HHS Public Access

Author manuscript

Results Probl Cell Differ. Author manuscript; available in PMC 2022 November 17.

Published in final edited form as:

Results Probl Cell Differ. 2022 ; 70: 295–311. doi:10.1007/978-3-031-06573-6_10.

Functional aspects of sperm chromatin organization

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Abstract

Sperm nuclei present a highly organized and condensed chromatin due to the interchange of histones by protamines during spermiogenesis. This high DNA condensation leads to an almost inert chromatin, with the impossibility of conducting gene transcription as in most other somatic cells. The major chromosomal structure responsible for DNA condensation is the formation of protamine-DNA toroids containing 25 to 50 kilobases of DNA. These toroids are connected by toroid linker regions (TLR) which attach them to the nuclear matrix, as matrix attachment regions (MAR) do in somatic cells. Despite this high degree of condensation, evidence shows that sperm chromatin contains vulnerable elements that can be degraded even in fully condensed chromatin, which may correspond to chromatin regions that transfer functionality to the zygote at fertilization. This chapter covers an updated review of our model for sperm chromatin structure and its potential functional elements that affect embryo development.

Keywords

Sperm; chromatin condensation; toroid linker region; matrix attachment region; double-stranded DNA breaks

1. Introduction

Spermatozoa are highly differentiated cells whose main function is to carry the paternal genetic content to the oocyte completely intact. To perform this function, evolution has provided mammalian sperm highly with specific physiological features, such as the shape, the presence of the acrosome, the midpiece and the flagellum, all designed to execute the

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CONFLICTS OF INTEREST DISCLOSURES

All authors declare no conflict of interest.

proper transport of the paternal genome through the female tract and provide the ability to penetrate the oocyte. Importantly, the sperm genome is almost completely transformed during final stages of spermatogenesis, leading to a highly condensed and transcriptionally inactive chromatin designed to protect the male genetic information during transport [1, 2].

Despite the vital importance of the sperm genome contribution to embryo development, research of the recent decades has shown that spermatozoa also deliver other components that may also be equally important for embryo development, presenting a much broader view of paternal inheritance beyond the genetic sequence of the paternal genome. These include a complex RNA population contained in the sperm nuclei or acquired through exosomes present in sperm maturation compartments that may regulate embryo gene expression [3], a DNA methylation profile that leads contributes to proper embryo progression [4], sperm proteins that may influence embryo epigenetic markers [5], or even the sperm centrioles [6]. In a similar manner, it is our view that the structural organization of sperm chromatin also has certain features that can be inherited by the paternal genome in the embryo and are required for proper development. In this chapter we describe these structural elements, how they are known to be moldable despite the highly condensed environment, and how they contribute to early embryonic evolution.

2. Sperm chromatin structure

2.1 Somatic chromatin

In eukaryotic somatic cells, DNA is associated with histones, which are positively charged proteins that are involved not only in chromatin condensation and organization, but also in several nuclear regulatory processes such as the recruitment of DNA polymerases or the assembly of complexes promoting gene transcription or repression. The first order of somatic DNA condensation is the nucleosome, in which about 150 bp DNA is wrapped twice around a histone octamer. Nucleosome formation shortens the chromatin length, and increases its average diameter to 10 nm. This filament is further coiled by linker histone H1 to form the 30 nm chromatin fiber. Finally, this 30 nm fiber is attached a specific sites to the nuclear scaffold, or nuclear matrix, through AT-rich DNA sequences named matrix attachment regions (MAR) [7, 8]. These attachments lead to the formation of chromatin loops, between 20 to 400 kilobases that may show an open/decondensed configuration in active transcription regions, and a more condensed estate in silent genes, thus generating euchromatin and heterochromatin regions [9, 10]. Somatic cell MARs perform well-documented transcriptional functions and are the sites of DNA replication origins [11]. Also, they present highly dynamic properties enabling chromatin to anchor different domains to the nuclear matrix, thus promoting physical interactions between distant genomic regions that may be located apart in the genetic sequence, for instance, bringing to close proximity distant gene promoters and their target genes, or generating gene clusters with related functions. Distal interactions lead to the generation of active or inactive foci that are dependent on the cell type and on the cell-cycle dependent manner [12, 13]. These interactions are important to understand gene expression clusters, and numerous studies are performed using advanced methods such as Hi-C [14, 15].

2.2 Sperm chromatin

The nuclear chromatin organization present in somatic cells is also present in early germ cells, such as spermatogonia through the first haploid cell type, round spermatogonia. As the round spermatids progress to spermatozoa, the chromatin becomes condensed as histones are interchanged by protamines at later stages of spermatogenesis [16, 17], through a process that involve transition proteins in some species such as humans, mouse, rat or sheep [18–20]. This leads to an extremely condensed nucleus that has with absolute transcriptional silence.

2.2.1 Condensing the DNA through protamines—Protamines are about half the size of histones, containing 50 to 110 amino acids. They also have a higher positive charge due to their higher content of lysine and arginine, allowing them to efficiently bind to the major groove of the DNA every 10 to 15 base pairs of DNA in each double helix turn [17, 21]. In the mammalian genome, two types of protamines (protamine 1 and protamine 2 family) are present. Whilst the first protein is present in all mammalian sperm, protamine 2 is only found in humans, primates, mouse, rabbits and stallions [18, 22–24]. The presence of protamine 2 in these species is essential for sperm formation, as knockouts for PRM2 resulted in infertility [25, 26], and an excess of protamine 1 expression resulted in sperm morphology alterations and premature DNA condensation [27, 28]. Thus, while protamine 1 is sufficient to fully condense sperm chromatin in some species, protamine 2 seems only to augment condensation by protamine 1 [23].

The binding of protamines confers a 44 fold smaller volume of the chromatin compared to liver cells [1]. This DNA condensation present in sperm cells leads to a strong protection of genetic content from genotoxic activity, a key feature to enable the delivery of an uninterrupted genetic information to the embryo, limiting mutations and ensuring the perpetuation of the species [29, 30]. However, upon fertilization, this highly condensed chromatin needs to be reestablished as a functionally active chromatin, thus requiring an interchange of protamines by histones in the male ponucleus, a process in which other proteins are also thought to be imported to paternal genome [31, 32].

2.2.2 Protamine-DNA Toroids—The binding of protamines to DNA leads to a major superstructure of DNA, the formation of toroidal DNA-protamine complexes of about 50 kilobases, estimated to measure between 60 and 100 nanometers diameter and 20 nanometers thickness [33–35]. The biochemical configuration of protamines and their cysteine content leads to the presence of one major post-translational modification: covalent disulfide bonds produced between neighbor protamines [22]. This inter-protamine binding leads to stabilization of toroidal structures rendering them resistant to genotoxic agents as nucleases [36] and more resistant to oxidative damage [37].

The consecutive toroidal structures remain linked through an uncondensed DNA, the toroid linker region (TLR). Moreover, toroid linker regions are regions that could remain condensed by histones, which some investigations have shown might have a role in early embryos, as they may be the source of epigenetic modifications that influence gene expression in early development [38, 39]. However, the specific localization of modified histones along the mature sperm genome is still a controversial topic, as some studies found

an increased presence in gene-rich regions [38, 40], whilst others have found the presence of histones in repetitive, intergenic regions [41]. Further experiments that do not rely on the use of external nucleases are needed in order to clarify these controversial results, as suggested by Yamaguchi, et al. [42]. Additionally, inter-specific differences may also be influencing these outcomes, as different histone levels have been found in humans (3% to 15%) [40, 41], mouse (1% to 15%) [18, 41], and marsupials (50%) [35, 41].

2.2.3 Sperm DNA loop domains—Despite the condensation of DNA into toroids by protamines, we and others have shown that sperm DNA is also organized into DNA loop domains that are attached at their bases to proteinaceous nuclear matrix [43–46]. The organization of the loops domains is altered during spermatogenesis, and may be inherited by the embryo. We have also provided evidence that the attachment of these loop domains is the site of DNA replication origins in the zygote, suggesting that the embryo inherits functional chromatin segments [47].

2.2.4 Tertiary structures of sperm chromatin—Some investigations conducted through freeze-fracture and atomic force microscopy have shown evidence of tertiary structures in sperm chromatin. These structures may be composed by parallel stacks of lamellar sheets parallel to the long axis in bulls, rabbits and men [18, 48]. Other studies conducted in the late nineties have shown the presence of nucleosome-like particles in the nuclear periphery [49], which is in accordance to the model of chromosome positioning in sperm, where telomeres would be condensed in histones and attached to the internal nuclear membrane towards the periphery of the cell [50–52]. Additionally, different chromosomal territories have been described in sperm nuclei, being the organization of chromosomes non-random, as it is also described for somatic nuclei [53–55]. Therefore, both the sperm chromatin structure and chromosomal organization may have implications for sperm function before and after fertilization.

3. The Toroid Loop Model for Sperm Chromatin Structure

We have proposed a model for mammalian sperm chromatin structure that incorporates several different aspects of sperm DNA packaging from many laboratories [56]. Here, we reiterate this model with an emphasis on which parts of the model have strong experimental support, and which components are hypotheses based on other considerations. The gist of the model is that it proposes that each DNA loop domain in the sperm cell is coiled into one protamine toroid (Figure 1A). However, there are many implications and hypotheses that remain to be tested that emerged from thinking about the Toroid-Loop model.

3.1. Aspects of the model that are supported by experimental evidence.

Figure 1C, right, lists the parts of the model for which supporting experimental evidence exists. (a) The depiction of large segments of sperm DNA the remain bound to histones, and not protamines, is based on the mapping of histones in human sperm by two different groups [40, 57]. The model therefore predicts that some entire loops are not coiled into toroids by protamines. (b) As discussed above, it is clear that when protamines bind to DNA, they form toroids that contain up to 50 kb of DNA [33, 58, 59]. These toroids are very

similar to toroids formed when DNA is incubated with divalent cations [60], suggesting that protamines induce a configuration in DNA that naturally forms when the negative charges on the phosphodiester backbone are neutralized with cations. (c) Evidence also suggests that protamine bound DNA is very resistant to nuclease digestion [36, 61]. This is not surprising given the very tight compaction of protamine-DNA toroids which probably does not allow access of the DNA fibers to enzymatic contact. (d) Our prediction that each protamine-DNA toroid is one loop is based on the finding that when sperm chromatin is digested to loop-sized fragments of 25 to 50 kb, the loops are released from the nuclear matrix [36]. (e) We and others have presented several lines of evidence to support that sperm DNA is organized into loops of between 25 to 50 kb that are attached at their bases to the nuclear matrix [43–46].

3.2. Aspects of the model that are proposed for which direct experimental data is yet to be acquired.

Our Toroid-Loop model for sperm chromatin structure contains several aspects that make sense to us based on existing data in sperm or other systems, but that have not yet been validated by experimental results (Figure 1C, left). (a) We have proposed that the protamine toroids are stacked, arranged as a role of life-saver candies, mainly because this arrangement makes the most logical sense to us. However, we have not seen electron microscopic evidence of this, except for one experiment we could not repeat. It is also important to remember that when considering the entire chromosome, the fully condensed chromatin fiber needs to bend or coil to fit inside the sperm nucleus, so there must be some flexibility in the arrangement of the packaged protamine-DNA toroids to do this. We have not accounted for this in our model. (b) We usually depict the nuclease sensitive TLRs as being bound to histones because we know that histone bound DNA is distributed throughout the sperm chromatin, and it is nuclease sensitive. However, we do not have any direct evidence to support this. (c) Based on the experimental evidence that when TLRs are digested with nuclease the DNA loop domains are released from the nuclear matrix [36], we predict that TLRs contain the matrix attachment regions (MARs), but, again, further experimentation will be required to firmly establish this. (d) Finally, and perhaps most importantly, we predict that TLRs are located in specific regions of the chromatin, and not randomly spaced along the DNA. This is based in part by experiments that suggest that sperm MARs are sequence specific [46, 62, 63]. We are currently attempting to map TLRs in mouse sperm to test this hypothesis directly.

4. Functional aspects of sperm chromatin structure: Sperm chromatin retains some active properties

As described above, when protamines replace histones in condensing the DNA, most of the chromatin is organized into highly condensed toroids. This condensation eliminates any activity involving gene transcription and translation and protects the protamine-bound regions from the activity of any protein such as nucleases, turning the DNA into an almost crystalline estate [56]. The biological significance of this inert status is to protect the genetic material during the transport towards the oocyte, preventing alterations in the genetic information.

Treatments that disrupt chromatin structure in somatic cells, such as dehydration or sonication, do not prevent the ability of the sperm to fertilize when injected into oocytes [64, 65]. This suggests that mouse sperm chromatin can retain its genetic, and, presumably, its structural chromosomal integrity under conditions that would severely degrade most other cell types, enough to fully participate in embryo fertilization. In fact, it is necessary to subject rodent sperm to a temperature of 125°C for 20 minutes in order to suppress its embryo development capacity [66]. Thus, during spermiogenesis, sperm chromatin is condensed to a state that protects the DNA from external assaults, but retains the ability to participate in embryogenesis. This is supported by the fact that mouse sperm nuclear “halos”, from which all histones and protamines have been extracted, retain enough structural information for the paternal genome to be replicated when injected into an oocyte [67].

However, the condensation by protamines, while protective, does not render sperm DNA completely impenetrable to damage. DNA oxidation is a common form of damage that occurs in mature spermatozoa. When reactive oxygen species damage DNA, the most common adduct formed is the 8-hydroxy-2’deoxyguanosine (8OHdG). The glycosylase OGG1 is the first enzyme to excise this DNA adduct, forming single strand DNA breaks. OGG1 was demonstrated to be present in human sperm [68], enabling this process in the fully compact chromatin. In a similar way, fully condensed sperm chromatin is also susceptible to nuclease digestion, but this is limited to those sites that are devoid of protamines, the TLRs. Degrading TLRs can be accomplished either with external nucleases or when endogenous nucleases present in the vas deferens luminal fluid are activated. In these cases, sperm DNA is degraded to loop-sized fragments with an average size of 25 kb, but no further degradation is observed [61].

4.1 DNA replication of the sperm chromatin

The first DNA replication occurring in mammalian zygote takes place a highly special environment, since both maternal and paternal genetic information remain contained in two separate pronuclei (Sirlin & Edwards, 1959). After fertilization, while the female genome completes metaphase II, extrudes the second polar body and decondenses to form the female pronucleus, the sperm genome interchange protamines with histones and decondenses to form the male pronucleus. Each pronucleus then replicates its DNA separately, then then the chromosomes condense to form the metaphase plate, when they will meet for the first time. Different studies have shown that this first DNA replication is asynchronous between both plonuclei, being delayed up to 2 hours in female pronucleus compared to male pronucleus [70–72].

In mammalian cells, DNA replication licensing may begin very soon, even after the M/G1 transition [73]. It is orchestrated by the recognition of the replication origins by the ORC (origin recognition complex) [74], that recruits CDT1 and CDC6, that finally recruit the MCM2–7 helicase complex at the S-phase. While growing somatic cells may retain ORC throughout the cell cycle, it is not clear when the gametes become licensed, and the differences in their chromosomal structure suggest differences in the timing of licensing. We previously demonstrated that meiotic maternal chromosomes may already be partially

licensed at the time of fertilization [75]. Oocytes are arrested at meiotic metaphase II, which has many structural similarities to mitotic metaphase, in which origins are thought to be fully licensed. However, due to the fact that the sperm genome needs to interchange protamines by histones after fertilization, replication licensing is delayed up to 4 hours after fertilization, and is added de novo to paternal pronuclei [75]. This de novo licensing is supported by other results from our lab, which showed that the removal of protamines and associated proteins through incubations with high-salt and DTT, which would remove any ORC proteins bound to DNA, did not prevent zygotes from replicating paternal DNA [47, 67], suggesting they were licensed in the oocyte, de novo. However, the presence of MAR regions attaching sperm chromatin to the nuclear scaffold is necessary to initiate DNA replication [47], which suggests that regions between toroids may also retain the factors for male pronucleus progression. In fact, in somatic cells, it is still not clear how DNA replication origins are identified by the cell, and there is evidence that different DNA sites can be used as origins in different cell types [76, 77]. In our most updated model of sperm chromatin, we have proposed that DNA replication origins (licensed sites) are located within the TLRs in sperm and that these TLRs/replication origins correspond to sperm nuclear matrix attachment regions (MARs). Our model also proposes that these TLRs also represent the small portions of DNA that remain bound to histones in fully condensed sperm (Figure 1).

4.2 Sperm chromatin fragmentation

As mentioned above, we have shown that despite its high degree of condensation, sperm chromatin has the capacity to be partially degraded by a mechanism we have termed sperm chromatin fragmentation (SCF) in which an endogenous nuclease enters the sperm nucleus and cleaves the DNA at TLRs [61, 78]. This process is driven by the presence of a luminal nuclease that enters the sperm when the membrane is damaged [78]. The DNA degradation is limited to the toroid linker regions, as the presence of toroidal structures condensed with protamines limited the presence of DSB to non-toroidal regions, leading mostly to toroid-sized DNA breaks (between 25 kb and 50 kb) [61, 79]. Moreover, further experiments also showed that these double-stranded DNA breaks may remain attached to the nuclear scaffold [80], further supporting the idea that TLRs are attached to the matrix. It also provides a structural rationale for the possible repair of some DSB in the oocyte after fertilization.

Our current model for SCF is that TLRs in fully condensed sperm chromatin provide vulnerable, nuclease sensitive regions that can be digested by external nucleases. The luminal fluid of the vas deferens contains a nuclease that is activated, either directly or indirectly, by Mn^{2+} ions and enters the sperm nuclease when the membranes are damaged. SCF appears to be a mechanism to prevent damaged spermatozoa from fertilizing an oocyte with a less than pristine copy of the paternal genome.

5. The impact of sperm organization to human infertility

It is now well established that oxidative stress is a major effector of physiological sperm perturbations, such as lipid peroxidation or protein modifications, and the main cause of DNA damage. Oxidative stress is produced by a misbalance of reactive oxygen species and

sperm antioxidant molecules [81, 82], and may have its origin in endogenous sources, as a by-product of sperm metabolism where hydroxyl radicals are generated by mitochondria in the respiratory electron chain [83]. However, exogenous sources such as toxicants, infections, leucospermia, heat stress or pollution, amongst others, have also been reported to affect sperm [84–86]. In all cases, due to their nature and size, reactive oxygen radicals may have much greater access to the DNA in condensed protamine toroids than nuclease. They are likely, therefore, to cause DNA breaks or DNA adducts in a much wider range of sperm DNA. Due to the presence of oxidative stress in human sperm, it been extensively studied as a cause of male infertility. It is well known that oxidative stress during spermatogenesis causes a reduction of sperm quality, and is a contributing factor to low sperm counts, reduced sperm motility and impaired morphology or membrane integrity [87]. In fact, in pathologies like varicocele where high oxidative damage is present, degraded sperm cells are more prevalent [88]. It is also accepted that these affectations in sperm physiology and quality by oxidative stress are correlated to a decrease of natural fertility rates both in humans and animals [89, 90].

This correlation, however, is not as clear for intracytoplasmic sperm injection (ICSI). Different studies have found controversial results regarding the clinical effects of oxidative stress in infertile patients, and it is still unclear whether oxidative DNA damage leads into observable effects in embryo development and pregnancy rates after ICSI [91]. This disparity suggests that ICSI may be a suitable treatment for patients with high oxidative damage. This may be due in part to the fact that ICSI includes the selection of the most motile and morphologically normal sperm which might be associated to less oxidative damage. In fact, correlations between sperm motility and oxidative DNA damage, and recent data obtained applying the alkaline Comet assay in ICSI-like selected sperm support the presence of this bias between sperm samples and ICSI-selected sperm [92].

While there is little correlation with oxidative damage and ICSI outcomes, some studies show a decrease in pregnancy outcomes in sperm with DSB [93, 94]. Also, the presence of double-stranded DNA breaks in sperm from men whose couples suffered recurrent miscarriage [95] have been suggested to be a subtype of DNA damage with major implication on fertility outcomes. First, the resemblance of human sperm double-stranded breaks to mouse SCF (activated through Mn^{2+}) when assessed through pulsed-field gel electrophoresis has suggested that this subtype of breaks in humans have its origin in an enzymatic mechanism, limiting their presence to the TLRs [96, 97]. In contrast to oxidative-related DNA damage, these DSBs are thought to be limited to the TLRs and are, therefore, distributed punctually throughout the genome. In fact, since these double-stranded breaks may somehow remain attached to the nuclear matrix, which may provide an opportunity to zygotes to correctly repair the 5’ and 3’ end breaks, thus minimizing chromosomal alterations in developing embryos. Sperm DSBs that are not repaired may contribute to miscarriages [98]. In a cohort of recurrent miscarriage couples without a known female factor, sperm DSBs were correlated to repeated pregnancy loss during the first trimester of pregnancy [96]. Two other studies demonstrated that embryos produced from males with increased incidences of double-stranded breaks had lower implantation rates and a delay in embryo developmental kinetics, both at polar body extrusion and at the morula stage [93, 94]. The first delays may coincide to those observed in the paternal pronucleus

of mouse zygotes produced from SCF-induced sperm cells, where an increase of H2AX phosphorylation was shown [79]. Delays at later preimplantational stages may relate to the activation of G1/S and G2/M checkpoints [99, 100].

6. Conclusions

Mammalian sperm chromatin structure represents a unique conformation of the DNA, which has evolved to provide a high level of protection of the male germ cell genetic information, while retaining small pockets of accessible chromatin that may be involved in both DNA replication and DNA DSB repair.

FUNDING INFORMATION

This work received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 801342 (Tecniospring INDUSTRY; TECSPR-19-1-0003) to JRM, and from the NIH, grant number GM123048 to WSW.

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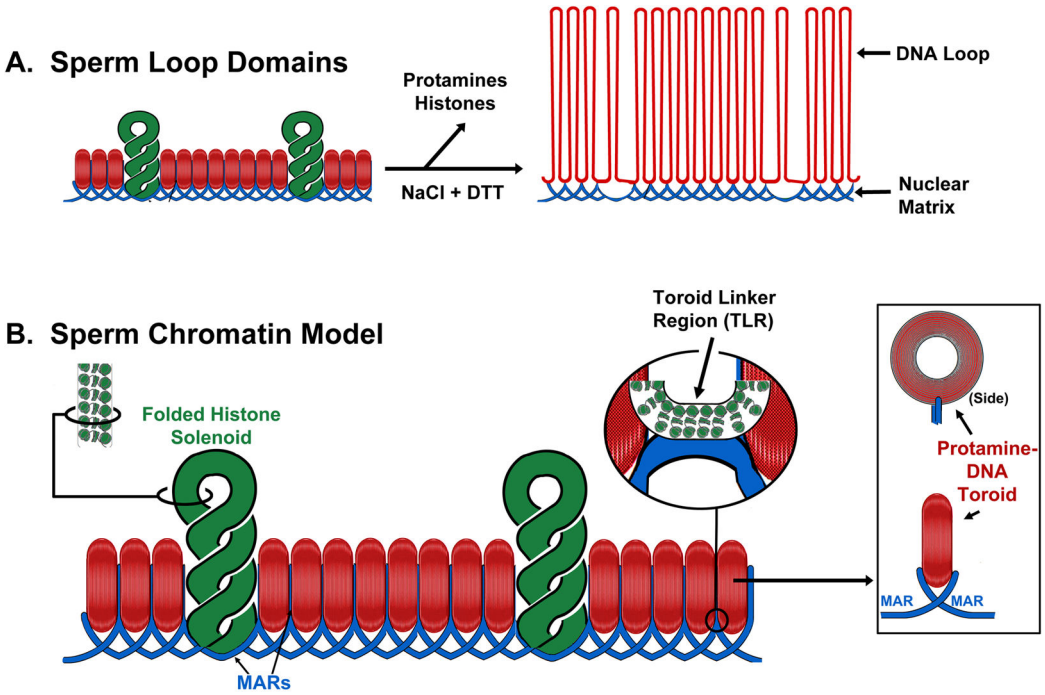

Figure 1.

The Toroid Loop Model for the sperm chromatin condensation. (A). Each DNA loop domain is coiled into a protamine-condensed toroid, suggesting that both structures are structurally related; (B). Adjacent toroids are linked by nuclease sensitive toroid linker regions (TLR), which are attached to the nuclear matrix; (C). Lists the different aspects of the model that are supported by experimental evidence (right), and the hypothesized aspects yet to be experimentally shown (left).