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Chromatin Organization During Spermiogenesis in *Octopus vulgaris*. I: Morphological Structures

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ABSTRACT In the process of the chromatin remodeling that occurs during spermiogenesis in some animal species, it is possible to distinguish between two separate aspects: the chromatin condensation pattern itself (granular, fibrillar, or lamellar), and the architecture of this pattern, that is to say, its arrangement within the nucleus. In the cephalopod *Octopus vulgaris* these two aspects are clearly differentiated. The condensation pattern develops from 25 nm fibers to fibers with a tubular aspect and with a progressively increasing diameter (40–60 nm and then to 80 nm), to end finally in the form of very thin fibers (3–5 nm) product of the coalescence and dissolution of the major fibers. The main directive force that governs this process lies in the global change that occurs in the proteins that interact with all (or the major part) of the genomic DNA. The condensation pattern by itself in this species does not present a fixed order: most of the fibers appear without any predominant spatial direction in the spermiogenic nuclei. However, as the nuclei elongate, the chromatin fibers arrange in parallel following the elongation axis. This parallel disposition of the chromatin fibers appears to be mediated by two specific areas, each of which we call a "polar nuclear matrix" (PNM). These matrices differentiate in the basal and apical nuclear poles adjacent to the centriolar implantation fosse and the acrosome, respectively. The areas that constitute the PNM have the following characteristics: (a) they are the only areas where DNA is found anchored to the nuclear membrane; (b) they are the zones from which the chromatin condensation pattern (fibers/tubules) begins; and (c) they are most probably the points through which the mechanical forces originating from nuclear elongation are transmitted to chromatin, causing the chromatin fibers/tubules to adopt an almost perfectly parallel disposition. Finally, we discuss the importance of the architecture of the chromatin condensation pattern, as it is one of the determining factors of the spatial organization of the mature sperm genome and chromosome positioning. *Mol. Reprod. Dev. 68: 223–231, 2004.* © 2004 Wiley-Liss, Inc.

Key Words: cephalopoda; *Octopus*; spermiogenesis; nuclear matrix; chromatin condensation

INTRODUCTION

During spermiogenesis the haploid genome of the early spermatid undergoes an important condensation that leads to the reduction of the nuclear volume. This condensation of the spermiogenic genome is due to the progressive change in the interactions between DNA and its associated proteins. In the most general case, histones are substituted by a class of specific protein that is very rich in basic amino acids. Such proteins are called sperm nuclear basic proteins (SNBPs) (see the reviews of Kasinsky, 1989; Chiva et al., 1995; Lewis et al., 2003). Protamines (Miescher, 1874; Bloch, 1969; Subirana, 1983) are a particular type of SNBP.

Interaction of SNBPs with DNA during spermogenesis accounts for the type of condensation shown by chromatin. Thus, for instance, some SNBPs promote a granular condensation (Saperas et al., 1993), while other SNBPs condense chromatin in fibers or lamellae (Maxwell, 1975; Càceres et al., 1999). DNA condensation by SNBPs can be considered as a first level of genome organization of the sperm nucleus. However, this is a nonspecific interaction that cannot explain, by itself, the higher orders of organization of the condensed sperm chromatin.

Ward et al. have described a second level of organization in the sperm nuclei of mammals. Similarly to what happens in the somatic genome, some parts of the sperm

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chromatin may be organized into loop domains attached at their bases to a sperm-specific nuclear matrix (Ward et al., 1989; Ward and Coffey, 1990, 1991). In this case, the interaction of the genome with the sperm nuclear matrix could be at least partially specific (Kramer and Krawetz, 1996) and necessary for the normal development of the early embryo in the stages after gamete fertilization (Ward et al., 1999).

A sperm nuclear matrix has been unequivocally described in mammals, *Xenopus* and some fish (Benavente and Krohne, 1985; Alsheimer and Benavente, 1996; Goldman et al., 1998; Glasenapp and Benavente, 2000; Hofemeister et al., 2002), although it is not yet known if occurs in sperm from other animals. However, the idea that there is an interaction between a rigid nuclear structure and specific DNA zones is very suggestive, as it leads to the question about the existence of a third level of DNA organization in the sperm nucleus: namely, the nuclear architecture; that is, the particular places that different genes or chromosomes occupy in the sperm nucleus.

Chromosomes occupy preferential positions in the somatic nucleus. The territories of the different chromosomes do not overlap; they change during the cell cycle in an ordered way, and are involved in the regulation of different nuclear functions, such as transcription and splicing (for more details see the reviews of Cremer and Cremer, 2001; Parada and Misteli, 2002). During the past decade, FISH techniques have been used to study if an architectural organization in the sperm nucleus also exists or whether chromosomes are randomly distributed in the nuclear volume (Zalensky et al., 1993; Haaf and Ward, 1995; Joffe et al., 1998; Solovei et al., 1998; Hazzouri et al., 2000). In these studies, genes, groups of genes, pieces of chromosomes, and centromeric and telomeric DNA are used as probes to check if a certain DNA sequence occupies the same or a similar position from cell to cell. Although the results are often partially contradictory (compare for instance Zalensky et al., 1993 with Hazzouri et al., 2000), it seems that in some species centromeric DNA tends to locate in the center of the sperm nucleus while telomers are preferentially found in the periphery, and regions of some chromosomes are found in similar positions in the different sperm nuclei of the same species. Thus, there does appear to be an overall architecture to the sperm genome.

Due to the high interspecific variability of nuclear spermiogenic phenomena, it is necessary to study several models so as to understand the fundamental processes that regulate the organization of DNA in sperm and the different solutions that have appeared in evolution. Spermiogenesis in the cephalopod *Octopus vulgaris* offers an appropriate model for these types of studies. In this article, we analyze by electron microscopy the nuclear processes that occur during the spermiogenesis of *O. vulgaris*. We describe the chromatin condensation pattern that occurs due to the progressive interaction of DNA with protamines and we describe the areas of the nucleus where chromatin begins to organize its condensation process. We call each of these areas the "polar nuclear matrix" (PNM). PNMs are located in the apical and basal poles of the nucleus, close to the acrosome and the implantation fosse, respectively. They are the structures that will allow the condensing chromatin fibers to adopt an ordered arrangement. We also discuss the analogy of these PNMs (apical and basal) with the single structure found in the basal pole of the hamster sperm nuclei, the "nuclear annulus" (Ward and Coffey, 1989), and their relationship with the organization of the genome.

MATERIALS AND METHODS

Animals

The specimens of *O. vulgaris* used in this work were collected along the Catalonian coast of Spain (Mediterranean sea). *O. vulgaris* belongs to the class Cephalopoda, order Octopoda (Ruppert and Barnes, 1994).

Electron Microscopy

Portions of gonads and epididymis or centrifuged sperm nuclei were prepared for electron microscopy by conventional methods. Samples were fixed in 2.5% glutaraldehyde, 0.1 M cacodylate buffer, pH 7.4, and postfixed in osmium tetroxide in cacodylate buffer. The fixed samples were dehydrated and soaked on Spurr's resin. Sections were stained with uranyl acetate and lead citrate and examined on a Hitachi 4–600 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

The quality of image shown in Figure 4E was improved using the image-analysis IMAGE PRO PLUS program.

Preparation of *O. vulgaris* Sperm Nuclei

Nuclei were prepared from ripe spermatozoa contained in spermatophores as in Giménez-Bonafé et al. (1999). Briefly, sperm were homogenized in isotonic buffer A (0.25 M sucrose, 5 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.4) containing 25 mM benzamidine chloride, and centrifuged (5,000*g*, 5 min). The pellet was collected with buffer A and these operations were repeated twice. The last pellet was incubated 30 min (4°C) in buffer A plus 1% Triton X-100, 25 mM benzamidine chloride, homogenized and centrifuged in the same conditions three times. The last pellet contained the mature sperm nuclei and some contamination of sperm tails.

Chemical Treatments of *O. vulgaris* Sperm Nuclei

The pellet of sperm nuclei was distributed into several aliquots. Some of these aliquots were resuspended with buffer A and used as a control.

Other aliquots were submitted to a hypotonic shock and to a chemical reduction by incubation with a high volume of 1 mM Tris-HCl, pH 8.0, 1 mM EDTA, 10 mM DTT (TE-DTT buffer) for 15 min (4°C). Some aliquots of reduced nuclei were then made 1 M NaCl or 2 M NaCl by adding solid salt, and incubated for 30 min at 4°C.

Treatment with 1 M NaCl or 2 M NaCl solubilizes protamines from the sperm nuclei. The amount of solubilized protamine was then measured by absorbance at 230 nm (Prieto et al., 2002).

Visualization of Nuclei by Fluorescence Microscopy

Aliquots of 3 μl from the nuclei suspension were placed on a slide and mixed with 3 μl of bisbenzamidine (100 μg/ml). Samples were observed and photographed with a fluorescent microscope ($\lambda = 360$ nm).

RESULTS

First Stages of the Spermiogenic Chromatin Remodeling of *O. vulgaris*

Figure 1A shows a longitudinal section of an *O. vulgaris* mature sperm cell nucleus obtained from the epididymis. The nucleus has a long shape (15 μm length approximately) and is slightly curved. It resembles a cylinder where the apical pole adjacent to the acrosome ("a" in Fig. 1A) has a diameter (0.35–0.4 μm) that is slightly smaller than the rest, as the mature sperm nucleus of this species tends to widen towards the basal pole ("b" in Fig. 1A). This basal enlargement may be due to an invagination (partially visible in Fig. 1A) that penetrates deeply into the sperm nuclei of many cephalopods and that contains a material of an unknown nature (see Fields and Thompson, 1976; Healy, 1989, for a discussion of the origin of this material). Figures 1B to 4 represent selected images that correspond to the most representative stages of the condensation of the *O. vulgaris* spermiogenic chromatin that leads to the mature sperm nucleus in Figure 1A.

Figure 1B represents a very early stage of spermiogenesis where the proacrosomal vesicle (bottom right) is not yet associated with the spermatidyl nucleus. However, all around the implantation fosse (top left) the nuclear envelope undergoes a densification that embraces a relatively large area of the nucleus during this first stage of spermiogenesis. We call this structure the *Lamina densa*. In this stage the beginning of spermiogenic chromatin condensation can be observed: chromatin agglutinates partially at the basal area (Fig. 1B,C) and it progressively fixes to the *Lamina densa*. Curiously, the attachment zone does not include all the *Lamina densa* but mainly the part that surrounds the centriole (Fig. 1C). It can be observed that in these attachment points (arrows in Fig. 1C) chromatin begins to adopt a fibrogranular aspect.

Figure 1D corresponds to a later stage where the nucleus is initiating its elongation. The proacrosomal

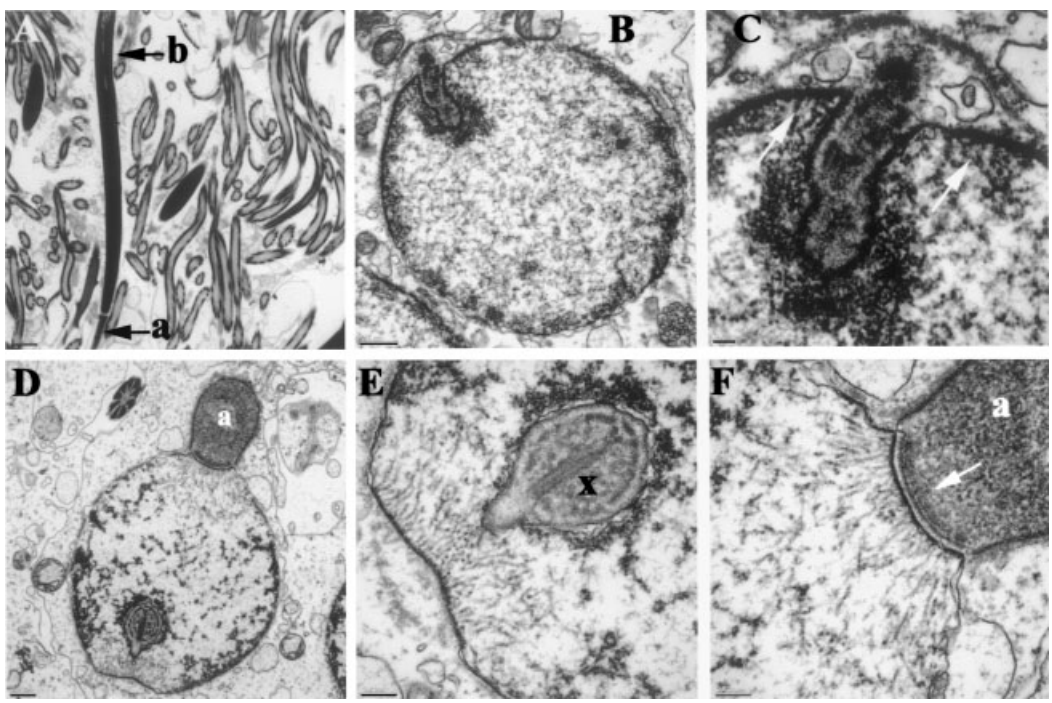

Fig. 1. Transmission electron microscopy images from *Octopus vulgaris* spermiogenesis. **A**: Longitudinal section of a mature sperm cell nucleus (a, acrosome; b, basal zone where the intranuclear invagination can be seen). **B**: Early spermatidyl nucleus. The implantation fosse and the surrounding nuclear *Lamina densa* can be observed at the **top left** corner. The multivesicular body that appears at the **bottom right** corner is the proacrosomal vesicle approaching the nucleus. **C**: Detail of the implantation fosse and the beginning of the relationship of chromatin (arrows) with the *Lamina densa*. **D**: Spermatidyl nucleus during the beginning of the elongation process (a, acrosome). **E**: Detail of the bottom part of (D). Chromatin is associated with the basal part of the *Lamina densa* and begins to organize into fibers (x, material of unknown origin found inside the invagination). **F**: Detail of the apical zone. Chromatin is also organizing in fibers in the areas of contact with the *Lamina densa* (a, acrosome; arrow, beginning of the internal organization of acrosome. Bars: A, 1 μm; B; and D, 0.5 μm; C, 0.1 μm; E and F, 0.2 μm.

vesicle is already found adjacent to the nucleus, and the basal invagination presents a more advanced development. Figure 1E shows a detail of how the membranes of the nuclear envelope are fused in the basal part, and how it is exclusively in this area where chromatin anchors, adopting a fibrillar structure 25 nm in diameter. In the apical pole (Fig. 1F), the same phenomenon can be observed, but this region is interesting for two additional reasons. First, the area of contact between the nucleus and the acrosome is the zone from which both the spermatic chromatin condensation and the layered organization of the acrosomal material simultaneously progress, although in opposite directions (see arrow in Fig. 1F). Second, the area of contact between nucleus and acrosome will determine the diameter of the apical transverse section of the mature sperm nucleus (see Fig. 1A).

From the stage depicted in Figure 1D, chromatin progressively condenses inside a continuously elongating nucleus. Condensation of chromatin in 25 nm fibers is produced from the poles (as shown in Figure 1E,F) and progresses towards the center of the nucleus where the chromatin is not yet packaged (Fig. 2A). Figure 2B can be considered as a detail of the basal part in Figure 2A where a complete section of the implantation fosse is shown. Fibrillar chromatin is found exclusively attached to the basal zone that surrounds the distal centriole. Figure 2C represents a transverse section ("c" in Fig. 2B) where it can be observed how the fibrillar chromatin is radially connected to the attachment zone, while the section shown in Figure 2D ("d" in Fig. 2B) shows the chromatin that is still not organized in fibers.

The difference between the concepts of "chromatin condensation pattern" and "spatial organization of the condensation pattern" can be appreciated in Figure 3. Figure 3A,B shows the apical half of a nucleus in a more advanced spermiogenic stage. All the nuclear chromatin is found condensed following a fibrotubular pattern, now of 40 nm in diameter. Chromatin fibers are anchored exclusively to the apical (Fig. 3B) and basal (not shown) zones, and only in the vicinity of these regions do they adopt a parallel distribution (Fig. 3B). The central part of the nucleus contains chromatin condensed with the

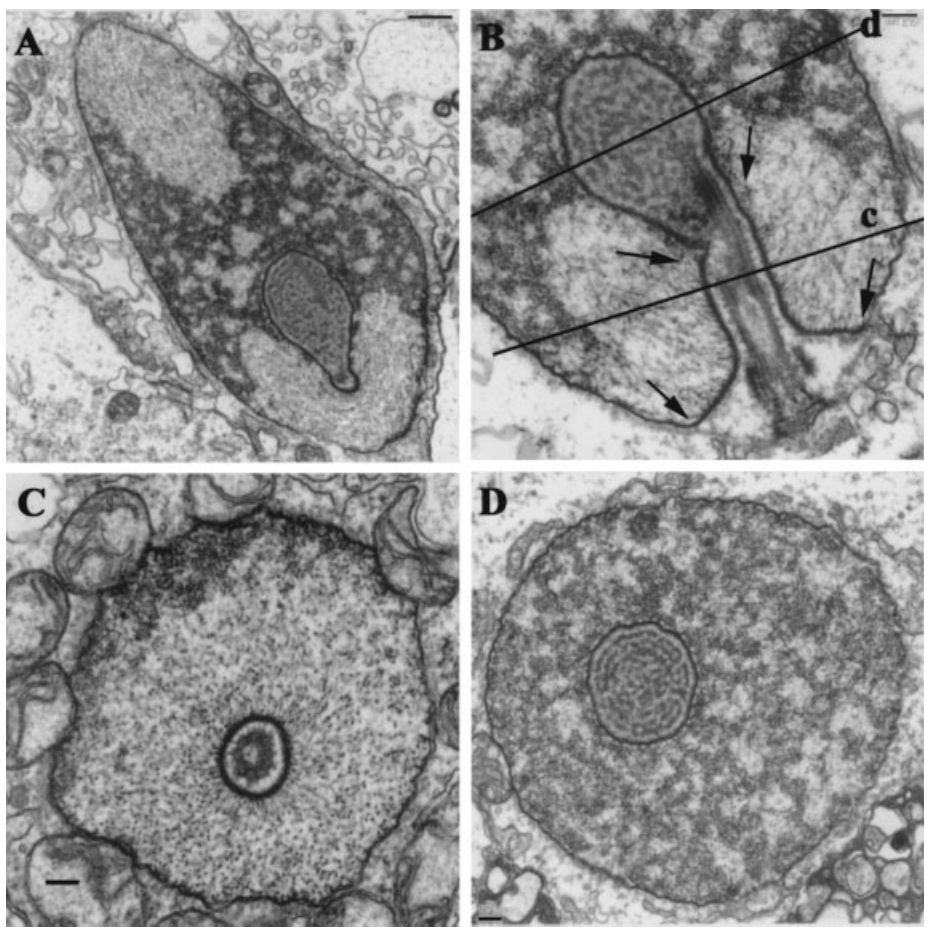

Fig. 2. A: Oblique section of an elongating nucleus. The fibrillar organization of chromatin progresses from the apical (**top left**) and basal (**bottom right**) poles towards the center. **B**: Detail of the implantation fosse in a spermiogenic stage similar to the one shown in (A). Chromatin appears anchored to the *Lamina densa* but only in the zone immediately adjacent to the centriole (region between each pair of arrows). (**C**, **D**) Transverse sections of a nucleus similar to the one shown in B. Although it is not treated in this work, it is worth noting the association that exists between mitochondrial and nuclear membranes. Bars: A, 0.5 μm; B; C; and D, 0.2 μm.

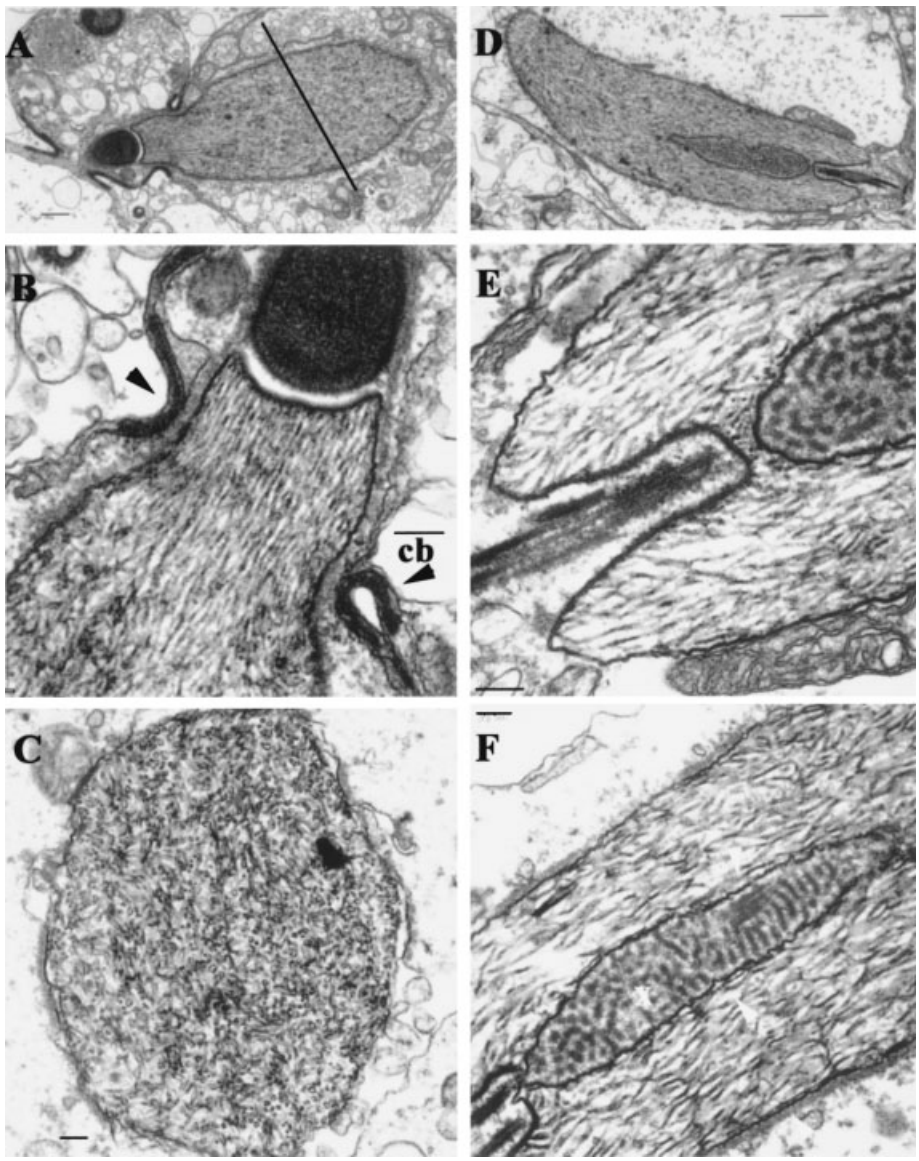

Fig. 3. A: Semi-longitudinal section of a spermatid nucleus in a stage where all the chromatin is in 40 nm fibers (a, acrosome). **B**: Detail of the apical zone. Chromatin appears clearly attached to the *Lamina densa* and fibers are already adopting a parallel distribution in this part (**top right**, acrosome; cb and arrowheads indicate edges of cytoplasmic bridges). **C**: Detail of the intermediate section of (A). Although the condensation pattern is the same as in the apical zone, the chromatin fibers are not seen to follow any preferential direction. **D**: Semi-longitudinal section of the basal pole of a nucleus in a later development stage. **E**: Detail of the zone surrounding the implantation fosse: the chromatin anchorage to the *Lamina densa* can be clearly appreciated. **F**: Detail of the area surrounding invagination (x) in a similar nucleus. Chromatin fibers are not associated with the nuclear membrane (arrowheads) except for the part of the lamina that surrounds the centriole (**bottom left**). In these nuclei, most of the chromatin fibers are found preferentially following the nuclear elongation axis direction. Bars: A and F, 0.5 μm; B; C; and E: 0.2 μm; D, 1 μm.

same pattern (40 nm fibers), but these fibers/tubules do not present any apparent order in their spatial disposition (see the bottom part of Fig. 3B, and Fig. 3C, which represent the section indicated in Fig. 3A). Later, as the nucleus continues its elongation process (Fig. 3D), the fibers/tubules of chromatin adopt a parallel distribution following the nuclear elongation axis. Figure 3E,F shows a magnification of a region equivalent to the bottom half of Figure 3D. Again, it can be verified that the chromatin is exclusively attached to the basal *Lamina densa* and not to the membrane that surrounds the material of the inner part of the invagination, nor to the peripheral nuclear membrane (arrowheads in Fig. 3F). Figure 3D–F also demonstrates that chromatin fibers adopt a parallel orientation and that this happens progressively as the nuclear elongation becomes more evident. Our interpretation is that this is due to the stretching forces to which chromatin is submitted during the nuclear elongation process through the anchorage points on the *Lamina densa*.

Final Stages of Spermiogenesis: Origin of the PNM From the *Lamina densa* and Demonstration of the PNM as the Chromatin Anchorage Point

In very advanced stages of *O. vulgaris* spermiogenesis, the 40 nm fibrilar structures associate to form progressively larger fibers (60–80 nm) which appear to be attached exclusively to the polar zones of the *Lamina densa* (Fig. 4A,B). The *Lamina densa* appears at the beginning of spermiogenesis as a simple fusion of the nuclear membranes of the apical and basal zones (see Fig. 1). However, as spermiogenesis advances, it progressively thickens and acquires a well defined multilamellar structure that we call PNM.

By the final stages, the major chromatin fibers fuse, driving towards the uniform thread-like appearance of chromatin. These chromatin threads contain protamines (as is shown in the accompanying article) and, as can be appreciated in Figure 4C–E, they adopt a quasi-parallel disposition.

Figure 4E shows a detail of the PNM structure at the basal zone as compared with the membranes that externally limit the nucleus (and the cell). The complexity of the PNM structure is easily appreciated here. The same figure shows a transverse section of the threads of mature chromatin, which present an approximate diameter from 3 to 5 nm. It is important to remark here that in all the micrographs that we have obtained for this study, chromatin appears to be anchored exclusively to the apical PNM, and the basal PNM that surrounds the centriole, but never to the inner membrane that coats the invaginated basal material (see also below).

In order to demonstrate the attachment of chromatin with the PNM, we have prepared *O. vulgaris* sperm cell nuclei from spermatophores and we have incubated them in 10 mM DTT, 1 mM Tris, pH 8.0, 1 mM EDTA (TE-DTT buffer) for 15 min at 4°C. As shown in Figure 5A, most of the nuclei undergo a slight swelling under these conditions. However, nuclei so treated retain all of the protamine in their chromatin. One aliquot of these nuclei was then incubated in 1 M NaCl, 10 mM DTT, 1 mM Tris, pH 8.0 for 30 min at 4°C. This treatment involves the loss of approximately 93% of the protamine associated with the sperm DNA, but even so nuclei do not lose their identity (Fig. 5B); i.e., they appear swollen but their DNA remains fixed to two areas, thus preventing their dissolution. The areas with the maximum DNA concentration (arrowheads in Fig. 5B) coincide precisely with the apical and basal regions of the nuclei where the PNMs are located. A second aliquot of the TE-DTT treated nuclei was incubated with 2 M NaCl, 10 mM DTT, 1 mM Tris,

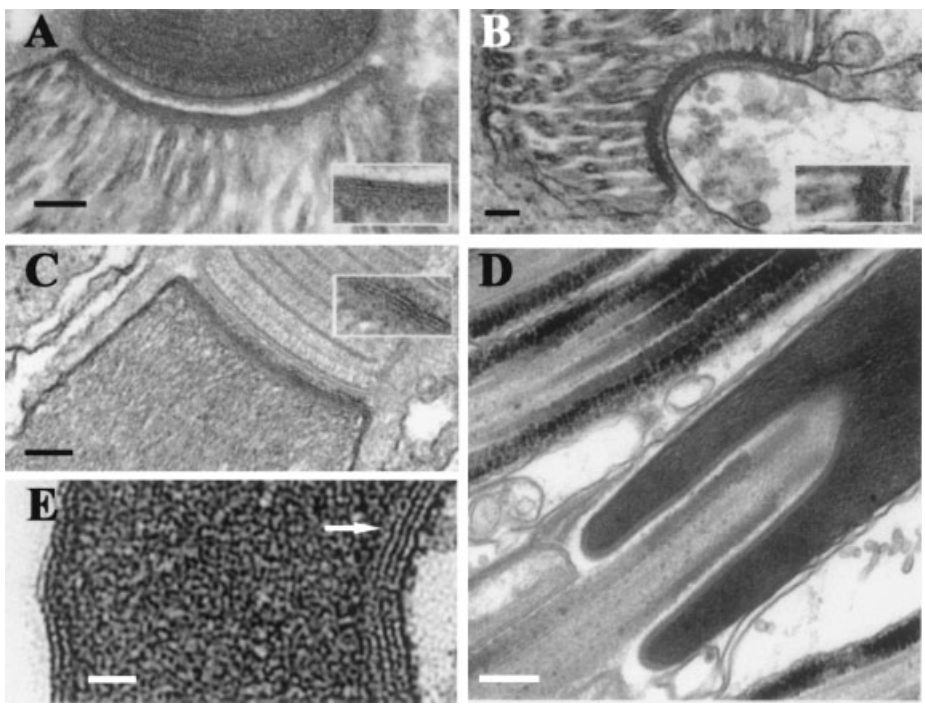

Fig. 4. Longitudinal apical (**A**) and basal (**B**) sections of advanced *O. vulgaris* spermiogenic nuclei. Chromatin fibers appear now with an approximate diameter of 60–80 nm resulting from the fusion of the 40 nm fibers. Chromatin is anchored to the apical and basal densifications of the membrane. These densifications adopt a multilamellar structure (insets). From this stage we call these structures the PNM; (**C**) and (**D**) Apical and basal longitudinal sections, respectively, of a nucleus corresponding to a later stage in spermiogenesis. Chromatin, bound to the PNM, adopts now the appearance of thin parallel fibers which are formed after the coalescence of the 80 nm fibers. **E**: Shows a transverse section of the basal zone where a detail of the PNM multilaminar structure (arrow), as well as the fine chromatin fibers, can be observed. Bars: A; B; and C, 0.1 μm (insets, 0.05 μm); D, 0.2 μm; E, 27 nm.

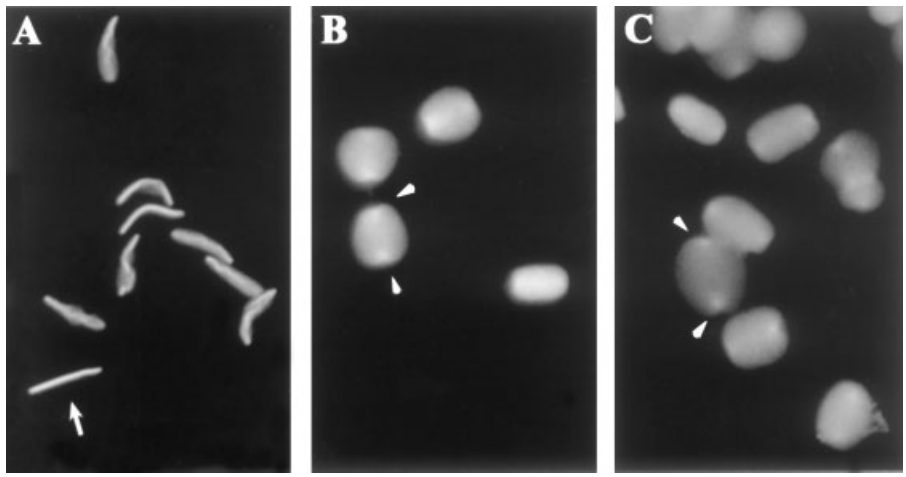

Fig. 5. *O. vulgaris* sperm cell nuclei incubated with a hypotonic and reductive medium (TE-DTT) for 15 min. This treatment promotes the swelling of most of the nuclei (**A**). The arrow shows one nucleus that has not yet reacted to the treatment; it serves as an example of an untreated control nuclei. TE-DTT treated nuclei were then washed with either 1 M NaCl (**B**) or 2 M NaCl (**C**). Although these treatments solubilize 93% and 97%, respectively, of the nuclear protamines, nuclei maintain their integrity as DNA is anchored to the nuclear edges (arrowheads).

pH 8.0 for 30 min at 4°C. Again, although this treatment eliminates approximately 97% of the protamine associated with the sperm DNA, individual nuclei can still be seen with their DNA anchored to the PNMs (Fig. 5C).

Electron microscopy of the 10 mM DTT/2 M NaCl treated nuclei (Fig. 6) demonstrates how, in fact, the PNM is the DNA anchorage point. Figure 6 shows that in both the apical (A) and the basal (B) areas, the PNMs retain a considerable amount of condensed chromatin, thus impeding the solubilization of the nucleus. In these images, the PNM does not show the laminar structure mentioned above, probably because of the DTT and/or NaCl treatment. However, this observation points to the PNMs being rigid structures, resistant to NaCl treatment, that maintain DNA strongly attached.

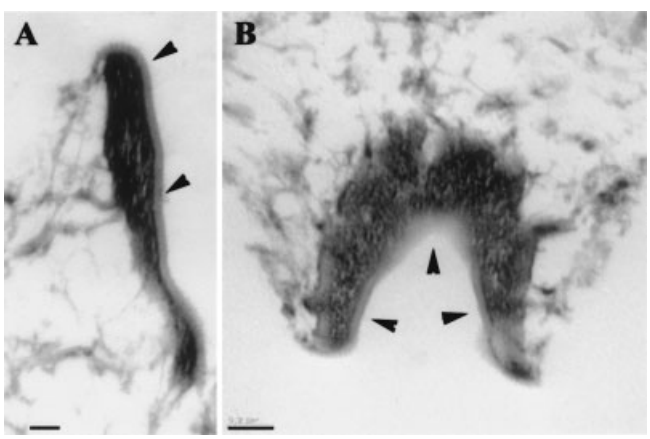

Fig. 6. Transmission electron micrographs of the apical (**A**) and basal (**B**) poles of the TE-DTT-2 M NaCl treated nuclei. The images show how, even after the chemical reduction with DTT and the extraction of the DNA-associated proteins by the high concentrations of NaCl used (2 M), chromatin is still anchored to the PNM (arrowheads), where it remains partially condensed. Bars: A and B, 0.2 μm.

DISCUSSION AND CONCLUSIONS

The observations presented in this work about the cephalopod *O. vulgaris* indicate, firstly, that at the beginning of spermiogenesis and in the regions neighboring the acrosome and the centriole, the inner and outer membranes of the nuclear envelope fuse to form an electrodense lamina. Part of the chromatin appears to anchor to the electrodense apical and basal laminas. Chromatin begins to condense from these zones, following a complex fibrillar pattern that evolves from 25 nm to 40 nm to 60–80 nm fibers as spermiogenesis advances. The reunion of the main fibers (not shown in detail in this work) leads to the final appearance of chromatin as a bundle of fine and compact parallel fibers (see Fig. 4C–E) that follow the axis of nuclear elongation.

We think that nuclear regions near the acrosome and the centriole may represent general areas where chromatin initially begins to be condensed and organized not only in the sperm cells of *O. vulgaris*, but also in other animals. A large number of electron microscopy observations in mammals demonstrate that the nuclear membrane adjacent to the acrosome undergoes an early differentiation during the first stages of spermiogenesis, acquiring a thickened and more electrodense aspect (see for instance Daudone and Alfonsi, 1986 and the review by Fawcett, 1975). Courtens et al. (1991) demonstrated that this electrodense lamina is not only formed by the nuclear membranes, but it also contains a larger DNA (chromatin) concentration as compared with other regions of the spermiogenic nuclei. However, although it appears to be a fairly common structure in these species, the origin and differentiation of the nuclear *Lamina densa* found in the acrosomal pole is not known. Even so, one can propose that it could be influenced by the activity of molecules that would occupy the space between acrosome and nucleus.

On the other hand, several authors have also described an electrodense lamina and/or chromatin accumulations in the nuclear regions adjacent to the centriole during the beginning of spermiogenesis. This lamina has been observed both in invertebrates (insects, Chevaillier and Gusse, 1975; molluscs, Ribes et al., 2001) and vertebrates (Gusse and Chevaillier, 1978).

A second interesting aspect is that the electrodense lamina (which in *O. vulgaris* will develop into the PNM) not only represents the point where chromatin initiates its fibrillar pattern of condensation, but it is also the point of chromatin anchorage. This fact illustrates the interdependence between the extranuclear and the intranuclear processes during the nucleomorphogenesis of the sperm cell. It has been proposed that in cephalopods, the cytoplasmic microtubules are responsible for the elongation of spermiogenic nuclei (Maxwell, 1974, 1975; Ribes et al., 2001). Thus, in the case of *O. vulgaris*, it seems very probable that the stretching of the nucleus provoked by the microtubules would also promote the distribution of chromatin fibers in parallel due to their anchorage to the PNMs.

The third aspect of this discussion refers to the relationship between these rigid structures and the architecture of the spermatic nucleus. The most similar structure to *O. vulgaris* PNM that we have found in the literature is the *nuclear annulus* studied by Ward and Coffey (1989) in the hamster spermatozoa. These authors find that the hamster *nuclear annulus* is located exclusively in the implantation fosse region. They propose that it can play an important role in the organization of condensing chromatin and also that every chromosome binds to this structure. In the genome of *O. vulgaris*, it may be that every chromosome presents at least two particular points of attachment to a PNM: one in the basal pole and the other in the apical pole (see Fig. 5). If it was also the case that these anchorage zones represented specific DNA sequences located in particular positions of chromosomes, these zones would determine the architecture of the entire sperm nucleus; i.e., the places in the sperm nucleus that would be occupied by the remainder of the genome. As already commented in the "Introduction," there are several studies showing that some genome regions are not distributed at random in the sperm nucleus (Zalensky et al., 1993; Joffe et al., 1998; Hazzouri et al., 2000), although this is not necessarily a universal fact (Solovei et al., 1998).

From our observations, we consider that the spermiogenic nuclei of *O. vulgaris* constitute an adequate model to study the DNA organization and architectural disposition of the genome. In the accompanying article, we present the study of the basic proteins that interact with DNA to determine the fibrillar type of condensation shown by chromatin. On the other hand, we are currently making progress in our laboratory in the study of the structure of the PNM.

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