Hypothesis

# Chromatin remodeling during spermiogenesis: a possible role for the transition proteins in DNA strand break repair

Guylain Boissonneault*

*Département de Biochimie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, QC, Canada J1H 5N4*

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**Abstract An important chromatin remodeling process is taking place during spermiogenesis in mammals and DNA strand breaks must be produced to allow the accompanying change in DNA topology. Endogenous DNA strand breaks are indeed detected at mid-spermiogenesis steps but are no longer present in mature sperm. Both in vitro and in vivo evidence suggests that the DNA-binding and condensing activities of a set of basic nuclear 'transition proteins' may be crucial to the integrity of the chromatin remodeling process. We propose that these proteins are necessary for the repair of the strand breaks so that DNA fragmentation is minimized in the mature sperm.** 

*Key words:* Transition protein; DNA strand break; Chromatin remodeling; Spermiogenesis

## 1. Introduction

The chromatin packaging of the sperm cells is strikingly different from that of the somatic cells [1,2]. This important change in chromatin structure takes place during the haploid phase of spermatogenesis termed 'spermiogenesis' where most features of the mature sperm are acquired including the species-specific shape of the sperm head. The transition in chromatin structure is not yet fully understood but involves the replacement of the somatic nucleosomal histones by a set of basic proteins, known as the 'transition proteins', which are being replaced by the protamines in subsequent steps [3,4]. Other well-documented nuclear events associated with modifications of the chromatin structure include an increase in histone acetylation [5], an increase in the activity of the ubiquitin system [6–8] and a change in DNA topology resulting from the elimination of the negative supercoiling [9,10]. The complete process results in a chromatin packaging that is about six times more condensed than mitotic chromosomes and where no DNA transaction can take place. Interestingly, the assessment of sperm chromatin integrity emerges as an important tool in discriminating between fertile and infertile patients [11–14] lending support to the concept that the chromatin remodeling process must be orchestrated adequately in order for the male gamete to achieve its full fertilizing potential. It is likely that a sizable number of single or double DNA strand breaks must be induced during spermiogenesis to eliminate supercoiling. We hypothesize that the small basic nuclear proteins, promoting DNA condensation, may contribute to repair these strand breaks therefore preventing this DNA damage to persist in the mature sperm.

## 2. DNA strand breaks and change in DNA topology

The change in DNA topology is of particular interest and is likely to be a critical and finely regulated mechanism leading to the formation of the sperm chromatin. The elimination of nucleosomes during spermiogenesis presumably leaves a great number of unconstrained DNA supercoils in the spermatid's haploid genome. The molecular mechanism at the origin of the nucleosome disruption is not known but may involve a combination of the destabilizing factors outlined above. Assuming that most of the supercoils in mammalian cells are constrained by nucleosomes at an average periodicity approaching 200 bp, a gross estimate the complete nucleosome removal would leave $7.5\times10^6$ free supercoils in the haploid genome of the spermatid. This is assuming that the nucleosome constrains the topological equivalent of only one supercoil instead of almost two because of the so-called linking number paradox [15]. The elimination of these unconstrained DNA supercoils must be performed by the introduction of single or double strand breaks in DNA. An estimate of the theoretical number of strand breaks required to eliminate the supercoiling cannot be made since we do not know if the DNA is topologically constrained at many sites within a DNA loop domain in the spermatid [16]. In theory, the swivel provided by one single strand breakage could eliminate all the supercoils of a loop domain if the domain is constrained only at both ends and if the nucleosomes present on a loop domain are dismantled at the same time exposing a large region of free DNA. This situation, however, appears unlikely as DNA must be anchored at several locations within a large loop domain (see Fig. 1). The estimated size of a DNA loop domain in sperm nuclei, although smaller than in somatic cells, remains very large indeed reaching around 50 kb [17]. A single nick in DNA may therefore not be sufficient to relieve the helical tension over such a great distance in the spermatid karyoplasm. However, if the supercoils are eliminated progressively as they become unconstrained (exposed) by the nucleosome loss then a much greater number of single strand

*Fax: (1)-819-564 5340.
*E-mail address:* gboisson@courrier.usherb.ca (G. Boissonneault).


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breaks should be expected and, as a result, the topological change is likely to be far more efficient. Experimental evidence suggests that a large number of DNA strand breaks are indeed detected at mid-spermiogenesis steps [18–20]. In fact, quantitative analysis of the fluorescence from terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate-biotin end labeling (TUNEL) analysis of isolated cells from the seminiferous epithelium indicates that the transient increase in the number of strand breaks can be far greater than previously thought (G. Boissonneault, unpublished observations). The origin of the transient increase in DNA strand breaks is not known but would require an endogenous nuclease activity present up to the late spermiogenesis steps. It has been proposed that topoisomerase II may play such a role being able to both create and ligate the DNA nicks during spermiogenesis [21,22]. However, as outlined below, naturally occurring alterations of the DNA condensing process or targeted deletions of some of the major basic proteins in mice are associated with an increase in DNA fragmentation. In these experimental models the topoisomerase II status should not be altered suggesting that some other mechanisms may contribute to the transient character of the endogenous DNA nicks.

## 3. The DNA packaging provided by the transition proteins and protamines plays a key role in the repair of DNA strand breaks

Upon elimination of supercoiling by the strand breaks an efficient mechanism must be required in order to 'seal' or 'repair' the DNA phosphate backbone. We surmise that the integrity of the DNA condensing process could play a key role in the elimination of the strand breaks since these DNA lesions appear transiently and are no longer detected at later steps of the spermiogenesis process where the nuclear protein transition is completed. The lack of strand break detection at later spermiogenesis steps does not appear to result from the shielding of DNA against the TdT used in the end labeling technique since labeling is obtained following treatment of these late spermatids with DNase I [19] (G. Boissonneault, unpublished observations). Therefore, the possibility exists that the DNA condensation process initiated by the transition proteins and completed by the protamines contributes to repair the DNA strand breaks. In accordance with this assumption, an alteration in the condensation state (chromatin packaging) of the sperm head has been previously correlated with an increase in DNA fragmentation [23–26] in the mature sperm. In addition, a correlation has also been established between underprotamination and nicking of DNA [27]. In vitro, the transition protein 1 (TP1) was found to stimulate the repair of a nick circular plasmid [28] and both TP1 and TP2 as well as protamines stimulate oligomerization of short DNA fragments in the presence of T4 DNA ligase [29,30]. The process by which the DNA condensation provided by the transition proteins and the protamines can improve the ligation efficiency of free DNA ends can be related to the capacity of these basic proteins to neutralize the negative phosphate groups allowing the free DNA ends to be brought into close juxtaposition without interfering with the ligase activity [29]. In the case of single strand breaks, the protein would increase the ligation efficiency by bridging the free DNA ends created at the break point [31]. A possibility also exists that the stimulation of single strand break ligation may be the consequence of a better alignment of the free DNA ends. The transition proteins or protamines would therefore act as 'alignment factors' which is reminiscent of the DNA bridging activity of the heterodimeric Ku protein, a subunit of the DNA protein kinase involved in non-homologous end joining. If the proteins involved in chromatin remodeling and condensation are important for the repair of strand breaks in vivo, one would expect that the targeted deletion of their respective gene or alteration in their sequential appearance would lead to the persistence of strand breaks up to much later steps of the spermiogenesis process or even in mature sperm. Targeted deletions of the mouse *Tnp1* or *Tnp2* gene is compensated for by an increase in the expression of the other gene so their function is apparently redundant [32,33]. As a consequence, these mutations did not result in major sperm head abnormalities although alterations in the condensation state of the nuclei were nevertheless observed in both cases. For instance, the sperm chromatin of the *Tnp2*-null mice was more accessible to intercalating dye and more susceptible to acid denaturation [34]. Based on the established correlation between chromatin structure assays and the presence of endogenous DNA nicks outlined above, these observations strongly suggest that an increase in DNA strand breaks could be detected in these animal models. Similarly, an alteration in sperm chromatin integrity based on acridine orange staining has been observed in sperm from mice with haploinsufficiency for either of the protamine genes [35]. The direct assessment of DNA strand breaks in these mouse strains by a technique such as TUNEL is therefore of prime importance.

Although the DNA-binding properties of the abundant basic nuclear proteins of the elongating spermatids may stimulate the re-joining of the strand breaks, neither the transition proteins nor the protamines possess an intrinsic DNA ligase activity to catalyze the formation of phosphodiester bonds. A DNA ligase activity must therefore be required during the condensation process. Aside from topoisomerase II, a likely candidate is an alternatively spliced, testis-specific isoform of DNA ligase III (DNA ligase IIIβ [36,37]). Although the DNA ligase IIIβ isoform mRNA has been detected in the round spermatids, evidence for the presence of the DNA ligase III protein up to the elongating spermatid steps must be obtained. Using an antibody against DNA ligase III, we recently provided preliminary evidence, by immunocytochemistry, that the protein can be detected in the nuclei of elongating spermatids [38] and that DNA ligase activity is present in nuclear extracts of sonication-resistant spermatids at steps coincident with the nuclear presence of transition proteins and protamines.

## 4. Conclusion

At present, the consequences of unrepaired DNA strand breaks for fertilization or embryo development are not known. In the rat, drug-induced single strand breaks in the spermatozoa of the cauda epididymidis altered their decondensation [39] and increased postimplantation loss and teratogenic effects in the embryo [40]. In humans, an increase in DNA damage above 25% in sperm population has been correlated with the probability that the fertilization rate is decreased under 20% [41]. In addition, it has been suggested that

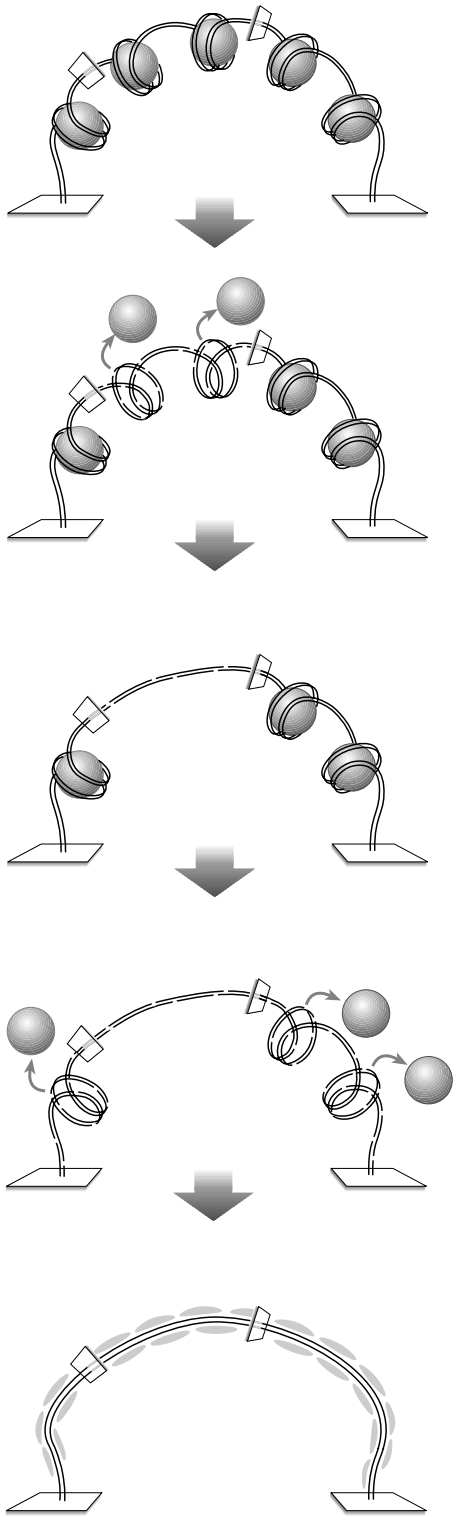

Fig. 1. Hypothetical model showing the sequential steps likely involved in the removal of DNA supercoiling during spermiogenesis. Considering one DNA loop domain, the nucleosome withdrawal leaves unconstrained (free) DNA supercoils between anchoring points within a DNA loop domain. The supercoils between these anchoring points are removed by single or double strand breaks produced by an as yet unknown mechanism. The subsequent removal of nucleosomes in other topologically constrained regions of the loop leaves additional free supercoils that are removed in a similar manner. Finally, the deposition of transition proteins and the protamines stimulates the repair of the DNA strand breaks while stabilizing the DNA in a more stable, non-supercoiled state.

DNA fragmentation may affect fertilization as well as formation of the pronucleus [42,43]. Up until now, only indirect evidence correlates the alteration in the chromatin remodeling process and the increase in DNA strand breaks in the mature sperm. A direct determination of DNA fragmentation by various techniques such as the TUNEL or the COMET assays therefore needs to be performed in sperm with altered chromatin condensation [44,45]. Moreover, the available knockout mouse models for both the transition proteins and the protamines should make it possible to establish the contribution of the major basic nuclear proteins of the spermatid to the repair of the DNA strand breaks and lend strong support to the hypothesis that is presented in this paper. If alteration in condensation and DNA strand breaks are indeed closely related phenomena it will be crucial to establish whether DNA fragmentation itself, which appears as a consequence of perturbed chromatin remodeling and condensation, can affect embryo development and fertility or if the altered complement of nuclear proteins is the major cause of impaired conception.

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