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Analysis of the high mobility group proteins associated with salt-soluble nucleosomes

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ABSTRACT

Two methods have recently been described for the isolation of monomer nucleosomes enriched in transcribed sequences which depend on their solubility in 0.1 M NaCl (Levy, W.B. and Dixon (1978), Nucleic Acid Res., 5, 4155-4163) or solutions containing divalent metal ions (Bloom, K.S. and Anderson, J.N. (1978), Cell, 15, 141-150). Using these procedures the proteins associated with such nucleosomes from rabbit thymus, calf liver and hen oviduct nuclei were isolated and analysed. Increased amounts of proteins HMG14 and HMG17 and small amounts of HMG1 and HMG2 were found associated with the four core histones H2A, H2B, H3 and H4 in these nucleosomes. HMG14 and HMG17 were found to be enriched 2 - 7 fold, suggesting an involvement of these two proteins with transcribed sequences.

0.1 M NaCl-soluble monomer nucleosomes prepared by the method of Levy and Dixon were analysed by polyacrylamide gel electrophoresis and found to be composed of principally two types of particle:

1. Core particles of 145 base pairs of DNA associated with the four core histones only.
2. Nucleosomes with 160 base pairs of DNA associated with the four core histones, increased amounts of HMG14 and 17, and no H1. Small amounts of HMG1 and HMG2 are also detected.

These results suggest that HMG14 and HMG17 might be interacting with the 15 base pair linker DNA. A model is presented for the structure of transcriptionally active chromatin.

INTRODUCTION

Recently much attention has been focussed on a group of chromosomal proteins called the high mobility group (HMG) proteins because of their possible role in gene transcription (1,2). In mammals and birds four HMG proteins have been identified, isolated and sequenced (for a review, see ref. (3)). These proteins are best considered as two pairs of closely related proteins: HMG1 and 2 forming one pair, HMG14 and HMG17 forming the other. In trout testis two proteins, HMG-T and H6, have been described in detail (4,5). HMG-T in fish is analagous to the HMG1 and 2 pair in mammals and birds. H6 probably corresponds to the HMG14/17 pair.

The four HMG proteins HMG1, 2, 14 and 17 have been found associated with rabbit thymus monomer nucleosomes (6,7), though not all the HMG1 and 2 may be firmly attached to the chromatin since about half the HMG1 and HMG2 is readily washed out of nuclei with saline or by very brief digestions with micrococcal nuclease (7). After extensive nuclease digestions HMG14 and 17 are found still associated with the monomer nucleosomes and it was concluded that HMG14 and HMG17 were bound to the nucleosome core particle. Levy et al (1,8) have also described the isolation from trout testis of 140 base pair particles which are soluble in 0.1 M NaCl (i.e., core particles) which, in addition to having the four core histones, had 1 - 2 molecules of H6. The DNA of these core particles was enriched 7 - 9 fold in transcribed sequences. The implication of these results was that the H6-containing nucleosomes were specifically associated with transcribed sequences. This conclusion appears to be confirmed by the recent findings of Weisbrod and Weintraub (2), that HMG14 and HMG17 in erythrocytes are required to maintain the DNAse I sensitivity of the globin gene. However, in our studies on the digestion of nuclei with DNAse I (9) we have not been able to demonstrate that the HMG proteins are specifically associated with DNAse I sensitive regions (i.e., transcribed sequences).

In this report we have investigated in some detail the monomer nucleosomes in the 0.1 M NaCl soluble fraction obtained by brief micrococcal nuclease digestion of rabbit thymus nuclei. We have found, first, that there is a 2 - 4 fold enrichment of HMG14 and HMG17 (relative to the core histones) in this fraction, suggesting an involvement of these two proteins with transcribed sequences. Secondly, polyacrylamide gel electrophoresis of the 0.1 M NaCl soluble nucleosomes reveals that the HMG14 and HMG17 are bound to nucleosomes lacking H1 but having 160 base pairs of DNA. This suggests that HMG14 and 17 interact with the 15 base pairs of DNA immediately contiguous to the 145 base pair of the core particle.

Nucleosomes enriched in HMG14 and HMG17 are also found to be released from nuclei during brief micrococcal nuclease digestions of rabbit thymus and hen oviduct nuclei, i.e., they appear in the 1SF supernatant fraction described by Bloom and Anderson (10) as being enriched in transcribed sequences. This finding supports the notion that HMG14 and HMG17 are associated with transcribed sequences.

MATERIALS AND METHODS

(a) Fractionation of rabbit thymus micrococcal nuclease digests by precipitation with 0.15 M NaCl:

Rabbit thymus nuclei were prepared and digested with micrococcal nuclease as described previously (6,7) to give approximately 5% acid-soluble nucleotides. The reaction was stopped by the addition of EDTA to a final concentration of 2mM. The solution was centrifuged at 30,000 g. for 50 min. The supernatant containing the solubilised nucleosomes and polynucleosomes was then dialysed overnight versus 0.15 M NaCl, 1mM EDTA, 0.5 mM PMSF (pH 7.6). Precipitated polynucleosomes and most of the H1-containing nucleosomes were pelleted by centrifugation at 30,000 g. for 40 min. The supernatant was made 1% SDS and extracted with phenol as described previously (7). Similarly the pellet was resuspended in 1% SDS and phenol extracted. Protein was recovered from the phenol phase by acetone precipitation. DNA was recovered from the aqueous phase by ethanol precipitation.

(b) Isolation of the 0.1 M NaCl-soluble fraction by the method of Levy and Dixon (1):

Rabbit thymus nuclei were prepared (7), and suspended at 10 mg/ml DNA in digestion buffer. Nuclei were digested for 15 min. at 37^{o} with 150 units per ml of micrococcal nuclease to give a 4% acid-soluble nucleotides digestion. The nuclei were pelleted, resuspended in two volumes of 2 mM EDTA (pH 7.6), and centrifuged at 12,000 g. for 20 min. to give a pellet (P2) and a supernatant. The supernatant was made 0.1 M NaCl by the addition of 4 M NaCl. After stirring for 1 h. the precipitated polynucleosomes and H1-containing monomers were removed by centrifugation at 12,000 g. for 20 min. The material in the supernatant was precipitated with two volumes of ethanol and the protein and DNA isolated from it by phenol extraction as described above.

(c) Fractionation of 0.1 M NaCl-soluble fraction by polyacrylamide gel electrophoresis:

Rabbit thymus nuclei were digested with micrococcal nuclease to give 2% and 4% acid-soluble nucleotides. The 0.1 M NaCl fractionation scheme described in the preceding section (b) was carried out and the 0.1 M NaCl supernatant collected. This was dialysed versus 2 mM triethanolamine (TEA)-HCl, 2mM EDTA, 10% glycerol, 0.5 mM PMSF (pH 7.6). The solution containing about 2 mg DNA was then loaded onto a 5% polyacrylamide gel and

electrophoresis carried out as described previously (7). The monomer nucleosome bands MN1 and MN2, visualised by ethidium bromide staining, were cut out of the gel and the DNA and protein extracted as described previously (7). The DNA was freed of acrylamide by hydroxyapatite chromatography (11).

(d) Isolation of the proteins from the 1SF supernatant fraction (10):

Rabbit thymus nuclei were digested with micrococcal nuclease (2% DNA rendered acid-soluble) and the nuclei pelleted by centrifugation at 2,000 g. for 10 min. The supernatant (1SF) was collected, made 1% SDS and phenol extracted. The protein was recovered from the phenol phase by acetone precipitation. The same procedure was carried out on nuclei prepared from oviducts of laying hens as described by Bloom and Anderson (10). The nuclei (2 mg/ml DNA) were digested for 4 min. with micrococcal nuclease (150 units/ml) giving a 4% digestion. The nuclei were pelleted and the proteins in the 1SF supernatant extracted with phenol.

(e) Preparation of hen oviduct nucleosomes enriched in ovalbumin sequences (10):

Oviduct nuclei (7 mg DNA) were prepared (10) and digested with micrococcal nuclease (150 units/ml) for 1.5 min. giving a 4% digestion. After centrifugation (90,000 g., 5 min.) the 1SF supernatant was made 10 mM EDTA pH 7.6, 0.5 mM PMSF, dialysed versus 10 mM TEA, 2 mM EDTA, 0.5 mM PMSF (pH 7.6), and then clarified by centrifugation (90,000 g., 30 min.). The supernatant containing 0.8 mg DNA was concentrated by Amicon ultrafiltration and loaded onto a 20 ml 5 - 20% sucrose gradient containing the TEA - EDTA - PMSF buffer. Centrifugation was carried out for 22 h. at 90,000 g. Protein and DNA was isolated from the monomer nucleosome peak (shaded area, Fig. 9) and from the material at the top of the gradient by phenol extraction.

(f) Electrophoretic analyses of DNA and protein:

DNA was analysed on 5% polyacrylamide gels as described previously (6) with Hae III restriction fragments of SV40 or PM2 DNA.

For qualitative purposes protein was analysed on SDS slab gels (7) or acetic acid-urea slab gels (12). To quantify proteins, protein mixtures were analysed as described previously (13) by electrophoresis on acetic acid 20% polyacrylamide tube gels followed by staining with Procion navy. Gels were scanned and peak areas determined by weighing the paper cut-outs.

(g) DNA analysis:

For rabbit thymus experiments DNA was measured by its U.V. absorption at 260 nm. Total DNA in 1 N NaOH has an extinction coefficient of 26.5 litres $cm^{-1} g^{-1}$. PCA-soluble DNA has an extinction coefficient of 33.9 litres $cm^{-1} g^{-1}$. In the oviduct experiments DNA was measured by the indole method (13 or 14).

RESULTS

Rabbit thymus salt-soluble nucleosomes:

Nucleosomes and polynucleosomes produced by micrococcal nuclease digestion followed by solubilisation with low-ionic-strength-EDTA buffers can be fractionated into two populations by the addition of 0.15 M NaCl; polynucleosomes and H1-containing monomer nucleosomes are precipitated with 0.15 M NaCl, whilst monomer nucleosomes lacking H1 (i.e., core particles) remain insolution (15,16). An examination of the HMG content of these two fractions (Fig 1) from rabbit thymus nuclei shows an enrichment relative to the histones of the four HMG proteins HMG1, 2, 14 and 17 in the salt-soluble fraction. However, much of the HMG1 and 2 may not actually be bound to the DNA in this fraction since if it is passed down a Bio-gel A-5m column the monomer peak contains HMG14 and 17 plus four core histones and only small amounts of HMG1 and 2 (not shown). In a control experiment with no nuclease digestion essentially only HMG1 and 2 (about half of the total HMG1 and 2) were found to be released from the nuclei into the 0.15 M NaCl supernatant (not shown). This enrichment of HMG proteins in the salt-soluble fraction has also been found by Levy et al (1,8) for the HMG protein H6 in trout testis chromatin (H6 in fish being very similar to HMG 14 and 17 in mammals and birds) and they have extensively characterised this fraction. In particular, they have found that the 140 base pair DNA in the salt-soluble fraction is 7 - 9 fold enriched in transcribed sequences which implies that H6 may be specifically associated with transcribed DNA sequences. We have analysed this salt-soluble fraction from rabbit thymus in some detail and in the following experiments we have followed the methods of Levy and Dixon (1) as closely as possible.

First of all we wished to measure the factor of enrichment of HMG14 and 17 in the salt-soluble fraction. We have done this by measuring HMG14 and 17 relative to the core histones in rabbit thymus nuclei and in the salt-soluble fraction by scanning Procion navy stained polyacrylamide gels of the total phenol-extracted protein from the two. Since HMG14 and 17

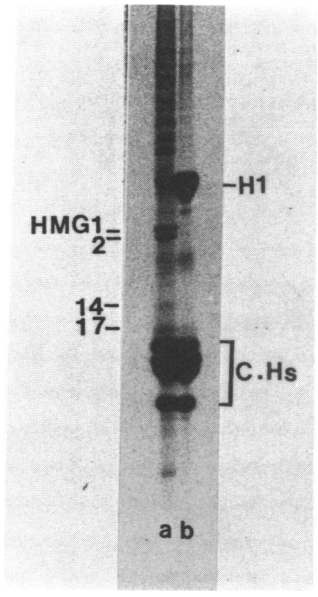

Figure 1

SDS-polyacrylamide gel electrophoresis of total proteins from (a) 0.15 M NaCl-soluble fraction. (b) 0.15 M NaCl precipitated fraction. The four core histones are abbreviated C.Hs.

constitute about only 0.3 - 1% of the total chromosomal protein, two loadings onto the gels were employed, one high (200 - 300 µg) in order to measure HMG14 and 17, and one low (20 - 50 µg) to measure the core histones. It should be pointed out that we have used Procion navy previously for quantifying histones and the stain uptake varies linearly with protein loaded in the range that we are working (13). Also the method was found to be very reproducible; for example, the HMG17/core histone ratio in total rabbit thymus nuclei protein was measured several times on different occasions and all gave a figure of about 1:100. HMG14 in the total chromatin protein is more difficult to measure since it is present in smaller quantities than HMG17 and it does not always completely separate from H4 (See Figs. 2A and 6B). Nevertheless, reasonably consistent values of about 0.3:100 were obtained for the HMG14/core histone ratio (Table 1).

The polyacrylamide gel electrophoretic analysis of the proteins of the salt-soluble fraction (prepared by the method of Levy and Dixon(1)), and of the total thymus nuclear proteins are shown in Fig. 2. From the scans

Table 1 : Quantitative analyses of HMG14 and HMG17 in various rabbit thymus nucleosomal fractions

	$\frac{\text{HMG14}}{\text{Core Histone}} \times 100\%$	HMG14 Enrichment	$\frac{\text{HMG17}}{\text{Core Histone}} \times 100\%$	HMG17 Enrichment
A. Total thymus nuclei	0.3 ($\pm$ 0.06)*	-	1.0 ($\pm$ 0.1)	-
B. Salt-soluble fraction (4% digestion)	1.2 ($\pm$ 0.3)	4.0	2.2 ($\pm$ 0.5)	2.2
C. Salt-soluble MN2 monomers from DNP gel (4% digestion)	2.5 ($\pm$ .005)	8.3	5.9 ($\pm$ 0.3)	5.9
D. Salt-soluble MN2 monomers from DNP gel (2% digestion)	3.3	11.0	6.1	6.1
E. 1 SF supernatant nucleosomes (2% digestion)	1.2	4.0	3.6	3.6

* Where values are given with standard deviations in parentheses these were obtained from three independent determinations.

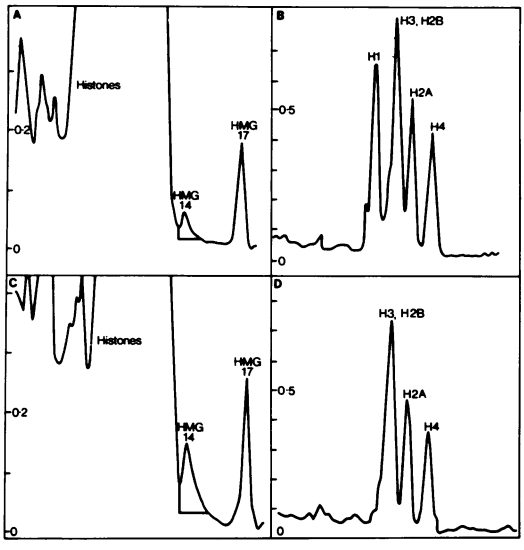

Figure 2

Scans of acetic acid polyacrylamide gel electrophoretic analyses of total phenol extracted protein from rabbit thymus nuclei (A, 300 µg; B, 30 µg) and from 0.1 M NaCl-soluble fraction prepared by the method of Levy and Dixon (1) (C, 250 µg; D, 25 µg). The ordinates give the absorption at 580 nm of the Procion navy stained gels. Migration was from left to right.

of these gels the quantities of HMG14 and 17 relative to the core histone were determined for the two samples (Table 1, rows A and B). It can be seen that the salt-soluble fraction has 2 - 3 times more HMG17 (relative to the histones) than total chromatin. The approximate enrichment of HMG14 appears to be somewhat higher (four-fold). We have carried out one similar experiment on calf liver, the nuclei of which we find to have about three times the level of in vitro RNA synthesis as rabbit thymus nuclei (unpublished results). A comparison of the HMG17/core histone ratios in the 0.1 M NaCl-soluble fraction and the 0.1 M salt-insoluble fraction from a 2 % nuclease digest gave an enrichment factor of 6.6 for HMG17 in the salt-soluble fraction (gel scans not shown). Thus the enrichment of HMG proteins in monomer nucleosome fractions may depend on levels of RNA synthesis.

In order to characterise this HMG14- and 17- enriched fraction in more detail, we have carried out polyacrylamide gel electrophoresis of the thymus salt-soluble nucleosomes. As described in our previous paper (7), when a total nuclease digest of rabbit thymus nuclei is separated electrophoretically on a preparative gel the monomers separate basically into two main bands, MN1 and MN2. This is shown diagramatically in Fig. 3A. MN1 is the core particle of 145 base pairs of DNA associated with the four core histones H2A, H2B, H3 and H4. MN2 is heterogenous (two bands are often seen running close together in this part of the gel), and larger, having 160 - 170 base pairs of DNA combined with all five histones and it also has HMG14 and 17 and

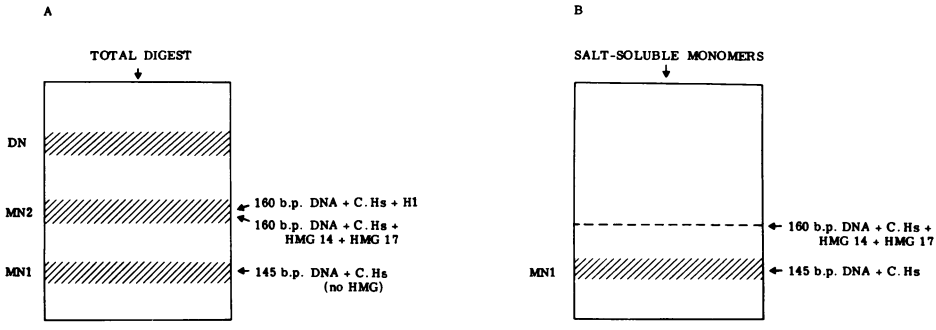

Figure 3

Diagrams showing the electrophoretic separation of nucleosomes from (A) a total rabbit thymus nuclear digest (see Figure 6 of ref (7)) and (B) 0.1 M NaCl-soluble nucleosomes. Core histones are abbreviated C.Hs.

variable amounts of HMG1 and 2. If, instead of loading the total digest, the salt-soluble material of Levy and Dixon is loaded onto the gel, one strong MN1 band and a fainter band behind in approximately the MN2 position are seen (Fig. 3B). These two bands were cut out and the protein (Fig. 4) and DNA (Fig 5) analysed. It can be seen that MN1 again is composed of 145 base pairs of DNA and just the four core histones. The minor, MN2, band now contains 160 base pairs of DNA complexed with the four core histones and increased amounts of HMG14 and 17, but contains no H1. Small amounts of HMG1 and 2 are also to be seen (Fig. 4, b,g), and also a band running just ahead of HMG2 which could be A24 or micrococcal nuclease since these run in approximately this position. Thus, the salt precipitation has removed the H1-containing nucleosomes. This experiment demonstrates that the salt-soluble material is basically composed of two types of particle; a core particle of 145 base pairs of DNA bound to the core histones and having no (or little) HMG proteins and a 160 base pair particle with the four core histones plus HMG14 and HMG17 (and some HMG1 and 2). The quantitative

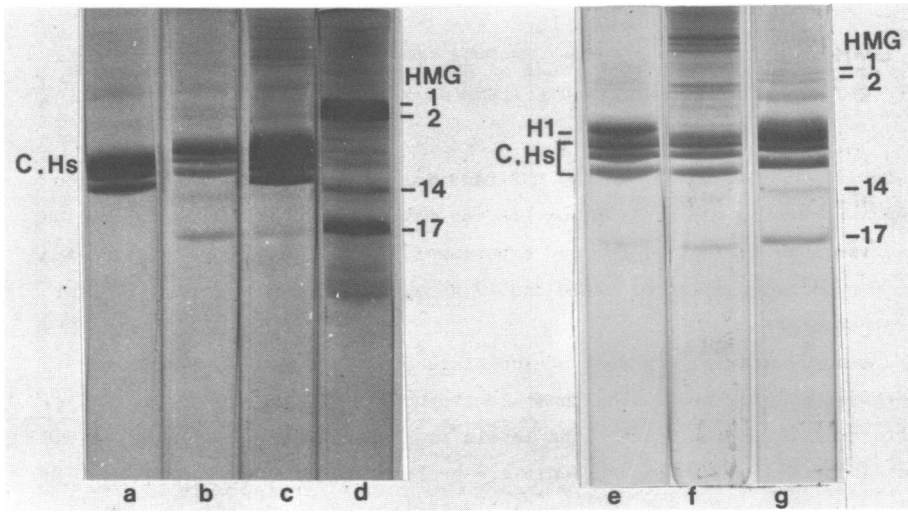

Figure 4

Acetic acid polyacrylamide gels of:-
(a) Total protein from MN1 band of Figure 3B
(b), (g) Total protein from MN2 band of Figure 3B
(c), (e) Total rabbit thymus nuclear protein
(d) Calf thymus HMG proteins
(f) Total protein from 1SF supernatant fraction from rabbit thymus

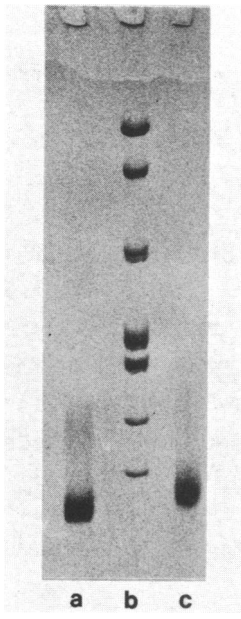

Figure 5

Polyacrylamide gel electrophoresis of DNA from (a) MN1 band of Figure 3B, (b) SV40 Hae III fragments, and (c) MN2 band of Figure 4B.

analysis of HMG14 and 17 in the MN2 band following the salt fractionation step was carried out by scanning Procion navy stained gels (Fig.6) and the results given in Table 1 for two experiments (rows C and D). This shows the further enrichment of HMG14 and 17 as a result of the preparative gel electrophoresis.

A more detailed analysis of the histones of the two salt-soluble nucleosomes using two dimensional electrophoresis (Fig. 7) revealed no differences. In particular, the levels of H4 acetylation appear to be the same and also the histone variants are present in the same quantities. The pattern of histones seen in these two gels is exactly the same as the pattern seen for total rabbit thymus nuclear histone (not shown).

It is, thus, apparent that when the salt-fractionation procedure of Levy et al (1,8) for isolating nucleosomes enriched in transcribed sequences is applied to thymus it gives at least two types of nucleosomes in the salt-soluble fraction, one containing HMG14 and 17 and the other having no (or very little) HMG protein. These nucleosomes in the 0.1 - 0.15 M NaCl super-

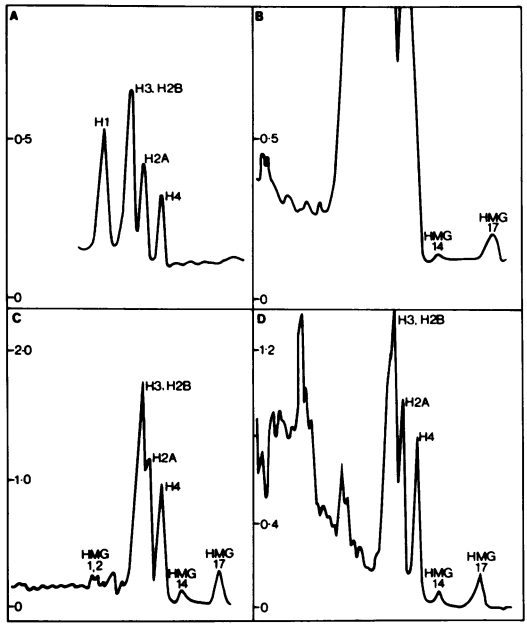

Figure 6

Scans of acetic acid polyacrylamide gels of total phenol-extracted thymus nuclear protein (A, 20 µg; B, 200 µg), protein from MN2 band of Figure 3B (C) and protein from 1SF fraction (D). Ordinates give absorption at 580 nm. Migration from left to right.

natant remain soluble even when divalent metal ions are present (data not shown). As a consequence of this solubility in divalent metal ion solutions, some of these nucleosomes are released from nuclei during the digestion with micrococcal nuclease.* Thus, when thymus nuclei are briefly digested with micrococcal nuclease and then centrifuged down, the resulting supernatant (1SF) is found to be enriched in HMG14 and 17 (Fig. 4f). The quantitative analysis of the proteins associated with the material released from nuclei (Fig. 6D) again reveals enrichments of HMG17 and HMG14 (3 - 4 fold) (Table 1, row E). This first supernatant fraction corresponds to the 1SF fraction described by Bloom and Anderson (10) which was found by these workers to contain nucleosomes enriched 5 - 6 fold in ovalbumin sequences when hen oviduct nuclei are digested briefly with micrococcal nuclease. When the proteins from the 1SF fraction from oviduct nuclei were analysed (Fig, 8)

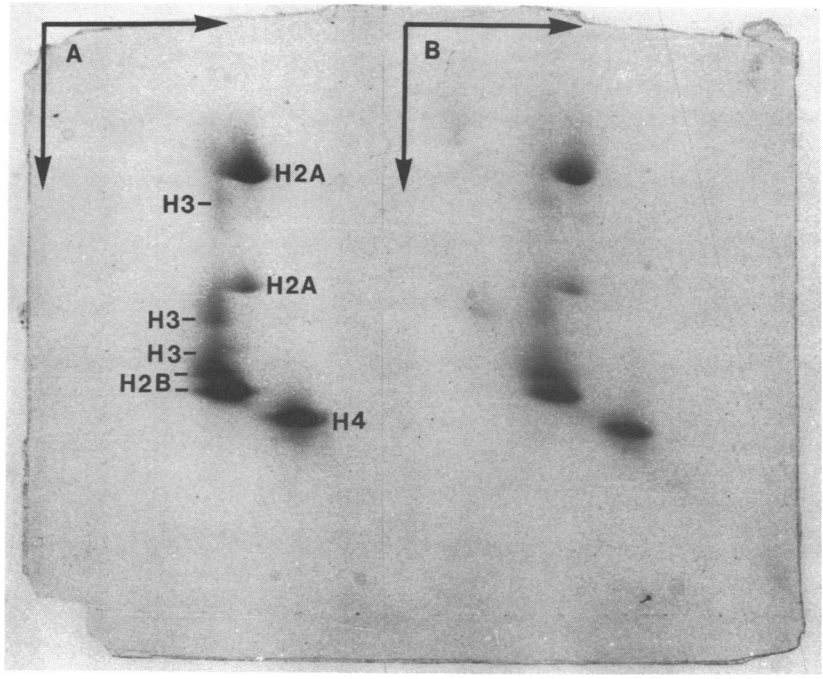

Figure 7

Two-dimensional gel electrophoresis of the proteins from (A) the MN1 and (B) the MN2 band of Figure 3B. First dimension (left to right) is in acetic acid-urea (12); the second dimension (top to bottom) is acetic acid-urea (6 M) plus 6 mM Triton X-100.

one again sees enriched quantities of HMG14 and HMG17 relative to the core histones. A quantitative analysis like those carried out for rabbit thymus nucleosomes showed that the enrichment of HMG14 was 3.5 and HMG17 was 2.1. We have isolated the monomer nucleosomes in the 1SF fraction from oviduct nuclei by sucrose gradient centrifugation (Fig. 9) and have analysed the DNA (Fig. 10) and protein (Fig. 11). It can be seen that these monomers have 150 - 160 base pairs of DNA combined with the four core histones, HMG14 and 17, and a number of non-histone proteins running behind the histones. A doublet of proteins in the HMG1 and 2 position are also seen. The protein running between HMG1 and 2 and histone H3 is not histone H1 and is probably micrococcal nuclease or A24. No HMG protein was detected in the material at the top of the sucrose gradient.

Since H1 was incompletely separated from the core histones in the

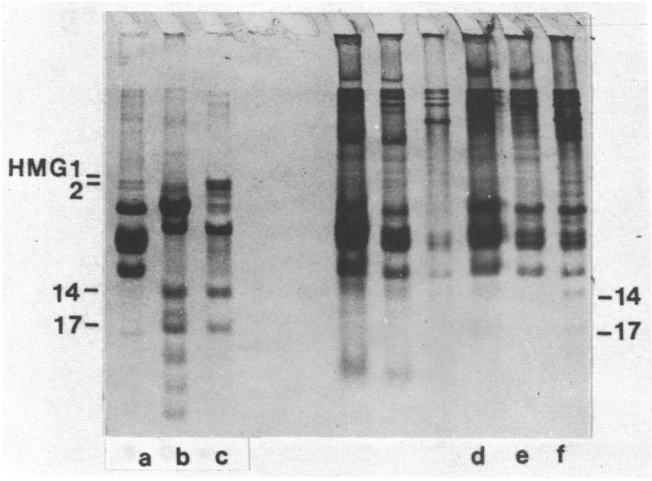

Figure 8

Acetic acid-urea gel electrophoresis of:-
(a) Rabbit thymus whole histone
(b) Hen oviduct total PCA extract
(c) Chicken erythrocyte HMG (which includes some H5)
(d) Total hen oviduct nuclear protein
(e) Same as (d) but $^{1}/_{5}$th the loading
(f) Hen oviduct 1SF proteins
Samples loaded in the other slots are not relevant to the paper.

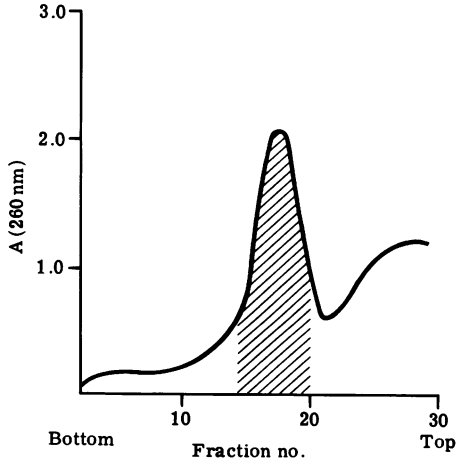

Figure 9

Sucrose gradient fractionation of the hen oviduct 1SF fraction. The hatched area is the monomer nucleosomes.

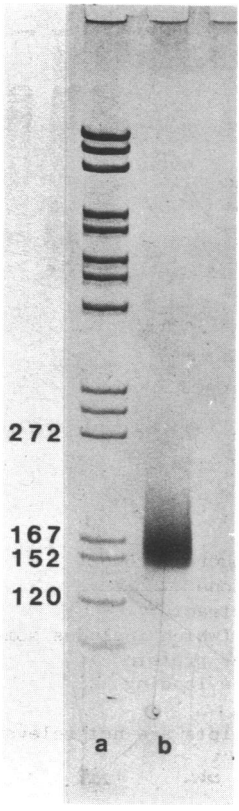

Figure 10

Polyacrylamide gel electrophoresis of (a) PM2 Hae III fragments, (b) DNA from the nucleosomes from the sucrose gradient of Figure 9 (hatched area).

total oviduct nuclear proteins (Fig. 11A) on these particular gels, it was not possible to measure the HMG14 and HMG17/core histone ratios with any accuracy. Instead, the quantities of HMG14 and 17 in the total oviduct nuclear protein and the 1SF monomers were measured relative to histone H4. There are proteins running on either side of HMG14 making the measurement of HMG14 in the total nuclear protein difficult, but from the hatched peak areas in Fig. 11A and 11B one obtains an approximate enrichment of 2.5 for HMG14 and 1.6 for HMG17 in the 1SF monomers.

Presumably the 1SF nucleosomes contain MN1 nucleosomes (core particles) as well as HMG-containing nucleosomes but attempts to fractionate 1SF

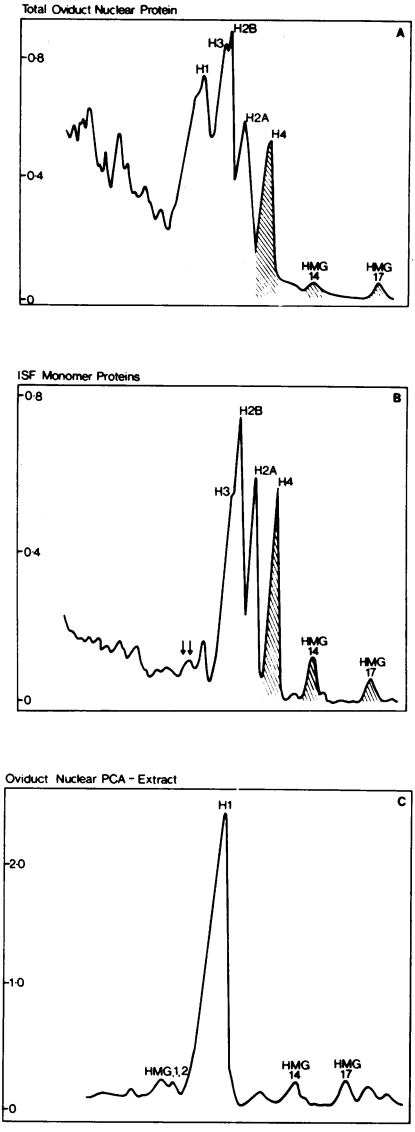

Figure 11

Scans of acetic acid polyacrylamide gels of (A) total phenol extracted oviduct nuclear protein, (B) protein from the 1SF monomer nucleosomes of Figure 9 (hatched area), and (C) total PCA-extracted protein from oviduct nuclei. The pair of arrows on B indicates a doublet running in about the HMG1 and 2 position. Ordinates give absorption at 580 nm. Migration from left to right.

nucleosomes on polyacrylamide gels were unsuccessful due to limited amounts of materials and aggregation of nucleosomes, and we therefore cannot confirm this.

DISCUSSION

The results of this paper show that HMG14 and HMG17 can be found associated with nucleosomes lacking H1 and thus confirms the results of Levy et al (1,8) that H6-containing nucleosomes in trout testis do not have H1. As a result of this absence of H1, HMG14- and HMG17- containing nucleosomes are found in the 0.1 - 0.15 M NaCl supernatant fraction and also are released into the incubation medium during nuclease digestions, appearing in the 1SF supernatant fraction. In the investigation of rabbit thymus nucleosomes by preparative gel electrophoresis we have found that, after removal of the H1-containing nucleosomes by 0.1 M NaCl precipitation, the monomer nucleosomes running in the MN2 position of the gel are composed of 160 base pairs of DNA, the four core histones, increased amounts of HMG14 and HMG17, and small amounts of HMG1 and 2. Since H1 is missing from these MN2 nucleosomes and since we have previously (7) presented evidence that HMG14 and 17 are bound to a nuclease resistant part of the nucleosome (leading us to suggest HMG14 and 17 are bound to core particles), the results of this paper could be interpreted as indicating that core particles with HMG14 and 17 bound are larger, having 160 base pairs of DNA rather than the normal 145 base pairs. Alternatively, it is possible that HMG14 and 17 are bound to a normal 145 base pair core particle but the extra 15 base pairs of DNA is linker DNA which has been protected from nuclease attack by some other proteins (e.g., HMG1 and 2) but these proteins have been subsequently stripped off by the exposure to 0.1 M NaCl and by the electrophoresis. The salt-soluble MN2 particles do have small amounts of HMG1 and 2 which could have been protecting this linker. The small quantity of HMG1 and 2 remaining on these particles may be explained by the fact that much of the HMG1 and 2 is very loosely bound within the nucleus (7) and is easily washed off with salt. A third possibility is that HMG14 and 17 are interacting with the 15 base pair DNA contiguous to the 145 base pairs of the core particles (i.e., replacing H1 on the linker) but the HMG14- and 17- bound linkers are more resistant to attack than H1 linkers. We note in this connection that Todd and Garrard (18) have described a particle (M11) which lacks H1 but appears to have a strong nuclease resistant barrier at 160 base pairs of DNA. It is, therefore, possible that these M11 nucleosomes are the HMG14-

and/or HMG17- containing nucleosomes. Further work is required to distinguish between these possibilities but the third explanation (that HMG14 and HMG17 are bound to the 15 base pair linker DNA) is the most attractive especially in view of the similarities between HMG14, HMG17 and H1. We have pointed out before the resemblances of these three proteins (3). Firstly, they are all rich in lysine, alanine and proline. Secondly, the basic N-terminal two-thirds of HMG14 and 17 have regions of sequence homology (19,20) with parts of the N-terminal and C-terminal of H1 (i.e., the random coil 'nose' and 'tail' regions of H1 (21)). Thirdly, HMG14 and HMG17 are random coil proteins in solution like the 'nose' and 'tail' regions of H1. Thus, HMG14 and 17 might be considered to be an H1 molecule minus the globular central region. Bakayev et al (22) have also found HMG-like proteins in the MN2 (and MN3) region of the gel electrophoresis of nucleosomes and have also interpreted this as indicating that HMG proteins are associated with linkers, though the presence of H1-nucleosomes in the MN2/MN3 region made this interpretation less clear. We have previously shown that the N-terminal basic regions of HMG14 and 17 can bind to DNA (23,24). The model we therefore propose is one in which the N-terminal basic regions of HMG14 and HMG17 are bound to the 15 base pair DNA immediately contiguous to the 145 base pairs of the core particle. Also, since we have previously found that HMG14 and 17 still remain attached to extensively digested nucleosomes (i.e., core particles), we also propose that the acidic C-terminals of HMG14 and HMG17 are interacting with the core particle. This could explain how HMG14 and HMG17 confer DNAse I sensitivity on the nucleosomes of transcribed genes (2). The replacement of H1 by HMG14 and HMG17 in specific regions of the genome also explains how in the reconstitution experiments of Weisbrod and Weintraub (2) the HMG14 and HMG17 proteins were able to reassociate with 0.35 M NaCl washed chromatin in a specific manner, regenerating the DNAse I sensitivity of the globin gene. In this experiment 0.35 M NaCl would have extracted HMG14 and HMG17 exposing their linker DNA but leaving the H1 linkers covered with H1. In the reconstitution the HMG14 and 17 would then simply recognise the exposed linker DNA and bind back to it, thus making it unnecessary to invoke specific receptors (2) for binding the HMG proteins.

In this paper we have shown that, as a result of the absence of H1, HMG14- and HMG17- containing nucleosomes are found in the 0.1 M NaCl-soluble fraction of Levy and Dixon (1) and in the 1SF supernatant fraction of Bloom and Anderson (10). Both these fractions contain variable amounts of HMG1 and HMG2 bound to the nucleosomes also. Both these nucleosome fractions have

been found to be enriched in transcribed sequences and therefore, since we find enrichments of HMG14 (2.5 - 4 fold) and HMG17 (1.6 - 6.6 fold) in both fractions, it does suggest that HMG14 and HMG17 are specifically associated with transcribed sequences, confirming the results of Levy and Dixon (1) and Weisbrod and Weintraub (2). A rough calculation shows that there are just about sufficient molecules of HMG14 and HMG17 to account for the DNAse I sensitivity of most of the transcribed genes in a particular tissue. There are approximately 3 x 10^7 nucleosomes per mammalian nucleus (assuming all the DNA is in nucleosomal structures). If 10% of the chromatin is transcriptionally active then there are about 3 x 10^6 'transcriptionally active' nucleosomes. From yields of proteins HMG14 and HMG17 from preparations of these proteins there are about 10^6 molecules of each per cell nucleus and thus there is almost sufficient HMG14 and HMG17 for there to be one molecule of HMG14 or HMG17 per transcribed nucleosome. However, our yields may be underestimates and there may be more per nucleosome. The N-terminal basic regions of HMG14 (residues 1 - 61) and HMG17 (residues 1 - 58) (19, 20) are only about half the length of the basic regions of H1 (residues 19 - 35 and 118 - 213)(25). Thus, since H1 is, like HMG14 and 17, protecting 15 - 20 base pairs of DNA (24) then it would seem more likely that there are two molecules of HMG14(or 17) per nucleosome, each protecting 7 - 10 base pairs of DNA. Simpson has shown that the H1-protected 15 - 20 base pairs of DNA are probably composed of two lengths, each about ten base pairs long, on each end of the 160 base pair 'chromatosome'(26). Likewise, we suggest that the HMG14 and 17- containing nucleosomes have an analagous structure in which, instead of having one H1 molecule protecting the two ends of the 160 base pair particle, there are two molecules of HMG14 (or 17), one for each 7 - 10 base pairs length of DNA at the two ends.

From the results of this paper and other workers (1,2,22) the most satisfactory description of transcriptionally active chromatin is one in which HMG14 and HMG17 are specifically associated with this region of the chromatin, the H1 on the linker DNA being replaced by the binding of the N-terminal basic regions of HMG14 and HMG17. Since the packing of the bulk of the nucleosomes in chromatin into the 200 - 300 Å solenoid structure (28,29) is considered to be brought about by interactions between H1 molecules on neighbouring nucleosomes (the interaction being presumably between the globular "head" regions of the H1 molecules (30)) it is apparent that this replacement of H1 by HMG14 and HMG17 on transcribed genes could result in the local unfolding of the solenoid to a less compact form to facilitate trans-

cription. Thus a gene with say 20-40 nucleosomes bound on to it may loop out as a 100 Å "beads-on-a-string" nucleofilament. Also, since there is no H1 spanning the nucleosome (26) holding the two ends of the nucleosomal DNA together, the nucleosome itself can unfold more easily to again facilitate transcription. Transient acetylation of H3 and H4 may also be obligatory for transcription to take place. However, we should like to stress that the model presented may be too simple a picture for there are a number of other facts which have to be taken into account, some of which are not entirely consistent with the model outlined. These are:

(a) We have been unable to show in previous experiments a specific association of HMG14 and 17 proteins with DNAse I sensitive regions (9). Thus, HMG proteins may also be associated with non-transcribed sequences.

(b) Levy and Dixon (27) have shown that H1-containing monomer nucleosomes, which are insoluble in 0.1 M NaCl, are enriched in transcribed sequences but that these nucleosomes are devoid of HMG proteins (8).

(c) In addition to the HMG14 and 17- containing nucleosomes, the 0.1 M NaCl soluble fraction contains core particles (MN1) which have no HMG proteins. These are produced even at the earliest stages of digestion (2%) and are probably derived from a class of nucleosomes having a small linker and no H1 (17,18). Are these nucleosomes also associated with transcribed sequences?

(d) Villeponteaux et al (30) have shown that extraction of H1 and H5 from erythrocyte nuclei with 0.6 M NaCl (pH3) does not destroy the higher DNAse I sensitivity of the globin gene. This extraction procedure would probably have removed HMG proteins also.

The location of HMG1 and HMG2 (or HMG-T) in chromatin is still not clear. Levy et al (8) have suggested that HMG-T is bound to a very nuclease-sensitive linker DNA. We have no evidence in thymus that this is so (7). We have postulated that there are two populations of HMG1 and 2; one population loosely bound within the nucleus, the other more firmly bound to the nucleosomes. This second population is found in the MN2 region of the gel electrophoresis (7) and may therefore, like HMG14 and HMG17, be bound to the relatively nuclease resistant linker DNA immediately adjoining the core particle. Although there is no evidence from the results of this paper that HMG1 and 2 are specifically bound to transcribed sequences, it may be significant that tissues with low RNA synthesis rates (thymus and erythrocytes) appear to have more HMG1 and 2 than tissues with high transcriptional rates (e.g. rat liver and hen oviduct). (See for example slots

a, b and c of Figure 8.) It is therefore possible that the concentration of HMG1 and 2 in the cell nucleus plays a role in modulating RNA synthesis levels.

ABBREVIATIONS

HMG : High Mobility Group
C. Hs : Core Histones, H2A, H2B, H3, H4
SDS : Sodium dodecylsulphate
PMSF : Phenylmethyl sulphonylfluoride
TEA : Triethanolamine
PCA : Perchloric Acid
DNP : Deoxyribonucleoprotein

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