REPRODUCTION
POINT OF VIEW

Does single-strand DNA break repair capacity influence oocyte maintenance and quality?

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Abstract

Human genome-wide association studies and evidence from animal models link ovarian ageing to double-strand (ds)DNA break repair capacity. Is there a connection between single-strand (ss)DNA repair mechanisms and ovarian function? We hypothesize that endogenous cellular processes subject oocytes to ssDNA lesions, and thus, ssDNA repair capacity is fundamental to their survival and maintenance.

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Introduction

Female mammals are born with a finite supply of oocytes for their entire lifetime. Immature oocytes are maintained in structures called primordial follicles in the ovary and are non-dividing, long-lived, and the storage unit of the female germline (Findlay *et al.* 2015). The prevailing consensus is that primordial follicles are not replenished after birth if the initial reserve is depleted; therefore, their DNA integrity must be preserved to safeguard oocyte quality and number and ensure fertility and offspring health.

The loss of oocyte number and quality can be caused by exposure to exogenous factors such as air pollutants, heavy metals, herbicides and pesticides, carcinogens/mutagens, and anti-cancer therapies. Indeed, primordial follicles appear to have a particularly low tolerance for DNA damage, as those that have sustained damage are rapidly identified and removed by apoptosis. This is likely a mechanism to prevent the transmission of genetic mutations to offspring. Thus, oocyte DNA damage leads to a decline in the number of available oocytes. On the other hand, in 2012, Kerr *et al.* demonstrated that when the apoptotic response is experimentally blocked in mice, oocytes that had sustained severe DNA damage could give rise to healthy offspring (Kerr *et al.* 2012). This provided some of the first evidence to suggest that oocytes can be safeguarded by the rapid detection and repair of DNA damage. Now, detailed information on the mechanisms and efficacy of DNA repair in prophase-arrested oocytes has emerged. Recent studies from our lab discovered that when double-strand (ds)DNA breaks are introduced by exogenous insults, but apoptosis is blocked, primordial follicle oocytes are capable of efficient dsDNA break repair, via the homologous recombination (HR) repair pathway (Stringer *et al.* 2020). However, less is understood about the mechanisms required for ensuring oocyte genome integrity under physiological conditions.

DNA lesions occur in living cells daily and can be generated by endogenous alkylating agents, hydrolytic deamination, non-enzymatic methylations, and oxidation. Notably, sperm are prone to mitochondrial and nuclear DNA damage induced by excessive ROS produced during spermatogenesis. Sperm, however, are incapable of repairing DNA damage in contrast to oocytes. This characteristic highlights the importance of maternal DNA repair factors, not only for maintaining oocyte number and quality but also in zygotes post-fertilization. Furthermore, despite being arrested during the diplotene stage of the first meiotic prophase over a long period (years or even decades in some species), primordial follicle oocytes remain metabolically active and, consequently, are sensitive to the accumulation of DNA damage. Based on this, we hypothesize that endogenous cellular processes can subject primordial follicle oocytes to single-strand (ss)DNA lesions and that ssDNA break repair mechanisms are thus critical in the maintenance and survival of female gametes.

Single-strand DNA break repair mechanisms: base-excision repair pathway

The key pathway responsible for ssDNA repair is the DNA base-excision repair (BER) pathway. BER proteins, including APE1, PARP1, XRCC1, Polβ (POLB), and LIG3, are highly conserved among mammals (Fig. 1). They

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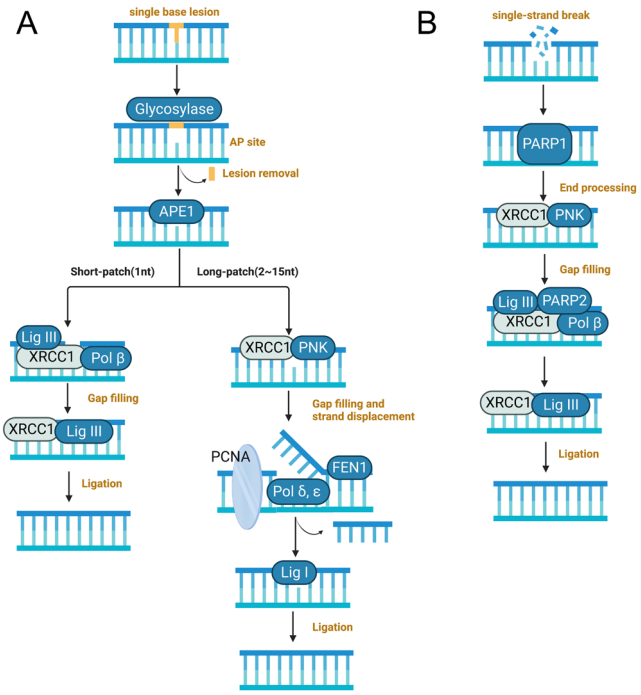

Figure 1 (A) In mammals, the base excision repair (BER) process involves the excision of the abasic site by DNA glycosylase and incision of phosphodiester backbone by AP endonuclease 1 (APE1). The ssDNA break intermediate with terminal sugar phosphate is removed by DNA polymerase beta and further replaces missing nucleotide in a short-patch-BER (SP-BER) process. The nick is ligated by DNA ligase 1 (LIG1) or ligase 3 (LIG3). A backup repair pathway termed long patch-BER (LP-BER) is employed to incorporate more than one nucleotide or when SP-BER fails to remove 5'-abasic sugar. This process uses extended DNA synthesis to form a 5' single-strand flap intermediate of up to 20 nucleotides, removed by flap endonuclease 1 (FEN1) and involves several other factors such as proliferating cell nuclear antigen (PCNA), replication factor C (RFC), Polβ and delta, and LIG1. (B) A specialized sub-pathway of BER is single-strand break repair consists of break detection by PARP1 and recruitment of scaffold protein XRCC1 and its downstream effectors Polβ, LIG3, and PARP2 for gap filling and ligation. The localization and function of these repair factors are not completely understood in the ovary, although we propose they likely play a fundamental role in the maintenance of prophase-arrested oocyte genome integrity.

are critical for restoring small lesions in the individual DNA strands, such as uracil incorporation, oxidized or reduced bases, non-bulky adducts, and ssDNA breaks derived from endogenous oxidative and hydrolytic decay (Abbotts & Wilson III 2017). A meta-analysis of GWAS for single nucleotide polymorphisms (SNPs) from 70,000 women at the age of natural menopause identified a link between BER repair genes and ovarian ageing (Day *et al.* 2015).

The chief regulators of BER are poly (ADP-ribose) polymerase-1 (PARP1) and x-ray cross-complementing protein 1 (XRCC1), as they are activated first upon exposure to oxidative stress. Upon DNA damage, PARP1 rapidly localizes to chromatin, catalyses PARylation of nuclear proteins, and relaxes chromatin structure near DNA lesions, promoting access to signalling and repair enzymes. PARP1 forms the major nuclear enzyme responsible for PARylation activity following ssDNA or dsDNA breaks and indeed localize at the nuclei of oocytes throughout the follicular development. The DNA repair factors such as XRCC1, APE1, LIG3, and Polβ, which play a central role in the BER pathway, also possibly act in concert with PARPs, although this remains to be investigated.

What is the evidence for BER function in oocytes?

Parp1−/− mice are fertile with an average lifespan; however, they show increased chromosomal instability, sister chromatid exchanges, and telomere shortening. PARP2 is also activated in response to DNA damage along with PARP1 and is required for ssDNA break repair. Interestingly, *Parp2*−/− mice exhibit severely impaired spermatogenesis. *Parp1*−/− and *Parp2*−/− double mutant mice die at the onset of gastrulation, suggesting the importance of both PARPs during early embryogenesis. A recent study in mice from our lab showed that inhibition of both PARP1 and 2 led to the depletion of the ovarian reserve (Winship *et al.* 2020). The findings from this study support our hypothesis by pointing to a possible role for ssDNA break repair in safeguarding oocytes from endogenous DNA damage that occurs as a consequence of normal cellular metabolism. Furthermore, the ssDNA breaks, when left unrepaired, eventually form dsDNA breaks, which are highly detrimental to oocytes. Therefore, it has been discovered that PARPs contribute to the repair of both ssDNA break and dsDNA break.

In addition to PARP1, damage-induced PARylation co-localizes with XRCC1, a molecular scaffold protein that facilitates and assembles other DNA repair factors at the damage site. In male primordial germ cells, it has only recently been established in *in vivo* studies that the BER pathway and XRCC1, in particular, are chiefly responsible for maintaining genomic integrity and stability. Similar to PARP, XRCC1 deficiency leads to impaired spermatogenesis and male infertility. Indeed *Xrcc1*−/− mice exhibited smaller-sized testes, low sperm count, and reduced motility (Xu *et al.* 2019). Furthermore, recent findings demonstrate the importance of XRCC1 during zygotic reprogramming after fertilization. Interestingly, mice with a heterozygous deletion of XRCC1 are fertile with an average life span, in contrast to embryonic lethality observed in mice with homozygous loss of XRCC1, suggesting that haploinsufficiency provides sufficient protein to maintain genomic stability. XRCC1-interacting partners, Polβ and LIG3, are recruited to the site of DNA damage by XRCC1 and are shown to prevent excessive engagement and activity of PARP1 during BER, which is attributed to PARP1 trapping at

the DNA break lesion site, causing the impediment to repair. The sensitivity of XRCC1 deficient cells to alkylating agent methyl methane sulfonate (MMS) was restored upon PARP1 deletion, illustrating the importance of XRCC1 in the BER process. So far, XRCC1 has been identified in Xenopus egg extracts (Cupello *et al.* 2019), but expression studies have not yet been undertaken in mammalian female germ cells. Evidence from the literature points to a fundamental role for XRCC1 in oocyte genome stability, but this remains to be determined.

In a recent study, the XRCC1-binding partner, Polβ, which is essential to maintain the ligation efficiency of the XRCC1–-LIG3 complex during BER, exhibited decreased expression within ageing mouse oocytes. Heterozygous Polβ expression in mice led to oocyte attrition and depletion of the ovarian reserve, increased detectable 8-oxoG levels, and increased sensitivity to alkylating agents. Moreover, overexpression of Polβ in aged murine oocytes by microinjection of Polβ cDNA increased oocyte survival, supporting the importance of Polβ function for oocyte maintenance (Hua *et al.* 2020). Although evidence from the literature points to a fundamental role for XRCC1 and Polβ in oocyte genome stability, their function in primordial follicle oocytes remains to be determined.

BER functions in other non-renewable cell types: implications for oocytes?

Notably, DNA repair was found to be efficient in yet another non-renewable, terminally differentiated cell type in the human body apart from germ cells – neurons. Ageing-associated ROS lesions constantly challenge the neuronal cell's genome integrity and thus initiate neurodegenerative disorders (Singh *et al.* 2019). Hence, neurons must be protected by tightly regulated DNA repair mechanisms. Indeed, BER is of significant importance for maintaining the genomic integrity in neurons. BER pathway protein, PARP1, is important for protection against neurodegenerative diseases and cellular ageing and mice with XRCC1-defective neurons exhibit dysfunctional presynaptic calcium signalling, cerebellar ataxia, and fatal seizures. These studies provide evidence for the crucial role of BER pathway proteins in terminally differentiated cell types, as well as diseases associated with ageing.

Conclusions

Based on the available evidence, an efficient DNA repair system is indispensable for policing mammalian oogenesis and folliculogenesis so that meiotically and developmentally competent oocytes can develop. Many studies have focused on the dsDNA capacity in the context of genome stability in female gametes. In this point of view, we propose that ssDNA break repair is also essential for optimal ovarian function, especially during ageing. The precise mediators of ssDNA repair in the survival and maintenance of primordial follicle oocytes remain unknown and thus warrant further study. Unravelling the mechanisms of the ssDNA break repair proteins in the DNA damage response will promote our understanding of how the diplotene stage-arrested oocytes cope with the DNA lesions built up in them with ageing. This knowledge may lead to new insights into the development of the targeted intervention in increasing oocyte survival and female fertility.

Declaration of interest

Karla J Hutt is on the editorial board of *Reproduction*. Karla was not involved in the review or editorial process for this paper, on which she is listed as an author. The other authors declare no competing financial, or other interests.

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Author contribution statement

All authors wrote and edited the manuscript and contributed equally.

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