EFFECTS OF AGE AND HYPOPHYSECTOMY UPON RELATIVE PROPORTIONS OF VARIOUS HISTONES IN RAT TESTIS

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SUMMARY. Various histone fractions of seminiferous epithelial cells of the testis of rats change in relative proportions during development and maturation of the testis with increasing age after birth, and these changes in the histones occur in the reverse direction in testis of mature rats during involution of the testis resulting from hypophysectomy. The changes are particularly striking in histone sub-fractions, designated X_1, X_2, and X_3, which are especially characteristic of testis and may be involved in meiosis.

The nuclei of seminiferous epithelial cells of the testis of the mature rat contain, in addition to the five usual histone fractions, three histone sub-fractions (designated X_1, X_2, and X_3) which are not observed (except in traces) in nuclei of liver cells (1,2). Histone sub-fraction X_1 is related in amino acid content and size to histone F1, but the electrophoretic mobility of X_1 on polyacrylamide gels at pH 2.7 is less than that of F1 (1,2). Histone sub-fraction X_2 accompanies histone F3 in the fractionation by the method of Johns (3), and it has an electrophoretic mobility slightly less than that of F3. Sub-fraction X_3 is related to histone F2b, but its mobility at pH 2.7 is between that of F3 and F2b (1,2). We have reported (4) that the relative amounts of the different histone fractions change considerably during various stages of spermatogenesis in the mature rat, and the changes are particularly large in sub-fractions X_1, X_2, and X_3. The present study was performed to determine the changes in relative proportions of the histone fractions during development and maturation of the testis at various ages after birth for correlation with the data of others (5,6) concerning the time of appearance of different cellular types in the seminiferous tubules of the developing testis. Conversely, we have studied the changes in proportions of the histones during involution of the testis in hypophysectomized rats for correlation with the

histologic studies of Clermont and Morgentaler (7). This research constituted a portion of the doctoral dissertation of S. R. Grimes (University of North Carolina, 1973). Kistler and Geroch (8) have recently confirmed the presence in rat testis of the histone fraction which we have designated as X_1.

METHODS

Male Sprague-Dawley rats were used in the experiments on changes in testis histones at various ages after birth. Hypophysectomized, male Sprague-Dawley rats (200-250 g) were obtained from Zivic-Miller Co., Allison Park, Pa.. The rats were maintained on Purina Lab Chow, and the hypophysectomized rats received 0.9% NaCl in 5% sucrose as drinking water.

Nuclei of the seminiferous epithelial cells of the testis were isolated and purified, and the histones were extracted from the chromatin as described previously (2,4). Electrophoresis of the histones was performed on 15% polyacrylamide gels at pH 2.7 (15 cm. gels, at 1 mA per gel) by the method of Panyim and Chalkley (9) as applied in the previous papers (2,4). After electrophoresis the stained gels were scanned at 600 nm in a Gilford model 2000 recording spectrophotometer, and the amounts of the individual histone fractions relative to total histones were calculated from the photometric scans as described (4). The histone bands or peaks were designated as X_1, F1, X_2, F3, X_3, F2b, F2a2, F2ala, and F2alb in the order of increasing electrophoretic mobility (see Fig. 1). The trailing shoulder on the F1 peak observed in the testis histone patterns at 7 d and 14 d was designated as F1'. Fractions F3, X_3, and F2b were not adequately resolved by electrophoresis of total histones, and consequently the combined proportions of these fractions are presented as percent of the total histones. However, in view of the particular interest in fractions F2b and X_3, these histones were separated from the total histones by the method of Johns (3) as described previously (2), and F2b and X_3 were separated electrophoretically (2) for photometric scanning and calculation of the ratio X_3/F2b.

RESULTS AND DISCUSSION

Clermont and Perey (5) and Vernon et al. (6) have reported that spermatogonia of types A and B predominate and primary spermatocytes are absent from testes of rats at 7 days of age. At 14 d spermatogonia predominate (68% of cells), but leptotene + zygotene (14%) and pachytene + diplotene (2%) primary spermatocytes are present. At 22-26 d primary spermatocytes predominate, but approximately 12% of the cells are young spermatids (stages 1-7). These data can be used to interpret the changes in relative amounts of the histone fractions in relation to events in spermatogenesis.

The gel photographs and photometric scanning curves (Fig. 1 and Table 1) reveal that sub-fraction X_1 is present only in small proportion in the testis

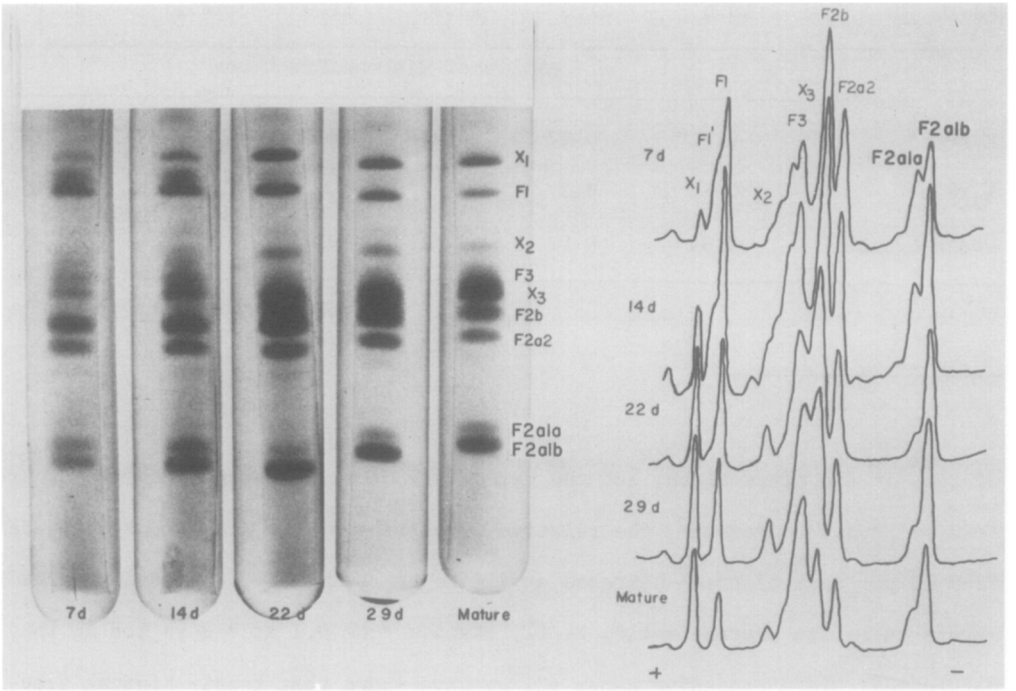

Fig. 1 Electrophoretic patterns of histones of seminiferous epithelial cells of the testis of rats at various time intervals (days) after birth. The photometric scans of the gels are shown on the right.

Table 1.

Average Relative Proportions of Various Electrophoretically Separated Histone Fractions of Nuclei of Testicular Seminiferous Epithelial Cells of Rats of Various Ages. (The data are averages obtained with 5 rats for each time period after birth).

Histone Fraction	Relative Proportions of Histone Fractions (Per Cent of Total Histones)					
	7 d	14 d	15 d	22 d	29 d	Mature
X_1	2.0	3.9	5.6	8.5	8.3	9.4
F1 + F1'	18.3	14.6	12.5	10.4	6.9	5.1
X_2	1.6	3.2	3.4	4.4	3.6	2.9
F3 + X_3 + F2b	45.9	48.8	49.2	52.6	54.0	54.7
F2a2	13.8	11.1	11.9	9.6	9.7	9.9
F2ala	8.5	6.8	5.1	2.6	3.4	5.2
F2alb	10.0	11.6	12.3	11.9	14.1	12.8
	Ratios of Histone Fractions					
X_1/F1	0.11	0.27	0.45	0.82	1.2	1.8
X_3/F2b	0	0.3	--	0.4	0.9	1.8
F2ala/F2alb	0.85	0.59	0.41	0.22	0.24	0.41

of rats at 7 d after birth, but the percentage of this histone increases four-fold by 22 d. Conversely, the relative proportion of the F1 histone decreases from 18 per cent of total histones at 7 d to approximately 5 per cent in the mature rat. The average ratio, X_1/F1, changes from 0.1 at 7 d to 1.8 in the mature rat. In a preceding paper (2) we have shown that testis histone fractions F1 and X_1 are closely related lysine-rich histones. The fact that the relative amount of X_1 increases while the relative proportion of F1 decreases suggests the possibility that F1 is converted into X_1, but this hypothesis

was not supported by data for the relative rates of incorporation of labeled amino acids into these histone fractions (10). Also, treatment of F1 and X_1 with alkaline phosphatase (2) did not provide any evidence that X_1 is a phosphorylated form of F1. However, the trailing shoulder on the F1 peak, designated in Fig. 1 as F1', apparently corresponds to phosphorylated forms of F1 since this shoulder disappears when the testis histones are treated with E. coli alkaline phosphatase prior to gel electrophoresis, and there is a corresponding increase in the principal F1 band. The F1' shoulder is prominent at 7 and 14 d, but it is present only in a trace at 22 d and is virtually absent at 29 d and in the mature rat (Fig. 1).

Histone fraction X_2 is scarcely detectable at 7 d, but this fraction increases in relative amount to a peak at 22-29 d and then declines to a smaller relative amount in the testis of the mature rat (Fig. 1). Histone fraction X_3 is not detectable in testis of the 7 d rat, but this fraction is found at 14 d where the ratio X_3/F2b is 0.3 (Fig. 1 and Table 1). This ratio rises progressively to a value of 1.2 at 29 d and 1.8 in the mature rat. Previous work (2) has shown that histone fraction X_3 is not a phosphorylated form of F2b.

Previous studies by Candido and Dixon (11) and Grimes et al. (10) have shown that acetylated species of histone fraction F2a1 are found in spermatogonia and primary spermatocytes and later in spermatids in the testis of the trout and the rat. In Fig. 1 the acetylated species, F2a1a, is the trailing member of the F2a1 doublet and F2a1b is the leading (non-acetylated) member. The ratio F2a1a/F2a1b is high at 7 d, declines to a minimum at 22 d, and then rises again in the mature rat (Table 1).

In summary, the spermatogonial stages, which are characterized by a high rate of mitosis and cell division, exhibit a high degree of phosphorylation of histone F1 and a high degree of acetylation of histone F2a1. At this stage histones X_1 and X_2 are present only in relatively low amounts. At 14 d when the pre-meiotic phases of primary spermatocytes (leptotene, zygo-

Table 2.

Average Relative Proportions of Various Electrophoretically Separated Histone Fractions of Nuclei of Testicular Seminiferous Epithelial Cells of Rats 29 and 44 d after Hypophysectomy

Histone Fraction	Relative Proportions of Histone Fractions (Per Cent of Total Histones)		
	Normal	29 d	44 d
X_1	11.8	6.7	5.9
F1 + F1'	6.2	10.9	12.8
X_2	3.1	2.9	1.8
$F_3 + X_3$ + F2b	50.1	52.2	51.7
F2a2	10.3	8.4	8.2
F2ala	5.4	4.5	4.3
F2alb	13.1	14.4	15.3
	Ratios of Histone Fractions		
X_1/F1	1.9	0.61	0.46
X_3/F2b	1.9	0.83	0.7
F2ala/F2alb	0.41	0.31	0.28

tene, pachytene, diplotene) are appearing, histone fraction X_3 is observed, and there are increases in the relative amounts of X_1 and X_2. Fraction X_2 reaches a peak at 22-29 d when young spermatids are beginning to appear, and fractions X_1 and X_3 continue to increase in relative amounts as the testis matures. Acetylation of histone F2a1 again becomes prominent in the spermatid stages (10,11). These data and conclusions are in agreement with our previous studies on changes in histones during spermatogenesis in the mature rat (4).

After hypophysectomy there are rapid decreases in relative numbers of all stages of spermatids and mature spermatocytes between 7 and 28 d after

surgery, and the relative proportions of the different germinal cells become constant by 35 d (7). In the regressed, hypophysectomized rat spermatogonia are the most numerous of the cell types in the seminiferous tubules, but approximately 9% of the cells are pachytene primary spermatocytes and 2% are early spermatids (7). The data of Table 2 indicate that after hypophysectomy the changes in histone fractions of the seminiferous epithelial cells of the testis are the opposite of the changes which occur in the developing rat, viz. the relative amount of X_1 declines while that of F1 increases; X_2 declines; the ratios X_3/F2b and F2ala/F2alb decrease.

The rapid increases of histone fractions X_1, X_2, and X_3 during the prophases of the first meiotic division in the developing rat testis (and their decline in the regressing testis) suggest that these histone fractions may play roles in meiosis. Their involvement in synapsis of the chromosomes is an intriguing but untested hypothesis. Involvement of these fractions in repression of DNA synthesis in primary spermatocytes is also a possibility.

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