmic representation of pF0108A H4 histone mRNA to basal level (5%). In constrast, following cycloheximide treatment, the cytoplasmic level of pF0108A H4 histone mRNA increased to 140% of control at

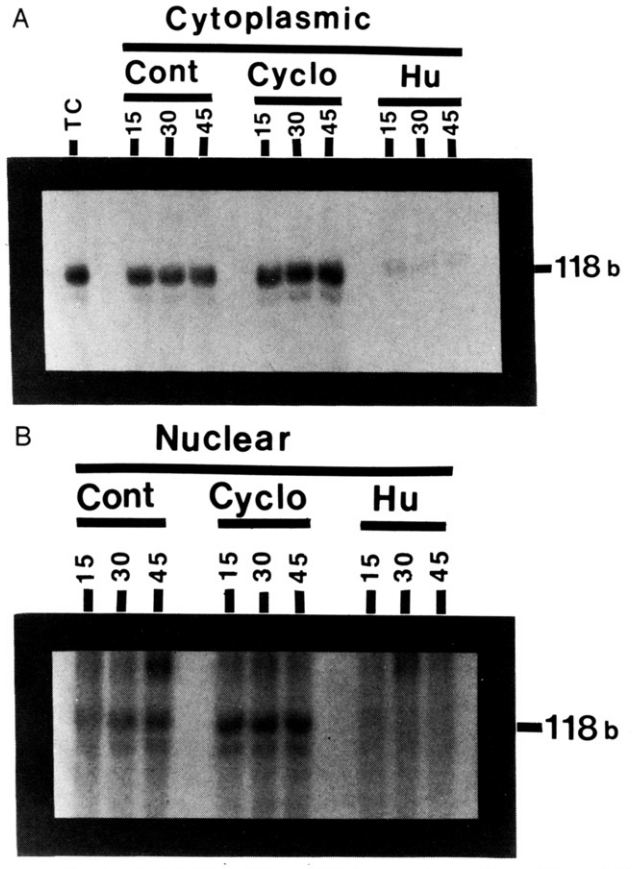

FIGURE 5: Levels of histone H4 mRNA in cytoplasm (A) and in nuclei (B) of exponentially growing HeLa S3 cells treated with hydroxyurea (HU) or cycloheximide (Cyclo) for different lengths of time (15, 30, and 45 min). Fifty micrograms of total cytoplasmic RNA and 100 μg of total nuclear RNA from untreated (control), HU-treated, and Cyclo-treated cells was probed with 160 ng of 3′ end-labeled *Nco*I–*Sac*II fragment of 108A. S1 nuclease digested samples were resolved on 6% polyacrylamide–7 M urea denaturating gels. TC represents the lane where total cellular RNA (50 μg) from untreated cells was analyzed. The autoradiograms of nuclear and cytoplasmic samples represent different exposure times.

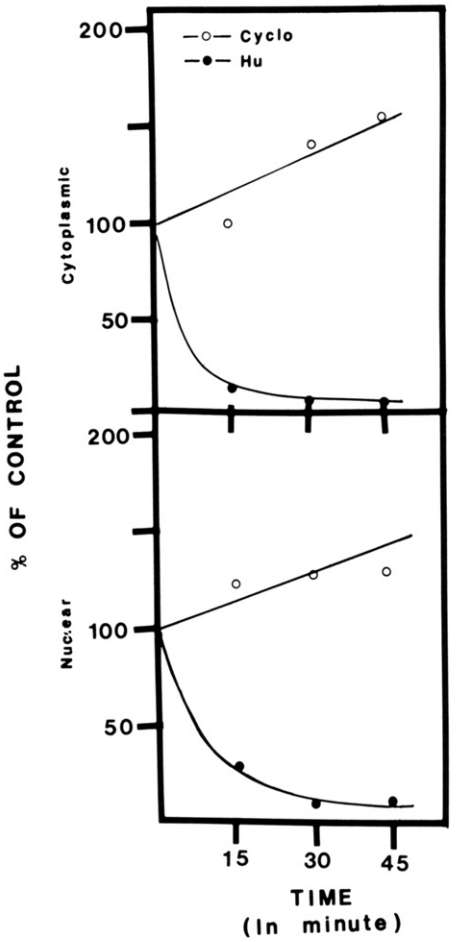

FIGURE 6: Graphical representation of histone H4 mRNA levels present in cytoplasm and in nuclei of hydroxyurea-treated (●) or cycloheximide-treated (○) cells as analyzed in Figure 5A, B. Values at each time point were averages obtained by densitometric scan of the autoradiograms of two separate S1 analyses. The levels of RNA in treated cells have been plotted as a percent of control (untreated cells).

30 min and to 153% of control at 45 min. These results are in agreement with findings from previous studies in which H4 histone mRNA metabolism was examined by Northern blot analyses of polysomal or total cellular RNA following DNA synthesis and protein synthesis inhibition (Heintz et al., 1983; Plumb et al., 1983a,b; Baumbach et al., 1984). In nuclei, the decrease of pF0108A H4 histone mRNAs following hydroxyurea treatment and the accumulation of these mRNAs following cycloheximide treatment are similar to those observed in the cytoplasm. As shown in Figures 5B and 6, the level of nuclear H4 mRNA decreased to 26% of control at 15 min of hydroxyurea treatment and increased to 130% of control following 45 min of cycloheximide treatment. Taken together, these results suggest that two key aspects of histone mRNA metabolism, selective turnover following inhibition of DNA replication and stabilization following inhibition of protein synthesis, take place with equal efficiency in both the nucleus and cytoplasm of HeLa S3 cells.

For comparison and to serve as a control for the effects of hydroxyurea and cycloheximide on RNA metabolism in general, we examined the influence of these inhibitors of DNA and protein synthesis on 18S ribosomal RNA. S1 nuclease analysis was carried out by using a 5′ end-labeled (^{32}P) 800 bp *Xba*I–*Sal*I fragment of a cloned 18S ribosomal RNA gene designated LS-2 (Figure 1). This fragment contains 200 bp of an 18S ribosomal RNA coding region. Cytoplasmic RNA

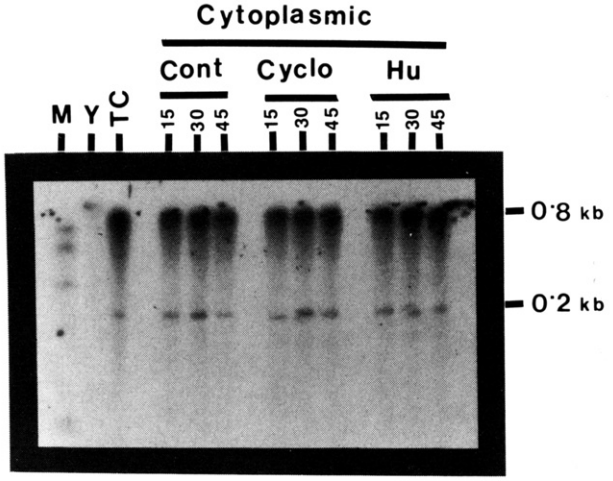

FIGURE 7: Representation of ribosomal RNA in hydroxyurea-treated (HU) or cycloheximide-treated (Cyclo) and in untreated (control) cells at various times after drug treatment. The autoradiogram shows an S1 analysis with the 0.8 kilobase *Xba*I–*Sal*I probe and total cytoplasmic RNA as described under Materials and Methods. The expected size of the protected probe was 0.2 kilobase. TC, Y, and M represent analysis of HeLa total cellular RNA, yeast RNA only, and *Hin*fI-digested pBR322 marker, respectively.

preparations were diluted to a level that allowed for DNA excess hybridization conditions. As shown in Figure 7, the intensities of the band generated by protection of the ribosomal

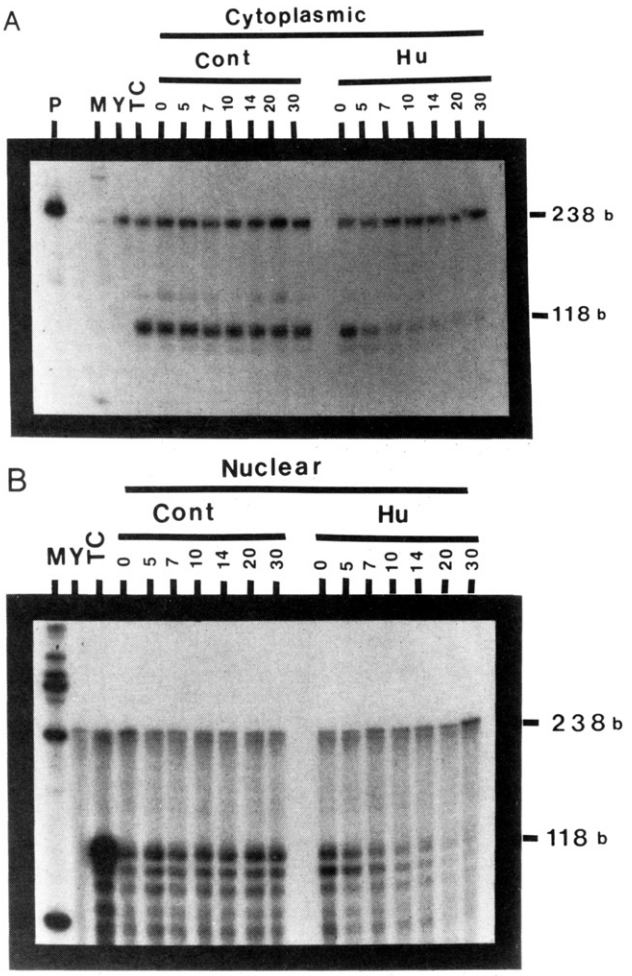

FIGURE 8: Time course of decay of histone H4 mRNA from cytoplasm (A) and the nucleus (B) of HeLa cells after DNA synthesis inhibition by hydroxyurea. Fifty micrograms of cytoplasmic and 100 μg of nuclear RNA from both untreated (Cont) and hydroxyurea-treated (HU) cells at each indicated time point were probed with the 3′ end-labeled *Nco*I–*Sac*II fragment of 108A. 238b and 118b show the reannealed probe and the S1-protected fragment of the probe, respectively. P, M, Y, and TC represent analysis of denatured probe only, *Hin*fI-digested pBR322 marker, and S1 mapping of probe with yeast RNA and with 50 μg of total cellular RNA, respectively.

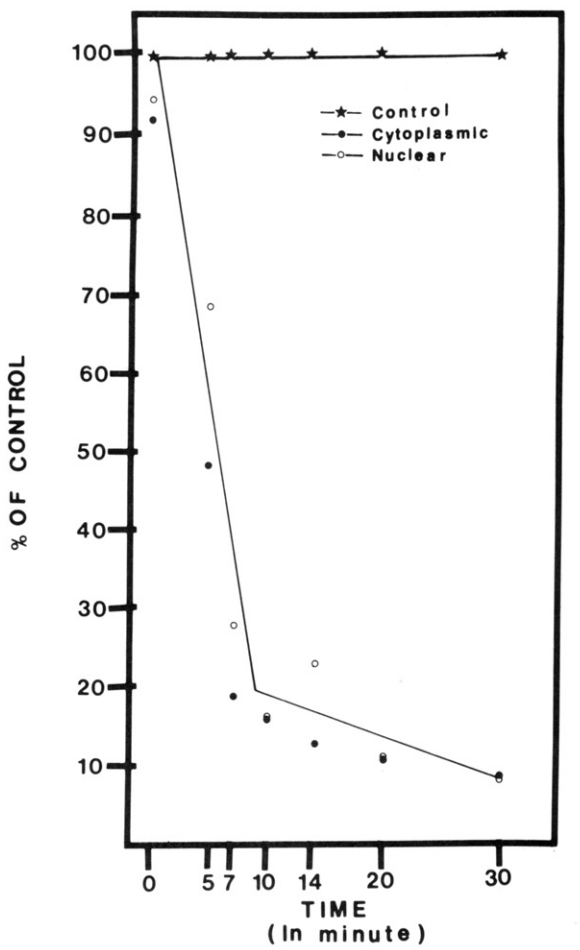

FIGURE 9: Decay curve for histone H4 mRNA from cytoplasm (●) and from nuclei (○). The curve was constructed on the basis of the data obtained by densitometric scans of autoradiograms of two separate S1 analyses. Values are expressed as a percent of control.

gene probe from S1 nuclease digestion do not vary significantly among control, hydroxyurea-treated, and cycloheximide-treated samples at different times following treatment. Therefore, the 18S RNA species remains relatively unaffected when DNA or protein synthesis is inhibited.

Kinetics of H4 Histone mRNA Turnover in the Nucleus and Cytoplasm following DNA Synthesis Inhibition. To further address the stability properties of pF0108A H4 histone mRNA in the nucleus and cytoplasm of HeLa cells, a kinetic analysis of the turnover of this H4 mRNA following hydroxyurea (HU) treatment was carried out. The rationale for these studies was to consider the possibility that mechanisms and/or factors involved in histone mRNA destabilization are similar in both cellular compartments. Exponentially growing HeLa S3 cells were treated with 1 mM hydroxyurea, and cells were harvested at 0, 5, 7, 10, 14, 20, and 30 min after treatment, along with untreated controls. Cytoplasmic (50 μg) and nuclear (100 μg) RNAs from each HU-treated and control sample were subjected to S1 nuclease analysis using a 3′ end-labeled *Nco*I–*Sac*II fragment of the pF0108A H4 histone gene as a ^{32}P-labeled probe. It is evident from the autoradiograms (Figure 8) that there is a rapid disappearance of pF0108A H4 mRNAs from both the cytoplasm and nucleus following hydroxyurea treatment that does not appear to be preceded by a lag period. From these results, a half-life for pF0108A H4 histone mRNA of approximately 6 min can be determined.

When S1 analysis was carried out with the *Nco*I–*Sac*II fragment as a 3′ end-labeled probe, the expected 118-base segment of the gene was protected by both cytoplasmic and nuclear RNAs. This segment corresponds to a region of the gene extending from the *Nco*I site to the ACCA motif which is the 3′ terminus of the mature pF0108A H4 mRNA. S1 analysis of cytoplasmic and nuclear RNAs also yielded a protected fragment of 238 bp (Figure 8) which resulted from reannealing of the probe. This interpretation is supported by the migration of radiolabeled, denatured probe (Figure 8A, lane P) and S1 analysis carried out on yeast RNA (Figure 8, lane Y). In the case of nuclear RNAs (Figure 8B), a series of protected fragments smaller than 118 bases is reproducibly evident, and the time course of disappearance for these smaller fragments following DNA synthesis inhibition is similar to that observed for the 118-nucleotide band. While a definitive interpretation of the origin of these bands remains unresolved, they may reflect protection of the probe by intermediates in the degradation of the H4 mRNA or, alternatively, may reflect overdigestion with the S1 nuclease.

The rates at which nuclear and cytoplasmic levels of pF0108A H4 histone mRNAs decline following inhibition of DNA synthesis can be evaluated from the data presented in Figure 9, where histone mRNA levels in the nucleus and cytoplasm of hydroxyurea-treated cells are expressed as a

percentage of those observed in untreated control cells. While the loss of pF0108A H4 mRNAs appears to be slightly more pronounced in the cytoplasm than in the nucleus during the initial 7 min of hydroxyurea treatment, by 10 min H4 mRNA levels in both cellular compartments were reduced to the same extent. For both the nuclear and cytoplasmic fractions, the same straight line can account for loss of pF0108A histone mRNAs during the first 10 min of hydroxyurea treatment (the kinetics of H4 mRNA loss are exponential), and while the decline in message levels continues at a reduced rate during the subsequent 20 min, the plots of the data for nuclear and cytoplasmic samples remain superimposable. Even at 45 min (Figure 5A,B) and at longer times following hydroxyurea treatment, low levels (less than 10%) of pF0108A H4 histone gene transcripts persist in the nucleus and cytoplasm, constituting a "basal level" that appears to be refractory to DNA synthesis inhibition.

Discussion

A key in the temporal and functional coupling of histone gene expression with DNA replication is the rapid and selective destabilization of histone mRNA that occurs at the natural termination of the S phase or following inhibition of DNA replication at any point during the S phase of the cell cycle. Hence, it is necessary to address mechanisms operative in histone mRNA turnover to understand the relationship between DNA replication and expression of histone genes.

In human and in mouse cells, it has been well established that histone mRNA destabilization and inhibition of DNA replication occur coordinately and stoichiometrically (Plumb et al., 1983a; Rickles et al., 1982; Heintz et al., 1983; Sittman et al., 1983; DeLisle et al., 1983; Alterman et al., 1984). This relationship is reflected by the parallel decline in DNA replication and cellular histone mRNA levels that is associated with both chemical [drugs that affect deoxynucleotide metabolism (Jacobs-Lorena et al., 1972; Stein et al., 1975; Parker & Fitschen, 1980; Heintz et al., 1983; Plumb et al., 1983a; Sittman et al., 1983) and DNA polymerase (Baumbach et al., 1984)] and biological [adenovirus infection (Flint et al., 1984) and shifts of temperature-sensitive mutants to nonpermissive temperatures (Hirschhorn et al., 1984)] perturbations of DNA synthesis. It has been shown that histone mRNA turnover does not require transcription and that it depends on protein synthesis (Baumbach et al., 1984). Additionally, recent results suggest that both the 5′ and 3′ regions of histone mRNAs may be involved in the coupling of histone mRNA stability with ongoing DNA synthesis (Morris et al., 1986; Lüscher et al., 1985). In this paper, we have presented results which suggest that during inhibition of DNA synthesis, histone mRNA degradation occurs in both the cytoplasm and the nucleus and that the kinetics and extent of cytoplasmic and nuclear histone mRNA degradation are similar.

The extent to which histone mRNA turnover occurs in the cytoplasm as well as in the nucleus was examined to begin addressing whether cellular structures and macromolecules required for histone mRNA degradation are found in both cellular compartments and possible similarities or differences in cytoplasmic as compared to nuclear histone mRNA turnover. Only 2% of the cellular histone mRNA is found in the nucleus, but the turnover that occurs following DNA synthesis inhibition and the accumulation that accompanies protein synthesis inhibition are similar to those observed for histone mRNA in the cytoplasm. Within 15 min after hydroxyurea treatment, there is a 75% reduction in the level of H4 histone mRNA in the nucleus; this corresponds to the 85% decrease observed in the cytoplasm. The kinetics of nuclear and cytoplasmic H4 mRNA turnover following hydroxyurea treatment are also similar. When protein synthesis is inhibited, a 1.3-fold increase in nuclear H4 mRNA levels and a 1.5-fold increase of H4 mRNA in the cytoplasm are observed after 45 min of treatment. Together, these results indicate that nuclear and cytoplasmic H4 histone mRNAs respond similarly to metabolic perturbations that influence message stability.

The similar efficiency of nuclear and cytoplasmic histone mRNA catabolism seems to indicate that association of the mRNA with polysomes actively engaged in histone protein synthesis is not a requisite for histone mRNA turnover. Also, because histone mRNAs are rapidly transferred to the cytoplasm upon completion of transcription, our results suggest that the newly synthesized nuclear histone mRNAs and those already in the cytoplasm are equally vulnerable to degradation following inhibition of DNA synthesis. These observations are consistent with but do not unequivocally establish that a similar mechanism for histone mRNA turnover is operative in both the cytoplasm and nucleus. We cannot eliminate the possibility that, at least to some extent, turnover mechanisms in the cytoplasm and nucleus are different.

Whether or not histone mRNA turnover is autogenously regulated (Butler & Mueller, 1973; Stein & Stein, 1984), whereby accumulation of histone protein selectively mediates degradation of the histone message, remains to be established. However, on the basis of our results, if such autogenous feedback control is operative, it is necessary to postulate that nuclear histone mRNAs (nuclear mRNPs) as well as cytoplasmic histone messages (polysome associated) are targets for selective turnover in the absence of DNA synthesis.

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Structural Studies of Acetylated and Control Inner Core Histones†

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ABSTRACT: The role of acetylation on the conformation and association state of the inner core histone octamer isolated from HeLa cells was examined. Preparation of suitable quantities of pure acetylated and control inner core histones from HeLa cells required the development of a new preparative procedure. The results from size-exclusion high-performance liquid chromatography and sedimentation equilibrium studies indicated that acetylated inner core histones associate to species larger than the octamer and form a more stable complex. Circular dichroism studies demonstrated that the amount of α-helix increases with increasing association of the histones. Furthermore, acetylation results in an increase in the amount of α-helix, perhaps coupled through its effect on the association state. At high protein concentration and elevated temperature, the acetylated sample displays a greater increase in β-sheet content, relative to the control sample. This increase in β-sheet content may be induced during the association of the acetylated sample to species larger than the octamer. There is a marked effect on the conformation of both acetylated and control inner core histones as a function of protein concentration, ionic strength, and temperature. The difference in conformational flexibility and association state of the acetylated vs. the control inner histone core may play a significant role in the control of transcription in the nucleus.

Despite considerable effort, the effect of acetylation on the properties of chromatin is not clear. The original observation that histones H3 and H4 were acetylated (Allfrey et al., 1964) suggested that acetylation might be a mechanism for modulation of transcription. Hyperacetylation of histones H3 and H4 has been correlated with increased periods of transcription during development (Ruiz-Carrillo et al., 1974, 1976; Sung & Dixon, 1970; Christensen et al., 1984; Christensen & Dixon, 1982; Oliva & Mezquita, 1982; Grimes & Henderson, 1983; Burdick & Taylor, 1976). A number of schemes are available to fractionate active from inactive chromatin, and the active fractions seem to be enriched in acetylated material (Levy-Wilson et al., 1979; Davie & Candido, 1980; Davie & Saunders, 1981).

Conversely, the transcriptional activity of chromatin has been reported to have decreased (Mathis et al., 1978) or not to be affected (Lilley & Berendt, 1979) by acetylation of the histones.

The action of DNase I on acetylated chromatin is reported to be preferential and much more rapid than control chromatin (Mathis et al., 1978; Nelson et al., 1978; Simpson, 1978; Sealy & Chaulkley, 1978; Vidali et al., 1978; Davie & Candido, 1980), suggesting that acetylation alters chromatin conformation. The DNase digestion experiments, and the observed

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