

·Review·

## Relationship between chromatin organization, mRNAs profile and human male gamete quality

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### Abstract

Spermiogenesis is a complex process leading to the formation of motile spermatozoa characterized by a highly stable chromatin compaction that transfers the paternal genome into the oocyte. It is commonly held that these haploid cells are devoid of transcriptional and translational activities and that the transcripts represent remnants of stored mRNAs. Recently, the chromatin organization of mature spermatozoa has been revisited as a double nucleoprotamine-nucleohistone structure possessing less-condensed regions sensitive to nuclease activity, which could be implicated in the expression of genes involved in the early embryo development. The existence of a complex population of mRNAs in human sperm is well-documented, but their role is not yet elucidated. Evidence for a latent transcriptional capacity and/or a potential *de novo* translation in mature spermatozoa from fertile men are essential for understanding the last steps of sperm maturation, such as capacitation and acrosome reaction. As such, we have documented the relationship between sperm quality and the distribution of sperm RNAs by showing divergent levels of transcripts encoding for proteins involved in either nuclear condensation (protamines 1 and 2) or in capacitation (eNOS and nNOS, *c-myc*) or in motility and sperm survival (aromatase) between low and high motile sperm issued from the same sample. Therefore, analyzing the profile of mRNAs could be helpful either as a diagnostic tool for evaluating male fertility after spermatogenesis or for prognosis use for fertilization. (*Asian J Androl* 2007 Sep; 9: 587–592)

**Keywords:** chromatin; fertility; gamete quality; man; spermatozoa; transcripts

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### 1 Introduction

Mammalian ejaculated spermatozoa comprises highly differentiated cells that originate from the complex pro-

cess of spermatogenesis, involving three major steps: the proliferation and differentiation of spermatogonia; the meiotic divisions during the spermatocyte stage; and, finally, the spermiogenesis. The transformation of spermatids into spermatozoa entails major morphological and molecular changes concerning acrosome and flagellar formation, cytoplasm elimination and mitochondria rearrangement and, finally, nuclear reshaping. Over the past few decades, spermatozoa have been considered just as mobile units for transferring the paternal genome from

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the testis to the oocyte, demonstrated by the histone-to-protamine exchange and, consequently, the resulting chromatin condensation, which finally leads to the shut-down of nuclear transcription. Indeed round spermatids store messages as ribonucleoprotein particles for long periods [1] and the particles are then translated in elongating spermatids [2]. The stability of mRNA resulting from the package and the severe loss of spermatozoal cytoplasm during spermiogenesis have resulted in spermatozoa being considered as biologically inert cells without transcriptional and translational potencies. However, recently, in relation to a better understanding of chromatin organization [3] and a possible *de novo* translation of mRNAs in spermatozoa [4], new insights about putative functions of spermatozoa should be considered.

## 2 Chromatin organization

### 2.1 Chromatin condensation players

The basic unit of somatic chromatin is the nucleosome in which DNA is coiled around a histone octamer, including H2A, H2B, H3 and H4 histones. Adjacent nucleosomes are connected by histones of the H1 linker class. The combination of covalent processes, such as methylation, acetylation and ubiquitination, are thought to contribute to chromatin organization and gene expression. The testis-specific histones H1A and H1B are found in pre-meiotic male germ cells, but a higher number of histone variants have been identified in male germ cells than in somatic cells. The displacement of histones is concomitant to the appearance of more basic nuclear proteins, such as tH2A, tH2B, H1T, spermatid-specific H2B, testis specific HMG, histone H1-like proteins in spermatids (Hils 1) and transition proteins (TNP) (see [5] for review).

Understanding the correct implication of such molecular variants during the histone/protamine exchange could help to define the expression pattern of testicular genes essential for the formation of normal spermatozoa. A novel histone H1 variant, H1T2, selectively and transiently expressed in round and elongated spermatids [6], seems to play a major role in chromatin reorganization by initiating and directing chromatin condensation. Null mutation in H1T2 demonstrates the critical role of H1T2 in spermiogenesis, with delayed nuclear condensation and reduced fertility. The epididymal sperm are morphologically and functionally abnormal, leading to a deficient motility and an inability to fertilize eggs under *in vitro* fertilization (IVF) conditions [7]. Moreover, Hils1 is a

histone restricted to elongating spermatids and is tightly implicated in chromatin condensation [8, 9].

TNP1 and TNP2 are the predominant nuclear proteins representing 55% and 40%, respectively, of the total nuclear proteins in spermatids [10]. Male mice with null mutations in either TNP1 or TNP2 are subfertile and are able to produce offspring [11, 12], although male mice with null mutations in both TNP1 and TNP2 are sterile [13]. In contrast with histone H1, protamines contain a very low amount of lysine but arginine is the most important residue (> 50%), resulting in higher DNA-binding affinity. Mice, horses and humans express two structurally distinct forms of protamines: Prm-1 and Prm-2. Only Prm1 is found in other mammals. Protamine haploinsufficient mice are infertile following the absence of chromatin assembly [14]. Consequently, changes in the expression of Prm 1 and Prm2 could increase DNA damage in spermatozoa and have been related to human male infertility (see [15] for a review).

### 2.2 Chromatin structure

Chromatin condensation is based on the presence of tightly packed toroids containing up to 60 kb of DNA resistant to DNA I digestion. The cessation of transcriptional activity in rat intermediate spermatids is concomitant with the disappearance of DNase I-hypersensitive regions normally present in transcriptionally active genes. In hamster spermatozoa, each protamine toroid is a single DNA loop domain interspersed by DNase I sensitive chromatin segments called toroid-linker regions, which are also the sites of matrix attachment regions [16]. The formation of less-condensed regions from this “donut loop” model leads to the creation of sites of chromatin digestion by endogenous nucleases present in hamster, mouse and human spermatozoa [17].

The replacement of histones by protamines is incomplete in human sperm, which retains roughly 15% of histones, unlike the sperm of other mammals [18]. The core histone fraction remaining is enriched in histone H2B, with a selective distribution of H2B variants in sperm nuclei [19]. This double structure nucleoprotamine/nucleohistone could correspond to the differential rearrangement of chromatin regions in which histone-enriched regions might possess specific functions, such as enhanced nuclease sensitivity and, therefore, could be implicated in the formation of a specific subset of genes involved in early embryogenesis [20]. This nuclease activity could be mediated by a nuclear matrix, including

topoisomerase II B (TOP2B) interacting with an extracellular  $Mn^{2+}/Ca^{2+}$ -dependent nuclease to promote DNA fragmentation and degradation [21].

The relation between function and nuclear architecture is well-known in somatic cells, but needs to be clarified in spermatozoa. Classically, chromatin compaction following spermiogenesis leads to the arrest of active transcription. Ribosomal 18 and 28S proteins have been only detected until the elongating spermatid stage in mice. Therefore, spermatozoa does not have sufficient 80S cytoplasmic ribosomal complexes, excluding, therefore, the support of any RNA translational process. However, new insights into chromatin organization lead us to the view that potential translational/transcriptional events exist in some circumstances.

### 2.3 Evidence for *de novo* translation and/or latent transcriptional capacity in mature spermatozoa

The potential involvement of mRNAs in functional spermatozoal activities is currently subject to debate [22–25], but the idea of a complete inactive transcription/translation in mature spermatozoa is discussed here in light of most recent data. An SAGE analysis of ejaculates from fertile men has revealed 25 functional gene groups: the most important one includes 96 nuclear protein genes involved in transcription and a second group comprises 84 ribosomal subunit genes interacting with protein synthesis [26]. Evidence of sperm endogenous reverse transcriptase activity [27] and the incorporation of foreign DNA sequences into sperm issued from reverse transcription of RNA molecules then transmitted to IVF embryos suggest that reverse transcribed products are fully active in spermatozoa being translated and expressed in offspring [28]. Very recent experiments described either by Pittoggi *et al.* [29], showing the correct outsplice of an intronic sequence incorporated in a DNA construct, or by Shaman *et al.* [21], who reported the demonstration of an active topoisomerase TOP2B associated with nucleases, are in favor of a less inert activity of chromatin than previously evoked. Despite the presence of a high degree of chromatin compaction in mature spermatozoa, these new data fit with the existence of isolated domains in more DNase-I-sensitive open conformations, indicating a potential transcriptional state for specific genes involved in early embryogenesis. Translational repression could be lifted in capacitating spermatozoa, as demonstrated by the incorporation of labeled amino acids into polypeptides [4] or the decrease

of *c-myc* transcripts in capacitated spermatozoa [30]. The first report describes an endogenous inhibition of the amino acids uptake only by mitochondrial translation inhibitors targeting mitochondrial 55S ribosomes [4], whereas the second work shows a restoration of the amount of *c-myc* transcripts in the presence of cycloheximide, a cytoplasmic 80S ribosome inhibitor [30]. Despite the relative absence of ribosomal complexes, the understanding of the translation mechanisms must be further clarified. In spermatozoa, the potential translation of mRNAs into proteins would be essential for the final events of the sperm maturation, such as capacitation and acrosome reaction.

The chromatin organization could be also responsible for modifications of the epigenome transmitted to embryos and involved in the early steps of embryonic development. Classically, changes in DNA and histone methylation profiles represent the main process of epigenetic regulation [31]. Rassoulzadegan *et al.* [32] reported an unexpected model of epigenetic inheritance by zygotic transfer of RNA molecules (RNA-mediated non-mendelian inheritance), which leads to the modification of phenotypic expression of the wild type allele of the Kit receptor gene in the progeny of heterozygote mice, suggesting a role of RNA molecules in the establishment of epigenetic states.

## 3 Significance of RNAs in mature spermatozoa

### 3.1 RNAs in human sperm

Most of the RNA synthesized is heterogeneous nuclear RNA, U1 and U2 small nuclear ribonucleoproteins [33, 34]. Until now, the remaining cytoplasmic mRNAs have been considered as negligible compared to the intranuclear and mitochondrial mRNAs. New investigations using various techniques such as reverse transcription polymerase chain reaction (RT-PCR) [24], microarray technology [35], *in situ* hybridization [5], confocal microscopy, serial analysis of gene expression [26] have discovered complex and specific populations of RNAs in mature spermatozoa, including microRNAs [36]. Whereas the average spermatozoon contains 0.015 pg total mRNA, a somatic cell contains 600-fold more mRNA [24]. Consequently, mRNA contamination by somatic cells or cytoplasmic droplets should be excluded by using a double swim-up or centrifugation on density gradients followed by a hypotonic treatment [35] and/or analysis of somatic cell markers [30].

Using array-based tests, up to 3 281 and 2 780 RNA transcripts have been detected from a pool of ejaculate samples and a single individual ejaculate sample, respectively [35]. The presence of various transcripts in mature sperm of rodents and men including, for example, *c-myc*, protamines 1 and 2, heat-shock proteins 70 and 90,  $\beta$ -integrins, phosphodiesterase isoforms, and numerous receptors have been listed (for reviews see [5, 24, 25]).

Among the complex population of mRNAs in spermatozoa, anti-sense microRNAs have been identified in human spermatozoa. Their similarity with siRNAs involved in RNA-mediated regulation suggest that they could influence early embryonic development [36, 37].

We and others have provided data on the presence of aromatase and estrogen receptors both in human immature germ cells and ejaculated spermatozoa [38, 39]. Spermatozoa functions, such as motility, could also be related to the mRNA profile. Therefore, we have compared the levels of different transcripts coding for molecules involved in nuclear condensation (Prm-1 and Prm-2), capacitation (eNOS, nNOS and *c-myc*), motility and sperm survival (P450 arom) using semi-quantitative RT-PCR in high and low motile fractions from normospermic patients [30]. Indeed, we have observed differential mRNAs distribution between the two fractions. No significant change in the *c-myc*/Prm-2 ratio between the two populations of spermatozoa was observed. Conversely, the amount of Prm-1 mRNA was significantly higher in low motile than in high motile fractions; in most of high motile sperm samples analyzed, eNOS and nNOS transcripts were undetectable, whereas they were in low motile sperm. In contrast, a 30% decrease of aromatase mRNA amount was observed in immotile sperm fraction, and was recorded in all samples studied. Moreover, the aromatase activity determined *in vitro* was also diminished by 34%. In addition, we observed amplified aromatase mRNA using real-time RT-PCR in asthenospermic infertile men and recorded a 44% decrease of the amount of transcripts as compared to normospermic controls (Saïd, Galeraud-Denis and Carreau, unpublished data).

Another study based on the array-predicted differential expression of genes in the two infertile (impaired motility) and fertile populations confirmed that spermatozoal RNA profile [40] could provide new information about the relation between the gamete quality and the environmental conditions of spermatogenesis and/or spermiogenesis in testis.

### 3.2 RNAs and male gamete quality

The existence of a potential translational activity in mature spermatozoa is obvious. The transcripts present in ejaculated and uncapacitated spermatozoa might be remnants from post-meiotically active genes, especially from round spermatids, which contain numerous RNAs either produced in the early stages of spermatogenesis [41] or during spermiogenesis, such as protamines and transition proteins [2].

Analysis of spermatozoal mRNA could represent the fingerprint for monitoring past events, especially the development profile of gene expression during spermatogenesis or spermiogenesis. In fact, impaired functions of reproduction have been reported above in knockout experiments performed on histone variants leading to abnormal gene expression during meiosis. Consequently, we can hypothesize the existence of an impaired mRNA profile in human spermatozoa.

The use of a cDNA microarray technique for qualitative and quantitative gene expression has shown the presence of thousands of mRNA species from testis and from pooled or individual spermatozoa, some of which are delivered to the oocyte upon fertilization [42]. The genome-wide approach between fertile and infertile patients could provide information about testis gene expression during spermatogenesis and help us to understand the mechanisms involved in the control of either normal or pathological spermatogenesis [43].

However, the study of individual mRNAs in fertile and idiopathic infertile men could also contribute to the knowledge of pathways playing a role during capacitation/acrosome reaction and/or fertilization. The accumulation of high amounts of transcripts, such as eNOS or Prm1, in low motile spermatozoa could be the consequence of an altered translation during spermiogenesis along with either a defective histone/protamine exchange and/or an impaired chromatin condensation. It would be interesting to study the putative relation between the chromatin composition, such as the histone/protamine ratio, and the wrong translation of some transcripts during spermiogenesis. Contrary to eNOS or Prm-1 transcript, aromatase RNA levels are reduced in low motile spermatozoa fraction. The relationship between the active synthesis of estrogens in mature spermatozoa and the amount of aromatase transcripts must be further investigated in pathological situations (asthenospermic and teratospermic patients) to elucidate the implication of estrogens in events such as spermatozoa survey and/or capacitation.

Furthermore, the stability of the wide range of spermatozoal RNAs seem to be different from each other: some of them could be degraded following damage occurring during successive freeze–thaw cycles, but others remain stable during similar treatment [44].

To conclude, all these observations reflect the complexity and heterogeneity of the RNA transcripts present in spermatozoa (Figure 1). Further investigations are necessary to understand the significance and the differential role of these mRNAs present in ejaculated and uncapacitated spermatozoa, particularly in relation to pathological sperms. Some of them might only be the fingerprint of spermatogenesis and/or spermiogenesis events. Others could be important for the final events just before and after fertilization. Analysis of the profile of mRNAs using a genome-wide approach applying microarray techniques or the evaluation of individual transcripts using real-time RT-PCR in fertile and infertile patients could be helpful either as diagnostic tools for evaluating male fertility [45], for the prognosis of fertilization [35] or for use in embryo development [44].

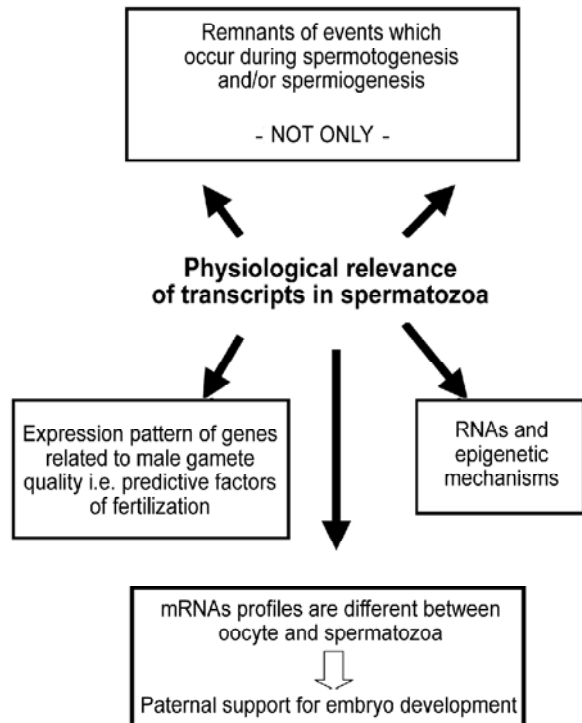


Figure 1. Putative significance of mRNAs in male gamete.

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