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Mammalian Spermatogenesis, DNA Repair, Poly(ADP-ribose) Turnover: the State of the Art

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1. Introduction

In the last decade an extensive and exhaustive research has focused on morphological and physiological features of mammalian male germ cell development defining a strict correlation between structural and molecular changes occurring from spermatogonia to spermatids and spermatozoa (Hermo et al., 2010 a-e; Inselman et al., 2003). The proliferation and meiotic phases whereby spermatogonia undergo several mitotic divisions to form spermatocytes and then haploid spermatids through two further meioses, have been studied in detail and the morphological, structural and functional features that are common to all generations of germ cells, elucidated (Hermo et al., 2010 a-e). This review will discuss only of those general features strictly related to the molecular events to be treated and will focus on the cell development arising from the dramatic changes in chromatin density and composition taking place during the differentiation process up to the late stages of spermatid maturation (spermiogenesis). The progression from diploid spermatogonia to haploid spermatozoa involves stage- and testis-specific gene expression, mitotic and meiotic division, and the histone-protamine changes (Grimes & Smart, 1985; Inselman et al., 2003; Meistrich et al., 1992, 2003). Alterations in DNA topology that occur in this process require both an exchange of histones to transition proteins and then to protamines, and the formation of DNA strand breaks (Aitken, 2009; Marcon & Boissonneault, 2004; Meistrich et al., 2003; Sakkas et al., 1995, 2010). The high frequency of DNA strand breaks during spermatogenesis needs a finely regulated DNA repair process, involving poly(ADP-ribosyl)ation of proteins among other mechanisms (Aitken & De Juliis, 2010; Meyer-Ficca et al., 2009) as the function of specific histone modifications, chromatin modifiers, DNA repair, DNA methylation, also the knowledge of the meaning of germ cell protein poly-ADP-ribosylation and of its relationship with DNA repair has made a great progress and here will be summarized and discussed.

2. Mammalian spermatogenesis

Mammalian spermatogenesis is an ordered and well-defined process occurring in seminiferous tubules of the testis. It is characterized by mitotical spermatogonia divisions to produce spermatocytes that proceed through meiosis to form a population of haploid cells (spermatids) over a period of several weeks (Hermo et al., 2010 a,b). The three specific

functional phases, proliferation, meiosis, and differentiation of spermatogenesis, involve three different germ cell populations, spermatogonia, spermatocytes, and spermatids. At different steps of development germ cells form various cellular patterns or stages, with 6, 12, and 14 specific stages in various mammals as human, mouse, and rat, respectively. These stages form a cycle of the seminiferous epithelium with a temporarily defined period for a given species (Hermo et al., 2010 a,b).

In the rat, the different classes of spermatogonia are dependent on a specific microenvironment contributed by Sertoli, myoid, and Leydig cells for proper development (Hermo et al., 2010 a, b).

In particular Sertoli cells provide the physical support, nutrients and hormonal signals to get a correct spermatogenesis, thus controlling germ cell proliferation. In the testis, cell adhesion and junctional molecules permit specific interactions and intracellular communication between germ and Sertoli cells. Germ cells are linked to one another by large intercellular bridges which serve to move molecules and even large organelles from the cytoplasm of one cell to another (Hermo et al., 2010 a, b).

With meiosis, spermatocytes go through chromosomal pairing, synapsis, and genetic exchange to be transformed into haploid cells. The synaptonemal complex and sex body are specific structural entities of the meiotic cells (Hermo et al., 2010 a).

Spermiogenesis is the haploid phase of spermatogenesis transforming spermatids into spermatozoa (Hermo et al., 2010 a,b). During this phase of germ cell development, spermatids undergo striking morphological transformations leading to the formation of highly specialized spermatozoa. It is a long process subdivided into distinct steps with 19 being identified in rats, 16 in mouse and 8 in humans. Spermiogenesis extends over 22.7 days in rats and 21.6 days in humans. Several structural and functional key events take place during the development of spermatids. During early spermiogenesis, morphological changes are evident: the Golgi apparatus turns into the acrosome, the endoplasmic reticulum forms the radial body and the annulate lamellae; mitochondria change shape, features and arrangement of location within cells; the chromatoid body develops, the shape of the spermatid head is structurally remodelled in a species-specific manner, and the nuclear chromatin becomes compacted to accommodate the fiber-shaped sperm head. Microtubules are described as forming a curtain to maintain sperm head shape and trafficking of proteins in the spermatid cytoplasm (Fouquet et al., 2000; Hermo et al., 2010 c, e).

At molecular level, during spermatogenesis, germ cells express many proteins involved in balance of water, pH, ion transport, etc.. In the nucleus, germ cells contain specialized transcription complexes able to perform the differentiation program of spermatogenesis, with cell-specific differences in the components of this machinery (Hermo et al., 2010, d-e). In mouse spermatocytes expression and localization of proteins critical to events of the meiotic cell division occur, with a temporal order for chromosomal pairing and recombination proteins, kinases and substrates that mediate the cell cycle transition (Inselman et al., 2003). Distinct and protein-specific patterns occur with respect to expression and localization throughout meiotic prophase and division and dramatic relocalization of proteins occurs as spermatocytes enter the meiotic division phase. Such a framework can clarify mechanisms of normal meiosis as well as mutant phenotypes and aberrations of the meiotic process (Inselman et al., 2003; Li et al., 2008; Zhao et al., 2004). Classifying proteins of spermatogenic cells with a view of their functions, and their applications in the regulation of fertility has made it possible to understand the molecular

biology of male gametogenesis in great detail, with the description of specialized proteins, which are dominantly and/or specifically expressed in germ cells and localized in spermatozoa (Gupta, 2005).

At certain periods before and during meiosis, one of the most conspicuous changes involves remodelling of the nucleosomal chromatin into a highly condensed chromatin (Figure 1). The structural reorganization and packaging of the DNA is concomitant with two sequential replacements of spermatid-specific basic nuclear proteins (Oko et al., 1996).

Mammalian, especially rat spermatogenesis involves a progressive and transient replacement of the classic histones by arginine-rich proteins (Kistler, et al., 1996; Meistrich et al., 1992, 2003). H1t, the testis-specific linker histone, appears in germ cells during the meiotic prophase of mammalian spermatogenesis, when the other variants have already disappeared or are present in traces, except H1a, which is the most abundant somatic subtype in rat testis proteins (Kistler et al., 1996; Meistrich et al., 1992, 2003).

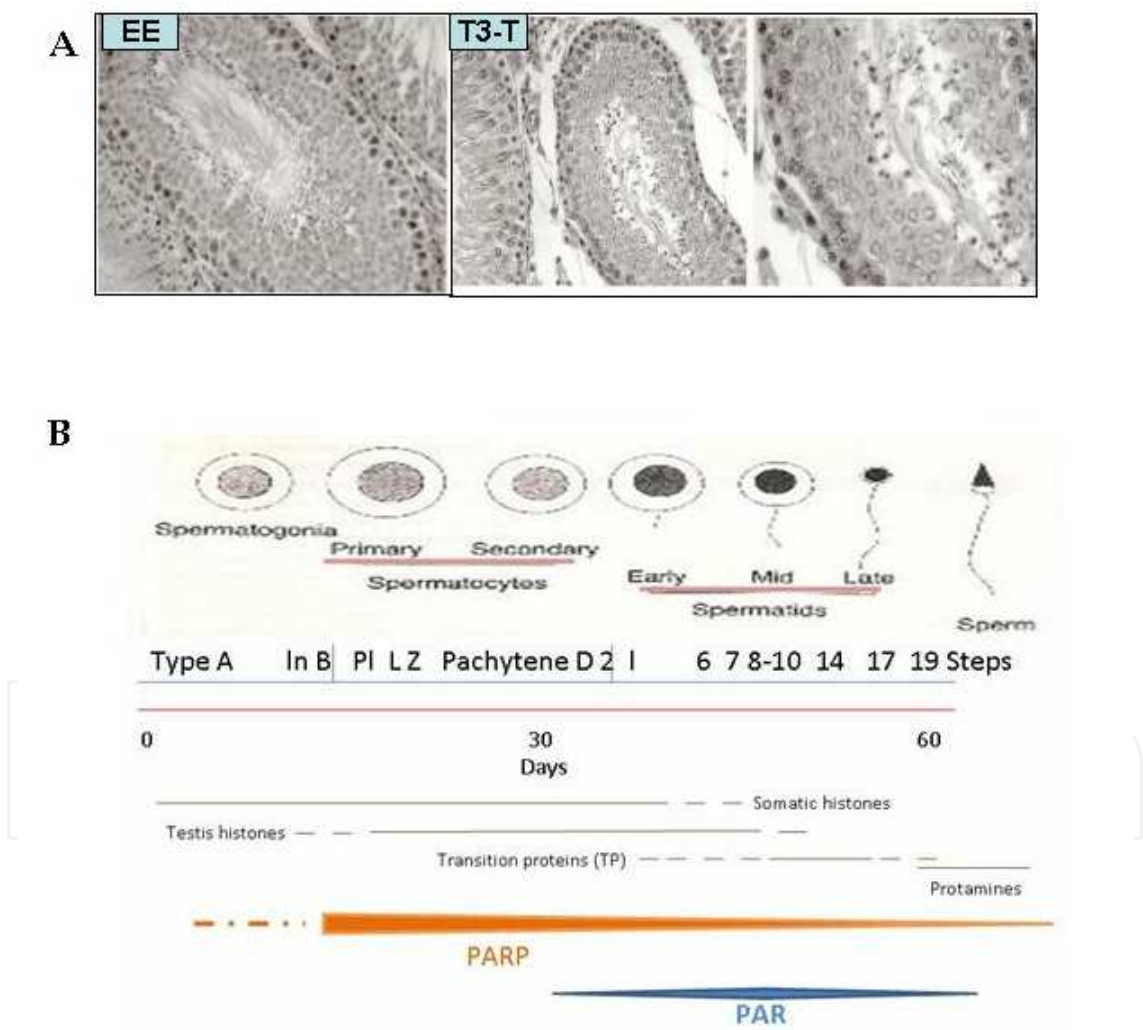


Fig. 1. Rat testis spermatogenesis. A) Optic microscopy of testis sections from euthyroid (EE, control) and hyperthyroid (T3-T) rats. T3-T rats were treated three weeks with daily administration of thriiodothyronine (Faraone Mennella et al., 2005a). Hormonal stimulus affected normal germ cell differentiation, by reducing spermatozoa in the tubule lumen. B) PARPactivity, PAR, and nuclear proteins during male rat germ cell differentiation.

The transition proteins replace histones during the initial stages of chromatin condensation of spermiogenesis and are later replaced by protamines, which are the only basic nuclear structural proteins in the sperm of most mammals (Dadoune, 2003; Meistrich et al., 1992; Ullas & Rao, 2003). The transition proteins, including the TP family, presumably mediate the replacement of histones by protamines (Kistler et al., 1996; Meistrich et al., 1992; Ullas & Rao, 2003).

The sequential synthesis and replacement of histones and testis specific proteins with protamines must be highly regulated in order to produce large number of spermatozoa with intact and competent DNA. Epigenetic regulation of gene expression and nucleoprotein transition is critical during spermatogenesis. In germ cell nucleus, epigenetic regulation include protein modifications methylation, acetylation, phosphorylation, ubiquitination, poly(ADP-ribosyl)ation, each signaling changes in chromatin structure (Carrel et al., 2007; Godmann et al., 2009; Hermo et al., 2010, d-e; Ullas & Rao, 2003; Yu et al., 2000; Zamudio et al., 2008).

3. Chromatin remodelling and the role(s) of DNA repair during spermatogenesis

Gene expression and other DNA metabolic events involving chromatin are organized specifically within the space of the cell nucleus and are related to nuclear architecture. Local chromatin structures are devoted to maintain genes in an active or silenced configuration, to accommodate DNA replication, chromosome pairing and segregation, and to maintain telomeric integrity. All these processes are highly regulated by chromatin remodelling (Ehrenhofer-Murray, 2004; Falbo, 2006; McNairn, 2003; Morrison, 2004; Phillips & Shaw, 2008; Saha et al., 2006).

Related to chromatin remodelling a large number of modifications are known as signals for the binding of specific proteins and many of them are associated with distinct patterns of gene expression, DNA repair, or replication (Deal et al., 2010; Rajapakse et al., 2010; Talbert & Henikoff, 2010).

Spermatogenesis provides an excellent example of roles for histone variants, post-translational modifications of histone and non-histone proteins, specifically poly (ADP-ribosyl)ation in regulating chromatin structure and function (Faraone Mennella et al., 1999; Govin et al., 2004; Grimes and Smart, 1985; Meyer-Ficca et al., 2005; Nair et al., 2008; Ullas & Rao, 2003).

Histone variants are especially prevalent during the development of germ cells and some of them play a role to compact DNA less tightly to facilitate rapid nuclear division, DNA replication and access to trans-acting factors (De Lucia et al., 1994; Faraone Mennella et al., 1999; Lewis et al., 2003).

The dual role for H1 in chromatin structure and gene regulation defines different heritable epigenetic states of gene activity which are maintained through mechanisms independent of gene sequence (Zamudio et al., 2008). Linker histone H1 exerts synergistic effects by modulating modifications of core histones either in the presence or absence of its own modification in man and mouse (Yan et al., 2003).

The best example of reversible compaction of DNA by multiple pathways concerns the condensation of DNA into sperm nuclei during spermiogenesis (Govin et al., 2004; Laberge et al., 2005 a,b). Chromatin remodelling is a major event that occurs during mammalian spermiogenesis. Nuclear condensation during germ cell differentiation is accomplished by

replacing somatic histones (linker and core histones) and the testis-specific H1t with transition proteins and, finally, with protamines, Figure 1 (Green et al., 1994; Grimes & Smart, 1985; Meistrich et al., 1992). The transition proteins, the TP family, are mediators in the replacement of histones by protamines (Green et al., 1994; Grimes & Smart, 1985; Meistrich et al., 1992).

Transition proteins and the tail regions of histones are sites of post-translational covalent modifications (Pirhonen et al., 1994; Ullas & Rao, 2003).

Methylation of position-specific lysine residues in the histone H3 and H4 amino-termini has linked with the formation of constitutive and facultative heterochromatin as well as with specifically repressed single gene loci (Cremer et al., 2004). Furthermore ubiquitylation of H2B might be involved in double strand break formation during meiosis (Agarwal et al., 2009).

Core histone acetylation occurs during the late stage of spermatogenesis in several organisms, allowing the removal of histones and their replacement by protamines (Grimes & Smart, 1985). Acetylation of rat testis H3, H4 and of testis histone variants TH2B and TH3 were observed in pachytene spermatocytes and round spermatids, in line with the hypothesised roles of acetylation in the deposition of histones onto DNA (early spermatogenesis) and replacement of histones by protamines in spermiogenesis (Grimes & Smart, 1985). In general core histone acetylation has important consequences for the organization of DNA in a nucleosome, loosening interactions at the periphery of the structure. In fact it has been reported that histone acetylation leads to a substantial decrease in nucleosome rigidity. Concurrent with histone acetylation are other post – translational modifications. Simbulan-Rosenthal et al. (1998) reported that acetylated core histones may also be subjected to (ADP-ribosyl)ation. Bouliskas et al. (1992) got evidence that acetylated H4 subspecies are predominantly tri- and tetra- (ADP-ribosyl)ated. He proposed that DNA strand breaks induce the formation of poly(ADP-ribosyl)ated species of histones, mainly H1, whereas in the absence of DNA strand breaks histones are mono- and oligo-(ADP-ribosyl)ated. This author hypothesized that newly synthesized core histones may be reversibly oligo(ADP-ribosyl)ated in order to facilitate their assembly into histone complexes and their deposition onto DNA at replication fork, and suggested that the observed simultaneous occurrence of acetylation and oligo (ADP-ribosyl)ation correlates with changes in chromatin structure.

The change in germ cell chromatin architecture requires a global but transient appearance of endogenous stage-specific DNA strand breaks (Laberge and Boissonneault, 2005 b; Leduc et al., 2008, a,b).

Controlling genome integrity is essential to guarantee the fidelity of DNA inheritance. Therefore, maintaining the integrity of sperm DNA is vital to reproduction and male fertility. Sperm contain a number of molecules and pathways for the repair of base excision, base mismatches and DNA strand breaks (Leduc et al., 2008, a,b).

In the mouse, elevated and global increase in DNA strand breaks levels are present in nuclei of round-shaped spermatids when chromatin starts to re-organize.

DNA strand breaks are also detected in the whole population of elongating spermatids (stages IX-XI) of the mouse seminiferous epithelium, coincident with histone H4 hyperacetylation during chromatin remodelling (Marcon & Boissonault, 2004).

In addition to the nuclear protein exchange, the chromatin remodelling process leading to the precise packaging of the paternal genome during spermiogenesis, involves the elimination of the free DNA supercoils created by the nucleosome removal. To reduce the

torsional stress induced by change in DNA topology, DNA strand breaks provide the swivel effect, with the contribution of topoisomerases, to both create and seal DNA nicks, and providing the controlled increase of linking number to relax DNA (Boissonault, 2002). The origin of the transient increase in DNA strand breaks would require an endogenous nuclease activity present up to the late spermiogenesis steps (Boaz et al., 2008). Topoisomerase II may play such a role being able to both create and ligate the DNA nicks during spermiogenesis (Boissonault, 2002; Chen and Longo, 1996; McPherson et al., 1993; Roca & Mezquita, 1989; Shaman et al., 2006; Yamauchi et al., 2007).

Topoisomerase II beta (TOP2B) is the type II topoisomerase present in elongating spermatids between steps 9 and 13, co-existing with tyrosyl-DNA phosphodiesterase 1 (TDP1), an enzyme known to resolve topoisomerase-mediated DNA damage, and gamma-H2AX (also known as H2AFX), triggered as a DNA damage response (Boissonault, 2002; Shaman et al., 2006; Yamauchi et al., 2007).

During the normal developmental program of the spermatids, dramatic consequences for the genomic integrity of the developing male gamete may arise from any unresolved double-strand breaks resulting from a failure in the rejoining process of TOP2B (Leduc et al., 2008, a,b).

A correct DNA condensing process is likely to play a key role in the elimination of the strand breaks since DNA breaks appear transiently and are no more present once the nuclear protein transition is completed. A current hypothesis links the DNA condensation process (from the transition proteins to the protamines) with the repair of the DNA strand breaks. An altered sperm chromatin packaging was already correlated with an increase in DNA fragmentation in the mature sperm. In addition, underprotamination seems to be related with DNA nicking, and the transition protein 1 (TP1) stimulates *in vitro* the repair of a nicked circular plasmid, whereas TP1, TP2 and protamines stimulate oligomerization of short DNA fragments in the presence of T4 DNA ligase (Adham et al., 2001; Carrell et al., 2007; Kierszenbaum, 2001; Leduc et al., 2008 a; Zhao et al., 2001). The transition proteins or protamines would therefore act as alignment factors by bridging the free DNA ends created at the break point.

Most of DNA damage in mid-spermatogenesis is attributed to physiological apoptosis of germ cells (Leduc et al., 2008, a,b; Sinha Hikim et al., 2003).

Apoptosis regulates germ cell over proliferation and eliminates defective germ cells. It is a normal event and occurs to select only high quality germ cells. Uncorrect cells do not achieve maturity; they undergo spontaneous cell death through apoptosis. In somatic cells, the apoptotic cascade involves the formation of apoptotic body; however, in highly differentiated spermatozoa, the sequence of events may differ as a result of the highly condensed sperm nucleus (Leduc et al., 2008, a,b; Sinha Hikim et al., 2003). In adult rat, most apoptotic cells are among spermatogonia (75%) and occur to a lesser extent during maturation divisions of spermatocyte and spermatid development. Increase of apoptotic germ cell death can be triggered by various regulatory stimuli, including deprivation of gonadotropins and intratesticular testosterone by GnRH antagonist, or by hormone treatment (Figure 1A), exposure to local testicular heating, Sertoli cell toxicants, and chemotherapeutic agents (Faraone Mennella et al., 2005a, 2009; Leduc et al., 2008, a,b; Sinha Hikim et al., 2003).

Recent studies have demonstrated that both spontaneous and increased apoptotic programmed cell death in abnormal spermatogenesis play a main role in male fertility (de Boer et al., 2010). Appropriate epigenetic regulation is needed throughout all phases of

spermatogenesis for imprinting, chromatin remodelling, the histone-protamine transition, etc.. Strikingly, aberrant epigenetic profiles, in the form of anomalous DNA and histone modifications, are characteristic of cancerous testis cells. Germ cell development is a critical period during which epigenetic patterns are established and maintained (de Boer et al., 2010).

Some questions about epigenetic modifications regulating these events are still unanswered, as the exact functions, the impact and the order of occurrence of the epigenetic modifications associated with spermatogenesis. Environmental factors may influence the epigenetic state that may be inherited through the male germ line and passed onto more than one generation (Agarwal et al., 2009; de Boer et al., 2010; Godmann et al., 2009; Patrizio et al., 2008).

Origin of DNA damage in human spermatozoa can occur by abortive apoptosis, abnormal chromatin packaging, generation of reactive oxygen species and premature release from Sertoli cells (Leduc et al., 2008 b). For a hypothesis explaining experimental data, de Boer and co-workers (2010) propose that regulation of chromosome structure in the germline, by the occupancy of matrix/scaffold associated regions, contains molecular memory function. The male germline is strikingly dynamic as to chromatin organization. To be installed, such memory requires both S-phase and chromatin reorganization during spermatogenesis. and in the zygote, that likely also involves reorganization of loop domains, where replication occurs.

The authors underline that nuclear structure, chromatin composition and loop domain organization are aspects of human sperm variability that in many cases of assisted reproduction is increased due to inclusion of more incompletely differentiated/matured sperm nuclei (de Boer et al., 2010).

New work on the function of specific histone modifications, chromatin modifiers, DNA methylation, and the impact of the environment on developing sperm suggests that the correct setting of the epigenome is required for male reproductive health and the prevention of paternal disease transmission (de Boer et al., 2010).

It is clear from the above data that programmed DNA fragmentation and DNA damage response take place during the chromatin remodeling steps in spermatids and are not necessarily synonymous with apoptotic degeneration. Chromatin-remodeling steps in spermatids may be intrinsically mutagenic and is an important source of genetic instability, that can be further enhanced by internal and external factors (De Iuliis et al., 2010; Sakkas & Alvarez, 2010).

4. Poly-ADP-ribosylation in mammalian spermatogenesis

4.1 The scenario of poly(ADP-ribose)polymerases

The modification of proteins by ADP-ribose polymers (PAR) is a reversible process in which the synthesis of PAR from NAD⁺ is catalyzed by poly(ADP-ribose) polymerases (PARPs) and polymer catabolism is due to poly(ADP-ribose) glycohydrolase (PARG) and ADPR-protein lyase (D'Amours et al., 1999; Faraone Mennella, 2005; Hassa & Hottiger, 2008). The PARP family has eighteen members that share the highly conserved PARP catalytic domain, but vary widely in other parts of the proteins (Hottiger et al., 2010). The different PARPs are grouped in subfamilies and are involved in various events mediated by their variable domain structures. Hassa and Hottiger (2008), on the basis of PARPs catalytic domain sequences have identified 3 separate groups, but other classifications can be made

on their different subcellular localization patterns, or on different composition in functional domains (ankyrin repeats, CCCH-, WWE- and macro-domain, etc) and precise functions (regulation of vault proteins, telomere length, DNA protection, etc.) (Citarelli et al., 2010; Hottiger et al., 2010; Otto et al., 2005). More recently it has been found that proteins within the PARP superfamily have altered catalytic sites, and have mono(ADP-ribose) transferase (mART) activity or are enzymatically inactive. These findings suggest that the PARP catalytically active region has a broader range of functions than initially predicted. Human PARP10 has transferase activity rather than polymerase activity, and enzymes where the catalytically important residues are present, may not act as PARPs. For example, human PARP3 has been reported to act in poly(ADP-ribosylation) (Augustin et al., 2003), and mono(ADP-ribosylation) (Loseva et al., 2010).

Despite of these recent findings, poly(ADP-ribosylation) is the second very important post-translational modification which mostly affects different nuclear acceptor proteins. It is involved in the regulation of several cellular functions related to the maintenance of genomic integrity (DNA repair, gene amplification, apoptosis) and to the expression and propagation of the genetic information (DNA transcription and replication, differentiation, neoplastic transformation) (Hassa & Hottiger, 2008). The synthesis of PAR is an immediate response to DNA damage and is the first step in a cascade of events leading to either DNA repair or apoptosis (Burkle, 2001; Malanga & Althaus, 2005). PARP-1 and PARP-2 are so far the only PARP enzymes whose catalytic activity has been shown to be induced by DNA-strand breaks, providing strong support for sharing key functions in the cellular response to DNA damage. Recent data suggest unique functions for PARP-2 in specific processes, such as genome surveillance, spermatogenesis, adipogenesis and T cell development (Yélamos et al., 2008).

4.2 PAR turnover and spermatogenesis

Since the early discovery in the '80-'90, that mammalian testes are enriched of PARP (Concha, 1989; Corominas & Mezquita, 1985; Farina et al., 1979 a, b), and the identification of *in vitro* and *in vivo* poly(ADP-ribosylated) testis-specific proteins (Corominas & Mezquita, 1985; Faraone Mennella et al., 1982, 1984, 1988, 1999), it was clear that this reaction is a metabolic event highly involved in mammalian male germ cell differentiation. At that time the presence of more than the 116kDa PARP was unconceivable, being the second enzyme, PARP2, discovered at the end of '90s (Ame' et al., 1999; Babiychuck et al., 1998). In rat testis most PARP activity was found in isolated seminiferous tubules (Quesada et al., 1989) and among linker histone variants, the rat testis specific H1t was preferentially modified with poly(ADP-ribose) (Faraone Mennella et al., 1999; Malanga et al., 1998).

In a study with differently-aged rats, it was found that in isolated intact nuclei of testis from 8-day-old animals (only spermatogonia present in seminiferous tubules), poly(ADP-ribosylation) of nuclear proteins was very low, increased significantly by 16-day (pachytene spermatocytes appear) and reached adult proportions by 32 days (condensing spermatids present), Figure 1B (Quesada et al., 1989). It was concluded that poly(ADP-ribosylation) of nuclear proteins in rat testis is closely correlated with spermatogenesis and was inferred that it is particularly active in the early stages of meiosis, where DNA breaks are frequently produced during DNA replication and transcription. The subcellular distribution of both PARP and Poly(ADPR)glycohydrolase (PARG) was also determined after separation of different germ cell populations, and the results showed that the maximum of both PARP

amount and PARP activity can be detected on tetraploid spermatocytes which undergo meiotic division, whereas PARG activity does not differ in germinal cells (Di Meglio et al., 2003). The authors concluded that regulation of PAR turnover, variations of PARP amount, as well as changes of PARP transcription level, accompany germinal cell differentiation, possibly being implicated in DNA replication, repair and other related events (Quesada et al., 2003; Di Meglio et al., 2003).

The advance in the knowledge of poly(ADP-ribosyl)ation reaction and the discovery of a number of enzymes defined as PARP family led to a great progress of research on PARPs and spermatogenesis (Ame' et al., 2004). Among PARP family members, PARP1 and PARP2 are the two enzymes demonstrated to be directly involved in base excision DNA repair, the former being modulated by PARP3, described as a newcomer in genome integrity and mitotic progression as it is stimulated by DNA double-strand breaks (Rulten et al., 2011; Boehler et al., 2011).

In a study by Tramontano et al. (2007) examining rat primary spermatocytes it was found that both PARP1 and PARP2 are present in these germ cells. However, the vast majority of PAR in these rat primary spermatocytes is produced by PARP1 suggesting possibly different roles of PARP1 and PARP2 in spermatogenesis.

Meyer-Ficca et al. (2005) showed for the first time that poly(ADP-ribose) formation, mediated by poly(ADP-ribose) polymerases (PARP-1 and PARP-2), occurs in spermatids of steps 11-14, steps that immediately precede the most pronounced phase of chromatin condensation in spermiogenesis. High levels of ADP-ribose polymer were observed in spermatid steps 12-13 in which the highest rates of chromatin nucleoprotein exchanges take place. They also detected gamma-H2AX, the histone variant indicating the presence of DNA double-strand breaks during the same step, and hypothesize that transient ADP-ribose polymer formation may facilitate DNA strand break management during the chromatin remodeling steps of sperm cell maturation.

Interestingly, other authors provided *in vivo* evidence for the pleiotropic involvement of Parp-2 in both meiotic and postmeiotic processes (Dantzer et al., 2006). They showed that Parp-2-deficient mice exhibit severely impaired spermatogenesis, with a defect in prophase of meiosis I characterized by massive apoptosis at pachytene and metaphase I stages. Although Parp-2^{-/-} spermatocytes exhibit normal telomere dynamics and normal chromosome synapsis, they display defective meiotic sex chromosome inactivation associated with dis-regulation of histone acetylation and methylation and up-regulated X- and Y-linked gene expression. These findings give evidence that chromatin remodeling steps during spermiogenesis trigger poly(ADP-ribose) formation. Knockout mice deficient in PARP1, PARG (110-kDa isoform), or both display morphological and functional sperm abnormalities that are dependent on the individual genotypes, including residual DNA strand breaks associated with varying degrees of subfertility. The data presented highlighted the importance of PAR metabolism, particularly PARG function, as a prerequisite of proper sperm chromatin quality. PARG is involved in DNA repair by regulating the amount of PAR synthesized in response to DNA damage since excessive accumulation of PAR may result in cell death (Meyer-Ficca, 2009).

In vivo evidence showed that Parp-2^{-/-} spermatids are severely compromised in differentiation and exhibit a marked delay in nuclear elongation (Dantzer et al., 2006).

Altogether, in addition to its well known role in DNA repair, Parp-2 exerts essential functions during meiosis I and haploid gamete differentiation (Dantzer et al., 2006).

The activity of PARP during chromatin remodeling steps of spermatogenesis in terms of repairing double stranded breaks and the poly (ADP-ribosyl)ation of histones, is critical and dysregulation of the chromatin remodeling steps of spermiogenesis could have serious consequences for the male gamete. Meyer-Ficca et al (2005) demonstrated the presence of poly (ADP-ribose) in elongated spermatids of rat. They showed that during these steps when a high number of DNA breaks occur directly preceding nuclear condensation, there is correspondingly a higher amount of PAR in rat germ cells. Greater PAR formation through PARP1 and PARP2 action occurs during this phase of spermatogenesis that includes a great deal of chromatin condensation (steps 11-14 of rat spermatogenesis); PAR levels decrease only when protamines appear in the chromatin (Meyer-Ficca et al., 2010). Thus, PAR formation could be important for repairing DNA strand breaks during these crucial chromatin remodeling steps of spermatogenesis. Furthermore, PAR formation could also be important for histone modification because not only is there auto-modification of PARP during spermatogenesis, but much of PARP activity is targeted towards the testes-specific histone, H1t (Agarwal et al., 2009; Malanga et al., 1998).

The presence of poly (ADP-ribose) polymerase and its homologues has been shown specifically during stage VII of human spermatogenesis. High PARP expression has been reported in mature spermatozoa of proven fertile men (Agarwal et al., 2009).

In a recent study, using human testicular samples, the strongest levels of PARP1 were found in spermatogonia. Presence of poly (ADP-ribose) differed slightly with the stage of spermatogenesis. Poly (ADP-ribosyl)ation was strongest in human round and elongating spermatids as well as in a subpopulation of primary spermatocytes. In contrast, mature spermatids had no PARP expression or poly (ADP-ribosyl)ation (Agarwal et al., 2009).

Origin of DNA damage in human spermatozoa can occur by abortive apoptosis, abnormal chromatin packaging, generation of reactive oxygen species, hormone stimuli, all events involving in some way PARP and its reaction (Godman, et al., 2009; Maymon et al., 2006)

In human testis, an increase in DNA strand breaks occurs in 100% of elongating spermatids becoming critical for human fertility (Agarwal et al., 2009). Focus on genomic integrity of the male gametes has increased to relate DNA integrity in mature ejaculated spermatozoa and male infertility with a growing concern about the role of PARP as a DNA damage repair protein (Agarwal et al., 2009).

4.3 PARP and epigenetic state in spermiogenesis

It is widely recognized that environmental factors may influence the epigenetic state and that these epigenetic modifications may be inherited through the male germ line and passed onto more than one generation (Godman et al., 2009).

Since genomic stability of cells is linked to their poly(ADP-ribosyl)ation capacity, the patterns of poly(ADP-ribosyl)ation during human spermatogenesis were studied (Maymon et al., 2006). By testicular biopsy immunohistochemistry evaluation of PARP-1 expression and of poly(ADP-ribose), the detection of PAR expression in germ-line cells and its subcellular localization in meiotic and postmeiotic prophase were demonstrated to link with chromatin modifications occurring during spermatogenesis and confirmed a key role for poly(ADP-ribosyl)ation in germ cell differentiation, to preserve DNA integrity.

Deduction of a mechanism in male transmission is difficult because of the specialized nature of the sperm cell, which requires very compact chromatin to enable transport and protect DNA against oxidative stress (Aitken & De Iuliis, 2009).

In a recent study by Jha et al. (2009) several isoforms of PARP were detected in ejaculated spermatozoa including PARP1, PARP2, and PARP9. Immunolocalization patterns showed that PARP was found near the acrosomal regions in sperm heads. Furthermore, a direct correlation was seen between sperm maturity and the presence of PARP, i.e., an increased presence of PARP1, PARP2, and PARP9 was seen in mature sperm when compared to immature sperm.

Inside seminiferous tubules hyperactivation of PARP and its cleavage accompany the morphological and functional changes induced by apoptotic stimuli (hormonal, oxidative, chemical, etc.) (Atorino et al., 2001; Boissonault et al., 2002; Faraone Mennella et al., 2009; Sinha-Hikkim et al., 2003). In human sperm, in the presence of a PARP inhibitor, 3-aminobenzamide, chemical and oxidative stress-induced apoptosis was reported to increase by nearly two-fold (Argawal et al., 2009). This novel finding suggests that PARP could play an important role in protecting spermatozoa subjected to oxidative and chemical damage (Argawal et al., 2009).

An age-related increase in DNA break repair and apoptosis was also demonstrated in human testicular germ cells. DNA repair markers (PARP-1, PAR, XRCC1, and apoptosis-associated markers (caspase 9, active caspase 3, and cleaved PARP-1) were detected in these cells (El-Domyati et al., 2009).

In summary, DNA damage in spermatozoa can be induced by events involving PARP as regulatory factor and occurring within the testis as apoptosis, and remodelling of sperm chromatin during the process of spermiogenesis, or in the post-testicular phase as induced mainly by radical oxygen species (ROS) and nitric oxide (NO), or by endogenous caspases and endonucleases; or by environmental factors.

To the latter refers epigenetic transmission of information from one generation to the next during chromatin replication in combination with posttranslational histone modification (the histone code) as demonstrated in parental imprinting (de Boer et al., 2010).

An epigenetic memory for male genetic transmission resides in a link between DNA replication and matrix associated DNA repair (Hatch et al., 2007).

Some authors propose that regulation of chromosome structure in the germline, by the occupancy of matrix/scaffold associated regions, contains molecular memory function. Nuclear structure, chromatin composition and loop domain organization are aspects of human sperm variability that in many cases of assisted reproduction is increased due to inclusion of more incompletely differentiated/matured sperm nuclei.

The association of DNA repair proteins with the nuclear matrix has been demonstrated by a number of authors, as well as topoisomerase II species constitute a significant component of the nuclear matrix (Roca & Mezquita, 1989; Quesada et al., 2000).

For adaptation of loop domain structure during chromatin remodeling at spermatid nuclear elongation, the activity of TopoIIB is essential for removing supercoiling from nucleosomal DNA in transit to protamine toroid chromatin (Leduc et al., 2008 a,b). In rat testes there is evidence that some of PARP activity is associated to nuclear matrix, thus becoming one of those tightly bound components which are not solubilized from chromatin by high salt treatment. By the use of DNA and protein cross-linking reactions, more evidences were provided about the association of PARP-1, PARP-2, and PARPs related proteins with the nuclear matrix. These findings confirmed that nuclear matrix could be seen as a fraction greatly enriched in transcription factors (i.e., C/EBP-beta) and enzymes (DNA Topo II, DNA PK) that co-localize with PARP-1 and -2 at the matrix associated regions (MARs) of chromatin. Moreover, PAR contributes to PARP-1 localization at the nuclear matrix,

showing that PARP(s) activity co-operates to the functions of this nuclear fraction. (Tramontano et al., 2005). Topo II is constantly present as a component of chromatin remodelling. As described in the previous section, alterations in DNA topology that occur in the extreme condensation of the spermatid nucleus have been shown to require the controlled formation of DNA strand breaks to allow the transition from a supercoiled form of DNA to a non-supercoiled form. Supercoiled DNA relaxes by transient formation of physiological strand breaks that spermatids, being haploid, cannot repair by homologous recombination. These DNA strand breaks trigger the activation of poly(ADP-ribose) polymerases PARP1 and PARP2 and any interference with PARP activation causes poor chromatin integrity with abnormal retention of histones in mature sperm and impaired embryonic survival (Meyer-Ficca et al., 2011). In this context, the activity of topoisomerase IIbeta (TOP2B), an enzyme involved in DNA strand break formation in elongating spermatids, is strongly inhibited by the activity of PARP1 and PARP2 in vitro and is restored by the PAR degrading activity of PAR glycohydrolase (PARG). Moreover, genetic and pharmacological PARP inhibition both led to increased TOP2B activity in murine spermatids in vivo, measured as covalent binding of TOP2B to the DNA (Meyer-Ficca et al., 2011). These data suggest a functional relationship between the DNA strand break generating activity of TOP2B and the DNA strand break-dependent activation of PARP enzymes which in turn inhibits TOP2B. Because PARP activity also facilitates histone H1 linker removal and local chromatin decondensation, cycles of PAR formation and degradation may be necessary to coordinate TOP2B dependent DNA relaxation with histone-to-protamine exchange necessary for spermatid chromatin remodelling (Meyer-Ficca et al., 2011). In the light of their own results and those from other groups, Meyer-Ficca et al. (2011) suggest that the activities of the DNA relaxing enzyme TOP2B, and the DNA strand break dependent enzymes PARP1 and PARP2 may be able to directly and dynamically regulate each other via the formation of DNA strand breaks and poly(ADP-ribose) to mediate simultaneous DNA relaxation and histone H1 removal as essential steps of spermatid chromatin remodeling necessary for sperm function (Meyer-Ficca, 2011).

5. Conclusions

In this review the possible biological significance of PARP in mammalian germ cells has been summarized focusing on the role played by PARP during spermatogenesis and sperm maturation, and on recent findings in ejaculated spermatozoa. It is widely demonstrated that molecular events leading to the high condensation of the spermatid nucleus, include an exchange of histones to transition proteins and then to protamines, that replace all other nuclear proteins in sperms, and alterations in DNA topology that require both the controlled formation of DNA strand breaks, and protein modifications, such as poly(ADP-ribosyl)ation. As discussed above, the sequential synthesis and replacement of histones and testis specific proteins with protamines found indeed in the poly(ADP-ribosyl)ation reaction a further regulatory process to control and to produce large number of spermatozoa with intact and competent DNA. Poly(ADP-ribosyl)ation involves the automodified PARP as the main actor in DNA protecting function, and the free enzyme as regulator of most of nuclear proteins demonstrated to be involved in chromatin remodelling, either as modifier enzyme or as a recruiter of partner proteins. PARP is activated whenever there are strand breaks in sperm DNA due to oxidative stress, chromatin remodeling or cell death. The fact that PARP and PAR localize at MARs, recognized as a site of “memory” for transmission of information from one generation to the next, gives further support to the role(s) of PARP in

essential steps of germ cell development. Male germ cells are exposed to a wide variety of endogenous and exogenous genotoxic agents, most of which involve PARP as a common player. Recent findings confirm the occurrence of PARP in ejaculated spermatozoa and the presence of higher levels of caspase 3-cleaved PARP in sperm of infertile men adds a new proof for the correlation between apoptosis and male infertility. In the light of these observations PARP can be regarded as an hallmark of the actual state of germ cells able either to counteract DNA damage or to give a signal of death upon high DNA abnormalities.

6. References

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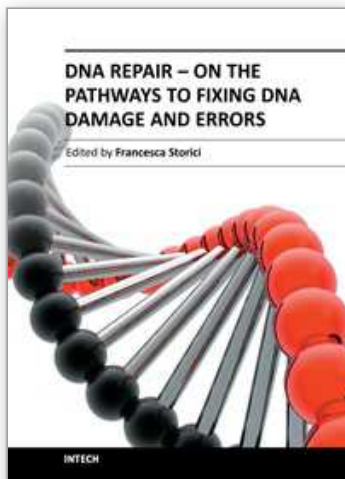
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DNA repair is fundamental to all cell types to maintain genomic stability. A collection of cutting-edge reviews, DNA Repair - On the pathways to fixing DNA damage and errors covers major aspects of the DNA repair processes in a large variety of organisms, emphasizing foremost developments, questions to be solved and new directions in this rapidly evolving area of modern biology. Written by researchers at the vanguard of the DNA repair field, the chapters highlight the importance of the DNA repair mechanisms and their linkage to DNA replication, cell-cycle progression and DNA recombination. Major topics include: base excision repair, nucleotide excision repair, mismatch repair, double-strand break repair, with focus on specific inhibitors and key players of DNA repair such as nucleases, ubiquitin-proteasome enzymes, poly ADP-ribose polymerase and factors relevant for DNA repair in mitochondria and embryonic stem cells. This book is a journey into the cosmos of DNA repair and its frontiers.

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