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Use of a Cell Cycle Mutant to Delineate the Critical Period for the Control of Histone mRNA Levels in the Mammalian Cell Cycle

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Temporal analysis of DNA replication and histone mRNA accumulation in a hamster fibroblast cell cycle mutant (K12) showed that histone mRNA accumulates periodically during the cell cycle and reaches its highest level in the S phase. The direct correlation between the initiation of DNA synthesis and the accumulation of histone mRNA to high levels in S phase demonstrated the strict interdependence of these two events. Moreover, a critical period necessary for histone mRNA accumulation occurred late in G1 phase. If cells were incubated at the nonpermissive temperature during this critical period, the amount of histone mRNA remained at the basal level. Transcription rate measurements indicated that the triggering of histone mRNA synthesis occurred in late G1 and this mRNA was synthesized at its maximal rate 3 to 5 h before its peak of accumulation. However, if cells were prohibited from synthesizing DNA as a consequence of the temperature-sensitive block in G1, the synthesis of histone mRNA was not initiated.

K12 is a temperature-sensitive (*ts*) cell cycle mutant which grows normally at 35°C, but is arrested in late G1 when incubated at 40.5°C (2, 25, 36). These fibroblast cells, grown as monolayers, can be synchronized by serum deprivation since the rate of cell proliferation in culture is dependent on both the concentration of different factors present in the medium (18) and the cell density of the cultures (9). Previously, it was demonstrated that histone protein synthesis was inhibited in K12 cells grown at the nonpermissive temperature (29). Subsequently, we described the use of this *ts* hamster cell cycle mutant to show quantitatively that histone and DNA syntheses were coupled (6). Results obtained from pulse-labeling of synchronized K12 cells with [^{3}H]lysine and analysis of the relative rates of histone synthesis throughout the cell cycle demonstrated that these hamster cells were actively synthesizing four- to fivefold more histone proteins at the peak of DNA synthesis than at the basal level. These observations raised two important questions: (i) was the increased histone synthesis during S phase due to the new transcription of histone genes, and (ii) is there any specific signal which triggers histone synthesis and, if there is, during which phase of the cell cycle does this crucial event occur?

The approach we have used to define the point in the cell cycle at which these regulatory processes may be activated is to determine the effect of the K12 *ts* mutation on the rate of histone mRNA synthesis and accumulation. By using two strains of yeast carrying *ts* cell division cycle mutations in late G1 or S, it has been shown that the activation of histone mRNA synthesis occurs in late G1 (16, 17). In view of these results, it was of interest to determine the timing of histone mRNA synthesis in growing mammalian cells which have a considerably longer cell cycle.

In this report, we present data comparing the temporal events of DNA synthesis and histone mRNA accumulation throughout the K12 cell cycle. In particular, we examined in detail the rate of transcription and histone mRNA levels during the G1 and early S periods when K12 cells were incubated at both the permissive and nonpermissive temperatures. Our results demonstrate that, whenever steady-state levels of histone mRNA drop dramatically to the basal level as a result of the mutation, there is always a parallel decrease in the histone transcription rate. In addition, we show that histone mRNA accumulates to its peak level 3 to 5 h after the maximal rate of its transcription, which in turn precedes the peak of DNA synthesis by 4 to 6 h. As a result of various temperature shift experiments, we define a critical period in mid- to late G1 crucial for the synthesis and accumulation of histone mRNA in S phase.

MATERIALS AND METHODS

Cell culture. Conditions for culturing and synchronization of the K12 cells have been described previously (6). Essentially, K12 cells were maintained in Dulbecco modified Eagle medium containing 4.5 mg of glucose (GIBCO Laboratories, Grand Island, N.Y.) per ml, supplemented with 10% cadet calf serum (Biocell, Carson, Calif.). For synchronization, cells were seeded in 150-mm-diameter culture dishes at a density of 10^4 cells per cm^2 in 25 ml of Dulbecco modified Eagle medium containing 1 mg of glucose per ml and 10% cadet calf serum and incubated for 4 days without a change of the medium. As a result, most cells were arrested in G1 phase by serum deprivation. Addition of fresh medium caused the cells to proliferate synchronously throughout the cell cycle.

DNA synthesis. The rate of DNA synthesis during the cell cycle was monitored by pulse-labeling synchronized cells with 0.25 μCi of [*methyl*-^{3}H]thymidine per ml for 30 min. The amount of incorporated thymidine was measured by the radioactivity recovered in the trichloroacetic acid-precipitable material (6, 26). It has been shown previously that this method of measuring the rate of DNA synthesis is comparable to that of labeling of the cell nuclei (7).

Cytoplasmic RNA extraction. The procedures for preparation of total cytoplasmic RNA were as described previously (22) with the following modifications. About 4×10^7 cells obtained from one 150-mm-diameter culture dish were pelleted and lysed with 0.5% Nonidet P-40 in isotonic high-pH buffer (0.14 M NaCl, 0.01 M Tris-hydrochloride [pH 8.4], 0.0015 M $MgCl_2$). Total cytoplasmic RNA was extracted from the pooled Nonidet P-40 supernatants. To 2.8 ml of cell supernatant, 0.8 ml of NETS buffer (0.1 M NaCl, 10 mM

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Tris-hydrochloride [pH 8.4], 1 mM EDTA, 1% sodium dodecyl sulfate) was added at 4°C. After two extractions with phenol-chloroform (1:1) preequilibrated with 10 mM Tris (pH 7.4)–1 mM EDTA, the aqueous phase was extracted with chloroform and adjusted to 0.3 M NaOAc (pH 5.4) and the RNA was precipitated at −20°C with 95% ethanol.

RNA blot hybridization. Conditions for analyzing the RNA samples on denaturing formamide-formaldehyde gels were described before (22). After blot transfer, hybridization was performed with either ^{32}P-labeled histone gene probes or mouse actin cDNA probe prepared by nick-translation. The specific activity of the labeled DNA ranged from 0.5×10^8 to 1×10^8 cpm/μg. Generally, about 1×10^7 to 5×10^7 cpm of labeled probe was used in each hybridization, which was carried out for 16 to 18 h at 42°C. The blots were exposed to Kodak XAR-5 film at −70°C, using a Lightning-plus intensifying screen, for 1 to 5 days. Autoradiographs of gels were quantitated by scanning the film at 650 nm with a Gilford spectrophotometer interfaced with a Bascom Turner Data Center (series 8000; Bascom Turner Instruments, Newton, Mass.) which was used to integrate the peak area.

Histone gene probes. The mouse histone plasmid subclone pMH3.2 was provided to us by W. E. Marzluff and D. B. Sittman (Florida State University, Tallahassee). This subclone contained a portion of the mouse *H3.2* gene and was derived from λ phage MM221 (33).

Using the mouse histone subclone pMH3.2 as hybridization probe, we isolated several hamster genomic sequences from a Charon 4A Chinese hamster genomic library (gift from P. Jackson, Los Alamos National Laboratory, Los Alamos, N.M.) which cross-hybridized with pMH3.2. One of these hamster genomic sequences contained a 3.7-kilobase *Eco*RI fragment which was subcloned into the *Eco*RI site of pUC8 (37). This plasmid, designated pAAD3.7, was shown to contain the hamster *H3.2* gene by the criteria of restriction mapping and DNA sequencing (L. K. Kung and A. S. Lee, unpublished data).

Actin cDNA probe. The mouse actin cDNA plasmid was obtained from S. Sharp, R. LaPolla, and N. Davidson (California Institute of Technology, Pasadena). This plasmid is similar to the actin plasmids previously described (27).

In vitro labeling of nuclear RNA. The procedure used for in vitro labeling of nuclear RNA is a modification of previously described methods (5, 19, 24). Synchronized K12 cells were released from serum block with a change of fresh medium. At various times after the addition of fresh medium, cells in 150-mm-diameter culture dishes were rinsed with cold phosphate-buffered saline and suspended in reticulocyte standard buffer (10 mM Tris [pH 7.4], 10 mM NaCl, 3 mM $MgCl_2$, 0.1 mM $CaCl_2$). Cells were lysed with 0.5% Nonidet P-40 and the nucleus pellet was suspended in nuclei storage buffer (50 mM Tris [pH 8.4], 5 mM $MgCl_2$, 0.1 mM EDTA, 40% glycerol). The transcription reaction mixture contained the following: nuclei (equivalent to 200 μg of DNA), 30% glycerol, 2.5 mM dithiothreitol, 1 mM $MgCl_2$, 70 mM KCl, 0.25 mM GTP, 0.25 mM CTP, 0.5 mM ATP, and 250 μCi of [α-^{32}P]UTP (3,000 Ci/mmol). The mixture was incubated at 37°C for 15 min and terminated by addition of reticulocyte standard buffer. The nuclear RNA was extracted as described previously (13, 23). The nuclei were suspended in 1.5 ml of high-salt buffer containing 30 μg of pancreatic DNase. The solution was incubated at 65°C for 15 s with agitation, and 30 μl each of 20% sodium dodecyl sulfate, 0.5 M EDTA, and 3 M NaOAc (pH 5.4) and 2.5 ml of 60 mM NaOAc (pH 5.4)–10 mM EDTA were added. A 200-μg portion of yeast tRNA was added as carrier and the solution was extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) preequilibrated with 60 mM NaOAc (pH 5.4)–10 mM EDTA and once with chloroform. After alcohol precipitation, the RNA pellet was suspended in 500 μl of 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Sigma Chemical Co., St. Louis, Mo.).

Measurement of transcriptional rate. Conditions for hybridization of the in vitro labeled nuclear RNA to excess DNA were as described before (22). In all experiments, 100 μg of cesium chloride gradient-purified plasmid pAAD3.7 DNA was baked onto each 25-mm nitrocellulose filter (type HA; Millipore Corp., Bedford, Mass.). The amount of radiolabeled RNA used in each hybridization ranged from 1.3×10^6 to 2.8×10^6 cpm. One-quarter of each filter containing 25 μg of plasmid DNA was used for each hybridization reaction. The background hybridization to filters with no DNA was about 15 cpm.

RESULTS

Histone mRNA accumulation is cell cycle dependent. First, we determine the relationship between the histone mRNA levels and the rate of DNA synthesis during the K12 cell cycle. DNA synthesis was monitored by pulse-labeling the cultures with [*methyl*-^{3}H]thymidine. At the same time, total cytoplasmic RNA was extracted. Equal amounts of each RNA sample were electrophoresed on denaturing formamide-formaldehyde gels and blotted onto nitrocellulose papers. To detect the hamster histone transcripts, we used as hybridization probes pMH3.2 containing the mouse *H3.2* gene and pAAD3.7 containing the hamster *H3.2* gene. Both the mouse and hamster *H3.2* probes hybridized to a hamster transcript of about 930 nucleotides. The relative levels of histone *H3.2* mRNA were quantitated by densitometric scanning. A comparison of the rate of DNA synthesis and the histone mRNA levels at various times during the cell cycle is shown in Table 1. It is evident from these data that as cells progress through their cycle, which is reflected in the enhanced rate of [^{3}H]thymidine incorporation, the amount of histone *H3.2* mRNA also increases in parallel, reaching its maximal level during the S phase.

Given that histone mRNA accumulation is cell cycle dependent, it was of interest to us to investigate the possibility that within the cell cycle there might be a shorter period which alone is responsible for the increase in histone mRNA content.

Effect of *ts* mutation on histone mRNA level. By using temperature shift experiments with synchronous cultures, the period at which the mutated function is required for the

TABLE 1. DNA synthesis and relative histone mRNA levels during incubation at 35°C

Time (h)	[^{3}H]thymidine incorporation (cpm)[a]	Relative histone *H3* mRNA content[b]
0	41	16
8	287	41
14	574	75
16	527	110
18	520	88
20	289	51
22	186	41
24	176	20

[a] Average of two experiments.
[b] Determined by densitometric scanning.

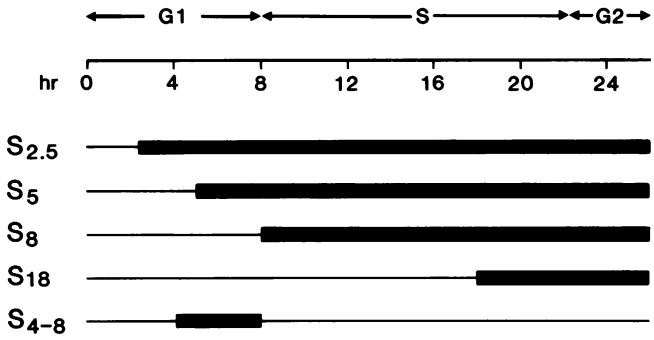

FIG. 1. Experimental design of temperature shift experiments. In each experiment, two sets of synchronized cells were prepared. One set was subjected to the temperature shift(s) indicated. The other set was incubated continuously at 35°C; DNA or RNA samples extracted from these dishes served as controls for the experimental points obtained at 40.5°C. The subscript refers to the time during the cell cycle at which the cells were shifted from 35 to 40.5°C. The thin line represents the incubation period at 35°C, and the thick line represents that at 40.5°C.

K12 cells to progress through the cell cycle was determined to be 1.8 to 4 h preceding the initiation of DNA synthesis (2, 25, 36). As a consequence, these cells are arrested in the second half of G1 phase when incubated at the nonpermissive temperature (40.5°C). We then asked whether any of the signals (transcriptional or post-transcriptional) affecting the temporal coordination of histone mRNA occurred within this period.

By shifting K12 cells to 40.5°C at various times during the cell cycle, we determined whether the levels or kinetics of accumulation of histone mRNA were affected in any way. The schemes for the five temperature shift experiments described below are outlined in Fig. 1. Initially, we synchronized parallel cultures of K12 cells at 35°C by serum deprivation. Upon addition of the fresh medium, both sets of K12 cells progressed normally through G1. At 2.5 h, one set of cells was shifted to 40.5°C and continued to be incubated at 40.5°C for the rest of the cell cycle, whereas the other set served as a control and was incubated continuously at 35°C. At various times during the cell cycle, the rate of DNA synthesis, as well as histone mRNA levels, was determined. The rationale for this experiment is that, if histone mRNA were to accumulate in the cell cycle when the cells are kept at 35°C for only 2.5 h, that would demonstrate that the message is made in early G1. The RNA blot hybridization pattern for both sets of cells is shown in Fig. 2A. It is clear that the cells which were shifted to 40.5°C in early G1 did not accumulate histone mRNA to the high levels as seen in the control cells. In a similar experiment in which the cells were shifted to 40.5°C after 18 h of incubation at 35°C, normal levels of histone mRNA were observed (Fig. 2B). Correspondingly, no DNA synthesis was detected for cells shifted to 40.5°C at 2.5 h, whereas control levels of DNA synthesis were observed in cells shifted to 40.5°C after 18 h (data not shown). Thus, it can be concluded that the events leading to the accumulation of histone mRNA in early S occur between 2.5 and 18 h after release of cells from quiescence.

Critical period of histone mRNA accumulation. To more precisely define the period necessary for histone mRNA accumulation, we incubated K12 cells for 5 or 8 h at 35°C and then shifted to 40.5°C for the rest of the cell cycle. We monitored simultaneously the rate of DNA synthesis and histone *H3.2* mRNA levels at various times; the results are shown in Fig. 3. It is evident that incubation of K12 cells at 35°C for the first 5 h after serum release was not sufficient to produce increased levels of histone *H3.2* mRNA in S phase (Fig. 3A). In contrast, when K12 cells were incubated for 8 h at 35°C before shifting to 40.5°C, a substantial accumulation of histone mRNA was observed (Fig. 3B). This indicated that, in our cell system, some critical event must have occurred between the 5- and 8-h period in G1 to allow histones to accumulate to high levels in early S.

The slight decrease in the peak levels of both DNA synthesis and histone mRNA observed in cells incubated for 8 h at 35°C (Fig. 3B) as compared with the control cells was probably due to a small subpopulation of K12 cells which was slightly out of synchrony. Since there is a natural variation in the rate of cell transversing through the cell cycle, the slower growing cells might still be in mid-G1 during the shift to 40.5°C 8 h after serum release. This small subpopulation of cells would be arrested in G1 and would not be able to initiate DNA synthesis or accumulate histone mRNA to high levels in S phase.

To further support our findings concerning the significance of this late G1 phase, we subjected a set of K12 cells to incubation at 40.5°C only during the 4- to 8-h period after release from serum deprivation. If this period is indeed important in histone mRNA accumulation we should expect significant inhibition in histone mRNA production. As in the previous experiments, we monitored the rate of DNA synthesis and the histone mRNA level. The results are shown in Fig. 3C. There is a three- to fourfold reduction of both the rate of DNA synthesis and histone mRNA levels as compared with the control samples.

The residual peak of DNA synthesis and the small amount of histone mRNA accumulated at around 18 h after serum release can again be explained by the presence of a small subpopulation of slower growing K12 cells. By the end of the 4-h incubation period at 40.5°C, these cells will emerge unaffected by the *ts* mutation block and, upon reincubation at 35°C, will produce a certain amount of histone mRNA later in the cell cycle, as compared with the control set. This delay does occur and is reflected in both DNA synthesis and mRNA accumulation (Fig. 3C). Moreover, this delay of about 4 h is equal to the time period of incubation at 40.5°C. On the other hand, faster growing cells and the majority of the cell population growing at the "normal" rate will be at this critical stage in their cycle when subjected to the nonpermissive temperature from 4 to 8 h. As a result, these

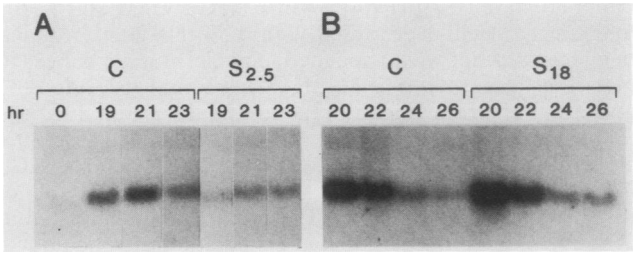

FIG. 2. Histone *H3.2* mRNA levels after temperature shift. Synchronized K12 cells were shifted from 35 to 40.5°C at either 2.5 ($S_{2.5}$) or 18 (S_{18}) h after addition of fresh medium. At the times indicated, total cytoplasmic RNA was isolated from these cells and control cells which had been incubated continuously at 35°C (C). About 10 μg of RNA was applied to each gel lane and, after electrophoresis, was blotted and hybridized with nick-translated pMH3.2. The autoradiograms are shown.

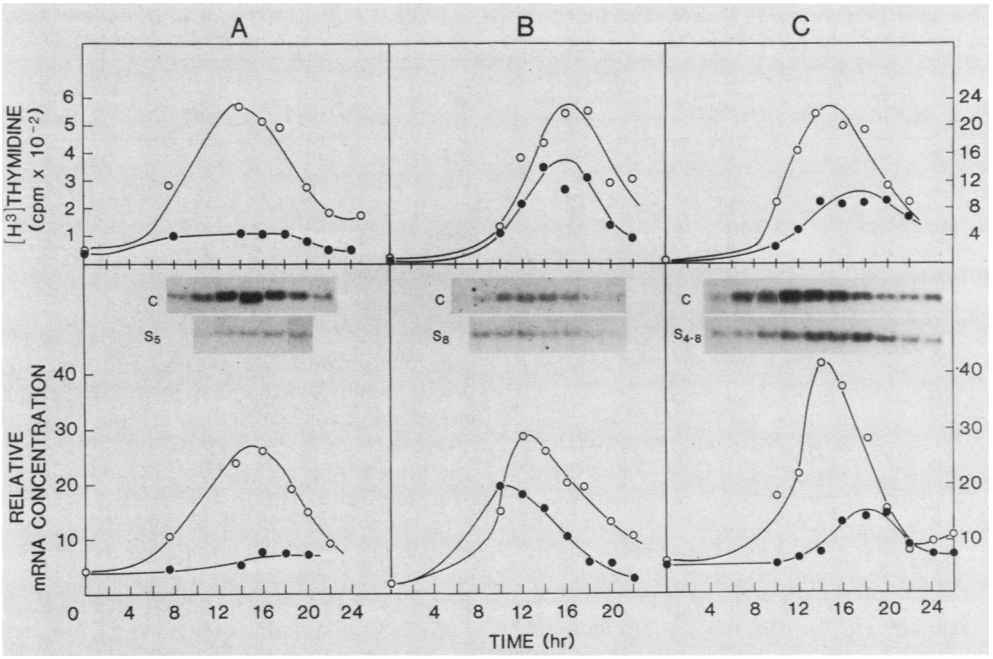

FIG. 3. Effect of K12 *ts* mutation on DNA synthesis and histone *H3.2* mRNA levels. Duplicate sets of synchronized K12 cells were assayed for DNA synthesis (top) and for histone *H3.2* mRNA levels (bottom) as described in the legend to Fig. 1. The amount of [*methyl*-^{3}H]thymidine counts indicated represents 2% of the total incorporation into a 60-mm-diameter dish. The scale on the left was used for (A) and (B), and the scale on the right was used for (C). Radiolabeled pMH3.2 was used as hybridization probe for histone *H3.2* mRNA. Symbols: ○, values obtained for control cells incubated continuously at 35°C; ●, values obtained from cells shifted to 40.5°C after 4 (A) or 8 (B) h of incubation at 35°C. In (C), the cells were incubated at 40.5°C from 4 to 8 h after addition of fresh medium.

cells will be prevented from synthesizing DNA as well as histone mRNA.

In summary, the data obtained from this experiment reaffirm our previous results that within the period of 5 to 8 h after stimulation of growth a critical event must occur which allows histone mRNA to accumulate 6- to 10-fold higher than the basal level. Furthermore, once this critical event is effected, continued incubation at the nonpermissive temperature is not necessary to cause the dramatic decrease in histone mRNA levels.

To demonstrate that this block in G1 specifically affects the histone genes, a control experiment was performed. The actin cDNA probe was used for determining the levels of actin mRNA under the conditions when cells were blocked in G1 (5 h of incubation at 35°C followed by placing cells at the nonpermissive temperature). Actin mRNA levels, unlike histone mRNA, were not affected by the block in G1 (Fig. 4). Similar levels of actin mRNA were observed in cells progressing through the cell cycle at 35°C (data not shown).

Since in all of the experiments described we have consistently observed that, when DNA synthesis was not being initiated, histone mRNA levels failed to increase above the basal level, a crucial question arises: was this due to the failure of the cells to initiate transcription of histone genes or to the rapid degradation of newly synthesized histone mRNA as the result of the inhibition of DNA synthesis?

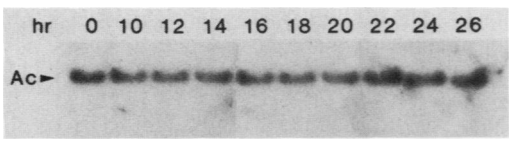

FIG. 4. Actin mRNA levels in cells blocked in G1. Synchronized K12 cells were placed at 40.5°C after 5 h of incubation at 35°C following serum release (time zero). Total cytoplasmic RNA was isolated at the indicated time points. About 10 μg of RNA per lane was used. Hybridization was performed with nick-translated actin cDNA plasmid. The position of actin mRNA (Ac) is indicated.

Effect of *ts* mutation on rate of transcription. To distinguish between the two possibilities given above, we examined the rate of transcription of histone genes under the conditions when no accumulation of histone mRNA was observed (that is, by shifting K12 cultures to 40.5°C 5 h after release from serum starvation). At the same time, we measured the rate of transcription in K12 cells which were incubated continuously at 35°C after serum release.

Nuclei isolated from synchronized K12 cells at various times after serum release were labeled for 15 min with [α-^{32}P]UTP. Nuclear RNA was extracted and hybridized to pAAD3.7 immobilized on nitrocellulose filters. The amount of radioactivity bound to the filter was determined after extensive washing.

For the control cells which were progressing through the cell cycle at 35°C, we observed a three- to fourfold increase in the rate of transcription of the histone *H3* gene, which reached its maximum at about 10 h after serum release (Table 2). Two points are worthy of note. (i) The extent of transcriptional rate increase is lower than histone mRNA accumulation during the cell cycle. Therefore, this increase in transcription does not fully account for the observed steady-state levels of histone mRNA in S phase. (ii) The rate of histone mRNA synthesis peaks 3 to 5 h before its maximal accumulation.

In contrast to cells incubated at 35°C, measurement of the transcription rate of *H3* genes under the conditions when cells were shifted to 40.5°C h after incubation at 35°C revealed only a minor increase at 10 h (Table 3). This small

TABLE 2. In vitro transcription rates of the *H3* gene during continuous incubation at 35°C

Time (h)	Input (10^6 cpm)	cpm bound[a]		*H3* mRNA hybridized (avg cpm)[b]
		pBR322	pAAD3.7	
3	1.3	0	38	26
	2.6	0	64	
7	1.3	2	54	42
10	1.3	10	108	83
12	1.3	0	60	43
	2.6	4	107	
16	1.3	0	40	31

[a] Calculated by subtracting background counts bound to blank filters.
[b] Represent parts per million of input radioactivity hybridized to pAAD3.7.

increase was likely to be caused by the small subpopulation of slower growing cells which had escaped the block of the *ts* mutation. Therefore, under the conditions when steady-state levels of histone mRNA do not accumulate appreciably higher than the basal level (see Fig. 3A), histone gene transcription apparently was not triggered. Further, we demonstrated that continuous incubation of cells at 40.5°C from the moment of their release from the serum block did not result in the initiation of histone transcription (Table 4). In this case, since all the cells were blocked in mid-G1, they were incapable of initiating histone mRNA synthesis.

DISCUSSION

The level at which histone genes are regulated during the cell cycle has been investigated previously by many research groups (4, 10, 11, 15, 20). Recently, with the availability of cloned, homologous histone gene probes, direct measurements of the rates of synthesis, accumulation, and turnover of histone mRNA in HeLa, mouse myeloma, mouse erythroleukemia, and mouse 3T6 fibroblast cells have been reported (1, 7, 8, 12, 14, 28, 34). From a variety of experiments using different methods of cell synchronization and of assaying RNA levels, it has been generally observed that there is a periodic accumulation of histone mRNA during the mammalian cell cycle and that both transcriptional and post-transcriptional processes regulate the histone mRNA. Our results obtained with the hamster mutant cell line K12 appeared to be in agreement with the previous observations that histone *H3* mRNA levels accumulated gradually and reached a maximum during S phase. In addition, we showed that in K12 cells the triggering of histone mRNA synthesis occurred in late G1 and that this mRNA was synthesized at its maximum rate 3 to 5 h before its peak of accumulation, which in turn preceded the peak of DNA and histone

TABLE 3. In vitro transcription rates of the *H3* gene when cells were shifted to 40.5°C 5 h after incubation at 35°C

Time (h)	Input (10^6 cpm)	cpm bound[a]		*H3* mRNA hybridized (avg cpm)[b]
		pBR322	pAAD3.7	
7	1.3	0	44	27
	2.6	3	61	
10	1.3	1	50	35
	2.6	7	88	
12	1.3	0	44	29
	2.6	0	70	
16	1.3	6	38	29

[a,b] See footnotes *a* and *b*, Table 2.

TABLE 4. In vitro transcription rates of the *H3* gene during continuous incubation at 40.5°C

Time (h)	Input (10^6 cpm)	cpm bound[a]		*H3* mRNA hybridized (avg cpm)[b]
		pBR322	pAAD3.7	
7	2.8	0	60	21
10	1.4	18	28	20
	2.8	0	58	
13	1.4	8	44	31

[a,b] See footnotes *a* and *b*, Table 2.

syntheses (6). Also, our measurement of the rate of histone gene transcription during the cell cycle suggested that the three- to fourfold increase in the transcriptional rate could not account for the six- to tenfold increase in the mRNA levels during its peak level. Therefore, our results, as well as others, are indicative of other control mechanisms which operate on the histone mRNA (1, 7, 12, 14).

In spite of the rapid advance made in measurement of histone mRNA levels and their rates of transcription during the cell cycle, relatively little is known concerning the events during the late G1 and S period which may be crucial for the periodic accumulation of histone mRNA. This lack of knowledge is partly due to the fact that many previous experiments were performed with cells synchronized at the G1/S border and partly due to the unavailability of well-characterized mammalian cell cycle mutants blocked at various points during the cell cycle.

The hamster mutant cell line K12 used in this study was originally isolated from an established line of Chinese hamster fibroblasts, Wg1A, after treatment of the cells with ethyl methane sulfonate (30, 31). K12 was selected because it grows normally at 35°C but is arrested in G1 upon incubation at 40.5°C. The shift-up time of K12 cells was found to be at 1.8 h before S phase (2). In another investigation, the step at which the *ts* mutation is expressed has been mapped at 4 h preceding the initiation of DNA synthesis (25). The K12 *ts* mutation is most likely to affect the commitment of the cells to initiate DNA synthesis. The phenotypes of the K12 cells at 40.5°C have been well characterized (21–23, 26). From reversion analysis, it has been suggested that the K12 mutation is of a regulatory nature (32). Therefore, this conditional cell cycle mutant is useful for analysis of the coupling of histone gene transcription and DNA synthesis since it provides the unique opportunity of obtaining a homogeneous population of cells blocked in G1. The use of a conditional cell cycle mutant eliminates the use of DNA synthesis inhibitors such as hydroxyurea and 1-β-D-arabinofuranosylcytosine which are commonly used to artificially uncouple the process of DNA and histone synthesis (12, 34).

The results we obtained with this *ts* mutant indicated that a critical period necessary for histone mRNA accumulation occurred late in G1 phase. If the cells were unable to synthesize DNA, the amounts of histone mRNA never accumulated to levels higher than the basal level and histone mRNA synthesis was not being initiated. Thus, we observed a strict coupling between the ability of cells to initiate DNA synthesis and the accumulation of histone mRNA to high levels. Our results obtained with K12 differed from those observed in the yeast cell cycle mutant *cdc7*, which initiated histone mRNA synthesis in spite of a block in DNA replication (17). A possible explanation is that the step at which the K12 *ts* mutation is expressed occurs before the signalling event of both histone gene transcription and DNA replication. To further clarify whether it is possible to dissociate the

signals governing initiation of DNA and histone syntheses, more detailed analysis of histone mRNA regulation in other conditional mammalian cell cycle mutants is needed.

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