CELL CYCLE STAGE-SPECIFIC TRANSCRIPTION OF HISTONE GENES

G. S. Stein, W. D. Park, C. L. Thrall, R. J. Mans, and J. L. Stein
Department of Biochemistry, University of Florida
Gainesville, Florida 32610

Received February 21, 1975

DNA complementary to histone messenger RNAs was utilized to assay the in vitro transcripts from chromatin of G_1 and S phase HeLa S_3 cells for the presence of histone specific sequences. The complementary DNA was prepared by transcribing, with an RNA-dependent DNA polymerase, histone messenger RNAs isolated from the polyribosomes of S phase HeLa S_3 cells to which adenylic acid residues had been enzymatically added. While the RNA transcripts of S phase chromatin contained histone specific sequences, such sequences were not detected in the RNA transcripts from G_1 chromatin. These results suggest that transcription of the genes for histone polypeptides is restricted to the S phase of the cell cycle.

INTRODUCTION

Several lines of evidence suggest a functional relationship between histone synthesis and DNA replication. Synthesis of the five histones--F_1, F_3, F_{2b}, F_{2a_2}, and F_{2a_1}--is restricted to the S phase of the cell cycle (1-4) and association of the messenger RNAs for these proteins with a specific class of polysomes occurs only at this time (2-4). Furthermore, if DNA replication is inhibited a rapid shutdown of histone synthesis is observed (1,2). While these findings support the contention that the genes for histones are transcribed exclusively during the S phase of the cell cycle, they do not preclude the possibility that regulation may reside at a post-transcriptional level.

We have recently added adenylic acid residues to the 3'-OH ends of histone messenger RNAs and using RNA-dependent DNA polymerase synthesized a complementary DNA (cDNA) (5). This high resolution probe was employed in the present studies to ascertain whether the transcription in vitro of histone messenger RNA sequences is restricted to the period of DNA replication.

MATERIALS AND METHODS

Exponentially growing HeLa S_3 cells were maintained in suspension culture in Joklik-modified Eagle's minimal essential medium supplemented with 3.5% each of calf and fetal calf serum. Cells were synchronized as previously des-

cribed (6). S phase cells were obtained by synchronization with 2 cycles of 2mM thymidine block. Three hours after release from the second thymidine block cells were harvested; at this time 98% of the cells were in S phase. G_1 cells were obtained 3 hours after selective detachment of mitotic cells from semi-confluent monolayers; 97% of the cells were in the G_1 phase of the cell cycle and S phase cells were not detected.

Nuclei were obtained by washing cells 4 times in 80 volumes of Earle's balanced salt solution and lysing the cells in 80 volumes of 80mM NaCl-20mM EDTA-1% Triton X-100 (pH 7.2). The nuclei were washed three times with the lysing medium and then twice with 0.15M NaCl-0.01 M Tris (pH 8.0). Nuclei isolated in this manner are free of cytoplasmic material when examined by phase-contrast and electron microscopy. Lysis of nuclei was achieved by suspending the nuclear pellet in triple glass-distilled water with several strokes of a wide-clearance Dounce homogenizer. The chromatin was allowed to swell at 4°C for 30 minutes, pelleted at 20,000 xg for 15 minutes, resuspended in distilled water, and again pelleted at 20,000 xg.

RNA was transcribed from chromatin using Fraction V *E. coli* RNA polymerase prepared according to the method of Berg *et al* (7). Transcription was carried out for 70 min at 37°C in a Dounce homogenizer fitted with a wide clearance pestle, and the reaction mixture was periodically homogenized to maintain chromatin solubility. The incubation mixture in a final volume of 10 ml contained: 0.04M Tris (pH 8.0); 4mM $MgCl_2$; 1mM $MnCl_2$; 0.02mM EDTA; 0.008% β-mercaptoethanol; 0.4mM each of ATP, CTP, UTP, and GTP; 150μg/ml of DNA as chromatin; and 600 units of RNA polymerase. RNA was extracted as follows. The reaction was brought to a concentration of 1% SDS-0.1M NaCl-0.01M sodium acetate-1mM EDTA (pH 5.4) and incubated at 37°C for 15 minutes. Following two extractions with equal volumes of phenol and chloroform-isoamyl alcohol (24:1, v/v) and two extractions with chloroform-isoamyl alcohol, nucleic acids were precipitated with 3 volumes of ethanol. The pellet was resuspended in 10mM Tris-0.1M NaCl-5mM $MgCl_2$ (pH 7.4) containing 40μg/ml of DNase I and incubated at 37°C for 60 minutes. Following one extraction with phenol-chloroform-isoamyl alcohol and two with chloroform-isoamyl alcohol, the aqueous phase containing the RNA transcripts was chromatographed on Sephadex G-50 fine and eluted with 50mM Tris-0.1M NaCl-1mM EDTA (pH 7.2). RNA was precipitated with 3 volumes of ethanol and resuspended in 25mM HEPES-0.5M NaCl-1mM EDTA, pH 7.0. More than 90% of the RNAs synthesized under these conditions migrate in the 4-14S region of SDS polyacrylamide gels reflecting the synthesis of RNA molecules which are 75-1500 nucleotides in length.

Histone mRNAs were isolated from the polyribosomes of S phase HeLa S_3 cells and ^{3}H-complementary DNA (^{3}H-cDNA) was synthesized as previously described (5) using ^{3}H-dCTP and ^{3}H-dGTP. Poly A was added to the 3'-OH termini of the histone mRNAs with an ATP:polynucleotidylexotransferase isolated from maize seedlings, and the polyadenylated mRNAs were then transcribed with RNA-dependent DNA polymerase from Rous sarcoma virus, using dT_{10} as a primer.

^{3}H-cDNA and unlabeled RNA were hybridized at 52°C in sealed glass capillary tubes containing in a volume of 15μl:50% formamide, 0.5M NaCl, 25mM HEPES (pH 7.0), 1mM EDTA, 0.37 ng cDNA, and RNA as indicated. The reaction mixtures were assayed for hybrid formation using Fraction IV single strand-specific S_1 nuclease isolated from *Aspergillus oryzae* (8). Each sample was incubated for 20 min in 2.0ml of 30mM sodium acetate, 0.3M NaCl, 1mM $ZnSO_4$, 5% glycerol (pH 4.6) containing S_1 nuclease at a concentration sufficient to degrade at least 95% of the single stranded nucleic acids present. The amount of radioactive DNA resistant to digestion was determined by trichloroacetic acid precipitation.

RESULTS AND DISCUSSION

Hybridization of histone cDNA to RNA was carried out in RNA excess in 50% formamide-0.5M NaCl-25mM HEPES (pH 7.0)-1mM EDTA. To determine the optimal

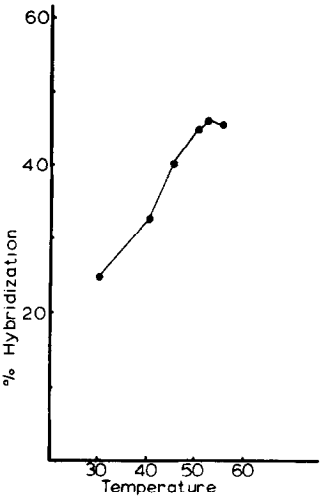

Figure 1

Rate of histone messenger RNA-cDNA hybridization as a function of temperature. 0.19 µg of histone mRNA was annealed with 0.37 ng of ^{3}H-cDNA for 30 minutes ($Cr_{o}t = 6.25 \times 10^{-2}$). Each reaction mixture also contained 3.75 µg of E. coli ^{o}RNA.

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temperature for the annealing reaction (9), we assessed the rate of hybridization by measuring the extent of hybrid formation between histone mRNA and cDNA at a $Cr_{o}t$ of 6.25×10^{-2}. The temperature-dependent rate curve shown in Figure 1 indicates that the rate of the hybridization reaction is maximal at 52° under these conditions. Therefore 52° was the temperature of choice for the RNA-DNA hybridization studies reported in this communication.

To directly ascertain if the genes which contain the information for histone synthesis are transcribed during a restricted period of the cell cycle, the following approach was pursued. Chromatin from G_1 and S phase cells was transcribed in a cell-free system, the RNAs were isolated and their ability to form S_1 nuclease resistant acid-precipitable hybrids with histone cDNA was determined. The kinetics of hybridization of histone cDNA and RNA transcripts from G_1 as well as S phase chromatin are shown in Figure 2. While transcripts from S phase chromatin hybridize with histone cDNA at a $Cr_{o}t_{1/2}$ of 2×10^{-1}, compared with a $Cr_{o}t_{1/2}$ of 1.7×10^{-2} for the histone mRNA-cDNA hybridization reaction, there is no evidence of hybrid formation between histone cDNA and

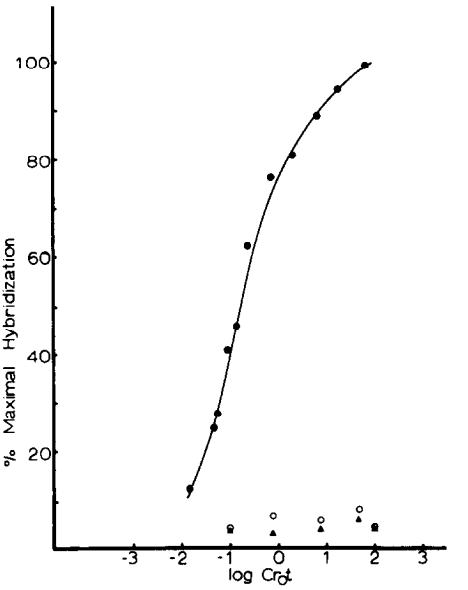

Figure 2

Kinetics of annealing of histone cDNA to in vitro transcripts from chromatin of G_1 and S phase HeLa S_3 cells. 0.37 ng of ^3H-cDNA (27,000 dpm/ng) was annealed at 52° to either 0.15 or 1.5 µg of RNA transcripts from G_1 (O O O) or S phase (● ● ●) chromatin. 0.37 ng of cDNA was also annealed to 1.5 µg of E. coli RNA isolated in the presence of S phase chromatin (▲ ▲). E. coli RNA was included in each reaction mixture so that the final amount of RNA was 3.75 µg. Cr_0t = moles of ribonucleotides x sec/liter.

G_1 transcripts, even at a Cr_0t of 100. The maximal level of hybrid formation between histone cDNA and S phase transcripts was 63% -- the same maximal level as was observed between histone cDNA and histone mRNA (data not shown). Fidelity of the hybrids formed between histone cDNA and transcripts from S phase chromatin is suggested by the fact that the T_m of these hybrids is identical to the T_m of histone mRNA-cDNA hybrids (65°). It should be noted that the T_m obtained under these conditions is consistent with an RNA-DNA hybrid having a GC content of 54%. This figure is in agreement with the nucleotide composition of histone mRNA reported by Adesnik and Darnell (10).

RNAs synthesized in intact S phase cells may remain associated with chromatin during isolation and in part account for histone specific sequences which are detected in S phase transcripts. To eliminate this possibility, E. coli RNA (an amount equivalent to the RNA transcribed under our in vitro

conditions) was added to S phase chromatin and RNA was isolated by the same procedure utilized to isolate in vitro transcripts. Since this RNA preparation did not exhibit any significant extent of hybrid formation with histone cDNA (Fig. 2), it is reasonable to conclude that the histone sequences present in S phase transcripts can be totally accounted for by in vitro synthesis.

The present results clearly indicate that histone sequences are available for transcription during S phase and not during G_1. These findings are consistent with the restriction of histone synthesis to the S phase of the cell cycle (1-4,6) and the association of histone messenger RNAs with polyribosomes only during S phase (2-4). Taken together this evidence suggests that the expression of histone genes is regulated at the transcriptional level and that the readout of these genetic sequences occurs only during the period of DNA replication.

These studies were supported by the following research grants: GB 38349 from the National Science Foundation, GM 20535 from the National Institutes of Health and ORO-3982-36 from the Atomic Energy Commission.

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