Heterogeneous Binding of High Mobility Group Chromosomal Proteins to Nuclei

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ABSTRACT A dramatic difference is observed in the intracellular distribution of the high mobility group (HMG) proteins when chicken embryo fibroblasts are fractionated into nucleus and cytoplasm by either mass enucleation of cytochalasin-B-treated cells or by differential centrifugation of mechanically disrupted cells. Nuclei (karyoplasts) obtained by cytochalasin B treatment of cells contain >90% of the HMG 1, while enucleated cytoplasts contain the remainder. A similar distribution between karyoplasts and cytoplasts is observed for the H1 histones and the nucleosomal core histones as anticipated. The presence of these proteins, in low amounts, in the cytoplast preparation can be accounted for by the small percentage of unenucleated cells present. In contrast, the nuclei isolated from mechanically disrupted cells contain only 30–40% of the total HMGs 1 and 2, the remainder being recovered in the cytosol fraction. No histone is observed in the cytosol fraction. Unlike the higher molecular weight HMGs, most of the HMGs 14 and 17 sediment with the nuclei after cell lysis by mechanical disruption. The distribution of HMGs is unaffected by incubating cells with cytochalasin B and mechanically fractionating rather than enucleating them. Therefore, the dramatic difference in HMG 1 distribution observed using the two fractionation techniques cannot be explained by a cytochalasin-B-induced redistribution. On reextraction and sedimentation of isolated nuclei obtained by mechanical cell disruption, only 8% of the HMG 1 is released to the supernate. Thus, the majority of the HMG 1 originally isolated with these nuclei, representing 35% of the total HMG 1, is stably bound, as is all the HMGs 14 and 17. The remaining 65% of the HMGs 1 and 2 is unstably bound and leaks to the cytosol fraction under the conditions of mechanical disruption. It is suggested that the unstably bound HMGs form a protein pool capable of equilibrating between cytoplasm and stably bound HMGs.

Evidence has accumulated that the high mobility group (HMG) chromosomal proteins are required for transcriptionally active chromatin structure. Incubation of chromatin with DNase I preferentially digests transcriptionally active sequences (4, 16, 34), while selectively releasing the HMGs (15, 17, 18, 33, 35). The preferential DNase I sensitivity of the globin genes in erythrocyte mononucleosomes is abolished by the selective extraction of the nonhistone fraction containing the HMGs, while readdition of purified HMGs restores it (35, 36). The HMGs may also be involved, at least indirectly, in the regulation of differential gene activity during cytodifferentiation since they are themselves developmentally regulated proteins. Gordon and colleagues (7) have shown that the proportions of HMGs 1, 2A, and 2B vary between different chicken tissues and that quantitative changes in HMG proportions are observed during in vivo[1] and in vitro[2] terminal myogenesis. Quantitative HMG changes also occur during spermiogenesis (37).

Recent evidence indicates that the HMGs are distributed between the cytoplasm and the nucleus. Rechsteiner and Kuehl (27) have shown that most of the ^{125}I-labeled HMG 1 injected into a cell is immediately sequestered in the nucleus. It is, however, capable of reentering the cytoplasm because the nucleus of an uninjected cell is eventually labeled upon fusion to an injected cell. The last observation is consistent with the majority of the HMGs being localized in the nucleus, but in

[1] Gordon, J. S., R. Kaufman, and B. I. Rosenfeld. Manuscript submitted for publication.
[2] Gordon, J. S., D. Levy, and E. Winter. Unpublished data.

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equilibrium with a small cytoplasmic pool. This is supported by the immunofluorescent studies of Smith et al. (32), who have observed nuclear, but not cytoplasmic, staining with antibodies against the HMGs. In contrast, Bustin and Niehart (2) report that antiserum prepared against HMG 1 stains the cytoplasm more extensively than nuclei and that HMG 1 is found in the cytosol upon the mechanical disruption and fractionation of cells.

One explanation for these conflicting results is that the HMGs found in the cytosol after mechanical disruption of cells leak from the nucleus. This is tested in the present study by quantitatively comparing the proportions of the HMGs found in cytosol of homogenized chick embryo fibroblasts (CEFs) with that found in the cytoplasts after mass enucleation of cytochalasin-B-treated cells (20, 26). Enucleated cytoplasts contain ~60–70% of the total cytoplasmic content (26, 39). They can be rapidly prepared without subjecting the nuclei to large mechanical shearing forces, dilution in large buffer volumes, or fixation. Thus, leakage of the nuclear contents can be minimized. That this technique causes minimal damage to the nuclei is supported by the observations that the karyoplasts (containing the nuclei surrounded by a shell of cytoplasm and plasma membrane) produced by this method can be used to reconstruct viable cells by fusion to cytoplasts (26), and that a portion of them are capable of regenerating to reform whole viable cells (21).

It is found that the cytoplast fraction contains <5% of the total cellular HMGs 1, 2A, and 2B. On the other hand, the cytosol obtained after mechanical disruption of cells contains 60% of the HMGs 1, 2A, and 2B, but no HMGs 14 or 17. Thus, all the HMGs appear to be predominantly localized in the nucleus, but a major proportion of the HMGs 1, 2A, and 2B, (but not HMGs 14 and 17) apparently are unstably bound and diffuse out of the nucleus during mechanical cell disruption.

MATERIALS AND METHODS

Preparation of Biological Material

Embryonic skeletal muscle was dissected from the legs of 18-d-old White Leghorn chicken embryos obtained from fertilized eggs (Spafas, Inc., Norwich, Conn.) incubated at 37°C. Secondary CEFs were prepared and cultured using techniques described by Rubin (29).

Mechanical Cell Fractionating

The 18-d embryonic skeletal muscle was fractionated into cytosol and nuclei using the following modifications of Gordon et al.'s (7) adaption of the techniques of Zak et al. (38) and Kuehl (13). The salt concentration originally used to relax the muscle extracts nearly all of the HMGs 1, 2A, and 2B from the nuclei (7). Consequently, the muscle tissue was washed in phosphate-buffered saline (PBS) rather than the relaxing solution (7) before passing it through a Latapie mincer. (Arthur H. Thomas Co., Philadelphia; No. 3437-N10). It was then suspended in 3.5 vol of 250 mM sucrose, 5 mM EGTA, 50 mM KCl, 5 mM $MgCl_2$, 5 mM sodium pyrophosphate, 50 mM sodium bisulfite, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol all in 25 mM Tris-HCl, pH 8.0, before homogenizing in a motorized Potter-Elvejhem tissue grinder with seven strokes of a loose-fitting pestle followed by 15 strokes with a tight-fitting pestle. Again the ionic strength of the solution was reduced relative to that originally used (7) to minimize HMG extraction. The homogenate was sequentially filtered through two layers of four-ply cheesecloth and two layers of Miracloth (Chicopee Mills, Inc., Milltown, N. J.). The nuclei and remaining contractile apparatus were separated from the cytosol by centrifugation at 800 *g* in a Sorvall HL-8 rotor (DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.) for 10 min. For those experiments to determine the effect of homogenization conditions on the distribution of the HMGs, the ionic strength was reduced by lowering the KCl concentration to 10 mM, the sodium pyrophosphate and $MgCl_2$ concentrations to 3 mM, and the sodium bisulfite concentration to 25 mM. The pH was raised from 5.8 to 8.0 by omitting the sodium bisulfite. None of these modifications effected nuclear morphology as observed by phase microscopy.

Mechanically disrupted CEFs were similarly fractionated into cytosol and crude nuclei by low-speed centrifugation. 12–24 100-mm-diameter dishes of near confluent CEF cells were washed with PBS, before dislodging the cells with a rubber policeman in 8 ml of PBS. The cells were sedimented at 800 *g* for 6 min in an HL-8 rotor and resuspended in 10 vol of RSB (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 300 mM $MgCl_2$) (34) containing 0.1% Trition X-100. After allowing the cells to stand for 15 min, they were homogenized with 25 strokes of a motorized Potter-Elvejhem tissue grinder. Complete cell disruption was ensured by monitoring with phase microscopy. After removal of one-fourth of the homogenate for the extraction of the total cellular HMGs, the nuclei were sedimented at 1,000 *g* for 10 min in an HL-8 rotor. The cytosol was removed with a Pasteur pipette and it, along with the nuclei, was stored in liquid nitrogen until extraction. In controls testing the effect of cytochalasin B on the distribution of the HMGs, the cells were incubated with 10 μg/ml of cytochalasin B for 90 min at 37°C before homogenization as described above.

In later experiments, the washes and homogenization buffers also contained 10 μg/ml cytochalasin B. In both cases, the results were similar.

Cell Enucleation

Cell monolayers on 60-mm tissue culture dishes were incubated in growth medium containing 10 μg/ml of cytochalasin B for 30 min at 37°C. The dishes were then placed, cell side down, into plastic bottles containing medium with cytochalasin B and centrifuged for 45 min at 37°C at 10,000 rpm in a Sorvall GSA rotor (19).

The extent of enucleation was monitored by treating cultures with Giemsa stain after fixation with methanol and directly enumerating the number of nucleated cells, as described previously (20). In all experiments, the efficiency of enucleation was >90%. The cytoplast cultures retained the same morphology and cell density as untreated control cultures. Extensive examination of another cell type, mouse L929 cells, showed that karyoplast cultures were contaminated with ~0.1% whole cells and that cytoplasts contained ~60–70% of the cytoplasm of whole cells (20, 21, 39). Because both the enucleation conditions used and the morphology of the bodies were similar, these figures were assumed to be applicable to the CEF cultures.

Extraction and Analysis of the Acid-Soluble Proteins

The 2.0% perchloric acid (PCA) -soluble proteins were extracted from whole cells, tissues, cytosols, nuclei, cytoplasts, and karyoplasts by the technique of Goodwin et al. (6), modified by Gordon et al. (7) for small amounts of tissue. The samples were brought to 2.0% PCA and 100 μg/ml protamine (added as carrier) and centrifuged for 10 min in a Beckman microfuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The acid-soluble proteins were then precipitated with 5 vol of acetone and 0.2 vol of HCl. The precipitate obtained by centrifugation for 10 min in a microfuge was washed three times with acetone and vacuum dried.

The 0.4N H_2SO_4-soluble proteins were isolated from whole CEF cells and CEF cytoplasts by the technique of Panyim et al. (24). All centrifugations were for 10 min in the microfuge.

The proteins were resolved by $NaDodSO_4$ polyacrylamide gel electrophoresis using the discontinuous buffer system of Laemmli (14), employing a 17% resolving gel. The muscle extracts were dissolved in 62.5 mM Tris-HCl, pH 6.8. Approximately 20 μg of proteins were electrophoresed. The extracts of the CEF fractions were dissolved in 30 μl of a concentrated loading buffer (170 mM Tris-HCl, pH 6.8, 27% glycerol, 13.7% β-mercaptoethanol, 5.5% SDS) by incubating at 45°C for 15 min followed by a 15-min incubation at 100°C. Except where noted, the complete sample was diluted to 7 μl with H_2O before electrophoresis at 130 V for 7 h. The gels were stained with 0.25% Coomassie Blue in 50% methanol and 10% acetic acid and then destained with 50% methanol and 10% acetic acid. The HMGs were identified by comigration with known HMGs. The HMGs 1 and 2 were extracted with 0.35 M NaCl from purified chicken erythrocytes as described by Vidali et al. (33). Calf thymus HMGs were prepared as described by Goodwin et al. (6). Chicken HMGs 14 and 17 were a gift from Dr. Stuart Weisbrod.

The HMG 1 and H1 were quantitated by scanning a Polaroid transparency of the gel with a microdensitometer (Joyce, Loebl and Co., Ltd., Gateshead-on-Tyne, England) and integrating the area under the bands, using a sonic digitizer (Scientific Accessories model NT/501). Controls were run to ensure that the staining intensity of each HMG band was linearly related to protein concentration (5).

DNA was measured by the microspectrofluormetric technique of Kissane and Robins (12) as modified by Santoianna and Ayala (30). Protein was assayed by the technique of Lowry et al. (22).

RESULTS

Distribution of HMGs after Mechanical Cell Fractionation

Fractionation of mechanically disrupted 18-d embryonic skeletal muscle into nuclei and cytosol reveals that a substantial proportion of the high molecular weight HMG fraction, including HMG 1 and both HMG 2 subfractions, 2A and 2B (7) is found in the cytosol, which contains no detectable H1 (Fig. 1). Consistent with partitioning of the HMGs to nucleus and cytosol is the obvious decrease in the amounts of HMG 1 relative to the H1s in nuclei as compared to whole cells (Fig. 1). The HMGs are present in the cytosol of skeletal muscle disrupted under a wide variety of conditions including ionic strengths between 68 and 155 mM (Fig. 1 *A*), the presence and absence of the detergent Triton X-100 (Fig. 1 *B*), at pH's between 5.8 and 8.0 (data not shown), and the presence or absence of the Ca^{++}-specific chelator, EGTA (data not shown). Proteins comigrating with chicken HMGs 14 and 17, however, appear in the cytosol only after cell fractionation at the higher ionic strength (Fig. 1 *A*).

Similary, on mechanical fractionation of fibroblasts a substantial proportion of the HMG 1 and the HMG 2 fractions are found in the cytosol (Fig. 2 *A*, lanes 2 and 4). The vast majority of the protein comigrating with chicken erythrocyte HMGs 14 and 17 is found in the nuclear fraction. This is more evident when the fractions are extracted with 5.0% PCA (Fig. 2 *B*) than when they are extracted with 2.0% PCA because there is a group of peptides in cytosol extracts having similar but not identical mobilities to the HMGs 14 and 17 which are soluble in 2.0% but not 5.0% PCA.

The proportion of HMG 1 in the cytoplasm is quantitated by determining the staining intensity of that band in the cytosol and nuclei lanes, as described in Materials and Methods, and dividing the amount of cytosol HMG 1 by the sum of the cytosol plus nuclear HMG 1. On the average, 60% of the total HMG 1 is found in the cytosol (Table I). When the nuclei are reextracted and sedimented under the same conditions, only

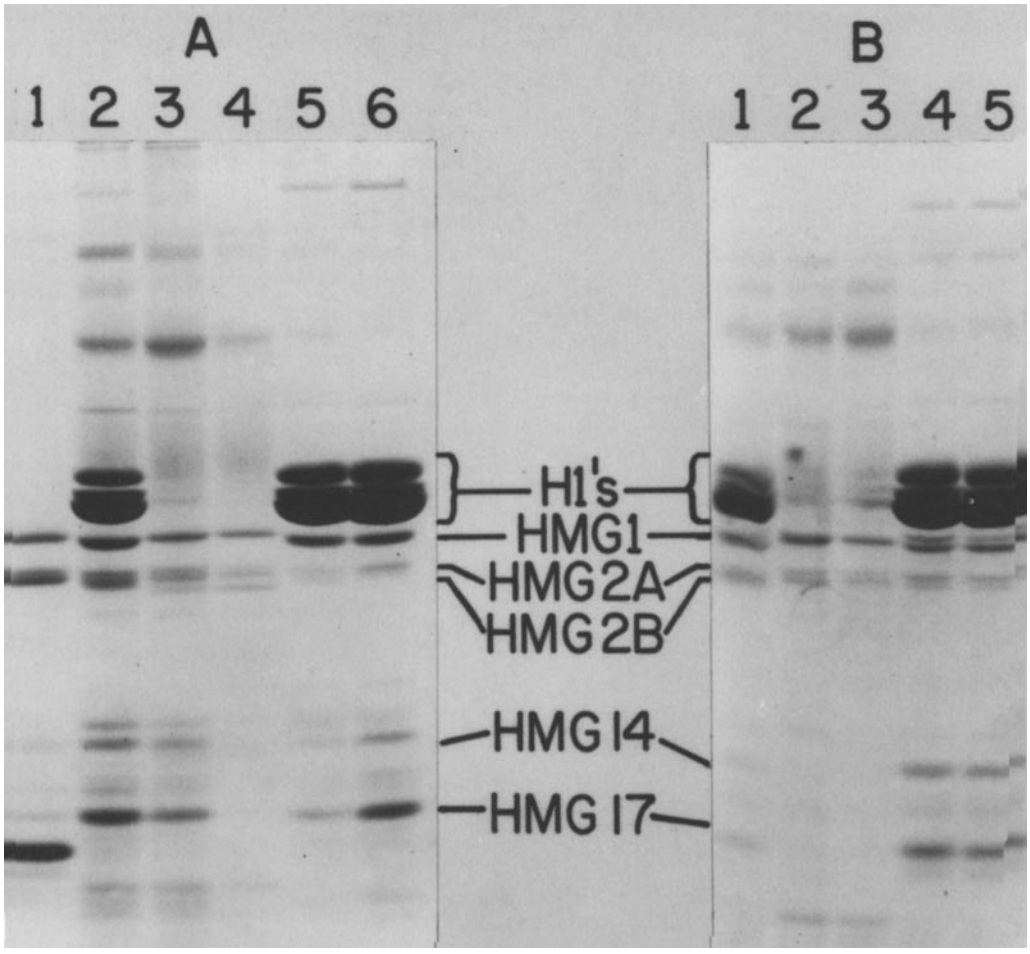

FIGURE 1 Distribution of HMGs in mechanically fractionated skeletal muscle. The 2.0% PCA-soluble proteins were isolated from the cytosol and nuclear fractions prepared, as detailed in Materials and Methods, from embryonic skeletal muscle homogenized under varying conditions. They were resolved by electrophoresis on $NaDodSO_4$ containing 17% polyacrylamide gels. (*A*) The effect of ionic strength on the distribution of the proteins. The proteins extracted from whole cells (lane *2*) were compared to those extracted from the cytosols of muscle homogenized in 155 mM (lane *3*) and 68 mM (lane *4*) salt and the proteins extracted from the nuclei of muscle homogenized in 155 mM (lane *5*) and 68 mM salt (lane *6*). The HMGs were identified by comigration with HMGs 1, 2A, and 2B (lane *1*) and HMGs 14 and 17 (not shown, see Fig. 2) isolated from the 0.35-M extract of chicken erythrocyte nuclei. (*B*) The effect of Triton X-100 on the distribution of the proteins. The proteins extracted from unfractionated muscle (lane *1*) were compared to those extracted from the cytosols of muscle homogenized in the presence (lane *2*) and absence (lane *3*) of 0.1% Triton X-100 and to the proteins extracted from nuclei of muscle homogenized in the presence (lane *4*) and absence (lane *5*) of detergent. The HMGs were identified as in *A*.

8% of the remaining HMG 1 is released (Fig. 2*A*, lanes 6 and 7; and Table I). Thus, ~36% of the HMG 1 appears to be stably bound to the nuclei.

The presence of 10 μg/ml of cytochalasin B does not affect the distribution of HMGs 1 and 2 between cytosol and nucleus in such a fractionation experiment. As in the untreated cells ~60% of the HMG 1 is recovered in the cytosol. Because cytochalasin B does not cause a major redistribution of the HMGs, a comparison of the HMG intracellular distribution after mass enucleation in the presence of cytochalasin B is feasible.

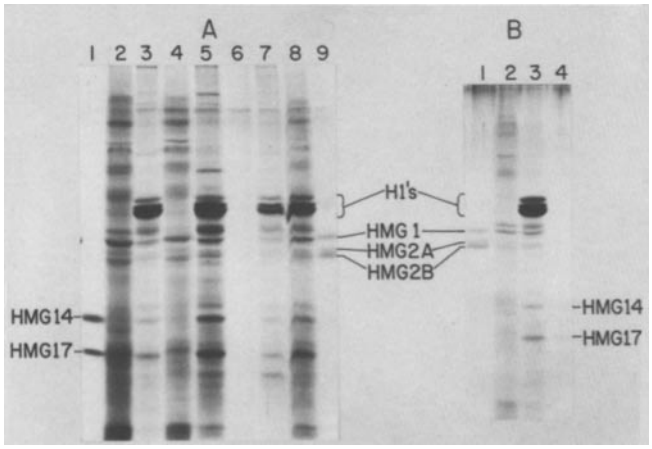

FIGURE 2 The distribution of the PCA-soluble proteins in mechanically disrupted CEFs. (*A*) The 2.0% PCA-soluble proteins were extracted from the cytosol (lane *2*) and nuclei (lane *3*) of mechanically disrupted CEFs and the cytosol (lane *4*) and nuclei (lane *5*) of cells incubated and lysed in the presence of 10 μg/ml of cytochalasin and resolved on $NaDodSO_4$ containing 17% polyacrylamide gels. A portion of the nuclei were reextracted in the same buffer (see Materials and Methods) and sedimented. The proteins in the resulting supernate (lane *6*) and nuclear pellets (lane *7*) were similarly compared. The proteins from the various fractions were also compared to those extracted from unfractionated cells (lane *8*). The HMGs were identified by comparison with the mobility of known erythrocyte HMGs 14 and 17 (lane *1*) and HMGs 1, 2A, and 2B (lane *9*). (*B*) The distribution of the 5.0% PCA-soluble proteins to cytosol (lane *2*) and nucleus (lane *3*) of mechanically lysed CEFs were compared as above. Chicken erythrocyte HMGs 1, 2A, and 2B (lane *1*) and HMGs 14 and 17 (lane *4*) are electrophoresed for comparison.

TABLE I

Presence of HMG 1 in the Cytosol of Mechanically Lysed CEFs *

	Supernatant HMG 1
	%
+Cytochalasin B	60.0‡ ± 10.0
−Cytochalasin B	58.0 ± 10.0
On rehomogenization	8.0§ ± 3.0

* CEFs were incubated in the presence and absence of 10 μg/ml of cytochalasin B for 90 min before mechanically lysing the cells and fractionating them into nucleus and cytosol. The HMGs were extracted from both fractions, resolved on SDS gels and quantitated as described in Materials and Methods.

‡ The percent of the total HMG 1 in the cytosol, determined from the ratio of HMG 1 in cytosol to the sum of HMG 1 in cytosol and nuclei ± the average error of the mean (AEM) is from five separate experiments, three in which the fractions were extracted with 2.0% PCA, and two in which the fractions were extracted with 5.0% PCA.

§ The percent released from rehomogenized nuclei was calculated, upon resedimentation of the nuclei obtained above, from the ratio of the HMG 1 in the supernate to the sum of the HMG 1 in the supernate and pellet. These values ± AEM represent the average of three separate experiments.

Distribution of HMGs after Cytochalasin-B-Induced Cell Enucleation

Over 90% of the CEFs incubated for 90 min in the presence of 10 μg/ml of cytochalasin B are enucleated on centrifugation (see Materials and Methods). After such a fractionation, nearly all the HMG 1 is recovered in the nuclei-containing karyoplasts. It is estimated, based on experiments with L-929 cells (21, 39), that the resulting enucleated cytoplasts contain 60–70% of the cytoplasm; but only 7% of the HMG 1 or H1 found in whole cells (Fig. 3, Table II) is recovered in the cytoplast fraction. The amount of HMG 1 and H1 recovered in the karyoplasts (per microgram of DNA) is nearly equal to that found in whole cells. The presence of small amounts of both of these nuclear proteins in the cytoplast preparation can best be explained by the contamination of the cytoplasts by a small fraction of unenucleated cells. This is confirmed by the identification of a relatively small amount of nucleosomal core histones in the cytoplast preparations as well (Fig. 4). The percent of the other HMGs found in cytoplasts is not determined because they are present in such small amounts relative to HMG 1 that the microdensitometer could not consistently resolve them above background. Where visually detected they also appear to be present in cytoplasts in the same amount

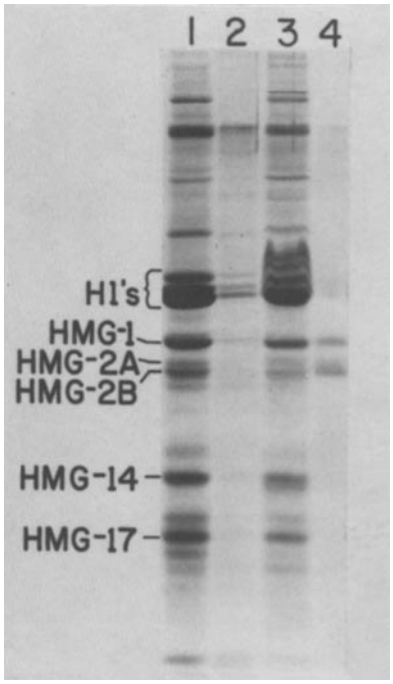

FIGURE 3 Distribution of 4.0% PCA-soluble proteins after mass enucleation of CEFs. The 4.0% PCA-soluble proteins were extracted as detailed in Material and Methods from unfractionated CEFs (lane *1*), from preparations of enucleated cytoplasts (lane *2*), and karyoplasts (lane *3*) generated from cytochalasin-B-treated fibroblasts, as detailed in Materials and Methods. They were then resolved on $NaDodSO_4$ containing 17% polyacrylamide gels. The HMGs were identified by a comparison of their migration with that of known erythrocyte HMGs 1, 2A, and 2B (lane *4*) and HMGs 14 and 17 (see Fig. 2).

TABLE II

Distribution of 2.0% PCA-soluble Proteins after Enucleation of Cytochalasin-B-Treated CEFs *

	Cytoplasts	Karyoplasts
	%	
HMG 1‡	7.0 ± 6.0	106 ± 30
H1	7.0 ± 5.0	116 ± 20

* Unenucleated cells plus the cytoplasts and karyoplasts obtained from an equal number of cytochalasin-B-treated cells, as described in the legend of Fig. 3, were extracted with between 2.0 and 5.0% PCA. The HMG 1 and H1, resolved on $NaDodSO_4$ polyacrylamide gels, were quantitated as described in Materials and Methods.

‡ The percent found in the cytoplasts, determined from the ratio of the proteins in cytoplasts to that in whole cells, represents the average ± AEM for eight separate preparations. The percent in the karyoplasts was determined for three of these preparations from the ratio of the amount of each protein per microgram DNA in the karyoplasts to that per microgram DNA in whole cells.

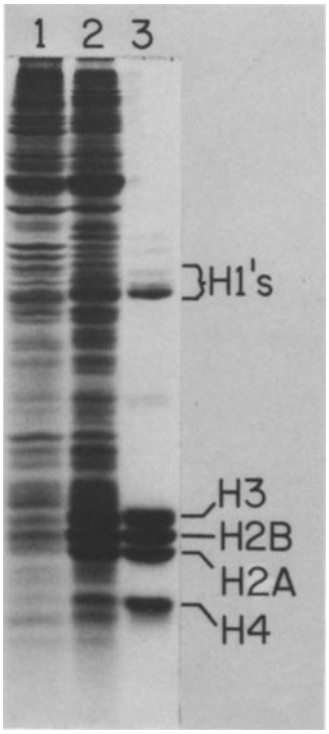

FIGURE 4 The presence of nucleosomal core histones in cytoplast preparations. The mobilities in $NaDodSO_4$ containing 15% polyacrylamide gels of the proteins extracted with 0.4N H_2SO_4, as described in Materials and Methods, from cytoplasts (lane *1*) prepared as in Fig. 3 and whole chicken fibroblasts (lane *2*) were compared to that of histones (lane *3*) isolated from chicken embryonic skeletal muscle nuclei.

relative to HMG 1 as they are in the whole cells and karyoplasts (Fig. 3).

The low molecular weight proteins that are extracted from the cytosols of mechanically disrupted cells with 2.0% but not 5.0% PCA are present only at low levels in the 2.0% PCA extracts of the cytoplasts. They are recovered primarily from the 2.0% PCA extracts of karyoplasts (data not shown). This suggests that they behave similarly to the larger HMGs. The effect is specific; other presumably cytoplasmic proteins which are soluble in 0.4 N H_2SO_4 are more highly enriched relative to the nucleosome core histones in cytoplasts than whole cells (Fig. 4).

The recovery of small amounts of HMG 1 in cytoplasts is not caused by differential recovery from small amounts of material. Equal numbers of cells are used for enucleation and mechanical fractionation. In addition, protamine is included in the extractions to act as a carrier during precipitation. Finally, extraction of various dilutions of cytosols from mechanically disrupted cells results in a linear recovery of HMG 1 down to the limits of detection (Fig. 5).

DISCUSSION

When CEFs are mechanically disrupted, 60% of the HMG 1 is found in the cytosol. On the other hand, when the same cells are enucleated after treatment with cytochalasin B, only 7% of the HMG 1 is in the cytoplasts, while the majority of the HMG 1 is recovered in the karyoplasts. The karyoplasts contain only ~10% of the cytoplasm of the cells (20). The 7% of the HMG 1 in the cytoplast fraction presumably reflects the contamination by unenucleated cells (observed to be 5–10%), as an equal proportion of the H1 is also found in the cytoplast fraction.

Thus, at least in CEFs, HMG 1 appears to be almost completely localized in the nucleus. It may be that the substantial proportion of HMG 1 found in the cytosols of mechanically disrupted CEFs and skeletal muscle leak from the nucleus during cell fractionation. Because Bustin and Niehart (2) report similar results with other tissues and cells, this may be a general phenomenon. The nuclear localization of the HMGs is supported by immunocytochemical results of Smith et al. (32) who observed no cytoplasmic staining by antisera specific to the

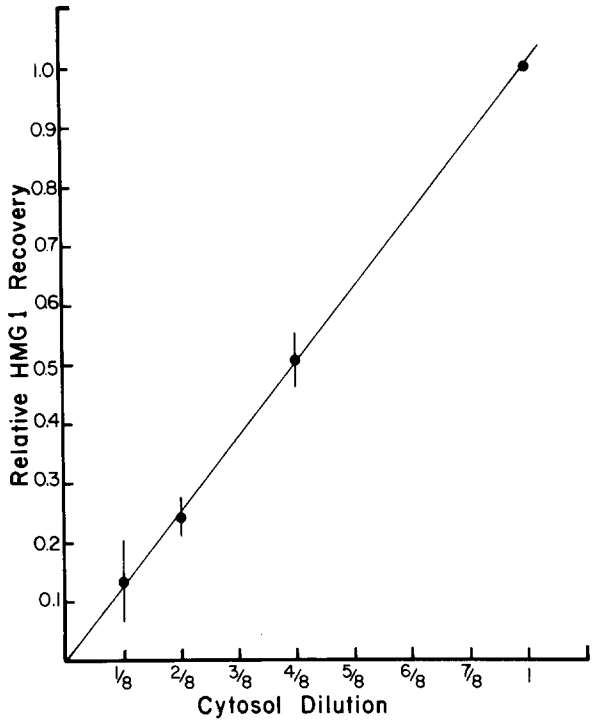

FIGURE 5 The linear recovery of HMG 1 with cytosol concentration. The HMGs were isolated from four different serially diluted cytosol preparations of mechanically lysed CEFs as detailed in the legend for Fig. 2. The proteins were resolved by electrophoresis on $NaDodSO_4$ containing polyacrylamide gels. The staining intensity of the HMG 1 band was quantitated as described in Materials and Methods. The amount of HMG recovered from each dilution was expressed as a fraction of that recovered from the undiluted cytosol. Bars indicate average error of the mean.

HMGs and by the rapid sequestering of injected HMG into the nucleus (26). It is still possible that the cytoplasm contains a very small percentage of the HMGs which cannot be resolved above the contribution because of whole cell contamination of the cytoplast preparations. If so, it cannot exceed a few percent of the total cellular HMGs.

Mass enucleation, as used in this study, is a useful technique to firmly establish the localization of proteins found in both the cytosol and nuclei (see reference 8). A number of proteins with obvious nuclear functions, other than the HMGs, apparently leak from the nucleus during mechanical cell lysis and fractionation. RNA polymerase I (28) and α-DNA polymerase (8, 31) are prominent examples. A number of other proteins with no known function which are detected in both nuclei and cytosol have been interpreted to be cytoplasmic contaminants (3, 25). Like the HMG 1, they could be nuclear proteins that leak to the cytosol during nuclear isolation. Mass enucleation could be used to reinvestigate the intracellular distribution of such proteins.

In addition to HMG 1, HMGs 2A and 2B are also found in the cytosols of mechanically disrupted fibroblasts and muscle. While the relative amounts of these other proteins found in cytosols is not extensively quantitated, inspection of the electrophoretic gels suggests that they are distributed between cytosol and nuclei, as well as between cytoplasts and karyoplasts, in the same relative amounts as is HMG 1.

This is not the case for the lower molecular weight HMGs 14 and 17, which like the nucleosomal core histones, are almost completely retained in the nucleus after mechanical disruption or after mass enucleation. This is another example of differences in behavior and properties of the high molecular weight HMGs (1 and 2) and low molecular weight HMGs (14 and 17) which have been shown to differ in their interaction with DNA (9–11) and in their immunospecificity (1, 32). These differences suggest different modes of binding to chromatin for the two HMG classes. The finding in the present study that the higher molecular weight HMGs are less stably bound to intact nuclei than the low molecular weight HMGs suggests that HMGs 14 and 17 are found in the interior of the chromatin fiber, probably as an intimate part of the nucleosomes (23) encompassing active genes (36), while the majority of the HMGs 1 and 2 may be at more superficial locations.

The diffusion of the majority of the HMG 1 and probably HMGs 2A and 2B from the nucleus is consistent with heterogeneous binding of these HMGs to nuclear sites. In CEFs, it appears that 60% of the HMG 1 is unstably bound. The biological significance, if any, of this apparent heterogeneous binding of high molecular weight HMGs in nuclei is not clear. The possibility that the unstably bound HMGs passed through the intact envelope of muscle nuclei prepared in the absence of detergents may suggest that they are part of a pool of nuclear proteins capable of transiently entering the cytoplasm. Recently, Rechsteiner and Kuehl (27) have shown that the vast majority of the injected ^{125}I-labeled HMG 1 is immediately sequestered in the nucleus. Part of it is not permanently fixed to the nucleus, however, as the nucleus of an uninjected cell eventually became labeled on fusion with a previously injected cell. Thus, it is reasonable to suggest that the unstably bound HMGs are in equilibrium with a minor cytoplasmic pool. This would, of course, place them in a unique position to mediate cytoplasmic influence on gene expression.

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