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HISTONE-PROTEIN TRANSITION IN *DROSOPHILA MELANOGASTER*

I. CHANGES DURING SPERMATOGENESIS[1]

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As is well known, in the course of development of the spermatozoon, a transition from a typical somatic or lysine-rich histone to a highly arginine-rich type occurs at the spermatid stage, and this change in the basic nuclear protein is often followed by a further alteration to protamine in the maturing spermatozoon. While the biological implications of such chemical transformations remain largely unknown, a precise definition of the nature of the changes provides a framework for evaluating the early speculations of Stedman and Stedman [19] about the possible functional involvement of basic proteins in the regulation of gene activity. In this communication we shall describe the pattern of transitional sequences of chromosomal proteins in the spermatogenic cycle of *Drosophila melanogaster* as determined by cytochemical methods (rather than purely chemical ones), using the criteria of Alfert and Geschwind [2], and Bloch and Hew [5]. The results discussed below indicate that the basic protein is changed from a lysine-rich to an arginine-rich histone during the final stages of spermiogenesis.

MATERIALS AND METHODS

The staining methods used for the demonstration of basic proteins consisted mainly of the trichloroacetic acid (TCA)-alkaline fast green method of Alfert and Geschwind [2] and the picric acid-bromophenol blue method of Bloch and Hew [5]. The former, in which the dye is used at a pH of 8.0 to 8.2, is designed specifically for detection of the histones of the cell nucleus, since any protamines that were present in the living cell would be extracted by hot TCA [2]. The method of Bloch and Hew, on the contrary, serves to reveal those protamines and histones that are extractable by hot TCA but not by picric acid, as well as the histones stainable by the method of Alfert

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and Geschwind. In order to determine the specific nature of histones, with respect to lysine or arginine content, the deamination procedure of Van Slyke [20] and the acetylation method of Monné and Slautterback [16] were used. Both of these procedures affect primarily the amino groups of lysine and not the guanidine groups of arginine [12, 17], so that stainability with alkaline fast green of those histones that are rich in the amino acid arginine is not effaced by pretreatment involving acetylation or deamination, whereas stainability of histones "rich in lysine" is inhibited.

The Feulgen procedure of Bloch and Godman [4], in which HCl is replaced by TCA mole for mole in all steps of the technique, and whereby the basic proteins are retained and can be restained by the Alfert-Geschwind method, was also employed to permit, through sequential staining, a comparison of the deoxyribonucleoproteins of the various cells during spermatogenesis of *D. melanogaster*.

In preparation for the cytochemical studies, testes from larvae, pupae, and adult males, seminal vesicles from males, and spermathecae from adult females of *D. melanogaster* were fixed for about 8 hr in 10 per cent neutral buffered formalin at room temperature, and were then processed for paraffin embedding and sectioning. Testes of the spawning rainbow trout, *Salmo gairdneri* (which were obtained through the courtesy of the Fish Division of the Institute for Fisheries Research, Michigan Department of Conservation) were fixed and processed in a similar manner, so that a direct comparison could be made between the mature spermatozoa of *D. melanogaster* and those of a species belonging to a genus in which chemical analysis had shown protamine to be present.

RESULTS AND DISCUSSION

The course of spermatogenesis in *D. melanogaster* in so far as it is related to histological differentiation of the testis and the production of mature

Fig. 1.—Section of the testis of *D. melanogaster* (1–2 weeks old) stained with fast green after deamination. Bundles of mature sperms stain intensely indicating the sperm protein to be an arginine-rich histone. × 1200.

Fig. 2.—Section of the testis of 1–2-day-old adult fly stained with fast green after deamination. Only a few of the sperm bundles stain and the rest, presumably those containing immature spermatozoa and elongating spermatids, remain unstainable. × 1200.

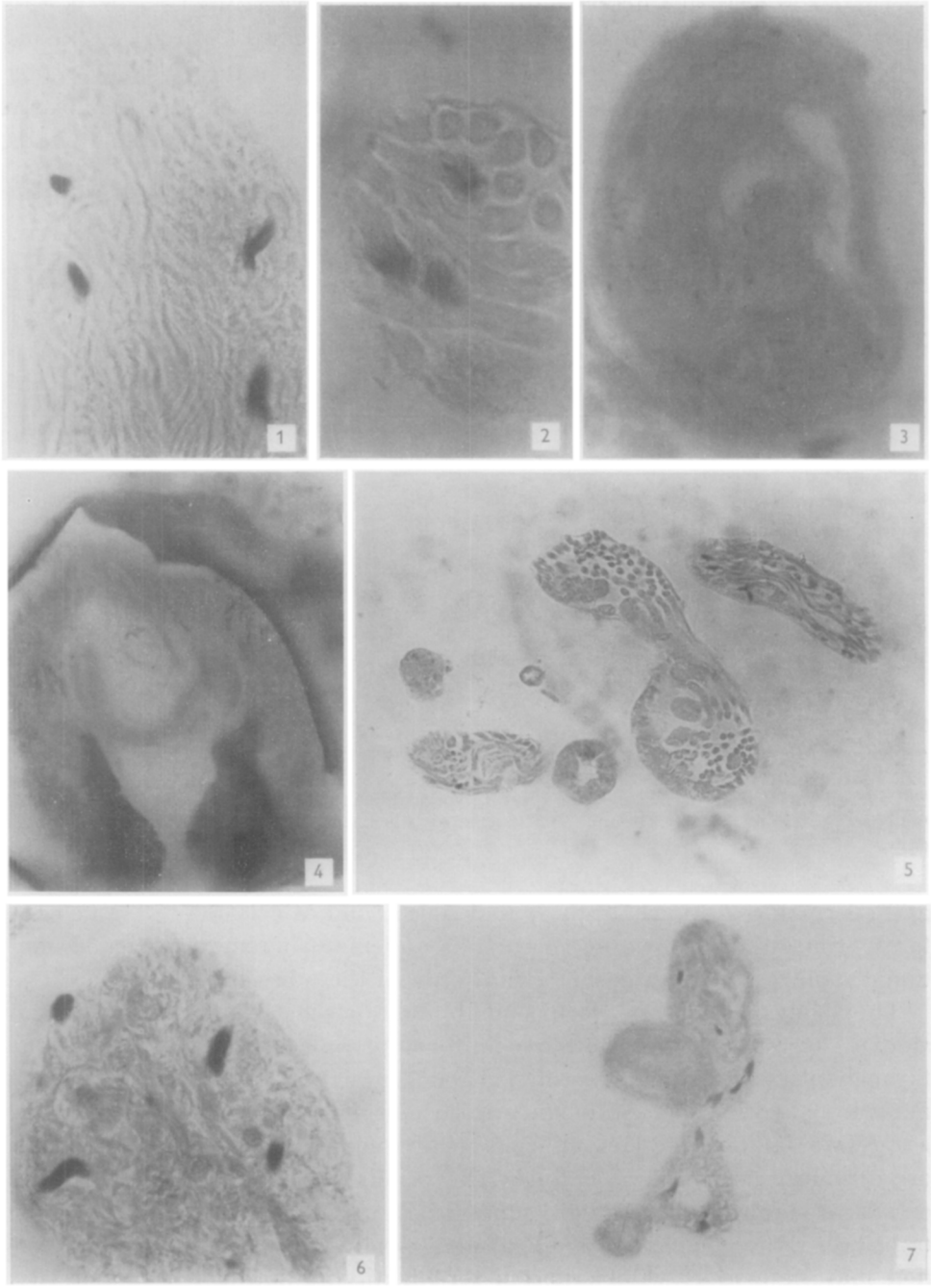
Fig. 3.—Section of seminal vesicle (above) and part of the testis. The scattered black dots in the seminal vesicle represent cross sections of sperm heads. The sperms stain characteristically with the TCA-fast green procedure and this may be taken to indicate that the mature sperms of *Drosophila* contain an arginine-rich histone that is not protamine. × 1200.

Fig. 4.—Section of the spermatheca of the adult female. The dark spots represent cross sections of the heads of spermatozoa transmitted during copulation. The sperms continue to stain with alkaline fast green after hydrolysis in hot TCA, and so the mature functional sperms of the fruit fly are devoid of protamine. × 1200.

Fig. 5.—Section of adult testis at eclosion stained with bromophenol blue after acetylation; except for a few sperm bundles, the rest of the tissue is unstained. The inconsequential effect of blocking is due to the presence of arginine-rich histones in the maturing spermatozoa. × 1200.

Fig. 6.—Section of testis of 1–2-week-old adult treated with picric acid-bromophenol blue procedure after acetylation; this treatment has not prevented staining of the mature sperm. × 1200.

Fig. 7.—Sections of adult testis showing fast-green-positive mature sperms characterized by dense heads and wavy tails. × 360.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spermatozoa has been described or reviewed by several workers [6, 9, 15]. Briefly stated, a sequential change in the frequency of spermatogeneous cells of different stages is correlative with the progress of the life cycle of this insect; for example, only spermatogonia are present in the testis of early larvae, primary spermatocytes appearing shortly before pupation; spermatids

TABLE I. *Summarized account of the histone transition during spermatogenesis in Drosophila melanogaster.*

Cell types	Stages in life-history	Fast green	Fast green after deamination	Bromophenol blue	Bromophenol blue after acetylation	Nature of basic protein
Spermatogonia	Early pupa through 1–2-week adult	+	−	+	−	Lysine-rich histone
Spermatocytes I and II		+	−	+	−	
Spermatids early and late		+	−	+	−	
Spermatozoa immature		+	−	+	−	
Spermatozoa maturing	Early pupa	+	−	+	−	
	Late pupa	+	−	+	−	
	Adult at eclosion	+	+	+	+	Arginine-rich histone
	Adult (1–2 weeks old)	+	+	+	+	
Spermatozoa[a]		+	+	+	+	

[a] Fully mature from sem. ves. and spth.

Conclusion: Histone transition occurs during sperm maturation, shortly before eclosion, and the basic protein of the mature functional spermatozoa is an arginine-rich histone and not protamine.

and spermatozoa appear in the pupal stages, and at eclosion the testis contains primarily transforming spermatids and maturing spermatozoa. Functional mature spermatozoa are found only in the adult testis.

The Feulgen and fast green stainabilities detected in our cytochemical studies suggest that there is a fairly uniform distribution of DNA and histone throughout most of the stages of spermatogenesis, with the exception of the maturing spermatozoa. Although precise morphological criteria for judging a sperm as mature are lacking, cells with condensed heads and coiled tails are ordinarily regarded as mature. The results of our cytochemical studies on sperm maturation, which are summarized in Table I, suggest that histone transition rather than microscopical identification of structural transformation may be a more valid criterion for identifying the degree of maturity.

In sections of the fruitfly testes that had been deaminated with nitrous acid or acetylated with acetic anhydride (as prescribed in references [16] and [20] to block lysine staining with fast green) only a small proportion of the sperm bundles in the adult testis (among those showing an increased stainability prior to deamination or acetylation of the tissue) revealed any color, but they stained intensely (Figs. 1, 2). None of the spermatozoa at earlier stages of spermiogenesis or nuclei in cells at any other stages of spermatogenesis revealed comparable stainability. The sperms in question are characterized, therefore, by the presence of histones that are rich in arginine, in contrast with the lysine-rich histones that are present in all other types of spermatogenous cells. This observation indicates that a transition (actually a synthesis as will be described in another publication) from a lysine-rich to an arginine-rich histone occurs during the later stages of spermiogenesis in *D. melanogaster.*

According to our cytochemical criteria there is no subsequent shift to a protamine. As stated by Alfert and Geschwind [2], the protamines are labile enough to be leached out from the cell during hydrolysis in hot TCA—a procedural step needed for extraction of DNA before staining with alkaline fast green. In our experiments, mature sperms of *Salmo gairdneri* reacted negatively to the Alfert-Geschwind technique, suggesting that in this species as in others of the genus, the mature sperms contain protamine. With respect to the spermatozoa of *D. melanogaster*, however, it should be noted that spermatozoa that remain stored in the seminal vesicles of the male (presumably mature and functional) and sperms contained within the spermathecae of the female (certainly mature and functional, since they were transferred in copulation), exhibit similar staining patterns; that is, they stain intensely with alkaline fast green both before and after acetylation and deamination (Figs. 3, 4). Formalin-fixed sectioned material, stained by the picric acid-bromophenol blue procedure [5] revealed a similar staining pattern (Figs. 5, 6). Apart from this measure of correspondence in cytochemically-determined staining properties, the arginine-rich spermatozoa of testis, seminal vesicle and spermatheca are morphologically indistinguishable; all of them are convoluted with compact heads and are, to all appearances, mature functional elements (Figs. 7, 8, 9, 10). It should be stated, however, that functional spermatozoa, conventionally adjudged by the property of motility are necessarily mature by morphological criteria, whereas the reverse may not be true. In *Drosophila virilis*, for example, mature spermatozoa can be observed at the end of the 4th day after emergence of the flies, but functionality of the sperm is not attained until the 6th day after emergence [8].

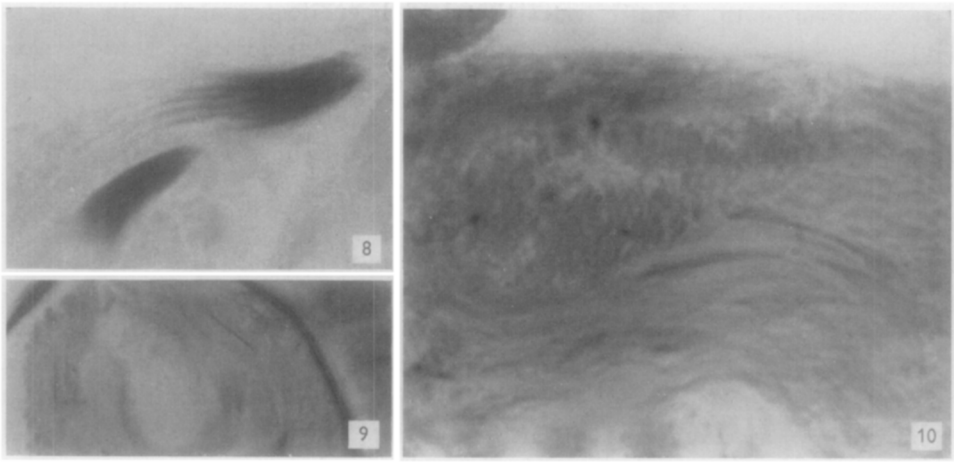

Fig. 8.—Enlargement of two bundles of sperms figured at right in Fig. 7. ×2000.

Fig. 9.—Section of spermatheca: the elongated dark structures represent the heads of sperms, and are comparable with those of testis and seminal vesicle. The convoluted tail is not readily evident. ×1600.

Fig. 10.—Section of seminal vesicle. The dark dots and streaks represent heads of mature sperms and are comparable with those of the testis. The coiled nature of the tail can be seen. ×1600.

It would seem from these observations that in *Drosophila melanogaster* the mature sperm can best be identified by the nature of its histone content. To be more precise, the time of "switch-over" to an arginine-rich histone presumably coincides with the attainment of maturity by the sperm, and judging from the fact that the histone shift is observed only in the spermatozoa of individuals shortly before eclosion (within 24 hr or so at 29°C ± 1), the sperms of the early pupal testis, which are not ordinarily distinguished from those of the adult [6, 9], can no longer, in our opinion, be considered completely differentiated entities. Furthermore, since the spermatozoa stain just as intensely after their transfer to the spermatheca of the female as they do in the adult testis, the basic protein of the mature sperm of *Drosophila* appears to be histone and not a protamine.

On entering the ovum, however, the sperm histone presumably becomes altered since the male pronucleus does not stain with alkaline fast green. This situation parallels that described for the mouse [1]. The early cleavage nuclei also remain nonstainable with fast green until the approach of blastulation in the developing egg of *Drosophila* [10]. These nuclei do stain, however, with the more sensitive picric acid-bromophenol blue procedure [5], but the stainability is lost on deamination or acetylation. The stainable

complex is presumably a histone, the so-called "cleavage" histone [5], as distinguishable from the fast-green-staining adult histone. Details about the nature of these changes and an assessment of their significance will be presented in another publication [11].

The functional properties of basic proteins are poorly understood. The hypothesis [19] that histones and protamines serve as regulators of gene action, has been opposed on stoichiometric grounds [18]. However, experiments recently conducted by Huang and Bonner [14], by Bonner and Huang [7], and by Allfrey, Littau and Mirsky [3] have brought into sharp focus the extent of involvement of histones in the regulation of cell activity. The results of these experiments clearly show among other things that (*a*) in cell free preparations of pea seedling nuclei, the histone when fully complexed with DNA does not permit the latter to act as a primer for the synthesis of chromosomal RNA; (*b*) the chromatin of pea embryos after removal of histone as well as the histone-depleted nuclei isolated from calf thymus do exhibit an increased rate of DNA-dependent RNA synthesis; (*c*) the arginine-rich histones are strong inhibitors while the lysine-rich fractions are non-inhibitory or at best weakly inhibitory.

These findings are of fundamental interest when we consider the fact that mature sperms in the great majority of known cases [13, 18] contain a protamine. Its presence in the sperm nucleus may, therefore, "stabilize" the nucleus so that it is incapable of synthesis of chromosomal (messenger) RNA and expression of the coding potential of the DNA. The protein transition during spermiogenesis (whereby arginine-rich histones replace lysine-rich histones) would in these terms appear to be an effective adaptive mechanism for preserving "packaged" genetic information and suppressing its capacity for directing synthetic activities during the period that the cell is being prepared for its role in fertilizing the egg.

SUMMARY

The basic protein of the mature spermatozoa of *Drosophila melanogaster* is an arginine-rich histone but not a protamine, as determined by cytochemical criteria.

The histone shift during spermatogenesis occurs during maturation of the sperm rather than at the spermatid stage.

"Maturity" of a spermatozoon should not be defined purely on morphological criteria. Adjudged cytochemically, the spermatozoa of the early pupal

testis of *Drosophila* cannot be considered as mature; mature sperms first appear shortly before eclosion.

The sperm nucleus, by the very nature of its nucleohistone complex, is assumedly devoid of any synthetic activity. This appears to be an evolutionary adaptation for insulating the genetic information during the period of its transit from one generation to the next.

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