
Synthesis of histone messenger RNAs by RNA polymerase II in nuclei from S phase HeLa S₃ cells

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ABSTRACT

Nuclei were isolated from synchronized HeLa S₃ cells and transcribed utilizing their endogenous RNA polymerases. Our data suggest that S phase nuclei are capable of synthesizing histone mRNA sequences while nuclei from G₁ phase cells are not. Transcription of histone mRNA sequences by S phase nuclei can be abolished completely by low levels of α -amanitin (1.0 μ g/ml, a concentration which completely inhibits RNA polymerase II). From these results it appears that transcription of the histone mRNA sequences occurs during the S phase but not during the G₁ phase of the cell cycle and that RNA polymerase II is responsible for histone gene readout.

INTRODUCTION

Eukaryotes possess three classes of nuclear DNA-dependent RNA polymerase, each believed to be responsible for the synthesis of specific classes of RNA. The class I polymerase is responsible for ribosomal RNA (rRNA) synthesis. This polymerase is found in high yields in nucleoli (1), the site of rRNA synthesis (reviewed by 2). Furthermore, conditions which inhibit the class II and III enzymes but favor the activity of the class I enzyme also favor the transcription of rRNA (3).

The class III enzyme synthesizes 5S rRNA and transfer RNA (tRNA) (4,5), as well as a small, nontranslated adenovirus RNA (6). The inhibition of synthesis of these RNAs by α -amanitin is directly proportional to the inhibition of the class III enzyme.

Polymerase II is believed to be responsible for the transcription of messenger RNA (mRNA). The class II enzyme is located in the nucleoplasm (7,8) purported to be the site of non-rRNA synthesis. In addition, α -amanitin at a level sufficient to inhibit the class II but not the class I or III enzymes inhibits the synthesis of heterogenous RNA (9), presumed to be the precursor of messenger RNA (reviewed by 10,11). Recently, it has been shown that RNA polymerase II is indeed responsible for the synthesis of specific

mRNAs. Low levels of α -amanitin inhibit not only the class II polymerase but also the synthesis of silk fibroin mRNA in the moth, *Bombyx mori* (12). Similar results have been obtained for the transcription of the ovalbumin gene of chick oviduct (G. Schutz, personal communication).

In contrast to the fibroin and ovalbumin genes which are represented as unique sequences in silk moth (13,14) and chick oviduct DNA (15,16), respectively, histone genes are represented as moderately reiterated sequences in the DNA of HeLa cells and other cell types (17-19). The question then arises as to which RNA polymerase transcribes histone genes - polymerase I or III, the products of which have been shown to be transcribed from reiterated sequences, or polymerase II, which has only been shown to transcribe unique sequence mRNAs. Evidence is presented which suggests that in nuclei from S phase HeLa cells histone genes are transcribed by a class II RNA polymerase. Consistent with other data from our laboratory which indicate that histone mRNA sequences are transcribed from chromatin of S phase but not G₁ cells, histone mRNA sequences are not transcribed in isolated nuclei from G₁ HeLa cells. These results taken together support a model for regulation of histone gene expression during the cell cycle of HeLa cells in which control resides at least part at the transcriptional level.

MATERIALS AND METHODS

Materials Ribonuclease-free sucrose was purchased from Schwarz/Mann. Nucleoside triphosphates were obtained from Sigma Chemical Company as were the α -amanitin and Hepes. (³H)UTP (25 Ci/mole) was purchased from New England Nuclear. All other reagents unless otherwise noted were analytical grade obtained from Mallinckrodt.

Cells HeLa S₃ cells were grown at 37°C in suspension culture on Joklik-modified Eagle's minimal essential medium supplemented with 7% calf serum. Cells were synchronized as described by Stein and Borun (20). S phase cells were harvested 2.5 hours after release from the second of two 2 mM thymidine blocks. Greater than 99% of these S phase cells are engaged in DNA synthesis as determined by ³H-thymidine labeling and autoradiography. G₁ phase cells were harvested 2 hours after selective detachment of mitotic cells from semiconfluent monolayers. ³H-thymidine labeling and autoradiography indicate that less than 0.1% of the G₁ cells are synthesizing DNA.

Isolation of nuclei Nuclei were isolated by a modification of the procedure of Sarma *et al.*, (21). Cells were harvested by centrifugation for 5 min at 350 xg at 37°C. All further steps in the isolation procedure were

conducted at 0-4°C. The cells were washed twice with 150 mM NaCl and suspended at 1×10^7 cells/ml in Hypo buffer (0.3 M sucrose, 2 mM Mg acetate, 10 mM Hepes (pH 7.6), 0.1% (v/v) Triton X-100, 0.04% (v/v) 2-mercaptoethanol) for 10 min. The cells were then lysed by 20-30 strokes with a Dounce homogenizer using a tight fitting B pestle. To the lysed cell suspension was added 1 volume of buffer A (2 M sucrose, 5 mM Mg acetate, 10 mM Hepes (pH 7.6), 0.04% (v/v) 2-mercaptoethanol). This suspension was underlaid with solution A and centrifuged for 45 min at 53,000 xg in a Beckman SW 27 rotor. The nuclear pellet was suspended in a small volume of 25% (v/v) glycerol, 5 mM Mg acetate, 50 mM Hepes (pH 7.6), 0.04% (v/v) 2-mercaptoethanol. Nuclei were used immediately or stored in liquid nitrogen.

In situ nuclear transcription Unless otherwise noted in the figure legends, the reaction mixture contained the following: 0.4 mM GTP, CTP, UTP and ATP, 5 mM Mg acetate, 70 mM KCl, 25 mM Hepes (pH 7.6), 0.04% (v/v) 2-mercaptoethanol, 12.5% (v/v) glycerol and $1-5 \times 10^7$ nuclei/ml. Incubation was at 25°C for 45 min in a Forma shaker bath. Samples were also manually shaken every 5-10 min to ensure suspension of the nuclei.

Isolation of RNA Transcription was stopped by addition of one ninth volume of ten times concentrated pH 5.4 buffer (1 M NaCl, 100 mM Na acetate, 10 mM EDTA (pH 5.4)) and one twenty-fourth volume of 25% (w/v) sodium dodecyl sulfate. The reaction mixture was extracted at ambient temperature twice with equal volumes of phenol and chloroform-isoamyl alcohol (24:1 v/v) and once with one volume of chloroform-isoamyl alcohol (24:1 v/v). Nucleic acid was precipitated from the aqueous phase with 2.5 volumes of ethanol overnight at -20°C. The precipitated nucleic acid was collected at 4°C by centrifugation for 15 min at 2000 xg, dissolved in 1.5 ml of 10 mM Tris (pH 7.4), 100 mM NaCl containing 50 µg/ml DNase I (Sigma) and incubated at 37°C for 60 min. This solution was extracted once at ambient temperature with one volume of phenol and chloroform-isoamyl alcohol (24:1 v/v) and once with one volume of chloroform-isoamyl alcohol (24:1 v/v). The aqueous phase was chromatographed on Sephadex G-50 fine (1 x 26 cm, Pharmacia) and eluted with 100 mM NaCl, 10 mM Na acetate, 1 mM EDTA (pH 5.4). The void volume fractions containing the RNA were pooled and the RNA was precipitated by the addition of 2.5 volumes of ethanol at -20°C for 2-3 days. The precipitated RNA was collected by centrifugation at 15,000 xg for 1 hour at 4°C. The RNA pellet was dissolved in distilled water and stored at -20°C.

Hybridization of the RNA The isolation of histone mRNAs from S phase polyribosomes of HeLa S3 cells and the synthesis and properties of comple-

mentary (^3H)-DNA (cDNA) to the histone mRNAs were described previously (22-25). RNA and (^3H)-cDNA were hybridized at 52°C in sealed capillary tubes. The standard hybridization was conducted in $15\ \mu\text{l}$ volumes containing 50% formamide, 500 mM NaCl, 25 mM Hepes (pH 7.0), 1 mM EDTA, 40 pg of cDNA (27,000 DPM/ng) and RNA as indicated in the figure legends. Hybrid formation was determined by digestion of non-hybridized cDNA with single-strand specific S_1 nuclease (Sigma). Each hybridization sample was incubated for 20 min at 37°C in 2 ml of 30 mM sodium acetate (pH 4.6), 300 mM NaCl, 1 mM ZnSO_4 , 5% (v/v) glycerol and 80 units of S_1 nuclease. S_1 nuclease-resistant hybrids were precipitated by the addition of 20 μg of bovine serum albumin (Sigma) to serve as a carrier and one volume of 20% (w/v) trichloroacetic acid and were collected by filtration through a Millipore HA filter. The filters were washed with 50 ml of 10% (w/v) trichloroacetic acid and dissolved in 1 ml of cellusolve. Ten ml of cellusolve scintillation fluid were added (2356 ml toluene, 788 ml cellusolve, 122 ml Concifluor (Mallinckrodt)) and radioactivity was measured with a Beckman LS-230 scintillation counter.

Nucleic acid levels RNA content was determined by the orcinol reaction as described by Schneider (26). NaOH was used instead of KOH in the alkaline digestion. G_2 polysomal RNA was used for the standard. The hydrolysate was assayed for the presence of DNA by Burton's method (27); no DNA was detected. Calf thymus DNA was used as the standard.

RESULTS

Isolation and characterization of nuclei HeLa S_3 nuclei were obtained by a modification of the procedure of Sarma *et al.* (21). Routinely a 70-80% yield of nuclei was obtained. No intact cells were visible in the nuclear suspension when examined by phase contrast microscopy although 4-6% of the nuclei had small amounts of adhering cytoplasm. The inclusion of CaCl_2 in the cell lysis buffer did not appreciably increase the yield of the nuclei but did decrease their capacity for transcription by 30%. In addition, the percent of nuclei with adhering cytoplasmic material increased from 4% if nuclei were isolated in the absence of CaCl_2 to 20% if CaCl_2 was included at a concentration of 3 mM. Polyribosomes in the cytoplasm are an excellent source of histone mRNA (24); therefore, only minimal amounts of adhering cytoplasm could be tolerated since histone mRNA sequences contributed by the cytoplasm could be significant compared with those synthesized by the nucleus and thus make analysis exceedingly difficult.

The incorporation of (^3H)-UMP into RNA by isolated nuclei is linear for 45 min and decreases rapidly thereafter (Fig. 1). The lack of incorporation

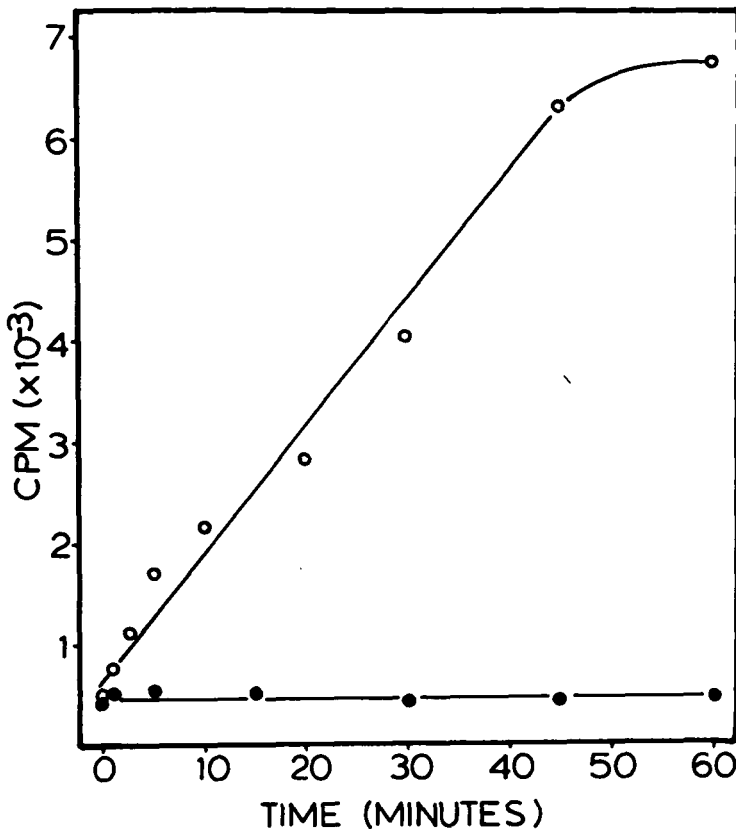


Fig. 1 Time-dependent incorporation of (³H)UMP into RNA by S phase HeLa nuclei. A 120 μ l reaction volume was incubated as described in Materials and Methods. The nonradioactive UTP was reduced to 0.05 mM and 24 μ Ci of (³H)UTP (evaporated to dryness under nitrogen) was included for transcription in the presence of nucleotides. To measure transcription in the absence of nucleotides, ATP, GTP, CTP and the non-radioactive UTP were omitted but the (³H)UTP was included. The concentration of nuclei in this reaction mixture was 3.5×10^7 nuclei/ml. At the indicated time, a 5 μ l aliquot was removed from the reaction volume and spotted on a 2.5 cm DE-81 filter paper disc (Whatman). At the conclusion of the assay, the filters were washed in a batch in the following manner: five times with 5% (w/v) NaH_2PO_4 for 5 min. each, twice with distilled H_2O for 30 sec each, and twice with 95% ethanol for one min each. The discs were dried under a heat lamp and inserted into scintillation vials containing 4 ml of Concifluor (Mallinckrodt)-toluene (42:1000) scintillation fluid. Radioactivity was determined in a Beckman LS-230 scintillation counter. Data points are averages of duplicate points. (○-○) Incorporation of (³H)UMP in the presence of ATP, GTP, and CTP. (●-●) Incorporation in the absence of ATP, GTP, and CTP.

of (³H)-UMP in the absence of three of the four ribonucleoside triphosphates supports the hypothesis that the (³H)-UMP is incorporated primarily into RNA. Nuclei from S phase cells synthesize 0.11 pg of RNA per nucleus in 30 min in

the standard reaction. S phase nuclei stored for at least three months in liquid nitrogen retain this transcriptional capacity as well as the specificity for histone gene transcription.

The nuclei retain activity representative of all three classes of DNA-dependent RNA polymerase. If transcription is inhibited by incubating the nuclei with increasing amounts of α -amanitin as shown in Fig. 2, a three component inhibition curve is obtained. The middle component, admittedly present in low amounts, was seen in four out of four nuclear samples which were tested for their α -amanitin response. Based on the known sensitivities of the solubilized polymerase from HeLa cells to α -amanitin (28-30), it appears that the nuclei possess all three classes of RNA polymerase. In the standard assay, the class I polymerase comprises 35% of the total RNA synthesizing activity, polymerase II, 58% and polymerase III, 7%. The presence of RNA polymerase III

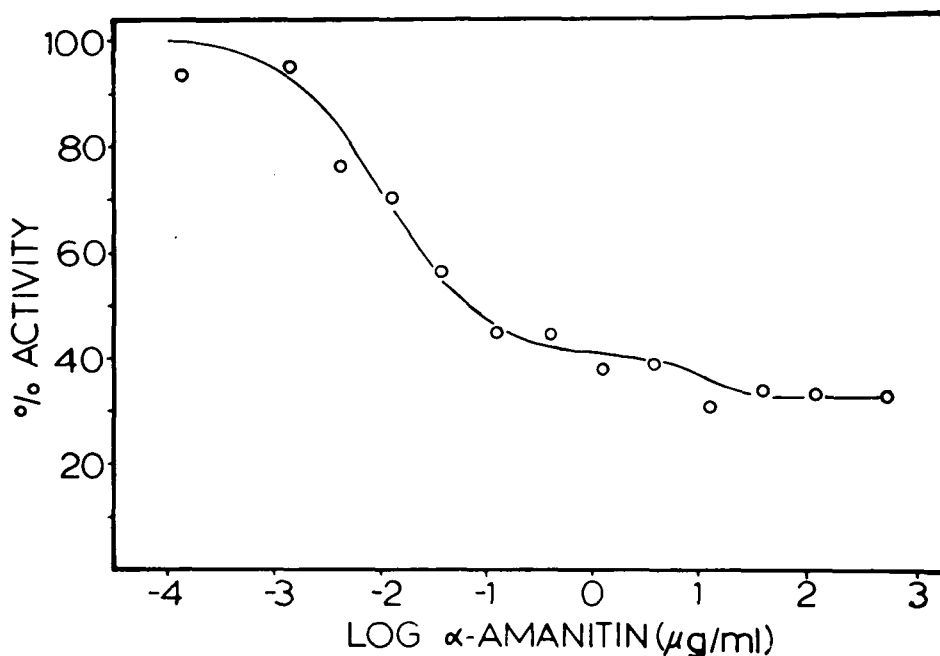


Fig. 2 Transcription by S phase HeLa cell nuclei in the presence of α -amanitin. A reaction volume of 40 μl for each α -amanitin concentration was incubated as described in Materials and Methods, except UTP was reduced to 0.05 mM and 2 μCi of $(3\text{H})\text{UTP}$ was included with each reaction volume. Alpha-amanitin was added to the reaction mixture prior to the addition of the S phase nuclei. Nuclei were incubated at a density of $2.4 \times 10^7/\text{ml}$. Thirty min after the addition of the nuclei, the reaction was stopped by pipetting a 10 μl aliquot onto a DE-81 filter paper disc. Discs were washed and prepared for scintillation counting as described in the legend of Fig. 1. Data points are the averages of duplicate samples. One hundred percent activity was 1184 cpm prior to a correction of 230 cpm for the blank.

in HeLa cell nuclei prepared by this procedure is further supported by the experiment of Sarma *et al.* (21) which demonstrated that polymerase III is active under the transcription conditions employed in our studies. Sarma *et al.* (21) showed that 5S rRNA and the putative precursor to 4S tRNA were synthesized in their nuclear transcription experiments; these two RNA species being the principal products of RNA polymerase III in intact cells.

Transcription of histone mRNA sequences from S phase nuclei Results from *in vitro* nuclear transcription experiments suggest that nuclei isolated from S phase HeLa cells are actively transcribing histone gene sequences. When RNA extracted from isolated S phase nuclei is hybridized in RNA excess with (^3H)-labeled DNA complementary to histone mRNAs, the kinetics of hybridization shown in Fig. 3 are observed. The RNA from nuclei incubated in the presence of all four ribonucleoside triphosphates, such that they are transcriptionally active, hybridizes with a $\text{Cr}_0\text{t}_{1/2}$ of 2.7, whereas RNA from nuclei which are not actively transcribing due to the absence of the four ribonucleoside triphosphates hybridizes with a $\text{Cr}_0\text{t}_{1/2}$ of 10. The increase in the representation of histone mRNA in nuclei which are transcriptionally active ranges from 2-4 fold. The histone mRNA sequences comprise 0.38-0.63% of the total RNA of the active nuclei and 0.13-0.17% of the endogenous pool of nuclear RNA (the endogenous RNA includes partially completed histone sequences as well as completed histone sequences which have not yet migrated from the nucleus). Although these nuclei possess the capability for *in situ* initiation of transcription (21), it is not known whether the observed stimulation is due to the *de novo* initiation of histone mRNA synthesis or whether we are merely detecting the completion *in situ* of pre-initiated histone mRNA.

The polymerase responsible for the synthesis of these histone messenger RNA sequences can be determined by incubating the nuclei in the presence of varying concentrations of α -amanitin. We can infer from the complete inhibition of the synthesis of histone messenger RNA, at as little as 1 μg of α -amanitin/ml (Fig. 3), that the class II polymerase is responsible for the transcription of histone genes. It is the only class of polymerase which is inhibited completely at this concentration, whereas the other two polymerase classes are inhibited only slightly or not at all. Since the $\text{Cr}_0\text{t}_{1/2}$ of the hybridization reaction between histone cDNA and RNA isolated from nuclei transcribed in the presence of either 1 μg or 100 μg of α -amanitin/ml is equal to 10, a value identical to that of endogenous RNA, no new histone mRNA sequences are synthesized in the presence of α -amanitin at a concentration of 1 $\mu\text{g}/\text{ml}$ or more. Thus the synthesis of histone messenger RNA appears to be performed by the class II polymerase.

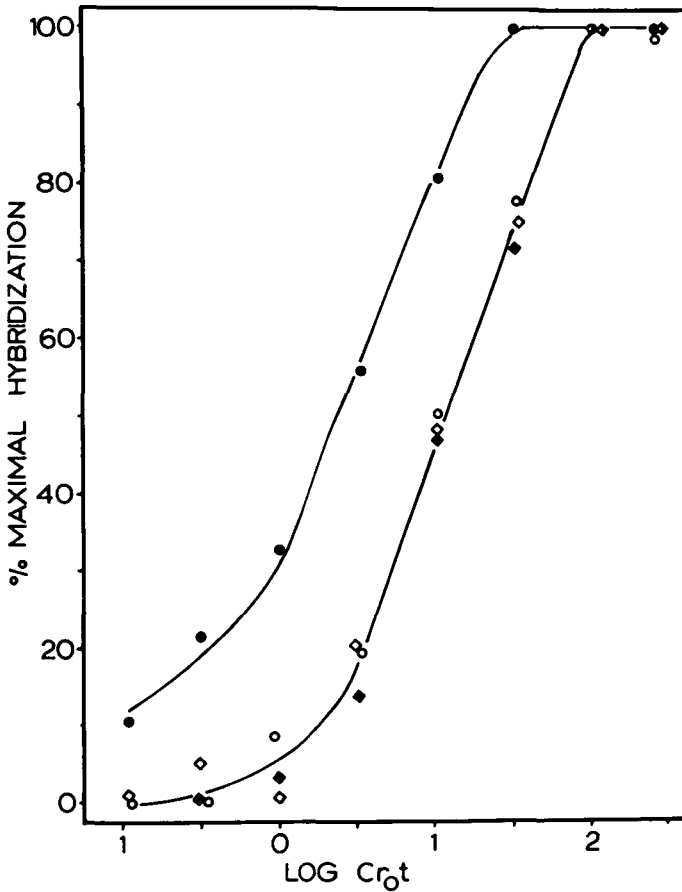


Fig. 3 Kinetics of the hybridization of S phase nuclear transcripts to histone cDNA. S phase nuclei were incubated in 1 ml of the transcription medium at a concentration of 4×10^7 nuclei/ml as described in Materials and Methods. Alpha-amanitin was added prior to the addition of the nuclei. RNA was isolated and 1.1 μ g of RNA was hybridized with histone (3 H)-cDNA for various time periods. Maximal hybridization was 60%. RNA from nuclei transcribed in the absence of α -amanitin (●-●). RNA from nuclei transcribed in the presence of α -amanitin at a concentration of 1 μ g/ml (◇-◇). RNA transcribed in the presence of α -amanitin at a concentration of 100 μ g/ml (◆-◆). RNA from nuclei transcribed in the absence of nucleotides and α -amanitin (○-○). $CrOt$ = moles ribonucleotides x time.

In order to rule out the possibility that a subclass of polymerase III which is inhibited at 1 μ g of α -amanitin/ml is responsible for transcription of histone mRNAs, we have also transcribed S phase HeLa cell nuclei in the presence of 0.01 μ g of α -amanitin/ml. From Fig. 2 it can be seen that the very α -amanitin-sensitive polymerase activity (which is putatively polymerase II) is inhibited 50% at 0.01 μ g of α -amanitin/ml. Therefore, if histone

mRNAs are synthesized by a class II enzyme, their synthesis should also be inhibited 50% at 0.01 μg of α -amanitin/ml. Indeed, this is the case. The amount of histone mRNA sequences synthesized above endogenous levels is proportional to the fraction of active class II polymerases (Table 1). Since no class III polymerase has yet been found which possesses the same α -amanitin response as a class II enzyme, we conclude that histone genes are transcribed solely by the class II polymerase.

We can calculate from the data of Fig. 3 and Table 1 that approximately one out of every five actively transcribing class II polymerases in S phase HeLa nuclei is involved in the transcription of histone genes. Histone mRNA

TABLE 1 The kinetics of hybridization of histone cDNA with RNA from S phase nuclei transcribed in the presence of low levels of α -amanitin.¹

Nucleotides ²	α -amanitin $\mu\text{g/ml}$	$\text{Cr}_0\text{t}_{1/2}$	% Total ³ histone sequences	% New ⁴ histone sequences	% Inhibition ⁵ of new histone synthesis	% Inhibition ⁶ polymerase II
+	0.0	4.5	0.38	0.25	0	0
+	0.01	7.1	0.24	0.11	56	50
+	1.0	13.2	0.13	0.00	100	100
-	0.0	13.2	0.13			

1. S phase HeLa nuclei were incubated at a concentration of 3.7×10^7 nuclei/ml in a 2 ml reaction volume as described in Materials and Methods. α -amanitin was added prior to the addition of the nuclei. RNA was isolated and 2 μg per hybridization was hybridized as described in Materials and Methods.
2. Transcription in the presence (+) or absence (-) of nucleotides.
3. The $\text{Cr}_0\text{t}_{1/2}$ of the histone mRNA - histone cDNA hybridization reaction is 1.7×10^{-2} . Therefore the percent histone mRNA sequence in an unknown sample = $(\text{Cr}_0\text{t}_{1/2} \text{ unknown} / \text{Cr}_0\text{t}_{1/2} \text{ histone mRNA})^{-1} \times 100$.
4. Percent total histone sequences - percent total histone sequences for nuclei transcribed in the absence of nucleotides.
5. $(1 - (\% \text{ new histone sequences} / \% \text{ new histone sequences transcribed in the absence of } \alpha\text{-amanitin})) \times 100$.
6. From Fig. 2 using that activity inhibited at 1 μg α -amanitin/ml as due solely to polymerase II.

hybridizes with histone cDNA with $C_{0}t_{1/2}$ of 1.7×10^{-2} (24). Since the RNA of actively transcribing nuclei hybridizes with histone (^3H)-cDNA with a $C_{0}t_{1/2}$ of 2.7-4.5, histone mRNAs represent 0.38-0.63% of the total nuclear RNA. Using a value of 5.4 pg of RNA per HeLa cell nucleus as determined in our laboratory, 0.17 pg for the amount of RNA synthesized per nucleus (assume UMP

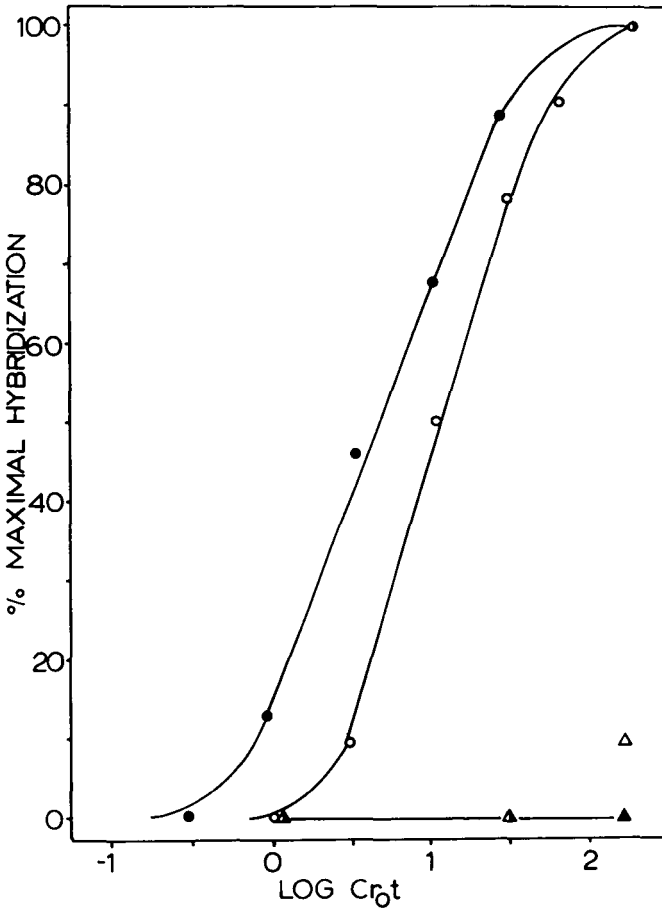


Fig. 4 Kinetics of the hybridization of the transcripts of S phase and G_1 nuclei to histone (^3H)-cDNA. S phase nuclei at a concentration of 3.8×10^7 nuclei/ml and G_1 nuclei at 4.6×10^7 nuclei/ml in individual reaction volumes were incubated as described in Materials and Methods. RNA was isolated and 3.5 μg of S phase RNA or 1.2 μg of G_1 RNA were hybridized for various time periods as described in Materials and Methods. Maximal hybridization for S phase nuclei was 50%. S phase nuclei transcribed in the presence of nucleotides (●-●). S phase nuclei transcribed in the absence of nucleotides (○-○). The percent maximal hybridization for G_1 nuclei transcribed in the presence (▲-▲) or absence (△-△) of nucleotides was calculated by dividing the percent of the hybridization of the G_1 RNA by the percent of maximal hybridization for RNAs of the S phase nuclei. C_0t = moles of ribonucleotides \times time.

comprises 25% of the total nucleoside monophosphates incorporated) in 45 min, and 0.13-0.17% for the endogenous histone mRNA content of S phase HeLa cell nuclei, we calculate that 15-25% of the RNA synthesized by the class II enzymes is histone mRNA. If we assume that the amount of RNA synthesized is proportional to the number of polymerases engaged in the synthesis of that RNA, then 15-25% of the class II polymerases are involved in the transcription of the histone genes at this particular point in the S phase of the HeLa cell cycle.

Transcription of G₁ phase nuclei The ability of isolated G₁ phase nuclei to synthesize histone mRNA was also examined. No significant difference was noted in the general transcriptional ability of G₁ and S phase nuclei, in their RNA content or in RNA recovery. G₁ nuclei, however, did not synthesize detectable amounts of histone mRNA sequences (Fig. 4). RNA of either the endogenous pool of G₁ nuclei or the RNA of active G₁ nuclei was not found to hybridize with the histone-cDNA probe, even at a C₀t of 320. It thus appears that the histone genes are transcribed during the S phase portion of the cell cycle but not during G₁. G₂ phase cells were not examined since 20% of the cells in a G₂ population obtained by synchronization with a double thymidine block are still synthesizing DNA (i.e., are in S phase) (20) and cell synchronization by mitotic selective detachment does not significantly enhance the purity of the G₂ population.

DISCUSSION

Based on the sensitivity of histone mRNA synthesis to α -amanitin inhibition, we conclude that histone mRNA sequences are transcribed by a class II DNA-dependent RNA polymerase. Although the histone genes are moderately reiterated as are the genes for ribosomal (31-33) and transfer RNA (33-35) which are transcribed by enzymes other than the class II polymerase, the histone genes code for an RNA which possesses a message function as do the single copy genes of ovalbumin (15,16), globin (36-38), immunoglobulin (39,40) and fibroin (13,14). Since fibroin (12) and ovalbumin (G. Schutz, personal communication) mRNAs are also known to be synthesized by a class II RNA polymerase, one might expect that mRNAs, regardless of the repetitiveness of the coding genes, are synthesized by a class II polymerase.

Whether or not all mRNAs are synthesized by the class II polymerase remains to be seen. The adenovirus genome is transcribed by both the class II and III polymerases (4). The class III polymerase is apparently responsible for synthesis of the viral 5.5S RNA. Although the function of this RNA is

not currently known, it is intriguing to speculate that it does not have a message function since it is approximately the same size as tRNA and 5S rRNA which do not have message functions. If this is indeed the case the adenovirus system lends further support to the hypothesis that mRNAs are synthesized by a class II polymerase.

We have found that synthesis of histone messenger RNA in isolated HeLa S₃ nuclei apparently occurs during the S phase of the cell cycle but not during G₁. This result is in agreement with the observation that the *in vitro* transcription of HeLa and human diploid fibroblast chromatin by the *E. coli* RNA polymerase yields histone messenger RNA sequences only if the chromatin is isolated from S phase cells (41,42), and the observation that histone mRNAs can only be isolated from cellular compartments of S phase cells (24,42-48). The evidence presented here that synthesis of histone mRNA occurs only in nuclei isolated from S phase bridges the gap between studies *in vivo* and those *in vitro* with chromatin.

Recently, Melli *et al.* (53) have reported that histone mRNA synthesis occurs throughout the cell cycle in HeLa cells. It is difficult to reconcile the apparent inconsistencies between our results and those of Melli *et al.* Obviously one must interpret with caution hybridizations performed between sea urchin histone DNA and HeLa cell RNAs, due to the limited extent of sequence homology. However, the results which Melli *et al.* present regarding the inability of cytosine arabinoside (an inhibitor of DNA replication) to inhibit histone mRNA synthesis are consistent with an earlier report from our laboratory (54) in which HeLa histone cDNA was used to assay histone mRNA sequences. Perhaps the most important difference between our experiments and those of Melli *et al.* is the method of cell synchronization used. We have obtained G₁ phase cells by mitotic selective detachment, a procedure which yields a G₁ population containing less than 0.1% S phase cells. In contrast, Melli *et al.* synchronized their cells by double thymidine block, a technique with which a background of 20-25% S phase cells (assayed autoradiographically) persists throughout the presumptive G₂, mitotic and G₁ phases of the cell cycle.

Although we cannot definitively eliminate the possibility that histone mRNAs are transcribed throughout the cell cycle and rapidly degraded by a specific nuclease during G₁, G₂ and M, this possibility seems unlikely. When histone mRNAs are added to G₁ chromatin (which is ineffective as a template for histone gene transcription) and subjected to transcription conditions, the added mRNAs can be quantitatively recovered (49).

Having identified the homologous RNA polymerase for the transcription of histone mRNA sequences, we can now assess the availability of histone genes for transcription from HeLa cell chromatin by the appropriate eukaryotic enzyme. Hopefully, use of the homologous polymerase will enhance the fidelity of the *in vitro* chromatin transcripts. Kostraba *et al.* (50) have demonstrated that a fraction of the nonhistone chromosomal proteins stimulates the transcription of homologous chromatin only when the homologous polymerase is used. The nonhistone chromosomal proteins have also been implicated in directing the correct expression of the rRNA genes by the class I eukaryotic RNA polymerase (51) and in the case of the eukaryotic 5S rRNA genes, the fidelity of *in vitro* transcription from chromatin requires that transcription be carried out with class III eukaryotic RNA polymerase (52).

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