RNA FROM HELA CELL MICROSOMES WITH PROPERTIES OF HISTONE MESSENGER *

Dieter GALLWITZ ** and Gerald C. MUELLER ***
McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

Received 6 December 1969

1. Introduction

DNA and histone synthesis are temporally closely coupled events in the reproduction of eucaryotic cells [1–5]. Using synchronized HeLa cells it has been shown that the bulk of histones is synthesized on polysomes in the cytoplasm during a 6–8 hr interval (i.e. S-phase) that DNA replication is occurring in the nucleus [3, 5, 6]. Interruption of DNA synthesis with hydroxyurea or with a high level of thymidine leads to a rapid decline of histone synthesis as measured both in living cells and in the isolated polysome fraction *in vitro*. Since reestablishing histone synthesis after restarting DNA replication depends on the synthesis of RNA, it appears likely that the coupling between these two processes is mediated in part through labile RNA species [7]. A rapidly labeled RNA with an approximate sedimentation coefficient of 7–9 S has already been detected on histone synthesizing polysomes from HeLa cells [7, 8]. We now describe the resolution of three RNA components which have the size and labeling properties expected for histone messenger RNA.

*This work was supported in part by National Institutes of Health Grant TO1 CA-5002.

**Present address: Physiologisch-chemisches Institut der Universität Marburg, Lahnberge, 355 Marburg (Lahn), Germany.

***Recipient of a Career Award from the United States Public Health Service, to whom reprint requests should be sent.

2. Methods

HeLa cells were grown in suspension cultures and synchronized for DNA synthesis with amethopterin as described earlier [9]. At the same time that DNA synthesis was initiated by the addition of thymidine (5 μg/10^6 cells), 600 μCi of 5-^{3}H-uridine (S.A. 25.1 Ci/mmole) were added to separate 400 ml cultures containing 125 X 10^6 cells. After 60 min of labeling with uridine, DNA synthesis was inhibited in one half of the cultures with 5 X 10^{-3} M hydroxyurea. The labeling with uridine was allowed to continue for an additional 45 min in the DNA synthesizing and inhibited cultures respectively. The cells were then harvested and lysed in 0.01 M tris-HCl (pH 7.5), 0.0015 M $MgCl_2$ and 0.005 M 2-mercaptoethanol using a Dounce homogenizer as previously described [5]. After removing the nuclei and large particulates by centrifugation at 20,000 *g*, the microsome fraction was pelleted by centrifugation at 110,000 *g* for 60 min. The microsomes were resuspended in 0.01 M tris-HCl (pH 7.4), 0.015 M KCl and 0.03 M EDTA and an equal volume of 0.15 M NaCl containing 0.4% sodium dodecyl sulfate and 0.02% polyvinyl sulfate added. The RNA was extracted with water-saturated phenol for 15 min at room temperature. After precipitation with 2.5 volumes of ethanol at –15°C the RNA was dissolved in 0.1 M NaCl, 0.001 M EDTA (pH 6.2) and reprecipitated with ethanol. The RNA was then dissolved in a small volume of the NaCl-EDTA solution and subjected to electrophoresis in the cold according to Dingman and Peacock [10] using 2.0 or 3.75% acrylamide gels containing 0.5% agarose or 4.0 and

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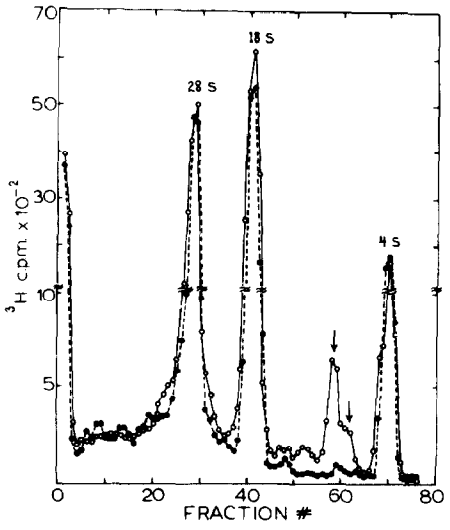

Fig. 1. Comparison of the labeled microsomal RNA isolated from HeLa cells in S-phase and RNA from cells in which DNA synthesis was blocked with hydroxyurea. 125 X 10^6 cells were synchronized with amethopterin and labeled with 600 μCi of 5-^{3}H-uridine (S.A. 25.1 Ci/mmole) for 100 min after initiating DNA synthesis with the addition of thymidine (open circles). 5 X 10^{-3} M hydroxyurea was added during the last 45 min of the labeling period in order to block DNA synthesis during this interval (closed circles). The RNA species were resolved electrophoretically in 2.0% acrylamide gels containing 0.5% agarose.

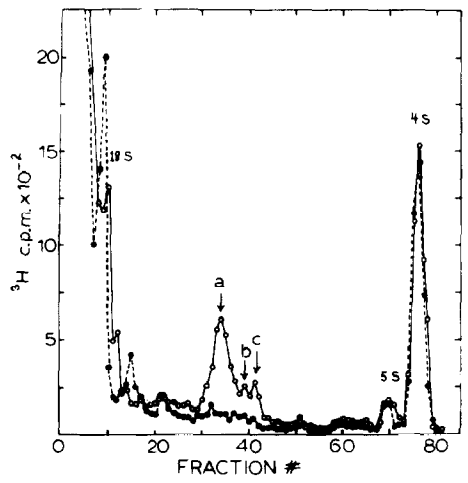

Fig. 2. Resolution of RNA species in a 3.75% acrylamide gel containing 0.5% agarose. The RNA from fig. 1 was used in this study; o = RNA from microsomes of DNA synthesizing cells; • = RNA from hydroxyurea inhibited cells. a, b, c refers to individual peaks.

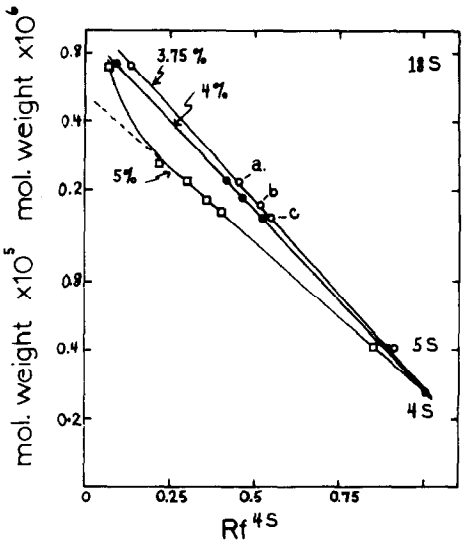

Fig. 3. An estimate of the molecular weights of the messenger RNA isolated from histone synthesizing microsomes. Electrophoresis was carried out in 3.75% acrylamide containing 0.5% agarose, 4% and 5% acrylamide gels respectively. The migration of messenger type RNA (a, b and c) is compared to that of 4 S, 5 S and 18 S in each gel system.

5.0% acrylamide gels without agarose. Electrophoresis was performed in 13 X 0.6 cm gel columns at 3 mA/tube for 165 to 200 min for the 2.0 to 5.0% acrylamide gels. Bromophenol blue was used as a tracking dye. The gels were fractionated mechanically and the radioactivity of the fractions measured as described previously [5, 9].

3. Results and discussion

Fig. 1 shows the radioactive pattern obtained in a 2.0% acrylamide gel containing 0.5% agarose. Besides the well defined 28 S, 18 S and 4 S RNAs there appears an additional RNA peak with an intimate shoulder between the 18 S and 4 S region. This fraction was not observed in RNA isolated from microsomes of cells in which DNA synthesis had not been allowed to start (i.e. unreversed thymidine deficient cells). When DNA synthesis was prevented during the final 45 min of the labeling period the extracted microsomal RNA also was deficient in this species of RNA. In other experiments it has been

shown that histone synthesis is nil in microsomes isolated from cells in both of these conditions whereas it is high when the micrsosomes are isolated from cells actively engaged in DNA synthesis [5–7]. In parallel control experiments it was shown that hydroxyurea did not interfere quantitatively with the gross incorporation of uridine into the RNA of whole cells or the microsomal fraction.

The RNA moving between 18 S and 4 S RNA could be further resolved reproducibly into three peaks using 3.75% acrylamide gels containing 0.5% agarose or using 4.0 and 5.0% acrylamide gels without agarose (fig. 2). From the linear relationship between the logarithm of the molecular weight of an RNA and the distance moved in acrylamide gels [10–12] the approximate molecular weight of these three peaks was calculated to be 0.22×10^6 (peak a), 0.18×10^6 (peak b) and 0.15×10^6 (peak c) (fig. 3). The assumed molecular weights used as reference were 1.9×10^6 and 0.71×10^6 for 28 S and 18 S RNA respectively [13], 0.41×10^5 for 5 S RNA [14] and 0.26×10^5 for 4 S RNA. Assuming that the different histone molecules consist of approximately 102 [15] to 216 [16] amino acid residues the minimal molecular weight for histone messenger RNAs would be expected to be $0.1–0.22 \times 10^6$. The three fractions, a, b, c, which were resolved on polyacrylamide gels fall into this range.

The fact that these three RNAs are found only on microsomes which are actively engaged in histone synthesis and that their molecular sizes correspond to the sizes needed to code for proteins of the histone class suggests that the RNAs may be messengers for histone synthesis. Proof of this conclusion awaits demonstration that the isolated RNAs can inform an *in vitro* protein synthesizing system in the synthesis of histones. In such a case the individual RNA species may provide a means for studying the genetic control for the synthesis of specific histones.

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