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Multiacetylated forms of H4 are found in a putative transcriptionally competent chromatin fraction from trout testis

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ABSTRACT

We have examined the distribution of acetylated histones derived from various trout testis chromatin fractions of different composition. Our results indicate that a chromatin fraction, preferentially solubilized by micrococcal nuclease, containing the bulk of the HMG proteins and similar to a fraction released from intact trout nuclei and previously shown to be enriched in transcribed DNA sequences also possesses high levels of multiacetylated species of H4. Histones 2A, 2B and 3 are also acetylated in this particular chromatin fraction. Monoacetylated species of the 4 inner nucleosomal histones appear to be characteristic of the nucleohistone portion of trout testis chromatin.

INTRODUCTION

Acetylation of histones is a known post-translational modification of nucleosomes whose role in chromatin function remains obscure. Two distinct types of reation are seen: in the first, the N^2-amino group of the amino-terminal serine in histones H1, H2A and H4 is acetylated, an event that is closely coupled to the synthesis of the polypeptide chain. This amino terminal acetyl group does not turn over. In contrast, there is extensive N^6-acetylation of lysine residues in the amino-terminal regions of all four nucleosomal histones H2A, H2B, H3 and H4 in several tissues as well as cells in tissue culture (1-6). The acetylation occurs largely in the S phase, is rapid, at multiple sites, and the turnover of acetyl groups is also rapid. The extent of acetylation is greatest for histones H3, H4 and H2B and in the case of trout testis cells (5) four different lysines are acetylated in each of these histones (H4: lysines 5, 8, 12 and 16; H3: lysines 9, 14, 18 and 23, and H2B: lysines 5, 10, 13 and 19) H2A has only one site of acetylation, lysine 5, in trout testis cells.

Numerous correlations have been observed between an increase in histone acetylation and gene activation. For example, histone acetylation precedes increases in RNA synthesis in lymphocytes following stimulation by mitogens

(7), in target tissues following hormone administration (8) and in the liver following partial hepatectomy (9). The transcriptionally active macronucleus of *Tetrahymena pyriformis* contains acetylated histones, whereas the genetically repressed micronucleus does not (10). Recently, several groups have demonstrated that the nucleosome structure in highly acetylated chromatin is altered in a manner that renders these nucleosomes selectively sensitive to DNase I (11, 12, 13). It is a reasonable hypothesis that since acetylation of the lysine residues in the basic amino terminal regions of the histones neutralizes their positive charges, their interactions with the phosphate groups of the DNA strand that surrounds the nucleosome core would be weakened thus leading to a more open structure.

In recent studies we have demonstrated that regions of trout testis chromatin enriched in their content of HMG proteins and transcribed DNA sequences can be purified by virtue of their rapid cleavage and release as mononucleosomes by micrococcal nuclease (14, 15). In an attempt to elucidate the role of histone acetylation in chromatin structure and function we have examined the distribution of acetyl groups within putative transcriptionally competent and inert chromatin regions in trout testis chromatin.

MATERIALS AND METHODS

Incubation of trout testis cells with C^{14} acetate

50 grams (wet weight) of fresh trout testis were minced with scissors and gently homogenized by hand in 4 volumes of Hanks balanced salt incubation medium (16) in which the $NaHCO_3$ was replaced by 5 mM Tris. The homogenate was filtered through 2 layers of cheese cloth and diluted to a final volume of 250 ml. Cycloheximide was added to a final concentration of 0.2 mM and the cell suspension was incubated with 5 mCi of ^{14}C sodium acetate (New England Nuclear) at 15°C for 1 hour. Following incubation, the cell suspension was frozen in dry ice.

Preparation of chromatin and nuclease digestions

Chromatin was prepared from the cells by a procedure described earlier (17), involving homogenization of the cells in a NaCl-EDTA solution and recovery of the chromatin pellet by centrifugation. The chromatin pellet was washed 3 times by gentle homogenization with RSB buffer (10 mM Tris pH 7.5; 10 mM NaCl; 3 mM $MgCl_2$) containing 1 mM Ca^{+2} followed by centrifugation at 100 x g in a Sorvall refrigerated centrifuge. The chromatin pellet was then resuspended in the RSB-Ca^{+2} buffer, at a concentration of 230 A_{260}/ml and incubated with 60 units/ml of micrococcal nuclease (Worthington) for 20 min. at

37°. The reaction was stopped by chilling the tube on ice followed by immediate centrifugation for 5 min. at 10,000 rpm in the HB-4 Sorvall rotor. The supernatant was designed S_1. The precipitate was resuspended in 1 mM EDTA (150 ml) by gentle homogenization and the soluble material recovered by centrifugation for 20 min at 10,000 rpm in the HB_4 rotor at 0°. The pellet, corresponding to the nucleoprotamine fraction, was designated P_2. The supernatant fraction (designated S_2) containing chromatin subunits was further fractionated into soluble and insoluble portions by the addition of NaCl to a final concentration of 0.1 M (14, 15). After 2 hr at 0°C the two fractions were separated by centrifugation at 10,000 rpm for 20 min in the HB-4 rotor. For the sake of simplicity and brevity, we shall design the fraction soluble in NaCl as cMN_1 and that insoluble in NaCl as cMN_2. These two fractions obtained by action of micrococcal nuclease on trout testis *chromatin* are not to be confused with MN_1 and MN_2 monosomes obtained when the same fractionation procedure is applied to intact trout testis nuclei (14, 15). A detailed characterization of each of the fractions that are obtained upon applying this fractionation procedure to chromatin will be the subject of another communication (Levy-Wilson and Dixon, manuscript in preparation).

Acid extraction of proteins and gel electrophoresis

Acid-soluble proteins were obtained from the various chromatin fractions by extraction with 0.2 M H_2SO_4 for 1 h at 0°, followed by centrifugation for 10 min at 10,000 rpm in the HB-4 Sorvall rotor. Proteins were recovered from the supernatant by precipitation with 2 volumes of ethanol for 16 h at -40°, the precipitate washed several times with ethanol and lyophilized. Electrophoretic analysis of the proteins was performed in acid urea polyacrylamide gels, containing 0.22% Triton X-100, as previously described (15).

Chromatography of basic proteins on Bio Gel P-60 columns

Protein samples, dissolved in 2 ml of 0.01 N HCl were layered onto the top of one of two columns (2 x 300 cm) connected in series and containing Bio Gel P-60 (100-200 mesh) equilibrated in 0.01 N HCl. The column was eluted with 0.01 N HCl at a flow rate of 50 ml/h. 5.0 ml fractions were collected. The absorbance of each fraction was monitored at 230 nm. An aliquot of 1.0 ml of every second fraction was counted in 10 mls of Oxifluor - H_2O oxidizer cocktail (New England Nuclear). Fractions comprising peaks of A and ^{14}C were pooled and lyophilized.

RESULTS

Distribution of C^{14} acetate among the various chromatin fractions

Trout testis chromatin from cells that had been labelled with ^{14}C-acetate *in vitro* as described in Methods, was digested with micrococcal nuclease to the extent of 24%, as determined by the A_{260} released into the first supernatant S_1 (see Methods). Subsequent treatment of the pellet fraction, P_1, with 1 mM EDTA released an additional 30% of the input A_{260} into the second supernatant, S_2. The remainder of the A_{260} corresponds to the nucleoprotamine fraction, P_2, which, in this particular batch of testis, accounted for 46% of the chromatin DNA. In Table I we summarize the distribution of ^{14}C acetate cpm and A_{260} obtained in the various fractions. Over 98% of the total ^{14}C acetate incorporated into chromatin were recovered in the nucleohistone fraction, sensitive to the nuclease (S_1 plus S_2) and only some 2% of the label remained bound to the insoluble nucleprotamine. Of the label bound to nucleohistone, 34% was in the first supernatant S_1 and 64% in the S_2 fraction. 75% of the label present in the S_2 fraction was recovered in the portion solubilized by 0.1 M NaCl and this fraction showed the highest specific activity (cMN1, Table I)

Electrophoretic analysis of the acid soluble proteins from the various chromatin fractions

In previous studies of a similar nature involving digestion of intact trout testis *nuclei* with micrococcal nuclease we had consistently found that, under the experimental conditions employed in our present studies, only 5-15% of the input A_{260} was released into the first supernatant, S_1 (14, 15). The S_1 fraction derived from nuclei is composed largely of very small DNA fragments (< 40 base pairs in length) together with the bulk of the HMG-T protein and variable amounts of H6 and ubiquitin depending on the batch of testis used (14, 15, 17, 18). Histones are *not* detectable in S_1 derived from nuclei. In view of the high percentage of A_{260} released into the fraction

TABLE I. Distribution of acetate groups in the various chromatin fractions.

Chromatin fraction	A_{260}	Total cpm ^{14}C - acetate	% A_{260}	%cpm	Specific activity (cpm/A_{260})
S_1	10870	100,000	23.6	34	9.20
S_2	14100	186,000	30.6	64	13.20
P_1	21030	5,000	45.7	1.7	0.24
cMN_1	8100	140,000	17.6	48	17.28
cMN_2	6000	46,000	13.04	16	7.67

S_1 obtained when the nuclease acted upon isolated *chromatin*, the complement of acid soluble proteins of each of the chromatin fractions obtained by the application of the procedure to chromatin was examined by electrophoresis in acid-urea-acrylamide gels containing 0.22% Triton X-100 (15). Fig. 1 (A and B) shows the protein patterns from fractions S_1 and S_2 run in two different gels for short (A) and long (B) times. In (A), all the proteins present in each fraction remain on the gel while in the second gel (Fig. 1B) run for a longer period of time, to allow a better separation of the histones, H6 and protamine have migrated

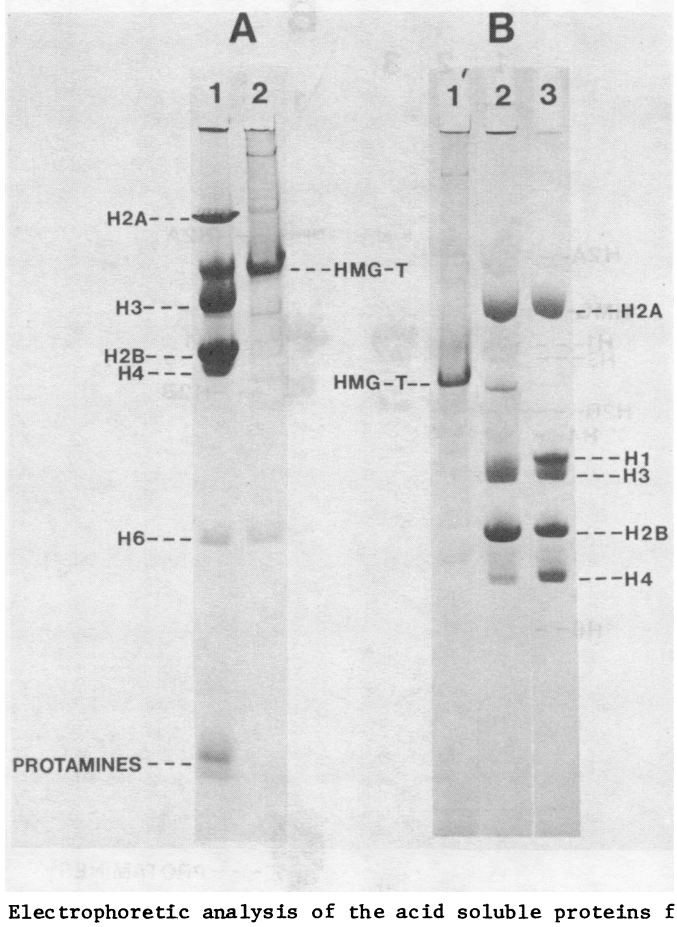

FIGURE 1: Electrophoretic analysis of the acid soluble proteins from fractions S_1 and S_2. In A, the gel was run for 20 hr at 300 volts; in B, a similar gel was run for 30 hr at 300 volts. Slots A1 and B1 display the proteins from fraction S_1 obtained from nuclei; slots A1 and B2 show the proteins from fraction S_1 derived from chromatin; slot B3 shows the proteins from fraction S_2 derived from chromatin.

off the gel. It is clear from Fig. 1 that fraction S_1 (A, slot 1; B, slot 2) contains, in addition to the major proportion of the HMG-T content of chromatin, the normal complement of histones H2A, H2B, H3 and H4 but lacks H1 which would normally migrate between H3 and HMG-T. S_1 also contains the bulk of the H6 content of chromatin and small amounts of protamine. Fraction S_2 contains the five histones (Fig. 1, slot B3; a trace of HMG-T and H6 can be seen in cMN_1 which is part of S_2, (Fig. 2, slot A1) and some protamine. We also examined the

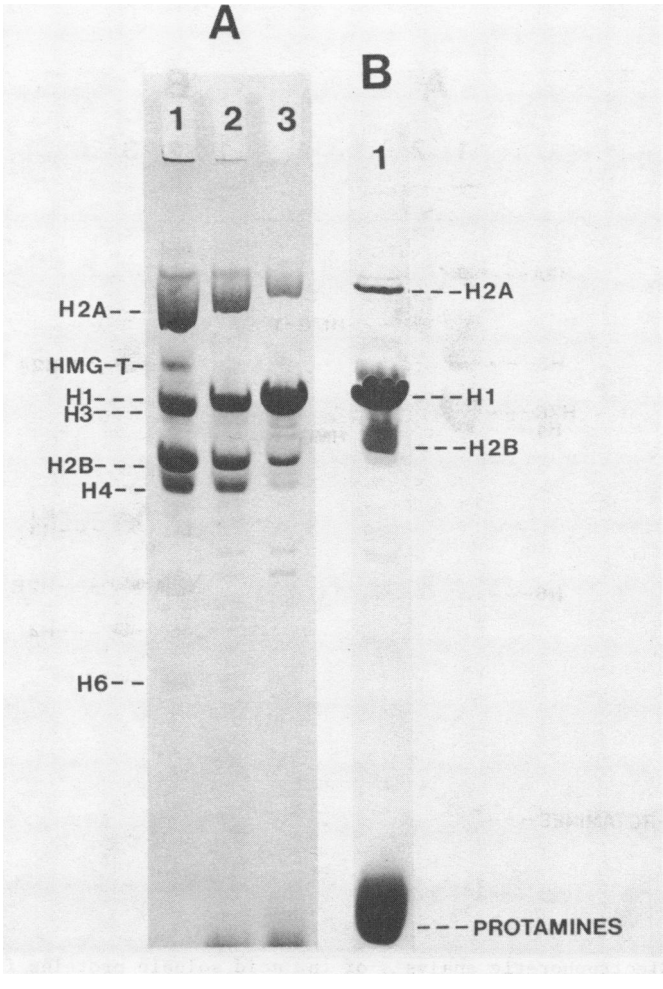

FIGURE 2: Electrophoretic analysis of acid soluble proteins from fractions cMN_1, cMN_2 and P_2, obtained from chromatin. Slot A1 displays the proteins from fraction cMN_1; Slot A2 displays the proteins from fraction cMN_2; Slots A3 and B1 display the proteins from fraction P_2.

proteins present in fractions cMN_1 (Fig. 2, slot A1) and cMN_2 (Fig. 2, slot A2) each of which contain the four inner histones. They differ in that cMN_1 fraction but not cMN_2 contains a small residue of HMG-T and H6. The nucleoprotamine fraction P_2 (Fig. 2, slots A3 and B1) consists mainly of protamine and H1 with smaller amounts of H2A and H2B and only traces of H3 and H4 as can be clearly seen when the sample is run for a longer period of time (data not shown). This is in agreement with previous data from our laboratory that demonstrates that H3 and H4 are the first two histones to be eliminated in the replacement of histone by protamines during the chromatin transformation process that occurs during testis development (29). An interesting observation which does not affect the significance in the interpretation of our present results emerges from the data in Fig. 2. It is apparent that histone H2A from fractions cMN_1, cMN_2 and P_2 (slots A1-3) has a different mobility in each case, being the fastest in cMN_1 and the slowest in P_2. It is likely that this behavior of H2A might be the result of differences in the levels of the poly-ADP-ribosylation (and perhaps also of phosphorylation) in H2A from the different fractions, since we have recently discovered that this histone is also modified by poly-ADP-ribosylation and that the bulk of the poly-ADP-ribosylation occurs in the condensed chromatin fraction (B. Levy-Wilson, unpublished results).

Bio Gel P-60 chromatography of the acid soluble proteins from fractions S_1, CMN_1 and CMN_2

Acetate-labelled testis histones were separated by chromatography on Bio Gel P-60 (19), in order to determine the extent of acetylation of individual histones and to compare the extent of acetylation in different chromatin fractions. Fig. 3 illustrates the profile obtained for fraction S_1. Although the resolution of the histones in the Bio Gel column illustrated in Fig. 3 was not as good as that obtained in Figs. 5 and 6, we observed that the acetate label was associated with a peak of A_{230} eluting at the void volume, which we labelled "X", with a broad peak in the general area of H2A, H2B and H3, and with a large peak associated with H4. Regions marked with ⊢——⊣, were pooled and the proteins in each pool identified by gel electrophoresis and comparison of the mobilities of each band with that of purified trout testis histone standards co-electrophoresed with the samples (data not shown). An absorbance peak (labelled X) eluting at the void volume of the column contained a significant fraction of the ^{14}C counts (Table II). When this material was electrophoresed in a Triton gel, the bulk of the sample remained at the origin and failed to show any histone bands. This material, eluting at the void volume may consist of either high molecular weight basic proteins or non-histone proteins of overall negative

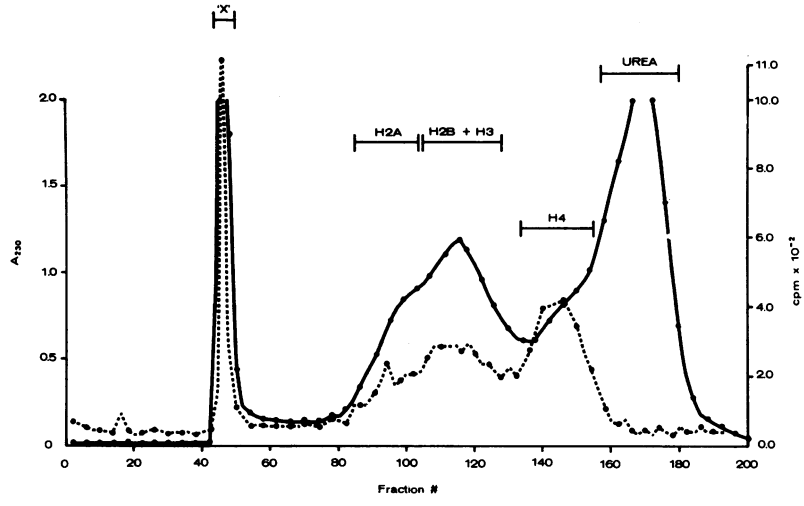

FIGURE 3: Bio Gel P-60 chromatography of the acid soluble proteins from fraction S_1 (●——●) A_{230}; (●----●) ^{14}C-acetate radioactivity.

charge unable to migrate in the gel system.

We wish to emphasize some features of the electrophoretic profile of the H4 peak derived from the column (Fig. 4). Multiple acetylated forms of H4 clearly resolved by electrophoresis are seen in this chromatin fraction S_1 which had been selectively solubilized by micrococcal nuclease and contained the bulk of the HMG-proteins, HMG-T and H6. There is an almost even distribution

TABLE II. Distribution of acetate groups among individual histone fractions derived from S_1

Protein Peak	Total A_{230} recovered	Total ^{14}C cpm recovered	Specific Activity (cpm/A_{230})
X	90	10,000	111
H3	64	10,600	165
H2A + H2B	90	14,900	165
H4	70	19,100	273

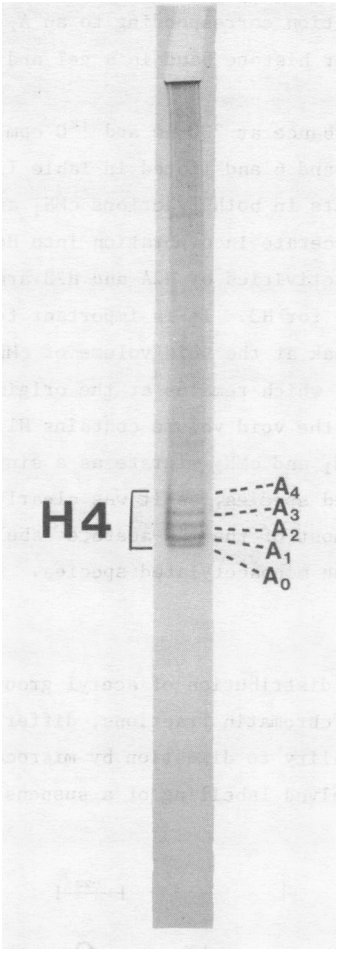

FIGURE 4: Triton-polyacrylamide gel electrophoresis of H4 from fraction S_1. Electrophoresis was performed for 30 hr at 300 volts as described in Methods. Migration is from top to bottom. A_0: non-acetylated parental H4; A_1: mono; A_2: di; A_3: tri, and A_4: tetra acetylated species of H4.

of stained protein in the mono-, di-, tri- and tetra-acetylated forms in Fig. 4. This result is consistent with the high level of acetate incorporation into H4 (Fig. 3) which leads to H4 showing the highest specific activity of acetate incorporation: 273 cpm/A_{230} (Table II). The Bio Gel P-60 profiles of the acid soluble proteins from fractions cMN_1 and cMN_2 were also compared (Figs. 5 and 6) and ^{14}C-acetate was seen to be associated with each of the histone fractions (20). Fractions including each peak of absorbance at 230 nm

were pooled and the purity of each histone fraction checked by electrophoresis on Triton gels. Each fraction corresponding to an A_{230} peak in Figs. 5 and 6 gave rise to a single major histone band in a gel and could thus be identified (data not shown).

The recovery of absorbance at 230 nm and ^{14}C cpm in each histone fraction was computed from Figs. 5 and 6 and listed in Table III. The H4 peak contains the highest number of counts in both fractions cMN_1 and cMN_2. Furthermore, the specific activity of acetate incorporation into H4 is the same in both fractions. The specific activities of H2A and H2B are higher in cMN_1 than in cMN_2; the opposite is true for H3. It is important to note that, as in the case of the S_1 fraction, the peak at the void volume of cMN_1 does not contain any histones but only material which remains at the origin of the gel. In the case of cMN_2, the peak at the void volume contains H1 (data not shown). Histones H3 and H4 derived from cMN_1 and cMN_2 migrate as a single band and show no evidence of multiacetylated species, as it was clearly the case in histones from fraction S_1. Therefore, most of the ^{14}C-acetate label in the histone from cMN_1 and cMN_2 represent the monoacetylated species.

DISCUSSION

We have examined the distribution of acetyl groups among histones derived from various trout testis chromatin fractions, differing in their protein composition and susceptibility to digestion by micrococcal nuclease. The experimental approach involved labelling of a suspension of trout testis cells

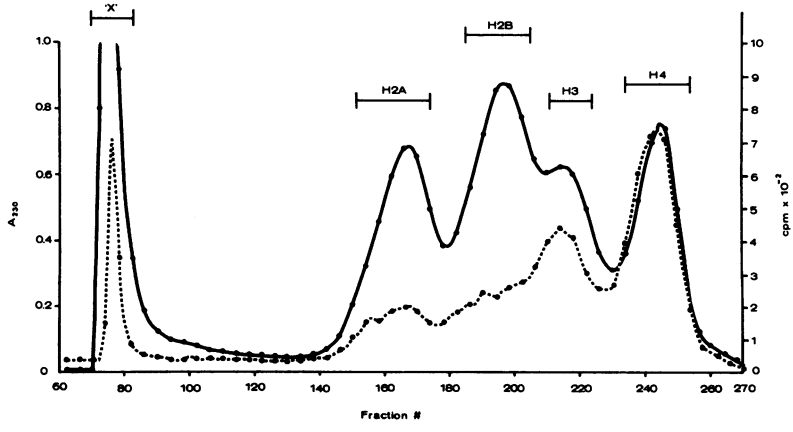

FIGURE 5: Bio Gel P-60 chromatography of two acid soluble proteins from fraction cMN_1. (●——●): A_{230}; (●- - -●) ^{14}C-acetate radioactivity.

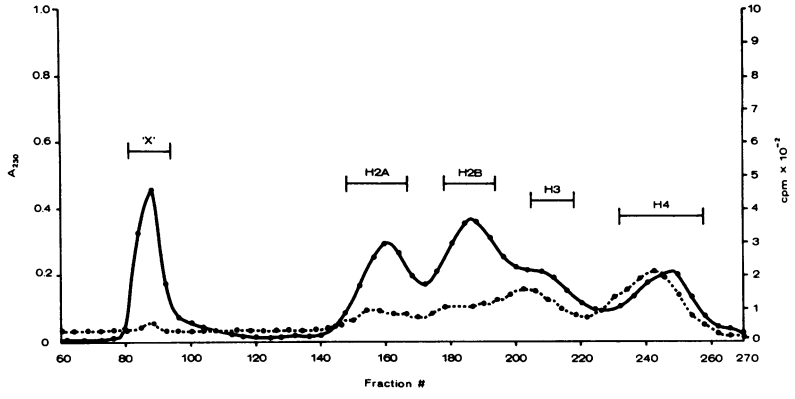

FIGURE 6: Bio Gel P-60 chromatography of the acid soluble proteins from fraction cMN_2. (●——●): A_{230}; (●----●): ^{14}C-acetate radioactivity.

derived from fresh testis with ^{14}C-acetate followed by digestion of the EDTA-treated chromatin with micrococcal nuclease. A fractionation procedure was

Table III. Distribution of acetate groups among individual histone fractions derived from: cMN_1 (Fig 5); cMN_2 (Fig 6).

Chromatin fraction	Protein sample	Total A_{230} recovered	Total cpm ^{14}C recovered	cpm/A_{230}	Specific activity relative to H4
cMN_1	X	60	8,000	13	0.20
	H2A	32	7,000	22	0.33
	H2B	40	18,000	450	0.67
	H3	28	13,000	464	0.69
	H4	36	24,000	666	1.0
cMN_2	X	13	800	61	0.09
	H2A	15	2,800	186	0.29
	H2B	16	4,000	250	0.39
	H3	10	5,500	550	0.87
	H4	12	7,600	633	1.0

employed which yields from testis nuclei, a mononucleosome subset enriched in its content of DNA sequences complementary to cytoplasmic polyadenylated RNA (14, 15) and also in its content of HMG-proteins.

By digesting the chromatin with the nuclease under conditions found to solubilize 5-15% of the input A_{260} from testis nuclei, 24% of the input A_{260} was recovered in the first supernatant S_1. When we analyzed the acid soluble proteins present in fraction S_1, we found that, in addition to the bulk of HMG-T and H6, the four inner histones H2A, H2B, H3 and H4 were also present, indicating that a chromatin subset containing both HMG proteins HMG-T and H6 had become soluble when 20% of the chromatin DNA was digested. Based on previous data from our laboratory which demonstrates that nucleosomes enriched in HMG proteins and selectively solubilized by micrococcal nuclease digestion of trout testis nuclei are enriched 10 fold in their content of sequences being transcribed into cytoplasmic polyadenylated RNA (14, 15), we expect that fraction S_1 is enriched in transcriptionally competent chromatin. The increased micrococcal nuclease sensitivity of transcriptionally active chromatin regions has now also been independently established for the ovalbumin coding sequences from oviduct (27, 28).

The release of histones into the supernatant fraction S_1 was somewhat unexpected, since in our studies with intact nuclei we had never observed histones in fraction S_1, with the exception of small and variable amounts of H1. Indeed it would appear that fraction S_1 from chromatin is equivalent in protein composition to the S_1 plus the MN_1 obtained from nuclei (15). However, in contrast to the MN_1 monosomes obtained from nuclei, the S_1 fraction when sedimented on sucrose gradients shows no peaks in the mononucleosome region but instead, most of the material remains at the top of the gradient (Beatriz Levy-Wilson, unpublished observation).

Furthermore, the protein composition of fractions S_2 and P_2 derived from nuclei and chromatin also differ (Table IV). What is the reason for these differences? The experimental procedure including the nuclease digestion is identical in both cases. The variation is in the manner in which the nuclear fraction of testis was processed prior to the nuclease step. On one hand, we routinely use purified trout testis nuclei, on the other, we have used chromatin, prepared from cells by vigorous homogenization in a NaCl-EDTA buffered solution. The exposure of the nuclei to EDTA apparently affects the nuclear membrane in such a manner that it becomes permeable to mononucleosomes containing histones that are selectively solubilized by micrococcal nuclease (21). In intact nuclei, the nucleosomes associated with the most susceptible HMG-T containing linker

Table IV. Composition of basic proteins of fractions derived from micrococcal nuclease action on intact nuclei or EDTA treated chromatin

Chromatin Fraction	Nuclei Protein composition	EDTA-treated chromatin Protein composition
S_1	HMG-T, HI, ubiquitin, H6	HMG-T, H2A, H2B, H3, H4 H6
MN_1	H6, H2A, H2B, H3, H4	H2A, H2B, H3, H4, trace of HI and HMG -T
MN_2	H2A, H2B, H3, H4, HI	H2A, H2B, H3, H4, HI, (small amt. Protamine)
Nucleoprotamine	H2A, H2B, (H3, H4), HI Protamines	HI, H2A, H2B, Protamines

regions cannot be solubilized until after EDTA treatment (15). Fraction S_2 obtained from EDTA exposed chromatin is also different from that derived from intact nuclei (Table IV) as we would expect since the H6 containing material from chromatin falls into the fraction S_1 while in nuclei it represents the NaCl-soluble portion of S_2.

Acid soluble proteins were extracted from fraction S_1 from chromatin and separated by chromatography on Bio Gel P-60 columns. Individual histone fractions were recovered from the various pools and their content of acetyl groups determined. ^{14}C-acetate was found associated with the four inner histones H2A, 2B, 3 and 4. When the purified H4 sample from S_1 was electrophoresed on an acid-urea-Triton gel, several slower-running bands representing multiacetylated forms of H4 were observed. The same phenomenon was not seen in the case of the H4 fractions derived from the other chromatin fractions. Since the multiple acetylated forms of H4 are most clearly resolved in our gel systems, we concentrated our analysis on this histone. However, we should keep in mind that similar observations might extend to H2A, H3 and H2B.

The data in Tables I and III and Figures 5 and 6 clearly indicate that histones in various regions of the nucleohistone portion of trout testis chromatin can also incorporate ^{14}C acetyl groups. Indeed, a substantial fraction of all the ^{14}C-acetate label incorporated into chromatin is found in fraction S_2 and the majority of these counts fractionate into the NaCl soluble fraction cMN_1. However, analysis of individual histones from these

fractions on gels does not reveal the presence of multiacetylated forms as in the case of H4 from S_1 fraction (as judged by the absence of bands stained with Amido-Black that migrate slower than the non-modified parental band) (B. Levy W., not shown). However, we cannot exclude the possibility that small quantities of multiacetylated histone bands are present in cMN_1 and cMN_2 at levels that are undetectable by our methods. At first sight, the lower specific activity of ^{14}C-acetyl groups in the H4 fraction from chromatin S_1 than that of the H4's from cMN_1 and cMN_2 (Tables II and III) appear at variance with the demonstration of high levels of multiacetylated species of H4 in S_1 (Fig. 4) and predominantly monoacetylated H4 in cMN_1 and cMN_2. We believe that the explanation lies in the observation by Jackson et al., (30) that there are two types of acetylation in high eukaryotic cells, one in which the acetyl groups turn over rapidly with a very short half life of 2-3 min. and a second type, with much slower turnover kinetics. We think it likely that the lower specific activity of acetylated H4 in chromatin fraction S_1 is due to this rapid turnover of acetyl groups. In contrast, we feel that the monoacetyl H4 seen in cMN_1 and cMN_2 from S_2 represents a more slowly-turning over component of the H4 acetyl groups, perhaps related to the demonstration (29) that enzymatic acetylation of the spermatid histones may be a prerequisite to their displacement by newly synthesized protamines. The binding of the amino-terminal regions of the histones to DNA is reduced after acetylation and they become susceptible to a chromatin-bound protease in the sperm nucleus which digests the loosened, acetylated histones into small fragments which are subsequently displaced by protamine (22). On the other hand it appears very clearly that few ^{14}C acetyl groups are associated with the condensed nucleoprotamine.

The finding of multiacetylated species of H4 associated with the chromatin fraction S_1, readily solubilized by micrococcal nuclease and also enriched in HMG proteins and likely to be enriched also in transcribed DNA sequences, is a significant one. It agrees very well with recent data from several laboratories demonstrating the association of multiacetylated forms of H4 with chromatin regions selectively digested by DNase I (11, 12, 13), an enzyme that acts as a structural probe in chromatin for transcriptionally competent genes (21, 23, 24). This observation is also consistent with recent work by Davie and Candido (25) demonstrating the presence of multiacetylated forms of H4 in a trout testis chromatin fraction selectively solubilized by DNase II and likely to be enriched in transcribed DNA sequences. The presence of multiacetylated H4 in the S_1 fraction in the present work is also consistent with the observation that nucleosomes containing highly acetylated histones are excised more rapidly

by digestion with micrococcal nuclease than are nucleosomes in which the histones are not acetylated (11). Multiacetylation of the N-terminal regions of H4 (and possibly also of H3, H2A and H2B) in this subset of nucleosomes would be expected to loosen the interaction of the encircling DNA with the histone core. This structural change, which is detected by DNase I (11, 12, 13), DNase II (25, 26) and micrococcal nuclease, may be a necessary stage in the conversion of the nucleosome to a transcribable form.

ACKNOWLEDGEMENTS

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