PARTIAL PURIFICATION AND CHARACTERIZATION OF INDIVIDUAL HISTONE MESSENGER RNAs FROM HELA CELLS

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SUMMARY

A polyribosomal 5-15 S poly(A)(-)-RNA preparation from S-phase HeLa cells, the only in vitro translation products of which were the five histones H1, H3, H2b, H2a and H4, was subjected to a new method of preparative polyacrylamide gel electrophoresis. With this method the mRNA for histone H4 (apparent molecular weight 1.4×10^5 daltons) was separated from the mRNAs coding for histones H3, H2b and H2a (m.w. $1.6 - 1.8 \times 10^5$ daltons) and from two different mRNAs coding for two histones H1 (m.w. 2.4×10^5 daltons). The mRNAs were identified by translation in a reticulocyte lysate.

INTRODUCTION

In cultured mammalian cells histone mRNAs enter the cytoplasm at the beginning of the DNA replication period (1-3). The relative abundance of these mRNA species during S-phase and their rapid transport from the nucleus to the cytoplasm made it possible to identify them by radioactive labelling (1-6). Labelled HeLa cell histone mRNA could be separated by polyacrylamide gel electrophoresis into three peaks having apparent molecular weights of 1.55×10^5, 1.8×10^5 and 2.1×10^5 daltons, respectively (3,5). By translation in cell-free protein synthesizing systems these mRNA species were shown to code for the five histones H1, H3, H2b, H2a and H4 (7-9).

We describe here the purification by preparative gel electrophoresis of the mRNAs for HeLa cell histones H4 and H1 from the messengers for histones H3, H2b and H2a. This work was done with the final goal to use their complementary DNAs

as probes in studies on the regulation of histone biosynthesis during the DNA replication period of the cell cycle. Furthermore, we present evidence to show that there exist in HeLa cells two different mRNAs which code for the two H1 histones that are separated in SDS-polyacrylamide gels.

MATERIALS AND METHODS

Histone mRNA was isolated from polyribosomes or from the 16,000 x g supernatant of S-phase HeLa cells which were synchronized by a double block with either 2 mM thymidine or 1.5 mM hydroxyurea as previously described (3,9). The RNA was fractionated on 15-30 % sucrose gradients (3) and the 5-15 S RNA fraction was passed over poly(U)-Sepharose to remove poly(A)-containing RNA (10). Transfer RNA and 5 S rRNA were removed from the poly(A)(-)-RNA by repeating washes with 3 M sodium acetate, 5 mM EDTA, pH 6.0 (11) and the RNA was recentrifuged on 15-30 % sucrose gradients. RNA analysis was performed on cylindrical 5 % polyacrylamide gels according to the procedure of Loening (12).

The RNA was further fractionated by a new method of preparative SDS-gel electrophoresis with continous elution. The method will be described in detail elsewhere (W. Roskam, manuscript in preparation). Briefly, after preelectrophoresis for 1 h at 10 mA, the RNA was loaded onto a 3 % (1.5 x 1.0 cm) spacer gel polymerized on a 5 % separating gel (4.5 x 1.0 cm) and electrophoresis was performed at 10 mA and 20°C. The flow rate of the elution buffer was 4 ml/h. The effluent was monitored at 260 nm and fractionated. The RNA was precipitated with 2.5 vol ethanol and washed several times with 3 M sodium acetate to remove coprecipitated phosphate. Then, the RNA was dissolved in 0.1 M LiCl, reprecipitated with ethanol and dissolved in bidistilled water.

The different RNA fractions collected were translated in a rabbit reticulocyte lysate and the amount of histone mRNA was quantitated as described (10). The ^{3}H-lysine-labelled translational products were analysed on SDS-polyacrylamide slab gels according to Laemmli (13) as modified by Weintraub *et al.* (14). Autoradiography of the gels was carried out as described by Bonner and Laskey (15). ^{14}C-lysine-labelled marker histones from HeLa cells were prepared as described (3) and further purified by chromatography on CM-cellulose (16).

RESULTS AND DISCUSSION

A typical analytical gel electrophoretic profile of 5-15 S poly(A)(-)-RNA from S-phase cells is shown in Fig.1. This RNA fraction contained the mRNAs for all five histones as was demonstrated by translation in the reticulocyte lysate and in a wheat germ system. The optical density profile of a RNA fraction similarly prepared but from hydroxyurea-blocked S-phase cells was almost indistinguishable from the profile of

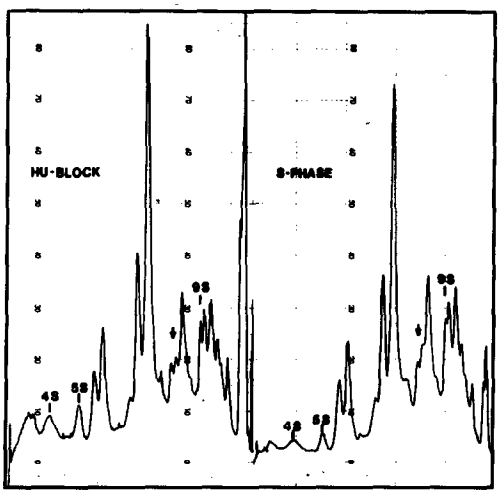

Figure 1. SDS-Polyacrylamide gel electrophoresis of 5-15 S poly(A)(-)-RNA from polyribosomes of S-phase cells and from hydroxyurea-blocked cells. Two A_{260} units of the RNAs were separated at room temperature on 10 x 0.6 cm cylindrical 5 % gels for 5.5 h at 3 mA/gel after preelectrophoresis under same conditions for 2 h. Gels were scanned at 260 nm.

S-phase RNA, although the histone mRNA activity as measured by translation was reduced to about 10 % of that of S-phase RNA. The only difference on gels which we observed repeatedly with RNA from hydroxyurea-blocked cells was a reduction of the absorbance in the region marked with an arrow in Fig.1. This, in fact, is the region where the mRNAs for histones H3, H2b and H2a migrate (see Fig.2 and 3). From the gel patterns shown in Fig.1 it is obvious that the histone mRNAs isolated as described are exceedingly impure, although the exclusive translational products of such RNA preparations from S-phase cells are the five histones.

In an attempt to further separate the histone mRNAs, 5-15 S RNA was fractionated by preparative gel electrophoresis. The elution profile is presented in Fig.2. As can be seen, there is a very good agreement of the optical density profile with that of the analytical gel shown in Fig.1. The effluent was divided into fractions and after ethanol precipitation and salt washings (see Materials and Methods) the RNA was translated and quantitated in the reticulocyte lysate as previously des-

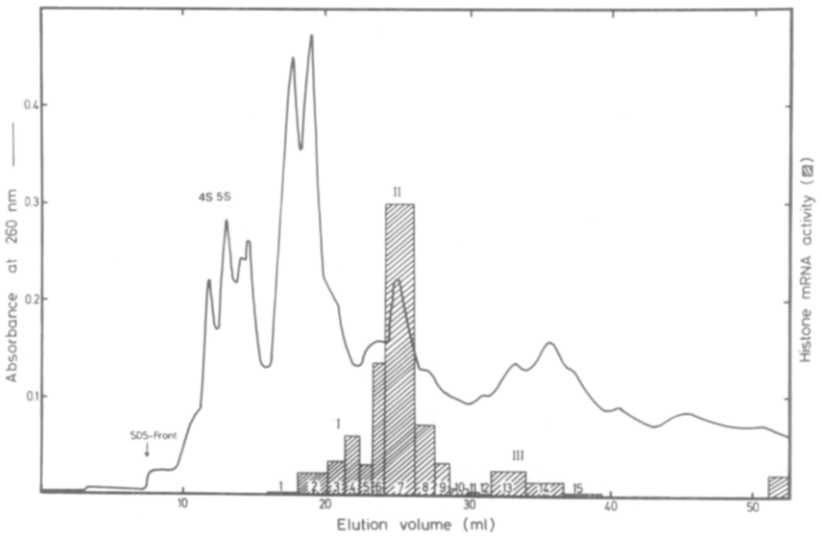

Figure 2. Preparative SDS-polyacrylamide gel electrophoresis of S-phase RNA shown in Figure 1. 30 A_{260} units of RNA were separated on a 5 % acrylamide gel with a 3 % spacer gel. Electrophoresis was performed at 10 mA and 20°C. The positions of 4 S and 5 S RNA are marked. Fractions were collected as indicated and histone mRNA was quantitated by translation in a reticulocyte lysate (10).

cribed (3,10). As shown in Fig.2, histone mRNA activity was separated into three peaks, designated as I, II and III in decreasing order of mobility. The translational products of the different RNA fractions were analysed on SDS-polyacrylamide slab gels (Fig.3). It cen be seen that peak I contains the mRNA for histone H4, peak II the messengers for histones H3, H2b and H2a, and peak III contains two partly separated mRNAs coding for the two histones H1 which are separated on SDS-gels. The molecular weights of the histone mRNAs were estimated by comparing the elution position of the messenger peaks with the corresponding absorbance peaks in the analytical gel profile. The apparent molecular weights were 1.4 x 10^5 daltons for H4 mRNA (about 420 nucleotides), 1.6 - 1.8 x 10^5 daltons (about 480 - 550 nucleotides) for the mRNAs coding for histones H3, H2b and H2a, and 2.4 x 10^5 daltons for the H1 mRNAs (about 720 nucleotides). The values are close to those which were previously found for radioactively labelled histone mRNA

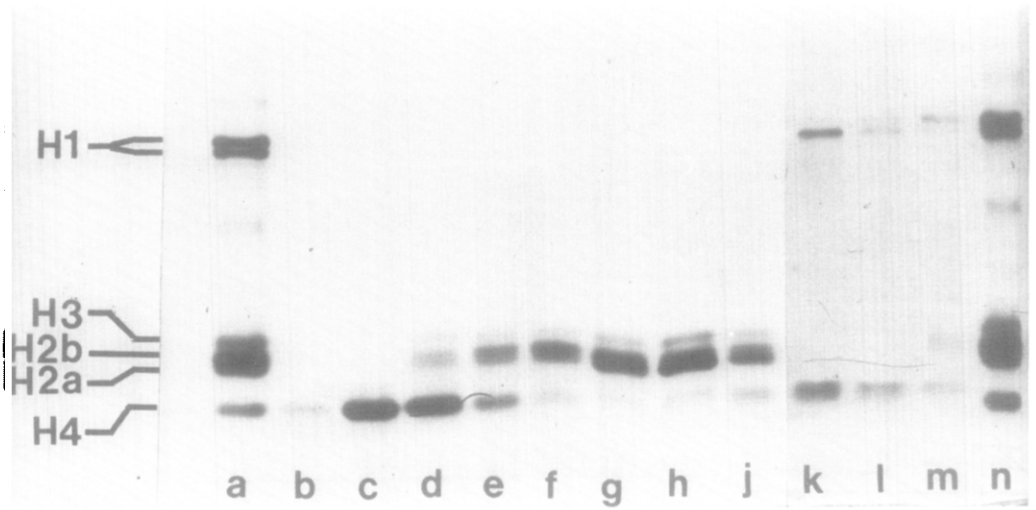

Figure 3. Translational products of mRNA fractions collected after preparative gel electrophoresis shown in Figure 2. After removing globin from the translational products of reticulocyte lysates, the ^{3}H-lysine-labelled *in vitro* products were separated by electrophoresis on 15 % SDS-polyacrylamide slab gels (10 cm x 15 cm x 0.13 cm). Electrophoresis was for 13 h at 50 V constant and room temperature. Radioactive bands were detected by fluorography (15). Slots a. and n.: marker histones from HeLa cells; slots b. to m.: translational products of RNA in fractions 2-9 and 13-15, respectively, shown in Figure 2. The faint band in slots f. to m. in the position of histone H4 represents the low amount of globin contaminating the translational products; the greater intensity of the globin band in slots k. to m. results from longer exposition of the film to this part of the gel.

separated in another gel system (3,5).

Our finding that in HeLa cells two mRNAs exist which differ in electrophoretic mobility and code for two histones H1 indicate that there are at least two different structural genes for histone H1. Since all histones, including the H1 histones, are synthesized coordinately during S-phase (2,10), it seems likely that the mRNAs are transcribed from a DNA repeat (10-20 repeat units per haploid genome in the case of HeLa cells (17)) similar to that described in sea urchins (18,19). If this turns out to be the case it will be of some interest to see whether in HeLa cells the DNA repeat units with different H1 coding sequences alternate or are separated.

The nature of the several RNA species found in the 5-15 S poly(A)(-)-RNA, besides 7 S rRNA and small amounts of tRNA and 5 S rRNA, is not clear. We prepared DNAs complementary to total 5-15 S RNA from S-phase cells and to the more purified RNA containing the mRNAs for histones H3, H2b and H2a (fraction II/7, Fig.2) after polyadenylation with a poly(A)-polymerase from E. coli (20). Both cDNAs hybridized extensively to 18 S ribosomal RNA from reticulocytes and furthermore, no differences were observed in the hybridization kinetics of the cDNA against 5-15 S S-phase RNA with polyribosomal RNA from S-phase cells and from hydroxyurea-blocked cells. Even the hybridization kinetics of cDNA against the purified RNA fraction containing the mRNAs for histones H3, H2b and H2a with S-phase and hydroxyurea-blocked polyribosomal RNA would suggest that instead of the expected 10-15 %, about 50 % of these three mRNAs are present in blocked cells (E. Bos and D. Gallwitz unpublished). This result is not compatible with experimental evidence obtained in several laboratories indicating that a block of DNA replication for more than 30 min results in the degradation of about 90 % of translatable as well as radioactively labelled histone mRNA (1-10).

Although with the preparative gel electrophoresis described in this paper a partial separation and purification of the individual histone mRNAs is achieved, the different mRNA fractions cannot be used to prepare satisfactory histone cDNAs. These results are in contrast to those published by Stein and coworkers (21). Certainly, for an extensive chemical purification of histone mRNAs other techniques have to be employed additionally.

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