Smerdon, M. J., & Lieberman, M. W. (1978a) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4238–4241.
Smerdon, M. J., & Lieberman, M. W. (1978b) in *DNA Repair Mechanisms* (Hanawalt, P. C., Friedberg, E. C., & Fox, C. F., Eds.) pp 327–336, Academic Press, New York.
Smerdon, M. J., Tlsty, T. D., & Lieberman, M. W. (1978) *Biochemistry 17*, 2377–2386.
Smith, C. A., & Hanawalt, P. C. (1976) *Biochim. Biophys. Acta 432*, 336–347.
Sollner-Webb, B., & Felsenfeld, G. (1975) *Biochemistry 14*, 2915–2920.
Tlsty, T. D., & Lieberman, M. W. (1978) *Nucleic Acids Res. 5*, 3261–3273.
Williams, J. I., & Cleaver, J. E. (1978) *Biophys. J. 22*, 265–279.
Worcel, A., Han, S., & Wong, M. L. (1978) *Cell 15*, 969–977.

Chromatin Fractionation Procedure That Yields Nucleosomes Containing Near-Stoichiometric Amounts of High Mobility Group Nonhistone Chromosomal Proteins†

James B. Jackson, James M. Pollock, Jr., and Randolph L. Rill*

ABSTRACT: Initial results of an approach to the isolation of functionally active chromatin are described. Slight digestion of mouse myeloma nuclei at 0 °C with micrococcal nuclease, followed by dialysis against near-physiological saline solution containing 1 mM Mg^{2+}, caused release of up to 17% of the nuclear DNA as soluble nucleoproteins. This soluble (S) fraction was relatively depleted in H1 histones and methylated DNA (5-methylcytosine) but highly enriched in RNA, single-stranded DNA, and nonhistone chromosomal proteins, particularly two species of the high mobility group identified as HMG 1 and HMG 2. The S fraction released most rapidly (6–8% of the total DNA) consisted mainly of mono- and small oligonucleosomes. The mononucleosomes appeared normal in terms of sedimentation behavior, DNA length, and content of histones H2A, H2B, H3, and H4, but lacked H1, and instead were associated with approximately stoichiometric amounts of HMG 1 and HMG 2. Studies using isolated, fluorescence-labeled, total mouse HMG proteins indicated that added HMG 1 and HMG 2 do not bind strongly to S-fraction nucleoproteins but that two smaller HMG species (probably HMG 14 and HMG 17) do bind preferentially to S-fraction mono- and dinucleosomes. These results argue against artifactual redistribution of HMG 1 and HMG 2 during this fractionation but suggest caution in interpreting the distribution of smaller HMG proteins after digestion of chromatin. The potential relationship of this soluble fraction to transcriptionally active chromatin is discussed.

The nucleosome model of chromatin structure has provided a unifying conceptual basis for understanding molecular aspects of genomic functions, but little is yet known about the structural distinctions between inactive chromatin and chromatin that is active in transcription or other nuclear events [reviewed by Felsenfeld (1978)]. Certain features of transcribing chromatin seem clear, such as its diffuse appearance in microscopic views [e.g., Faken (1978)]. In inactive chromatin, the ~100-Å diameter nucleosome fibers (Olins & Olins, 1974; Oudet et al., 1975) are further folded into thicker fibers of "solenoids" (Finch & Klug, 1976) or "superbeads" (Renz et al., 1977), at least partly through the influence of histone H1 and divalent cations. Thick fibers are not observed in active transcription units (Franke et al., 1978), and even the folded form of nucleosomes may be absent from exceedingly active genes (Franke et al., 1976; Laird et al., 1976; Woodcock et al., 1976). Structural alterations of nucleosomes in active regions are also indicated by their preferential degradation by DNase I and DNase II (Felsenfeld, 1978).

Active and inactive chromatin are expected to differ in both chemical composition (e.g., nonhistone proteins, histone modifications, and DNA modifications) and physical properties such as solubility and accessibility to nucleases. Differences of the latter type have been used with some success as the basis for isolating chromatin enriched in active gene sequences [reviewed by Gottesfeld (1978)].

We have attempted to improve these methods by taking fuller advantage of the natural solubility properties of chromatin, the influence of the native structure on the specificity of micrococcal nuclease cleavage, and the ability of the nuclear substructure to act as a sieving matrix. To preserve the nuclear matrix, thick chromatin fibers, and the distinctions between heterochromatin and euchromatin, we sheared mouse myeloma chromatin in situ by very slight digestion of nuclei at 0 °C under near-physiological ionic conditions (Ris & Kubai, 1970; Olins & Olins, 1972; Pooley et al., 1974). Fractionation on the basis of solubility was performed under similar conditions, and mechanical aggitation was avoided throughout the procedure. Micrococcal nuclease was used because it prefers nucleosome spacers, does not degrade active genes (Sollner-Webb & Felsenfeld, 1975; Garel & Axel, 1976; Felsenfeld, 1978), and may rapidly attack transiently single-stranded DNA in active chromatin (see Discussion). Very recent evidence has shown that this nuclease preferentially excises, but does not degrade, nucleosomes from transcrip-

† From the Department of Chemistry and the Institute of Molecular Biophysics, The Florida State University, Tallahassee, Florida 32306. *Received November 21, 1978; revised manuscript received March 29, 1979.* This work was supported by a grant from the U.S. Public Health Service (GM-21126) and a grant from the Department of Energy to the Institute of Molecular Biophysics.

* R.L.R. is the recipient of a U.S. Public Health Service Career Development award. Author to whom correspondence should be sent at the Institute of Molecular Biophysics.

0006-2960/79/0418-3739$01.00/0

tionally active regions of oviduct and trout testis chromatin (Bloom & Anderson, 1978; Levy et al., 1979).

This approach yields a soluble nucleoprotein fraction that is highly enriched in nucleosomes containing close to stoichiometric amounts of the high mobility group proteins HMG 1 and HMG 2 and has several other distinctive properties consistent with an origin from active chromatin.

Materials and Methods

Isolation of Nuclei. Nuclei were isolated from myeloma line 66-2 tumors grown in BALB/C mice. Immediately after excision tumors were disrupted in a hand-driven, glass–Teflon homogenizer containing 5–10 volumes of ice-cold medium (0.25 M sucrose, 50 mM Tris, 25 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% Triton X-100, and 0.1 mM PMSF,[1] pH 7.5). The homogenate was passed through eight layers of prewashed cheesecloth and subjected to 10 strokes each with the loose and tight pestles of a Vitro Dounce homogenizer. Nuclei were collected by centrifugation at 2000*g* for 10 min and washed twice with the above medium. Further purification was performed by centrifugation through dense sucrose of the above ionic composition as described previously (Rill et al., 1977, 1978). Nuclei were resuspended in digestion buffer (0.25 M sucrose, 60 mM KCl, 15 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM PMSF, and 15 mM Tris, adjusted to pH 6.5 with cacodylic acid), made 25% in glycerol, and stored at −25 °C. Alternatively, nuclei were isolated as above except that the homogenization medium was 0.34 M sucrose, 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 2 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 15 mM Tris-HCl, pH 7.4, and 0.1 mM PMSF [modified from Wallace et al. (1971)].

Fractionation of Chromatin. Nuclei were washed twice with nuclear digestion buffer and resuspended in the same buffer at a concentration of about 40 A_{260} units/mL. Digestion with micrococcal nuclease (Worthington; 2 units/A_{260} unit of chromatin) was performed in an ice–water bath and terminated by addition of $^1/_{20}$ volume of 0.1 M EGTA (pH 7.5). Nuclei were then dialyzed for 6–12 h against the fractionation medium (120 mM KCl, 30 mM NaCl, 3 mM cacodylic acid, 1 mM $MgCl_2$, 0.1 mM PMSF, 0.1 mM EGTA, and 15 mM Tris-HCl, pH 7.5). Insoluble chromatin ("P fraction") was pelleted by centrifugation at 30000*g* for 20 min, and the supernatant ("S fraction") was carefully removed with a Pasteur pipet.

Density Gradient Centrifugation. Samples were centrifuged on 12-mL linear gradients of 5–30% sucrose (w/w), or 6–24% glycerol (w/v), in an International SB-283 rotor at 30000 rpm for 11 h (4 °C). Gradients contained either 10 mM Tris-HCl, 1 mM cacodylate, 0.1 mM EDTA, and 0.1 mM PMSF (pH 7.5) or the fractionation medium, as noted.

Isolation of HMG Proteins from Mouse Myeloma Nuclei. Chromatin was prepared by washing purified nuclei three times with 10 mM Tris-HCl, 1 mM cacodylate, 0.1 mM EDTA, and 0.1 mM PMSF, pH 7.5 (centrifugation at 12000*g* for 10 min). HMG proteins were extracted with 0.35 M NaCl, low mobility group (LMG) proteins were removed by precipitation with 2% Cl_3AcOH as described by Goodwin et al. (1975) except that hand homogenization with a glass–Teflon homogenizer was substituted for mechanical blending, and trace amounts of histone–DNA complexes in the 0.35 M NaCl extract were removed by precipitation with 5 mM $MgCl_2$, followed by centrifugation at 30000*g* for 30 min.

Polyacrylamide Gel Electrophoresis. DNA was electrophoresed on 15-cm slab gels of 6% acrylamide prepared as described by Loening (1967) except that the tray buffer and gels were made 0.1% in $NaDodSO_4$ for nucleoprotein samples. Protein-free DNA was isolated by the method of Britten et al. (1974). Gels were stained with Stains-All (Eastman) as described by Dahlberg (1969) or with ethidium bromide.

Proteins were electrophoresed on a discontinuous gel system prepared as described by Laemmli (1970) and modified by Bonner & Pollard (1975) for histones. Gels were stained with coomassie blue in 5:4:1 methanol–H_2O–acetic acid, destained in the same solution, and scanned in a Gelman DCD-16 recording densitometer at 580 nm.

Protein electrophoresis in low-pH gels containing 6.25 M urea was by the method of Panyim & Chalkley (1969), with coomassie blue staining as described above.

Nucleoproteins were electrophoresed in 6% polyacrylamide slab or tube gels and stained with ethidium bromide as previously described (Rill & Nelson, 1978). For second-dimension analyses, bands or whole lanes were excised with a razor blade under UV illumination. After being soaked in water containing 0.1 mM PMSF for 1 h, gel strips were incubated for 20 min at 50 °C in 50 μL of 1.1% $NaDodSO_4$, 5 mM EDTA, 1 mM dithiothreitol, 1 mM PMSF, 0.005% bromophenol blue, and 10 mM Tris-HCl, pH 7.0, and then loaded onto the discontinuous Laemmli gel system as described above to analyze for proteins.

Fluorescent Labeling of HMG Proteins with Dansyl Chloride. Isolated HMG proteins were resuspended in 0.05 M triethanolamine hydrochloride (pH 8.0), 0.35 M NaCl, and 0.1 mM PMSF at a concentration of 0.16 mg/mL (room temperature). One-twentieth volume of 2.5% dansyl chloride in acetone was added, followed immediately by vortexing. After 2 min an additional $^1/_{20}$ volume of dansyl chloride solution was added, and the solution was again vortexed. After 5 more minutes, the protein was precipitated with 6 volumes of cold 0.01 M HCl in acetone and washed once with HCl–acetone and twice with acetone to remove unreacted dansyl chloride. The extent of dansylation was followed by determining the increase in absorption at 340 nm using an extinction coefficient of 3.4×10^3 M^{-1} cm^{-1} for the dansyl–protein conjugates (Hartley & Massey, 1956; Chen, 1968). To confirm the absence of unbound dansyl groups, and to quantitate the relative fluorescence of specific dansylated proteins, we electrophoresed samples on polyacrylamide gels containing $NaDodSO_4$, exposed them to UV illumination, and photographed them through a yellow filter. The negatives were scanned with a Joyce-Loebl recording microdensitometer. Free dansyl chloride hydrolysis products, which migrate with the tracking dye as a brightly fluorescent band, were absent from the dansylated HMG preparation.

Quantitative Determination of DNA, RNA, and Protein. DNA quantitation was accomplished by the diphenylamine method of Burton as modified by Giles & Myers (1965). Standards were prepared from RNase-treated calf thymus DNA (Worthington). RNA was assayed by the orcinol test (Schneider, 1957) using yeast soluble RNA, type III (Sigma), as the standard. HMG protein was quantitated by the commercial Bio-Rad dye binding method with the Bio-Rad bovine γ-globulin standard. Due to substantial uncertainty in relative dye binding constants, protein quantitation must be considered approximate.

[1] Abbreviations used: bp, base pairs; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; Cl_3AcOH, trichloroacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Mops, 3-(*N*-morpholino)-propanesulfonic acid; m^5Cyt, 5-methylcytosine.

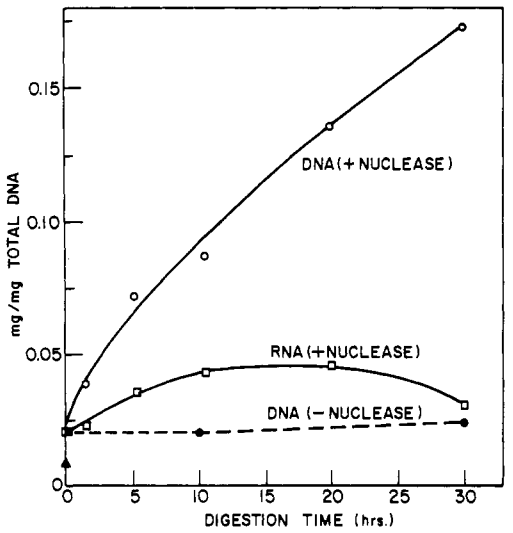

FIGURE 1: Release of nucleic acids into the soluble (S) fraction as a function of digestion time. Aliquots of nuclei were digested with micrococcal nuclease and fractionated as described under Materials and Methods. The ordinate represents the weight of S-fraction DNA or RNA, determined colorimetrically, divided by the weight of total DNA in each aliquot. The amount of DNA released from nuclei isolated in the absence of Ca^{2+} and Mg^{2+} without addition of nuclease is indicated by (▲).

Thermal denaturation of DNA in 0.12 M sodium phosphate buffer, pH 6.8, was monitored with a Beckman Acta CII spectrophotometer. A YSI thermistor probe and thermivolt thermometer were used to give a continuous record of absorbance at 260 nm vs. temperature on an X-Y recorder. The temperature was programmed to increase at about 1 °C/min by using a Neslab Model TP-2 programmer attached to a circulator bath.

Separation of single-stranded DNA from double-stranded DNA was achieved by hydroxylapatite (Bio-Rad; DNA grade) column chromatography as described by Britten et al. (1974). Samples were loaded onto hydroxylapatite columns in 0.12 M sodium phosphate buffer at 60 °C. Single-stranded DNA was eluted by washing with 5–10 volumes of the same buffer, and then double-stranded DNA was eluted by washing with 5–10 volumes of 0.4 M sodium phosphate buffer at 60 °C.

DNA Base Analysis: Quantitation of 5-Methylcytosine. DNA fractions (100 μg) were dialyzed into 0.1 × SSC (0.15 M NaCl and 0.015 M sodium citrate), lyophilized, and then hydrolyzed for 1 h with 88% formic acid in sealed ampules at 175 °C in an oil bath. Formic acid was removed in vacuo over KOH (Nass, 1973). The residue was dissolved in 100 μL of 20 mM $(NH_4)_2CO_3$ (pH 10.0), and 30 μL was applied to a 55 × 0.6 cm column of Aminex A-10 (courtesy of Bio-Rad Laboratories). The individual DNA bases are eluted with 20 mM $(NH_4)_2CO_3$ (pH 10.0) (Singhal, 1974) at a pressure of 170 psi and a flow rate of 0.5 mL/min. The effluent was monitored at 273 nm with a Spectromonitor II (Laboratory Data Control). All bases were completely resolved. Peak areas were determined from the recordings by using a planimeter. Base ratios were calculated from the appropriate molar extinction coefficients at 273 nm.

Amino acid analyses were performed on a Beckman 120B analyzer. Salt-free, lyophilized protein was hydrolyzed in vacuo at 110 °C for 24 h with constant-boiling HCl (Pierce; sequanal grade) containing a drop of 10% redistilled phenol.

Results

Slight Nuclease Digestion Releases a Salt-Soluble (S) Nucleoprotein Fraction. Mouse myeloma nuclei treated with micrococcal nuclease for 30 h at 0 °C released up to 17% of the input DNA as nucleoproteins after equilibration with nearly physiological saline solution. DNA release was nearly linear with time (Figure 1). The nuclear morphology, viewed by light microscopy after methyl green–pyronin B staining (Stern, 1968), appeared unaffected by this treatment. Considerable RNA was also released, but non-acid-soluble RNA in the soluble (S) fraction approached a plateau at ~0.04 mg/mg of total DNA after 10 h, when ~9% of the total DNA was solubilized (Figure 1). No acid-soluble oligonucleotides were detected above the control level (no nuclease) throughout the digestion, indicating that nucleosome trimming and degradation were minimal.

Table I: Comparisons of the Relative Electrophoretic Mobilities of Putative Mouse Myeloma HMG 1 and 2 and Calf Thymus HMG 1 and 2[a]

	rel mobilities	
	mouse myeloma[b]	calf thymus[c]
HMG 1/H3	0.764 ± 0.013	0.74 ± 0.01
HMG 2/H3	0.782 ± 0.012	0.77 ± 0.01
HMG 1/H4	0.687 ± 0.014	0.65 ± 0.01
HMG 2/H4	0.703 ± 0.014	0.67 ± 0.01

[a] Samples were electrophoresed on 18% polyacrylamide gels containing $NaDodSO_4$. [b] The average and standard deviation of measurements from five gels are reported. [c] Calculated from the published photographs of Bustin et al. (1978). The small differences observed between the mouse and calf HMG's may be due in part to differences in the gel systems.

Nuclei isolated in the presence and absence of Mg^{2+} and Ca^{2+} released about 2 and 1% of the input DNA, respectively, when incubated in fractionation medium without prior nuclease treatment, indicating that endogenous nucleases were active during isolations of nuclei. No additional DNA was released from nuclei incubated alone for 30 h in digestion medium containing Mg^{2+} and Ca^{2+} (Figure 1), demonstrating that release of the S fraction beyond control levels was due to micrococcal nuclease.

S Fraction Is Enriched in HMG and Other Nonhistone Proteins and Is Depleted in Histone H1. Proteins from soluble and pelleted (P) fractions obtained after various digestion times were examined by $NaDodSO_4$ gel electrophoresis (Figure 2). Soluble fractions released by endogenous nucleases (lanes 1, 2, and 5–7) contained at least 30 nonhistone proteins and relatively small amounts of histones. Nonhistone proteins were much more abundant in S than in P fractions at all digestion times, but some were solubilized more readily than others. Samples of S fractions used in lanes 5–12 contained approximately the same amount of DNA; thus, the band intensities reflect relative yields of released proteins. Comparisons of the relative abundancies of S-fraction proteins with increasing digestion indicate that histones were released by micrococcal nuclease, presumably in nucleosomes (see below), and many nonhistones were released nearly completely in the absence of digestion beyond control levels (solubilization of 2% of the DNA of nuclei isolated in the presence of divalent cations). However, a group of major, slowly migrating nonhistones and a pair migrating between H1 and H3 were released by digestion until >9% of the total DNA was solubilized. Gel scans showed that further digestion decreased the amount of this latter pair with respect to H1.

The two prominent proteins migrating between H1 and H3 were of particular interest because their mobilities were virtually identical with those of the HMG 1 and HMG 2 nonhistone proteins of calf thymus chromatin, as shown in Table I. Only four major proteins from 0.35 M NaCl extracts

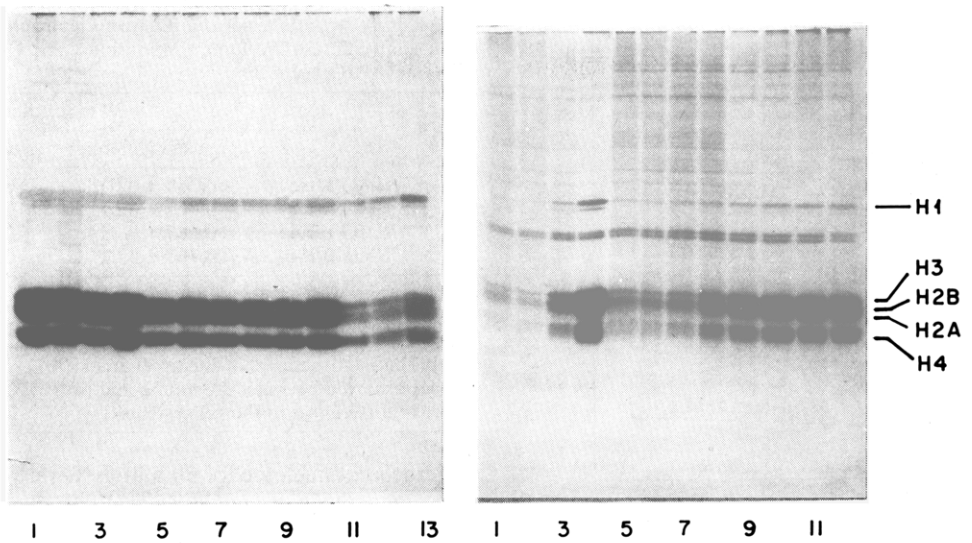

FIGURE 2: Electrophoretic patterns of proteins from S fractions (right) and P fractions (left) obtained after varying degrees of digestion of nuclei. Electrophoresis was on 14-cm slab gels of 18% polyacrylamide containing $NaDodSO_4$. Lanes 1–4 are proteins from nuclei isolated in the absence of divalent cations, then suspended in digestion medium containing Ca^{2+} and Mg^{2+}, and incubated at 0 °C. Lane 1: no nuclease added; EGTA added at time 0. Lane 2: no nuclease added; EGTA added after incubation for 22 h. Lane 3: digested 2.66 h. Lane 4: digested 22 h. Lanes 5–12: proteins from nuclei isolated in the presence of divalent cations. Lanes 5–7: no nuclease added; EGTA added at 0, 10, and 30 h. Lanes 8–12: digested for 1.5, 5.25, 10.5, 20, and 30 h, respectively. Lane 13 (left slab): displays proteins from unfractionated mouse myeloma nuclei.

Table II: Comparisons of the Amino Acid Compositions (Mole Percent) of Putative Mouse Myeloma HMG 1 + HMG 2 and Calf Thymus HMG 1 and HG 2

amino acid	calf[a]		mouse[b]
	HMG 1	HMG 2	HMG 1 + HMG 2
Asp	10.7	9.3	10.1
Thr	2.5	2.7	3.6
Ser	5.0	7.4	7.3
Glu	18.1	17.5	20.2
Pro	7.0	8.9	6.3
Gly	5.3	6.5	7.0
Ala	9.0	8.1	9.9
Val	1.9	2.3	3.6
Ile	1.8	1.3	1.9
Leu	2.2	2.0	3.6
Tyr	2.9	2.0	1.6
Phe	3.6	3.0	2.0
His	1.7	2.0	0.7
Lys	21.3	19.4	18.0
Arg	3.9	4.7	4.0

[a] Data from Goodwin & Johns (1977). [b] Total 2% Cl_3AcOH soluble proteins from a 0.35 M NaCl extract of mouse myeloma chromatin. This fraction contains mainly putative HMG 1 and HMG 2 (see Figure 4). Results are uncorrected for hydrolytic losses.

Table III: Tyrosine and Tryptophan Contents of Putative Mouse Myeloma HMG 1 + HMG 2 Determined by UV Spectral Analysis

	mouse myeloma[a] total HMG	calf thymus[b]	
		HMG 1	HMG 2
protein concn[c] (mg/mL)	0.879		
A_{280nm}	0.736		
A_{288nm}	0.469		
ϵ_{280} (M^{-1} cm^{-1})[d]	21 980	20 900	20 000
ϵ_{288} (M^{-1} cm^{-1})[d]	14 000		
mol of Tyr per mol of HMG[e]	6.6	7	6
mol of Trp per mol of HMG[e]	2.4	2	2

[a] Total 2% Cl_3AcOH soluble proteins from the 0.35 M NaCl extract of myeloma chromatin. This fraction contains mainly (putative) HMG 1 and 2 (see Figure 4). [b] Data reported by Baker et al. (1976). [c] Determined by the Bio-Rad assay. [d] A molecular weight of 26 250 was assumed. [e] Determined by the spectrophotometric method of Edelhoch (1967). The agreement between the Tyr and Trp contents of mouse and calf HMG's may be somewhat fortuitous because of uncertainties introduced by the protein assay. There is a discrepancy between the number of tyrosines indicated by spectral analyses of Baker et al. (1976) and amino acid analyses of Goodwin & Johns (1977). This discrepancy also exists in our data and is presently unexplained.

of calf or rabbit thymus chromatin are soluble in 2% trichloroacetic acid (Cl_3AcOH) (Goodwin et al., 1973; Goodwin & Johns, 1973, 1977). These proteins, termed HMG 1, HMG 2, HMG 14, and HMG 17, are of low molecular weight (<30 000), have high contents of acidic and/or basic residues, and migrate rapidly on $NaDodSO_4$– or acid–urea–polyacrylamide gels. We found that 0.35 M NaCl extracts of both whole mouse myeloma chromatin and S fractions contained only two major, 2% Cl_3AcOH soluble proteins. Proteins from both extracts migrated identically with one another and with the major S-fraction nonhistones on gels containing $NaDodSO_4$ (Figure 3), acetic acid–urea, or Triton DF16–acetic acid–urea (not shown). Thus, these S-fraction proteins operationally belong to the HMG group and were tentatively identified with HMG 1 and 2. Minor components of the 0.35 M NaCl extract of total mouse chromatin migrated slightly behind H3 on $NaDodSO_4$ gels, as expected for HMG 14 and 17. These did not seem enriched in S fractions.

S fractions consistently contained reduced levels of H1 relative to other histones, as is illustrated in Figure 3C. (Figure 2 does not accurately reflect the relative amounts of histones due to staining and destaining artifacts associated with overloading.) No evidence indicated that protein degradation contributed to the appearance of putative HMG's or depletion of H1 in S fractions. Myeloma tissue is low in proteinase activity, and residual activity should be inhibited by PMSF and the low pH of all solutions. Partial degradation products of H1 and HMG 1 and 2 typically migrate ahead of the parent proteins, but behind H3, while those of the other histones migrate ahead of H4. There were virtually no bands in these regions (excepting those attributed to HMG 1 and 2) in patterns of heavily loaded P and S fractions (Figure 2). Identification of these proteins with HMG 1 and 2 was

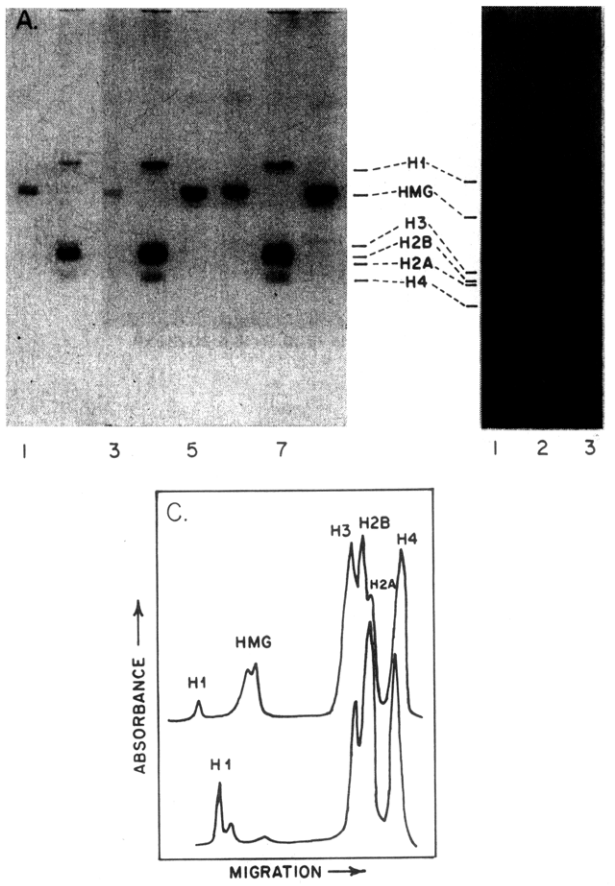

FIGURE 3: (A) Comparison of 0.35 M NaCl extractable, 2% Cl_3AcOH soluble proteins from mouse myeloma chromatin (lanes 1, 5, and 8) and from the soluble (S) fraction (lanes 3 and 6) by electrophoresis on 18% polyacrylamide gels containing $NaDodSO_4$. Whole mouse nuclear proteins appear in lanes 2, 4 and 7. The 0.35 M NaCl extractable proteins from the S fraction were obtained by sedimenting the S fraction on a sucrose gradient containing 0.35 M NaCl and collecting the upper (nonnucleosomal) fractions. LMG proteins were then precipitated with 2% Cl_3AcOH. (B) Comparison of whole mouse nuclear proteins (lane 1), whole S-fraction proteins (lane 2), and 0.35 M NaCl extractable, 2% Cl_3AcOH soluble proteins from myeloma chromatin (lane 3). (C) Comparison of the histone H1 content of the S fraction (upper scan) and whole mouse myeloma chromatin (lower scan). Samples were electrophoresed as above, stained with coomassie blue, and scanned with a Gelman DCD-16 densitometer. The recovery of the S fraction was 5% of the total DNA.

confirmed by amino acid analysis (Table II). In addition, UV spectral analyses for tyrosine and tryptophan content were in good agreement with data reported for calf thymus HMG 1 and 2 (Table III). No histones contain tryptophan, and H1 contains little tyrosine [0.9 mol/100 mol of total amino acids recovered; Johns (1977)].

Some S-Fraction DNA Is Single Stranded. Thermal denaturation showed that P-fraction DNA was fully double stranded, with a melting hyperchromicity approaching 41%. The hyperchromicity of S-fraction DNA from briefly digested nuclei was only 27%, and the denaturation curve was biphasic, with a broad low-temperature transition indicative of single-stranded DNA (Figure 4). Hydroxylapatite chromatography confirmed the presence of single-stranded DNA amounting to 35–40% of the total. The hyperchromicity of S-fraction DNA increased significantly with further digestion (Figure 4, inset), probably due to dilution with double-stranded DNA since no acid-soluble oligonucleotides were produced.

S-Fraction DNA Is Undermethylated. Base analyses were performed on S- and P-fraction DNA from three preparations of nuclei digested such that 2.8, 9.4, and 17.3% of the total

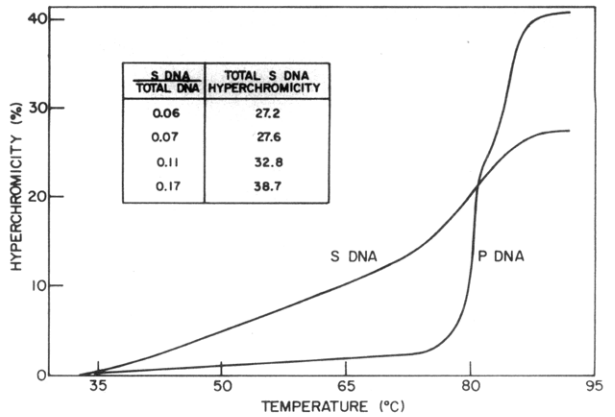

FIGURE 4: Thermal denaturation profiles of deproteinized, RNase-treated S- and P-fraction DNA illustrating the partial single-stranded character of S DNA. The S fraction was isolated after brief digestion (yield = 6% of the total DNA). Inset: summary of the total hyperchromicity upon melting of S DNA obtained from four different extents of digestion resulting in the indicated yields of the S fraction.

Table IV: Comparison of the 5-Methylcytosine Contents of S- and P-Fraction DNA

	m^5Cyt content (mol % of total bases)			
	% yield of S fraction[a]			
fraction	2.8	9.4	17.3	av
P	0.70	0.72	0.68	0.70 ± 0.02
S	0.60	0.59	0.53	0.57 ± 0.03

[a] Expressed as percent of total nuclear DNA.

DNA were recovered in S fractions. No uridine was detected in any samples. The average G+C content (40.5 ± 0.4 mol %) and m^5Cyt/Cyt ratio (0.036 ± 0.001) of P-fraction DNA agreed well with data of Solage & Cedar (1978) for mouse L-cell DNA (G+C ≃ 42%; m^5Cyt/Cyt = 0.042). The G+C contents of S and P DNA were the same within error, but all S-fraction DNA samples were undermethylated, with m^5Cyt contents averaging 18 ± 4% less than P-fraction DNA (Table IV).

S Fraction Contains Mainly Mono- and Small Oligonucleosomes. Both S-fraction DNA and P-fraction DNA from all digestion times were mainly of nucleosome (~200 bp) and oligonucleosome length (Figure 5). The repeat lengths in both fractions appeared identical, but S-fraction DNA (mainly less than 1000 bp) was always much smaller on the whole than P-fraction DNA. S fractions also contained small amounts of 40–60-bp DNA (less than 10% of the total). Thermal denaturation showed that this short DNA was highly single stranded (not shown). No systematic increases were noted in the mobilities of S- or P-fraction DNA bands with increasing digestion.

Sedimentation patterns of S-fraction nucleoproteins on sucrose gradients (Figure 6) were virtually identical with those of mono- and oligonucleosomes from digested myeloma chromatin and redigested P fractions. Examinations of the proteins across S-fraction gradients (Figure 6) showed that most large nonhistones cosedimented with DNA remaining near the top (fractions 5–7). HMG proteins also appeared in these fractions. These may have been DNA bound, since free HMG's sediment mainly in the top four fractions, and electrophoretic methods have revealed small nucleoproteins containing HMG's and other nonhistones (Figure 7). Histones H2A, H2B, H3, and H4 appeared in fraction 11, the trailing edge of the ~11S peak, and were present in subsequent

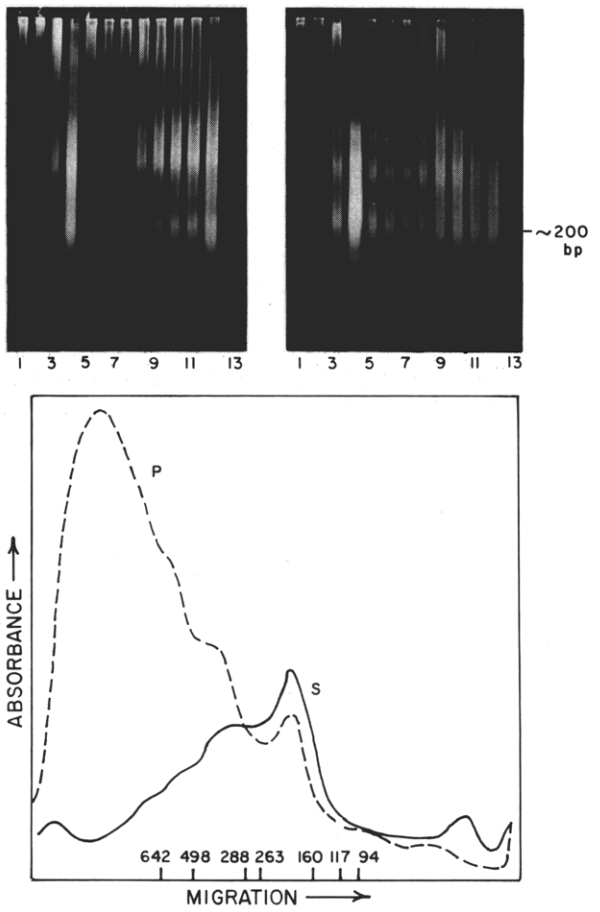

FIGURE 5: Electrophoretic analyses of DNA from the S fractions (upper right) and P fractions (upper left) obtained from nuclei digested to various extents with micrococcal nuclease (same samples as shown in Figure 2). Lanes 1–4 are from nuclei isolated in the absence of divalent cations. Lanes 1 and 2: no nuclease added; EGTA added after 0 and 22 h. Lanes 3 and 4: digested 2.66 and 22 h. Lanes 5–12: from nuclei isolated in the presence of divalent cations. Lanes 5–7: no nuclease added; EGTA added after 0, 10, and 30 h. Lanes 8–12: digested for 1.5, 5.25, 10.5, 20, and 30 h. Lanes 13 are markers (*Hae*III endonuclease R fragments of PM2 DNA). Electrophoresis was on 15-cm, 6% polyacrylamide slab gels containing $NaDodSO_4$; staining was with ethidium bromide. The relative size distributions of S and P DNA are illustrated better in scans of Stains-All stained gels (bottom). The S fraction was 11.2% of the total DNA in this case. Samples were deproteinized and electrophoresed on 6% polyacrylamide tube gels lacking $NaDodSO_4$. Vertical lines mark the locations and lengths of *Hae*III fragments of PM2 DNA.

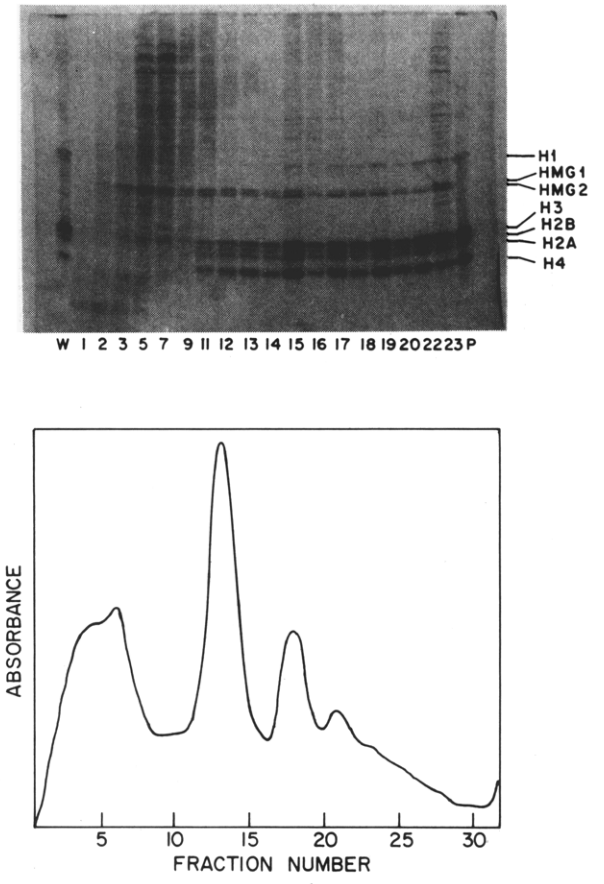

FIGURE 6: (Bottom) Sucrose density gradient sedimentation profile of S-fraction nucleoproteins. Yield of the S fraction = 5.6% of the total DNA. (Top) Electrophoretic analyses of proteins from the indicated gradient fractions (1–23), whole myeloma nuclei (W), and total P fraction (P). (Protein loading does not reflect protein quantities in the gradient fractions.) Electrophoresis was on a 15-cm, 18% slab gel containing $NaDodSO_4$.

fractions in approximately the stoichiometry expected for typical nucleosomes. [In many S-fraction preparations the amount of H4 seemed anomalously high. We believe that this was due to the presence of nonhistone protein(s). The protein migrating similarly to H3 in fractions 5–9 also is likely to be a nonhistone and/or micrococcal nuclease.] Most strikingly, the central region of the ~11S mononucleosome band contained no H1, but it contained amounts of putative HMG 1 and 2 that approached the staining levels of core histones. Both H1 and HMG proteins were found in the oligonucleosome fractions, and HMG 1 was specifically depleted from the largest particles. Though its significance is unclear, the latter phenomenon provides an argument against possible redistribution of HMG proteins, since HMG 1 binds much more strongly than HMG 2 to H1 subfractions (Smerdon & Isenberg, 1976; Yu & Spring, 1977).

To unequivocally demonstrate that HMG's were bound to nucleosomes, we electrophoresed a whole S fraction and S-fraction mononucleosomes isolated from sucrose gradients on low ionic strength polyacrylamide gels in the presence and absence of 3 M urea, respectively. A major, fast migrating band from the whole S fraction contained core histones plus significant amounts of HMG and other nonhistone proteins (Figure 7). A faster migrating particle contained mainly the HMG's and other nonhistones, while a slower band contained HMG's, H1, and core histones but little other nonhistone proteins. Electrophoresis of mononucleosomes from the S-fraction gradient yielded two bands on a broad background. One migrated identically with highly purified nucleosome cores (Figure 7B). Second-dimension analysis showed that this particle contained only core histones. The slower band contained core histones plus HMG proteins but not H1 (Figure 7C). Free HMG proteins migrated far behind the above particles on identical gels, as did virtually all P-fraction nucleoproteins.

A densitometer scan across the above second-dimension gel in the region of densest core histone staining (Figure 8) was very similar to those obtained for mononucleosomes derived from the whole S fraction by electrophoresis (Figure 7A) and gradient centrifugation (Figure 8). Integration of these patterns yielded a ratio of HMG/core histone staining of 0.11 (±5%). This was identical, within error, with the ratio of H1/core histone staining found for whole mouse chromatin electrophoresed in the same manner (Figure 8), strongly indicating that there was one HMG protein per S-fraction nucleosome.

Although we have focused attention on the common HMG proteins, a number of higher molecular weight nonhistone

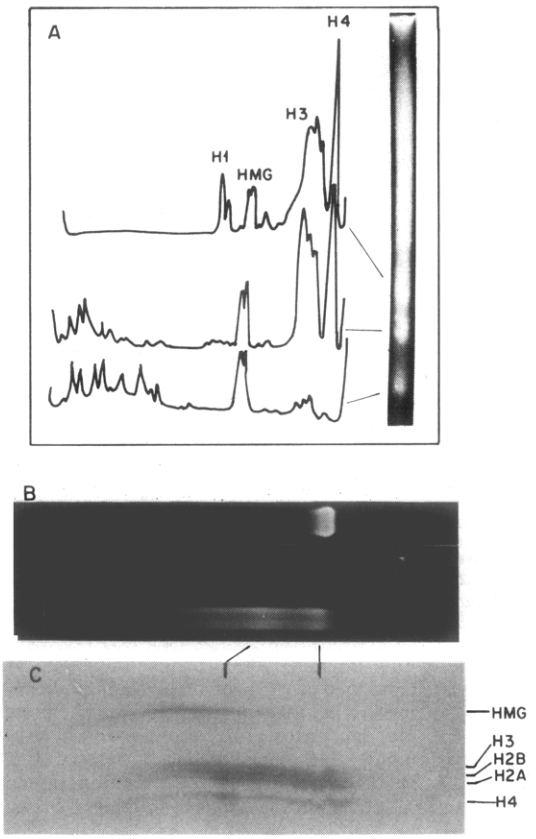

FIGURE 7: (A) Right: electrophoretic pattern of total S-fraction nucleoproteins on a 6% polyacrylamide gel containing 3 M urea, 10 mM Tris–Mops, and 2 mM EDTA (final pH 7.6). Gel was stained with ethidium bromide. Left: electrophoretic analyses on 18% gels containing $NaDodSO_4$ of proteins from the three nucleoprotein bands indicated. (B) Electrophoretic patterns of highly purified nucleosome cores obtained by micrococcal nuclease digestion of H1- and H5-depleted chicken erythrocyte chromatin (top pattern) and of S-fraction mononucleosomes isolated by density gradient centrifugation (lower two patterns). Samples were electrophoresed on a 15-cm, 8% polyacrylamide slab gel as described above except that urea was omitted from the gel and sample. (C) Analysis of the proteins contained in the nucleoproteins separated in (B) by electrophoresis on a second-dimension 18% polyacrylamide slab gel containing $NaDodSO_4$. Staining was with Uniblue A (Eastman) in methanol–acetic acid–water (5:1:4). [Note that the scales of (B) and (C) are different and that only the lower portion of the second-dimension gel is shown.]

proteins coisolate with S-fraction mononucleosomes. Collectively, these amount to a considerable weight fraction of the total protein (e.g., see Figure 7A).

Free HMG Proteins Do Not Bind Significantly to S-Fraction Nucleosomes. For investigation of the potential for redistribution of HMG proteins during the preparation of S-fraction chromatin, isolated HMG's were slightly labeled with dansyl chloride (about one dansyl moiety per protein molecule), and 0.75 mg was mixed with 2 mg (DNA equivalents) of S chromatin in the fractionation medium (near-physiological ionic strength). After concentration to 1 mL by ultrafiltration, the mixture was incubated for 6 h at 4 °C and then centrifuged on 6–24% glycerol gradients. Separate control gradients were loaded with S fraction alone and with dansylated HMG proteins alone. The resulting gradient fractions were made 1% in $NaDodSO_4$, and the fluorescence of each was quantitated.

Free HMG proteins sedimented as a skewed peak, with 82 and 97% of the total fluorescence in the top 5 and 10 fractions, respectively (Figure 9). The fluorescence profile of HMG's mixed with the S fraction was slightly shifted, in a manner consistent with rapid, reversible binding to faster sedimenting

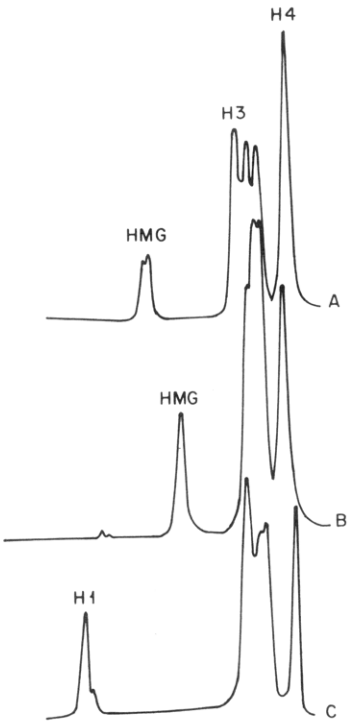

FIGURE 8: The ratio of HMG proteins to core histones in S-fraction mononucleosomes is similar to the ratio of H1 to core histones in whole mouse myeloma chromatin. (A) Densitometer scan of proteins from S-fraction mononucleosomes isolated by sucrose density gradient centrifugation after brief digestion (6% yield of the S fraction). (B) Densitometer scan across the region of highest protein staining in the second-dimension electropherogram shown in Figure 7C. (C) Whole mouse chromatin histones. Electrophoresis was on 18% gels containing $NaDodSO_4$. Gels in (A) and (C) were stained with coomassie blue.

nucleoproteins, yet 81% of the total fluorescence remained in the top 10 fractions (behind the mononucleosome peak). The continuous decline of the HMG fluorescence through the mono- and oligonucleosome region, and the failure of the fluorescence profile to follow the A_{260nm} profile, demonstrates that this binding was weak and that the previously bound, undansylated HMG proteins were not replaced by dansylated HMG's. These results are particularly striking since DNA can bind nearly 5 times its own weight of HMG proteins (Shooter et al., 1974) and isolated nucleosome cores can bind more than an equivalent amount of extra histones (Voordouw & Eisenberg, 1978).

The "specific fluorescence" of the two major HMG species in several gradient fractions was determined from measurements of the fluorescence and coomassie blue staining after electrophoresis. The specific fluorescence of HMG 1 + HMG 2 from the control gradient was 11, that of fractions 2, 5, and 12 from the mixing experiment was 8, and that of fraction 14 was 7. This decrease across the mononucleosome peak (fractions 12 and 14), though small, is consistent with the conclusion that the dansylated HMG 1 and HMG 2 appearing in this region were due to weak binding and spillover of unbound proteins, which were added in large excess over the endogenous HMG's and in twice the amount used for the control. Furthermore, examination of the proteins from different gradient fractions revealed that nearly half of the fluorescence cosedimenting with the mono- and oligonucleosomes was due to the binding of two smaller HMG proteins, most likely to be HMG 14 and 17 (Figure 9, inset). In contrast to the putative HMG 1 and HMG 2, these proteins were depleted in the upper gradient fractions and were preferentially bound to nucleosomes. This behavior is not

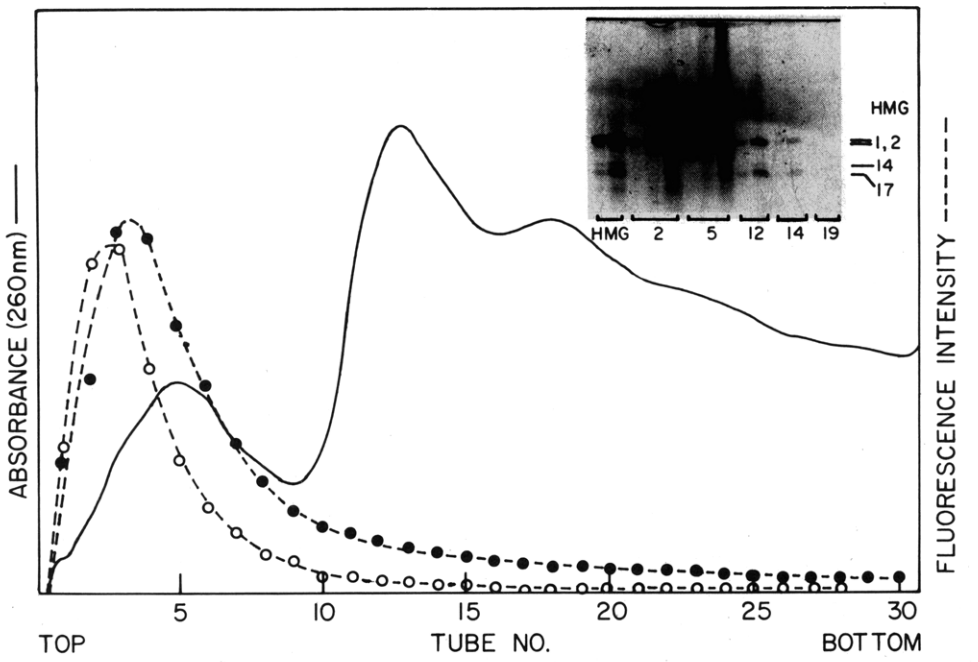

FIGURE 9: The interaction of added, fluorescence-labeled mouse myeloma HMG proteins with S-fraction nucleoproteins. Total 0.35 M NaCl extractable, 2% Cl_3AcOH soluble proteins from mouse myeloma chromatin were dansylated and then centrifuged on glycerol gradients in the presence (●) and absence (○) of the total S fraction, as described in the text. The amount of HMG protein sedimented in the presence of the S fraction was about twice that sedimented in its absence. Inset: fluorogram of the total dansylated HMG proteins and of dansylated proteins recovered from fractions 2, 5, 12, 14, and 19 of the gradient containing the S fraction. Samples were electrophoresed on an 18% polyacrylamide slab gel containing $NaDodSO_4$, illuminated with a UV (365-nm) lamp, and photographed by using Polaroid transparency film. (Printing therefore yields black protein bands.) Note the relatively high abundance of two rapidly migrating bands in fractions 12–19. These are tentatively assigned as HMG 14 and HMG 17.

particularly surprising, since HMG 17 is much more basic and histonelike than HMG 1 or HMG 2.

Discussion

After minimal digestion with micrococcal nuclease, mono- and small oligonucleosomes are released from nuclei under mild, near-physiological conditions, without mechanical shearing, chelating agents, or low ionic strength solutions. These conditions are often used to solubilize chromatin but disperse the nuclear matrix and heterochromatin and unfold thick chromatin fibrils. This release is not surprising since ribonucleoproteins must pass the nuclear membrane in vivo. The depletion of methylated DNA and accumulation of most nuclear nonhistone proteins, RNA, and single-stranded DNA in the fraction released show that the selection factors operating here yield a structurally unique subset of the chromosomal material that is presumably of functional significance. This significance cannot be fully assessed until hybridization studies are completed, but recent findings argue for a relationship to transcriptional activity.

Nucleases show a clear preference for transcriptionally competent chromatin. DNase I and DNase II rapidly *degrade* active genes in numerous chromatin types [reviewed by Felsenfeld (1978)]. Chromatin modestly enriched in active genes is obtained by brief DNase II digestion of dispersed chromatin, followed by partial precipitation of inactive chromatin with 2 mM Mg^{2+} (Gottesfeld, 1978). Although active genes are not degraded by micrococcal nuclease, mononucleosome-length DNA rapidly *released* from chromatin of hen oviducts (Bellard et al., 1978; Bloom & Anderson, 1978), *Drosophila* heat-shock embryos (Wu et al., 1978), and trout testis (Levy et al., 1979) is highly enriched in transcribed sequences, indicating that this nuclease preferentially cleaves spacer regions of active nucleosomes, without subsequently degrading the nucleosome core.

The preference of micrococcal nuclease for spacer DNA in chromatin may partially explain these differences, but its specificity for single-stranded DNA at moderate to high ionic strengths (Kacian & Speigelman, 1974) may contribute to early release of active chromatin. Endogenous single-stranded DNA (1.5–2% of the total) from human cells (Tapiero et al., 1974), normal embryonic chicken cells (Tapiero et al., 1976; Leibovitch & Harel, 1978), and leukemic chicken cells producing avian myeloblastosis virus (Leibovitch et al., 1977) was found to be enriched in sequences complementary to both the nonrepetitive portion of the total DNA and to homologous cellular RNA, suggesting an origin from active transcription sites. Single-stranded DNA from the myeloma S fraction, also about 2% of the total nuclear DNA, may be of similar origin.

Undermethylation of S-fraction DNA demonstrates that it represents a subfraction of the mouse genome. Additional significance of this result is difficult to assign since the function of methylation in eucaryotes is uncertain. The localization of m^5Cyt in highly repetitive DNA is well documented (Salomon et al., 1969; Miller et al., 1974; Harbers et al., 1975), and rapidly reassociating mouse liver DNA (33% of the total with C_0t = 50 M s) contains a disproportionate amount of m^5Cyt (48% of the total) (Solage & Cedar, 1978). Thus, undermethylation of S DNA could signify a depletion of redundant, untranscribed sequences.

Since completion of this work, two similar methods for fractionating chromatin on the basis of solubility after brief digestion of nuclei with micrococcal nuclease were reported. In general, our method appears more stringent with respect to the extent of digestion (production of acid-soluble material and loss of nucleosome spacer regions) and salt solubility, but the importance of these differences cannot be assessed with present data. Fractionation of hen oviduct chromatin under very similar conditions, after digestion to 2–3% acid solubility at 37 °C, yielded soluble nucleoproteins containing mainly

mononucleosome-length DNA (165–200 bp). This DNA was five- to sixfold enriched in ovalbumin coding sequences amounting to about 35% of the ovalbumin genes in the total chromatin (Bloom & Anderson, 1978). No other composition data were reported. Levy et al. (1979) have shown that brief digestion of trout testis nuclei causes the release of HMG-T (similar to calf HMG 1 and 2) into the supernatant and that homogenization of the nuclear pellet in 1 mM EDTA, followed by addition of salt, yields a 0.1 M NaCl soluble subfraction of nucleosomes. This contains all four core histones, no H1, and a stoichiometric amount of H6 (homologous to calf HMG 17) associated with 140 bp length DNA. This DNA represented a subset of the trout genome and was at least sevenfold enriched in sequences complementary to total cytoplasmic poly(A)-containing RNA. Limited digestion of trout testis chromatin with DNase II yielded a 2 mM $MgCl_2$ soluble subfraction of nucleosomes containing no H1 and both HMG-T and H6 associated with about 180 bp length DNA, suggesting that HMG-T is bound to nucleosome spacers.

The most distinctive feature of the S fraction described here is the extreme enrichment of HMG 1 and HMG 2 *bound to mononucleosomes* in near-stoichiometric amounts. Also noteworthy is the cosedimentation and coelectrophoresis of specific subsets of nonhistone proteins with mono- and oligonucleosomes and with subnucleosome-length DNA. Further two-dimensional electrophoresis studies have shown that specific subsets of nonhistones comigrate with unique, small lengths of DNA and that S mononucleosomes can be further fractionated into classes differing in nonhistone protein content (S. Chambers and R. L. Rill, unpublished experiments).

The extremely low degree of chromatin degradation required to yield these nucleosomes, the retention of HMG's on nucleosomes through several purification steps, including even electrophoresis in the presence of 3 M urea, and the absence of strong binding of free HMG 1 and 2 to nucleosomes argue against the possibility that HMG 1 and HMG 2 were released by digestion of underlying DNA and then bound to nucleosomes. On the other hand, the preferential binding of smaller HMG-like proteins (probably HMG 14 and 17) to nucleosomes raises the possibility of redistribution of these proteins among digestion products.

The HMG-containing nucleosomes appear normal in DNA size, core histone content, and sedimentation coefficient. They also exhibit a normal circular dichroism spectrum and digestion pattern with DNase I (Jackson and Rill, unpublished experiments). They differ from typical nucleosomes in their saline solubility and lack of histone H1. These two properties are undoubtedly linked and may relate to the in vivo function of HMG proteins.

A relationship between H1 and the insolubility of chromatin has long been recognized and is reflected by the fact that nucleosomes containing H1 are insoluble in 0.15 M NaCl or KCl solutions in which nucleosome cores lacking H1 are soluble (Olins et al., 1976). Studies of the effects of removing H1 on the structure of chromatin (Renz et al., 1977; Thoma & Koller, 1977) and SV40 minichromosomes (Christiansen & Griffith, 1977; Varshavsky et al., 1977) have shown that H1 is a major factor in the folding of ~100-Å diameter nucleosomal fibers into more compact forms.

The composition of S-fraction mono- and oligonucleosomes is most simply interpreted as a result of in vivo substitution of HMG 1 and 2 for H1, probably by direct binding to nucleosome spacers. Such substitution should affect nucleosome solubility and influence the local chromatin fiber folding because of marked sequence differences. H1 is well suited for cross-linking distant DNA segments because basic residues are concentrated in both ends of the chain, with the majority near the C terminus (Macleod et al., 1977). HMG 1 and HMG 2 have highly basic N-terminal regions that bind DNA but are enriched in acidic residues (~30% of the total) concentrated near the C termini (Walker et al., 1976). HMG 1 is most unique with 41 continuous acidic groups in the C-terminal 120 residues (Walker et al., 1978). One can easily imagine that replacement of bivalent H1 by proteins with a high negative charge density on one end could maintain active chromatin in a relatively unfolded, soluble state.

Alternatively, the HMG proteins may function in repeated, higher order structures such as solenoid turns or superbeads, since there is about one HMG protein per five to ten nucleosomes. (However, the preponderance of HMG's over H1 in soluble *oligo*nucleosomes suggests at least limited clustering of HMG-containing nucleosomes and argues against a simple periodic distribution.) The ability to isolate in a simple way chromatin fragments bound by HMG proteins or other nonhistone proteins will permit direct assessment of their relationships to specific DNA sequences.

References

Baker, C., Isenberg, I., Goodwin, G. H., & John, E. W. (1976) *Biochemistry 15*, 1645–1649.

Bellard, M., Gannon, F., & Chambon, P. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 779–791.

Bloom, K. S., & Anderson, J. N. (1978) *Cell 15*, 141–150.

Bonner, W. M., & Pollard, H. B. (1975) *Biochem. Biophys. Res. Commun. 64*, 282–288.

Britten, R. J., Graham, D. E., & Neufeld, B. R. (1974) *Methods Enzymol. 29E*, 363.

Bustin, M., Hopkins, R. B., & Isenberg, I. (1978) *J. Biol. Chem. 253*, 1694–1699.

Chen, R. F. (1968) *Anal. Biochem. 25*, 412.

Christiansen, G., & Griffith, J. (1977) *Nucleic Acids Res. 4*, 1837–1851.

Dahlberg, A. E., Dingman, C. W., & Peacock, A. C. (1969) *J. Mol. Biol. 41*, 139–147.

Edelhoch, H. (1967) *Biochemistry 6*, 1948–1954.

Fakan, S. (1978) *Cell Nucl. 5*, 3–53.

Felsenfeld, G. (1978) *Nature (London) 271*, 115–122.

Finch, J. T., & Klug, A. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1897–1901.

Franke, W. W., Scheer, V., Trendelenburg, M. F., Spring, H., & Zentgraf, H. (1976) *Cytobiologie 13*, 401.

Franke, W. W., Scheer, V., Trendelenburg, M., Zentgraf, H., & Spring, H. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 755–772.

Garel, A., & Axel, R. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3966–3970.

Giles, K. W., & Myers, A. (1965) *Nature (London) 206*, 93.

Goodwin, G. H., & Johns, E. W. (1973) *Eur. J. Biochem. 40*, 215–219.

Goodwin, G. H., & Johns, E. W. (1977) *Methods Cell Biol. 16*, 257–268.

Goodwin, G. H., Sanders, C., & Johns, E. W. (1973) *Eur. J. Biochem. 38*, 14–19.

Goodwin, G. H., Shooter, K. V., & Johns, E. W. (1975) *Eur. J. Biochem. 54*, 427.

Gottesfeld, J. M. (1978) *Methods Cell Biol. 16*, 421–436.

Harbers, K., Harbers, B., & Spencer, J. H. (1975) *Biochem. Biophys. Res. Commun. 66*, 738–746.

Hartley, B. S., & Massey, V. (1956) *Biochim. Biophys. Acta 21*, 58.

Johns, E. W. (1977) *Methods Cell Biol. 16*, 183–204.

Kacian, D. L., & Spiegelman, S. (1974) *Anal. Biochem. 58*, 534–540.
Laemmli, V. K. (1970) *Nature (London) 227*, 1–6.
Laird, C. D., Wilkinson, L. E., Foe, V. E., & Chooi, W. Y. (1976) *Chromosoma 58*, 169.
Leibovitch, S. A., & Harel, J. (1978) *Nucleic Acids Res. 5*, 777–787.
Leibovitch, S. A., Tapiero, H., & Harel, J. (1977) *Cell Biol. Int. Rep. 1*, 309–315.
Levy, B. W., Connor, W., & Dixon, G. H. (1979) *J. Biol. Chem. 254*, 609–620.
Loening, U. E. (1967) *Biochem. J. 102*, 251–257.
Macleod, A. R., Wong, N. C. W., & Dixon, G. H. (1977) *Eur. J. Biochem. 78*, 281–291.
Miller, O. J., Schnedl, W., Allen, J., & Erlanger, B. F. (1974) *Nature (London) 251*, 636–637.
Nass, M. M. K. (1973) *J. Mol. Biol. 80*, 155–175.
Olins, D. E., & Olins, A. L. (1972) *J. Cell Biol. 53*, 715–736.
Olins, D. E., & Olins, A. L. (1974) *Science 183*, 330–332.
Olins, A. L., Carlson, R. D., Wright, E. B., & Olins, D. E. (1976) *Nucleic Acids Res. 3*, 3271–3291.
Oudet, P., Gross-Bellard, M., & Chambon, P. (1975) *Cell 4*, 281–300.
Panyim, S., & Chalkley, R. (1969) *Arch. Biochem. Biophys. 130*, 337–346.
Pooley, A. S., Pardon, J. F., & Richards, B. M. (1974) *J. Mol. Biol. 85*, 533–549.
Renz, M., Nehls, P., & Hozier, J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1879–1883.
Rill, R. L., & Nelson, D. A. (1978) *Cold Spring Harbor Symp. Quant. Biol. 42*, 475–482.
Rill, R. L., Nelson, D. A., Oosterhof, D. K., & Hozier, J. C. (1977) *Nucleic Acids Res. 4*, 771–790.
Rill, R. L., Shaw, B. R., & Van Holde, K. E. (1978) *Methods Cell Biol. 18*, 69–103.
Ris, H., & Kubai, D. F. (1970) *Annu. Rev. Genet. 4*, 263–294.
Salomon, R., Kaye, A. M., & Herzberg, M. (1969) *J. Mol. Biol. 43*, 581–592.
Schneider, W. C. (1957) *Methods Enzymol. 3*, 680.
Shooter, K. V., Goodwin, G. H., & Johns, E. W. (1974) *Eur. J. Biochem. 47*, 263.
Singhal, R. P. (1974) *Sep. Purif. Methods 3*, 339–398.
Smerdon, M. J., & Isenberg, I. (1976) *Biochemistry 15*, 4242–4247.
Solage, A., & Cedar, H. (1978) *Biochemistry 17*, 2934–2938.
Sollner-Webb, B., & Felsenfeld, G. (1975) *Biochemistry 14*, 2915–2920.
Stern, H. (1968) *Methods Enzymol. 12B*, 107–108.
Tapiero, H., Monier, M. N., Shaool, D., & Harel, J. (1974) *Nucleic Acids Res. 1*, 309–322.
Tapiero, H., Leibovitch, S. A., Shaool, D., Monier, M.-N., & Harel, J. (1976) *Nucleic Acids Res. 3*, 953–963.
Thoma, F., & Koller, T. (1977) *Cell 12*, 101–108.
Varshavsky, A. J., Nedospasov, S. A., Schmatchenko, V. V., Bakayev, V. V., Chumackov, D. M., & Georgiev, G. P. (1977) *Nucleic Acids Res. 4*, 3303–3325.
Voordouw, G., & Eisenberg, H. (1978) *Nature (London) 273*, 446.
Walker, J. M., Goodwin, G. H., & Johns, E. W. (1976) *Eur. J. Biochem. 62*, 461–469.
Walker, J. M., Hastings, J. R. B., & Johns, E. W. (1978) *Nature (London) 271*, 281–282.
Wallace, P. G., Hewish, D. R., Venning, M. M., & Burgoyne, L. A. (1971) *Biochem. J. 125*, 47.
Woodcock, C. L. F., Frado, L. L., Hatch, C. L., & Riccardiello, L. (1976) *Chromosoma 58*, 33.
Wu, C., Livak, K., & Elgin, S. (1978) *Miami Winter Symp., 10th, Abstr.*, 119.
Yu, Sh. H., & Spring, T. G. (1977) *Biochim. Biophys. Acta 492*, 20.