A NEW HISTONE FOUND ONLY IN MAMMALIAN TISSUES WITH LITTLE CELL DIVISION

by

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Summary

The several criteria are discussed which support the idea that the new electrophoretic band, found in histones of non-replicating tissue, is indeed a previously unreported histone.

We have recently described (1,2) a polyacrylamide gel electrophoretic technique capable of resolving the five major groups of histone molecules. The purpose of this previous work was to define a method capable of rapidly analyzing histones isolated under conditions designed to minimize proteolysis from many different tissues and species. In the course of our studies with mammalian tissues, we have found that tissues which are primarily non-replicating contain a histone band of characteristic mobility which is totally absent from histone of more actively replicating tissues such as the thymus or tumor cells. So far as we are aware, the existence of this band has not been previously reported.

Materials and Methods

All tissues were rapidly dissected from freshly killed or anesthetized animals. The tissue was frozen on dry ice and stored at -30° prior to homogenization. The Ehrlich ascites tumor cell line was grown intraperitoneally in white mice (strain Swiss Webster).

Histones were isolated by extracting chromatin with 0.4 N H_2SO_4 and precipitated by adding four volumes of ethanol as previously described (1,2).

Acrylamide gel electrophoresis was performed as described recently (1). Densitometer traces of destained gels were obtained on a Gilford model 2000 and quantitation of histone in each band was determined with a Dupont curve analyzer.

Results and Discussion

In Fig. 1 is shown a typical separation of calf thymus histones using this electrophoretic system which is capable of resolving all five

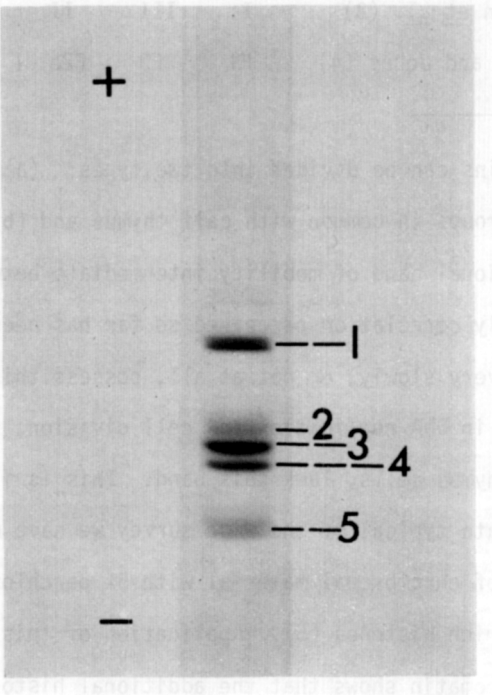


Fig. 1. Polyacrylamide gel electrophoretic pattern of calf thymus histones. The nomenclature is that defined by Panyim and Chalkley (2).

major groups of histone. The nomenclature we employ is compared with that of Rasmussen et al. (3) and of Phillips and Johns (4) in Table I. That of Rasmussen et al. is primarily based upon column chromatographic separations,

and that of Phillips and Johns upon chemical separations. In order to relate all the electrophoretic bands in our system to histones separated by other methods, we have prepared the histone fractions separately following the methods of Rasmussen et al. and of Phillips and Johns and then tested fractions so obtained in our electrophoretic system.

In the course of a survey of mammalian histones, we have observed

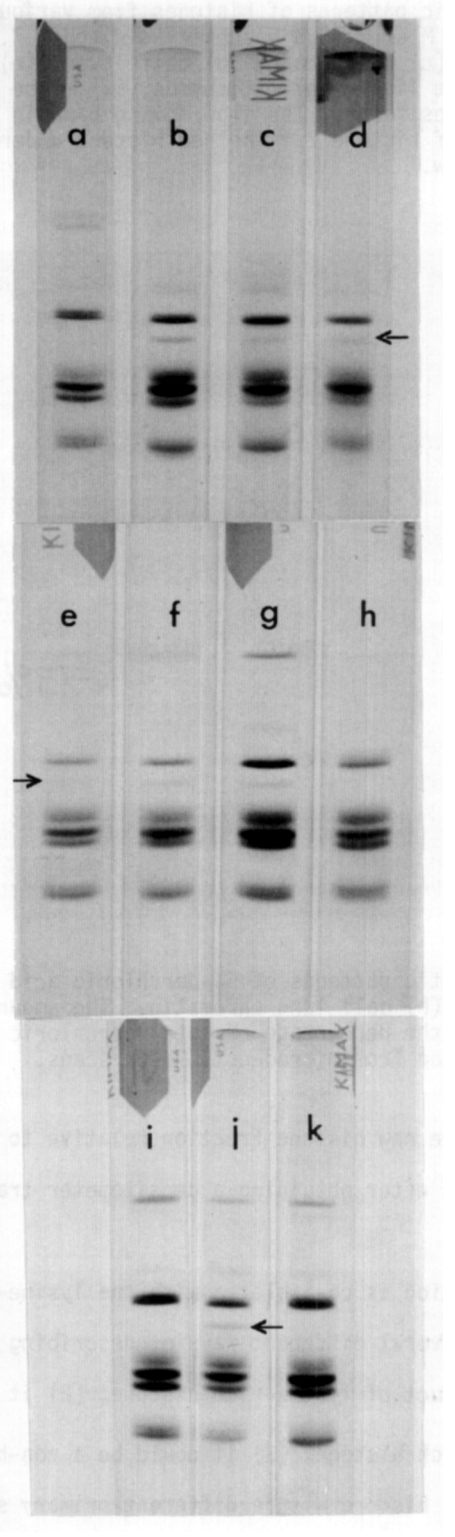
Table I

Comparison of Histone Nomenclature

This paper	1	2	3	4	5
Rasmussen et al. (3)	I	III	II	II	IV
Phillips and Johns (4)	F1	F3	F2b	F2a2	F2a1

that these proteins can be divided into two types: (a) those which have the five major groups in common with calf thymus and (b) those which possess an additional band of mobility intermediate between that of bands 1 and 2. The only correlation perceived so far has been that those tissues which replicate very slowly, or not at all, possess this extra band. Tissues which are active in DNA replication and cell division, such as ascites tumor cells or thymus cells, lack this band. This is illustrated in Fig. 2 which contains data typical of the wide survey we have made.

Extraction of chromosomal material with 5% perchloric acid specifically extracts lysine-rich histones (5). Application of this extraction technique to lung chromatin shows that the additional histone band is co-isolated with the lysine-rich histones indicative of a similar chemistry. The result of extracting calf thymus and calf lung chromatin with 5% perchloric acid is shown in Fig. 3. Sulfuric acid extraction of the residual histone which is insoluble in perchloric acid indicated that both the lysine-rich histone and the histone of the additional band had been quantitatively extracted.


a
b
c
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e
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g
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i
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Fig. 2. Electrophoretic patterns of histones from various tissues and animals in 15% acrylamide, 2.5 M urea, 0.9 N acetic acid, pH 2.7. (a) Calf thymus, (b) calf endometrium, (c) calf brain, (d) calf liver, (e) rat lung, (f) rat kidney, (g) rat liver, (h) rat thymus, (i) mouse thymus, (j) mouse liver, (k) mouse ascites tumor. The slow moving band in several samples is the oxidized form of histone 2. The new histone under study is indicated with an arrow.

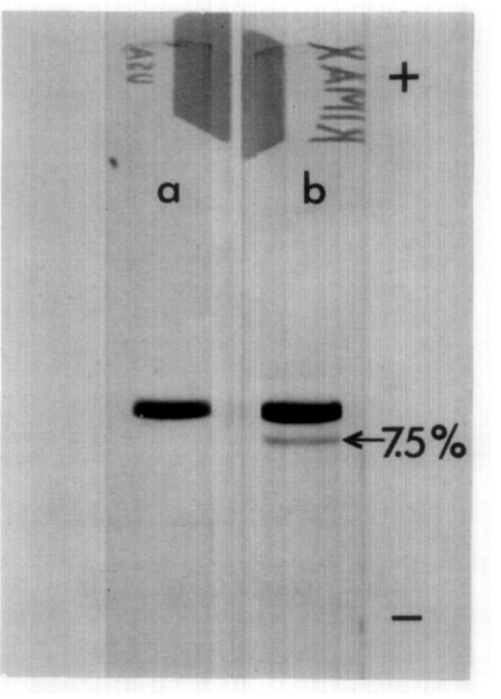

Fig. 3. Electrophoretic patterns of 5% perchloric acid extracts of (a) calf thymus chromatin and (b) calf lung chromatin. The amount of the new histone band is expressed as the percentage of total perchloric extract of calf lung chromatin as calculated from microdensitometer scans.

The amount of the new histone fraction relative to the lysine-rich fraction was computed after obtaining a densitometer trace of the gel of Fig. 3.

As the new fraction is co-isolated with the lysine-rich histone fraction, we have considered several different ways of describing it. (1) It could be a degradation product of lysine-rich histone; (2) it could be a modification of the lysine-rich histone; (3) it could be a non-histone contaminant; and (4) it could be a histone with a different primary sequence to the

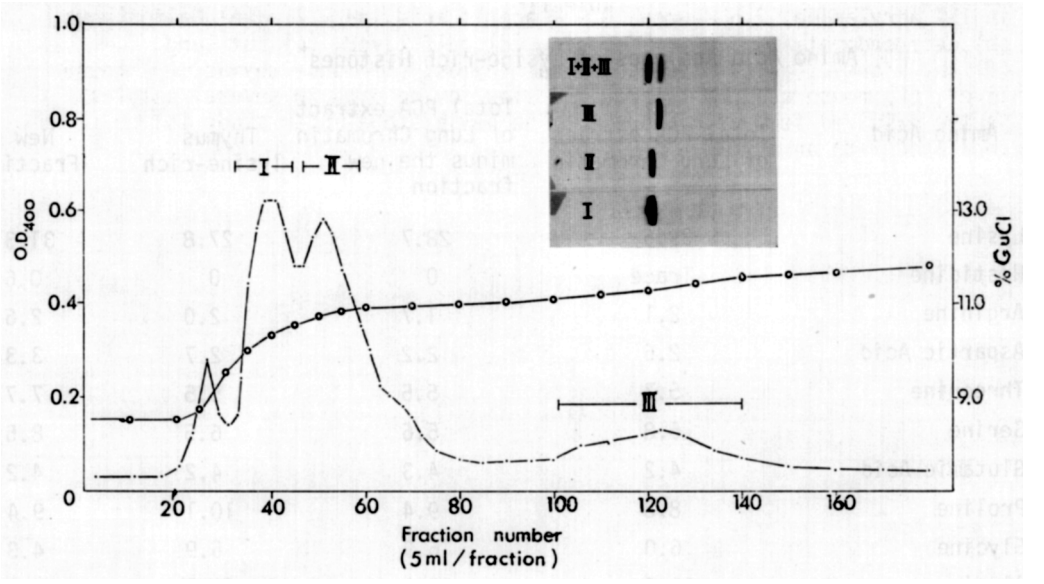

Fig. 4. Chromatography of perchloric acid extract of calf lung chromatin, and electrophoretic analysis of the eluant. The latin numerals refer to pooled eluant fractions. $O.D._{400}$, —·—·—· ; % GuCl, -o-o

lysine-rich histone, though with enough chemical similarity to account for the co-extraction.

The new fraction was separated from the lysine-rich histone by repeated chromatography on a column of CG-50 resin using a gradient of guanidine chloride as shown in Fig. 4. The fraction of interest is obtained after the bulk of the lysine-rich histone has been eluted. The amino acid analyses of the various lysine-rich histone fractions described in this paper are given in Table II. The new fraction is characterized by a prepondrance of basic amino acids, the absence of cysteine and tryptophan, the low level of the aromatic residues. Further, this fraction was an acid-soluble component of isolated chromosome material. On the basis of these criteria, we propose that this protein is a histone.

This protein can be described as a very lysine-rich histone and most likely it is this characteristic that governs its solubility in perchloric acid and permits its coextraction with the other very lysine-

Table II

Amino Acid Analyses of Lysine-rich Histones†

Amino Acid	Total PCA extract of Lung Chromatin	Total PCA extract of Lung Chromatin minus the new fraction	Thymus lysine-rich	New Fraction
Lysine	29.6	28.7	27.8	31.3
Histidine	Trace	0	0	0.6
Arginine	2.1	1.7	2.0	2.6
Aspartic Acid	2.6	2.2	2.7	3.3
Threonine	5.7	5.5	5.5	7.7
Serine	6.8	6.6	6.5	8.5
Glutamic Acid	4.2	4.3	4.2	4.2
Proline	8.8	9.4	10.1	9.4
Glycine	6.0	6.9	6.9	4.3
Alanine	23.8	25.0	23.3	16.8
Cysteine	0	0	0	0
Valine	4.4	4.4	4.9	5.2
Methionine	0	0	0	0
Isoleucine	1.0	0.9	0.9	1.9
Leucine	3.9	3.9	4.1	2.1
Tyrosine	0.6	0.3	0.6	1.1
Phenylalanine	0.6	0.5	0.6	0.9
*Tryptophan	0	0	0	0

†Mole % of total amino acid content.

*Analysis for tryptophan following the method of Barman and Koshland (6).

rich histone. It is clearly not a degradation product nor a modification of the usual lysine-rich histone. This is most critically seen in the presence of histidine only in the new fraction. Further, the amounts of lysine, arginine, threonine, serine and isoleucine are higher in the new fraction; glycine, leucine and particularly alanine are present in reduced amounts.

Acknowledgements

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ERRATA

Vol. 36, No. 2 (1969) in the Communication, "Induction of Respiratory Control by K^+ in Mitochondria," by A. Gómez-Puyou, F. Sandoval, M. Tuena, A. Peña, and E. Chávez, pp. 316-321.

Page 317, line four from top should read:

to pH 7.3 with tris) 0.001 M EDTA, 0.02 M tris-HCl (pH 7.3) and 0.1 M Sucrose. instead of

to pH 7.3 with Tris), 0.02 M Tris-HCl (pH 7.3), and 0.1 M sucrose.

Vol. 36, No. 6 (1969), in the Communication, "Muscle-Type Aldolase Isolated from a Liver Tumor," by L. W. Brox, A. G. Lacko, R. W. Gracy, R. C. Adelman, and B. L. Horecker, pp. 994-998:

The name "L. W. Brox" was correctly printed on p. 994. However, it is misprinted in the following places:

cover page 4 - Table of Contents;
cover page 3 - Author Index for Issue;
Volume Table of Contents;
page 1054 - Author Index for volume.

The Publishers regret the error.

Vol. 37, No. 5 (1969) in the Communication, "The Incorporation of p-Hydroxybenzoic Acid and Isopentenyl Pyrophosphate into Ubiquinone Precursors by Cell-Free Preparations of Rat Tissues," by Michael J. Winrow and Harry Rudney, pp. 833-840.

In Table II, The Incorporation of Labeled Precursors into Compound "R", the specific activities of the labeled compounds should be expressed as "μc/μmole" instead of "μc/ mole."