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THE HISTONES ASSOCIATED WITH CONDENSED AND EXTENDED CHROMATIN OF MOUSE LIVER

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ABSTRACT

Histones were extracted from isolated mouse liver nuclei, and from mouse liver condensed and extended chromatin. Mouse liver histones were found to be very similar to those of calf thymus in their solubility properties, relative electrophoretic mobilities, and molecular weights as determined on SDS-polyacrylamide gels. Quantitative analysis by high-resolution gel electrophoresis demonstrated a remarkable similarity between the histones of condensed chromatin and those of extended chromatin. However, minor differences were found. A unique subspecies was found only in condensed chromatin histone and the relative amounts of fractions F2A1 and F2A2 differed in the two types of chromatin. The ratio of the parental to the acetylated form of F2A1 was identical in the two chromatin samples. Since DNA extracted from the condensed chromatin fraction consisted of approximately 50% satellite DNA, the general similarities between the histones of condensed and extended chromatin make it likely that even this simple, highly repetitive DNA is complexed with a number of histone subfractions.

INTRODUCTION

In spite of the considerable progress in understanding the chemical and metabolic properties of histones in a variety of tissues and organisms (for review, see Phillips, 1971), the precise role played by histones (or by any histone subfraction) in the structure and function of chromatin in eukaryotic organisms is not clear. In an attempt to obtain additional information regarding the function of histones, we have compared the histones associated with condensed and extended chromatin isolated from mouse liver nuclei. In other cell types these two kinds of chromatin are markedly different in their structural and functional properties (see Frenster, 1969, for review). Extended chromatin has been found to contain those genes which are active in RNA synthesis. On the other hand,

condensed chromatin is genetically inactive (Frenster, 1969). It is likely (though not proven) that these functional differences also exist in the structurally different (condensed vs. extended) chromatin fractions isolated from mouse liver nuclei. In addition, it has been shown that the highly repetitive mouse satellite DNA, which is known to be localized in the centromeric heterochromatin of mouse chromosomes (Pardue and Gall, 1970; Jones, 1970), is preferentially localized in the condensed chromatin of interphase mouse cells (Yasminch and Yunis, 1970; Pardue and Gall, 1970). Thus, by comparing the histones associated with condensed chromatin, containing approximately 50% satellite DNA, and extended chromatin, containing no detectable satellite DNA, of

mouse liver, we are able, not only to compare the histones of two different types of chromatin, but also to study the histones associated with a simple (Waring and Britten, 1966; Southern, 1970), highly repetitive (Britten and Köhne, 1968) satellite DNA.

MATERIALS AND METHODS

Isolation of Nuclei

Nuclei were isolated from (10–25) mouse livers by a slight modification of the method of Yasminch and Yunis (1970) in which the minced livers were blended at low speed for 15 or 30 s in the semi-micro cup of a Waring blender, before homogenization.

Isolation of Condensed and Extended Chromatin

Nuclei were disrupted by sonification (10–12 s), and the condensed and extended chromatin fractions were separated by differential centrifugation according to the method of Yasminch and Yunis (1970).

Extraction and Analysis of DNA

DNA was extracted from the condensed and extended chromatin fractions by the Sarkosyl-Tris-EDTA method of Eckhardt and Gall (1971). Isolated DNA was characterized either by analytical ultra-

centrifugation in neutral cesium chloride gradients as previously described (Eckhardt and Gall, 1971), or by electrophoresis in composite 3-mm slab gels containing 2.5% acrylamide and 0.5% agarose, according to the methods of Peacock and Dingman (1968) and Dahlberg et al. (1969) as described by Zeiger et al. (1971). Only those condensed chromatin fractions whose DNA consisted of approximately 50% satellite DNA were used for histone analysis (Fig. 1).

Isolation of Histones

Histones were prepared from saline (0.15 M NaCl)-washed whole nuclei or subnuclear fractions (condensed and extended chromatin) by repeated extractions with 4 M urea (ultrapure, Mann Research Labs Inc., New York) and 1 N H₂SO₄ in 3:2 (vol/vol) ratio. All procedures were carried out at 0–5°C. The solubilized histones were precipitated with 4 vol 100% ethanol at –25°C for at least 12 h, then washed twice with acetone-0.01 N HCl or 95% ethanol and dried under vacuum before being stored in a dessicator at room temperature.

Histone Fractionation

The fractionation procedure used was essentially Johns' Method I (Johns, 1964) with minor changes necessitated by the fact that the starting material consisted of small amounts of isolated mouse liver nuclei instead of whole calf thymus tissue. All supernatants throughout the procedure were clarified by centrifuging at 12,000 *g* × 10 min. Histone fractions F2A1 and F2A2 were separated using Johns' F2A Fractionation Method II (Johns, 1967) on saline-washed nuclei.

Acrylamide Gel Electrophoresis

High-resolution acrylamide gel electrophoresis was performed according to the method of Panyim and Chalkley (1969 a), using 0.5 cm (ID) cylindrical gels containing 15% acrylamide (electrophoretically pure, Eastman Kodak Co., Rochester, N. Y.) and 2.5 M urea. Samples were dissolved in either 8 M urea –0.01 N HCl or 15% sucrose –0.9 N acetic acid –2.5 M urea, usually containing 0.1 M β-mercaptoethanol. Gels were 25 cm long and were preelectrophoresed at 100 V for 24 h, and run at 250 V for 16.5–17 h. All gel runs were performed at constant voltage and at 22°C.

Gels were stained in 1% fast green in 7% acetic acid as described previously (Gorovsky et al., 1970) and densitometry was performed immediately after destaining at 630 nm with a Gilford 2400 spectrophotometer. The densitometer scans were retraced and the areas under the curves were cut and weighed to obtain relative amounts of the histone fractions (Table II).

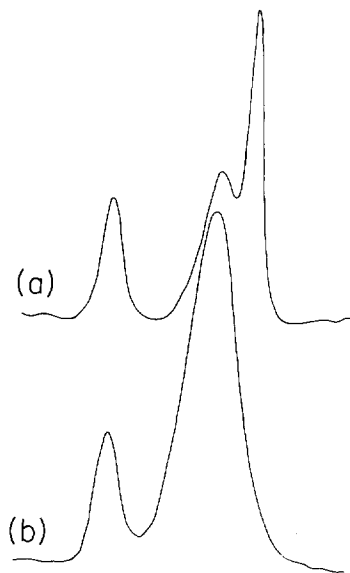


FIGURE 1 Densitometer tracings of DNA isolated from (a) condensed chromatin and (b) extended chromatin, after equilibrium centrifugation in neutral CsCl.

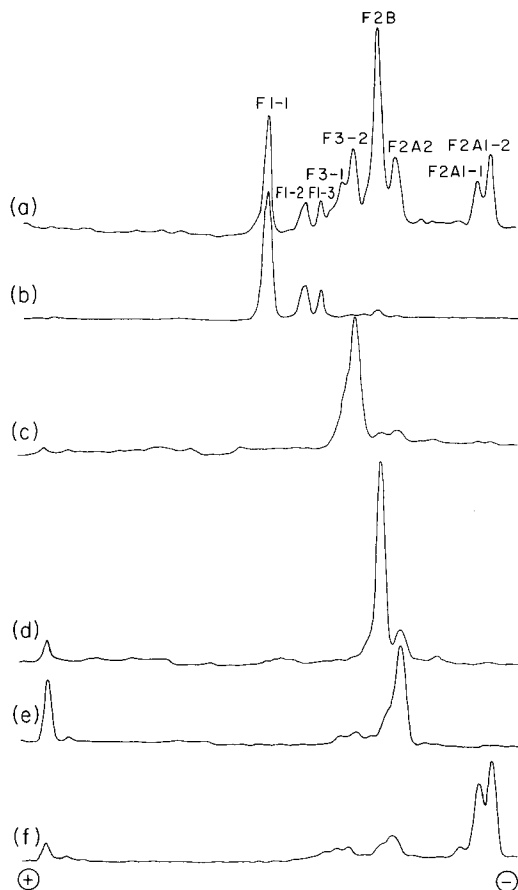


FIGURE 2 Densitometer tracings of polyacrylamide gels containing whole mouse liver histone and fractions. (a) Whole mouse liver histone, (b) F1, (c) F3 reduced, (d) F2B, (slightly contaminated by F2A2, as shown by its differential staining properties in amido black [Kaye and McMaster-Kaye, personal communication]), (e) F2A2, and (f) F2A1. The gels were electrophoresed at 250 V for 16.5 h at 22°C. The nomenclature used is basically Johns' (1964). The superscripts indicate the order of mobility ([+] to [-]) in this gel system.

SDS Gel Electrophoresis

Molecular weights were determined by electrophoresis on 15% polyacrylamide gels containing 0.05% SDS at pH 7.6 as described by Panyim and Chalkley (1971). Electrophoresis was carried out at 80 V for 6.5 h at 22°C. Gels were stained in 0.1% fast green in 20% acetic acid—50% methanol overnight and gradually destained by repeated changes in 7.5% acetic acid—5% methanol (Weber and Osborn, 1969). The gels were scanned at 630 nm and relative mobilities were calculated with respect to purified calf

thymus fraction F2A1 which was run as a marker on each gel.

RESULTS

Identification and Characterization of Histone Fractions

The histones isolated from mouse liver nuclei have been identified by the solubility criteria of Johns (1964) and by the electrophoretic criteria of Panyim and Chalkley (1969 a, 1969 b). Although completely pure histone fractions were not obtained, we were able to achieve marked enrichment for fractions with properties similar to those obtained by Johns and others with calf thymus histones. In all cases the identification of a particular fraction by its solubility properties agreed with the identification based solely on its electrophoretic properties. As might be expected from the remarkable similarities in the histones of a number of vertebrates (Panyim et al., 1971 a), the histones extracted from mouse liver nuclei are very similar to those of calf thymus in their solubility properties, mobilities on urea-acrylamide gels, and in their molecular weights as determined by SDS-acrylamide gel electrophoresis. Fig. 2 shows densitometer tracings of long polyacrylamide gels of whole mouse liver histone and of the isolated fractions. Fig. 3 compares the reduced form of whole mouse liver histone (Fig. 3 b) with the oxidized form (Fig. 3 a). The molecular weights of the major histone fractions are given in Table I.

The two minor bands of histone fraction F1 (Fig. 2 b) were consistently observed even when

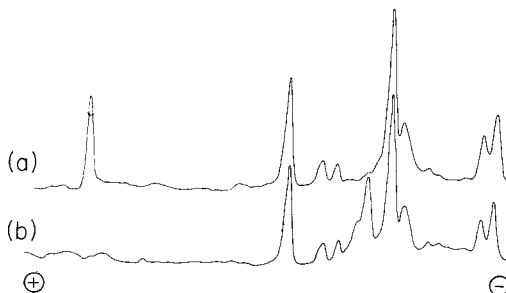


FIGURE 3 Oxidation of mouse liver histone. Densitometer tracings of whole mouse liver histone (a) oxidized during storage in 8 M urea — 0.01 N HCl and (b) subsequently reduced with 0.1 M β -mercaptoethanol for 36 h before electrophoresis on long gels. Mouse liver F3 migrates as a single band, suggesting that there is only one thiol group per F3 molecule (Panyim et al., 1971 b).

TABLE I
Molecular Weights of Mouse Liver Histone Fractions
on SDS-Polyacrylamide Gels*

Histone fraction	Molecular weight	
	Run 1	Run 2
F1 †	23,500	23,000
F1' †	22,000	22,000
F1'' †	21,250	20,000
F3 §	13,750	13,500
F2B	14,000	13,750
F2A2	12,750	12,500
F2A1	11,250	11,250

* The data presented here are from two independent runs. Included in each run was one gel containing whole calf thymus histone, from which a log molecular weight vs. mobility curve was generated. Molecular weights of calf thymus histones were taken to be: F1-21,000 and F2A2-12,750 (Panyim and Chalkley, 1971); F3-15,324 (DeLange et al., 1972); F2B-13,775 (Iwai et al., 1970, 1972); and F2A1-11,282 (DeLange et al., 1969). All molecular weights were rounded off to the nearest 250 daltons.

† Correspondence of the three F1 bands observed on SDS gels to those observed on urea-acrylamide gels has not been established.

§ The molecular weights of both calf thymus F3 and mouse liver F3 as determined by SDS-polyacrylamide gel electrophoresis are the same (approximately 14,000 daltons) but differ significantly from the value obtained by DeLange et al. (1972) from primary sequence analysis. The reason for this discrepancy is unclear.

nuclei and histones were isolated in 0.05 M bisulfite to inhibit proteolysis (Panyim et al., 1968; Bartley and Chalkley, 1970). Moreover, the relative amounts of all three F1 bands were found to be constant (Table II), suggesting that the minor bands are not the result of degradation. The rapidly migrating doublet of fraction F2A1 (Fig. 2f) coelectrophoreses with the two major subspecies of purified calf thymus F2A1 on urea-acrylamide gels. In addition, it migrates as a sharp, single peak on SDS gels, with a relative electrophoretic mobility identical to that of calf thymus F2A1. This suggests that, like calf thymus F2A1 (DeLange et al., 1969; Wangh et al., 1972), the two mouse liver F2A1 subfractions differ by the presence of one additional acetyl group on the slower migrating form.

Comparison of the Histones of Condensed and Extended Chromatin

The histones extracted from condensed and extended chromatin are qualitatively similar when compared by electrophoresis on long polyacrylamide gels (Fig. 4). Moreover, quantitative analysis (Table II) indicates that the relative amounts of the five major fractions and of the resolvable subfractions are also quite similar in condensed and extended chromatin. Each condensed chromatin DNA sample contained approximately 50% satellite DNA, while the extended chromatin DNA sample had little, if any, detectable satellite DNA. The relative amounts of total F1 (24%), total F3 (20%), and total F2B (30%) were equal in the two types of chromatin. In addition, the relative amounts of the subspecies of F1 and F3 were identical. The ratios of the subspecies of F2A1 in the two chromatin fractions were also the same. This suggests that unlike the correlation observed in a variety of other systems between either the rate (Allfrey, 1964, 1970, 1971; Wangh et al., 1972) or amount (Allfrey, 1970; Gorovsky et al., 1973) of F2A1 acetylation with RNA synthesis, there is no apparent relationship between the amount of acetylated subspecies of F2A1 and the probable genetic activity of these two mouse liver chromatin fractions. However, significant differences were found: (a) a minor, unidentified fraction (X, Fig. 4 a) always constituting approximately 1% of the total histone (Table II, $1.2 \pm 0.1\%$) associated with condensed chromatin was never present in histone extracted from extended chromatin (results based on five independent isolations) and (b) approximately 50% more F2A1 and 30% less F2A2 were present in histone associated with condensed chromatin than with extended chromatin.

DISCUSSION

The Histones of Condensed and Extended Chromatin

The histones associated with condensed and extended chromatin have been investigated previously (Frenster, 1965 a, b; Comings, 1967; Pallotta et al., 1970). However, recent improvements in separating histone subfractions by electrophoresis in long polyacrylamide gels (Panyim and Chalkley, 1969 a) have led us to reinvestigate this problem in greater detail. Moreover, the

TABLE II
Quantitative Analysis of Mouse Liver Histone of Condensed and Extended Chromatin

Histone fraction	Condensed chromatin	Extended chromatin
F1-1	15.8 ± 0.8*	16.2 ± 0.6
F1-2	3.3 ± 0.3	3.1 ± 0.1
F1-3	4.5 ± 0.3	4.8 ± 0.5
Total F1	23.6 ± 0.7	24.2 ± 0.7
x	1.2 ± 0.1	—
F3-1	7.0 ± 0.9	6.7 ± 0.6
F3-2	13.2 ± 0.5	12.8 ± 0.6
Total F3	20.2 ± 1.0	19.5 ± 0.7
F2B	29.7 ± 1.1	30.7 ± 0.7
F2A2‡	9.8 ± 0.4	12.7 ± 0.8
F2A1-1	5.1 ± 0.3	3.8 ± 0.3
F2A1-2	7.2 ± 0.4	4.9 ± 0.5
Total F2A1	12.3 ± 0.6	8.7 ± 0.8
F2A1-2§/F2A1-1	1.4 ± 0.1	1.3 ± 0.1
F2A2-E /F2A2-C		1.31 ± 0.11
F2A1-C/F2A1-E		1.48 ± 0.11

* Mean percentage ± standard error from five independent isolations. Each histone sample was completely reduced.

‡ Not included in this analysis is heterogeneous material, presumably the result of proteolytic degradation, which migrated between F2A2 and F2A1, and comprised less than 4% of total histone in all samples.

§ Ratios were calculated for each isolation, then treated as separate data points.

|| Abbreviations: *C*, condensed chromatin; *E*, extended chromatin.

specific localization of the satellite DNA in condensed chromatin provided an independent means for assessing the purity of our chromatin fractions. In general, our results suggest that even at high resolution, the histones isolated from the condensed and extended chromatin fractions are remarkably similar. However, we have observed the presence of a unique, minor subspecies of histone (X) in condensed chromatin, as well as quantitative differences in the amounts of fractions F2A1 and F2A2 in the two types of chromatin.

The DNA isolated from the condensed chromatin fractions which we studied was consistently found to contain 45–50% satellite DNA, while DNA isolated from the extended chromatin fractions contained little or no detectable satellite DNA. Therefore, it is reasonable to assume that if one (or a few) histone fraction(s) was preferentially associated with satellite DNA, the relative amount

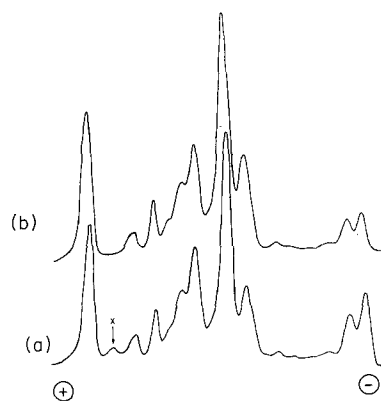


FIGURE 4 Densitometer tracings of long gels containing whole mouse liver histone from (a) condensed chromatin and (b) extended chromatin. The arrow indicates the unidentified fraction (x) which is associated only with condensed chromatin, and never with extended chromatin.

of that fraction in whole histone isolated from the two types of chromatin should parallel the relative amounts of satellite DNA. It is clear from our quantitative analysis (Table II), however, that none of the major histone fractions are enriched in the condensed chromatin fraction and deficient in the extended chromatin fraction to the same extent as satellite DNA. We conclude that this simple (Waring and Britten, 1966; Southern, 1970), highly localized (Pardue and Gall, 1970; Yasminch and Yunis, 1970) family of DNA sequences is complexed with more than one type of histone and, most likely, with the entire complement of histone fractions.

The Role of Histones in Chromatin Structure

Histones associated with chromatin differing cytologically, as well as functionally, in different tissues (Panyim and Chalkley, 1969 b), in polytene and nonpolytene chromosomes (Cohen and Gotchel, 1971), and in condensed and extended chromatin (this report) have been shown to be remarkably similar. This suggests that histones may not function in whatever mechanisms are responsible for morphological differences among chromatin or chromosome types. It is still possible, of course, that histones act at a finer level of chromatin structure, i.e. in the attainment and maintenance of the primary supercoiling of DNA in nucleohistone complexes (Bradbury, 1969; Pardon and Wilkins, 1972). It should be pointed out, however, that any theory regarding the role of histones in eukaryotic chromatin must account for the qualitative similarities, as well as the small quantitative differences and the presence of specific minor subfractions in the histone complement of different species (Panyim et al., 1971 a), of different tissues (Panyim et al., 1968; Panyim and Chalkley, 1969 b), and of different types of chromatin (this report).

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