ISOLATION AND CHARACTERIZATION OF HISTONE f1 IN RIBOSOMES*

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SUMMARY

A protein has been isolated from purified cytoplasmic polysomes and ribosomes which appears to be histone f1 based on its electrophoretic mobility, extraction properties, amino acid analysis, and color when stained with alizarin black in polyacrylamide gels. It was found in large and small polysomes, ribosomes, and ribosomal subunits. It remained attached to polysomes after treatment with 0.25 M NH_4Cl or with 0.01 M EDTA in 0.2 M NaCl, which remove extraneously adsorbed proteins.

INTRODUCTION

Recent studies made in this Laboratory (1-4) have contributed to the accumulating evidence that the metabolism of the very lysine-rich histone f1 differs markedly from that of the other histone fractions. For example, histone f1 was shown to turn over significantly in the chromatin of exponentially-growing Chinese hamster cells, while the other histones were conserved for several generations (2,3). A possible correlation between this f1 turnover and RNA synthesis was observed, suggesting that the continual replacement of f1 in the chromatin might somehow be involved in stable RNA synthesis (2,3). It was also suggested in these reports that there existed a nonchromatin pool of f1 (2,3). These studies led us to search for f1 in cytoplasmic polysomes which contain a large portion of the cell's stable RNA in their ribosomes. The following report describes the isolation and identification of histone f1 in ribosomes and polysomes.

METHODS

Chinese hamster cells (line CHO) grown in suspension culture (5) were harvested by sedimentation, washed with cold ribonuclease-free 0.25 M sucrose, and suspended in 4 ml of 0.13 M NaCl-RSB (0.13 M NaCl; 0.0015 M $MgCl_2$; 0.01 M Tris, pH 7.4) at 1-3°C. The cells were then treated with Nonidet P-40 (6) and

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sodium deoxycholate as previously described (7), which solubilized the cytoplasm without breaking the nuclei. The nuclei were removed by centrifuging 10 minutes at 800 x g. Smaller particulate material was then removed from the cytoplasm by centrifuging it 5 minutes at 20,000 x g. This procedure, which employed no mechanical devices to break the cells, produced a cytoplasmic preparation free from contamination with nuclei, broken nuclear fragments, or DNA extracted from nuclei (Table I).

Two ml of cytoplasm were layered over a 7.5-35% sucrose gradient made in 0.13 M NaCl-RSB and centrifuged 4 hours at 25,000 rpm (75,500 x g) in a Spinco SW-25.2 rotor. Ribosomal subunits, ribosomes, and polysomes containing 2-9 ribosomes were collected from fractions of the sucrose gradient. Larger polysomes were collected as a pellet (Fig. 1a). The sucrose gradient fractions were centrifuged 15 hours at 140,000 x g to recover the particulates as pellets.

In experiments where large quantities of ribosomal particles were needed, the large volume of cytoplasm from 3 x 10^9 cells was first layered over 2.2 M sucrose in 0.13 M NaCl-RSB and centrifuged 17 hours at 140,000 x g. The resulting pellet was suspended in 0.13 M NaCl-RSB. Two ml of the concentrated suspension were layered over each of three 7.5-35% sucrose gradients and centrifuged 17 hours at 22,500 rpm in a Spinco SW-25.2 rotor. The 40S, 60S, and 80S regions were collected as fractions from the sucrose gradient as described above. The polysomes were collected as a pellet below the gradient (Fig. 1b).

Total acid-soluble proteins were extracted from polysomes or from chromatin (1) with 0.4 N H_2SO_4. The proteins were recovered as precipitates by adding 10 volumes of acetone. Histone f1 was extracted from the polysomal and ribosomal pellets or from chromatin with 5% $HClO_4$ by the method of Johns (8). The f1 was recovered from the extract by precipitation overnight in 18% trichloroacetic acid.

The preparations were subjected to electrophoresis as described by Panyim and Chalkley (9). After electrophoresis, the gels were stained overnight in a 0.5% alizarin black-7% acetic acid solution and then electrophoretically destained. In some cases, a thin strip of plastic was inserted 2 mm into the top of the gel so that two samples could be run in the same gel to compare their mobilities.

Nanomolar amino acid analysis was performed on 40 µg of protein using a Beckman Model 120-B amino acid analyzer equipped with high-sensitivity cuvettes using a method developed in this Laboratory by Shepherd and Roberts (10).

RESULTS

When histones are separated by electrophoresis and stained with alizarin black, histones f3, f2b, f2a2, and f2a1 stain a dark blue color, whereas histone f1 stains a strikingly different light blue-green color (gel a, Fig. 2). When the acid-soluble proteins of polysomes were subjected to the same treatment, a protein band having the same mobility and characteristic color as f1 was observed (gels b and c, Fig. 2). When polysomes were extracted with 5% $HClO_4$, a method which specifically extracts histone f1 from chromatin (8), this protein was extracted and recovered (gel d, Fig. 2). Split gel runs

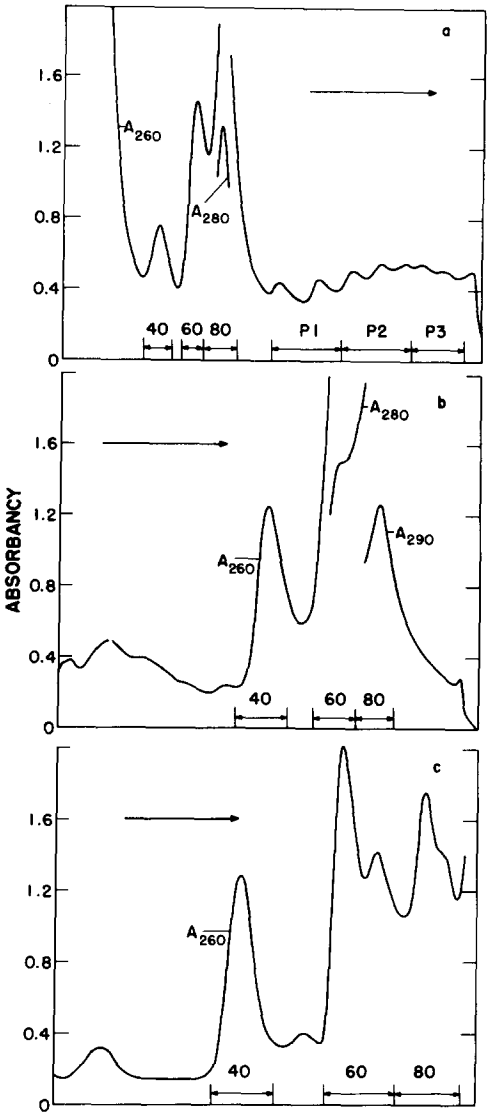

Fig. 1. Sucrose gradient zone sedimentation absorbancy profiles; top (left), bottom (right): (a) cytoplasmic polysomes from 1.5 x 10^8 cells; P1 region, 2-3 ribosomes per polysome; P2 region, 4-6 ribosomes per polysome; P3 region, 7-9 ribosomes per polysome; pellet, 10 or more ribosomes per polysome; (b) ribosomes from 5 x 10^8 cells; pellet contained all the polysomes; and (c) polysomes and ribosomes from 9 x 10^8 cells after treatment with 0.1 M EDTA in 0.2 M NaCl. Pellet contained all the polysomes; fractions were taken from the 40S, 60S, and 80S regions as shown.

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confirmed that this polysomal protein had the same mobility as the f1 in whole chromatin histone (gel e, Fig. 2) and as $HClO_4$-isolated chromatin f1 (gel f, Fig. 2). A typical recovery of polysomal and nuclear chromatin f1 was 103 and 797 µg/10^9 cells, respectively, the polysomal f1 making up 11.4% of the

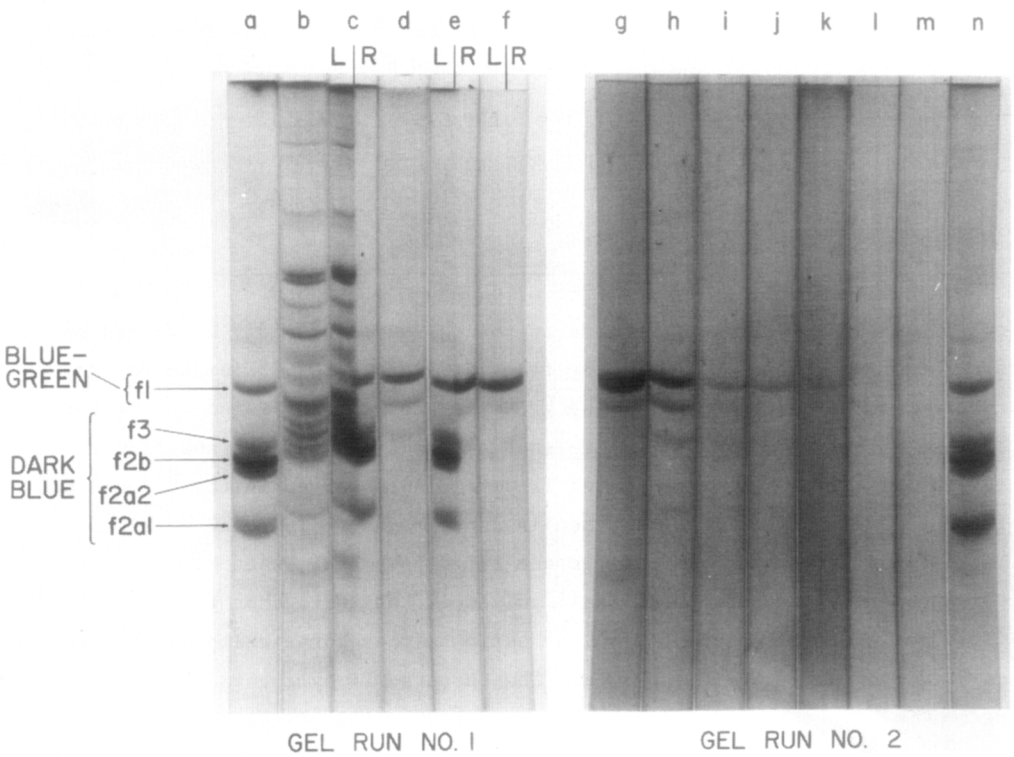

Fig. 2. Electrophoretic patterns in polyacrylamide gels (15% acrylamide; 2.5 M urea; 0.9 N acetic acid, pH 2.7) after 3.5 hours at 2 mamps per gel.

Gel Run No. 1: Various protein preparations (a) 100 µg whole CHO histone; (b) 100 µg polysomal acid-soluble protein; (c) split gel, same as (a) and (b); (d) 25 µg polysomal f1; (e) split gel, 50 µg whole histone (left) and 10 µg polysomal f1 (right); and (f) split gel, 5 µg nuclear f1 (left) and 10 µg polysomal f1 (right).

Gel Run No. 2: Histone f1 extracted from polysomes and ribosomes shown in Fig. 1a (total amount recovered was run); (g) 25 µg nuclear f1; (h) polysomal pellet, 10 or more ribosomes per polysome; (i) polysomal region P3, 7-9 ribosomes per polysome; (j) polysomal region P2, 4-6 ribosomes per polysome; (k) polysomal region P1, 2-3 ribosomes per polysome; (l) 80S region; (m) 60S region; and (n) 100 µg whole CHO histone.

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total cellular f1 and the ratio of chromatin f1 to polysomal f1 being 7.7.

Since histone synthesis had been reported to take place specifically on

TABLE I. Distribution of ^{3}H-Thymidine in DNA Isolated from Cell Fractions by a Modified Schmidt-Thannhauser Procedure (1)

	Whole Cells	Cytoplasm	Nuclei
dpm ^{3}H-Thymidine/10^{6} Cells	111,895	322	111,573
% DNA in Fraction	100	0.3	99.7

small polysomes (11), we fractionated the polysomes of 4.3×10^{8} cells as shown in Fig. 1a and extracted the fractions with $HClO_4$. Histone f1 was detected in all six fractions, the greatest amount being found in the large polysomes (gel h, Fig. 2) and the least amount in the dimers and trimers (gel k, Fig. 2). Trace amounts of f1 were also observed in the 80S and 60S ribosomal pellets [difficult to see in photographs (gels l and m, Fig. 2)]. The recovery of most of this f1 in the large polysomes, when histone synthesis apparently takes place on small polysomes, suggests that this polysomal f1 is not nascent histone. Also, we estimate that these cells synthesize f1 at a rate of 0.729 $\mu g/10^{9}$ cells/min [based on an f1 synthesis rate of 3.7 $\mu g/10^{9}$ S-phase cells/min (1,3) and the fact that 23% of the cells of an exponential culture are in the S-phase (12)]. This synthesis rate is orders of magnitude too slow to produce nascent f1 in the quantity we isolated from polysomes.

To determine if histone f1 was present in the various ribosomal forms, the cytoplasm of 2.9×10^{9} cells was fractionated as shown in Fig. 1b. Histone f1 was present in significant quantities in the 80S ribosomes (gel c, Fig. 3) and in the 60S subunit (gel d, Fig. 3). Trace amounts of f1 were observed in the 40S subunit (gel e, Fig. 3).

Treatment of polysomes with 0.25 M NH_4Cl has been shown to remove extraneously adsorbed proteins from polysomes without disrupting the polysome's structure or its capacity to synthesize protein (13). After such treatment, f1 was still extracted from the polysomes (gel g, Fig. 3). Polysomes and ribosomes were also treated with NEB-2 buffer (0.2 M NaCl; 0.01 M Tris, pH 7.4; 0.01 M EDTA), which removes adsorbed proteins (14). After such treatment, the preparation was fractionated by sucrose gradient zone sedimentation (Fig. 1c). Detectable amounts of f1 were isolated from the 40S, 60S, and 80S regions as well as from the polysomal pellet (gels h, i, j, and k, Fig. 3). Recovery of f1 after these treatments indicates that the f1 is not likely an extraneously adsorbed protein on the ribosomes.

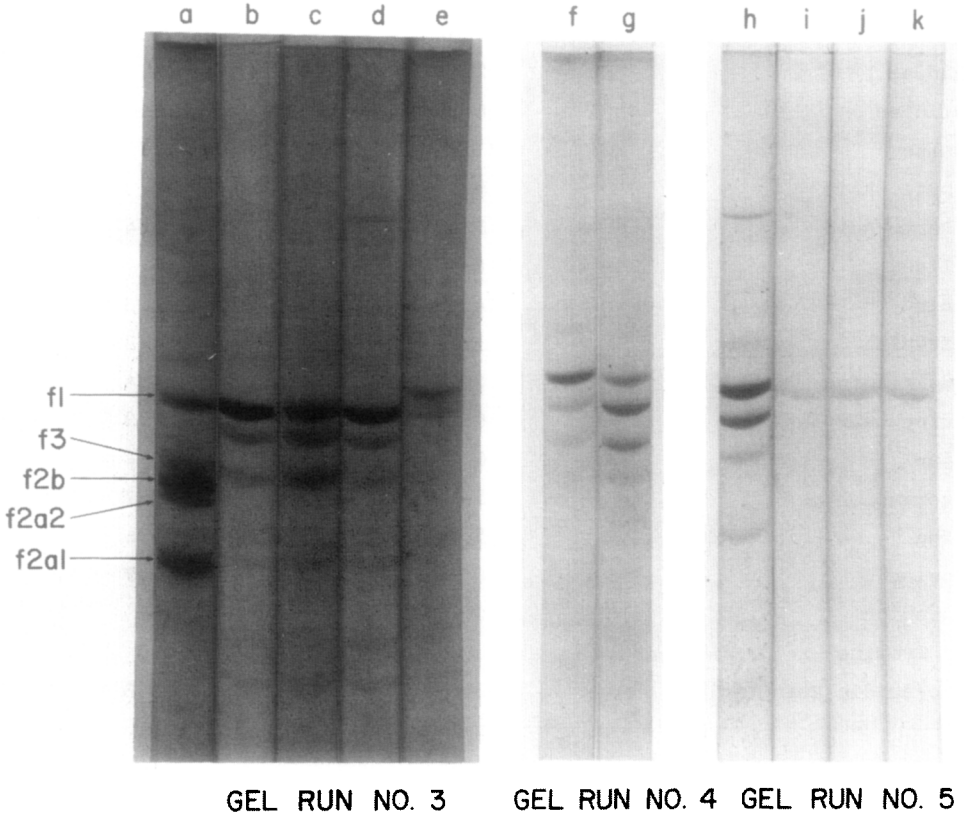

Fig. 3. Electrophoretic patterns of polysomal and ribosomal histone f1 (conditions same as in Fig. 2).

Gel Run No. 3: Histone f1 extracted from preparations shown in Fig. 1b (a) 100 µg whole histone; (b) 25 µg f1 extracted from polysomal pellet; (c) total f1 from 80S ribosomal region; (d) total f1 from 60S subunit region; and (e) total f1 from 40S subunit region.

Gel Run No. 4: (f) 25 µg polysomal f1; and (g) total f1 from polysomes treated with 0.25 M NH_4Cl.

Gel Run No. 5: Histone f1 extracted from polysomes and ribosomes (Fig. 1c) treated with 0.01 M EDTA in 0.2 M NaCl (total amount recovered was run); (h) polysomal pellet; (i) 80S ribosomal region; (j) 60S subunit region; and (k) 40S subunit region.

Finally, the amino acid composition of polysomal f1 (gel d, Fig. 2) was found to be very similar to that previously reported (1,3) for histone f1 extracted from chromatin (Table II).

TABLE II. Amino Acid Analysis of Chromatin and Polysomal Histone f1 Fractions

Amino Acid	Chromatin f1* (μmole %)	Polysomal f1 (nanomole %)
Lysine	27.6	30.0
Arginine	2.8	4.7
Histidine	0.3	0.8
Alanine	21.7	18.4
Aspartic Acid	3.1	5.3
Glutamic Acid	5.4	5.4
Proline	8.9	5.7
Leucine	4.1	4.3
Isoleucine	1.3	1.3
Phenylalanine	0.8	1.4
Glycine	6.2	7.3
Serine	6.8	5.4
Threonine	5.1	3.7
Valine	5.1	5.6
Tyrosine	0.7	0.9
Methionine	0.1	0
Half Cystine	0	0
Basic/Acidic Amino Acids	3.6	3.3

*Analysis for whole chromatin f1 previously reported (1,3).

DISCUSSION

A comparison of the electrophoretic patterns of unfractionated histones and ribosomal basic proteins has been made in several laboratories (15-22). Results of these comparisons varied widely, with some reports suggesting that all histones were present in ribosomes and others suggesting that none or only one or two major histones were present. The experiments reported above demonstrate that at least histone f1 is located on ribosomes of exponentially-dividing cells and suggest that the histone fraction which Beeson and Triplett (17) found in both nuclear histones and ribosomes was f1.

The possibility that histone f1 leaked from the nucleus and artifactually adsorbed to ribosomes during preparation is unlikely for several reasons: (a) cytoplasm was isolated by a very gentle method which does not break any

nuclei (23) and which does not extract any DNA from the nucleus; (b) a buffer was employed having an ionic strength and magnesium concentration which stabilizes nucleoprotein; (c) f1 was recovered from polysomes released from cells both before and after deoxycholate treatment (23); (d) f1 was recovered from polysomes and ribosomes after treatments known to remove extraneously adsorbed proteins; and (e) no H_2SO_4-extractable histones could be detectable in post-ribosomal particles or in the cytoplasmic-soluble fraction (23).

It is interesting to note that Dick (24) has found that, when bean root tips were incubated with various histone fractions, the most lysine-rich fraction entered the cells and localized in the cytoplasm while the other histones localized in the nucleus. Also of interest is the fact that Paik and Lee have found that rat kidney microsomes contain an enzyme which hydrolyzes histone f1 more actively than any other protein tested (25). These observations, along with those reported here, suggest that histone f1 may have a metabolic function in polysomes. Metabolic experiments are currently underway in an attempt to determine if the turnover of chromatin f1 (2,3) is related to the presence of f1 in polysomes.

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