Isolation and Characterization of TH2A, a Germ Cell-specific Variant of Histone 2A in Rat Testis*

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The histones of mammalian testis nuclei are much more complex than those of somatic tissue nuclei, and their resolution into variants can be greatly improved by polyacrylamide gel electrophoresis in the presence of Triton X-100. A previously unidentified germ cell-specific variant, TH2A, has been isolated in pure form and characterized by analysis of its amino acids and tryptic peptides as a variant of H2A. The electrophoretic mobility of TH2A is similar to H2A.1 on Triton-acid-urea and sodium dodecyl sulfate gels, but more like H2B on acid-urea gels. TH2A first appears in the testis of 16-day-old rats, a time that coincides with the appearance of primary spermatocytes in the maturing testis, and with further germ cell development the level of TH2A increases. Synthesis of testis histones, as measured by tritiated amino acid incorporation into histones in vivo, was studies using highly purified pachytene spermatocytes (98%) and early spermocytes (97%) obtained by elutriation and Percoll density gradient centrifugation. Negligible synthesis of any histone was detectable in the early spermatids. In contrast, high rates of synthesis of TH2A, as well as the somatic forms, H2A.1 and H2A.2, were observed in pachytene spermatocytes. Thus, it is apparent that TH2A synthesis in pachytene spermatocytes is not solely for replacement of the somatic H2A forms by a germ cell-specific variant in chromatin.


It is now well established that the basic structural unit of chromatin is a DNA-histone complex called the nucleosome (reviewed by Kornberg, 1977). The primary role of histone in the nucleosome is structural, but recently, histone modifications have been shown to be associated with chromatin function, such as transcription. For example, highly acetylated histones are preferentially associated with nucleosomes that contain DNA of active genes and are the most sensitive to nuclease digestion (Allfrey, 1977). Furthermore, following the discovery of histone variants (Franklin and Zweidler, 1977), it has been postulated that substitution of different histone variants could result in nucleosomes that differ in their structures or functions (Albright et al., 1980). During mammalian spermatogenesis, cells display an exceptionally wide variety of histone variants sequentially expressed in various stages, making it an excellent system for studying the role of histone variants in chromatin structure.

Spermatogenesis is the process by which a spermatogonial stem cell gives rise to a mature spermatozoon. During spermatogenesis, several interesting chromosomal events take place, including chromosome pairing, meiotic divisions, chromatin condensation, and nuclear elongation (Clermont, 1972). Parallel to these morphological changes, there are massive biochemical changes in the chromosomal proteins that involve several complete replacements of the basic nuclear proteins; including new germ cell-specific histones and, after meiosis, new sets of spermatid- and sperm-specific basic proteins.

DNA synthesis occurs only in spermatogonia and preleptotene spermatocytes. There do not appear to be any testis-specific histones present in these stages, only histone variants normally found in somatic cells (Grimes et al., 1975a). The cells then enter the meiotic prophase; at the zygote step, there is pairing of homologous chromosomes and shortening and thickening of the chromosomes. Genetic recombination occurs in the pachytene spermatocytes and the chromatin stays in this condition for a long period of time, during which the nuclear volume progressively increases. The amounts of testis-specific histone variants, TH1 and TH2B, are increasing at this time relative to their somatic counterparts (Brock et al., 1980). This stage is followed by the short diplote step, during which the homologous chromosomes separate except at points where crossing over has occurred. Finally, the nucleus goes through meiotic division to yield secondary spermatocytes, which have a short life span, and without duplicating their DNA, they enter the second maturation division.

The newly formed haploid spermatids are characterized by a small spherical nucleus, no change in the histone complement from the pachytene spermatocyte stage (Brock et al., 1980), and a decreasing rate of transcription. In Steps 9 to 11 of spermatid maturation, nuclear elongation occurs, condensation of the chromatin begins, and RNA synthesis (Soderstrom and Parvinen, 1976) is turned off. The nuclei of elongated spermatids (Steps 13 to 19) have the shape characteristic of the mature sperm, are highly condensed, and resistant to sonication. During these stages, the histones are replaced by

*This investigation was supported in part by Grants PCM-78-05997 from the National Science Foundation and CA-06294 from the National Cancer Institute, Department of Health, Education and Welfare. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

(Received for publication, November 30, 1981)
TP spermatid proteins (Grimes et al., 1975c). The nuclei of the mature spermatozoa condense into a very tight inactive mass and the TP proteins are replaced by the mammalian sperm protein S1 (Kistler et al., 1973; Grimes et al., 1977).

Several of the testis-specific histones in the rat, TH1, TH2B, and X2, have already been described (Branson et al., 1975; Shires et al., 1975) and are easily identifiable on acid-urea polyacrylamide gels (Panyim and Chalkley, 1969). The discovery of additional somatic histone variants has been reported using a Triton X-100-acid urea polyacrylamide gel system (Franklin and Zweidler, 1977; Zweidler, 1978), and using this system we have identified an additional variant of H2A in the rat testis. The biochemical characteristics, developmental distribution, and synthesis of this H2A variant during spermatogenesis are reported in this manuscript.

**EXPERIMENTAL PROCEDURES**

**Source of Animal Tissue**

Mature, 200- to 250-g Sprague-Dawley male rats were obtained from Harlan Sprague-Dawley, Madison, WI, and 8-, 12-, 15-, and 20-day-old immature animals were obtained from Timco Breeding Laboratories, Houston, TX. Histology of the immature testes showed that the nongerminal cells vastly outnumbered the germinal cells in 8- and 12-day-old animals and that the most advanced stages of germ cell development in the immature animals were early type A spermatogonia on day 8, late type A spermatogonia on day 12, zygotene spermatocytes on day 16, and late pachytene spermatocytes on day 20.

**Animal Treatments**

Radioactive Labeling—To measure incorporation of radioactive amino acids into histones, each mature rat was labeled by intratesticular injection of 50 µCi of L-[4,5-3H(N)]lysine (specific activity 60 to 80 Ci/mmol, New England Nuclear) plus 50 µCi of L-[2,3-3H]arginine (specific activity 15 to 30 Ci/mmol; New England Nuclear) in a volume of 100 µl given 90 min before they were killed. For histone acetylation experiments, rat testes were injected with cycloheximide and sodium [3H]acetate according to the method of Grimes et al. (1975b).

Sterilization of Rats by Irradiation—Two treatments of 1300 rad each of 60Co γ-irradiation were given locally to the testes 1 week apart to sterilize mature rats. The animals were killed 7 days after the last dose of XRT.1 Histological analysis confirmed that the testes were devoid of germ cells, and only somatic cells remained.

**Preparation of Rat Nuclei**

Nuclei from various tissues and cells were prepared according to the method of Platz et al. (1977), except that 0.4 M phenylmethylsulfonyl fluoride in 100% methanol was added to the cell lysing solution for a final concentration of 1 mM.

**Extraction and Fractionation of Histones**

Acid extraction of histones from purified nuclei was performed according to the method described by Platz et al. (1977). Histones from adult rat testes nuclei were separated into lysine-rich and arginine-rich fractions by differential extraction of nuclei according to the method of Johns (1964), as modified by Brock et al. (1980).

**Preparation of Highly Purified Cells**

Single cell suspensions of testes that had been injected with [3H]lysine and [3H]arginine were prepared with trypsin, as previously described (Meistrich, 1977). The cells were first separated by centrifugal elutriation, followed by density separation on Percoll gradients (Meistrich et al., 1982). In the purified fractions, pachytene spermatocytes represented 98% and early spermatids represented 97% of the nucleated cells. Cells were washed in phosphate-buffered saline before resuspension in lysing solution for nuclei preparation.

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1 The abbreviations used are: XRT, x-ray-treated; SDS, sodium dodecyl sulfate; BIS, N,N'-methylenebisacrylamide; TEMED, N,N,N',N''-tetramethylenediamine.
phoretically eluted through a 2-cm plug of acrylamide (12% acrylamide, 2.5 M urea) into a dialysis bag; this electrophoresis of the protein through the plug also served to separate the protein from the Triton X-100. The eluted protein was then dialyzed against 0.9 N acetic acid and lyophilized.

One-dimensional Sodium Dodecyl Sulfate Gels—SDS slab gels containing a gradient of 8% to 20% acrylamide with a 3% stacking gel were run using the buffer system of Laemmli (1970).

Peptide Mapping

The purified protein sample was dissolved in 0.25 N HCl, trypsinized, and the peptides were mapped by the two-dimensional (electrophoresis and thin layer chromatography) procedure of Bremer et al. (1981), except the reduction and carboxymethylation procedure was omitted.

Amino Acid Analysis

The protein was dissolved in 500 μl of constant boiling HCl (Pierce Chemical Co.) and hydrolyzed in vacuo at 105 °C for 20 or 70 h. Samples were then lyophilized from deionized, distilled water three times. They were finally dissolved in 300 μl of sodium citrate buffer (pH 2.2; Pierce) prior to analysis and 100-μl samples were used for injection. Samples were analyzed on a Glenco "60 single column amino acid analyzer, supplied with a cation-exchange resin (HP-C; Bio-Rad), and eluted with sodium citrate buffers (Pico Buffer System II; Pierce). Amino acids were monitored simultaneously at 440 and 570 nm using ninhydrin (Pierce) as the detecting agent.

RESULTS

Identification of Testis Histones on Triton Gels—To identify the histones of rat testis on the Triton X-100-acid-urea polyacrylamide gel system we used a two-dimensional electrophoresis procedure, which employed an acid-urea cylindrical gel in the first dimension and a Triton-acid-urea slab gel in the second dimension. Two-dimensional gels of somatic and testis histones, arginine-rich and lysine-rich fractions of testis histones, and fractions of testis histones further purified on Amberlite CG-50 (Bonner et al., 1968) were used for positive identification of testis histones in Triton gels. Electrophoresis of mouse somatic histones in 6 mM Triton-acid-9 M urea gels (Zweidler, 1978) corresponded to our identification of the rat somatic histones in this system. The polychromasia of histones stained with Amido black (that is, blue staining of arginine versus black staining of lysine-rich histones) also helped verify the correlation of the histone migrations between the acid-urea and the Triton gel systems.

The two-dimensional gel shown in Fig. 1 was not properly reduced before electrophoresis of the histone because it is not possible to prerun the stacking gel that holds the first dimensional gel in place. Therefore, the pattern shown contains oxidized histones. Elimination of histone oxidization requires a fully reduced stacking and running gel, as described under "Experimental Procedures." H2A.2, TH2B, and TH3 are the histones most susceptible to oxidation, which affects their mobility in Triton gels. The mobilities of these proteins in oxidizing gels was confirmed by purposely oxidizing histone samples with hydrogen peroxide (Blankstein et al., 1977).

The electrophoretic pattern of testis histones in a totally

![Fig. 1. Electrophoresis patterns of rat histones extracted with 0.25 N HCl. A, fully reduced one-dimensional Triton-acid-9 M urea gel of testis histones. Migration is from top to bottom. B, two-dimensional gel (unreduced) of testis histones. Migration in the first (horizontal, acid-urea) dimension is from left to right. The labels across the top designate the somatic histones. Migration in the second (vertical, Triton X-100) dimension is from top to bottom.](http://www.jbc.org/)

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reduced Triton gel is shown in Fig. 1A. In the control lanes of
the second dimensional gel in Fig. 1B, the relatively simple
electrophoretic pattern for somatic histones (rat brain tumor,
RT489) can be compared to the more complex pattern for
testic histones in Triton gels. The different electrophoretic
mobilities of H2A.2, TH2B, and TH3 in the reduced versus
oxidized gels are apparent.

Identification of the H2A Variants—To rule out that
TH2A was an acetylated modification of H2A, histones were
extracted from nuclei that had been prepared following [3H]
acetic acid injection of rat testes (Grimes et al., 1975b). The
radioactivity profile of the histones separated by electropho-
resis on Triton cylindrical gels showed no label in TH2A,
while there was extensive acetylation on Triton gels. The different electrophoretic
patterns for somatic histones (rat brain
tumor, RT489) can be compared to the more complex pattern for

Table 1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>H2A.1</th>
<th>H2A.2</th>
<th>TH2A</th>
<th>X2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>9.8</td>
<td>11.0</td>
<td>7.4</td>
<td>10.2</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.2</td>
<td>2.3</td>
<td>3.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Arginine</td>
<td>8.2</td>
<td>8.8</td>
<td>8.6</td>
<td>7.8</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>7.4</td>
<td>6.7</td>
<td>6.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.0</td>
<td>2.9</td>
<td>3.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Serine</td>
<td>6.4</td>
<td>3.6</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>10.8</td>
<td>11.2</td>
<td>11.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Proline</td>
<td>4.4</td>
<td>4.6</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>11.2</td>
<td>10.7</td>
<td>12.1</td>
<td>11.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>10.2</td>
<td>10.7</td>
<td>10.8</td>
<td>13.8</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Valine</td>
<td>5.4</td>
<td>5.1</td>
<td>5.6</td>
<td>7.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>(0.2)</td>
<td>0.8</td>
<td>(0.3)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.0</td>
<td>5.0</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Leucine</td>
<td>11.0</td>
<td>13.0</td>
<td>10.5</td>
<td>9.6</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3.0</td>
<td>2.8</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.8</td>
<td>1.1</td>
<td>1.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Methionine was detected in only one of the two samples.
A Germ Cell-specific Variant of Histone 2A

Histone patterns (H2A region) of various rat tissues and mouse testis separated in a fully reduced Triton gel that underwent electrophoresis for 72 h. Rat testis histones are labeled on the left.

Histone patterns (H2A region) of various rat tissues and mouse testis separated in a fully reduced Triton gel that underwent electrophoresis for 72 h. Rat testis histones are labeled on the left.

FIG. 4. Basic nuclear proteins extracted from testes of various age rats or from the liver. The separation is by one-dimensional Triton gel electrophoresis (partially reduced). Histones seen in both somatic and testis nuclei are labeled on the left and histones that are highly enriched or only seen in the testis are labeled on the right.

The developmental appearance of germ cell basic nuclear proteins was studied by comparing electrophoretic patterns of histones from nuclei of immature and adult testes. Fig. 4 shows this Triton gel, which was only partially reduced by a cysteamine prerun. Sterilized testes, which contain only somatic cells, have the same protein pattern as the liver, which serves to distinguish which histones are contributed by the germinal cells of immature and adult testes. Unirradiated adult testes contain testis-specific histones as well as somatic histones. Immature testes from 8- and 12-day-old rats do not contain TH2A and TH2B, but these two histones begin to appear in the 16-day-old rat, and their levels increase until maturity. The first appearance of TH2A and TH2B in the testes of 16-day-old rats coincides with the appearance of the first pachytene spermatocytes. An increase of X2 in the testis can be seen as early as 8 days, with a progressive accumulation with time until it reaches a peak on day 20. TH1 and TH3 are present in the 8-day-old rat testis and continue to increase until maturity. The testis-specific or testis-enriched variants TH2A, TH3, TH1, and TH2B increase with the maturity of the testis, and during the same time span their respective somatic forms decrease. The relative amounts of the testis histones from 16- and 20-day-old rats, and from purified pachytene spermatocytes (98%) and early spermatids (97%), were quantitated and compared (Table II). It is significant that the XRT-sterilized mature testes contain no TH2A and very little X2. The changes observed between days 16 and 20 parallel the increase in the number of pachytene spermatocytes. TH2A accumulation is further substantiated by the even higher level in isolated pachytenes.

It had been observed that when gels are run 40 h or longer to resolve completely the individual H2As, there is a secondary protein band following each of the H2As. These secondary bands are found in high proportion in early spermatids; they are essentially absent from pachytene spermatocytes. In Fig. 5, they are designated by the vertical dashed lines. We have not yet characterized these bands, but the possibility exists that they are phosphorylated modifications of each of the

examined; however, their relative proportions varied noticeably, a result consistent with previous observations (Zweidler, 1976; Blankstein and Levy, 1976; Franklin and Zweidler, 1977; and Russanova et al., 1980). In all of the adult testis tissues studied, H2A.2 is a minor histone except for XRT-sterilized testis, which contains only somatic cells. X2 was highly enriched in the testes in all species we studied, but it was also present in low levels in somatic tissues. TH2A is rat testis-specific; it is not detectable in rat somatic tissues or in the testes of any other species we examined.
A Germ Cell-specific Variant of Histone 2A

TABLE II
Quantitation of testis H2A proteins from Triton-acid-urea gels

The values of individual H2A proteins are expressed as percentages of total H2As and total H2A as the percentage of total H4.

<table>
<thead>
<tr>
<th>Histone</th>
<th>XRT</th>
<th>16-day</th>
<th>20-day</th>
<th>Adult</th>
<th>Purified cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pachytene</td>
</tr>
<tr>
<td>X2</td>
<td>4.4</td>
<td>13.3 ± 1.1</td>
<td>22.8 ± 0.3</td>
<td>17.0 ± 0.8</td>
<td>16.9</td>
</tr>
<tr>
<td>TH2A</td>
<td>0</td>
<td>4.7 ± 0.2</td>
<td>12.2 ± 0.1</td>
<td>24.5 ± 0.4</td>
<td>25.3</td>
</tr>
<tr>
<td>H2A.1</td>
<td>23</td>
<td>60.5 ± 0.8</td>
<td>48.1 ± 0.9</td>
<td>44.0 ± 0.9</td>
<td>40.8</td>
</tr>
<tr>
<td>H2A.2</td>
<td>72.0</td>
<td>21.3 ± 0.3</td>
<td>16.8 ± 0.7</td>
<td>14.5 ± 1.0</td>
<td>16.9</td>
</tr>
<tr>
<td>H2A/H4 × 100</td>
<td>103</td>
<td>99.4 ± 0.5</td>
<td>91.8 ± 5.5</td>
<td>106.4 ± 5.5</td>
<td>106</td>
</tr>
</tbody>
</table>

* Data are from one 28.5-h electrophoresis sample on one slab gel of 9 M urea, 15% acrylamide, and 0.37% Triton.

* Data are from one 46-h electrophoresis sample on three cylindrical gels of 8 M urea, 12% acrylamide, and 0.37% Triton.

* Data are from three samples, each undergoing electrophoresis on three cylindrical gels of 8 M urea, 12% acrylamide, and 0.37% Triton for 46 h.

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Fig. 5. Protein (---) and radioactivity (- - -) profiles of the histones (H2A region) of the total cell suspension, purified pachytene spermatocytes, and purified early spermatids, electrophoresed for 40 h in fully reduced cylindrical one-dimension Triton-acid-8 M urea gels. Radioactivity data is the average of at least three counts of 20 min each for each slice. Based on a background of 27 cpm and an efficiency of 25%, the 95% confidence limits for the radioactivity in each slice is less than 1.5 cpm or 6 dpm.

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Fig. 6. Same as for Fig. 5, except electrophoresis occurred for 20 h in partially reduced gel and the profiles include all the histones. In this figure, TH2A is designated X9.

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Major H2As, since, except for H1, H2A typically has the highest level of phosphorylation, and phosphorylated forms migrate slower than the original forms (Sherod et al., 1970; Dolby et al., 1979).

Histones from highly purified pachytene spermatocytes and early spermatids were prepared from rat testis following injection of tritiated lysine and arginine. The proteins were separated by electrophoresis for either 20 or 40 h in Triton gels, and in vitro incorporation of labeled lysine and arginine histones was examined. Fig. 6 shows the results from the 20-h gel, which shows high rates of incorporation of the TH2A, H2A.1, H2A.2, TH1, and TH2B in pachytene, while in early spermatids negligible incorporation is detectable. The radioactivity profiles indicate there are differences in the rates of incorporation of the different histone classes in the pachytene spermatocytes. The rates of incorporation of radioactivity into the histones TH2B, TH1, and H4 were, respectively, 2.2, 2.1, and 0.16 times the total H2A incorporation. The lysine-rich histones most actively incorporate labeled precursors. Since equal amounts of [3H]lysine and [3H]arginine were used, the differences in incorporation reflect probable differences in rates of synthesis. Even if, in the most extreme case, only [3H]lysine was incorporated, the differences between the lysine contents of the histones are not sufficiently large to account for the differences in incorporation rates. Thus, it appears that the incorporation of lysine and arginine into TH1, TH2B,
and the H2A variants accounts for over 95% of pachytene histone radioactivity. Gels run for 40 min in which the individual H2As were well resolved are shown in Fig. 5, and in this case the incorporation into all variants appears to occur. The specific activities of the variants (relative to TH2A) were 1.0, 0.7, and 0.6 for H2A.1, H2A.2, and X2, respectively. On the longer gel, the peak of radioactivity coincident with the X2 protein band is probably a combination of incorporation by X2 and a co-migrating protein; on the short gel there was not a distinct peak of radioactivity associated with X2. In addition, when nuclei were salt-treated (West and Bonner, 1980) to remove non-histone proteins prior to extraction of histones, the H2A protein profile on a long gel was essentially the same, but the specific activity of X2 was greatly reduced (0.3) relative to TH2A (data not shown).

**DISCUSSION**

We have identified and purified a testis-specific histone, TH2A, from the rat, which by amino acid composition and peptide maps is shown to be a variant of H2A, and, therefore, we conclude that TH2A is the product of a unique H2A gene. This new protein band identifiable in Triton gels is not only testis-specific but, as shown in immature and irradiated rats, is germ cell-specific. It is primarily synthesized in pachytene spermatocytes and its synthesis is essentially turned off by the early spermatid stage of germ cell development. Although TH2A increases with the appearance of pachytene spermatocytes in the testis, while the somatic forms of H2A decrease, the rates of synthesis of TH2A, H2A.1, and H2A.2 at this stage are all high. This result suggests that the H2A synthesis in pachytene is not solely for replacement of the somatic forms by a germ cell-specific variant. TH2A is associated with nucleosomal core particles, based on fractionation of chromatin from micrococcal nuclease-digested nuclei. The S3 fraction of Levy and Dixon (1978) that contains about 90% mononucleosomes contains TH2A in the same relative proportion to the other core histones, as it does in whole chromatin (data not presented).

Additionally, we have purified and performed amino acid analysis on X2, a major testis chromosomal protein first described by Branson *et al.* (1975). Mills *et al.* (1977) assumed that X2 in the rat testis was an H2A variant based only on histone stoichiometry. Our amino acid analysis of purified X2 confirms that it is, indeed, a variant of H2A. A protein with a similar electrophoretic mobility to X2 in acid-urea and Triton gels has been described in rats and other species. This protein was designated M2 in mouse and rat tissues (Zweidler, 1978) and chicken erythrocytes (Urban *et al.*, 1979) and H2A.X in mouse L1210 leukemia cells (West and Bonner, 1980). As we have observed for X2, the mobility of H2A.X on SDS gels is slightly slower than H2A.1 and H2A.2 (West and Bonner, 1980). Therefore, we conclude that X2, M2, and H2A.X are most likely the same protein. X2 is found in all rat tissues we have analyzed. However, the fraction of the total H2A protein that is X2 is highest in the testis (Fig. 3); hence, we refer to it as a testis-enriched histone. The enrichment of X2 in the testis increases in the maturing animals until it reaches its peak in the 20-day-old animal (Fig. 4) (Grimes *et al.*, 1975a; Mills *et al.*, 1977), and X2 levels are extremely low in XRT testes. Thus, the X2 concentration is very low in non-germinal cells, highest in spermatogonia and early primary spermatocytes, and intermediate in pachytene spermatocytes and early spermatids.

The developmental changes observed in the H2A variants during rat spermatogenesis parallel changes in the other histones. These include TH2B, a testis-specific variant of H2B; the TH1 band, which is made up of two proteins; Hlt and Hla (Seyedin and Kistler, 1980); and TH3, the testis-specific form of H3, which are now characterizing. In all cases, along with the formation of spermatocytes the somatic forms H2A, H2B, H1, and H3 simultaneously decrease as the testis-specific variants TH2A, TH2B, Hlt, and TH3 increase; H4 is invariant. However, the degree of replacement is different for different histones. TH2A accounts for only 25% of total H2A in the purified pachytene spermatocytes or spermatids, while X2 accounts for 20% and H2A.1 and H2A.2 account for 55%. In contrast, TH2B accounts for about 90% of the total H2B protein (Fig. 6). The situation with H1 is intermediate; the testis-specific form, Hlt, accounts for 55% of total H1, the testis-enriched form, Hla, accounts for 30%, and the somatic forms, Hlb,c,d,e, for 15% (Bucci *et al.*, 1982). We surmise the regulation of the replacement process must be complex.

The testis-specific forms, TH2A, TH2B, and Hlt, are all synthesized in the pachytene stage. However, while the synthesis of H2B and somatic H1 in pachytene is negligible, somatic histone synthesis in the form of H2A.1, H2A.2, and H4 does take place. Thus, the switch in gene expression during the primary spermatocyte stage from somatic to testis-specific forms is incomplete and is specific to certain histones.

X2, the testis-enriched form of H2A, reaches maximum levels at about day 20, which parallels the level of Hla, the testis-enriched form of H1 (Seyedin and Kistler, 1980). The levels of Hla are highest in the spermatogonia and/or early primary spermatocytes (Bucci *et al.*, 1982). Thus, the two testis-enriched forms appear to be regulated coordinately and are maximal at the earliest stages of spermatogenesis.

Spermatocyte-synthesized histones must be stable because no histone synthesis occurs during spermiogenesis (the development of the early spermatid into the spermatozoon) and all histone species are present in round spermatids. Both the somatic and testis-specific histones are lost from the spermatid nucleus during nuclear condensation and elongation, and are replaced by a set of low molecular weight basic proteins, designated TP proteins (Grimes *et al.*, 1977).

The biological role of these histone changes in the spermatocyte stage is as yet unknown. The result of these changes most likely contributes to the alterations in chromatin structure necessary for the various events associated with meiosis or spermiogenesis. Possible roles include genetic recombination, reprogramming the genome to allow the synthesis of many testis-specific enzymes and structural proteins, the initial steps in nuclear condensation, and allowing replacement by spermatidial basic proteins.

Acknowledgments—We are grateful to Dr. Ira R. Goldknopf, Department of Pharmacology, Baylor College of Medicine, for supplying...
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us with purified protein A24 and ubiquitin. Marcia Williams for technical assistance, Rozanne Goddard and Ann McCarver for typing this manuscript. Animals used in this study were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards of the United States Department of Agriculture and Department of Health, Education and Welfare, National Institutes of Health.

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