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Rapid Reversible Changes in the Rate of Histone Gene Transcription and Histone mRNA Levels in Mouse Myeloma Cells

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The levels of histone mRNAs are reduced 90 to 95% after treatment of mouse myeloma cells with inhibitors of DNA synthesis which disrupt deoxynucleotide metabolism. In contrast, novobiocin, which inhibits DNA synthesis but does not alter deoxynucleotide metabolism, did not alter histone mRNA levels. Upon reversing the inhibition by fluorodeoxyuridine by feeding with thymidine, histone mRNA levels are restored to control levels within 40 to 60 min. The rate of histone gene transcription is reduced 75 to 80% within 10 min after treatment with fluorodeoxyuridine and increased to control levels within 10 min after refeeding with thymidine. Inhibition of protein synthesis with cycloheximide or puromycin in cells which had been treated with fluorodeoxyuridine resulted in an increase of histone mRNA levels. This was partly due to an increase in the rate of transcription. The data indicate that both transcription and mRNA degradation are linked to deoxynucleotide metabolism. Continued protein synthesis is necessary for maintaining the inhibition of histone gene transcription.

Histone proteins are synthesized and incorporated into chromatin primarily during the S phase of the cell cycle (3, 10, 13, 30). There are different nonallelic histone variants which are under different regulation (8, 30). The predominant histones in cultured cells are the replication variants, which are the major histones synthesized during the S phase (30). The concentrations of the mRNAs coding for the replication-variant histone mRNAs are regulated in coordination with DNA synthesis (2, 4, 5, 9). There are at least two components of this regulation: (i) transcription of the histone genes and (ii) stability of the histone mRNAs (14, 24).

Histone mRNA concentrations are greatly reduced in mammalian cells after inhibition of DNA synthesis with compounds that interfere with deoxynucleotide metabolism (2, 4, 5). This reduction is due to a decrease in the half-life of the histone mRNA coupled with a decrease in the rate of transcription (14, 24). Inhibitors of protein synthesis block the reduction in histone mRNA levels (5, 26) primarily by stabilizing the mRNA but also by altering the rate of transcription (24).

Here we report that these changes in the rate of transcription of the histone genes are rapid and complete within 10 min of inhibition of DNA synthesis. The effects are also rapidly reversible, transcription being stimulated within 10 min after release from inhibition of DNA synthesis with fluorodeoxyuridine by adding thymidine. Inhibition of protein synthesis results in an increase in histone mRNA levels in cells treated with fluorodeoxyuridine (FUdR), by increasing the transcription rate of these genes as well as stabilizing the mRNA. Continued protein synthesis is required for maintaining the reduction in the rate of histone gene transcription.

MATERIALS AND METHODS

Cell culture. Mouse myeloma 66-2 cells were grown in suspension culture as previously described (16). Cells were used at a concentration of 4×10^5 to 5×10^5/ml. The cells were treated with the following inhibitors of DNA synthesis: hydroxyurea (5 mM), cytosine arabinoside (40 μg/ml), adenine arabinoside (40 μg/ml), mycophenolic acid (2 μM), methotrexate (2 μM), or FUdR (2 μM). To reverse the inhibition by FUdR, thymidine was added to a concentration of 10 μM. Protein synthesis inhibitors, puromycin (100 μg/ml) or cycloheximide (0.1 mM), were used in some experiments.

Assay for histone mRNA concentrations. Total cell RNA was prepared from 5×10^6 cells, and the histone mRNAs were assayed by an S1 nuclease assay as previously described (24). The probes used were the following H3 genes: H3.2 and H3.1, derived from phage MM221 (23, 25); H3.614 derived from MM614; and H3.291 derived from MM291. These represent different H3 genes which code for the H3.1(H3.1 and H3.291) or H3.2(H3.2 and H3.614) proteins and have very similar DNA sequences in the protein-coding region (25; unpublished data). All of these genes have a *Sal*I site at amino acid 58, and this site was labeled with [γ-$^{32}PO_4$]ATP and polynucleotide kinase. An H2b.1 gene derived from MM221 was labeled at the *Ava*I site at amino acid 92 (24). One microgram of total cell RNA from each sample was routinely used in the assays.

Assay for histone gene transcription. Nuclei were prepared and incubated with [α-^{32}P]GTP for RNA synthesis as previously described (15). The nuclear RNA was prepared by extraction with phenol at 55°C and pH 5 as previously described (15). The RNA was separated from remaining nucleotides by gel filtration on Sephadex G-50 and hybridized to DNA dots. Hybridization was for 72 h in 50% formamide–0.75 M Na^+ at 52°C as previously described. The probes used were a coding region fragment (amino acids 58 to 126) of the H3.2 gene and a mouse actin cDNA (18) clone. The hybridized RNA was detected by autoradiography and quantitated by liquid scintillation counting.

Determination of deoxynucleotide levels. The levels of deoxynucleotide triphosphates were determined by high-pressure liquid chromatography by a modification of the method described by Garrett and Santi (11). Briefly, cells were pelleted and suspended in water at a concentration of 5×10^8 cells per ml, 1/10 volume of cold 4 M perchloric acid was added and extracted for 5 min on ice, and the debris removed by centrifugation. The solution was neutralized to pH 6 with 3 M KOH–0.3 M KH_2PO_4, and the insoluble potassium perchlorate was removed by centrifugation. To measure ribonucleotide triphosphate levels, the sample was

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chromatographed directly on a Whatman Partisil-10 SAX/25 column (4.6 mm by 25 cm). The column was eluted with a 20-ml gradient of 180 mM KH_2PO_4 (pH 5.5) to 420 mM KH_2PO_4 (pH 5.5) at a flow rate of 1 ml/min, followed by continued elution with 420 mM KH_2PO_4 (pH 5.5) at 27°C. The nucleotides were detected with a Varian LC5000 liquid chromatograph equipped with a Varichrom UV-Vis detector at 260 nm. The identity of each peak was confirmed by recovering it, treating it with calf intestinal alkaline phosphatase, and then chromatographing it on a reverse-phase column as described below. For determining deoxynucleotides, the sample was treated with 20 mM sodium periodate for 2 min at 25°C. Methylamine was then added to a concentration of 0.2 M from a 4 M stock solution (pH 7.5), and the sample was incubated for 30 min at 37°C. Glucose was then added to a concentration of 0.01 M, and the sample was incubated for 2 min at room temperature. This procedure destroys the ribotriphosphates (11). The sample is then applied to the SAX column. The ATP/ADP ratio of all samples was greater than 9:1.

In an alternative procedure, we analyzed the amount of ribonucleosides and deoxynucleosides. The sample was adjusted to pH 8.5 with KOH and then treated with 20 U of bovine intestinal alkaline phosphatase (Sigma type VII-S) per ml for 30 min at 37°C. The sample was then adjusted to pH 6, and the nucleosides were resolved by chromatography on a Supelco LC-18-DB deactivated reverse-phase column (4.6 mm by 25 cm). The column was eluted with 6 ml of solvent A (0.05 M KH_2PO_4 [pH 4.0]), followed by a 20-ml gradient with 50% methanol-water as solvent B (0 to 20% solvent B) at 35°C and a flow rate of 1 ml/min. To analyze the deoxynucleosides, the ribonucleosides were removed by binding to Affigel 601 (boryl-agarose) before the chromatography to allow accurate quantitation of the deoxynucleosides. The sample was incubated with a small amount of Affigel 601 at 4°C for 15 min in a microfuge tube. The resin was removed by centrifugation, and the incubation was repeated with fresh Affigel 601. The sample was adjusted to pH 5.5 with phosphoric acid and applied to the deactivated reverse-phase column. At least 90% of the total deoxynucleosides were present as the deoxynucleotide triphosphates, and these two procedures gave essentially identical results.

Materials. Radioisotopes were obtained from ICN Pharmaceuticals, Inc. Restriction enzymes were obtained from Bethesda Research Laboratories.

RESULTS

Histone mRNA concentrations are rapidly reduced after treating myeloma cells with hydroxyurea and cytosine arabinoside, and a new steady-state level of histone mRNA is obtained at 5 to 10% of the original level (24). This shift is due to a decrease in the rate of transcription and a decrease in the half-life of the mRNA. Since we are interested in systems in which transcription is rapidly regulated, we have investigated the time required to obtain changes in rates of histone mRNA synthesis and the effect of protein synthesis inhibitors on histone mRNA levels.

Changes in cytoplasmic histone mRNA levels. In addition to testing the effect of hydroxyurea and cytosine arabinoside, we have tested other inhibitors of deoxynucleotide metabolism and, hence, DNA synthesis on the steady-state concentration of histone mRNA. An S1 nuclease assay was used to detect specific histone mRNAs. In this assay, a histone gene is cut in the protein-coding region with a restriction enzyme and labeled at the 5′ end. The labeled fragment is then hybridized with total cell RNA under conditions of DNA excess. The S1-resistant DNA is then analyzed by polyacrylamide gel electrophoresis to separate the protected DNA fragments and detected by autoradiography. Since the hybridization is done in DNA excess, the intensity of the band is proportional to the amount of RNA in the sample. Two types of protected fragments are observed in this assay. One results from the protection of the gene to the end of the coding region by a number of different RNAs, and the other results from protection of the gene by the mRNA derived from this gene. The identity of the protected fragments has previously been determined by analyzing the S1-resistant DNA in parallel with DNA-sequencing reactions (25).

To test the linkage of histone mRNA levels and DNA synthesis in mouse myeloma cells, exponentially growing cells were treated with a number of inhibitors of DNA synthesis, and the histone mRNA levels were measured by the S1 nuclease assay. Two different probes were used: an H3 gene from MM614 which was cut at the *Sal*I site at amino acid 58 (Fig. 1A) and an *Ava*I-*Sal*I fragment from MM221 containing an H2b gene cut at amino acid 92(*Ava*I) and an H3 gene cut at amino acid 58(*Sal*I; Fig. 1B). In addition to the fragments produced by cleavage at the end of the coding regions and the end of the mRNAs, the H2b gene also protects a fragment (H2b variant in Fig. 1B) which results from cleavage in the coding region at an amino acid change at position 18 (24, 25). We have observed that all four histone H3 genes we have isolated are regulated coordinately (unpublished data; see Fig. 4C). The results in Fig. 1 show that all of the inhibitors except novobiocin dramatically reduced histone mRNA levels. FUdR, adenine arabinoside, mycophenolic acid, and methotrexate all reduce histone mRNA levels to about the same extent 1 h after treatment with the inhibitors (Fig. 1A). All of these inhibitors reduced the levels of deoxynucleotides at least 75% in the myeloma cells (see below). In contrast, novobiocin, which inhibited DNA synthesis 90%, as measured by incorporation of [^{3}H]thymidine, did not have any effect on either histone H3 or H2b mRNA levels (Fig. 1B). To detect the same amount of histone mRNA in cells treated with hydroxyurea or cytosine arabinoside, 10 times as much RNA was used compared with control cells or cells treated with novobiocin (Fig. 1B, lanes 3 to 6).

Changes in deoxynucleotide levels. Since all of these inhibitors except novobiocin probably interfere with nucleotide metabolism, it was possible that the drop in histone mRNA levels was paralleled by changes in deoxynucleotide levels. To test this possibility, the levels of deoxynucleotides were quantitated after perchloric acid extraction by high-pressure liquid chromatography (Fig. 2). The mouse myeloma cells we used in these experiments have unusually high levels of dCTP so it was very easy to follow accurately changes in the levels of this nucleotide. All of the inhibitors used reduced the level of dCTP dramatically (Fig. 2) and altered the levels of the other deoxynucleotides. Except for mycophenolic acid which also lowered the GTP levels (6), the levels of ribonucleotides were not changed. In contrast, novobiocin, an inhibitor of mammalian DNA replication (17), had no effect on deoxynucleotide concentrations (Table 1). These results suggest that the alterations in deoxynucleotide levels are related to the changes in histone mRNA levels.

Protein synthesis is required for the drop in histone mRNA levels. It had been previously shown by a translation assay that histone mRNA levels were not decreased if HeLa cells were treated with DNA synthesis inhibitors in the presence of inhibitors of protein synthesis (4, 26). To confirm that this

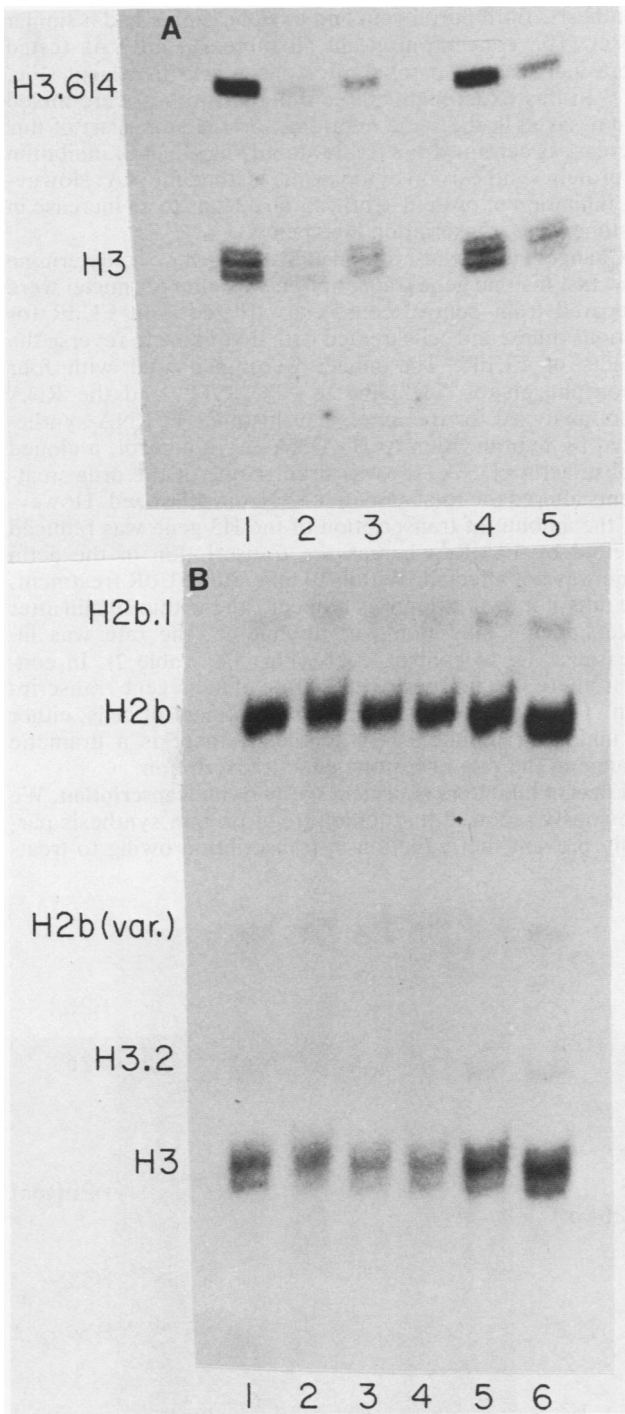

FIG. 1. Effect of different inhibitors of histone H3 mRNA levels. (A) Cells were treated for 1 h with various inhibitors, and total cell RNA was prepared. One microgram of RNA was hybridized to the H3.614 gene which had been labeled at the *Sal*I site at amino acid 58. The hybrids were treated with S1 nuclease, and the protected DNA fragments were resolved on a 6% polyacrylamide gel in 8.3 M urea and detected by autoradiography. The protected fragments are: H3 protection to the AUG codon by a number of H3 mRNAs and H3.614 protection by the H3 mRNA derived from this gene to the first nucleotide of the mRNA. Lane 1, control; lane 2, mycophenolic acid; lane 3, methotrexate; lane 4, control; lane 5, adenine arabinoside. (B) Cells were treated with various concentrations of novobiocin for 1 h (lanes 1 to 3) or with 5 mM hydroxyurea (lane 5) or 40 μg of cytosine arabinoside (lane 6) per ml, and total cell RNA was prepared from each sample. Either 0.5 μg (lanes 1 to 4) or 5 μg (lanes 5 and 6) of RNA was hybridized to an *Ava*I-*Sal*I fragment derived from phage MM221 (25) which contains a portion of a mouse H2b.1 gene and a mouse H3.2 gene (24). The fragment was end-labeled with [γ-$^{32}PO_4$]ATP, and polynucleotide kinase S1 nuclease-resistant DNA was detected and analyzed as described above. The bands are: H3 protection of the H3 gene to the AUG codon; H3.2 protection to the end of the H3.2 mRNA; H2b (variety) protection to the amino acid change at amino acid 18 in this gene (25); H2b protection to the AUG codon by a number of H2b mRNAs; and H2b.1 protection to the end of the H2b mRNA. The percentages in parentheses are the amount of inhibition of DNA synthesis observed. DNA synthesis was measured by incorporation of [^{3}H]thymidine into DNA for 15 min, 45 to 60 min after treatment with the inhibitor. Lane 1, novobiocin (500 μg/ml) (90%); lane 2, novobiocin (250 μg/ml) (74%); lane 3, novobiocin (100 μg/ml) (17%); lane 4, control; lane 5, hydroxyurea (98%); lane 6, cytosine arabinoside (95%).

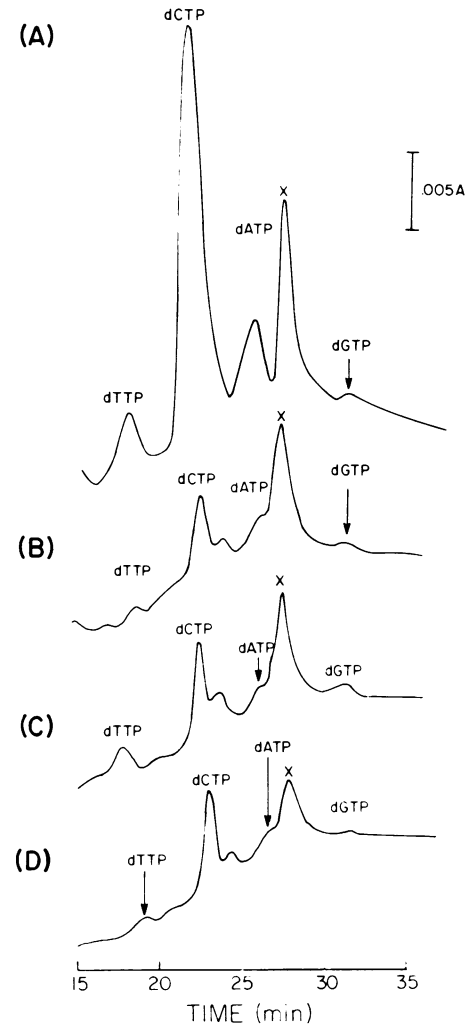

FIG. 2. Changes in deoxynucleotide triphosphate levels. Exponentially growing mouse myeloma cells were treated for 1 h with either 2 μM methotrexate, 2 μM FUdR, or 5 mM hydroxyurea. The perchloric acid-soluble fraction was analyzed for deoxynucleotide triphosphates by high-pressure liquid chromatography as described in the text. The position of the deoxynucleotide triphosphates as determined by standards is indicated. Peak X is a breakdown product of NADPH whose concentrations did not change significantly with drug treatment. A total of 5×10^6 cells were used in each assay. (A) Control cells, (B) FUdR, (C) hydroxyurea, (D) methotrexate.

TABLE 1. Levels of dCTP and histone mRNA in myeloma cells[a]

Treatment	dCTP	Histone H3 mRNA
Control	100	100
Hydroxyurea	19	8
FUdR	12	6
Cytosine arabinoside	18	6
Methotrexate	20	10
Adenine arabinoside	10	8
Mycophenolic acid	21	5
Novobiocin	105	90

[a] The levels of dCTP were determined in exponentially growing mouse myeloma cells by high-pressure liquid chromatography as described in the text. Equal amounts (5×10^6) of cells were assayed in each case. Before isolation of the nucleotides, cells were treated with the indicated inhibitor for 1 h. The levels of ribonucleotides were identical in each culture except when the cells were treated with mycophenolic acid which reduced the GTP levels and increased the UTP levels. The levels of histone H3 mRNA were determined by S1 nuclease analysis. The autoradiogram was scanned with a densitometer. The levels of dCTP and histone H3 mRNA were assigned an arbitrary value of 100 in exponentially growing (control) cultures.

was also the case in myeloma cells, we treated cells with both cycloheximide and either cytosine arabinoside, hydroxyurea, or FUdR. Figure 3 shows that both the H3 and H2b mRNAs are stable in the presence of DNA synthesis inhibitors if protein synthesis is inhibited. To show this response clearly, eight times as much RNA was analyzed for the drug-treated cultures (lanes 5 to 8) as in the control culture (lane 4).

Rapid changes in histone mRNA levels. Since FUdR has a very specific effect on thymidylate synthetase (22) and the block can be overcome simply by adding thymidine to the media without washing out the FUdR, this procedure was chosen for intensive study. Treatment of cells with FUdR results in a rapid drop in histone mRNA concentrations with kinetics similar to that that we had previously reported in these cells for hydroxyurea treatment (data not shown). Reversal of the FUdR-induced inhibition of DNA synthesis by adding thymidine to the media resulted in a rapid recovery of histone mRNA concentrations. An increase in cytoplasmic histone mRNA was detectable within 15 min after adding thymidine, and the control levels were reached within 1 h (Fig. 4A). The levels of H3.2 mRNA and H2b.1 mRNA increased in parallel. The H3 gene derived from MM614 codes for about 50% of the total H3 mRNA in the myeloma cells. The levels of this mRNA increased with the same kinetics, and this RNA represented the same fraction of total H3 mRNA throughout the recovery (Fig. 4B). This suggests that synthesis of many histone mRNAs is stimulated simultaneously and that the large amount of H3.614 mRNA present in exponentially growing cells is due to an increased rate of synthesis of this RNA.

Since inhibitors of protein synthesis prevent the degradation of histone mRNA (5, 26) and partially prevent the reduction in rate of histone gene transcription (24), we tested the effect of protein synthesis inhibitors on cells which had been treated with FUdR. Surprisingly, treatment with either cycloheximide or puromycin resulted in an increase in the concentration of histone mRNAs in cells which had been treated with FUdR for 45 min (Fig. 4). The increase is not as rapid (cf. Fig. 4B, lanes 2 to 5 and 7 to 10) as that observed after adding thymidine but levels close to control levels are reached within 60 to 90 min after inhibition of protein synthesis. Both puromycin and cycloheximide had a similar effect. The concentrations of all three H3 mRNAs tested were increased in parallel after puromycin treatment (Fig. 4C). In this experiment, three different probes were mixed and assayed in the same hybridization reaction. Part of this increase is certainly due to the stabilizing effect of inhibition of protein synthesis on cytoplasmic histone mRNA. However, inhibition of protein synthesis also leads to an increase in histone gene transcription (see below).

Changes in the transcription of histone genes. To determine how fast histone gene transcription was altered, nuclei were prepared from control cells, cells treated with FUdR for various times, and cells treated with thymidine to reverse the effects of FUdR. The nuclei were incubated with four ribotriphosphates, including [α-$^{32}PO_4$]GTP, and the RNA were analyzed for the amount of histone H3 RNA synthesized by hybridization to H3 DNA. As a control, a cloned mouse actin cDNA (18) was used. None of the drug treatments altered the total amount of RNA synthesized. However, the amount of transcription of the H3 gene was reduced fivefold by FUdR, whereas the transcription of the actin gene was not affected. Within 10 min after FUdR treatment, the rate of transcription was reduced, and within 10 min after reversing the inhibition with thymidine, the rate was increased close to control levels (Fig. 5A; Table 2). In contrast, there was no change in the rate of actin gene transcription. Thus, within 10 min after the treatment of cells, either to inhibit or initiate DNA synthesis, there is a dramatic change in the rate of histone gene transcription.

Effect of inhibitors of protein synthesis on transcription. We previously reported that inhibitors of protein synthesis partially prevent the reduction in transcription owing to treat-

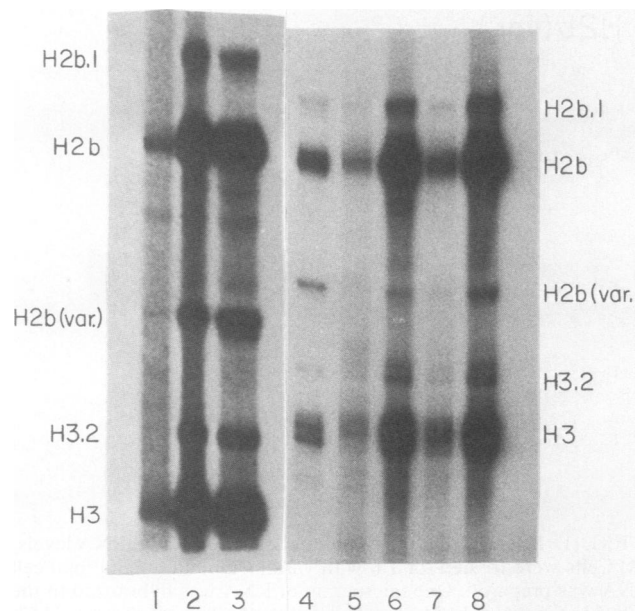

FIG. 3. Effect of cycloheximide on histone mRNA levels. Cytoplasmic mRNA was prepared from cells treated with various inhibitors of DNA synthesis for 40 min in the presence and absence of cycloheximide. The RNAs were analyzed as in Fig. 1B, and the protected fragments are identified as in Fig. 1B. Lane 1, FUdR (0.5 μg of RNA); lane 2, FUdR + cycloheximide (0.5 μg of RNA); lane 3, control (0.5 μg of RNA); lane 4, control (0.5 μg of RNA); lane 5, hydroxyurea (4 μg of RNA); lane 6, hydroxyurea + cycloheximide (4 μg of RNA); lane 7, cytosine arabinoside (4 μg of RNA); lane 8, cytosine arabinoside + cycloheximide (4 μg of RNA).

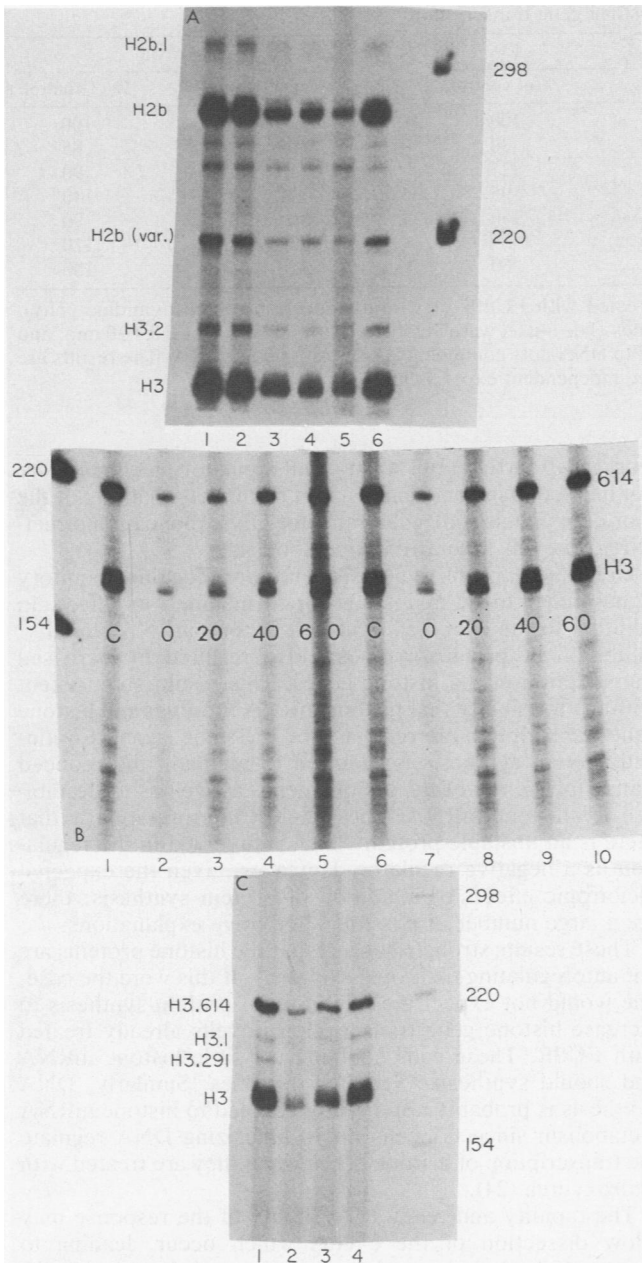

FIG. 4. Recovery of histone mRNA levels after inhibition by FUdR. (A) Cells were treated for 45 min with FUdR, and then thymidine was added to a final concentration of 10 M. RNA was prepared from cells at various times after refeeding thymidine and assayed for histone mRNA levels by using the *Ava*I-*Sal*I fragment containing the H3.2 and H2b.1 genes. The fragments are labeled as in Fig. 1B. The position of marker DNAs (a *Hin*fI digest of pBR322) is indicated. Lane 1, 60 min after adding thymidine; lane 2, 30 min after adding thymidine; lane 3, 15 min after adding thymidine; lane 4, duplicate culture at 15 min; lane 5, 45 min after treatment with FUdR; lane 6, control (no treatment). (B) Cells were treated as in (A) except that cycloheximide (0.1 mM; lanes 2 to 5) rather than thymidine (lanes 7 to 10) was added to half of the culture. The levels of H3 mRNA were assayed with the H3.614 gene as described in Fig. 1A. Lanes 1 and 6, control; lanes 2 and 7, 45 min of FUdR treatment; lanes 3 and 8, 20 min after addition of thymidine or cycloheximide; lanes 4 and 9, 40 min; lanes 5 and 10, 60 min. (C) Cells were treated with FUdR for 45 min, and then puromycin was added. RNA was prepared from cells at various times and then assayed for histone H3 mRNAs. Three different H3 genes were labeled at the *Sal*I site at amino acid 58 (to identical specific activities), and the DNAs mixed and hybridized to the RNA. The hybrids were analyzed as in Fig. 1A. The bands H3.1, H3.291, and H3.614 represent protection by the specific mRNA to each of the H3 genes assayed. The position of a marker DNA (pBR322 cut with *Hin*fI) is indicated. Lane 1, control; lane 2, FUdR (45 min); lane 3, FUdR (45 min) and puromycin (30 min); lane 4, FUdR (45 min) and puromycin (60 min).

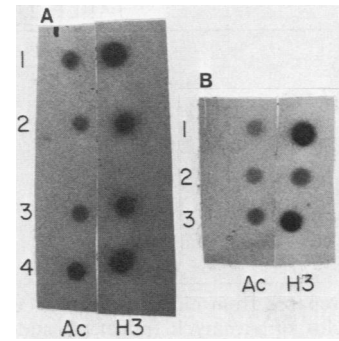

FIG. 5. Transcription of histone H3 mRNA. (A) Nuclei were isolated from control cells, cells treated with FUdR for 10 min, cells treated with FUdR for 45 min, and cells treated with FUdR for 45 min, and thymidine was added for 10 min. The nuclei were incubated with [α-$^{32}PO_4$]GTP for 30 min, and RNA was prepared. Equal amounts of RNA (2×10^6 cpm) were hybridized to 5 μg of H3 DNA (a cloned fragment coding for amino acids 58 to 126) and 5 μg of an actin cDNA (18) as described in the text. The hybrids were detected by autoradiography. Lane 1, control; lane 2, FUdR (10 min); lane 3, FUdR (45 min); lane 4, FUdR (45 min) and thymidine (10 min). (B) Nuclei were prepared from control cells, cells treated with FUdR for 45 min, and cells treated with FUdR for 45 min followed by puromycin for 25 min. RNA was synthesized by the isolated nuclei, and equal amounts of RNA (5×10^6 cpm) were analyzed as in (A), by hybridization to H3 DNA or actin DNA. Lane 1, control; lane 2, FUdR (45 min); lane 3, FUdR (45 min) and puromycin (30 min).

ment of cells with hydroxyurea (24). We tested the effect of cycloheximide and puromycin on transcription of the histone genes in cells which had been treated with FUdR to see whether the increase in histone mRNA levels was due to increased transcription as well as a decreased rate of turnover. Both inhibitors significantly stimulated histone gene transcription but had little effect on the transcription of the actin gene (Fig. 5). Transcription of the histone H3 genes was not increased to control levels but was similar to that observed in control cells treated with cycloheximide (24; Table 2). The slower recovery of histone mRNA levels after inhibition with protein synthesis relative to stimulation with thymidine is probably due to the smaller increase in rate of histone gene transcription. Thus, in addition to preventing the decrease in histone gene transcription and half-life when DNA synthesis is inhibited in cells synthesizing DNA, inhibition of protein synthesis increases the levels of histone mRNA in cells not synthesizing DNA, partly by increasing the rate of transcription.

DISCUSSION

Study of the mechanism of regulation of mRNA concentrations is facilitated if rapid changes in mRNA metabolism can be induced by defined treatments and if the effect is reversible. Regulation of the histone mRNA levels in cultured cells meets both these requirements. In addition, these

TABLE 2. Effect of FUdR on histone gene transcription[a]

Treatment (min)	H3			Actin	
	cpm	(% of input)	% of control	cpm	% of control
Control	600	(0.03)	100	120	100
FUdR (10)	180	(0.009)	30	103	85
FUdR (45)	150	(0.007)	25	110	90
FUdR (45) + Thy (10)	580	(0.029)	95	170	140
FUdR (45) + Thy (25)	920	(0.046)	150	105	90
FUdR (45) + cyclo (25)	325	(0.016)	55	135	110
FUdR (45) + puro (25)	360	(0.018)	60	120	100

[a] Nuclei were prepared from mouse myeloma cells which had been treated with FUdR for various times, and then thymidine (Thy), cycloheximide (cyclo), or puromycin (puro) was added for the indicated times. The nuclei were incubated with [α-$^{32}PO_4$]GTP for 30 min, and RNA was prepared. Equal amounts (2×10^6 cpm) of RNA were hybridized to DNA dots containing H3 DNA or actin cDNA. The results are from a single experiment but are representative of results obtained in three independent experiments.

genes are expressed at some point in the life of all cells. There is a rapid effect on both mRNA stability and gene transcription in this system induced by inhibition of DNA synthesis with agents that perturb deoxynucleotide metabolism. The effect is as rapid as that seen in other rapidly responding eucaryotic systems: the stimulation of transcription by ecdysone in *Drosophila* (1) and the stimulation of mouse mammary tumor virus transcription by hydrocortisone (21, 27).

Our results are consistent with the report of Groppi and Coffino (12) that in S49 lymphoma cells, there is significant histone synthesis in G1 cells which are not synthesizing DNA. This is certainly not the case in all cell types, as both G1 HeLa cells (20) and G1 3T6 cells (7) contain very low levels of histone mRNAs, although the histone genes are transcribed at a significant rate in G1 3T6 cells (7). Stimac and co-workers have shown (28) that when G1 HeLa cells, which contain very low levels of histone mRNA, are treated with cycloheximide, the concentration of histone mRNA in the G1 cells is dramatically increased. In view of these findings, it is not surprising that inhibiting protein synthesis in cells which have already been treated with FUdR increases histone mRNA levels. These divergent results can be understood if the molecules involved in regulating histone mRNA metabolism are not tightly coupled to DNA synthesis. The results we obtained with novobiocin support this possibility.

The mechanism of regulation of histone mRNA concentrations is not clear. Since there are coordinate changes in histone mRNA half-life in the cytoplasm and histone gene transcription in the nucleus, it is possible a single "signal" molecule is produced which affects both compartments simultaneously. This signal molecule could be coupled with deoxynucleotide metabolism. All of the inhibitors which affect histone mRNA levels also alter deoxynucleotide levels. Aphidicolin, which dramatically reduces histone mRNA levels (14) and also inhibits DNA replication has a similar effect on dCTP levels as the other drugs tested (unpublished data). Another inhibitor of DNA replication in mammalian cells, novobiocin (17, 19), did not alter either histone mRNA levels or deoxynucleotide levels. It should be pointed out, however, that novobiocin may also affect other cellular processes (29). In support of this model, we have observed that all the inhibitors tested alter the level of the deoxynucleotides (particularly dCTP) in a similar fashion (Marzluff and Henricks, unpublished data) and do not just affect the deoxynucleotide targeted by the particular drug. A close interelationship of the metabolism of all the deoxynucleotides is expected if the "replitase" model of Pardee and coworkers is correct (19). Most small regulatory molecules are not metabolic intermediates and it is unlikely that any of the four deoxynucleotide substrates for DNA replication directly regulates histone mRNA metabolism.

Continued protein synthesis is required for this inhibitory signal either to be maintained or to maintain its effect. In addition to the expected effect on histone mRNA stability, inhibition of protein synthesis also resulted in increased transcription of the histone genes. This result is consistent with the possibility that histone mRNA turnover and histone gene transcription are regulated by the same signal. Continued protein synthesis is required to maintain the reduced transcription rate of the histone genes as well as to destabilize the histone mRNA. The simplest interpretation is that there is an unstable protein which is involved in the regulation as a negative regulator. However, given the expected pleiotropic effects of inhibition of protein synthesis, there are a large number of possible alternative explanations.

These results strongly suggest that the histone proteins are not autoregulating their own synthesis. If this were the case, one would not expect the inhibitors of protein synthesis to increase histone gene transcription in cells already treated with FUdR. These cells contain very little histone mRNA and should synthesize very few histones. Similarly, DNA synthesis is probably not directly coupled to histone mRNA metabolism since G1 cells not synthesizing DNA regulate the transcription of histone genes when they are treated with hydroxyurea (24).

The rapidity and ready reversibility of the response may allow dissection of the events which occur, leading to changes in both the rate of transcription and the altered half-life. We are currently trying to identify the molecules involved.

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