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The Structure of the Sleeping Genome: Implications of Sperm DNA Organization for Somatic Cells

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Abstract The tertiary structure of the DNA that makes up the eukaryotic genome is remarkably plastic, taking many different forms in response to the different needs of the cell. During the cell cycle of one cell, the DNA is replicated, reorganized into mitotic chromosomes, and decondensed into interphase chromatin. Within one cell at any given point in time, the chromatin is divided into hetero- and euchromatin reflecting active and inactive states of the DNA. This organization varies within one organism since different parts of the genome are active in different cell types. This article focuses on the most dramatic cell-type-specific DNA organization, that found in spermatozoa, in which the entire genome is reorganized into an inactive state that is more highly condensed than mitotic chromosomes. This unique example of eukaryotic DNA organization offers some interesting clues to the still unanswered questions about the role that the three-dimensional packaging of DNA plays in its function. © 1994 Wiley-Liss, Inc.

Key words: nuclear matrix, sperm DNA, protamines, histone, supercoiling

Many researchers now view the structure of DNA within living organisms as constantly fluctuating, at all levels, in response to the various functions it performs. At the molecular level, the hydrogen bonds of a few base pairs of the DNA helix are broken and repaired for the transcription of an individual gene. At the other end of the spectrum are changes at the macromolecular level, such as the replication and condensation of an entire interphase chromatin into a mitotic chromosome. The magnitude and exact nature of these structural changes of DNA in response to function are still unclear, but several steps have been made towards understanding these complex events. DNA is molded into its various structures by the proteins of the nucleus, changing its tertiary shape as different programs within its information store are utilized by the cell. Histones in somatic cells [McGhee and Felsenfeld, 1980] and protamines in spermatozoa [Coelingh et al., 1969; Balhorn, 1982] fold the DNA into manageable units and confer upon it various degrees of supercoiling. At larger intervals the nuclear matrix organizes the chromosomes into functional domains, providing fixed sites for replication and transcription [Nelson et al., 1986; Pienta et al., 1991; Vogelstein et al., 1980; Vaughn et al., 1990].

This paper focuses on one of the most extreme examples of DNA reorganization, the packaging of DNA within the mammalian sperm nucleus. In these cells the entire genome is completely reorganized, becoming more tightly packaged than even the mitotic chromosome for efficient transport into another organism. This particular form of the eukaryotic genome is unique for several reasons. Sperm DNA is inactive in that there is no replication or transcription ongoing in the fully mature sperm nucleus [Stewart et al., 1984]. It may therefore be considered structurally more homogeneous than somatic DNA since the chromatin is not divided into active and inactive states. Furthermore, sperm DNA is coated by proteins called protamines that contain roughly twice the content of basic amino acids than do histones, and bind DNA in a very different manner [Coelingh et al., 1969; Balhorn, 1982]. When viewed by thin-section electron microscopy, sperm chromatin appears as an electron-dense material with no discernable structure [Fawcett, 1970; Lalli and Clermont, 1981]. If sperm DNA is inactive, however, it is awakened when the paternal genome is again reorganized in the zygote after fertilization. This suggests that sperm DNA may retain some spe-

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cific structural aspects during its condensation which the components in the egg can recognize to restructure a viable nucleus (although there are arguments counter to this suggestion, and these are addressed below; see Current Model for Sperm DNA Packaging).

Studying this extreme example of eukaryotic DNA organization which represents the limit of inactivity for complex eukaryotes may yield clues to those aspects of DNA organization that are most crucial to function. This review focuses on what such studies have taught us about the relationship between the structure of DNA and its various functions in higher excoriates.

THE LOSS OF NUCLEOSOMAL STRUCTURE WITH LOSS OF DNA ACTIVITY

One of the most interesting aspects of mammalian sperm chromatin organization is that the fundamental DNA packaging unit found in all other somatic cells, the nucleosome, is either completely absent or present in a very small percentage of sperm chromatin. A nucleosome is formed when DNA is wrapped twice around an octamer of histones, the major DNA binding proteins of eukaryotic DNA (Fig. 1B) [Olins and Olins, 1973; Woodcock, 1973; McGhee and Felsenfeld, 1980]. The vast majority of the DNA in a mammalian sperm nucleus is not bound to histones but to proteins that are much more basic, termed protamines [Coelingh et al., 1969; Balhorn, 1982]. Balhorn [1982] has proposed a model for protamine–DNA interactions in which the protamines exist as an extended string of amino acids in the minor groove of the DNA (Fig. 1G). The positive charges of the arginine residues bind to the negative charges of the phosphodiester backbone of the DNA, resulting in a completely neutralized chromatin fiber. These neutralized fibers then condense into a highly compact structure (Fig. 1H–I). The fundamental packaging unit of protamine-bound DNA has only recently been described by Hud et al. [1993] as a 90 nm toroid containing approximately 60 kb of DNA, a structure differing markedly from any chromatin structure defined in somatic cells. It is difficult to conceive of a model in which this toroid could be made up of nucleosomes.

Another piece of evidence indicating that protamines bind DNA in a markedly different structure than histones is that sperm DNA is not measurably supercoiled [Risley et al., 1986; Ward et al., 1989]. In the nucleosome configuration, DNA is wrapped twice around the histone octamer in a left-handed superhelix (Fig. 1B) [Richmond et al., 1984]. This has the effect of negatively supercoiling the DNA; that is, twisting it in a way that would unwind the double helix if the Watson–Crick base pairing were destabilized. Sperm DNA is not measurably supercoiled; this suggests that sperm DNA is not organized into nucleosomes.

The absence of nucleosome structure in sperm DNA suggests that nucleosomes are important in the packaging of active rather than inactive DNA. In support of this, recent data has demonstrated that histones function to expose or conceal regulatory sequences in DNA to or from transcription factors [Kornberg and Lorch, 1991; Adams and Workman, 1993]. Schmid et al. [1992] have recently demonstrated in yeast that nucleosome positioning may prevent transcription by preventing binding of the necessary initiation factors. The transcription factor TFIIIA will bind to its 5S RNA promoter sequence only when the nucleosome is positioned so that the sequence is free of histones [Lee et al., 1993]. It is clear from these experiments that histones play specific roles in the regulation of transcription by virtue of their positioning on the DNA, and that their positions can be modified by the cell. In contrast to histone binding, the model for protamine binding to DNA proposes that the DNA is almost completely coated by protamines with very little, if any, left exposed to the nucleus (Fig. 1G). It is thus doubtful that protamines play an important role in similar positioning effects.

Fig. 1. Comparison of DNA packaging models for somatic and sperm cells. **A–E:** Somatic cells. DNA is coiled twice around histone octamers to form nucleosomes (B), which are then coiled into a 30 nm filament, one configuration of which may be the solenoid (C). The 30 nm filament is organized into DNA loop domains which are attached at their bases to the nuclear matrix (D). The DNA loop domains can be visualized by extracting the histones with salt and staining with an intercalating dye. The loop domains extrude from the nucleus and the DNA appears as a halo of fluorescence surrounding the nucleus (E). **F–J:** Sperm cells. Protamines bind to DNA along the minor groove in a linear fashion, completely neutralizing the negatively charged DNA molecule (G). The protamines probably bend DNA very slightly, causing it to coil (H). The neutralized chromatin fiber condenses by binding to other fibers by Van der Waal's forces into a toroid structure (I). Protamine-bound DNA is also organized into loop domains within the sperm nucleus (J). (Reprinted with permission from Biology of Reproduction 48:1193–1201, 1993.)

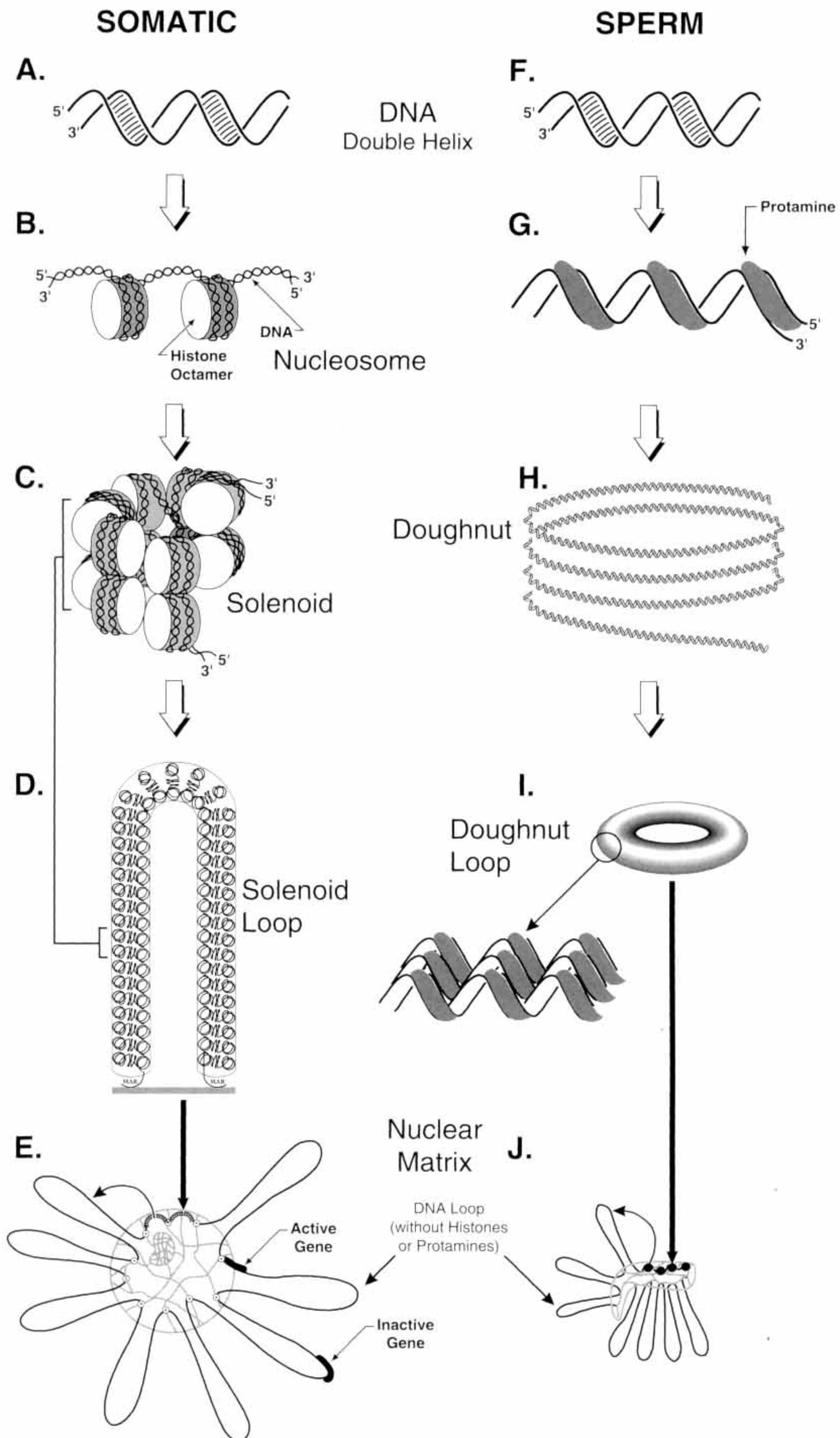

Figure 1.

A second potentially activating role of nucleosomes is that nucleosomal DNA is negatively supercoiled. It has been suggested that one function of the negative supercoiling in most DNA is to lower the energy of activation of denaturing the double helix during transcription and replication [Liu, 1983; Parvin and Sharp, 1993]. When the histones are removed, as they must be, during transcription, the torsional stress that results from the negative supercoiling decreases the energy required to unwind the DNA. This argument is difficult to prove experimentally, but at least two recent reports lend support for this hypothesis. Dunaway and Ostrander [1993] positioned transcription sites on a series of plasmids in different orientations with respect to each other in such a way that the transcription process itself would generate positive or negative supercoils. Genes that were present in the areas of negative supercoiling were more readily transcribed in vivo than DNA that was positively supercoiled or not supercoiled at all. Parvin and Sharp [1993] developed an in vitro transcription model with a minimal set of transcription factors. These authors discovered that the requirement for additional factors was decreased when the DNA was negatively supercoiled and suggested that the free energy of negative supercoiling promoted DNA denaturation. These data suggest that the negative superhelicity of somatic cell DNA, conferred at least in part by histones, does play an important role in the activation of transcription. The complete, or nearly complete, absence of supercoiling in mammalian sperm DNA indicates that negative superhelicity is not required for packaging of viable DNA into live nuclei, since sperm DNA is activatable upon fertilization. The lack of nucleosomal organization in sperm DNA is therefore consistent with (though not proof of) the importance of negative superhelicity for transcription and DNA replication.

Both of the structural components conferred on DNA by histones, negative superhelicity and nucleosome positioning, are lost when the DNA is packaged into spermatozoa. I believe that this loss attests to the importance of both of these factors in transcription and DNA replication, two functions absent in fully mature spermatozoa. It also suggests that these two structural entities are not required to maintain viable DNA; that is, the organization of eukaryotic DNA into nucleosomes and as highly negatively supercoiled molecules is not absolutely essential to the genetic information contributed to the embryo by the male parent.

Higher-Order Sperm DNA Structure

Given the complete reorganization of sperm DNA that occurs at the nucleosomal level during spermatogenesis, the question arises whether any structural organization of the DNA that is required for proper sperm function exists in the sperm nucleus. It is known that exogenous DNA added to *Xenopus* egg extracts will form nuclei that are indistinguishable from normal nuclei [Newport, 1987]. If one complete complement of the haploid paternal genome were therefore injected into an egg, it would stimulate the formation of a pseudopaternal pronucleus. Would an egg so injected produce a viable embryo, or is the nuclear structure that folds the DNA, which would be lost by the deproteination, necessary? For technical reasons this experiment is currently impossible to perform, but the question behind it challenges a fundamental biological concept: Is all the information required to build a multicellular organism from one cell contained within the two-dimensional, linear DNA, or does the three-dimensional packaging of that DNA by the nucleus contain additional, necessary information for proper embryogenesis?

Let us examine one specific example. A series of elegant experiments from the laboratory of Ronald Laskey has demonstrated that demembranated *Xenopus* sperm nuclei incubated in mitotic egg extracts will form 100–300 organizing centers for DNA replication, each containing 300–1,000 replicons [Mills et al., 1989]. These data suggest that all the enzymes necessary for the replication complex are present in the egg extracts, but what about the origins of replication, themselves? It is possible that the origins were already organized into clusters in the compacted sperm nucleus, and that upon fertilization the egg supplies the enzymes to build the replication foci around these centers. Indeed, DNA that is not packaged into nuclei before incubation with mitotic extracts is not replicated very efficiently [Newport, 1987; Blow and Laskey, 1986]. Sperm DNA is organized into loop domains that are attached at their bases to a nuclear matrix in a manner similar to that of somatic cells (Fig. 1J) [Ward et al., 1989]. In somatic cells these DNA loop domains are equivalent to the replicon, the DNA being replicated on the nuclear matrix at the base of each loop (Fig. 1E) [Vogelstein et al., 1980]. If sperm DNA is

also organized into loop domains, one might suggest that the replicons are attached to the sperm nuclear matrix, and act as organizing centers for replication upon fertilization.

Recent experiments using fluorescent in situ hybridization (FISH) have demonstrated that mammalian sperm DNA does exhibit some degree of specific organization. Zalensky et al. [1993] provided evidence that in human sperm nuclei the centromeres are positioned in the center of the nucleus, and that telomeres are located more peripherally. Data from our laboratory using hamster sperm nuclei provided evidence that the terminal telomeric repeat sequence $(TTAGGG)_n$ is bound to the sperm nuclear matrix [de Lara, et al., 1993]. While these data are far from providing conclusive answers to the question of the functional significance of the three-dimensional organization of sperm DNA, they do indicate that this organization does exhibit a degree of specificity. The challenge now is to understand why the sleeping sperm genome would have any degree of organization beyond simple packaging, since its only function is to transport the paternal genome to the egg.

CURRENT MODEL FOR SPERM DNA PACKAGING

Mammalian sperm nuclei provide a unique model for the study of eukaryotic DNA structures and their relationships to function. This DNA is at the same time homogeneously inactive, since it is not replicated or transcribed, and biologically viable, since it is capable of being incorporated into an embryo as half its genome. By examining what has been lost (nucleosomal structure and negative supercoiling) and what has been retained (DNA loop domain structure and specific three-dimensional organization), valuable information as to the relationships of the various structural conformations of DNA to function may be inferred.

A visual comparison of the current models for DNA packaging in somatic cells with that of sperm DNA is outlined in the accompanying diagram. It must be stressed that both models are constantly being modified as new data is obtained, and this diagram should be considered a working hypothesis. The current model of our laboratory for mammalian sperm DNA organization is based on data from several laboratories (depicted in Fig. 1F–J) [Ward, 1993]. The original protamine model proposed by Balhorn suggests that the protamines are bound to the minor groove of the DNA double helix (Fig. 1G) [Balhorn, 1982]. The fluorescent in situ hybridization data suggests that long stretches of individual DNA sequences are coiled into discrete foci [de Lara et al., 1993], suggesting that protamines must bend the DNA at least slightly. This was demonstrated by the recent data of Hud et al. [1993], who showed that protamine-complexed DNA formed toroids. Finally, previous work also demonstrated that sperm DNA is organized into DNA loop domains that are attached to the nuclear matrix by specific sequences (Fig. 1J) [Kalandadze et al., 1990; Ward and Coffey, 1990]. The model that is proposed in the accompanying diagram (Fig. 1F–J) predicts that each loop domain is condensed into a single toroid by protamines. In this model, sperm DNA is slightly supercoiled, retaining only about 15% of the total number of superhelical turns found in somatic cell DNA.

The comparison illustrates what aspects of eukaryotic DNA organization are retained and which ones are lost when sperm DNA is condensed into its inactive state. Such studies in sperm DNA are just beginning, and already important questions about somatic cell DNA function have been raised. The study of sperm DNA therefore promises to yield many exciting clues to the biology of DNA that could not have been obtained in other systems.

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