# AN UNUSUAL PATTERN OF LYSINE RICH HISTONE COMPONENTS IS ASSOCIATED WITH SPERMATOGENESIS IN RAT TESTIS

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Summary. Lysine rich histones from the testes as well as many other organs from sexually mature rats were examined by polyacrylamide gel electrophoresis. Extracts from testes contained a prominent species that was at most a minor component in the other tissues examined. Detailed comparison of lysine rich histones from testis and thymus by column chromatography revealed marked quantitative differences in three of the five resolvable components. Treatment with bacterial alkaline phosphatase did not alter the electrophoretic behavior of any of the fractions resolved from testis or thymus. The appearance of one of the components in the testis as a prominent band could be correlated with the occurence of the early phases of active spermatogenesis.

In the course of electrophoretic studies of a specific basic protein associated with spermatogenesis in the rat testis (1,2) we have observed a distinctive pattern of heterogeneity for lysine rich histones (f1 or KAP histones) (3-8) in this organ.

## EXPERIMENTAL PROCEDURES

Preparation of bulk lysine rich histones. The procedure of De Nooij and Westenbrink (9) was used.

Polyacrylamide gel electrophoresis. pH 3 system. The system of Panyim and Chalkley (10) was modified slightly. Cylindrical gels (0.5 x 5 or 10 cm) were polymerized from a solution consisting of 0.94 M acetic acid, 6 M urea, 20% acrylamide, 0.1% N,N'-methylenebisacyrlamide (BIS), 1.25% (V/V) N,N,N',N'-tetramethylenediamine (TMD), and 0.054% ammonium persulfate (APS). Impurities were removed by electrophoresis before use (10), and, after electrophoresis of samples, the protein bands were detected by staining with amido black 10B (1).

Polyacrylamide gel slabs (0.2 cm thick by 9.5 cm long) were formed from the same solution described above. Sample slots were formed above the main gel using a gel polymerized from a solution containing 0.94 M acetic acid, 6 M urea, 5% acrylamide, 0.25% BIS, 1.25% TMD, and 0.16% APS. After electrophoretic removal of impurities from the gel, protein samples were separated at a potential of 150 V for 17 hr. Protein bands were detected by staining with Coomassie brilliant blue (11).

pH 4.5 system. The composition of the main gel for the slabs and the composition of the chamber buffer have been described (1). The gel containing the sample slots was polymerized from a solution consisting of 5% acrylamide, 0.13% BIS, 0.12 M KOH, 0.725% (V/V) acetic acid, 0.018% TMD, and 0.09% APS. Electrophoresis of samples was for 16 hr at 125 V.

Ion-exchange chromatography. Bulk lysine rich histones (25 to 30 mg) were fractionated on a column (2.5 x 30 cm) of Bio Rex 70 (100-200 mesh) (Bio-Rad, Richmond, Calf.) at room temperature using a linear gradient of guanidine HCl (GCl) formed from 850 ml of 7% GCl, 0.1 M sodium phosphate of pH 6.8 (refractive index 1.3479) and 850 ml of 14% GCl, 0.1 M sodium phosphate of pH 6.8 (refractive index 1.3602) (12). Fractions of 5 ml were collected at a flow rate of 10 ml/hr.

## RESULTS AND DISCUSSION

For the rapid extraction of lysine rich histones, whole tissues were homogenized in cold 0.2 M $H_2SO_4$, and the proteins soluble in 3% trichloroacetic acid (TCA) were prepared as described previously (1). When testicular extracts were examined electrophoretically on polyacrylamide gels containing 20% acrylamide, 6 M urea, and 0.94 M acetic acid (pH 3 system), a distinctive doublet pattern in the f1 histone region was obtained even when migration was over a relatively short distance (Fig. 1). Electrophoresis for longer periods, coupled with the preparation of purified bulk f1 histones by a standard procedure (9), emphasized that the testis indeed contained a slowly migrating f1 histone component that was at most a minor component in the extracts from many other organs

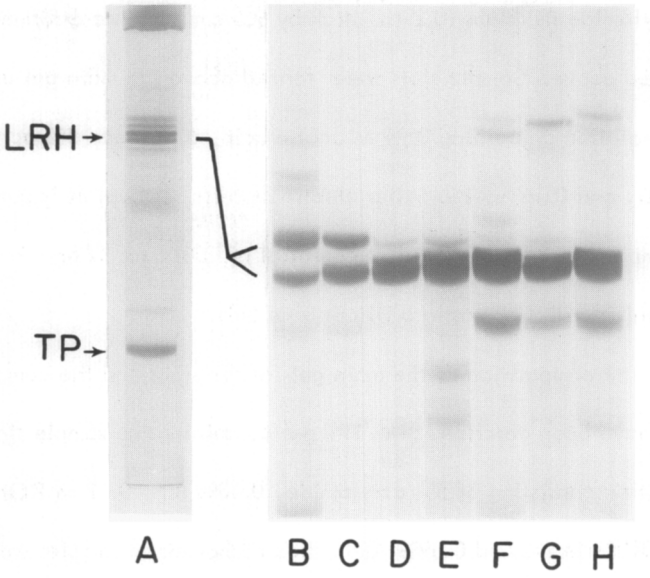


Fig. 1. Electrophoretic pattern of f1 histones from rat testis and other organs. All samples except that of gel C consisted of the fraction of total tissue protein that is soluble in 3% TCA(1). The tissues extracted were: A-testis, B-testis, D-thymus, E-small intestine mucosa, F-kidney, G-lung, H-liver. Gel C contained bulk testis f1 histones prepared as described in "Experimental Procedures". The composition of the polyacrylamide gels (pH 3 system) is described in "Experimental Procedures". Gel A (0.5 x 5 cm) was run for 3 hr at 100 V. The remaining gels (0.5 x 10 cm) were run for 15 hr at 150 V, and only the portion of these gels in the vicinity of the f1 histones is displayed. The location of f1 histones (LRH) and of the testis-specific basic protein (TP) (1) is indicated.

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(Fig. 1). The other tissues examined include thymus, small intestine mucosa, kidney, lung, liver, (Fig. 1) and spleen, ventral prostate, heart, brain, and skeletal muscle (not shown).

In other experiments, we prepared purified bulk f1 histones from rat testis and thymus, and then separated the various components by the highly sensitive chromatographic procedures described by Kinkade and Cole (12) (Fig. 2 and 3). It has been previously established that a number of rat organs including thymus contain a closely similar if not identical family of five resolvable f1 histone species (13). While the amino acid composition of each of the rat components has not yet been determined, studies of rabbit f1 components suggest that the five rat components all probably represent proteins of unique primary structure (14, 15).

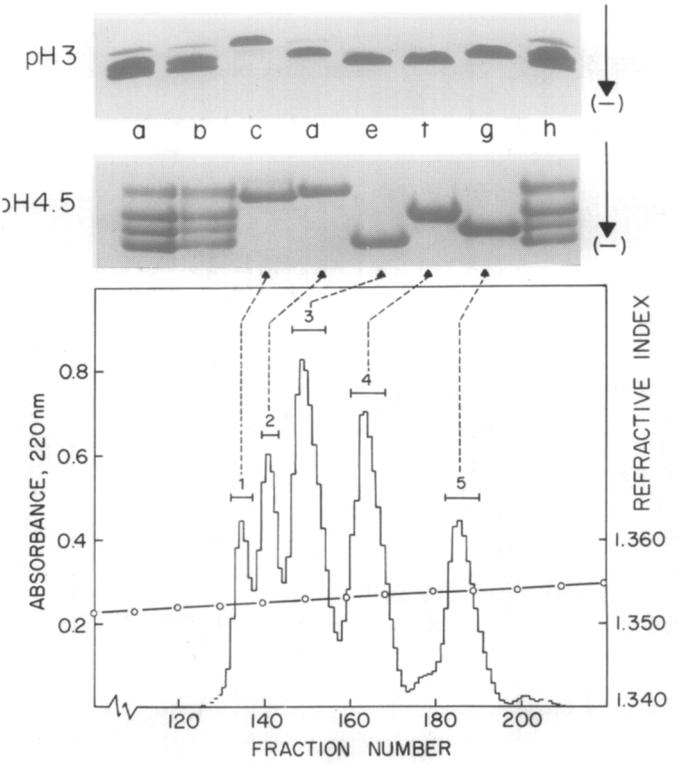


Fig. 2. Separation of thymus f1 histones by ion exchange chromatography and electrophoretic behavior of the resulting fractions on polyacrylamide gels. Preparation of bulk f1 histones and description of the chromatography are described in "Experimental Procedures". The indicated fractions were pooled, dialysed against distilled water, and lyophilized. The dry protein was dissolved in 0.01 M acetic acid, and samples of individual chromatographic fractions as well as samples of unfractionated f1 histones were examined electrophoretically in each of two different polyacrylamide gel slab systems. Only the region of each gel slab occupied by the stained f1 histone bands is reproduced. The samples applied to each gel slab were identical and were: A-27 µg of the proteins soluble in 3% TCA from whole thymus, B and H-9 µg bulk f1 histones, C through G-5 µg respectively of each of the 5 chromatographically resolved components as diagrammed.

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Chromatographic separation of thymus f1 histones (Fig. 2) revealed the expected five components with the approximate quantitative distribution observed by others (13). Separation of testis f1 histones under identical conditions conditions revealed a conspicuously different profile (Fig. 3). Each of the fractionated components from testis and thymus was examined under two different conditions of electrophoresis. Comparable chromatographic fractions from the two tissues invariably showed identical electrophoretic mobilities in each of the two systems (Fig. 2 and 3). Making the arbitrary assumption that the

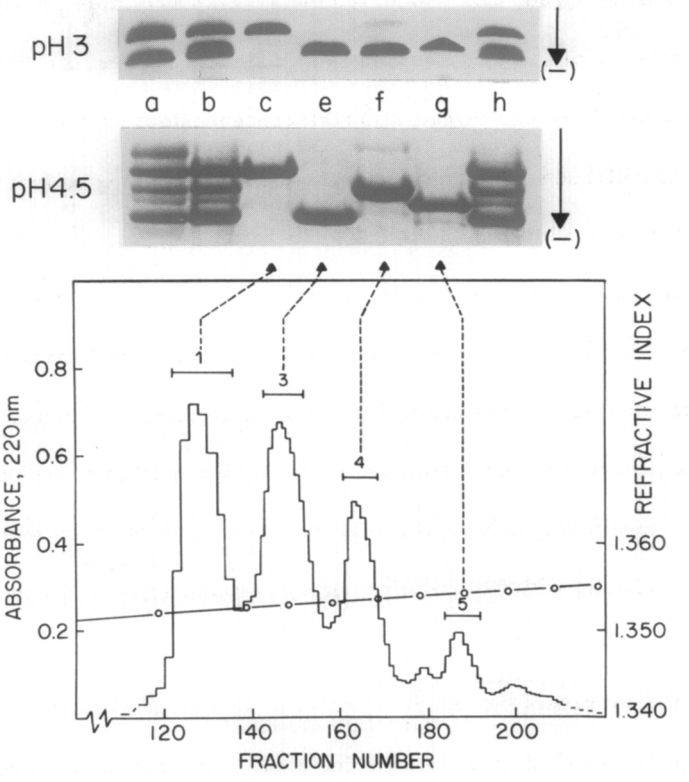


Fig. 3. Separation of testis f1 histones by ion exchange chromatography and electrophoretic behavior of the resulting fractions on polyacrylamide gels. For details see the legend to Fig. 2 and "Experimental Procedures". The samples applied to each of the gel slab systems were identical and consisted of: A-25 µg of the proteins soluble in 3% TCA from whole testis (the most slowly migrating band in the pH 4.5 system is not an f1 histone), B and H-16 µg of bulk f1 histones, C through G-5 µg of the indicated chromatographic fraction.

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corresponding components from the two tissues are in fact identical, it appears that component 1 constitutes the principal f1 histone in the adult testis but represents only a minor component in the thymus. As component 1 is also the sole f1 histone component accounting for the slowly migrating band in the pH 3 gel system, it is clear that this histone band is also a minor species in all of the other tissues examined. In contrast to the pronounced elevation of component 1 in adult testis, component 2, if present, is not readily discernible. Similarly, component 5 is reduced in amount relative to the pattern found for the thymus.

To test whether component 1 might represent a phosphorylated derivative of one or more of the other f1 components, we treated both bulk f1 histones and their fractionated components from both testis and thymus with high concentrations ( 1 mg/ml) of an active preparation of E. coli bacterial alkaline phosphatase (BAP) for long periods under several conditions of ionic strength and pH (16-18). In no case was component 1 converted to a more rapidly migrating species upon electrophoresis. While we can not exclude phosphorylation at a site insensitive to BAP, or indeed some other form of post-translational modification as accounting for the properties of this histone species, preliminary compositional studies indicate it to be a typical f1 histone but to be distinguished by an unusually high valine content. This finding is in accord with the observations of others that increased valine content correlates with early elution during chromatography under the conditions used (13, 14).

While it is not possible to assign any specific physiological correlation with the characteristic pattern of f1 histones found in the adult testis, two of our unpublished observations suggest that such specific correlations may exist. First, when spermatogenesis is arrested by surgical translocation of the testis of an adult rat to its abdomen [1], component 1 is reduced to a minor species by 13 days after surgery. Second, the testis of the neonatal rat (3 days of age) also lacks the intense peak of component 1. Although we have not determined in the developing rat testis the time when component 1 attains its adult prominence, we observed that the electrophoretic appearance of f1 histones in extracts from the testes of 21 day old rats is difficult to distinguish from the adult pattern. The lengthy prophase of the first meiotic division is well under way by 21 days of age, and whether or not the unusual elevation of component 1 is correlated with an early aspect of meiosis, or perhaps with the waves of mitotic division that precede meiosis, remains uncertain.

Branson et al. (19) have reported in abstract form the existence of 3 unusual

histone bands in adult rat testis. One of these (X1) was apparently similar to histone fraction f1 as a bulk fraction.

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## REFERENCES

1) Kistler, W.S., Geroch, M.E., and Williams-Ashman, H.G. (1973) J. Biol. Chem. 248, 4532-4543.
2) Kistler, W.S., Noyes, C., and Heinrikson, R.L. (1974) Biochem. Biophys. Res. Commun. 57, 341-347.
3) Johns, E.W. (1971) Histones and Nucleohistones (edit. by Phillips, D.M.P.) pp. 2-46, Plenum Press, London.
4) DeLange, R.J., and Smith, E.L. (1971) Annu. Rev. Biochem. 40, 279-314.
5) Hnilica, L.S. (1972) The Structure and Biological Function of Histones, CRC Press, Cleveland.
6) Olson, M.O.J., Starbuck, W.C., and Busch, H. (1974) The Molecular Biology of Cancer (edit. by Busch, H.) pp. 309-353, Academic Press, New York.
7) Louie, A.J., Candido, E.P.M., and Dixon, G.H. (1973) Cold Spring Harbor Symp. Quant. Biol. 28, 803-819.
8) Bonner, J., and Garrard, W.T. (1974) Life Sciences 14, 209-221.
9) De Nooij, E.H., and Westenbrink, H.G.K. (1962) Biochim. Biophys. Acta 62, 608-609.
10) Panyim, S., and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346.
11) Swank, R.T., and Munkres, K.D. (1971) Anal. Biochem. 39, 462-477.
12) Kinkade, J.M., Jr., and Cole, R.D. (1966) J. Biol. Chem. 241, 5790-5797.
13) Kinkade, J.M., Jr. (1969) J. Biol. Chem. 244, 3375-3386.
14) Bustin, M., and Cole, R.D. (1968) J. Biol. Chem. 243, 4500-4505.
15) Rall, S.C., and Cole, R.D. (1971) J. Biol. Chem. 246, 7175-7190.
16) Balhorn, R., Rieke, W.O., and Chalkley, R. (1971) Biochemistry 10, 3952-3959.
17) Lake, R.S., Goidl, J.A., and Salzman, N.P. (1972) Exptl. Cell Res. 73, 113-121.
18) Kabat, D. (1970) Biochemistry 9, 4160-4175.
19) Branson, R.E., Grimes, S.R., Jr., Yonuschot, G., and Irvin, J.L. (1973) Fed. Proc. 32, 588 Abs.