Sperm DNA fragmentation: awakening the sleeping genome

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Abstract
We have recently demonstrated that mammalian spermatozoa have the ability to degrade their DNA by a mechanism that is similar to apoptosis in somatic cells. When this mechanism is activated, the DNA is first degraded into loop-sized fragments by TOP2B (topoisomerase IIB). This degradation, termed sperm chromatin fragmentation, can be reversed by EDTA, which causes TOP2B to religate the double-stranded breaks it originally produced. Under certain conditions, a nuclease then degrades the sperm DNA further, digesting the entire sperm genome. When mouse spermatozoa which have been treated to induce TOP2B-mediated DNA breaks are injected into oocytes, the paternal DNA is specifically and completely degraded. This total digestion of paternal DNA occurs at the time of DNA synthesis initiation. In the present study, we explore the significance of an active TOP2B in the nucleus for mouse sperm function.

Topo II (topoisomerase II) in mammalian chromatin

Topo II is a large, relatively insoluble enzyme that alters the topological structure of DNA [1–3]. Topo II is a homodimer that can unwind DNA by first making double-stranded DNA breaks, passing another part of the same DNA strand through the break and, finally, religating the broken strands [1]. One unique feature of Topo II is that when the enzyme cleaves both strands of the double-helix DNA, each half of the homodimer is temporarily covalently bound to a 3'-end of the DNA. This intermediate is called the ‘cleavable complex’, because, if the cell is treated with the denaturing detergent SDS at this point, the Topo II is denatured and the double-stranded DNA breaks are permanent [3,4].

To understand the necessity for Topo II in mammalian cells, it is helpful to consider the physical size of the human genome. There is a total length of 2 m of DNA in each human somatic cell, which is divided into 46 individual DNA segments (chromosomes) that contain from 1.6 to 8.3 cm of DNA [5]. This means that the DNA in the longest chromosome is close to 100 times the length of the average somatic cell nucleus. Even with the condensation of this DNA that is mediated by histones and other chromosomal proteins, manipulating such a length of DNA causes significant topological problems for the cell. Topo II is a major contributor to manipulating the cell’s DNA. Eukaryotic DNA is organized into DNA loop domains in which the DNA is attached to the structural component of the nucleus, the nuclear matrix, every 20 kb to 120 kb [6,7]. Topo II is located at the bases of these loop domains on the nuclear matrix [7–9] and plays important roles in DNA synthesis and RNA transcription [10,11]. The ability of Topo II to pass one strand of DNA through another broken strand also allows this enzyme to untangle chromosomes that may become intertwined during mitosis. Indeed, Topo II mutants in yeast are arrested at metaphase, because the entangled chromosomes are unable to be resolved [12]. Finally, as described more fully in the next section, Topo II plays an important role in the degradation of DNA during apoptosis [13,14].

Topo II plays an important role in both the function and structure of eukaryotic DNA. However, the presence of this enzyme in mammalian spermatozoa has not been well established. Mammalian sperm chromatin is much more condensed than even mitotic chromosomes, because of the very basic protamines that replace histones during spermiogenesis [15]. Protamines condense the DNA into tightly packed toroids that contain approx. 50 kb of DNA [16,17]. We, and others, have shown that this highly condensed sperm DNA is also organized into loop domains by the sperm nuclear matrix [18–21]. We have also provided evidence to support a model for sperm chromatin organization, the Donut-Loop model (Figure 1A), in which each of the protamine toroids is a single DNA loop domain [22]. The protamine toroids are connected by short segments of chromatin called toroid linkers that are much more sensitive to nuclease digestion. These toroid linkers also contain the MARs (matrix attachment regions) for sperm chromatin, and this is the most likely location for Topo II in the sperm cell.

Sperm DNA is inert in the sense that it is not replicated in the sperm cell, nor is RNA transcribed from the fully condensed genome. However, it must be capable of initiating these functions shortly after fertilization. Hence, we previously described the sperm DNA as the ‘sleeping’ genome [23]. As described below, our analysis of the surprising finding that spermatozoa have the ability to degrade their own DNA led us to obtain evidence for active Topo II in fully condensed mammalian spermatozoa.
The ability of the sperm chromatin to degrade all its DNA through a Topo II-initiated mechanism may be related to an apoptotic-like mechanism in spermatozoa, although its exact role has yet to be determined. Similarly, the identity of the nuclease(s) that works in concert with TOP2B in the sperm cell will have to wait for further investigation. However, our work on SCF has allowed us to demonstrate that the mature sperm cell does have a functioning Topo II in its nucleus.

The Donut-Loop model for sperm chromatin structure suggests that at least a small component of the highly condensed sperm chromatin is more accessible to DNA-manipulating enzymes than the DNA that is condensed into toroids. The DNA in protamine toroids is condensed into an almost crystalline state with most of the DNA completely surrounded by other chromatin fibres [16]. We have provided evidence that this DNA is much less susceptible to exogenous DNase I digestion than the matrix-associated DNA [22]. The toroidal linker regions probably reflect a much more open chromatin structure, possibly bound to histones rather than protamines. If so, these matrix-associated DNA segments may provide the newly fertilized embryo with nucleation sites for sperm chromatin decondensation. Just after fertilization, when protamines are removed and replaced with histones, the sperm DNA must be negatively supercoiled, and it is possible that the TOP2B provided by the sperm could provide this topological change.

These MARs may also be the initiation points for paternal DNA replication. In somatic cells, DNA is replicated at the bases of the loop domains on the nuclear matrix [30,31]. Also, it was recently demonstrated that sperm chromatin is organized into DNA loop domains that are competent for embryonic DNA synthesis [32]. Furthermore, our recent study suggests that functional TOP2B is located at the bases of sperm DNA loop domains [28]. Taken together, these results suggest the possibility that the sperm cell provides at least one enzyme, TOP2B, which may be used in the first round of DNA synthesis of the male genome. It is important to note that TOP2A is more clearly associated with DNA replication than TOP2B, so there may not be a direct role of sperm TOP2B in paternal DNA replication. But the presence and location of an active Topo II in the sperm nuclear matrix suggests that this possibility must be tested.

Conclusions

Many questions have yet to be resolved about the roles of TOP2B in spermatozoa. Our recent results have provided evidence for the existence of this enzyme in mammalian spermatozoa. Furthermore, we have provided evidence that this TOP2B can be activated, resulting in cleavage of sperm DNA into loop-sized fragments, thereby suggesting that the sperm cell with its highly condensed chromatin has the capability of manipulating its own DNA. It also suggests that the paternal chromatin may play a more active role in its own fate during the early stages of embryogenesis than previously thought.
References


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