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Chromatin Condensation During Spermiogenesis in Rats

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ABSTRACT The process of chromatin condensation during the transformation from spermatids to spermatozoa in rat was observed by transmission electron microscopy. In Golgi and cap phase spermatids (stages 1-7), 2 sizes of chromatin fibers are evenly distributed in the nuclei, 10 nm and 30 nm thick (level 1 and 2). The latter are uniform fibers that appear as dense dots in cross section, while the former are thin zigzag fibers that link between 30 nm fibers. In earlier stages, level 1 fibers tend to predominate, while in later stages level 2 fibers do. In early acrosome phase spermatids (stages 8-9), the nuclei transform from round to pear shape with partially formed acrosomes covering the anterior ends, and the chromatin fibers, which are mostly at level 2, are packed closely and evenly together. There are increasing number of larger fibers about 40 nm in diameter (level 3) in the subacrosomal region of the nuclei. In mid acrosome phase spermatids (stages 10-12) the 40 nm fibers appear to grow in width to 50 nm (level 4) which transform into long straight fibers that are interlaced together in several directions, and become distributed evenly throughout the nuclei. In late acrosomal phase spermatids (stages 13-14) the chromatin appears as 60 and 70 nm thick knobs and branching cords (levels 5 & 6) in the anterior part of the nucleus, which becomes highly tapered, while the posterior part still contains mainly 50 nm straight fibers. The wave of transformation to thick chromatin cords continues from the anterior to posterior regions, until in maturation phase spermatids (stages 15-17) when chromatin in the anterior halves of some nuclei is completely condensed and the rest of chromatin appears as 90-100 nm thick (level 7) branching cords with narrow intervening light spaces. In immature spermatozoa (stages 18-19) the chromatin becomes completely condensed with only few pale spots scattered widely throughout each nucleus. The three main types (30, 50, 100 nm) of chromatin fibers observed during spermiogenesis correlate well with the sequence of the development of the spermatid to spermatozoa when histones, transition proteins and protamines are bound to DNA.

KEYWORDS: chromatin, condensation, rat, spermatids.

INTRODUCTION

During the process of spermiogenesis of most mammals, DNA in the haploid male germ cells are progressively condensed by the gradual replacement of histones with transitional proteins. These proteins are replaced in turn by protamines, which are the most basic nuclear proteins.[1,2] Concurrent with the histones' replacement, the original nucleosomal-based chromatin fibers transform into different organization and become condensed into various patterns which finally form completely electron-opaque mass as seen in the nuclei of the fully mature spermatozoa.[3] The degree of chromatin compactness in mature sperm of various species depend on the amount of histones being replaced by protamines. Rat spermatozoa exhibit denser chromatin than human spermatozoa, which is probably due to the lower percentage of histones remaining in their chromatin.[4] Whereas nucleohistone chromatin fibers in spermatids of all mammals examined so far appear to have nucleosomal organization which may be arranged in form of 30 nm solenoid fibers, nucleo-protamine chromatin fibers may vary in forms. In rat spermatozoa, they may exist in the form of large fibers or cords with thickness about 80-100 nm that are aligned in parallel.[5] In this study we have identified the different levels of higher-ordered chromatin fibers in rat spermatids and the pattern of their condensation.

MATERIALS AND METHODS

Adult Wistar rats age 10-12 weeks were obtained from National Center for Experimental Animals, Salaya Campus, Mahidol University, Bangkok,

Thailand. They were anesthetized by ether inhalation, and the testes were removed, sliced into small pieces, fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 4^0C overnight, and postfixed in 1 % OsO_4 in the same buffer for 2 hours. Subsequently, the tissue blocks were dehydrated by ethanol and embedded in Araldite 502 resin. Ultrathin sections were cut and stained with lead citrate-uranyl acetate and examined under a Hitachi TEM H-300 at 75 kV. Catalase crystal (Agar Aids) with the lattice spacing of 87.5 A° were photographed at various magnifications and used for comparison with the measurement of chromatin fiber sizes. The dimensions of chromatin fibers appearing in TEM negatives were projected and measured under a Nikon 6 C profile projector with a secondary magnification of 10X to 20X.

Results

The organizational levels of chromatin fibers in various stages of spermatids were examined by TEM and their thickness measured as mentioned above.

Round spermatids These cells belong to the Golgi and cap-phase which, according to the classification proposed by Leblond and Clermont,[6] correspond to stages 1 to 7 (Fig 1A; 6). The nuclei of these cells, which are round, have two levels of chromatin fibers (Table 1). Level 1 are fine zig-zag fibers that are dispersed throughout the nucleus, each with the thickness about 10 nm. Level 2 are thicker fibers having about 30 nm in diameter, which usually appear as dense dots in cross section, and correspond to the fundamental chromatin fibers that are present in the nuclei of various stages of spermatocytes, as well as in somatic cells. Level 2 fibers may aggregate tightly together to form small blocks or narrow strips of heterochromatin close to the nuclear envelope (Fig 1B). Only a small number of heterochromatin blocks were observed in the nuclei of round spermatids, as the 10 nm and 30 nm fibers constitute the majority of the euchromatin.

Early acrosome phase spermatids These cells correspond to stage 8 and 9 of the cycle of the seminiferous epithelium[6] (also see Fig 6). The nuclei of these cells are transformed into oval or pear shape, and the acrosome becomes a definitive structure covering the anterior part of the nucleus. The nuclear membrane under the acrosome is thickened and the nuclear ring together with the manchette, consisting of 4-5 layers of longitudinally oriented microtubules, are formed (Fig 1C, D). The main mass of cytoplasm moves to the posterior end (Fig 1C). Within the nucleus, in addition to the two levels of chromatin fibers, thicker fibers which appear in cross section as larger and denser dots, each with diameters of about 40 nm (level 3), could also be observed (Fig 1C, D; Table 1).

Mid acrosome phase spermatids These cells correspond to stages 10 to 12 (Fig 6). Their striking feature is the elongation of the nuclei, which start to assume falciform shape. The anterior region is covered by the completely formed acrosome (Fig 2A). The centriolar apparatus is localized in the implantation fossa contiguous to the electron-dense caudal plate of the nucleus (Fig 2A, B). Towards the end of stage 10 and in stage 11 chromatin fibers increase in size to 50 nm in diameter (level 4), which are distributed chiefly in the subacrosomal area, and in the caudal part close to the dense plaque on the posterior nuclear membrane (Fig 1E, F; Table 1). Besides the difference in diameter, the appearance of these fibers is also drastically different: while level 2 and 3 fibers appear as dot-like, which is interpreted to be cross sections of highly convoluted fibers, level 4 fibers appear as thick and straight fibers with long sections appearing even in thin sections (Fig 1E, F). Some level 4 fibers might also be seen as dot-like features, which is interpreted to be the cross sections of the straight fibers, but with less frequency (Fig 1F). From this observation, it is possible that during stage 10 the chromatin fibers begin to change in size as well as conformation. While the first 3 levels of chromatin fibers may be randomly coiled, the 4^{th} level fibers, in addition to becoming thicker, also transform into straight threads which are extensively aligned in parallel. In stage 11 the nucleus is uniformly filled with level 4 fibers (Fig 1E, F). The next levels of organization are highly condensed chromatin fibers appearing as large knobs (level 5) and branching cords (level 6) with the thickness varying from 60-70 nm (Fig 2B, D; Table 1). These start to form in the subacrosomal portion of the nucleus. This culminates in the initiation of chromatin condensation in the anterior part of the nuclei in stage 12 (Fig 2A-D). These levels 5 and 6 fibers may achieve their thickness and density as the result of lateral association and coalescence of several level 4 fibers. Interestingly, the width of the anterior part of the nucleus is also substantially reduced, while the mid and caudal portions of the nucleus are still filled with level 4 fibers and appear rather wide (Fig 2A, B, F), suggesting the possibility that the nuclear tapering occurs as the result of this initial chromatin condensation.

Late acrosome phase spermatids These cells

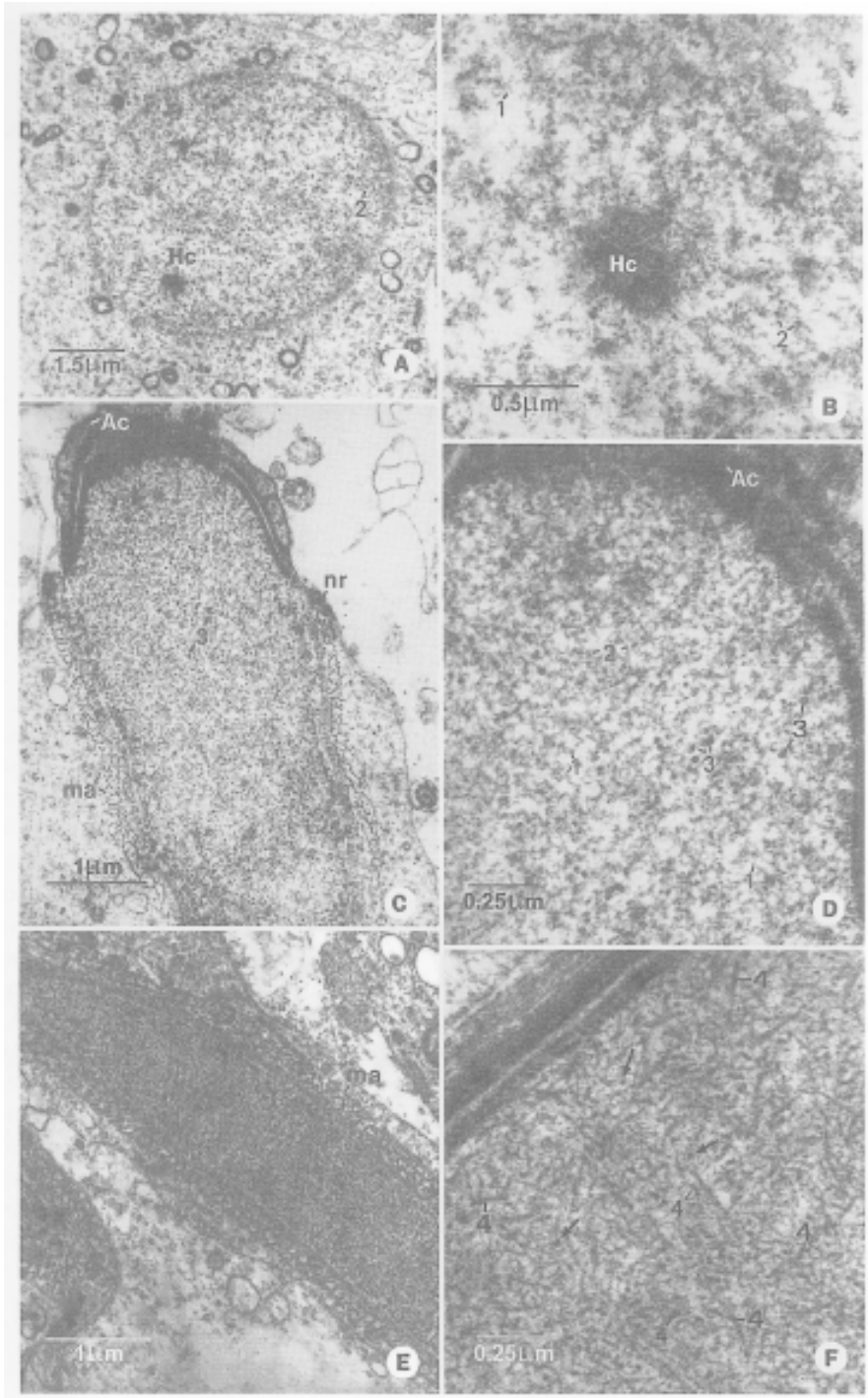

Fig 1.

A, B Stage 1 rat spermatid, showing the nucleus containing mostly euchromatin which consists of two levels of chromatin fibers: level 1 appear as thin zigzag fibers with the thickness about 10 nm (1), and level 2 appear mostly in cross sections as dense dots about 30 nm in diameter (2). Few blocks of heterochromatin (Hc) consisting of tight aggregations of 30 nm fibers are also present. Magnifications of all electron micrographs are indicated by micron bars (mm).

C, D Stage 9 spermatid, showing elongated nucleus with completely formed acrosome (Ac) at the anterior, nuclear ring (nr) and manchette (ma) in the cytoplasm that becomes localized towards the posterior part of the cell (in C). In D, a few large dense dots about 40 nm in diameter (3), which may represent cross sections of larger chromatin fibers, are present in the subacrosomal area and the anterior part of the nucleus.

E, F Stage 11 spermatid, showing 50 nm straight fibers interlacing with each other; short straight segments (4) as well as dots (arrows) which represent the cross sections of these fibers could also be observed.

Table 1. The sizes of chromatin fibers during condensation in various stages of rat spermatids. Levels 1 and 2 are thought to be fibers before histones are replaced, while levels 4, 5, 6, and 7 are fibers after histone replacement. At level 4, chromatin fibers significantly increase in size, as well as change from coiled to straight conformation. Level 7 is the highest ordered structure when the chromatin appears as branching cords. The measurements of chromatin fibers were performed from TEM negatives of at least 10 cells from each stage. Values are expressed as mean ± SD.

STAGES OF MALE GERM CELLS	DIAMETERS OF CHROMATIN FIBERS (nm)						
	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Golgi & cap phase spermatids (Stages 1-7)	10.01 ± 0.02	30.03 ± 0.13	-	-	-	-	-
Early acrosome phase spermatids (Stages 8-9)	10.03 ± 0.06	30.02 ± 0.04	40.03 ± 0.03	-	-	-	-
Mid acrosome phase spermatids (Stages 10-12)	10.02 ± 0.05	30.04 ± 0.04	-	50.01 ± 0.11	-	-	-
Late acrosome phase spermatids (Stages 13-14)	-	-	-	50.05 ± 0.24	61.88 ± 2.50	68.94 ± 1.44	93.13 ± 4.86
Maturation phase spermatids (Stages 15-17)	-	-	-	-	-	68.39 ± 1.19	94.72 ± 4.48

correspond to stages 13 and 14 (Fig 6). Level 5 and 6 chromatin fibers could be found throughout the nucleus (Fig 3A-F). The gradient of chromatin condensation progresses from the anterior part until it covers the whole nucleus. In some spermatids the anterior half of the nuclei may contain completely condensed chromatin.

Maturation phase spermatids These cells correspond to stages 15 to 17 (Fig 6), where the acrosomes are fully developed and covers the anterior two-third of the nuclei. Still attached to the posterior end of the acrosome are the nuclear rings and manchettes (Fig 4A-E; 5A, B). Gradually, the manchettes disappear by being shed with the residual cytoplasm. The chromatin in the anterior half of the stage 15 nucleus becomes almost completely condensed, except for small electron-lucent slits or channels between very thick branching chromatin cords, each about 100 nm in width (level 7) (Fig 4B, D; 5A, B; Table 1). However, in the posterior half of the stage 15 nucleus may still contain level 5 and 6 and a small amount of level 4 fibers (Fig 4A, B, E). The midpiece of the tail is formed between the manchette (Fig 4F). In stage 16 and 17 the chromatin assumes level 7 organization throughout the nucleus (Fig 5A, B).

Immature spermatozoa These cells correspond to stages 18 and 19, when their chromatin becomes almost completely condensed and electron opaque, except for small circular spots which are widely scattered throughout the nucleus (Fig 5C-F).

Discussion

Leblond and Clermont[6] have classified rat spermatids into 19 stages based mainly on the acrosome and tail formations. While many investigators have studied the sequence of replacement of histones by transitional proteins and protamines,[1,7-10] actual morphometric data concerning the change in the higher orders of chromatin fibers and the pattern of their condensation in various stages of spermatids of rats have still not been reported. The present study has shown that the nuclei of round spermatids (stages 1-7) have mostly 10 nm-thick chromatin fibers (level 1), and a certain amount of 30 nm-thick fibers (level 2), and the latter may also aggregate together to form small heterochromatin blocks. The dehydration process during tissue preparation may not decrease the actual size of the chromatin fibers, because they are already composed of very tightly packed macromolecules. In early acrosome phase spermatids (stages 8-9), the chromatin fibers increase to 40 nm, which is designated as level three. These fibers appear as dense dots in cross-sections which could be interpreted that they are randomly coiled. In mid-acrosome phase spermatids (stages 10-12), further enlargement of chromatin into 50 nm-thick straight fibers (level 4) which assume more parallel orientation were observed. Thus, 40 nm-thick fibers were no longer observable. The size and conformation change of chromatin fibers during these stages may be the consequence of the loss of histones, including various variants of H1 and other core histones, and/or their replacement by transition proteins (TP) as

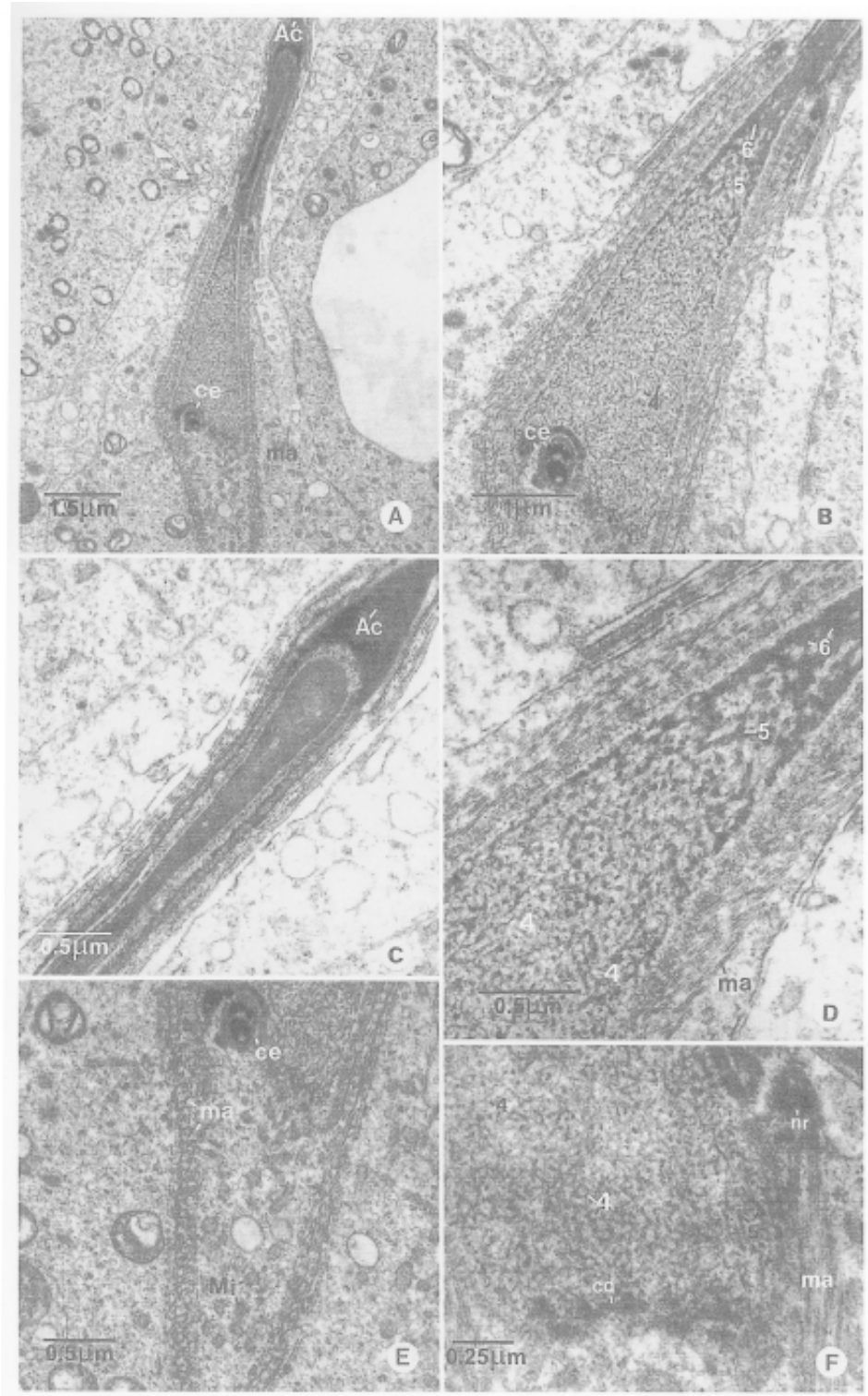

Fig 2.

A-D Stage 12 spermatid: the anterior part of the nucleus contains thick branching chromatin cords with variable thickness at 60 nm (5) and 70 nm (6), which may represent the higher-ordered structures formed by the lateral aggregation of smaller level 4 fibers. The most anterior part of the nucleus usually contains completely condensed chromatin (in C) while the middle and posterior parts still contain 50 nm straight fibers (4) (in B, D).

E, F The posterior end of the same nucleus showing the implantation fossa occupied by the centriole (ce), manchette (ma) attaching to the nuclear ring (nr) and surrounding the nucleus, and the caudal condensation (cd) on the posterior nuclear membrane.

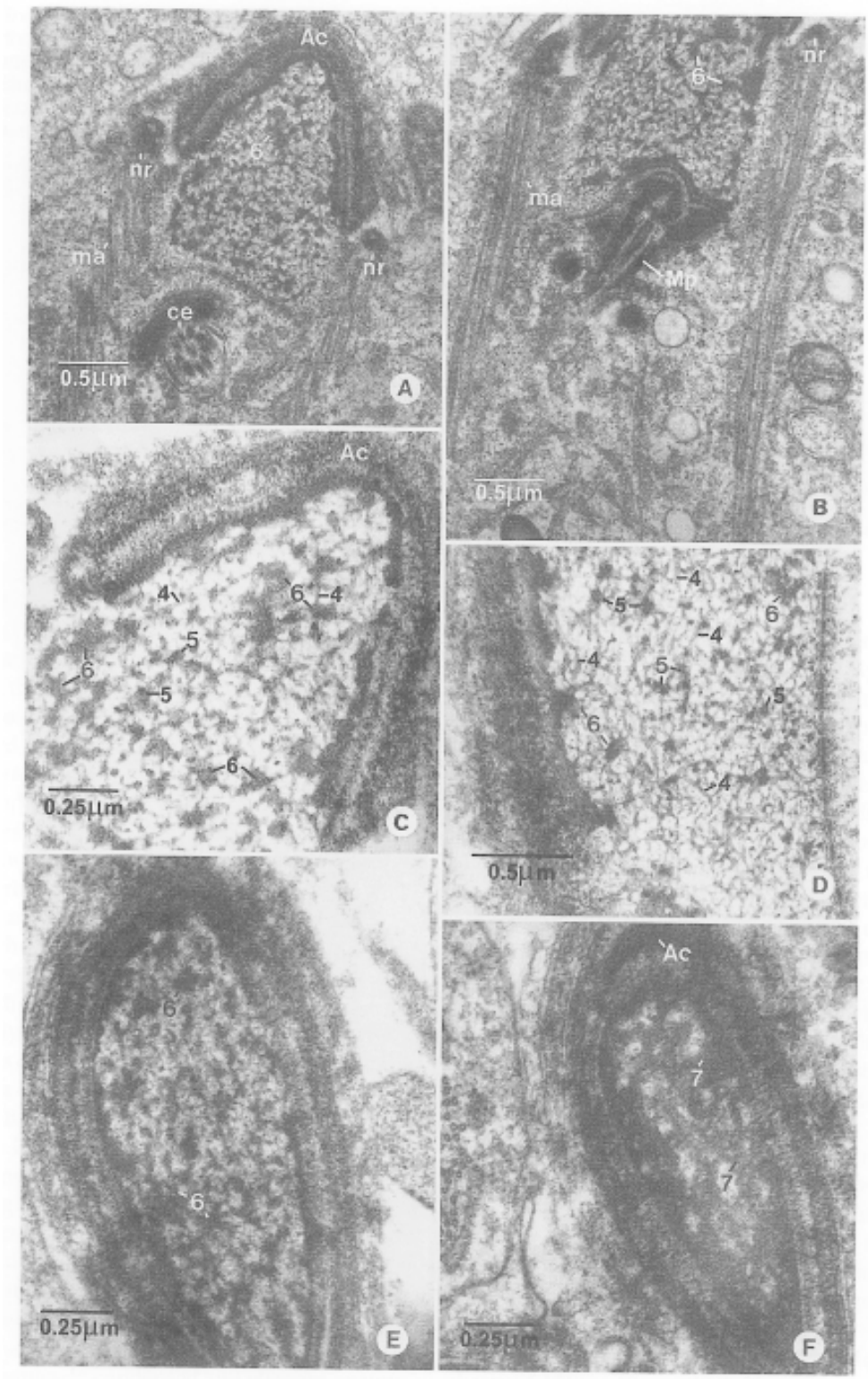

Fig 3.

A-D Stage 13 spermatid, showing the coalescence of 50 nm fibers (4) to form chromatin cords of larger sizes at 60 nm (5) and 70 nm (6) which are spreading throughout the nucleus (in A, C, D). In B, the midpiece of the tail (Mp) is formed from the centriole (ce) in the implantation fossa. Nuclear ring (nr) and manchette (ma) are present on both sides of the nucleus.

E, F Stage 14 spermatid, showing the formation of highly electron dense chromatin cords at 70 nm (6) and 90-100 nm (7) throughout the nucleus.

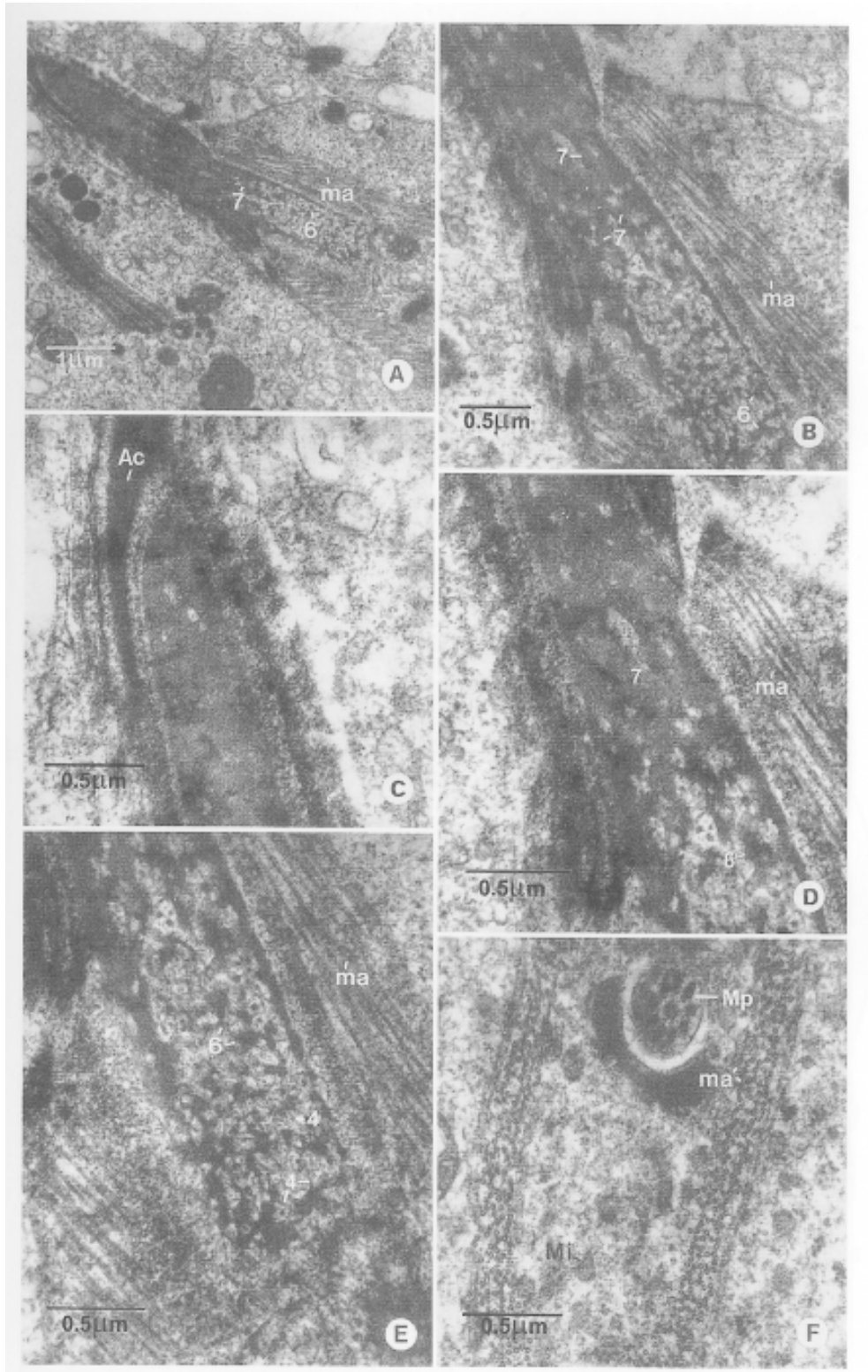

Fig 4.

A-E Stage 15 spermatid, showing completely condensed chromatin in the anterior part of the nucleus (in A, C), and the presence of branching 90-100 nm chromatin cords (7) in the middle portion (in B, D), while the posterior portion (in E) still contains 70 nm (6), 60 nm (5), and 50 nm (4) fibers.

F The midpiece of the tail (Mp) and a group of mitochondria are present between the manchettes (ma).

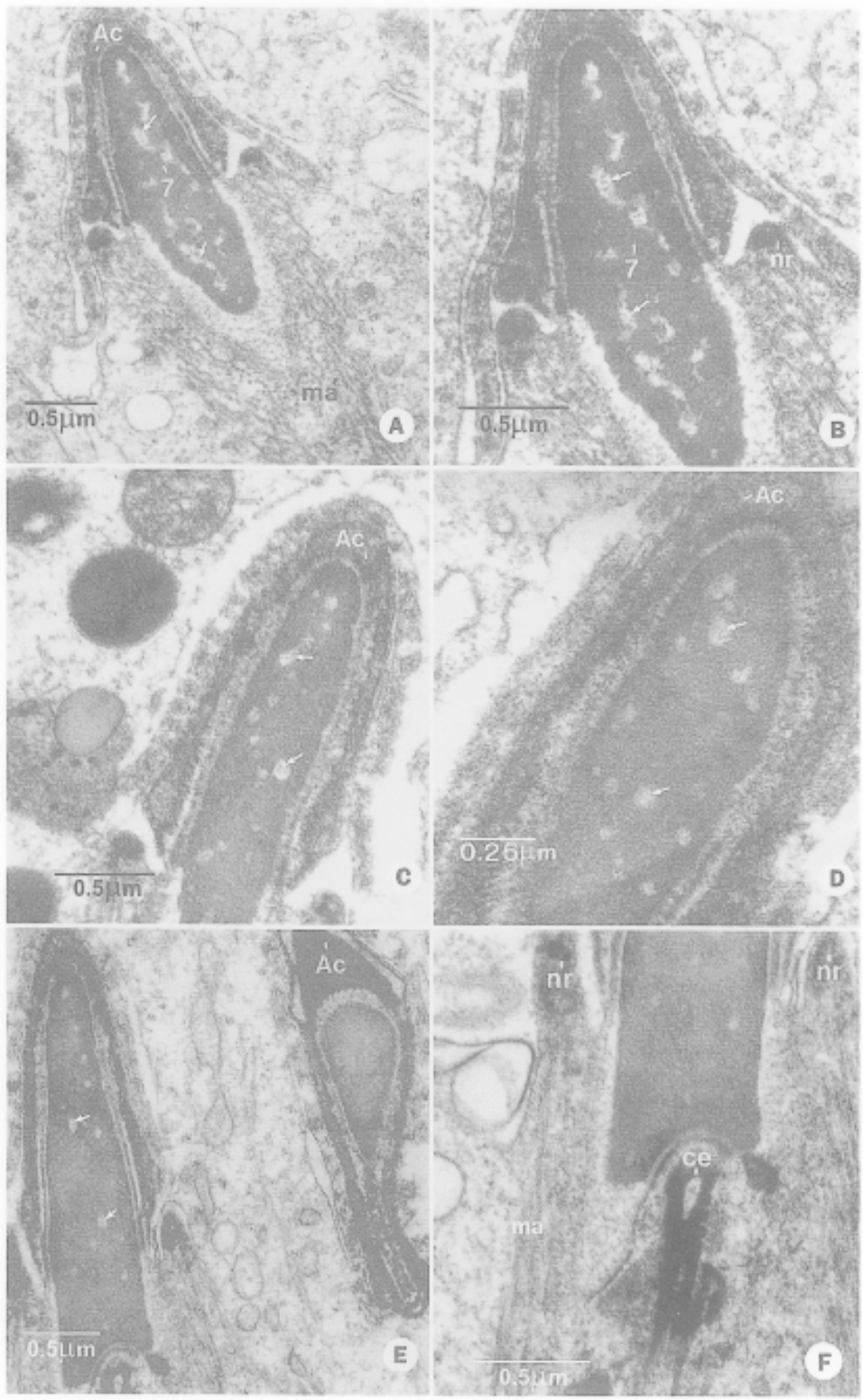

Fig 5.
A, B Stage 17 spermatid, showing the highly condensed chromatin throughout the nucleus which still appear as branching cords, each about 90-100 nm thick (7), separated by narrow clefts (arrows) which are completely electronlucent.
C-F Stage 18 (C, D) and stage 19 (E, F) spermatids, showing completely condensed chromatin in all areas of the nucleus, except for very small vacuoles which appear as electronlucent spots (arrows).

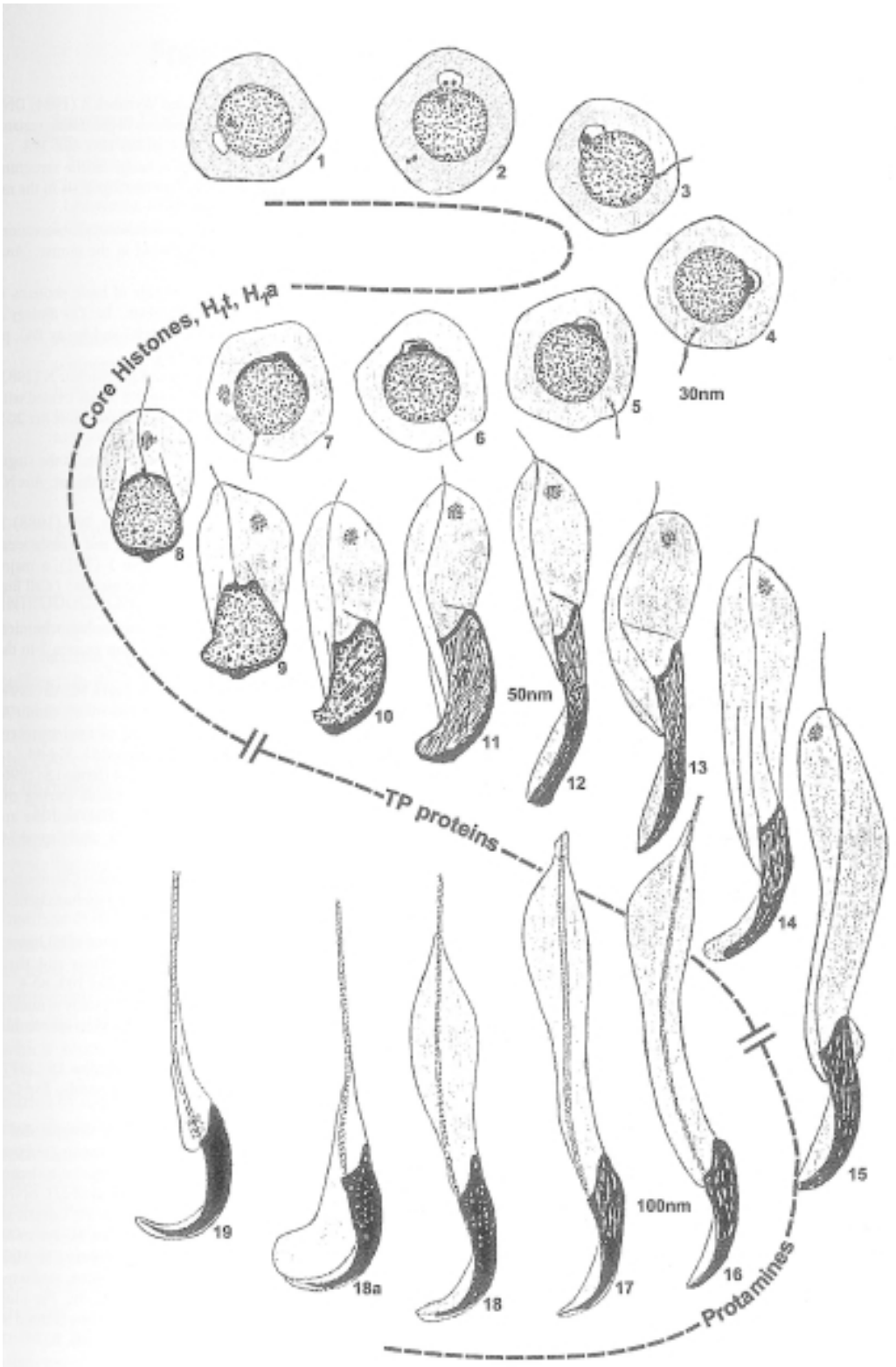

Fig 6. A diagram summarizing the changes in sizes and conformation of chromatin fibers and the pattern of chromatin condensation in correlation to the sequence of replacement of histones, transitional (TP) proteins, and protamines.

reported earlier.[9-12] In late acrosome phase spermatids (stage 13-14), chromatin fibers increase in thickness to 60-70 nm, starting from the subacrosomal and spreading to the caudal regions of the nucleus. Data in Table 1 indicate that the 50 nm fibers in stages 11 and 12 spermatids may be gradually transformed into large intermediate fibers (62 nm and 70 nm) in stages 13-14 spermatids, and finally converted to 95 nm fibers in mature spermatids. This initial chromatin condensation may also be brought about by TP proteins which are found to be the prevalent lysine-rich proteins in these stages[7, 8, 10, 13] (see Fig 6). The large chromatin cords in maturation phase spermatids (stages 15-17) have similar appearance to chromatin remaining within the heads of rat spermatozoa after being decondensed with urea-DTT. This decondensed chromatin appeared as thick branching cords about 100 nm in width linked together by thin zig-zag fibers about 30 nm in diameter.[5] The latter fibers disappeared upon subsequent treatment with micrococcal nuclease. From these results, it is likely that the thick chromatin cords represent the highly packed nucleoprotamines linked together by smaller and loosely packed nucleohistones, which could contain the remaining histones in the rat sperm head, and that these nucleohistone fibers were preferentially digested away by micrococcal nuclease. This interpretation is consistent with the pattern of chromatin condensation in late spermatids where the chromatin fibers gradually increase in size, starting from 50 nm parallel straight fibers in stages 11 and 12 to 90-100 nm branching cords in stages 15 to 17. The increase in thickness of chromatin cords could correspond to the lateral or parallel association of neighboring 50 nm fibers, which later become coalesced and tightly packed together. This final step of condensation could be brought about by protamines, which have been shown to replace TP proteins in stage 15 to 17 spermatids[9,14] (see Fig 6). The pattern of chromatin condensation in rat spermatids may differ from that proposed for human sperm chromatin, where, after histones are replaced by protamines, the randomly coiled 30 nm nucleohistone fibers turn into 50-100 nm beaded fibers that may be formed by the packing together of compact toroidal-shaped nucleoprotamine beads.[15,16]

Acknowledgements

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