Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1849–1853, April 1983
Biochemistry

Histone mRNA concentrations are regulated at the level of transcription and mRNA degradation

(DNA synthesis inhibitors/isolated nuclei/histone variants/RNA synthesis)

DONALD B. SITTMAN, REED A. GRAVES, AND WILLIAM F. MARZLUFF

Department of Chemistry, Florida State University, Tallahassee, Florida 32306

Communicated by J. Herbert Taylor, December 30, 1982

ABSTRACT The levels of histone mRNA are rapidly reduced after treatment of cultured cells with hydroxyurea or cytosine arabinonucleoside. The histone mRNA for the replicative histone variants is destroyed rapidly, with a half-life of 10–15 min. The levels of mRNA coding for the replacement histone variant H3.3 were unchanged after treatment with DNA synthesis inhibitors. In addition to the rapid destruction of histone mRNA, there was a reduction to 1/5th in the rate of transcription of the histone genes. Lymphoma cells (S49) arrested in G1 by cyclic AMP produce and contain significant levels of histone mRNA. Hydroxyurea reduces the rate of transcription and the levels of histone mRNA in the G1-arrested cells.

Histones are the basic proteins that are complexed with DNA to form the eukaryotic chromosome. It is generally thought that histones are synthesized when DNA is synthesized (1, 2). However, in one mammalian cell line, S49 mouse lymphoma cells, histones are also synthesized in G1 and incorporated into chromosomes during the subsequent S phase (3).

The levels of histone mRNA are also regulated, both during the cell cycle and in response to DNA synthesis inhibitors. In yeast cells (4) and HeLa cells (5), there is little, if any, histone mRNA present just after mitosis, and the histone mRNA accumulates in the cytoplasm when the cells enter S phase. When S phase cells are treated with DNA synthesis inhibitors the level of histone mRNA drops rapidly (6–10). Whether this rapid drop in histone mRNA level is accompanied by changes in the rate of histone RNA synthesis is not known.

An additional aspect of histone mRNA metabolism in mammalian cells is the presence of histone protein variants (11, 12). One class, replication variants, predominates in rapidly dividing cells. Another class of histones, replacement variants, do not appear to be coupled to DNA replication and are present in increased levels in differentiated, nondividing, tissues (13).

The isolation of mouse histone genes (14) allows us to directly measure both synthesis and degradation of histone mRNA. We report here that treatment of mouse cells with inhibitors of deoxynucleotide synthesis reduces the level of replication variant histone mRNAs, but not the level of a replacement variant mRNA, H3.3. This reduction is due to both a decreased rate of histone mRNA synthesis and an increased rate of degradation. In G1 lymphoma cells, which are not synthesizing DNA but are synthesizing histone mRNA, the inhibitors also reduce the rate of histone mRNA synthesis.

METHODS

Cell Growth. Mouse myeloma 66-2 cells were grown, synchronized by isoleucine starvation (15), and treated with DNA synthesis inhibitors as described in the figure legends. When double drug treatments with cycloheximide and a DNA synthesis inhibitor were performed the cycloheximide was added 5 min prior to the addition of the DNA synthesis inhibitor. The final concentration of cycloheximide was 0.1 mM. Cells washed with fresh medium after drug treatment retained their viability. "Deathless" S49 mouse lymphoma cells were grown and synchronized as described by Lemaire and Coffino (16).

Nuclear Transcription. Nuclei were isolated and incubated for RNA synthesis essentially as described by Marzluff (17). The final salt concentrations in the transcription were 70 mM KCl and 6 mM $MgCl_2$. We used 100 μCi (1 Ci = 3.7×10^{10} Bq) of [α-^{32}P]GTP (ICN, 450 Ci/mmol) with a final concentration of 50 μM per 200-μl reaction mixture. All other nucleoside triphosphates were at a final concentration of 500 μM. Incubation was for 30 min at 25°C. Incorporation was linear for this time. Reactions were stopped by a 5-min incubation with an equal volume of DNase I (Sigma) at 25 μg/ml, 0.6 M NaCl, 50 mM Tris·HCl at pH 7.5, and 20 mM $MgCl_2$. $NaDodSO_4$ and EDTA were then added to final concentrations of 1% and 10 mM, respectively, followed by the addition of ammonium acetate to 0.3 M. RNA was extracted with an equal volume of water-saturated phenol at 55°C for 5 min, precipitated with ethanol, and washed three times with 75% (vol/vol) ethanol. The RNA was separated from unincorporated nucleoside triphosphates by chromatography through a Sephadex G-75 column packed in a 1-ml disposable pipette.

DNA Dot Preparation. The DNA was made linear by restriction enzyme cleavage. It was then extracted with neutral phenol, precipitated with ethanol, and washed three times with 75% ethanol. The DNA was applied to nitrocellulose filter paper with a Hybri-dot apparatus (Bethesda Research Laboratories) by the procedure of McKnight and Palmiter (18) at 6.5 μg of DNA per dot.

The following plasmid DNAs were derived from the λ phage MM221 (14): pMH3.1, a 2.4-kilobase (kb) *Eco*RI fragment that contains a histone H3.1 gene; pMH3.2, a 0.9-kb *Eco*RI/*Sal* I fragment that codes for amino acids 58–135 of a histone H3.2 gene; pMH2a, a 1.1-kb *Eco*RI fragment that contains amino acids 93–125 of a histone H2a.1 gene. Plasmid pHU-4A, which contains a complete human histone H4 gene (19), was a gift of N. Heintz and R. Roeder. Plasmid pHU3.3, which contains a human histone H3.3 gene, was a gift from R. Cohn. Plasmid pκ24^{11}, a cDNA clone of the immunoglobulin κ light chain, was a gift of R. Perry (20). Plasmid pU1.1 codes for a mouse U1 small nuclear RNA gene (unpublished results).

Hybridization. The filters were incubated for 1 hr at 52°C in hybridization buffer [50% (vol/vol) deionized formamide/0.75 M NaCl/0.075 M sodium citrate/10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.1% $NaDodSO_4$/10 μg of poly(A) per ml/

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: kb, kilobase.

0.4% polyvinylpyrrolidone/0.4% Ficoll]. The filters were then hybridized with the appropriate RNA samples in 0.5 ml of hybridization buffer for 3 days at 52°C. Filters were washed six times with an excess of 0.15 M NaCl/0.015 M sodium citrate/2 mM EDTA/10 mM Tris·HCl, pH 7.5/0.1% $NaDodSO_4$ at 52°C for 30 min per wash followed by one wash with the above buffer minus $NaDodSO_4$.

The amount of RNA hybridized to a particular DNA dot was detected by autoradiography. A quantitative determination of the amount of RNA bound to a dot was made by cutting out the dot and measuring the radioactivity with a scintillation counter. When low levels of radioactivity were bound that could not be accurately determined with scintillation counting but could be seen with long exposure to autoradiography, then the relative amounts of bound radioactivity were determined by scanning the autoradiograph with a Gelman DCD-16 densitometer. With the appropriate autoradiograph exposure the densitometer scans gave results identical to those of scintillation counting. All hybridizations were done in DNA excess. Identical results were obtained when the amount of DNA bound was reduced to 50%. The level of hybridization for any given sample was proportional to the input RNA.

S1 Nuclease Protection Assays. Steady-state histone mRNA levels were determined by S1 nuclease protection assays using mouse histone probes. The histone genes were cleaved with a restriction enzyme at a site within the coding region. The DNA was labeled at the 5′ end with [γ-^{32}P]ATP and polynucleotide kinase. The labeled DNA (50–70 fmol) was hybridized to either cytoplasmic or total cell RNA (0.5–5.0 μg) in 5 μl of 80% (vol/vol) formamide/0.4 M NaCl/40 mM 1,4-piperazinediethanesulfonic acid (Pipes), pH 6.4, at 58°C for 3 hr as described by Weaver and Weissmann (21). The S1 nuclease-resistant DNA was denatured and analyzed by electrophoresis on a 6% polyacrylamide gel in 8 M urea. The probes used were as follows: H2b.1, the histone H2b gene was cleaved with *Ava* I at amino acid 93; H3.2, the histone H3.2 gene was cleaved with *Sal* I at amino acid 58 [these genes were derived from MM221 (14)]; H3.3, the histone H3.3 gene was cleaved at amino acid 80 with *Bgl* II (R. Cohn, personal communication).

Pulse Labeling. Cells (2 × 10^7) were suspended in 5 ml of medium with 10% horse serum plus 2 mCi of [^{3}H]uridine (ICN, 30 Ci/mmol). Pulses were for the indicated time at 37°C. The pulse was stopped by pouring the cells into an equal volume of phosphate-buffered saline plus 100 mM uridine. Five milliliters of 2.5% $NaDodSO_4$/0.025 M EDTA was added and the mixture was homogenized in a Dounce homogenizer, 10 strokes with a B pestle. Ammonium acetate was added to 0.3 M and the RNA was extracted with hot phenol (55°C) and precipitated with ethanol. The RNA was washed with 75% ethanol and incubated in 0.5 ml of 10 mM Tris·HCl, pH 7.5/5 mM $Mg(OAc)_2$/2 μg of DNase I at 37°C for 30 min. The RNA was again extracted with phenol, precipitated with ethanol, and washed three times with 75% ethanol. The [^{3}H]RNA samples were then hybridized to DNA dots.

RESULTS

In order to study the levels of regulation of histone mRNA, we used hybridization of steady-state and newly synthesized histone RNA to isolated mouse histone genes. The genes used code for the H3.1 and H3.2 histones, an H2b.1 histone, and an H2a.1-like histone which has a proline substituted for an alanine at position 126 [nomenclature of Franklin and Zweidler (11)]. These genes are all derived from a single histone gene cluster contained in the λ bacteriophage MM221 previously described (14). The RNAs coded for by the H3.1, H3.2, and H2b.1 genes hybridize to the major fraction of H3 and H2b mRNAs present in mouse myeloma cells. The sequences of these genes have been completely determined (unpublished data). We also used heterologous histone genes as probes. One is a human H3.3 replacement variant gene (R. Cohn, personal communication). The other is a human H4 gene, pHu4a (19).

Histone mRNA Levels After Inhibition of DNA Synthesis. When exponentially growing mouse myeloma cells are treated with hydroxyurea the level of histone RNA is rapidly reduced to a new steady-state level, approximately 10% of the original, within an hour (Fig. 1). The kinetics of the change in the H2b mRNA levels was determined by an S1 nuclease assay and the

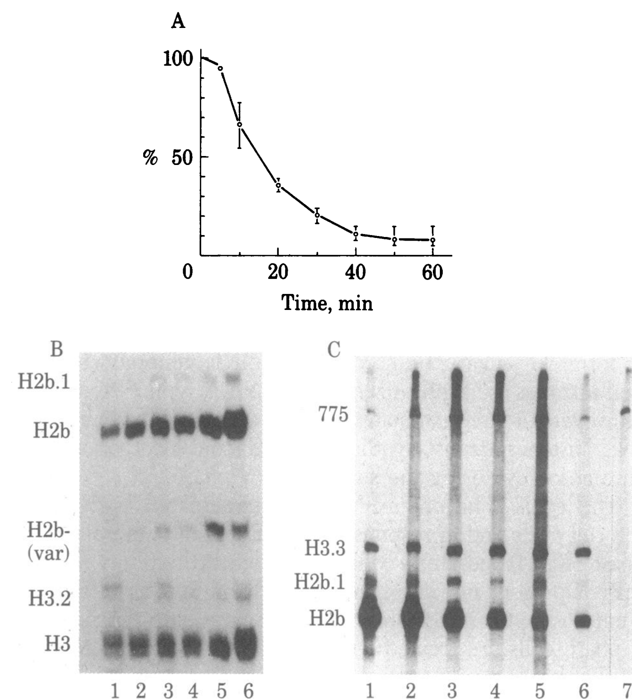

FIG. 1. (*A*) S1 nuclease determination of histone RNA levels after inhibition of DNA synthesis with hydroxyurea. Total RNA was isolated at various times after addition of 5 mM hydroxyurea to exponentially growing mouse myeloma cells and was assayed for its ability to protect 5′-end-labeled histone clones from S1 nuclease. The amount of H2b RNAs that protect the H2b.1 gene to the AUG codon is presented as the percentage of starting RNA level. The amount of protection was quantitated by scanning the autoradiograms with a Gelman DCD-16 densitometer. The error bars indicate the range of values determined for three separate experiments. Identical results were obtained when fluorodeoxyuridine or cytosine arabinonucleoside was used. (*B*) Autoradiogram of the hybridization of various amounts of cellular RNA to 20 fmol of H2b.1 and H3.2 probe. The samples and amounts used were as follows: lane 1, 2.5 μg, 50 min after hydroxyurea; lane 2, 2.5 μg, 40 min; lane 3, 2.5 μg, 30 min; lane 4, 1 μg, 20 min; lane 5, 0.5 μg, 10 min; lane 6, 0.5 μg, no hydroxyurea. Three bands are due to hybridization of H2b RNA: H2b(var), an H2b variant with an amino acid substitution at amino acid 17; H2b, the family of H2b mRNAs with common coding region sequences to the AUG codon; H2b.1, the mRNA derived from this gene. Two bands are due to hybridization of H3 mRNA: H3, a family of mRNAs with a common coding region sequence to the AUG codon, and H3.2, the mRNAs derived from this gene. (*C*) Autoradiogram of S1 nuclease assay of the hybridization of 5 μg of total cell RNA isolated at various times after the addition of hydroxyurea. A mixture of 5′-end-labeled probes containing 20 fmol of pmH2b.1 and 10 fmol of the human H3.3 gene was used. Autoradiography was overnight with a Lightning Plus screen (DuPont) at −70°C. The band at 775 is an undigested parent fragment. Lane 1, exponential cells; lane 2, 5 min after the addition of hydroxyurea; lane 3, 10 min; lane 4, 20 min; lane 5, 30 min; lane 6, 40 min; lane 7, tRNA control.

results are shown in Fig. 1. After cells were treated with hydroxyurea the steady-state histone mRNA levels decayed with an apparent half-life of 10–15 min after a 5-min lag. A plot of the decay of H2b mRNAs is shown in Fig. 1*A*. Identical results were obtained when the H3.2 gene was used as a probe. Fig. 1*B* shows an autoradiograph of the results obtained when RNA was incubated with a mixture of end-labeled H2b.1 and H3.2 probes. The H2b.1 band was formed by protection of the RNA encoded by the gene used in this experiment. The H2b band represented protection by a family of H2b mRNAs similar to this gene up to the AUG codon. The H3.2 band was due to protection of the DNA by the mRNA derived from this gene. The H3 band is due to a family of mRNAs that are similar in the coding region extending to the AUG initiation codon. The positions and identity of the protected bands have been determined in separate experiments by DNA sequence analysis (unpublished data). Fig. 1*C* shows the results obtained when a mixture of the H2b.1 and H3.3 genes was used as a probe. The levels of H3.3 mRNA were not altered by the DNA synthesis inhibitor.

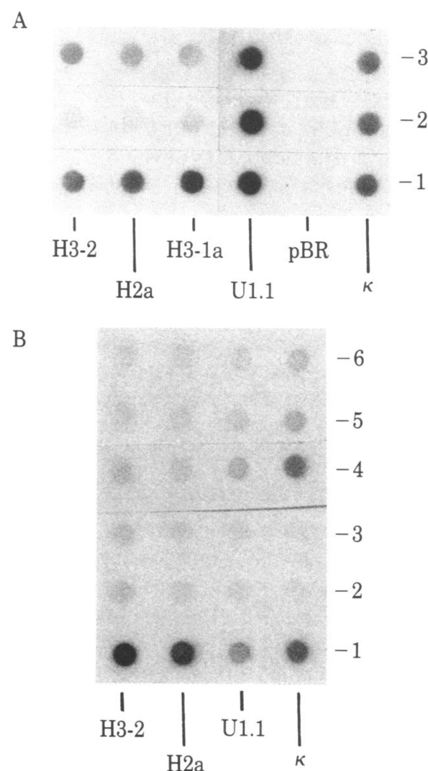

FIG. 2. Autoradiogram of hybridization of *in vitro* transcribed RNAs to DNA dots. The recombinant plasmids containing the genes indicated were linearized with *Eco*RI and bound to nitrocellulose. κ indicates the plasmid containing a κ immunoglobulin light chain, pκ24[11] (20), and pBR indicates pBR322. The other DNAs are described in the text. RNA was synthesized in nuclei in the presence of [α-^{32}P]GTP isolated from mouse myeloma cells. Cells were treated with inhibitors for 1 hr prior to isolation of the nuclei. (*A*) Row 1, exponentially growing cells; row 2, cells treated with cytosine arabinonucleoside (40 μg/ml); row 3, cells treated with cytosine arabinonucleoside and cycloheximide. In each experiment 7.6×10^6 cpm of RNA was hybridized. (*B*) Row 1, exponentially growing cells; row 2, same nuclei as row 1 incubated in the presence of α-amanitin at 1 μg/ml; row 3, same as row 2 incubated with α-amanitin at 90 μg/ml; row 4, nuclei from cells treated with cytosine arabinonucleoside. In each experiment 6.4×10^6 cpm of RNA was hybridized. Row 5, nuclei (row 4) were incubated for 10 min with [α-^{32}P]GTP and RNA was prepared for half of the reaction mixture; row 6, GTP was added to a final concentration of 500 μM to the other half of the reaction mixture (row 5) and incubated for an additional 20 min prior to isolation of the RNA. For rows 5 and 6, 4.2×10^6 cpm of RNA was hybridized.

Effect of DNA Synthesis Inhibitors on Transcription. The effect of DNA synthesis inhibitors on histone gene transcription was determined by using RNA synthesis in isolated nuclei and by pulse-labeling. Nuclei isolated from cultured myeloma cells and incubated *in vitro* reflect the RNA synthetic activity of the cells from which they were derived (22, 23). The RNA made in isolated nuclei was hybridized to immobilized histone DNA. Nuclei were isolated from exponentially growing mouse myeloma cells and from cells previously treated for 1 hr with the DNA synthesis inhibitors cytosine arabinonucleoside or hydroxyurea. Nuclei isolated from cells treated with DNA synthesis inhibitors transcribed histone mRNA 1/5th as efficiently as nuclei from untreated cells. The inhibitory effect of cytosine arabinonucleoside on histone mRNA synthesis is shown in Fig. 2 and Table 1. H2a and H3 histone RNA was detected by hybridization to subcloned genes. Similar results were obtained with a human H4 gene (see Fig. 3). The histone probes used do not contain highly repeated sequences and detect only histone RNA when hybridized to total cellular RNA. Upon Southern analysis both the H3.2 and H2a subclones hybridized to only 15 bands, which correspond to 10–20 copies of mouse histone genes (ref. 24; unpublished results). In these experiments we also measured the rate of synthesis of mouse κ immunoglobulin light chain RNA by using the cDNA clone pκ24[11] (20). As expected, the levels of this RNA were not affected by inhibition of DNA synthesis. We also probed hybridization to a clone coding for mouse U1 RNA. There was no detectable hybridization to pBR322. The residual histone RNA transcription seen after DNA synthesis inhibition was not due to hybridization of H3.3 RNA to the H3.2 genes, because these two genes do not crossreact significantly under the conditions used. We also obtained identical results when we hybridized to a human H4 gene (see Fig. 3). As seen in Fig. 2*B*, rows 2 and 3, the RNAs that hybridized to these clones are RNA polymerase II products. Their synthesis was inhibited by low concentrations of α-amanitin.

Fig. 2*B*, rows 5 and 6, and Table 1 also show that the reduction of histone RNA synthesis in nuclei isolated from cytosine arabinonucleoside-treated cells was not due to the degradation of histone RNA during the time of incubation. When the nuclei were allowed to incorporate [α-^{32}P]GTP for 10 min and then incubated for a further 20 min in an excess of unlabeled GTP, there was no decrease in the amount of histone

Table 1. Transcription in nuclei *in vitro*

	RNA hybridized, cpm			
Treatment	H3.2	H2a	U1.1	κ
Control, 30 min	1,265	1,007	371	680
(% of input)	(0.020)	(0.016)	(0.006)	(0.011)
araC, 30 min	266	149	211	624
(% of control)	(21)	(15)	(57)	(92)
araC + CHX, 30 min	823	509	277	1,326
(% of control)	(65)	(51)	(75)	(195)
araC, 10 min	259	213	189	430
araC, 10 min + 20-min chase	242	197	209	387

Nuclei were isolated either from exponentially growing myeloma cells (control) or from cells treated for 1 hr with cytosine arabinonucleoside (araC, 40 μg/ml) or cytosine arabinonucleoside plus cycloheximide (CHX). Nuclei were incubated with [α-^{32}P]GTP for 10 or 30 min. Identical amounts of RNA (6.4×10^6 cpm) were hybridized for lines 1–3 and 4.2×10^6 cpm were hybridized for lines 4 and 5. There was no effect on total RNA synthesis when cells were treated with any of the inhibitors prior to isolation of the nuclei.

RNA during the chase period. In addition, the same proportion of total transcripts are histone RNA whether the RNA was isolated after 10 or 30 min of continuous incubation with [α-^{32}P]GTP. The factors responsible for degradation of histone mRNA were not present in the isolated nuclei.

We also investigated the transcriptional activity of the histone genes by an alternative procedure: [^{3}H]uridine pulse labeling. The total cell RNA labeled during a short pulse was isolated and hybridized to DNA dots, and specific dots were cut out and their radioactivities were measured. Table 2 shows that during a 10-min pulse the same relative amount of histone RNA was synthesized as determined by transcription *in vitro*. The amount of histone RNA synthesized in 10 min in cells treated with cytosine arabinonucleoside or hydroxyurea was reduced to 20% of the amount synthesized in untreated cells. The reduced amount was not due to extremely rapid turnover of RNA in the treated cells. Cytosine arabinonucleoside inhibited histone RNA synthesis in a 5-min pulse to approximately the same extent as in a 10-min pulse. A larger relative amount of histone RNA synthesis in the shorter pulses would have been observed if extremely rapid turnover were affecting the results.

Effect of DNA Synthesis Inhibitors on S Phase Myeloma Cells. To demonstrate that the residual 20% level of histone RNA synthesis was not due to the G1 phase cells in the exponentially growing population we tested the effect of DNA synthesis inhibitors on S phase myeloma cells. Mouse myeloma cells were synchronized by isoleucine starvation. Fig. 3 shows that hydroxyurea inhibited histone mRNA synthesis in S phase cells 70–80%, the same amount as in exponentially growing cells. Similar results were obtained with cytosine arabinonucleoside. Synthesis of histone RNA is down only 30% in G1 phase myeloma cells (Fig. 3), consistent with the significant levels of histone mRNA present in these cells (unpublished results).

Histone RNA Synthesis in Synchronized Lymphoma Cells. A derivative of S49 lymphoma cells, the "deathless" mutant, is arrested in G1 phase after treatment with cyclic AMP for 25 hr (16). These G1-arrested cells (less than 5% of cells synthesizing DNA) contain a significant level of histone mRNA, 25–50% of that in exponentially growing cells (Fig. 4*A*), and they are also synthesizing histones (V. Groppi, personal com-

Table 2. Pulse labeling *in vivo*

Treatment	Exp.	RNA hybridized, cpm H3.2	H2a	U1.1	κ
Control, 10 min	1	1,732	1,282	475	3,392
(% of input)		(0.029)	(0.022)	(0.008)	(0.058)
	2	1,137	976	391	2,244
(% of input)		(0.016)	(0.014)	(0.006)	(0.032)
araC, 10 min	1	345	212	387	2,890
(% of control)		(30)	(17)	(85)	(85)
	2	200	170	211	1,827
(% of control)		(18)	(17)	(54)	(81)
OH-urea, 10 min	1	301	181	436	2,974
(% of control)		(17)	(14)	(95)	(88)
araC + CHX, 10 min	2	633	363	272	2,439
(% of control)		(56)	(37)	(70)	(100)
Control, 5 min	2	851	658	356	1,939
(% of input)		(0.012)	(0.009)	(0.005)	(0.028)
araC, 5 min	2	208	114	274	1,745
(% of control)		(24)	(17)	(77)	(90)

Exponentially growing mouse myeloma cells were labeled with [^{3}H]uridine for 5 or 10 min. Exponentially growing cells were treated with inhibitors for 1 hr prior to labeling. araC, cytosine arabinonucleoside; OH-urea, hydroxyurea; CHX, cycloheximide. Total RNA was isolated and hybridized to the DNA dots. Identical amounts of RNA were used in all hybridizations in each experiment (5.9×10^{6} cpm of RNA in experiment 1 and 7×10^{6} cpm in experiment 2). There was no effect on the total RNA synthesis when cells were pretreated with any of the inhibitors.

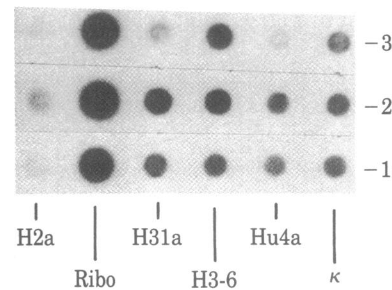

FIG. 3. Hybridization of RNAs transcribed *in vitro* from synchronized myeloma cells. The probes used were the same as Fig. 2 plus three additional plasmids. Ribo indicates the plasmid pLV1334 (25), which contains the sea urchin 18S rRNA gene and part of the 26S rRNA gene. Hu4a indicates the plasmid pHu4a (19), which contains a human H4 gene. H3-6 (14) hybridizes to a small unknown RNA synthesized constitutively (unpublished results). Mouse myeloma cells were synchronized by isoleucine starvation. Nuclei were isolated from G1 cells (1.5 hr after isoleucine was restored) and S phase cells (8 hr after isoleucine was restored). Half of the S phase culture was treated with hydroxyurea 1 hr prior to isolation of the nuclei. Row 1, G1 cells; row 2, S phase cells; row 3, S phase cells plus 5 mM hydroxyurea.

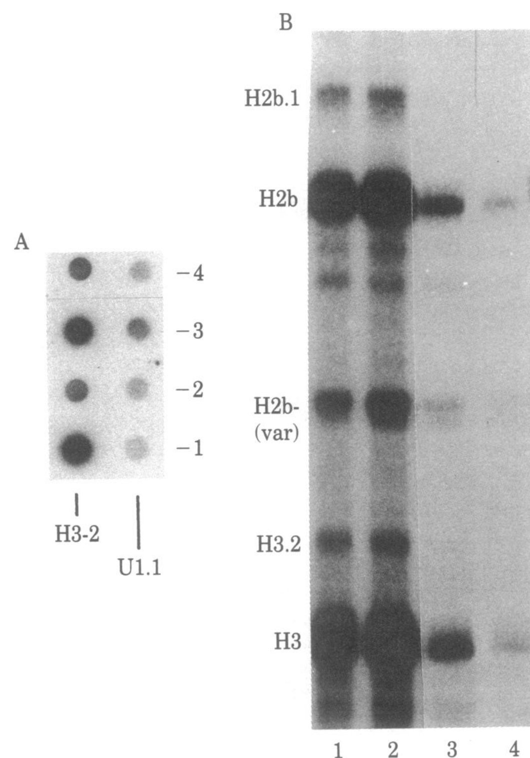

FIG. 4. (*A*) Nuclei isolated from mouse lymphoma cells were incubated with [α-^{32}P]GTP for 30 min. RNA was isolated and hybridized to the various DNAs. The input RNA was 5.4×10^{6} cpm in each experiment. Row 1, exponentially growing cells; row 2, exponentially growing cells treated with 1 mM hydroxyurea 1 hr prior to isolating nuclei; row 3, cells arrested in G1 by 0.5 mM cyclic AMP; row 4, G1 cells treated with 1 mM hydroxyurea. (*B*) Levels of histone mRNA were measured by S1 nuclease as described for Fig. 1*C*. The bands are identified as in Fig. 1*B*. Lane 1, G1 cells arrested by cyclic AMP; lane 2, exponentially growing cells; lane 3, exponentially growing cells treated with hydroxyurea for 1 hr; lane 4, G1 cells treated with hydroxyurea for 1 hr.

munication). As with the synchronized myeloma cells, the relative amount of histone RNA synthesis in G1 phase cells was about 70% that of S phase cells as can be seen in Fig. 4*B*. Hydroxyurea reduced the synthesis of histone RNA to the same basal level in both G and S phase, reducing the transcription to about 20% that of S phase cells. The levels of histone mRNA in the cytoplasm, as determined by S1 nuclease analysis, were reduced by the same amount (Fig. 4*B*).

Effect of Cycloheximide on the Effect of DNA Synthesis Inhibitors. Butler and Mueller (8) reported that cycloheximide, an inhibitor of protein synthesis, prevents the degradation of histone mRNA that occurs after treatment with DNA synthesis inhibitors. The effect of cycloheximide on histone gene transcription was examined in mouse myeloma cells. Prior treatment (5 min) of cells with cycloheximide partially prevented the drop in histone RNA synthesis after treatment with cytosine arabinonucleoside. This effect was seen with both nuclear transcription *in vitro* and pulse labeling as shown in Fig. 2*A*, lane 3, and Tables 1 and 2. The same effect was seen with hydroxyurea (data not presented). Similarly, S1 nuclease analysis showed that treatment with cycloheximide completely prevented the disappearance of histone mRNA from the cytoplasm (data not shown). Cycloheximide by itself reproducibly affected transcription of some specific genes, although it did not alter the total rate of RNA synthesis. It increased the rate of κ light chain transcription and reproducibly decreased the rate of synthesis of the histone genes about 30–40%, to the same level observed in cells treated with both protein synthesis inhibitor and DNA synthesis inhibitor. It is likely that cycloheximide completely prevents the reduction in histone mRNA synthesis by DNA synthesis inhibitors.

DISCUSSION

The results presented show that the DNA synthesis inhibitors, cytosine arabinonucleoside and hydroxyurea, cause a very rapid reduction in the steady-state level of histone RNA. These results also show, by both nuclear transcription *in vitro* and pulse labeling, that a component of this alteration is a reduction to 1/4th to 1/5th in the transcription of the histone genes. We believe that the regulation of histone RNA synthesis takes place at two levels: (*i*) mRNA turnover and (*ii*) transcription. The half-life of mRNA after cells are treated with hydroxyurea is less than 15 min. This is in good agreement with the results of Gallwitz (26), who calculated a half-life of 13 min with a lag phase of less than 5 min. A similar reduction in steady-state levels is seen with cytosine arabinonucleoside, fluorodeoxyuridine, and mycophenolic acid (unpublished results). After treatment with these drugs there is also a decrease in the histone gene transcription to 20% of the control values. Our preliminary evidence indicates that the half-life of histone mRNA normally is slightly less than 1 hr in exponentially growing cells (unpublished results). Thus there is a decrease in the transcription rate and an increase in the degradation rate of histone mRNA, each by a factor of 4–5.

In the cell types studied here the level of histone RNA and the rate of transcription after inhibition of DNA synthesis is always at least 20% of those in S phase cells. This is true even with cyclic AMP-treated G1-arrested lymphoma cells in which DNA synthesis is reduced at least 95%. Most histone RNAs detected, which presumably code for the replication variants expressed in these cells, are reduced equally. An exception is the levels of histone H3.3. The incorporation of this protein into chromatin persists in hydroxyurea-treated cells (27). This is not unexpected, because these histones predominate in fully differentiated tissues compared to rapidly cycling cells and have subsequently been named replacement variants (13). There may also be other specific histone RNA populations that are not affected by the inhibition of DNA synthesis.

The molecular basis of the regulation of histone mRNA levels is not known. The simplest assumption is that the same agent is responsible for both accelerating the degradation and reducing the synthesis of histone mRNA. The changes in synthesis and degradation rate of the histone mRNA are not obligatorily linked to DNA synthesis. Because the agents tested that inhibit histone mRNA synthesis also affect deoxynucleotide metabolism it is possible that the two are related.

We are grateful to Phil Coffino and Vince Groppi for S49 cells and helpful discussions. We also thank Dr. Ron Cohn and Nat Heintz for providing plasmid DNAs. This work was supported by National Institutes of Health Grant GM28932 to W.F.M.; D.B.S. was supported by National Institutes of Health Fellowship GM07158.

1. Gallwitz, D. & Mueller, G. C. (1969) *Science* **163**, 1351–1353.
2. Gurley, L. R., Walter, R. A. & Tobey, R. A. (1972) *Arch. Biochem. Biophys.* **148**, 633–641.
3. Groppi, V. E. & Coffino, P. (1980) *Cell* **21**, 195–204.
4. Hereford, L. M., Ousley, M. A., Ludwig, J. R. & McLaughlin, C. S. (1981) *Cell* **24**, 367–375.
5. Rickles, R., Marashi, F., Sierra, F., Clark, S., Wells, J., Stein, J. & Stein, G. S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 749–753.
6. Borun, T. W., Schraff, M. D. & Robbins, E. (1967) *Proc. Natl. Acad. Sci. USA* **58**, 1977–1982.
7. Breindl, M. & Gallwitz, D. (1973) *Eur. J. Biochem.* **32**, 381–391.
8. Butler, W. B. & Mueller, G. C. (1973) *Biochim. Biophys. Acta* **294**, 481–496.
9. Stahl, H. & Gallwitz, D. (1977) *Eur. J. Biochem.* **72**, 385–392.
10. Gallwitz, D. & Mueller, G. C. (1969) *J. Biol. Chem.* **244**, 5947–5952.
11. Franklin, S. G. & Zweidler, A. (1977) *Nature (London)* **266**, 273–275.
12. Kinkade, J. M. & Cole, R. D. (1966) *J. Biol. Chem.* **241**, 5790–5797.
13. Zweidler, A. (1980) in *Gene Families of Collagen and Other Structural Proteins*, eds. Prokop, D. J. & Champe, P. C. (Elsevier, Amsterdam), pp. 47–56.
14. Sittman, D. B., Chiu, I.-M., Pan, C.-J., Cohn, R. H., Kedes, L. H. & Marzluff, W. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4078–4082.
15. Marzluff, W. F., Pan, C.-J., & Cooper, D. L. (1978) *Nucleic Acids Res.* **5**, 4177–4191.
16. Lemaire, I. & Coffino, P. (1977) *Cell* **11**, 149–158.
17. Marzluff, W. F. (1978) *Methods Cell Biol.* **14**, 317–331.
18. McKnight, G. S. & Palmiter, R. D. (1979) *J. Biol. Chem.* **254**, 9050–9058.
19. Heintz, N., Zernik, M. & Roeder, R. G. (1981) *Cell* **24**, 661–668.
20. Schibler, U., Marcu, K. B. & Perry, R. P. (1978) *Cell* **15**, 1495–1503.
21. Weaver, R. & Weissmann, C. (1979) *Nucleic Acids Res.* **7**, 1175–1193.
22. Marzluff, W. F., Murphy, E. C. & Huang, R. C. (1973) *Biochemistry* **12**, 3440–3446.
23. Manley, J. L., Sharp, P. A. & Gefter, M. L. (1979) *J. Mol. Biol.* **135**, 171–197.
24. Jacob, E. (1976) *Eur. J. Biochem.* **65**, 275–284.
25. Blin, N., Sperrazza, J. M., Wilson, F. E., Bieber, D. G., Michel, F. E. & Stafford, D. W. (1979) *J. Biol. Chem.* **254**, 2716–2721.
26. Gallwitz, D. (1975) *Nature (London)* **257**, 247–248.
27. Wu, R. & Bonner, W. (1981) *Cell* **27**, 321–330.