



The capacity of oocytes for DNA repair

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Abstract

Female fertility and offspring health are critically dependent on the maintenance of an adequate supply of high-quality oocytes. Like somatic cells, oocytes are subject to a variety of different types of DNA damage arising from endogenous cellular processes and exposure to exogenous genotoxic stressors. While the repair of intentionally induced DNA double strand breaks in gametes during meiotic recombination is well characterised, less is known about the ability of oocytes to repair pathological DNA damage and the relative contribution of DNA repair to oocyte quality is not well defined. This review will discuss emerging data suggesting that oocytes are in fact capable of efficient DNA repair and that DNA repair may be an important mechanism for ensuring female fertility, as well as the transmission of high-quality genetic material to subsequent generations.

Keywords Ovary · Primordial follicles · Folliculogenesis · Base excision repair · Mismatch repair · Nucleotide excision repair · Homologous recombination · Non-homologous end joining · Detection and response

Introduction

DNA is under constant assault by endogenous and exogenous agents that damage and alter the chemical structure and genetic sequence. It is predicted that every cell in the human body receives tens of thousands of DNA lesions per day, including thousands generated by endogenous alkylating agents, hydrolytic deamination, non-enzymatic methylations and oxidation [1–3]. Similarly, many chemicals and agents present in the environment (such as in food, air or water) have carcinogenic or mutagenic properties. These exogenous and endogenous reactions can lead to various types of lesions on the nucleotide heterocyclic base or phosphate backbone and can turn a legitimate base into either a mutagenic, miscoding or noncoding lesion and can even change one base into another [1, 2, 4, 5]. These adducts can impair base pairing, block DNA replication and result in nucleotide loss and DNA single-strand breaks (SSBs). Furthermore, when two SSBs arise in close proximity, or when

the DNA-replication apparatus encounters a SSB, double-strand breaks (DSBs) can form. Therefore, to ensure viability, cells have evolved multiple systems to detect and repair these different types of DNA damage.

Females are born with a finite supply of oocytes and it is imperative that the health of these oocytes is maintained throughout reproductive life to ensure that only the “best” possible oocytes are available for ovulation, fertilisation and subsequent embryonic development [6]. One mechanism by which oocyte number and quality may be safeguarded is the implementation of strategies to rapidly detect and repair DNA damage sustained as a consequence of normal metabolic activities, or through exposure to exogenous factors. Indeed, the ability of a cell to repair DNA damage is vital to the integrity of its genome and thus, to its normal functionality. It is particularly important for gametes to correct damage to their DNA, to avoid apoptosis and prevent the transmission of genetic mutations to offspring [6]. Surprisingly, however, detailed information on the ability of oocytes to undertake efficient DNA repair, and the contribution of DNA repair to oocyte quality, is only recently emerging as an essential area of research. This review will give an overview of the DNA repair pathways available to oocytes and discuss evidence from various animal models indicating that the capacity for DNA repair is vital for female fertility.

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The female germline is stored in structures called primordial follicles

The female germline is stored in the ovary in the form of primordial follicles, which are structures comprising an immature oocyte, surrounded by a single layer of supporting somatic cells, known as granulosa cells (Fig. 1) [7]. The enclosed oocytes are non-growing, non-proliferative and arrested part way through the first meiotic prophase at the diplotene stage. They may remain in this growth and meiotic arrest for several months (mouse) or up to 50 years (human) before being activated to begin the process of folliculogenesis, which is characterised by dramatic growth of the oocyte and resumption of meiosis, as well as the extensive proliferation of the granulosa cells (Fig. 1) [8]. The coordinated development of the oocytes and granulosa cells during folliculogenesis ultimately results in the ovulation of a meiotically mature and developmentally

competent oocyte (often referred to as MII stage), which can be fertilized and is able to support embryonic development (Fig. 1) [9, 10]. From birth onwards, the number of primordial follicles found in the ovary steadily declines until the pool becomes exhausted, leading to the end of fertility, followed by the beginning of menopause. Importantly, the entire complement of gametes available to the female is formed during fetal (human) or perinatal (mouse) development and new oocytes cannot be made after this point, even if the supply is prematurely depleted [11]. Thus, repair of those that are damaged is likely an important mechanism for ensuring that adequate numbers of primordial follicles are maintained in the ovary to support fertility and perpetuation of the species.

In comparison to somatic cells, oocytes in primordial follicles exhibit a number of unique properties that should be considered when evaluating their ability to undertake DNA repair: (1) they are meiotically arrested, with a diploid nucleus containing four copies of each chromosome ($2n$,

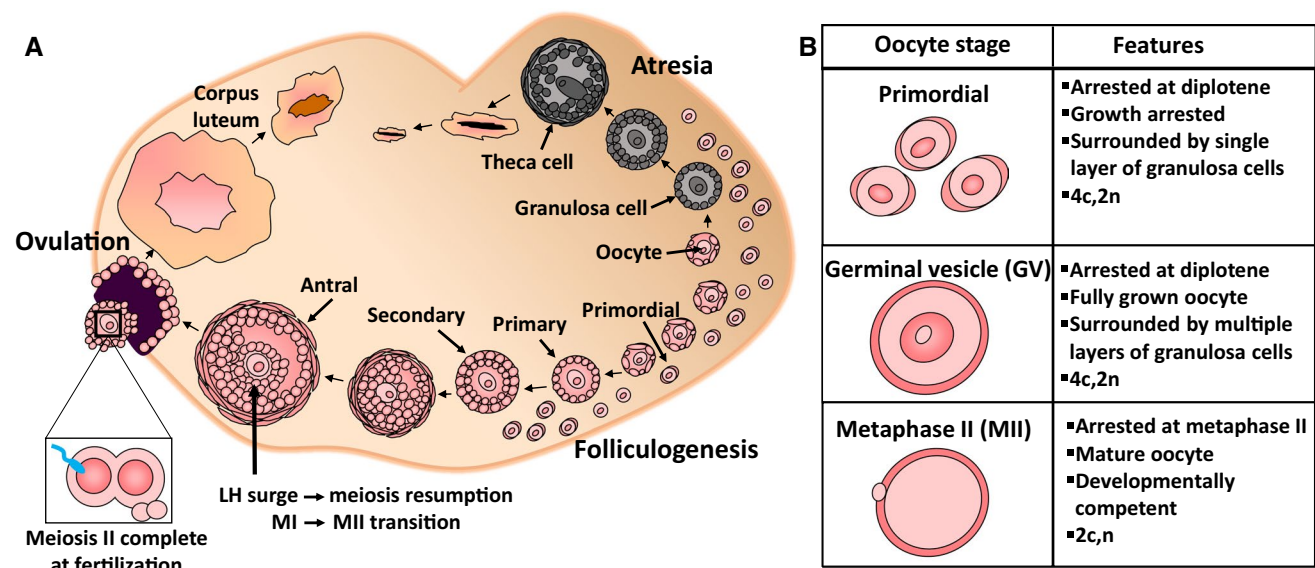


Fig. 1 Schematic representation of folliculogenesis in the mammalian ovary. **a** Follicles form the functional unit of the ovary and are comprised of the central germ cell (oocyte), surrounded by specialized somatic granulosa cells, as well as a theca cell layer, surrounding mature growing follicles only. A finite supply of non-growing, meiotically arrested primordial follicle oocytes form the ovarian reserve and once lost from the follicle pool, these cells cannot be replenished. From birth onwards, a limited number of primordial follicles are periodically activated to undergo folliculogenesis; a process in which primordial follicles mature through primary, secondary, and antral stages under the influence of follicle-stimulating hormone (FSH) which is produced at puberty. From the growing follicle pool in women, one dominant follicle is ovulated under the influence of luteinizing hormone (LH), leaving behind remnants to form the corpus luteum, required to hormonally support a potential conceptus. The remaining growing follicles undergo atresia, a hormonally regulated apoptotic process, and this is the fate of the majority of follicles. This cyclical

pattern continues until the pool of primordial follicles drops below a critical threshold and menopause ensues. **b** Schematic representation of different oocyte developmental stages. Primordial follicle oocytes are the most abundant form of oocyte in the ovary and are meiotically arrested at diplotene. They are non-dividing, so they can remain arrested in the ovary for months in mice, or decades in humans. They are distinguished morphologically by a surrounding single layer of squamous granulosa cells. Primordial follicle oocytes contain four chromatids (c) and are diploid ($2n$). Germinal vesicle (GV) oocytes remain meiotically arrested at diplotene, however, represent a fully grown oocyte. GV oocytes are $4c, 2n$. Germinal vesicle breakdown indicates that meiosis has resumed and the extrusion of the first polar body represents completion of meiosis I in humans. The oocyte is then arrested at metaphase II (MII), at which stage it is developmentally competent and haploid ($2c, n$), but will only complete meiosis if fertilization occurs

4c), (2) their condensed chromosomes have relaxed slightly to allow some transcription, but homologs remain in close association with each other, (3) they are non-growing but metabolically active and (4) they are amongst the most long-lived cells in the body and remain in this unusual stasis for many months or decades, depending on the species. During this time, they may be subjected to ongoing exogenous and endogenous stressors. Notably, oocytes in primordial follicles appear to be more susceptible to DNA-damaging agents compared to surrounding somatic cells and growing oocytes, and have a greater tendency to undergo apoptosis [12, 13]. Recent work described below provides compelling evidence that this is likely to be due to the evolution of a highly sensitive apoptotic response, rather than lack of DNA repair capacity.

Expression profiling indicates that oocytes should be well-equipped to repair DNA damage

Early studies demonstrated that damaged DNA is efficiently repaired when transferred to mammalian MII oocytes and zygotes, or *Xenopus* oocyte extracts, showing that factors accumulated in the mature oocyte are vital for maintaining genome integrity [14–18]. Since that time, advances in methods for RNA amplification, and the development of sequencing and microarray platforms have enabled the transcriptomic analysis of DNA repair factors in oocytes and preimplantation embryos. Several studies have used microarrays to profile RNA expression in germinal vesical (GV) oocytes, MII stage oocytes and embryos in mice [19–21] and humans [22–24]. Interestingly, components from all DNA repair pathways including direct lesion reversal, base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), homologous recombination (HR) and non-homologous end-joining repair (NHEJ) are represented in mouse, monkey and human MII oocytes and embryos [23–26]. At least two groups have successfully performed global gene expression studies using mouse oocytes from earlier stages of folliculogenesis, including primordial, primary, secondary, small antral and large antral follicles [27–29]. The result of these works indicate that there is an over-representation of transcripts involved in DNA repair observed throughout oocyte development, with at least 80% of the genes commonly detected between all developmental stages [27]. Proteomic analysis using liquid chromatography–mass spectrometry (LC–MS/MS) of 7000 GV, MII, and zygotes identified a total of 53 proteins involved in the DNA repair in both MII oocytes and zygotes [30]. Of these proteins, 35 were upregulated in the MII oocyte and included single-strand break repair and nucleotide-excision repair proteins [30]. Combined, these studies provide strong

evidence that oocytes from primordial follicles through to MII have the capacity to repair damaged DNA and maintain genome integrity (Table 1). Interestingly, a recent RNA-seq analysis suggested that there may be species differences in the ability of GV and MII oocytes to undertake DNA repair [31]. In this study, the overall expression patterns of genes involved in the repair of DNA double strand breaks were different between primates and mouse. Additionally, following *in vitro* exposure to etoposide, RAD51 (a key player in the homologous recombination repair pathway) was expressed and recruited to double strand break sites at much lower levels in primate oocytes than in mouse oocytes. Based on these data, it was proposed that rodent oocytes have a superior DNA repair competence to that of primates [31]. Of note, however, the rhesus monkeys used in these analyses were aged between 7 and 14 years, and females begin the process of maternal ageing as early as 10 years in this species [32]. Maternal ageing is associated with a decline in the expression of repair factors, including RAD51, and reduced DNA repair capacity in oocytes [29, 33–35], and it is not clear if maternal age was adequately controlled between species in this study. Thus, whether true inter-species differences in DNA repair capacity exist in oocyte requires further exploration.

DNA damage and repair mechanisms

Specific repair pathways have evolved to correct the many different types of DNA damage that can occur. These pathways employ a suite of different enzymes, including topoisomerases, nucleases, ligases, phosphatases, polymerases and helicases that work in a lesion-dependent manner, although some proteins may have important roles in more than one pathway (Fig. 2). Major lesions and repair mechanisms are described below, with relevance to oocytes and pathological consequence for fertility discussed where the data are available.

Alkylation of DNA, direct lesion reversal and base excision repair (BER)

Alkylating agents are reactive chemicals that transfer alkyl carbon groups (adducts) onto a broad range of biological molecules, including DNA, consequently altering their structure and function. Eukaryotic cells harbor endogenous alkylating agents and metabolites, including MGT1 O6-methylguanine DNA methyltransferase, MAG 3-methyladenine DNA glycosylase, and APN1 apurinic/apyrimidinic (AP) endonuclease that alkylate nuclear DNA at both oxygens and nitrogens [36]. Reactive species such as free radicals and oxidants are formed inside living cells during normal metabolic activities and can also react with different

Table 1 DNA repair pathways identified in oocytes

Repair mechanism	Type of damage	Common causes	Key components	Pathway overview	Oocyte function
Direct lesion reversal	Small base adducts	Alkylating agents	MGMT, ALKBH	[4]	Unknown
Mismatch repair (MMR)	DNA mismatches and insertion/deletion loops	DNA replication and recombination	MSH2-6, PMS1 and 2, PMS2L3 and 4 and MLH1 and 3	[179]	[18]
Base excision repair (BER)	Small DNA lesions, such as oxidized or reduced bases, non-bulky adducts and DNA single-strand breaks	Endogenous oxidative and hydrolytic decay	DNA glycosylases initiate this process by releasing the modified base. The DNA repair enzymes X-ray repair cross-complementing group 1 (XRCC1), 8-Oxoguanine DNA glycosylase (OGG1) and apurinic/apyrimidinic endonuclease (APE1) play a central role in the BER pathway	[180]	[17, 63–65]
Nucleotide excision repair (NER)	Repairs bulky DNA adducts, cross-links and oxidative damages	Environmental agents including UV radiation	XPA, XPC and XPD	[97]	[15, 16]
Trans-lesion bypass mechanisms	Base damage and gaps blocking replication forks	Unrepaired DNA damage	Mutagenic DNA polymerase such as pol η (XPV) four specialized Y-family DNA polymerases and six additional low-fidelity polymerases	[181, 182]	[117]
Non-homologous end-joining (NHEJ)	Double strand breaks (DSBs)	Ionizing radiation (IR) or chemically induced	Ku70/80, DNA-PKcs, end-processing enzymes, polymerases and DNA ligase IV	[183]	[139, 184]
Homologous recombination (HR)	DSB, stalled replication forks and interstrand DNA crosslinks	Meiotic recombination, ionizing radiation (IR)	MRN complex, BRAC2, RAD51	[185]	[31, 131, 141]
Fanconi anaemia (FANC) pathway	DNA interstrand crosslinks (ICLs)	Mitomycin C (MMC) or diepoxybutane (DEB) UV radiation, IR, hydroxyurea, replication	FA core complex (FANCA-G, FANCL, FANCM) FAAP24 and FAAP100, FANCD1/BRCA2, FANCD2, FANCF/BRIP1/BACH1, FANCG and FANCI	[108, 109, 186]	Unknown

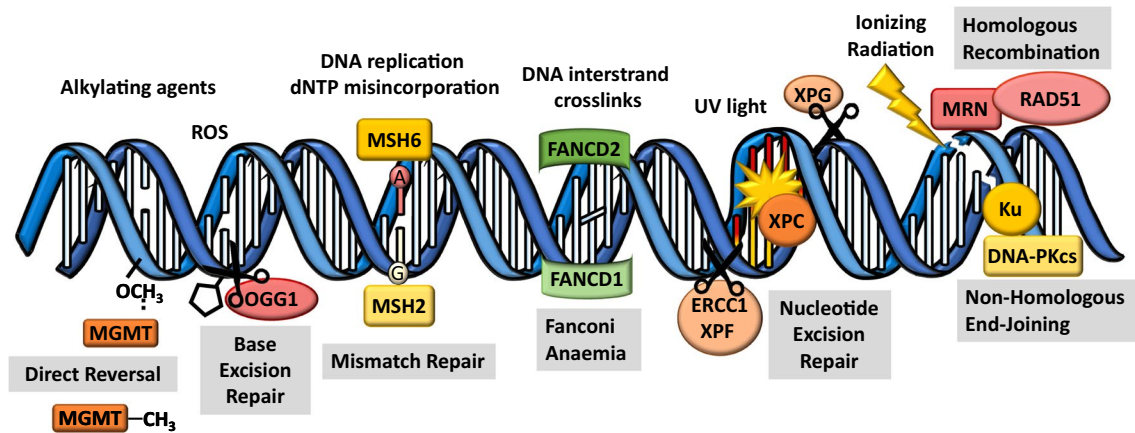


Fig. 2 Types of DNA damage and repair mechanisms. Alkylating agents can transfer alkyl carbon groups (CH_3) onto DNA and can lead to base-mismatched pairing. Some alkylated bases can be repaired by direct lesion reversal using dioxygenase enzymes e.g., MGMT. Other agents can cause more complex adducts such as reactive oxygen species (ROS) which causes 8-oxoguanine DNA lesions. The base excision repair pathway, via DNA glycosylases (e.g., OGG1), are responsible for replacing a single damaged nucleotide that cannot be repaired by direct reversal. Spontaneous DNA alterations can arise during DNA replication due to dNTP misincorporation, which are primarily repaired by the mismatch repair (MMR) pathway (e.g., MSH2–MSH6 complex initiates the repair of small nucleotide mismatches). DNA interstrand crosslinks are very toxic covalent links within the double helix that prevent DNA unwinding and affect both strands of DNA. The Fanconi anaemia pathway works to coordinate several distinct repair activities belonging to different classic repair

pathways. Complex (CPLX) 1 is formed in response to DNA damage recruits FANCD2 which, via interactions with FANCD1, promotes loading of complex 2 that includes components from other repair pathways. When DNA is severely damaged (e.g., UV radiation) and large sections of DNA need to be replaced the nucleotide excision repair pathway is activated, XPC recognizes and binds the helix-distorting lesion and then recruits endonucleases XPG and ERCC1–XPF to excise the damaged DNA fragment and allow polymerase to resynthesize the strand. DNA double strand breaks are caused following exposure to exogenous stressors (e.g., ionizing radiation). Homologous recombination (HR) and non-homologous end joining (NHEJ) are the two main pathways responsible for DNA DSB repair. KU70/KU80 and MRN complex and are involved in the detection of double strand breaks and recruitment of various repair factors (e.g., RAD51 is essential for HR repair and DNA-PKcs is essential for NHEJ repair

components of DNA and modify bases [37]. Reactive oxygen species (ROS) are produced within growing follicles and are required for oocyte maturation, the resumption of meiosis and ovulation in rats [38, 39]. Although they play important roles within the ovary, the cyclic production of these DNA damaging agents may lead to an increased cumulative risk of ovarian pathology if ROS-induced DNA damage is not repaired. Indeed, increased ROS levels in follicular fluid have been associated with poor oocyte and embryo quality, low conception and pregnancy rate in women [40–42].

In the clinical setting, alkylating agents are commonly used as anti-cancer compounds and exposure to these compounds is associated with damage to oocyte DNA and depletion of the ovarian oocyte supply, leading to infertility and menopause in female cancer survivors [43–48]. Notably, in the case where female cancer patients are fertile after treatment with alkylators, studies have convincingly shown that there is no increase in birth defects or chromosomal abnormalities in their children [49, 50]. Such studies provide indirect evidence that the oocytes that survive exposure to these compounds are able to repair any damage they sustained.

Some adducts can be removed through direct lesion reversal by the AlkB homologue (ALKBH) family of dioxygenase enzymes that use an oxidative dealkylation reaction

or *O*6-alkylguanine-DNA alkyltransferase (MGMT), which serves as an alkyl transfer protein [4, 51–53] (Table 1; Fig. 2). In mouse and human oocytes and early embryos, MGMT and other similar enzymes are highly expressed at the transcript level [23, 24, 27], suggesting that oocytes may be able to repair these adducts via direct lesion reversal, but direct functional studies to confirm this have not been undertaken.

Other bulky adducts must be repaired via the base excision repair (BER) pathway (Table 1), which removes and replaces a single damaged nucleotide by targeting the damaged base (such as an alkylated base). The initiation of BER occurs by the recognition and excision of a damaged DNA base by a DNA glycosylase (Fig. 2). Different DNA glycosylases recognize and remove different kinds of damaged bases (e.g., uracil glycosylase, 3-methyladenine glycosylase and UV-endonucleases), and the specificity of the repair pathway is determined by the type of glycosylase involved [54, 55]. In humans, there are currently 11 known DNA glycosylases [56]. 8-Oxoguanine is one of the most common DNA lesions resulting from reactive oxygen species (ROS) and is primarily repaired by the DNA glycosylase, OGG1 (Fig. 2). DNA glycosylases function by cleaving the *N*-glycosidic bond between the

base and the backbone of the nucleotide residue, leaving an AP site, also known as an abasic site. Once the base is removed, the AP site is removed by an AP endonuclease or an AP lyase, which nicks the DNA strand 5' or 3' to the AP site, respectively. The remaining nucleotide backbone is excised by a phosphodiesterase and the gap is filled by a DNA polymerase and sealed by DNA ligase [55, 57].

During development from primordial follicle to mature MII stage, it is proposed that the oocyte accumulates an abundance of DNA repair factor transcripts and proteins to repair any DNA damage encountered at fertilisation and during subsequent early embryogenesis. This is likely necessary for two reasons, (1) transcription is restricted in a conceptus until the two-cell stage in mice [58], or the four-cell stage in humans [59] and (2) sperm DNA is prone to oxidative damage and 8-Oxoguanine lesions [60]. In support of this hypothesis, all genes involved in the BER repair pathway are expressed in human and monkey MII oocytes and embryos [23, 24, 26]. In contrast, although OGG1 protein is present in human sperm, the subsequent enzymes of the BER pathway, APE1 and XRCC1, cannot be detected at the protein level [61]. Interestingly, OGG1 protein is expressed at very low levels in mouse oocytes, while APE1 and XRCC1 protein are readily detectable [62]. This inverse relationship suggests that some degree of functional co-operation between the oocyte and sperm may be required to repair 8-Oxoguanine lesions after fertilisation in mice [62]. While the efficiency of BER repair remains to be fully explored, the fact that oxidative damage to sperm DNA is associated with both miscarriage and developmental abnormalities in offspring [60] suggests that there may be inherent limits to the DNA repair capacity of the oocyte in this instance. In addition to the repair of oxidative damage to sperm DNA, new evidence also points to a functional role for BER, and oocyte-derived XRCC1 in particular, in the repair of lesions in the paternal DNA that are generated during zygotic reprogramming [63]. Importantly, the repair of these lesions is linked with timely mitotic entry as the zygote transitions into a two-cell embryo. Thus, oocyte-derived BER appears to be important for at least two different types of DNA damage post fertilisation.

In the case of other types of damage, functional studies in mature *Xenopus* oocytes and nuclear extracts have demonstrated that bulky DNA lesions and AP sites are efficiently repaired by alternative BER mechanisms, including the proliferating cell nuclear antigen-(PCNA) dependent pathway and the DNA polymerase β -(pol β)-dependent pathway [17, 64, 65]. These same pathways are conserved in mammalian cells [66, 67], although they remain to be investigated in mammalian oocytes. Moreover, whether the BER pathway functions in oocytes at the primordial follicle stage has not been determined.

DNA replication-induced errors and mismatch repair (MMR)

Spontaneous DNA alterations can arise during DNA replication due to dNTP misincorporation, which can distort the helical structure of DNA. These replication-dependent errors are predicted to occur at the rate of 10^{-6} – 10^{-10} mutations/base pair/cell generation [68–71]. Notably, base–base mismatches and insertion/deletion mispairs can also be generated during recombination, which has important implications for oocytes during meiotic recombination. The mismatch repair (MMR) pathway is primarily utilized to repair these dNTP misincorporations, as well as insertion and deletion loops (IDLs) that occur during DNA replication and recombination (Table 1; Fig. 2). Moreover, some of the MMR components have been reported to play a significant role in DNA damage signaling via direct interaction with ATM in response to non-replication-related DNA damage [72, 73]. Heterodimeric complexes of MutS-related proteins (MutS α and MutS β) target and repair base:base and IDL mispairs. MutS α (MSH2–MSH6 complex) initiates the repair of small 1–12 nucleotide mismatches and IDLs, whereas the repair of larger IDLs (2–16 nucleotides) is initiated by MutS β (MSH2–MSH3 complex) [74, 75]. The partial redundancy between MutS α and MutS β helps to explain the different phenotypes of the various MutS-related protein-null mice. *MSH2*, *MSH3* and *MSH6* mRNA are highly expressed in human GV oocytes [23]. Although MutS-related protein levels have not been measured in human oocytes, the presence of transcripts indicates that MMR may be required to maintain genome integrity. Indeed, *Xenopus* oocyte extracts have been shown to effectively repair different mismatches in vitro [18]. The other two eukaryotic MutS-related proteins, MSH4 and MSH5, are not involved in MMR, but have essential roles regulating chromosome pairing during meiotic recombination [76, 77]. In mice, loss of MSH4 and MSH5 leads to failed chromosome pairing during prophase I of meiosis, resulting in apoptosis and sterility in both males and females [78]. Indeed, in humans, mutations in *MSH5* are associated with ovarian insufficiency [79, 80]. Of the four MutL homologs, MLH1, MLH3, PMS1 and PMS2, three are involved in mismatch repair and MLH3, PMS2 and MLH1 are required for mammalian meiosis [81–86]. Mice null for *Mlh3*, *Pms2* and *Mlh1* are infertile, spermatocytes fail to progress normally beyond the pachytene stage of meiosis, while oocytes fail to complete meiosis after fertilization [81–83]. Combined, these findings suggest that MMR and its related proteins are vital for coordinating oocyte DNA damage signaling initially not only during recombination but also after meiotic arrest.

Ultraviolet radiation and nucleotide excision repair (NER)

The most pervasive environmental DNA-damaging agent is ultraviolet light, which can induce thousands of lesions per exposed cell, per hour. There are two major classes of mutagenic DNA lesions induced by UV radiation, including cyclobutane–pyrimidine dimers (CPDs), which are the most abundant and probably most cytotoxic lesions and 6–4 photoproducts (6–4 PPs, pyrimidine adducts), which exert potentially lethal, mutagenic effects [2]. Many organisms use the photolyase enzyme that specifically binds to CPDs (CPD photolyase) or 6–4 PPs (6–4 photolyase) and reverses the damage using the energy of light, a process known as photoreactivation (Table 1). These photolyase enzymes are either absent or non-functional in humans and are considered to be ancient repair proteins [2, 87]. Thus, the nucleotide excision repair (NER) and to some extent, BER and MMR are involved in repairing DNA lesions induced by UV radiation in humans (Table 1).

While oocytes are not normally exposed to UV light, the development of assisted reproductive technologies, such as in vitro maturation and in vitro fertilisation, mean that there are new situations in which oocytes are exposed to potentially damaging visible light [88]. Similarly, some environmental factors such as chemotherapeutic drugs can also cause bulky adducts and have been shown to activate NER [89, 90]. Furthermore, studies have shown overlapping functions of some components of the BER and NER pathways, especially the repair of oxidative DNA damage [91–93], suggesting that NER may also be involved in ROS-associated ovarian pathology. Therefore, NER is also likely to be essential for oocyte genome integrity. The NER repair pathway acts on a wide variety of lesions, but is especially important for bulky, helix-distorting lesions [94]. In eukaryotes, NER functions by excising an oligonucleotide fragment of ~30 nucleotides that contains the DNA lesion. Briefly, XPC recognizes and binds to the DNA lesion, then recruits endonucleases XPG and XPF, as well as TFIIH, which is a subcomplex of polymerase II transcription machinery that operates to uncoil the DNA fragment. XPA and XPD are recruited at the same time by TFIIH to coordinate single strand DNA-binding protein replication protein A and helicase activity, respectively [95, 96]. XPG endonuclease and ERCC1–XPF heterodimeric protein then make cuts 3' and 5' of the damaged strand, respectively, releasing the damaged DNA fragment (Fig. 2). The gap that is generated is then repaired by DNA synthesis using the opposite, normal DNA strand as a template.

Two different NER pathways exist, with one that operates on transcriptionally active DNA and the other on transcriptionally silent DNA [97]. Significantly, in humans and mice, transcription-coupled NER (TCNER) can proceed

in the absence of XPC, thus XPC may not be required for TCNER [98, 99]. In GV and MII, oocytes which are transcriptionally quiescent, *Xpc* is highly expressed along with most of the other genes involved in NER [23]. Studies utilizing the thymidine incorporation assay led to an increase in the number of radioactive [³H]thymidine nuclear incorporation in oocytes after UV irradiation [15, 16], demonstrating excision and replacement of damaged bases by repair enzymes in resting primordial and growing oocytes [15, 16]. Importantly, incorporation of [³H]thymidine was six times greater in growing oocytes versus primordial oocytes [15]. This suggests that repair mechanisms are not as efficient in primordial oocytes and may partially explain why they are more susceptible to DNA damage compared to growing oocytes. Although *Xpa*- and *Xpc*-deficient mice are fertile [100, 101], mice deficient in *Ercc1* or those carrying a point mutation in *XPD*, show decreases in fertility over time, indicative of premature ovarian failure [102–104]. Together, these findings suggest that some components of NER pathway are vital for protecting the ovarian reserve, and warrant further investigation.

Interstrand crosslinks (ICLs) and Fanconi anaemia (FA) pathway

DNA interstrand crosslinks (ICLs) are highly toxic covalent links within the double helix that prevent DNA unwinding, thereby blocking both DNA replication and transcription [105]. ICLs are caused by a variety of endogenous metabolites (i.e., nitric oxide, malondialdehyde), environmental exposures (i.e., acrolein, crotonaldehyde, formaldehyde and acetaldehyde) and cancer chemotherapeutic agents (i.e., chlorambucil, cisplatin, mitomycin C, psoralen) that have two reactive groups [106]. Indeed, female patients exposed to ICL-inducing drugs (i.e., chlorambucil, cisplatin, carboplatin and ifosfamide) have a significantly reduced rate of successful pregnancy, possibly due to direct damage to the primordial follicles [107]. As ICLs affect both strands of DNA, the Fanconi anaemia (FA) pathway works to coordinate several distinct repair activities belonging to different classic repair pathways, including NER, translesion synthesis (TLS), and HR, to remove crosslinks [108]. The FA pathway was identified by cloning genes commonly mutated in Fanconi anaemia patients, which is an autosomal-recessive chromosomal instability disorder. Cells derived from FA patients are hypersensitive to DNA cross-linking agents, ionizing irradiation and oxygen radicals, which lead to increased apoptosis or growth arrest. Clinical manifestations of FA include various hematological deficiencies, developmental abnormalities, infertility and increased cancer susceptibility.

There are 11 identified FA proteins that form into two core complexes in response to stalled replication forks

[109]. Complex 1 is formed in response to DNA damage and monoubiquitinates and recruits FANCD2 to chromatin containing the cross-link. Monoubiquitinated FANCD2 then promotes chromatin loading of the second complex via interactions with FANCD1/BRCA2, which includes components from other repair pathways including HR (e.g., MRN complex and Rad51). Recruitment of both complexes is required for the repair of the cross-link, and is presumably repaired through HR and translesion synthesis (TLS) [109]. Interestingly, FANCD2 has been shown to bind to synaptonemal complexes of meiotic chromosomes during spermatogenesis [110]. In particular, this pathway appears to have important roles in the repair of DNA during the proliferation of the embryonic precursors of oocytes (known as primordial germ cells and oogonia) and during meiotic recombination [111–113]. Moreover, male and female mice genetically null for FA factors *Fac* and *Fanca* have significantly reduced fertility, with premature reproductive senescence in females attributed to premature ovarian failure [111–113], thus demonstrating a role for FA in maintaining the ovarian follicular reserve.

Translesion synthesis and template switching

If the cell is to survive in a situation where repair cannot occur in time for replication, the only option is to bypass the lesion using polymerases with less stringent base-pairing requirements than replicative polymerases. At the site of a lesion, the high-fidelity DNA replication polymerase is transiently switched for low-fidelity DNA polymerase, but is returned once the replication fork passes the site of the lesion to continue as normal. Alternatively, the DNA lesion is completely bypassed at the replication fork, leaving a single-strand gap in the synthesized sequence, which is subsequently repaired using the sister chromatid as a template, as in HR repair [114]. TLS is unlikely to play a significant role in maintaining the genome integrity of diplotene-arrested oocytes because of their lack of mitotic activity. However, sperm production is associated with many rounds of DNA replication and cell division, and in agreement with this, all known TLS polymerases show higher expression in testis compared with tissues that contain only somatic cells, with high levels of polymerase expression particularly evident in round spermatids [115, 116]. Interestingly, growing oocytes accumulate polymerases involved in TLS [23], which may point to an important role for this pathway in the development embryo after fertilization [117].

DSB repair: homologous recombination (HR) and non-homologous end-joining (NHEJ) repair

Similar to somatic cells, DNA DSBs are generated in oocytes following exposure to exogenous stressors, such as

ionizing radiation, chemotherapeutic drugs, and environmental toxicants [13, 118–120]. Recent studies have also reported the spontaneous induction and accumulation of DSBs in oocytes as a consequence of endogenous oxidative stress and maternal aging [34]. Furthermore, unlike somatic cells, DNA DSBs are intentionally induced and repaired in oocytes during meiotic recombination.

Unrepaired or incorrectly repaired DSBs can result in chromosome breaks, translocations, deletions and point mutations [121], ultimately leading to oocyte apoptosis, impaired oocyte growth and maturation, infertility, miscarriage or genetic defects in offspring [6, 119, 122, 123]. Indeed, chromosomal aberrations such as deletions, translocations and inversions are detected in ~2–6% of human oocytes [124, 125] and miscarriages [6, 126, 127]. While large deletions are lethal, small chromosomal losses underlie numerous rare human genetic disorders characterised by intellectual disability, growth retardation and/or congenital malformations. One example is 1p36 deletion syndrome, which affects 1/5000–10,000 births, making it one of the most common mental retardation syndromes in humans [128]. The majority of the deletions contributing to this syndrome arise de novo in the oocyte, possibly resulting from unequal recombination or erroneous repair of spontaneously occurring DNA DSBs [128]. Clearly, it is essential for oocytes to undertake efficient repair of DNA DSBs to avoid infertility and transmission of germline defects to the next generation. Indeed, studies in apoptosis-deficient mice showing that oocytes that have sustained DSBs as a consequence of chemotherapy or radiation can give rise to healthy offspring and provide strong evidence that oocytes can repair significant levels of DNA damage despite being predisposed to activate cell death [119, 129].

HR and NHEJ are the two main pathways responsible for DNA DSB repair [130]. In somatic cells, DNA DSBs lead to the phosphorylation of histone H2AX (γ H2AX) by ATM kinase [130]. This results in the formation of discrete foci at the sites of DNA damage that facilitate the recruitment and targeting of repair factors. HR is considered to be the most error-free pathway for DSB as it uses the undamaged sister-chromatid as a template. As such, HR is likely to be the pathway of choice for oocytes because sister chromatids are present (at least before the resumption of meiosis), which can serve as a template for accurate, error-free repair. *RAD51* and *BRCA2* are essential for HR repair and are among the few specific repair factors that have been identified to be transcribed in primordial follicle oocytes [34, 131]. Interestingly, *Rad51* is detected at higher levels in primordial follicle oocytes from young mice compared to old mice [29, 33, 34, 131], suggesting that primordial follicle oocytes lose the capacity to undertake efficient DSB repair as they age. In mammals, there is some evidence that GV and MII oocytes may utilize HR-repair enzymes (i.e., RAD51) in response

to exogenous DNA-damaging agents in culture, possibly to modulate cell death [31, 131]. Indeed, microinjection of mature oocytes with a neutralizing RAD51 antibody increases their susceptibility to doxorubicin-induced death, presumably by reducing the ability of the oocyte to undertake timely DNA repair [31, 131]. Another key player in the repair response is the MRN complex, comprising MRE11, RAD50 and NBS1 [132]. It is one of the earliest responders to DNA DSB, acting as a sensor of DNA damage and mediating the activation of ATM. MRE11 also possesses nuclease activity and directly participates in the repair process. In mice, MRE11 is essential for DNA DSB repair during early meiosis I, but its expression and function has not been previously characterised within oocytes of primordial follicles [133–135]. However, evidence suggests that MRE11 is required to detect DNA damage in mature oocytes, as blocking MRE11 in the presence of DNA damage increases γ H2AX foci and results in compromised DNA integrity in MII oocytes [135].

NHEJ repair tends to be error prone because the break ends are directly ligated without the use of a homologous template. Repair involves the binding of KU70/KU80 to each break end. DNA-PKcs then forms a connecting bridge, while XRCC4 acts as a scaffolding protein that allows Ligase IV to ligate the ends. While it is not yet clear what role NHEJ plays in oocytes, Ku70 and Ku80 mutants have been described as fertile, but rarely produce offspring [136, 137]. As these mice exhibit similar early aging without substantially increased cancer levels [136], it would be interesting to determine if these animals also suffer premature ovarian failure.

Interestingly, studies in somatic cells suggest that the choice between DSB repair pathways differs with increasing age [138]. The transcription of gene-encoding proteins involved in both HR and NHEJ have been detected in mammalian oocytes, although it is not clear under which circumstances they might be differentially activated [23, 24, 26, 131, 139]. One hypothesis, based on evidence from *Xenopus* oocytes, is that pathway choice is influenced by stage of oocyte development, with diplotene-arrested oocytes employing HR, while fully grown, meiotically mature oocytes may preferentially store NHEJ proteins for use in the developing embryo after fertilisation [140, 141].

Detection and response to DNA damage

In somatic cells, although different lesions are detected and repaired by different repair pathways, they all trigger common upstream signaling pathways. These pathways include slowing or arresting of cell-cycle progression [142, 143], changes in chromatin structure at the damage site and the transcriptional induction and posttranslational modification

of various proteins involved in DNA repair [144, 145]. The protein kinases ATM, ATR and DNA-PKcs, and members of the poly(ADP-ribose) polymerase (PARP) family are considered to be the master regulators of these signaling pathways, acting together or independently to coordinate the responses to specific types of DNA damage [3, 146].

DNA-PKcs is essential for DSB repair, as deletion in mice results in severe immunodeficiency and radiation hypersensitivity [147]. Specifically, DNA-PKcs mediates the formation of the synaptic complex that brings together the two DNA molecules at the site of DNA DSBs and its kinase activity is only activated once this complex is formed [148]. Interestingly, both male and female DNA-PKcs-deficient mice are fertile [149], suggesting that DNA-PKcs has a non-essential role during meiosis.

Both *Atm* and *Atr* are highly expressed in mammalian oocytes [23, 24, 26] and mutation of ATM in humans gives rise to ataxia telangiectasia, a debilitating neurodegenerative disease with symptoms including hypersensitivity to agents that cause DSBs and infertility [150, 151]. Studies using mice and arabidopsis suggest conserved roles in meiosis, particularly during homologous chromosome recombination, and disruption results in abnormal chromosomal synapsis and subsequent chromosome fragmentation [152–156]. Indeed, the failure to repair the developmentally induced DSBs generated during meiotic recombination in ATM-deficient mice results in the complete absence of ovarian follicles and infertility [153, 154, 157]. Phosphorylated-ATM (activated) has also been identified in primordial follicle oocytes within 3 h of exposure to radiation, highlighting its role in the response of oocytes to exogenous sources of DNA damage [119]. Interestingly, low to moderate levels of DNA damage in fully grown prophase-arrested mouse oocytes do not prevent the resumption of meiosis because ATM is not effectively activated [158]. This means that oocytes can re-enter meiosis, even in the presence of DNA damage. However, an ATM-independent pathway involving the spindle assembly checkpoint (SAC) is activated prior to anaphase, which blocks the segregation of chromosomes and the development of mature oocytes [159–161]. In contrast, high levels of DSBs do activate an ATM/Chk1-dependent checkpoint, which maintains oocytes in G2/prophase arrest until repair of the damage occurs [158]. Thus, while ATM is essential for detecting DNA damage in immature oocytes, its role in fully grown GV oocytes appears to be dependent on the extent of damage.

Less is known about the function of PARPs in immature and mature oocytes. PARP-1 is a nuclear enzyme that is activated in response to DNA damage and has a key role in the organization of DNA repair by regulating chromatin structure and DNA metabolism by the poly(ADP-ribosylation) of numerous repair proteins and histones [162, 163]. *Parp-1* knockout cells and mice are hypersensitive

to DNA-damaging agents, including alkylating agents and γ -irradiation [164], but are resistant to oxidative stress-induced cell death [165]. *Parp-1* null mice also show enhanced genomic instability and high frequencies of chromosome aberrations, sister chromatid exchanges and telomere shortening, but are still fertile [166–169]. PARP-2 is also activated in response to DNA damage and is required for the repair of single strand DNA breaks [162, 170, 171]. Interestingly, PARP-proteins are expressed in oocytes and have a critical role in the maintenance of chromosome stability during meiosis [172, 173]. *Parp-1*-deficient oocytes exhibit incomplete homologous chromosome synapsis and persistent histone H2AX phosphorylation [172] and *Parp-2*-deficient mice exhibiting severely impaired spermatogenesis [173]. Thus, PARP-proteins may also respond to and initiate repair of DNA damage in both resting and growing oocytes.

Repair or apoptosis?

After the initial detection of DNA damage, the cell must ‘decide’ whether to undergo apoptosis, or to repair the damaged DNA. The p53 protein is a transcription factor that is a key regulator of this ‘decision’ and in somatic cells it can initiate four main outcomes after activation; DNA repair, cell-cycle arrest, senescence and apoptosis [174]. In response to DNA damage ATM, CHK1 and CHK2 phosphorylate and activate p53, which then can initiate transcription of, amongst other targets, the CDK inhibitor, p21, to regulate cell-cycle and/or the pro-apoptotic BAX/BAK and PUMA proteins [174]. The choice between these outcomes depends on a number of other variables, indicating that the p53 pathway is responsive to activities of other signaling pathways within the stressed cell [174]. Notably, immature oocytes are already arrested, so presumably can only initiate apoptosis or DNA repair. Interestingly, a number of studies have failed to detect p53 in oocytes [119, 175, 176]. However, the expression of TAp63 α , an isoform of p53’s homolog, p63, can be detected at high levels in the immature oocyte nucleus [175, 176]. TAp63 α is vital for monitoring the integrity of the female germline, and DNA damage induces its phosphorylation [177] and results in its binding to p53-related DNA sites [175]. Thus, TAp63 α represents a specific responder to DNA damage in immature prophase-arrested oocytes [178].

Conclusions

In summary, transcriptional profiling, the study of DNA repair-deficient knockout mouse models and in vitro assays, suggest that oocytes are equipped to execute the repair of a

variety of different DNA lesions. However, further understanding of the functional roles of specific proteins at each stage of oocyte development, and the overall contribution of the DNA repair to the maintenance of oocyte quality throughout reproductive life, require further investigation. Such studies will provide insight into the role that DNA repair plays in determining the number and quality of oocytes available to females, which has implications for the length of the fertile lifespan, the age the menopause begins or reproductive senescence and the mechanisms utilized by the female germline to ensure transmission of high fidelity genetic information to future generations.

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