SPERMATOGENESIS IN THE MOUSE

I. Autoradiographic Studies of Nuclear Incorporation and Loss of $^3$H-Amino Acids

J. F. MAYER, Jr. and B. R. ZIRKIN

From the Division of Reproductive Biology, Department of Population Dynamics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

ABSTRACT

Autoradiographic and electron microscope methods were used to correlate changes in nucleoproteins with nuclear fine structure during spermatogenesis in the mouse. Testes were fixed at daily intervals after intratesticular injection with labeled amino acid. $[^3]$H]Arginine, lysine, valine, and proline were rapidly incorporated into primary spermatocyte nuclei, retained through subsequent spermatocyte divisions and through spermatid differentiation to step 12 of spermiogenesis, but were lost with spermatid differentiation beyond step 12. Arginine and lysine (not valine or proline) also were rapidly incorporated into certain elongated spermatid nuclei but differed strikingly in their distribution and fate. Nuclei of late step-12 through step-15 spermatids were initially labeled with arginine. This label was retained through subsequent spermatid differentiation and sperm maturation in the epididymis. By contrast, lysine was initially incorporated only into late step-12 and step-13 spermatid nuclei, and was retained only to early step 14 of spermiogenesis. Spermatid incorporation of lysine coincided with the initiation of chromatin condensation in late step-12 nuclei, and loss of lysine coincided with the completion of condensation in step-14 nuclei.

KEY WORDS spermatogenesis · nuclei · nucleoprotein · autoradiography · ultrastructure

In eutherian mammals, the somatic histones present in nuclei of cells in early stages of spermatogenesis are ultimately replaced by protamines, highly basic proteins rich in arginine and cysteine (1, 2, 14, 23). Replacement of the histones involves a series of nuclear protein changes rather than a direct replacement of one protein by another. For example, recent biochemical studies of isolated testicular cells of the rat have demonstrated that at least three histonelike proteins first appear in primary spermatocyte nuclei (4, 9, 13, 17, 27) but are not retained in late spermatid nuclei (10). These results are consistent with previous autoradiographic studies which have demonstrated the uptake of $^3$H-amino acids into pachytene spermatocyte nuclei of mouse (12, 22), rat (28), and ram (19). Biochemical (3, 8-11, 14, 15, 17, 18, 20, 26, 27) and autoradiographic (12, 19, 22) studies also have indicated that a number of new basic nucleoproteins are synthesized in mammalian spermatids. In the rat, only one of these spermatid proteins, protamine, is retained through spermiogenesis and sperm maturation in the epididymis (10, 14). As yet, however, the exact stages of spermatogenesis in which basic protein replacements occur and in which transient proteins are lost have not been determined. Ad-
ditionally, the relationship of basic protein changes to the morphology of the nuclei in which they occur is not known.

MATERIALS AND METHODS

Tissue Preparation

Sexually mature Swiss albino mice (25-30 g) were injected intratesticularly (0.02 ml) with 20-μCi [3H]arginine (sp act, 23 Ci/mM), [3H]lysine (sp act, 60 Ci/mM), [3H]proline (sp act, 60 Ci/mM), or [3H]valine (sp act, 11.5 Ci/mM) (New England Nuclear, Boston, Mass.). In some experiments, the initial injection with labeled amino acid was followed by an additional injection (chase) containing 1,000-fold higher concentration of unlabeled amino acid. The results were identical with or without a cold chase, and the data shown in this paper were obtained from experiments without a chase. At various intervals (2 h-23 d) after injection, testes and epididymides were fixed for 1.5 h at 4 °C or 23 °C with 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. The resulting samples were then washed several times with cacodylate buffer, postfixed in 1% osmium tetroxide for 1 h, dehydrated in ethanol, and embedded in Epon-Araldite.

Autoradiography

Serial sections (0.5, 1.0, and 1.5 μm) were cut with an ultramicrotome (Sorvall MT1 DuPont Instruments-Sorvall, DuPont Co., Newtown, Conn.), mounted on subbed slides, and dipped in Kodak NTB2 emulsion. After exposure for 1, 2, 3, 4, or 8 wk at 4 °C, the slides were developed in Kodak D-19 (4 min at 19 °C) and fixed in Kodak Ektathio (7 min). After they were washed and dried, the sections were either stained with 1% toluidine blue in 1% borax or viewed unstained under a phase-contrast microscope. Specific stages of spermatogenesis were determined by cell association criteria established for the mouse by Oakberg (24).

To determine whether the grains seen in autoradiographs represented label incorporated into proteins, [3H]arginine, [3H]lysine, or [3H]proline was injected into control (untreated) testes or testes which had received prior (0.5 h earlier) treatment with cycloheximide (Boehringer Mannheim Biochemicals, Indianapolis, Ind.: 100 μg in 0.02 ml). 2 h after injection with a given amino acid, testes were homogenized in 0.15 M Tris buffer (pH 8.0) containing 1 M NaCl, 0.1 M 2-mercaptoethanol, 6 M urea, and 1% Triton X-100 (21). The samples were then centrifuged at 20,000 g for 20 min to pellet DNA and insoluble debris. The resulting supernates were treated (0 °C, 20 min) with an equal volume of 20% TCA to precipitate proteins. Incorporation of label into TCA-soluble and TCA-insoluble fractions of testis homogenates was then measured in the control and cycloheximide-treated testes.

Grain counts were performed on autoradiographs exposed for 1-2 wk. At least 50 randomly selected nuclei were counted for each cell type. Grains were counted over spermatocyte or round-spermatid nuclei showing maximal cross sections, and over elongated and mature spermatids showing full sagittal sections.

RESULTS

Incorporation into Protein

With each amino acid tested, >97% of the total radioactivity of the testis was found in TCA-insoluble fractions of homogenized testes (Table I). These results indicated that labeled amino acids were incorporated into protein. Additionally, when testes were treated with cycloheximide be-

| Treatment* | [3H]Arginine | [3H]Lysine | [3H]Proline |
|------------|--------------|------------|------------|
| No inhibitor | 913 ± 225$ | 7,798 ± 2,909 | 2,061 ± 362 |
| TCA soluble | 142,491 ± 54,268 | 232,272 ± 82,042 | 112,508 ± 45,081 |
| TCA insoluble | 2,572 ± 593 | 13,048 ± 1,341 | 8,264 ± 1,176 |

* 2 h after injection with a given 3H-amino acid (20 μCi in 0.02 ml), testes were homogenized on 0.15 M Tris buffer (pH 8.0) containing 1 M NaCl, 0.1 M 2-mercaptoethanol, 6 M urea, and 1% Triton X-100. The samples were then centrifuged at 20,000 g for 20 min to pellet DNA and insoluble debris. The resulting supernates were treated (0 °C, 20 min) with an equal volume of 20% TCA to precipitate proteins. TCA-soluble and -insoluble fractions were assayed by scintillation counting.

$ All values represent an average of 3-5 determinations ± SEM.

§ Cycloheximide (100 μg in 0.02 ml) was injected 0.5 h before 3H-amino acid injection.
fore injection of label, the level of radioactivity in TCA-insoluble testis fractions was reduced by 75–99% (Table I). This further suggested that the label was incorporated into protein, although the possibility that the cycloheximide treatment might inhibit cellular amino acid uptake was not ruled out.

**Incorporation into Spermatocytes**

The initial incorporation of [3H]arginine, [3H]lysine, [3H]proline, and [3H]valine into spermatocyte nuclei, and the subsequent appearance of these amino acids in nuclei of successive stages of spermatogenesis, were identical. In testes fixed 2 h after a single injection of any one of the four amino acids, primary spermatocyte nuclei were labeled, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a). Nuclei of cells fixed 2 h after injection, with pachytene spermatocytes labeled most heavily (Figs. 1A–D and 2a). Nuclei of secondary spermatocytes were only sparsely labeled (Figs. 1A–D and 2b) as were nuclei of early spermatids (steps 1–11; Figs. 1A–D and 2a).

In testes fixed at daily intervals after injection, labeled amino acids appeared in nuclei of successive stages of spermatogenesis. The daily shifts seen in the populations of cells with labeled nuclei coincided exactly with the rate at which spermatocytes would be expected to complete spermatogenesis (5, 25), indicating that the nucleoprotein initially labeled in spermatocyte nuclei was retained as the spermatocytes completed meiosis and entered spermiogenesis (Fig. 3A–D). Label first appeared in nuclei of secondary spermatocytes 1 d after injection (Figs. 1A–D and 2a) but was not seen in spermatids beyond early step 14 on this or subsequent days (Fig. 3B and C). Thus, in striking contrast to [3H]arginine, epididymal spermatozoa showed little or no [3H]lysine nuclear labeling, even 13 d after injection (Fig. 3E).

In contrast to both arginine and lysine, little or no [3H]proline or [3H]valine was incorporated into spermatid nuclei (Fig. 1c and d). Additionally, these amino acids were not observed in epididymal spermatozoa even 9 d after label was introduced. This is consistent with the fact that the protamine of mouse spermatozoa does not contain valine or proline residues and that protamine is the major basic nuclear protein of mouse spermatozoa (1, 2).

**Chromatin Condensation**

Electron microscope studies demonstrated that the nuclei of spermatids at steps 1 through early 12 (Fig. 4a) contain large amounts of dispersed chromatin. Chromatin condensation is initiated in late step-12 nuclei (Fig. 4b) and continues through early step 14 (Fig. 4c). Condensation is essentially completed by late step 14 and undergoes no apparent change through step 16 (Fig. 4d). These observations are consistent with those made previously (6).

**DISCUSSION**

The studies presented here show that primary
FIGURE 2 Autoradiographs showing incorporation of [3H]lysine into spermatocyte nuclei and subsequent loss of label. Testes were fixed at intervals after injection with [3H]lysine. Results were similar with [3H]arginine, [3H]valine, or [3H]proline. Bar, 10 μm. All micrographs × 1200. (a) Testis fixed 2 h after injection. Nuclei of pachytene spermatocytes (arrow) are heavily labeled (Fig. 2a). Note that step-6 spermatid nuclei (arrow head) show little or no label at this time (Fig. 2a). Stained with toluidine blue. (b) Testis fixed 2 h after injection. Nuclei of secondary spermatocytes show little or no label. Stained with toluidine blue. (c) Testis fixed 1 d after injection. Nuclei of secondary spermatocytes are now labeled. Stained with toluidine blue. (d) Testis fixed 5 d after injection. Nuclei of spermatids through step 6 (shown) are now labeled. Stained with toluidine blue. (e) Testis fixed 11 d after injection. Nuclei of step-12 spermatids are now labeled. Unstained phase-contrast micrograph; silver grains appear as white dots. (f) Testis fixed 13 d after injection. Nuclei of step-13 spermatids (shown) and later steps of spermatid development show little or no label. Phase-contrast micrograph.

FIGURE 1 Distribution of [3H]-amino acids in spermatocyte (●), spermatid (○), and caput epididymal sperm (□) nuclei after intratesticular injection with (A) [3H]arginine, (B) [3H]lysine, (C) [3H]proline, or (D) [3H]valine. Fixation 2 h after injection. Spermatogenic cell types (abscissa): leptotene (L), zygotene (Z), pachytene (P), secondary spermatocytes (S), spermatid steps (1-16), and caput epididymal spermatozoa (E). Roman numerals (abscissa) indicate stages of the cycle of the seminiferous epithelium (24). Grain counts were performed on at least 50 randomly selected nuclei for each cell type. Mean numbers of grains are plotted (ordinate). SE (not plotted) were not >± 1.8 for any cell type.
spermatocyte nuclei incorporate the intratesticular injected amino acids [3H]arginine, [3H]lysine, [3H]valine, and [3H]proline, thus confirming previous autoradiographic studies of mammalian spermatogenesis (12, 19, 22, 28). Our studies further demonstrate that, at intervals after injection, the initially incorporated labeled amino acids appear in nuclei of successive stages of spermatogenesis.

Figure 3 Distribution of label in secondary spermatocytes (S), spermatids (1-16), and caput epididymal sperm (E) nuclei after intra-testicular injection with [3H]lysine or [3H]arginine. Fixation at intervals after injection. Label which originally (2 h after injection) had been incorporated into spermatocyte and spermatid nuclei is shown by closed (●●●) and open (○○○) circles, respectively, as in Fig. 1. For explanation of symbols used, see Fig. 1.
Fig. 4 Electron micrographs showing the nuclear fine structure of various spermatid steps. Bar, 1 μm. All micrographs × 5600. (a) Early step-12 spermatid nucleus. The chromatin is uniformly dispersed. (b) Late step-12 spermatid nucleus. Initial chromatin condensation occurs in the anterior portion of the nucleus. (c) Early step-14 spermatid nucleus. Only the posterior region of the nucleus remains uncondensed. (d) Step-16 spermatid nucleus. The chromatin of late step-14 through step-16 spermatids is completely condensed.

Spermatogenesis. These results indicate for the first time that nucleoprotein initially labeled in primary spermatocyte nuclei is retained through subsequent meiotic stages and through spermatid differentiation to step 12 of spermiogenesis, but is lost with spermatid differentiation beyond step 12. Interestingly, loss of this protein occurred at the time of the initiation of chromatin condensation, in step-12 nuclei.

Intratesticularly injected [3H]arginine and [3H]lysine, but not [3H]valine or [3H]proline, also were rapidly incorporated into certain elongated spermatid nuclei, but differed strikingly in their initial distribution and in their fate. [3H]Arginine was initially incorporated into nucleoprotein(s) of late step-12 through step-15 spermatids. As expected from previous studies (2, 7, 16), the labeled arginine appeared in successively more mature spermatid steps at intervals after initial injection but was simultaneously lost from nuclei of earlier steps. Ultimately, only epididymal spermatozoa appeared labeled. Unexpectedly, and in marked contrast to arginine incorporation, [3H]lysine was initially incorporated into nucleoprotein(s) of late step-12 and step-13 spermatids, retained only through early step 14, and then lost.
REFERENCES

1. Balhorn, R., L. P. Gledhill, and A. J. Wiebe. 1977. Mouse sperm chromatin proteins: Quantitative isolation and partial characterization. Biochemistry. 16:6774-6780.

2. Bellve, A. R., A. Anderson, and L. Hanley-Bowdoin. 1975. Synthesis and amino acid composition of basic proteins in mammalian sperm nuclei. Dev. Biol. 45:349-365.

3. Bonner, D. 1977. Chemical aspects of histone acetylation and replacement in mouse spermatids at different stages of maturation. Cytobiologie. 15:420-437.

4. Brodsky, R. E., S. R. Geremes, G. Yoruscso, and J. L. Irving. 1975. The histones of rat testis. Arch. Biochem. Biophys. 168:403-412.

5. Clermont, Y., and M. Troth. 1969. Duration of the cycle of the seminiferous epithelium in the mouse and hamster determined by means of $[3H]$thymidine and radioautography. Fertil. Steril. 20:805-817.

6. Dooner, G. B., and D. Bennett. 1973. Fine structural observations on the development of the sperm head in the mouse. Am. J. Anat. 136:339-362.

7. Ehrlich, P. S., and L. Levine. 1975. Mouse sperm basic nuclear protein. Electrophoretic characterization and fate after fertilization. J. Cell Biol. 66:251-262.

8. Goldberg, H. B., R. Geremes, and W. R. Bruce. 1977. Histone synthesis and replacement during spermatogenesis in the mouse. Differentiation. 7:167-180.

9. Geremes, S. R., Jr., C. B. Chao, and J. L. Irving. 1975. Effect of age and hypophysectomy upon relative proportion of various histones in rat testis. Biochem. Biophys. Res. Commun. 64:991-997.

10. Geremes, S. R., Jr., M. L. Meistrick, R. D. Plate, and S. L. Hensica. 1977. Nuclear protein transitions in rat testis spermatids. Exp. Cell Res. 106:31-39.

11. Geremes, S. R., Jr., R. D. Plate, M. L. Meistrick, and S. L. Hensica. 1975. Partial characterization of a new basic nuclear protein from rat testis elongated spermatids. Biochem. Biophys. Res. Commun. 67:182-189.

12. Kirschbaum, A. L., and L. L. Tivel. 1975. Structural and transcriptional features of the mouse spermated genome. J. Cell Biol. 65:238-270.

13. KIffler, W. S., and M. E. Geremes. 1975. An unusual pattern of histone-rich histone components associated with spermatogenesis in rat testis. Biochem. Biophys. Res. Commun. 63:378-384.

14. KIffler, W. S., M. E. Geremes, and H. G. Williams-Adamson. 1973. Specific basic proteins from mammalian testes. Isolation and properties of small basic proteins from rat testis and epididymal spermatids. J. Biol. Chem. 248:4532-4543.

15. KIffler, W. S., M. E. Geremes, and H. G. Williams-Adamson. 1975. A highly basic, small protein associated with spermatogenesis in the human testis. Invest. Urol. 12:346-350.

16. Koren, V., and A. Panyk. 1975. Autoradiographic study of mouse spermatid arginine-rich nuclear protein in fertilization. J. Exp. Zool. 191:65-96.

17. Kur, K. K., G. Jahnke, and J. L. Irving. 1975. Changes in basic chromosomal proteins during spermatogenesis in mature rat. Arch. Biochem. Biophys. 168:413-424.

18. Lam, D. M. K., and W. R. Bruch. 1971. The biosynthesis of protein during spermatogenesis of the mouse: Extraction, partial characterization, and site of synthesis. J. Cell. Physiol. 87:13-24.

19. Lor, M. 1972. Metabolisme de l'acide ribonucleique et des proteins dans les spermatocytes et les spermatides du Belier (Ovis aries): Etude de l'incorporation et de la degradation du $[3H]$thymidine, du $[3H]$histidine, et de la $[3H]$arginine. Ann. Biol. Anim. Biochim. Biophys. 12:411-429.

20. Lor, M., and M. Lanneau. 1975. An electrophoretic analysis of the basic nuclear proteins of rat spermatozoa. Exp. Cell Res. 92:510-512.

21. Markushin, Y., and K. Marushkin. 1974. Properties of chromatin isolated from bull spermatozoa. Biochem. Biophys. Acta. 340:498-506.

22. Moses, V. 1965. Synthetic activities during spermatogenesis in the mouse. Exp. Cell Res. 38:197-224.

23. Myszkowski, C. H., R. Schornick, T. H. Rosen, and E. F. Stempapf. 1973. Amino acid composition and carboxyl-terminal structure of some basic chromosomal proteins of mammalian spermatozoa. Biochem. Biophys. Acta. 322:173-177.

24. Oakberg, E. F. 1956. A description of spermogenesis in the mouse and its use in analyses of the cycle of the seminiferous epithelium and germ cell renewal. Am. J. Anat. 99:391-413.

25. Oakberg, E. F. 1956. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. Am. J. Anat. 99:507-516.

26. Plate, R. D., S. R. Geremes, M. L. Meistrick, and S. L. Hensica. 1975. Changes in nuclear proteins of rat testis cells separated by velocity sedimentation. J. Biol. Chem. 202:5791-5800.

27. Sheehy, A. M. P. Carpenter, and R. Chalkley. 1975. New histones found in mature mammalian testes. Proc. Natl. Acad. Sci. U.S.A. 72:2714-2718.

28. Vaughan, J. C. 1966. The relationship of the “Sphere Chromatophile” to the fate of displaced histones following histone transition in rat spermatogenesis. J. Cell Biol. 31:257-278.