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Multiple forms of H4 histone mRNA in human cells

(HeLa cells/electrophoresis/translation)

A. C. LICHTLER*, S. DETKE*, I. R. PHILLIPS*, G. S. STEIN*, AND J. L. STEIN†

*Department of Biochemistry and Molecular Biology, and †Department of Immunology and Medical Microbiology, University of Florida, Gainesville, Florida 32610

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ABSTRACT **Two species of H4 histone mRNA were isolated from the polysomes of S phase HeLa S_3 cells. Electrophoresis under denaturing and nondenaturing conditions indicates that the two H4 mRNAs differ in size. Both mRNAs translate H4 histones *in vitro*, lack poly(A) at their 3′ termini, and are capped at their 5′ termini. Polyacrylamide gel electrophoresis and tryptic peptide analysis suggest that the polypeptides synthesized by the two mRNAs are indistinguishable.**

Histones play an important role in structural and transcriptional properties of the eukaryotic genome (reviewed in refs. 1–10). Hence, over the past several years a considerable amount of attention has been focused on histone genes and on the regulation of histone gene expression. Histone genes are intriguing from a biological standpoint for a number of reasons. Of particular importance are the presence of five classes of histone polypeptides and the transcription of histone mRNAs from reiterated genes. The fact that there are multiple copies of histone genes raises important considerations regarding the identity and functional significance of the histone sequences. Have all copies of the genes coding for individual histones been conserved? Are all copies of the histone genes expressed simultaneously? While fractionating histone mRNAs with the objective of isolating mRNAs for individual histones, we isolated from the polysomes of S phase HeLa cells two mRNAs that code for the arginine-rich H4 histone. We have therefore been addressing the questions of whether the two mRNAs are distinctively different species and whether there are variations in the proteins coded by the H4 histone mRNAs.

MATERIALS AND METHODS

Preparative Polyacrylamide Gel Electrophoresis of HeLa Cell Histone mRNA. ^{32}P-Labeled polysomal RNA was isolated from 1 liter of S phase HeLa cells synchronized by double thymidine block (11). Cells were lysed in 20 mM Tris·HCl, pH 7.5/25 mM NaCl/5 mM $MgCl_2$/0.1 mM spermidine/1% deoxycholate/1% Triton X-100 and centrifuged at 8000 × *g* for 10 min. The supernatant was centrifuged through a cushion of 2 M sucrose in lysis buffer without detergent at 100,000 × *g* for 2 hr, and the polysomal RNA pellet was extracted with phenol/chloroform/isoamyl alcohol (12). 7–11S RNA was isolated on a 5–30% sucrose gradient as described (12). Unlabeled 7–11S RNA was isolated by using similar procedures from 30 liters of S phase HeLa cells, mixed with ^{32}P-labeled RNA, and electrophoresed on a 6% polyacrylamide slab gel polymerized in 36 mM Na_2HPO_4/30 mM Tris·HCl/2 mM EDTA/0.2% $NaDodSO_4$ (at pH 7.8) as described (12, 13). Bands were located by autoradiography and excised and the RNA was eluted electrophoretically (13).

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Cell-Free Protein Synthesis. Cell-free translation of the excised bands was carried out according to Weber *et al.* (14) with 20 μCi of [^{3}H]lysine and 1–5 μg of RNA per 50-μl reaction mixture. Aliquots of the translation system were mixed with unlabeled HeLa cell marker histones, dialyzed against 0.9 M acetic acid/2.5 M urea/0.4% 2-mercaptoethanol, and electrophoresed on 15% acetic acid/urea/polyacrylamide gels (15). The gels were stained with amido black to locate unlabeled markers and fluorographed (16, 17), and the x-ray films were scanned with a Joyce–Loebel densitometer.

Denaturing Polyacrylamide Gel Electrophoresis of H4-1 and H4-2 Histone mRNAs. Electrophoresis in polyacrylamide gels containing formamide was carried out according to Maniatis *et al.* (18). ^{32}P-Labeled RNAs eluted from a nondenaturing polyacrylamide gel were precipitated with ethanol. The RNA pellet was resuspended in deionized formamide, heated at 100°C for 2 min, quick cooled on ice, and placed onto an 8% acrylamide/98% formamide slab gel buffered with 20 mM sodium phosphate (pH 7.5). The gel was run at 200 V/cm and autoradiographed while wet.

Electrophoresis in polyacrylamide gels containing glyoxal was carried out as described (19). RNA and DNA samples for glyoxal gels were precipitated with ethanol, dried, and resuspended in 1.0 M deionized glyoxal/50% dimethyl sulfoxide/10 mM sodium phosphate (pH 7.0) and incubated at 50°C for 1 hr. The samples were then electrophoresed on a 6% polyacrylamide gel buffered with 10 mM sodium phosphate (pH 7.0). The running buffer also contained 10 mM glyoxal. Electrophoresis was at 180 V for 15 hr; the gel was dried and autoradiographed according to Laskey and Mills (17).

Cap Analysis of H4-1 and H4-2. ^{32}P-Labeled RNAs eluted from gels were digested with ribonuclease T1 (350 units/ml), T2 (400 units/ml), and A (50 μg/ml) in ammonium acetate (pH 6) for 6 hr at 37°C according to Levis and Penman (20) and then with alkaline phosphatase according to Haegeman and Fiers (21) in 0.1 M NH_4HCO_3 at 37°C for 40 min. Chromatography was carried out on polyethyleneimine-cellulose in 1.3 M formic acid/pyridine (pH 4.3) according to Haegeman and Fiers (21). After autoradiography, the spots that migrated more slowly than the P_i spot were eluted with 30% triethylamine/CO_2 (pH 7.7) (21), digested with P1 nuclease [1 mg/ml in 50 mM ammonium acetate (pH 6)] for 1 hr at 37°C, and then spotted on glass-backed cellulose thin-layer plates (Macherey and Nagel Cel 400-10) along with unlabeled core caps and methylated and unmethylated nucleotides (P-L Biochemicals). Two-dimensional chromatography was carried out with isobutyric acid/concentrated NH_4OH/H_2O, 66:1:33 (vol/vol), in the first dimension and *n*-propanol/concentrated HCl/H_2O, 68:17.6:14.4 (vol/vol) (22) in the second dimension. The thin layers were autoradiographed at −70°C with intensifying screens for 3 weeks; unlabeled markers were located under ultraviolet light.

Two-Dimensional Thin-Layer Separation of Tryptic Peptides from Translation Products of H4-1 and H4-2 Histone mRNAs. H4-1 and H4-2 mRNAs were translated *in vitro* with the wheat germ translation system of Weber *et al.* (14). One microgram of mRNA was translated for 1 hr in a 50-μl assay containing 20 μCi of either [^{3}H]lysine (80 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) or [^{3}H]leucine (62 Ci/mmol). At the conclusion of the translation, 100 μg of HeLa histone H4 (isolated as described in ref. 23) was added to the translation assay, which was then dialyzed overnight against water. The dialyzed translation assay was dried under reduced pressure, suspended in 50 μl of 1% NH_4CO_3, digested for 2 hr at 37°C with 3 μl of *N*-tosylphenylalanine chloromethyl ketone (TPCK)-treated trypsin (20 mg/ml, Sigma), digested for a further 2 hr with another 3 μl of trypsin, dried under reduced pressure, and suspended in water. The samples were spotted on a thin-layer cellulose plate (20 × 20 cm, Eastman) and electrophoresed for 80 min at 400 V with pyridine/acetic acid/water, 1:10:289 (vol/vol). The thin-layer cellulose plates were dried and subjected to chromatography in the second dimension with *n*-butanol/acetic acid/H_2O, 4:1:5 (vol/vol) (24). The thin-layer cellulose plates were dried, stained with ninhydrin (0.2% ninhydrin/1% pyridine/1% acetic acid in acetone), and prepared for fluorography by dipping in 7% (wt/vol) 2,4-diphenyloxazole (PPO) and anhydrous diethyl ether. Radioactivity was detected with sensitized X-RP1 x-ray film (17) at −70°C for 20–40 days.

RESULTS AND DISCUSSION

Preparative scale electrophoresis of unlabeled 5–18S polysomal RNA from S phase HeLa cells in the presence of ^{32}P-labeled 5–18S tracer RNA gave the pattern shown in Fig. 1*A*. The individual bands were excised and the RNAs were eluted and then translated in a wheat germ cell-free protein-synthesizing system (14). The translation products were electrophoresed with unlabeled HeLa cell marker histones on acetic acid/urea/polyacrylamide gels (15); no preliminary purification to separate the histones from other translation products was carried out prior to electrophoresis. The gel was analyzed by fluorography (16, 17), and the results from translation of the RNAs from the faster (H4-1) and more slowly (H4-2) migrating H4 bands of Fig. 1*A* are shown in Fig. 1 *B* and *C*. The amount of [^{3}H]lysine label incorporated into hot acid-resistant, trichloroacetic acid-precipitable material was proportional to the amount of RNA added to the translation mixture. A similar procedure was followed to assign the coding properties of the other bands shown in Fig. 1*A*. For both bands designated H4 (Fig. 1*A*), the majority of the labeled translation product comigrated with purified marker HeLa cell H4 histone (Fig. 1 *B* and *C*). Based on the assumption that each of the protein products has a similar specific activity and that the area of each peak is proportional to the amount of radioactivity in the gel band, planimetric integration of the peaks indicates that greater than 95% of the translation product of the faster migrating H4 band (Fig. 1*A*) and approximately 85% of the translation product of the more slowly migrating H4 (Fig. 1*A*) constitutes H4 histone. Peak WG (Fig. 1 *B* and *C*) is a wheat germ protein (based on translation in the absence of added mRNA); therefore, its contribution to the total amount of protein has been subtracted in the above calculation. The fast migrating material in band A most likely represents incomplete polypeptides, but has been included in the purification calculations. Our estimations of purities for the mRNAs are therefore somewhat conservative. The efficiency of translation of both H4 histone mRNAs was similar. Neither of the RNA bands identified as H4 histone mRNAs was synthesized in cells treated with cytosine arabinoside or hydroxyurea.

Because the buffer system used in the electrophoretic fractionation shown in Fig. 1*A* contained 76 mM Na^+, which would allow RNA to have considerable secondary structure, it is possible that the two mRNA species have the same molecular weight but different secondary structure. It is also possible that the more slowly migrating H4 band (Fig. 1*A*) is a complex of the faster migrating band with some other RNA species. We therefore eluted the two H4 histone mRNA bands from an aqueous gel, denatured the RNAs in hot 98% formamide, and compared their electrophoretic mobilities in 8% polyacrylamide gels containing 98% formamide (18, 25). Under these conditions, RNAs migrate solely as a function of molecular weight and differences in secondary structure or aggregation of RNA molecules should be eliminated. It can be seen in Fig. 2*A* that the two H4 histone mRNAS have distinctly different mobilities.

Further support for the contention that the two H4 mRNAs differ in molecular weight rather than in degree of secondary structure can be gleaned from their distinctly different electrophoretic migration in 6% acrylamide gels containing 10 mM glyoxal (Fig. 2*B*). Glyoxal interacts preferentially with guanosine residues of the RNA molecules and the glyoxalated nucleic acid, essentially irreversibly denatured, migrates as a function of molecular weight alone (19). *Hin*dIII fragments of ^{32}P-labeled DNA from simian virus 40 were present in an ad-

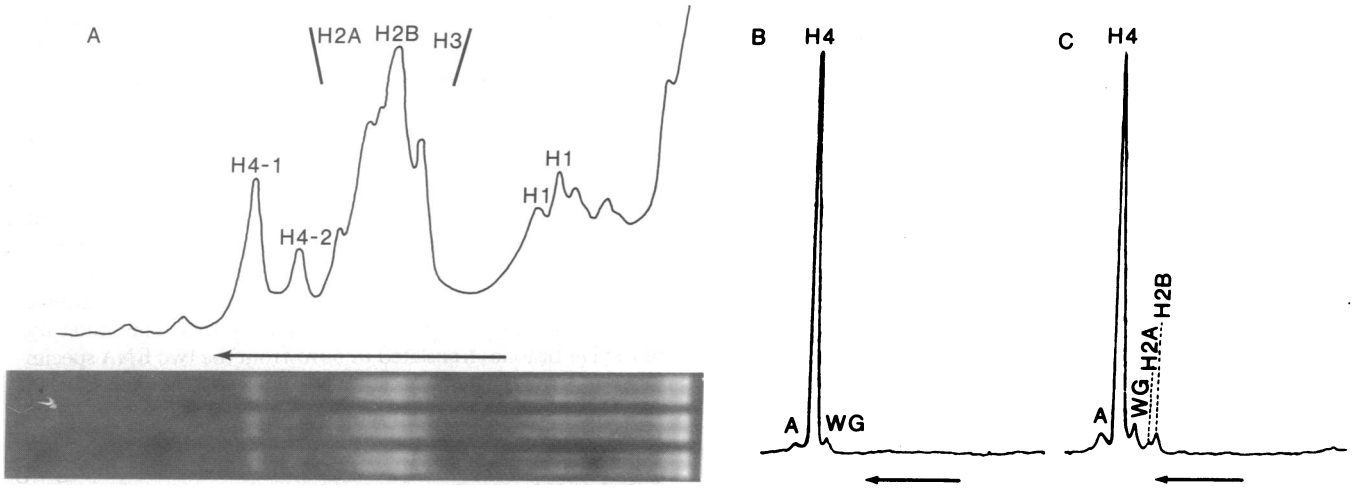

FIG. 1. (*A*) Preparative polyacrylamide gel electrophoresis of HeLa cell histone mRNA. (*B*) Translation products from band labeled H4-1. (*C*) Translation products from band labeled H4-2. WG, wheat germ protein.

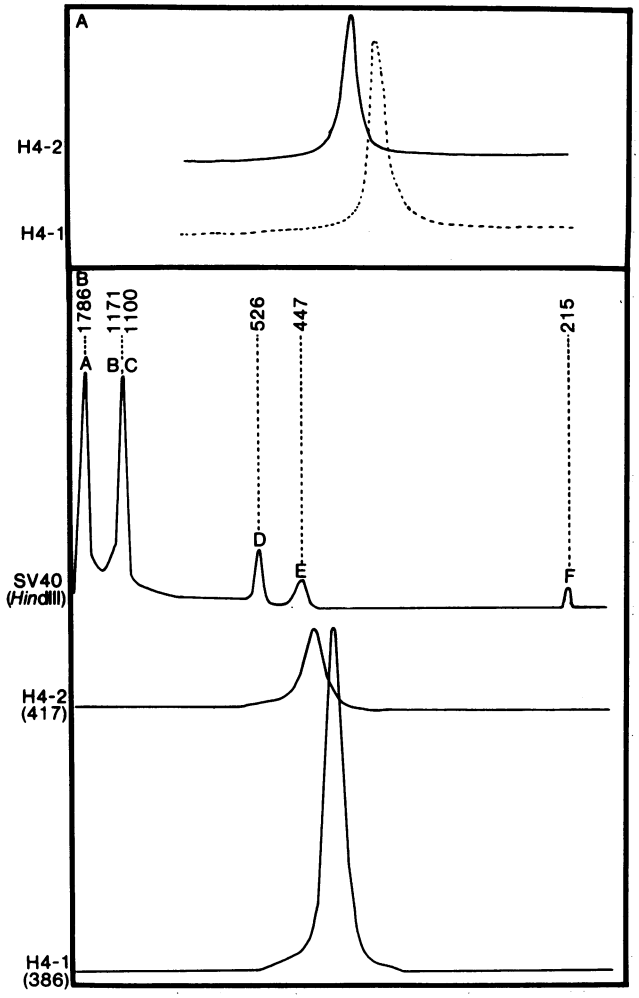

FIG. 2. Denaturing polyacrylamide gel electrophoresis of H4-1 and H4-2 histone mRNAs. (*A*) Electrophoresis of ^{32}P-labeled H4-1 and H4-2 histone mRNAs was done as described by Maniatis et al. (18) and was followed by autoradiography. —, Optical density scan of H4-2 autoradiogram; - - -, optical density scan of H4-1 autoradiogram. Direction of migration is from left to right. (*B*) Electrophoresis of ^{32}P-labeled H4-1 and H4-2 histone mRNAs was carried out as described by McMaster and Carmichael (19) and was followed by autoradiography. (*Upper*) Optical density scan of autoradiogram of size markers, which were *Hin*dIII restriction fragments of simian virus 40. Size is indicated in number of bases. (*Lower*) Optical density scan of H4-1 and H4-2 autoradiograms.

jacent well of the slab gel during electrophoresis and were used as molecular weight markers. Because the molecular weights of DNA and RNA are directly comparable in glyoxal gels (19), it can be calculated that HeLa cell H4-1 histone mRNA contains 386 nucleotides—approximately 80 nucleotides (20%) more than required to code for H4 histone. H4-2 histone mRNA contains 417 nucleotides. Similarly, H4-1 and H4-2 histone mRNAs migrate independently in denaturing agarose gels containing methylmercury (data not shown).

Evidence for the presence of two distinct H4 histone mRNAs in S phase HeLa cells also came from experiments in which the H4 histone mRNA region of a gel as shown in Fig. 1*A* was serially sectioned and extracted RNAs from H4-1 and H4-2 bands as well as from the region between the bands were translated *in vitro;* the translational activity per μg of RNA for H4 histone was significantly greater for the H4-1 and H4-2 RNA bands than for the region between the bands. The presence of two H4 histone mRNA species was further substantiated by the presence of H4-1 and H4-2 histone mRNAs in these gels when RNAs were labeled *in vitro* by addition of ^{32}P-labeled pCp to the 3′ termini with T4 RNA ligase.

The 5′ and 3′ termini of the two H4 histone mRNAs have been partially characterized. When chromatographed twice on oligo(dT)-cellulose, both H4 mRNAs eluted with the high salt wash and maintained unaltered electrophoretic mobilities (data not shown). The latter results indicate that neither RNA species contains a poly(A) region long enough to allow it to be retained on an oligo(dT)-cellulose column. Furthermore, these findings suggest that the difference in electrophoretic mobilities of the two H4 histone mRNAs is not attributable to poly(A) at the 3′ terminus of one of the mRNA species.

Analysis of the 5′ termini of the two H4 histone mRNAs indicates the presence of cap structures. H4-1 and H4-2 histone mRNAs eluted from a gel were digested with a mixture of ribonucleases A, T1, and T2, which together hydrolyze every RNA phosphodiester bond unprotected by 2′-*O*-methylation, to give 3′-mononucleotides and cap structures of the form m^{7}G5′ppp5′NmpMp (cap 1) and m^{7}G5′ppp5′NmpMmpZp (cap 2). These were further digested with alkaline phosphatase to remove the terminal phosphate from the caps and from the mononucleotides. The products were fractionated on polyethyleneimine-cellulose according to Haegeman and Fiers (21). As shown in Fig. 3*A* for each histone mRNA, there are three spots (labeled A, B, and C) that migrate in the region between the origin and location of inorganic phosphate. Spot A was eluted separately, whereas spots B and C were eluted together because they contained very low levels of radioactivity. The eluted material was further analyzed by digestion with *Penicillium* nuclease P1, which cleaves RNA nonspecifically to yield ribonucleoside 5′-monophosphates irrespective of ribose 2′-*O*-methylation and, thus, will release structures of the form m^{7}G5′ppp5′Nm (core caps). The P1-digested caps were fractionated in the presence of unlabeled markers by two-dimensional thin-layer chromatography (22) on cellulose thin-layer plates followed by autoradiography. This chromatography system is capable of separating all the common methylated and unmethylated nucleotides found in cap structures, as well as the core cap structures m^{7}G5′ppp5′Gm and m^{7}G5′ppp5′Am, which we have previously shown to be the only core cap structures found in a preparation containing H3, H2A, H2B, and H4 histone mRNAs (26). Radioactive material associated with spots A, B, and C of H4-1 and H4-2 histone mRNAs (Fig. 3*A*) comigrated with unlabeled m^{7}G5ppp5Gm (Fig. 3*B*). Radioactive material associated with spot A of both histone mRNAs also comigrated with unlabeled 5′-UMP. Our inability to detect labeled mononucleotides in material eluted from spots B and C is probably due to the extremely low levels of radioactivity present and to the expected reduced amount of radioactivity in mononucleotides compared to core caps. We interpret our results to indicate that three types of cap structures, all containing 2′-*O*-methyl-G as the penultimate base, are associated with H4-1 and H4-2 histone mRNAs; spot A is cap 1 and spots B and C are cap 2 structures.

As shown in Fig. 1, the polypeptides translated *in vitro* from the two H4 histone mRNAs fractionated identically in acetic acid/urea/polyacrylamide gels and electrophoresed coincidentally with H4 histone marker. Under these conditions, fractionation is by charge and molecular weight. The similarity of the H4 histones translated *in vitro* from the two RNA species was confirmed by digesting [^{3}H]leucine- or [^{3}H]lysine-labeled polypeptides with trypsin and carrying out two-dimensional peptide mapping with ninhydrin-stained peptides from *in vivo* synthesized HeLa H4 histone as markers. As shown in Fig. 4, radiolabeled peptides translated by H4-1 and H4-2 histone mRNAs fractionated identically in a two-dimensional thin-layer

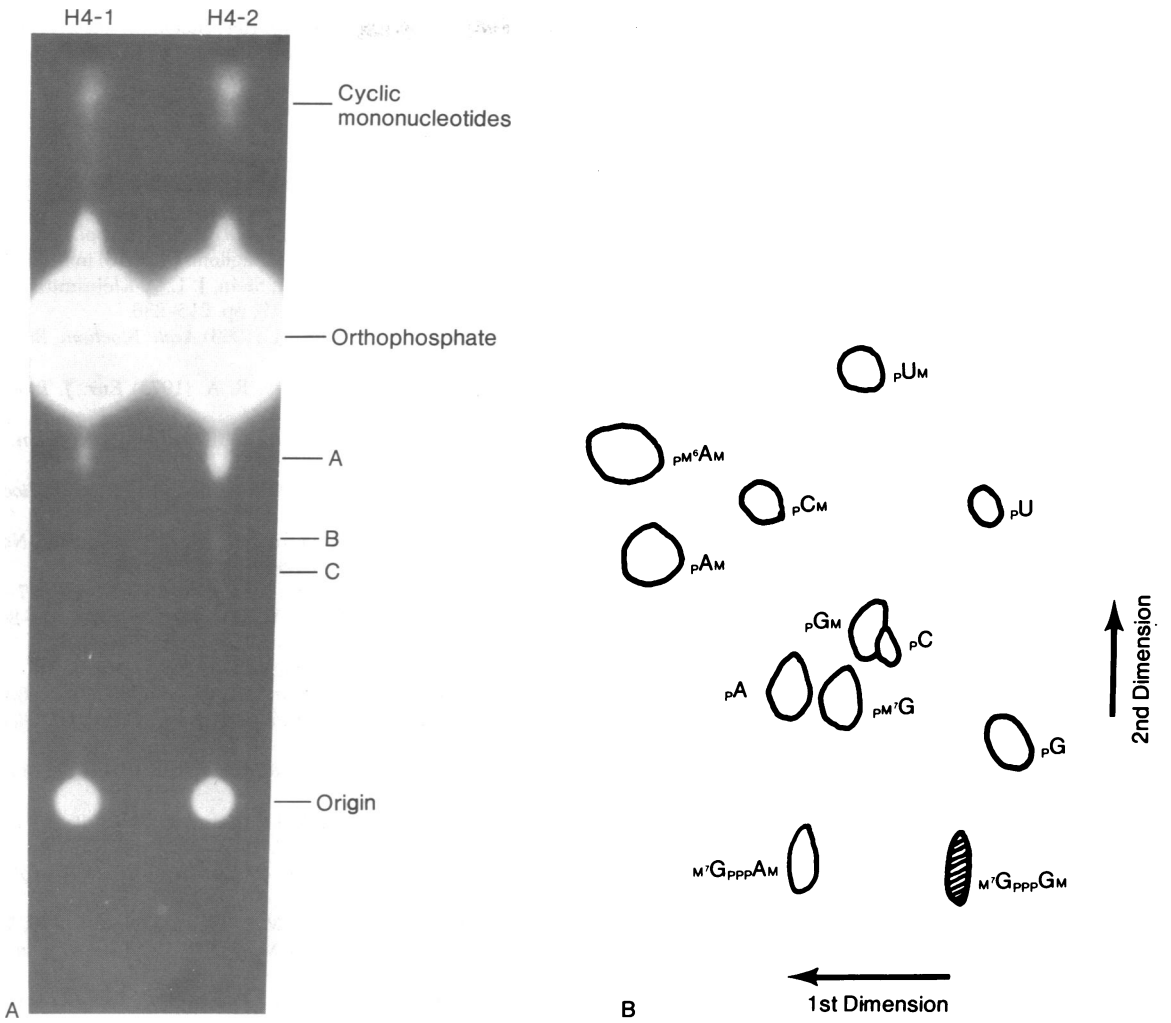

FIG. 3. Cap analysis of H4-1 and H4-2. (*A*) Thin-layer chromatography of T1, T2, and A nuclease digestion products from H4-1 and H4-2 histone mRNAs. (*B*) Two-dimensional chromatography of P1 nuclease-digested caps from H4-1 and H4-2 histone mRNAs. Circles show location of unlabeled markers; cross-hatching shows location of ^{32}P label. The chromatograms from digests of H4-1 and H4-2 histone mRNAs are identical.

chromatography/electrophoresis system (24). We have also shown that proteins translated from H4-1 and H4-2 histone mRNAs are identical by using another radiolabeled amino acid (glycine) and fractionating tryptic peptides by the two-dimensional chromatography/electrophoresis procedure of Katz *et al.* (27).

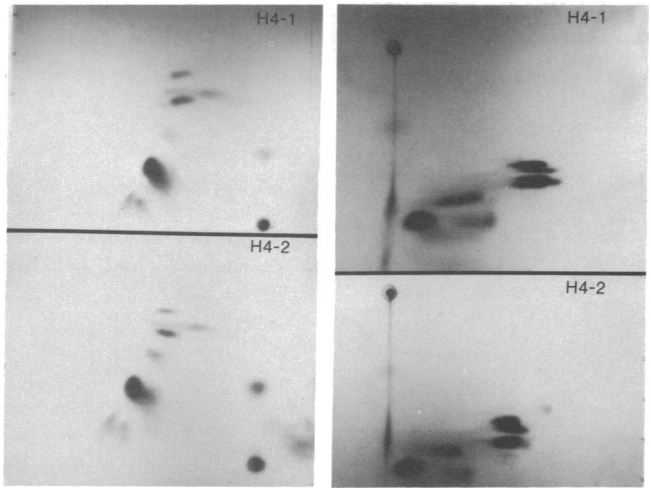

FIG. 4. Two-dimensional thin-layer separations of (*Left*) [^3H]-leucine- and (*Right*) [^3H]lysine-labeled tryptic peptides from the translation products of H4-1 and H4-2 histone mRNAs.

In summary, our results suggest that there may be at least two size classes of mRNA on the polysomes of S phase HeLa cells that code for identical H4 histone proteins. Both H4 histone mRNA species lack poly(A) at the 3′ terminus and contain the same cap structure, $m^7G5'ppp5'Gm$, at the 5′ terminus.

There are several possible explanations for the presence of multiple forms of H4 histone mRNA in HeLa S_3 cells. One is a precursor–product relationship between a larger and smaller H4 mRNA species with at least one processing step needed to give the final product, H4-1. Because both RNAs were isolated from polysomal RNA, it would be necessary to postulate that the final processing step takes place in the cytoplasm and is not absolutely necessary for production of functional mRNA. This possibility is consistent with the data of Hackett *et al.* (28) showing that the UV target size for the HeLa H4 mRNA is close to 1000 bases, although such target size could also reflect the binding of RNA polymerase molecules at a promoter that is a considerable distance from the initiation of transcription. Several eukaryotic mRNAs are transcribed in precursor form, and this may also be true of HeLa histone mRNAs. The processing events could be simple cleavages of sequences at the 3′ or 5′ ends. If the 5′ end were cleaved, the cleavage site would

have to produce a 5′-G sequence which would then be recapped. Alternatively, there could be internal splicing at a 5′ or 3′ noncoding region of the RNA.

HeLa histone genes are reiterated 30- to 40-fold (29), raising the possibility that the H4-1 and H4-2 mRNAs represent transcripts from different copies of the human histone genes derived from the same parent. Alternatively, the two RNA species could represent allelic differences in the genes contributed by the two parents. By analogy, sea urchin histone genes are reiterated (30, 31), and sequence analysis of cloned genes and restriction enzyme analysis of cellular DNA show that they are arranged in clustered, tandem repeat units (32–34). Variations in the sea urchin gene repeat units that are transcribed at different stages of development have been observed, yielding multiple forms of histone mRNAs or histone proteins or both (35–37). Of particular interest is the evidence of Grunstein (38) showing H4 histone mRNAs that differ in length by 40 bases and also show considerable sequence divergence even within the coding region, although this is believed to reflect changes in the third bases of codons that code the same amino acid. Although it is not known if HeLa histone genes are arranged in clustered repeat units, the fact that HeLa histone genes are reiterated raises the possibility that there may be different classes of human histone genes that are under different regulatory control. These classes could conceivably be regulated differentially during development, after chemical or viral transformation, or during neoplastic progression. The question of whether the different HeLa cell H4 histone mRNAs are in a precursor–product relationship or whether they represent transcripts from different classes of histone genes, as well as the implications thereof, can best be answered by sequence analysis of the two mRNAs.

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1. Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R.-C., Marushige, K. & Tuan, D. Y. H. (1968) *Science* **159,** 47–56.
2. Stein, G. S., Spelsberg, T. C. & Kleinsmith, L. J. (1974) *Science* **183,** 817–824.
3. Hnilica, L. S. (1972) *The Structure and Biological Function of Histones* (CRC, Cleveland).
4. Elgin, S. C. R. & Weintraub, H. (1975) *Annu. Rev. Biochem.* **44,** 725–744.
5. Busch, H. (1978) *The Cell Nucleus* (Academic, New York), Vols. 4–7.
6. Kornberg, R. D. (1977) *Annu. Rev. Biochem.* **46,** 931–954.
7. Felsenfeld, G. (1978) *Nature (London)* **271,** 115–122.
8. Kedes, L. H. (1979) *Annu. Rev. Biochem.* **48,** 837–870.
9. Stein, G. S., Stein, J. L. & Thomson, J. A. (1978) *Cancer Res.* **38,** 1181–1201.
10. Kedes, L. H. (1976) *Cell* **8,** 321–331.
11. Stein, G. S. & Borun, T. W. (1972) *J. Cell Biol.* **52,** 292–307.
12. Thrall, C. L., Lichtler, A., Stein, J. L. & Stein, G. S. (1978) in *Methods in Cell Biology,* eds. Stein, G. S., Stein, J. L. & Kleinsmith, L. J. (Academic, New York), Vol. 19, pp. 237–255.
13. Grunstein, M. & Schedl, P. (1976) *J. Mol. Biol.* **104,** 323–349.
14. Weber, L. A., Nilsen, T. & Baglioni, C. (1978) in *Methods in Cell Biology,* eds. Stein, G. S., Stein, J. L. & Kleinsmith, L. J. (Academic, New York), Vol. 19, pp. 215–236.
15. Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130,** 337–346.
16. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
17. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
18. Maniatis, T., Jeffrey, A. & Van de Sande, H. (1975) *Biochemistry* **14,** 3787–3794.
19. McMaster, G. K. & Carmichael, C. G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4835–4838.
20. Levis, R. & Penman, S. (1978) *J. Mol. Biol.* **120,** 487–516.
21. Haegeman, G. & Fiers, W. (1978) *J. Virol.* **25,** 824–830.
22. Hall, R. (1967) *Methods Enzymol.* **12,** 305–315.
23. Johns, E. W. (1967) *Biochem. J.* **105,** 611–614.
24. Bennett, J. C. (1967) *Methods Enzymol.* **11,** 330–339.
25. Pinder, J. C., Staynov, D. Z. & Gratzer, W. B. (1974) *Biochemistry* **13,** 5373–5378.
26. Stein, J. L., Stein, G. S. & McGuire, P. M. (1977) *Biochemistry* **16,** 2207–2213.
27. Katz, A. M., Dreyer, W. J. & Anfinsen, C. B. (1959) *J. Biol. Chem.* **234,** 2897–2900.
28. Hackett, P. B., Traub, P. & Gallwitz, D. (1978) *J. Mol. Biol.* **126,** 619–635.
29. Wilson, M. C. & Melli, M. (1977) *J. Mol. Biol.* **110,** 511–535.
30. Kedes, L. H. & Birnstiel, M. L. (1971) *Nature (London) New Biol.* **230,** 105–169.
31. Weinberg, E. S., Birnstiel, M. L., Purdom, I. F. & Williamson, R. (1972) *Nature (London)* **240,** 225–228.
32. Schaffner, W., King, G., Daetwyler, H., Telford, J., Smith, H. O. & Birnstiel, M. L. (1978) *Cell* **14,** 655–671.
33. Kedes, L. H., Chang, A. C. Y., Housman, D. & Cohen, S. (1975) *Nature (London)* **255,** 533–538.
34. Sures, I., Maxam, A., Cohn, R. H. & Kedes, L. H. (1976) *Cell* **9,** 495–502.
35. Overton, G. C. & Weinberg, E. S. (1978) *Cell* **14,** 247–257.
36. Kunkel, N. S. & Weinberg, E. S. (1978) *Cell* **14,** 313–326.
37. Newrock, K. M., Cohen, L. H., Hendricks, M. B., Donnelly, R. J. & Weinberg, E. S. (1978) *Cell* **14,** 327–336.
38. Grunstein, M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4135–4139.