BIOLOGY OF REPRODUCTION 48, 1193–1201 (1993)

MINIREVIEW
Deoxyribonucleic Acid Loop Domain Tertiary Structure in Mammalian Spermatozoa[1]

W. STEVEN WARD[2]

Division of Urology, Program in Cell and Developmental Biology, Robert Wood Johnson Medical School New Brunswick, New Jersey 08903–0019

ABSTRACT

In the four decades since the discovery of the basic structure of the DNA double helix, researchers have been investigating the more dynamic tertiary structures that DNA assumes in the various forms of chromatin. The tertiary structure of DNA is important because it is directly related to the function of the genome: for the cell to access the information that is present in the genome accurately and efficiently, the DNA must be in an organized form. This paper reviews the recent work on one particular enigmatic structural form of eukaryotic DNA, that of the highly condensed spermatozoa. Based on the literature and on recently completed experiments in the field, a new model for DNA packaging in sperm nuclei is presented. In this model, each individual DNA loop domain in the sperm chromatin is condensed into a toroid-shaped structure termed the DNA loop doughnut.

INTRODUCTION

It was the discovery of the structure of DNA that elucidated how DNA could function as the biochemical component of which genes were made. The double-helix structure made it immediately obvious how complex information could be stored, in a linear sequence of codes, and how the genome could be efficiently replicated and transferred to the next generation [1]. In the four decades since, much attention has been devoted to the more complex, tertiary structures the DNA assumes at various levels when it is packaged into chromatin. These higher-order structural motifs have proven to be just as important to DNA function as the double helix [2, 3]. Moreover, it is now becoming clear that the structural organization of DNA determines the functional fate, that is, the differentiation, of a cell [2, 4, 5].

While a consensus model for the organization of DNA in somatic cells is now beginning to form [3], it has been only partially defined for the more highly condensed DNA of spermatozoa [6, 7]. The well-documented hierarchy of DNA packaging for the somatic cell is a useful model from which to begin searching for the specifics of sperm DNA organization. Each level of DNA packaging in somatic cells has a specific functional jurisdiction. By identifying and comparing the homologous structural motifs in sperm DNA, we may discover more about the functions of both sperm nuclei and the mammalian genome.

Using somatic cell DNA organization as a paradigm for the study of sperm DNA is logical because spermatozoa are derived from germ cells that have a somatic cell-type DNA organization. Thus, whatever conformation sperm DNA ultimately assumes, it developed from the relatively well-understood somatic DNA chromatin structure and must be related, however distantly. Here, this approach has been used to derive a model for one level of sperm DNA organization that is not yet understood, the structure of the sperm DNA loop domain in its condensed form (Fig. 1).

THE HIERARCHY OF SOMATIC CELL DNA ORGANIZATION AND ITS RELATIONSHIP TO FUNCTION

To begin to understand the function of DNA organization in spermatozoa, we must first review the more fully defined structural hierarchy of DNA in somatic cells, from the DNA double helix to mitotic chromosomes.

Double Helix

The genetic information is coded in a four-letter, linear code in one strand of the DNA. The duplicity of the complementary strands allows for the perfect reproduction of a second copy of the entire genome during cell division [1].

Nucleosome

Every 200 bp of DNA are coiled twice around an octomer of histones to form a nucleosome [8]. This serves at least three functions. First, it shortens the meter of DNA that must be packaged into the average mammalian cell by a factor of 6 [3]. Second, the octomer may help to control the conformation of the DNA by opening into half-nucleosomes when genes are being transcribed [9]. Third, the DNA is wound around the histones in a direction that negatively supercoils the DNA, that is, in a direction that tends to unwind the double helix [10]. This negative superhelicity allows the double helix to be much more easily separated, exposing the template strand, for replication or for transcription upon removal of the histones [11].

Accepted February 10, 1993.
Received December 11, 1992.
[1]Supported by NIH grant HD28501 and by the Edwin A. Beer Award (NY Acad Med).
[2]Correspondence: Division of Urology, Program in Cell and Developmental Biology, MEB-588, Robert Wood Johnson Medical School, New Brunswick, NJ 08903–0019. FAX: (908) 418–8013.

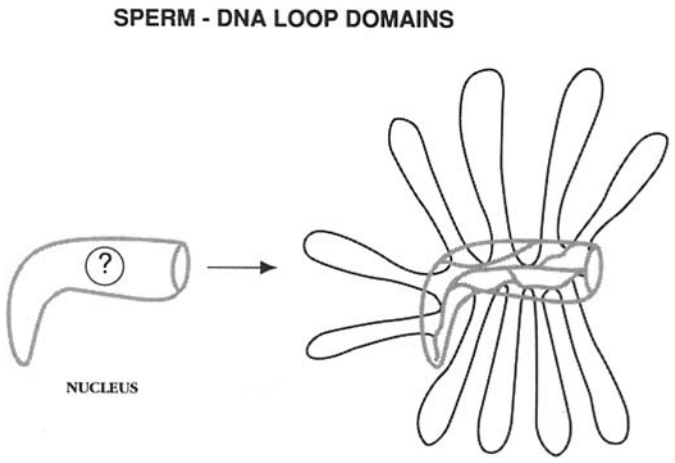


FIG. 1. DNA is organized into loop domains, anchored to the sperm nuclear matrix (right), diagrammed here for a hamster sperm nucleus. It is not known how these DNA loops are packaged in the fully condensed sperm nucleus.

The Solenoid

DNA coiled into nucleosomes is further compacted into a fiber with a diameter of 30 nm. The most accepted model for how DNA is packaged in this 30-nm filament is the solenoid configuration. Six nucleosomes are coiled around each other to form one turn that has a diameter of 30 nm [12]. This shortens the total length of the genome even further. The existence of the solenoid remains controversial, but it is the most probable conformation of the 30-nm filament of eukaryotic chromatin. The model for sperm chromatin put forth in this paper is presented with the solenoid as the parent structure (i.e., during spermiogenesis), but it is not dependent upon it. This will be discussed in greater detail below.

DNA Loop Domains

The 30-nm solenoid fiber of chromatin is arranged into loop domains of an average of 60 kbp in length, which are attached at their foundations to a structural component of the nucleus termed the nuclear matrix [13, 14]. They are attached by specific DNA sequences that are intimately related to function [2, 5]; active genes [2, 5, 15] and origins of DNA replication [14, 16] are associated with the nuclear matrix at the bases of the loop domains. The attachment sites that are related to gene transcription have been proposed to be transient [4], but this idea has been challenged [17]. Razin and colleagues [17, 18] have shown that the chicken α-globin gene is attached to the nuclear matrix at different sites when it is active and inactive. Finally, it has been shown that different cell types within the same organism have different genes associated with the nuclear matrix [2, 19], suggesting that organization of DNA on the nuclear matrix may play a significant role in cell differentiation.

Mitotic Chromosome

Exactly how the DNA loop domains are compacted into the mitotic chromosome remains controversial; the two models that exist have been compared by Pienta et al. [3]. Both contain all the structural motifs discussed above. The nuclear matrix condenses, coiling the associated DNA loop domains into a compact form. In the radial loop model, 18 loop domains are wound around a central core to form a miniband, whereas in the radial coil model, the loop domains are first coiled into a larger, 240-nm fiber that is then further coiled into stacks. The function of the short-lived chromosome configuration is to package the newly replicated DNA into units that allow for efficient separation of the sister chromatids into the two daughter cells, while retaining the specific organizational motifs present in the parent cell. The organized condensation of the loop domains accomplishes both tasks.

SPERM DNA ORGANIZATION AND ITS RELATIONSHIP TO FUNCTION

While many recent advances have contributed to defining the homologous hierarchy of DNA organization in mammalian spermatozoa, the complete hierarchy has not yet been elucidated. The levels of sperm DNA organization so far established are as follows.

Double Helix

The double helix in sperm DNA is almost identical to that of DNA in somatic cells; the genetic code is contained within the four-letter code of DNA. The only documented differences are the increased length of telomeres in sperm DNA [20] and the extent of DNA hypermethylation [21].

Protamine-DNA Complex

This level of sperm DNA organization is homologous to nucleosomes in somatic cells, but it is very different in structure. The DNA binding proteins of mammalian sperm DNA are protamines, highly basic proteins with long stretches of arginine residues [7]. They bind to DNA lengthwise along one of the grooves, with each positively charged arginine residue neutralizing one negatively charged residue of the phosphodiester backbone of the DNA. This transforms the polyanionic DNA into a neutral polymer, and the DNA-protamine complexes can then bind together by van der Waals forces in a linear fashion, at the molecular level [7]. This model, unlike the homologous structure in somatic cells, the nucleosome, does not confer any supercoiling on the sperm DNA [6]. This prediction has been confirmed by two independent laboratories, each of which provided evidence that protamine-bound sperm DNA was not highly supercoiled, if at all [22, 23].

The functional significance of this arrangement may be related to the inertness of sperm DNA. Since the sperm cell does not replicate its DNA or transcribe RNA, its DNA has

no teleological need to be supercoiled (there is one reason for sperm DNA to be very slightly supercoiled, and that is packaging, as discussed in the model below). The overriding evolutionary pressure on protamine is to condense the sperm genome to a tightly packaged, protected state, and the supercoiling can be temporarily sacrificed for the transit. The protamine DNA binding model [7] accomplishes this task.

Sperm Solenoid Equivalent

Very little published evidence addresses the sperm DNA equivalent of the solenoid in somatic cell DNA. Electron micrographic techniques routinely fail to elucidate any discernable structure in sperm chromatin because of its high electron density [24]. This level of sperm DNA packaging is the subject of the model described below.

Sperm DNA Loop Domains

Topologically constrained DNA domains were first described by Risley et al. [23] for amphibian spermatozoa. In their study, the size of the frog loop domains were approximately 25 kb, much smaller than those reported for somatic cells. Similarly, hamster sperm DNA has been shown to be organized into loop domains attached at their bases to a sperm nuclear matrix [22] (Fig. 1). These loop domains are about half the size (47 kbp) of the loops found in somatic cells of the same animal (76 kbp), but are otherwise very similar [22]. Moreover, loop domains in both the hamster [25] and the chicken [26] have been shown to be attached to the nuclear matrix by specific sequences. This suggests that sperm DNA is specifically organized rather than randomly packaged into the nucleus.

These experiments suggest that sperm DNA is organized as specifically as that in somatic cells, and that this organization may be related to function in the same manner. It is possible, for example, that the sperm nuclear matrix may provide a cell type-specific organization of the human genome needed for fertilization and embryonic development, in the form of DNA loop domain attachments.

Sperm Equivalent of the Mitotic Chromosome

There may not be a structure equivalent to the mitotic chromosome in sperm nuclei because spermatozoa do not undergo mitosis. However, the fact that they are the recent products of a meiotic division demands at least consideration of the possibility. It is known, for example, that human sperm DNA can be prematurely condensed into single chromatids with the same banding patterns as the mitotic chromosomes in somatic cells [27]. Also, fully condensed sperm chromatin retains some centromeric specific proteins also found in somatic cells [28]. These data suggest that at least some remnant of the mitotic structure is present in sperm DNA.

PROPOSED MODEL FOR THE SOLENOID EQUIVALENT IN SPERM CHROMATIN

Problem Being Addressed

Recent evidence, together with the published data on sperm chromatin structure discussed above, makes it possible to propose a model for the solenoid equivalent in mammalian spermatozoa. This structural motif is important because we do not yet understand how the DNA loop domains are packaged into the sperm nucleus (Fig. 1). Unlike the somatic cell histones, protamines do not seem to decrease the length of DNA at all, so how are the loops packaged into the nucleus? Are they simply laid down linearly beside each other along the entire length of the sperm nucleus, or are they folded or coiled into a novel macromolecular structure?

Evidence

The model must incorporate, and depends upon, all the available evidence for sperm DNA structure. This includes the data already discussed: that protamine binds to DNA lengthwise along one of the grooves [7], that DNA loop domains exist (Fig. 1), and that the DNA of sperm nuclei containing protamines is not supercoiled [22, 23]. There are three additional pieces of evidence that have been included in preparing the new model, discussed below.

Sperm DNA is packaged in discrete foci. The first piece of evidence comes from in situ hybridization [29] and immunohistochemical staining [28] experiments performed on sperm nuclei for centromeres. In every example, the centromeres were located at discrete foci within the sperm nucleus, rather than being spread out along its length. In situ hybridization experiments with probes specific for chromosome Y in human sperm have shown similar results [30, 31]. These data suggest that the DNA loop domains are folded or coiled into small domains within the sperm nucleus, rather than collapsing lengthwise with little or no bending.

Preliminary experiments from our laboratory using a telomere-specific repeat probe have given similar results. When condensed sperm nuclei were probed, a pattern of spots was seen, but decondensed nuclei demonstrated a series of linear streaks. These data support the idea that the DNA loops are folded or coiled into discrete foci in the sperm nucleus.

Sperm chromatin has the appearance of 60-nm nodules. Allen et al. [32] have recently developed a technique for examining biological structures by atomic force microscopy (AFM). Images of amembraneous bull and mouse sperm nuclei and partially decondensed mouse sperm chromatin provided by this new technique have revealed that the DNA is not organized as long, linear bundles of fibers. Instead, sperm DNA appears to be coiled into large nodules that are somewhat variable in size, and each is large enough to contain a replicon-equivalent of DNA. Koehler et al. [33]

noted a similar arrangement of rat sperm DNA into nodules, or beads, with a diameter of 13–25 nm. Arrays of the nodules are tightly packed inside the nucleus in a three-dimensional arrangement that has not yet been described.

Volume of the mammalian sperm nucleus is greater than previous data suggested. A previous discussion of sperm DNA organization [6] used volume measurements for sperm nuclei that were obtained by serial section electron microscopy. Allen et al. [34] have since discovered that these volumes represent the minimum possible volume for the nucleus, one that reflects the extensive dehydration required for embedding the sperm in plastic. New data, obtained by AFM of fully hydrated sperm nuclei, indicate that the volume of the nucleus is more than twice previous estimates. These studies have shown that sperm chromatin in vivo is extensively hydrated, and that the larger volumes are consistent with the proposed nodular organization or coiled sperm DNA. Using the previous, smaller measurements, it was calculated that the mouse sperm nucleus contained barely enough volume to contain the naked DNA, with very little room for the bulky three-dimensional structures found in chromatin [6]. These newer measurements allow the possibility that sperm chromatin also contains larger organizing structures.

THE SPERM DNA LOOP DOUGHNUT MODEL

Criteria for the Model

Given all the data discussed above, it is now possible to construct a model for sperm DNA structure intermediate between protamine binding and loop domain organization. The criteria for the model are twofold. The first is obvious: it must be consistent with all available data on sperm DNA structure. The second criterion is suggested by the fact that spermatozoa are derived from stem cells that contain histones, and the organization of sperm DNA must therefore be consistent with the traditional somatic cell hierarchy, including nucleosomes and solenoid formation. Therefore, the solenoid equivalent in sperm chromatin ought to contain as few changes as possible from the parent, solenoid structure of the stem cells.

First Step: Histone Replacement by Protamines

The basic tenet of the model is that the 60-nm nodules discussed above [32] each represent one DNA loop domain. This focal localization of a single loop domain is consistent with the in situ hybridization data already cited. Since we know that protamine binds DNA in a linear, side-by-side fashion, the most efficient way to package the DNA of a loop domain into a round structure is in wide, concentric, and overlapping circles, as in a wound ball of string.

For the purposes of this model, the solenoid configuration is assumed to be the major conformation of DNA in the somatic cell 30-nm chromatin fiber. In one turn of the solenoid configuration, there are 13 supercoils of DNA, two for each of the six nucleosomes and one for the solenoid itself (Fig. 2A). The first proposal was that as protamines replace the histones, the 12 nucleosomal supercoils are removed so that the DNA is less curved or more linear, as originally suggested [7]. But the criterion that requires the fewest changes necessary predicts that the single solenoid supercoil remains. This simplest model does not fit the data, however, because the 1200 bp of DNA in one solenoid supercoil would form a circle with a diameter of 129 nm, twice the size of the observed round structures. If, on the other hand, only 11 of the 12 nucleosomal supercoils were removed, the DNA would be coiled into two spiraling circles for each solenoid turn in the chromatin (Fig. 2). The two spiraling circles of DNA complexed with protamines would bind to each other by van der Waals forces, as Balhorn proposed [7].

This first step of the model predicts that sperm DNA is actually slightly negatively supercoiled, to a degree that is only 2/13, or 15%, of histone-bound DNA. This is consistent with the two published reports demonstrating little or no superhelical density in protamine-bound sperm DNA [22, 23] because the methods used were not sensitive enough to distinguish between a complete lack of superhelical density versus an 85% decrease. Such a tight regulation of the superhelical density of DNA during spermatogenesis requires the presence of topoisomerase. Morse-Gaudio and Risley [35] have recently demonstrated that topoisomerase II is present in all cell types during spermatogenesis except the spermatozoon itself.

Finally, the focal replacement of histones by protamines predicted by this model is consistent with the earlier model of Risley et al. [23], who suggested that the supercoiling domains might serve as units of structural transitions during spermiogenesis.

Second Step: Condensation of the DNA Loop Domain

The next step of the model is the formation of the circular structures seen by AFM [32] in sperm chromatin. The model predicts that each of these structures represents a collapsed DNA loop domain. A comparison of the parent solenoid loop conformation of DNA bound to histones and the final doughnut loop model of DNA bound to protamines is diagrammed in Figure 3. The schematic intermediate is drawn only as an instructive diagram and is not predicted as a real, functional intermediate that occurs during spermiogenesis. The actual transition must be much more complex, involving transitional proteins that are not considered in this model. The comparison of the histone-solenoid configuration and that of the sperm nuclei is diagrammed in Figure 3, for a loop domain of 47 kbp, the average size reported for hamster spermatozoa [22]. Each of the 39 solenoid turns (each solenoid turn = 13 supercoils of DNA) in the parent loop domain (Fig. 3, left) eventually becomes two spiraling circles of DNA when replaced

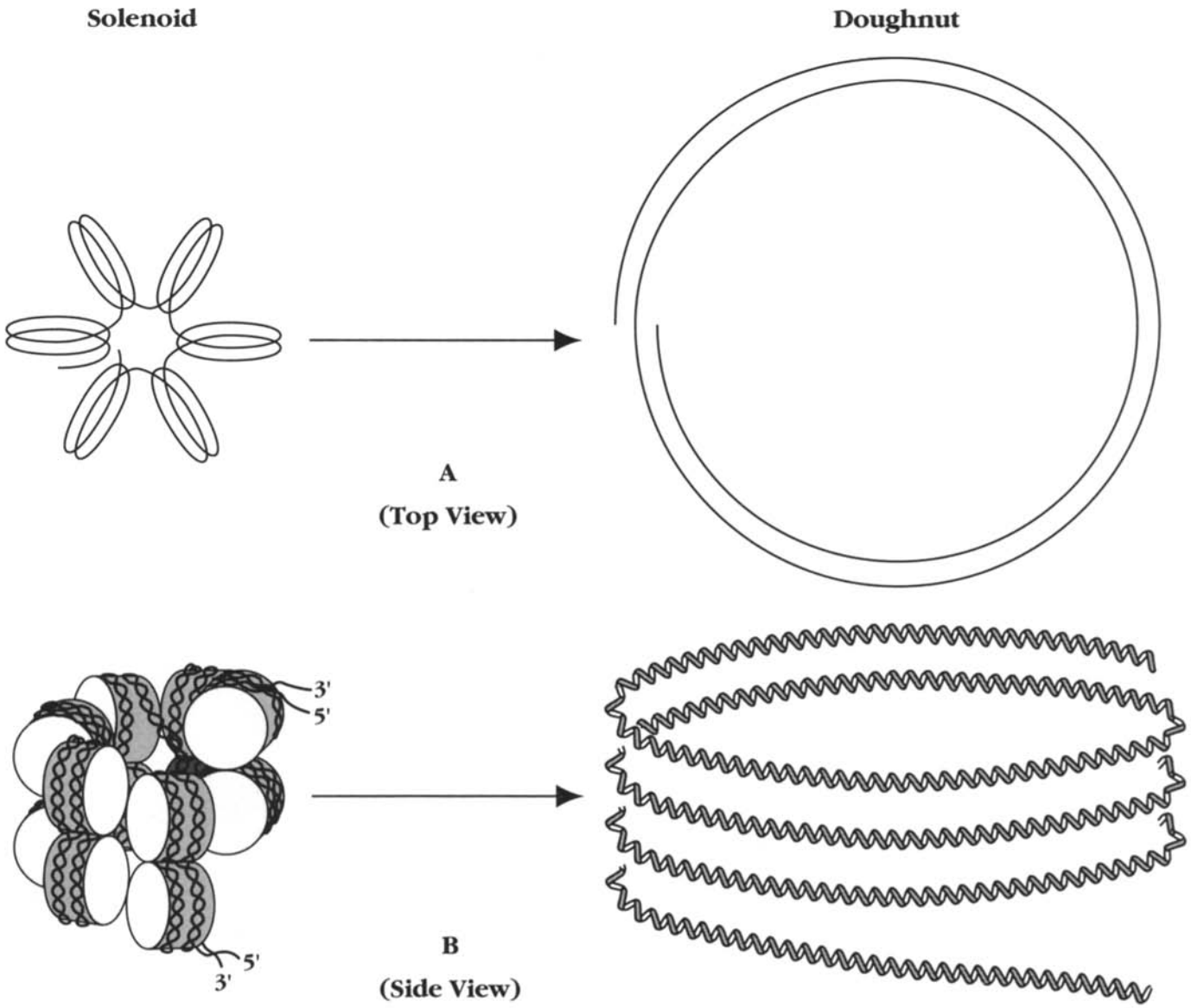


FIG. 2. Model of protamine replacement in mammalian sperm DNA. A) One coil of DNA in the histone-bound, solenoid configuration has 13 supercoils of double helix DNA, two for each nucleosome and one for the solenoid itself. The model proposed here predicts that as the protamines replace the histones during spermatogenesis, all but two of the 13 supercoils are uncoiled, as shown on the right (the DNA double helix has been drawn as a single line). B) On the left, two coils of DNA in the solenoid conformation (total of 26 superhelical turns) with DNA wrapped around the histone octomers are diagrammed. On the right, the same amount of DNA is shown bound to protamines (shaded) in an expanded doughnut configuration, drawn to the same scale (here, the 26 supercoils of the two solenoid turns become four doughnut supercoils). The histones keep the DNA in the relatively bulky solenoid configuration, while the protamine-complexed DNA concentric circles can bind together into much thinner structures (see Fig. 3C).

by protamines (Fig. 3, center). In the doughnut configuration, these protamine-bound DNA circles from each arm of the loop are collapsed into a doughnut-shaped nodule about 65 nm in diameter (Fig. 3, right).

The model, and the data previously cited, also predict concomitant changes in the relationship between the DNA and the nuclear matrix. First, at some point during spermiogenesis (whether before, after, or during protamine replacement is unknown) the number of DNA attachment sites must increase to decrease the average size of the loop domain [22, 24]. Second, the sperm nuclear matrix must also condense somewhat to accommodate the condensing chromatin (Fig. 3, right). This prediction is consistent with the condensation of sperm nuclei during spermatogenesis.

According to the model, this nodule is not a perfect cylinder, nor evenly and regularly composed. Rather, the model predicts that the protamine-complexed DNA circles (Figs. 2B and 3, center) are collapsed in a disorderly fashion, much like the coils of a wound garden hose, forming a three-dimensional structure shaped like a doughnut, or torus (Fig. 3, right). This torus has an outer diameter of 65 nm, an

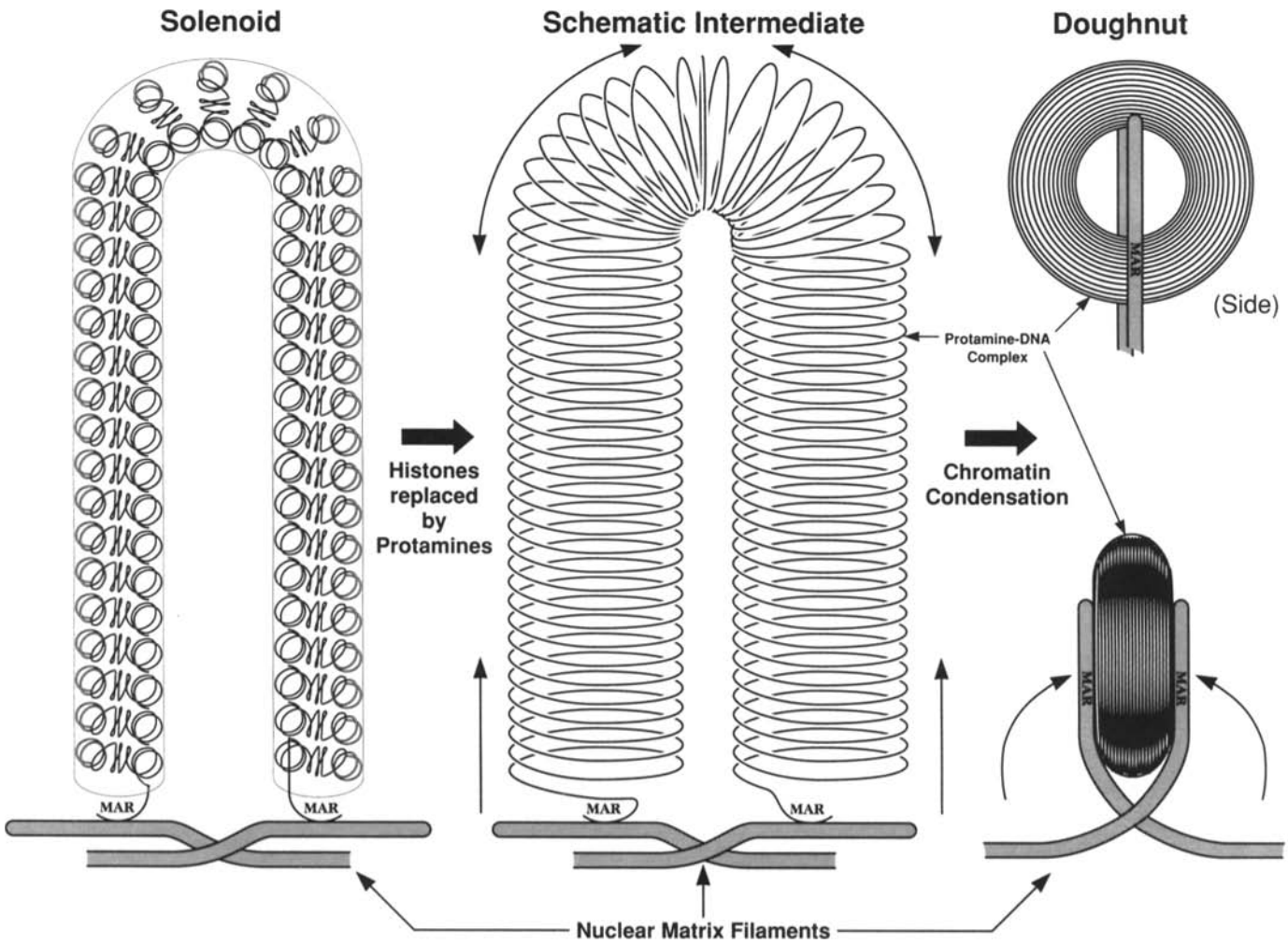


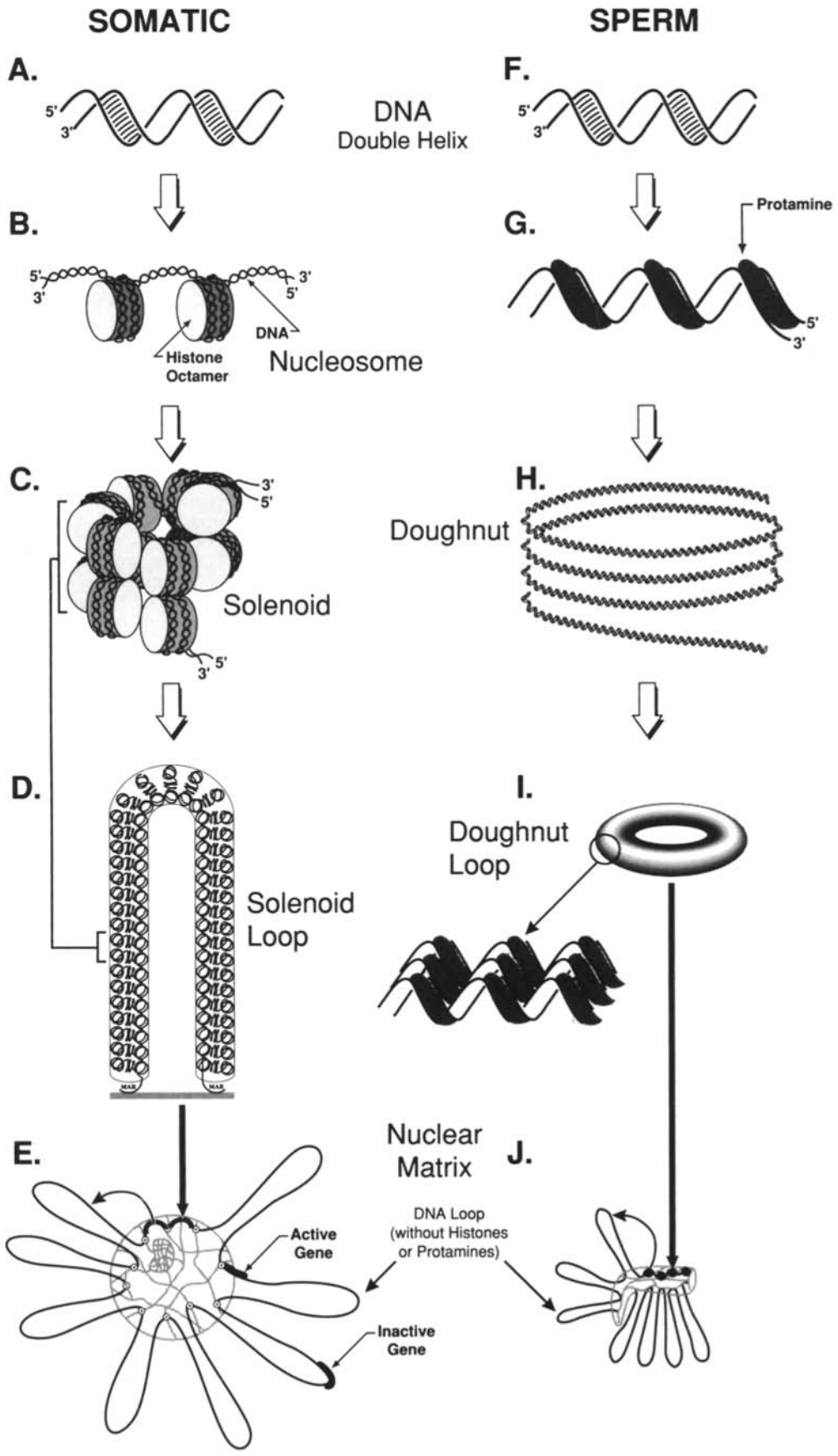
FIG. 3. Model of solenoid equivalent in one sperm DNA loop. The model is diagrammed for one loop of DNA that is 47 kbp in length, and the comparative structures are drawn to scale, viewed from above (top) and from the side (bottom). In the parent cell, the DNA is in the solenoid configuration (left). As the histones are replaced by the protamines, each turn of the solenoid becomes two concentric circles (center). In the doughnut structure, the protamine-bound DNA circles are collapsed into a toroid-shaped structure made up of 72 circles of DNA with an average diameter of 65 nm (right). The schematic intermediate (center) is drawn only as an instructive diagram, and is not predicted as a real, functional intermediate that occurs during spermiogenesis. The actual transition must be much more complex, involving transitional proteins that are not considered in this model.

inner diameter of 32.5 nm, and height of 25 nm (Fig. 3, right). Such a structure has recently been independently demonstrated experimentally by Hud and Balhorn (personal communication; Hud NV, Balhorn R, unpublished) using purified DNA and protamines in vitro. Note that the model also predicts that the exact dimensions of the doughnut would vary with the size of the DNA loop domain packaged within it. Sperm DNA loop domains are predicted to vary in size as do somatic cell loop domains, although this has not been directly addressed.

DISCUSSION

With the proposed model for the coiling of the DNA loop domains in mammalian sperm, it is now possible to construct a model for the packaging of the entire haploid genome into the sperm nucleus in which sperm DNA loop

FIG. 4. Equivalent levels of DNA packaging in somatic and sperm chromatin based on the doughnut-loop model. In somatic cells, DNA (A) is wound twice around histone octomers into nucleosomes (B), which then coil into solenoids with six nucleosomes per turn (C). DNA in solenoid form is attached at intervals of about 60 kb to the nuclear matrix at their bases to form DNA loop domains (D). In the somatic cell, these solenoid loop domains are contained within the nucleus, but when the histones are extracted, they can be visualized outside the nucleus (E). Active genes are more closely associated with the somatic nuclear matrix than inactive genes. In the sperm nucleus, highly positively charged protamines bind to DNA lengthwise along the double helix, neutralizing the negative charges of the DNA (G). The protamine-bound DNA is coiled with a very slight bend in the protamine DNA complex into concentric circles (H). These circles of one loop then collapse into a doughnut (I) in which the neutral DNA protamine complexes are tightly packed together by Van der Waal's forces (I, inset). Each doughnut represents one DNA loop domain attached to the sperm nuclear matrix (J). The DNA loop domains of sperm nuclei are smaller than those of somatic cells.


SOMATIC
SPERM
A.
5'
3'
DNA
Double Helix
F.
5'
3'
B.
5'
3'
3'
5'
DNA
Histone
Octamer
Nucleosome
G.
Protamine
5'
3'
C.
3'
5'
Solenoid
3'
5'
H.
Doughnut
D.
Solenoid
Loop
I.
Doughnut
Loop
E.
Nuclear
Matrix
J.
DNA Loop
(without Histones
or Protamines)
Active
Gene
Inactive
Gene

domains are packaged as doughnuts bound to the nuclear matrix (Fig. 4). The doughnut model allows sperm DNA to be tightly packaged (inset, Fig. 4I) compared to the relatively open configuration of somatic cell DNA at the same level of organization (Fig. 4D), and at the same time to be organized into discrete functional units, the DNA loop domains (Fig. 4J).

This model is only a first approximation of the data and it will undoubtedly progress through many revisions before a consensus, similar to that being formed for histone-bound chromatin, is reached. The loop doughnut model for the local packaging of DNA loop domains in spermatozoa still does not explain many unknown aspects of sperm chromatin structure. The next step, for example, is to ask how the loop doughnuts are arranged within the nucleus. If sperm chromatin is condensed in a fashion similar to that of mitotic chromosomes, it is likely that the loop doughnuts are arranged in a circular pattern around a central core [3]. This possible extension of the loop doughnut model is similar to a proposal Koehler et al. [33] made for rat sperm chromatin in 1983. Another aspect of sperm chromatin not yet fully addressed by this model is the presence of a small amount of histone proteins in fully condensed sperm nuclei [36]. It is possible that either the top of the sperm loop remains associated with histones, filling the center of the toroid loop with nucleosomes. Alternatively, or simultaneously, the DNA that is associated with the sperm nuclear matrix may remain in the form of nucleosomes to allow for greater freedom in bending at this region.

Regardless of the unanswered questions, the loop doughnut model for sperm DNA packaging is consistent with the data available, and it anticipates many possible aspects of sperm function. It is conceivable, for example, that one function of the spermatozoon is to transfer the paternal genome to the zygote in a organized but compact form. The relocation of the proper three-dimensional DNA organization may function to ensure that the developing embryo can access the paternal genetic information rapidly and efficiently. The condensed, rather than open, form of the DNA increases the chances that it will survive the voyage intact. The doughnut structure fulfills both requirements, keeping DNA sequences in the same focal packets found in somatic cells, localized DNA loop domains, in a more highly condensed form than is found in somatic cells. If this loop doughnut model for sperm DNA structure can be more fully verified, this aspect of sperm DNA tertiary structure will lead us to a more complete understanding of sperm DNA function.

ACKNOWLEDGMENTS

I would like to thank Dr. Rod Balhorn and Dr. Nick Hud of the Lawrence Livermore Laboratory for useful discussions and for sharing their recently submitted data. Dr. Hud's experimental results were obtained independently during the writing of this manuscript. I would also like to thank Mr. Michael Cooper for drawing all the figures.

REFERENCES

1. Watson JD, Crick FHC. Molecular structure of nucleic acids. Nature 1953; 171:737–738.
2. Getzenberg RH, Pienta KJ, Ward WS, Coffey DS. Nuclear structure and three-dimensional organization of DNA. J Cell Biochem 1991; 46:1–11.
3. Pienta KJ, Getzenberg RH, Coffey DS. Cell structure and DNA organization. Crit Rev Euk Gene Express 1991; 1:355–385.
4. Jackson DA. Organization beyond the gene. TIBS 1986; 11:249–253.
5. Mirkovitch J, Gasser SM, Laemmli UK. Relation of chromosome structure and gene expression. Philos Trans R Soc Lond 1987; 317:563–574.
6. Ward WS, Coffey DS. DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod 1990; 44:569–574.
7. Balhorn R. A model for the structure of chromatin in mammalian sperm. J Cell Biol 1982; 93:298–305.
8. McGhee JD, Felsenfeld F. Nucleosome structure. Annu Rev Biochem 1980; 49:1115–1156.
9. Chen TA, Smith MM, Le SY, Sternglanz R, Allfrey VG. Nucleosome fractionation by mercury affinity chromatography. Contrasting distribution of transcriptionally active DNA sequences and acetylated histones in nucleosome fractions of wild-type yeast cells and cells expressing a histone H3 gene altered to encode a cysteine 110 residue. J Biol Chem 1991; 266:6489–6498.
10. Liu L. DNA topoisomerases-enzymes that catalyze the breaking and rejoining of DNA. CRC Crit Rev Biochem 1983; 15:1–24.
11. Liu LF, Wang JC. Supercoiling of the DNA template during transcription. Proc Natl Acad Sci 1987; 84:7024–7027.
12. Finch JT, Klug A. Solenoid model for superstructure in chromatin. Proc Natl Acad Sci USA 1976; 73:1897–1901.
13. Berezney R, Coffey DS. Identification of a nuclear protein matrix. Biochem Biophys Res Commun 1974; 60:1410–1417.
14. Pardoll DM, Vogelstein B, Coffey DS. A fixed site of DNA replication in eucaryotic cells. Cell 1980; 19:527–536.
15. Cockerill PN, Garrard WT. Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. Cell 1986; 44:273–282.
16. Vaughn JP, Dijkwel PA, Mullender LHF, Hamlin JL. Replication forks are associated with the nuclear matrix. Nucleic Acids Res 1990; 18:1965–1969.
17. Razin SV. DNA interactions with the nuclear matrix and spatial organization of replication and transcription. Bioessays 1987; 6:19–23.
18. Razin SV, Rzeszowska-Wolny I, Moreau J, Scherrer K. Localization of points of DNA attachment to the nuclear skeleton inside the domain of chicken α-globin genes in functionally active and functionally inactive nuclei. Mol Biol 1985; 19:456–466.
19. Robinson SI, Nelkin BD, Vogelstein B. The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. Cell 1982; 28:99–106.
20. de Lange T, Shiule L, Myers RM, Cox DR, Naylor SL, Killery AM, Varmus HE. Structure and variability of human chromosome ends. Mol Cell Biol 1990; 10:518–527.
21. Groudine M, Conkin KF. Chromatin structure and de novo methylation of sperm DNA: implications for activation of the paternal genome. Science 1985; 228:1061–1068.
22. Ward WS, Partin AW, Coffey DS. DNA loop domains in mammalian spermatozoa. Chromosoma 1989; 98:153–159.
23. Risley MS, Einheber S, Bumcrot DA. Changes in DNA topology during spermatogenesis. Chromosoma 1986; 94:217–227.
24. Fawcett DW. A comparative view of sperm ultrastructure. Biol Reprod 1970; 2(suppl):90–127.
25. Ward WS, Coffey DS. Specific organization of genes in relation to the sperm nuclear matrix. Biochem Biophys Res Commun 1990; 173:20–25.
26. Kalandadze AG, Bushara SA, Vassetzky YS Jr, Razin SV. Characterization of DNA pattern in the site of permanent attachment to the nuclear matrix in the vicinity of replication origin. Biochem Biophys Res Commun 1990; 168:9–15.
27. Martin RH. A detailed method for obtaining preparations of humans sperm chromosomes. Cytogenet Cell Genet 1989; 35:252–256.
28. Haaf T, Grunenberg H, Schmid M. Paried arrangement of nonhomologous centromeres during vertebrate spermiogenesis. Exp Cell Res 1990; 187:157–161.
29. Moens PB, Pearlman RE. Satellite DNA I in chromatin loops of rat pachytene chromosomes and in spermatids. Chromosoma 1989; 98;287–294.
30. Guttenbach M, Schmid M. Determination of Y chromosome aneuploidy in human sperm nuclei by nonradioactive in situ hybridization. Am J Hum Genet 1990; 466:553–558.
31. Wyrobek AJ, Alhborn T, Balhorn R, Stanker L, Pinkel D. Fluorescence in situ hybridization to Y chromosomes in decondensed human sperm nuclei. Mol Reprod Dev 1990; 27:200–208.

32. Allen MJ, Pogany GC, Siekhaus W, Balhorn R. Atomic force microscopy studies of mammalian sperm chromatin. J Cell Biol 1990; 111:110 (abstract 613).
33. Koehler JK, Wurschmidt U, Larsen MP. Nuclear and chromatin structure in rat spermatozoa. Gamete Res 1983; 8:357–370.
34. Allen MJ, Hud NV, Lee C, Pogany G, Siekhaus WJ, Balhorn R. Analysis of sperm nuclear volumes and extent of chromatin compaction by atomic force microscopy. J Cell Biol 1991; 115:50 (abstract 289).
35. Morse-Gaudio M, Risley MS. Topoisomerase expression during spermatogenesis. J Cell Biol 1991; 115:50 (abstract 286).
36. Tanphaichitr N, Sobhon P, Taluppeth N, Chalermisarachai P. Basic nuclear proteins in testicular cells and ejaculated spermatozoa in man. Exp Cell Res 1978; 117:347–356.