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Genome Instability and DNA Repair in Somatic and Reproductive Aging

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Abstract

Genetic material is constantly subjected to genotoxic insults and is critically dependent on DNA repair. Genome maintenance mechanisms differ in somatic and germ cells as the soma only requires maintenance during an individual's lifespan, while the germline indefinitely perpetuates its genetic information. DNA lesions are recognized and repaired by mechanistically highly diverse repair machineries. The DNA damage response impinges on a vast array of homeostatic processes and can ultimately result in cell fate changes such as apoptosis or cellular senescence. DNA damage causally contributes to the aging process and aging-associated diseases, most prominently cancer. By causing mutations, DNA damage in germ cells can lead to genetic diseases and impact the evolutionary trajectory of a species. The mechanisms ensuring tight control of germline DNA repair could be highly instructive in defining strategies for improved somatic DNA repair. They may provide future interventions to maintain health and prevent disease during aging.

1. INTRODUCTION

As a storage mechanism of all heritable information, the genome is surprisingly unstable. Tens of thousands of lesions are estimated to be inflicted on DNA within each cell of the human body on a daily basis. DNA repair mechanisms evolved early during evolutionary ancestry as they are essential to facilitate the maintenance and inheritance of the genetic information. Genetic defects in DNA repair genes in humans lead to a wide spectrum of pathologies that can be classified into three disease categories: (*a*) The most severe types of dysfunctions lead to developmental abnormalities, while (*b*) a wide spectrum of progeroid syndromes is characterized by segmental aging, which describes pathologies that are typically found during normal aging but occur in some but not all tissue types. Such progeroid syndromes can also be associated with (*c*) cancer susceptibility that is typically associated with a specific spectrum of tumor types.

The maintenance of a stable genome is prerequisite for heritability, and germ cells have a particularly high degree of DNA repair proficiency and a mutation rate that is more than an order of magnitude lower than the somatic mutation rate (1). Indeed, somatic DNA repair only needs to maintain a functional genome during the lifespan of an individual. In stark contrast, germ cells perpetuate the genetic information indefinitely. Recently, significant progress has been made in the determination of somatic mutations, mostly due to improved methodologies for single-cell sequencing. Mutations can be unequivocally identified by DNA sequencing. By contrast, the identification of DNA damage is technically far more challenging and poses a major obstacle to better understanding the degree of genome damage. Mutations are a consequence of DNA damage and/or replication errors and thus could be considered a proxy for the degree of experienced DNA damage. Somatic mutation rates are significantly correlated with the lifespan of a range of species that have been investigated, further supporting an important role for DNA repair in determining a species-specific lifespan and pace of aging (2).

Nuclear DNA experiences a wide range of damaging events, and the distinct types of physical and chemical alterations of the DNA structure require specific sets of damage recognition and removal mechanisms (**Figure 1**). Distinct cell and tissue types differ in their propensity for being subjected to specific genotoxic sources. For instance, ultraviolet (UV)-induced helix-distorting lesions are hazardous to the skin, while the liver is particularly exposed to toxins contained in the diet, requiring detoxification. The distinct parts of the exposome partially explain the specific

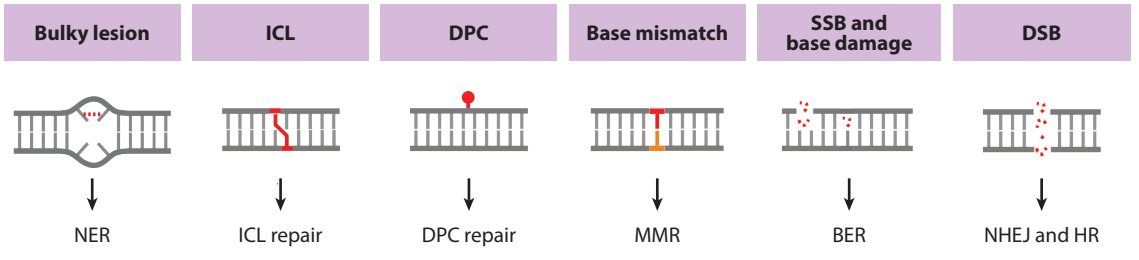

Figure 1

Different types of DNA lesions and their corresponding DNA repair pathways. DNA is constantly exposed to endogenous and exogenous genotoxic agents, which induce distinct types of DNA lesions. Different DNA lesions then trigger specific DNA damage signaling and repair pathways. Bulky, helix-distorting lesions such as cyclobutane pyrimidine dimers and 6-4 pyrimidine-pyrimidone photoproducts are repaired via NER. By contrast, DNA ICLs trigger ICL repair, DPCs trigger DPC repair, and base mismatches trigger DNA MMR. Lastly, base damage and DNA SSBs activate BER, while DNA DSBs are repaired via either NHEJ or HR. Abbreviations: BER, base excision repair; DPC, DNA–protein crosslink; DSB, double-strand break; HR, homologous recombination; ICL, interstrand crosslink; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; SSB, single-strand break. Figure adapted from images created with BioRender.com.

pathologies associated with congenital DNA repair defects. While environmental genotoxins have increasingly been identified, the sources of endogenous DNA damage types remain less characterized. Recently, metabolic formaldehyde was shown to trigger a range of pathologies, including neurodegeneration (3). Formaldehyde is also an excellent example of a chemical that may induce a variety of genotoxic effects. The most toxic consequences of formaldehyde exposure are DNA–protein crosslinks that require a complex set of removal mechanisms (4). Moreover, this chemical can also trigger an imbalance of the reactive oxygen detoxification system, leading to oxidative stress (5).

In contrast to the accumulation of somatic mutations, the accumulation of DNA damage has, thus far, mostly been shown indirectly. For instance, the most widely used marker of sites of DNA double-strand breaks (DSBs), phosphorylated H2AX, has been observed to increase during aging in a wide range of tissues in mice (6). The direct measurement of DNA lesions has been hampered by the dearth of methodologies to detect the many different, highly diverse types of lesions. The exception is cyclopurines, which cause bulky distortions of the DNA helix. They have directly been shown to increase during aging (7).

While the pathological consequences of DNA damage have been clearly established, therapeutic strategies for their mitigation have remained largely elusive. Nonetheless, the reduction of genotoxins has made a major contribution to reduce avoidable cancers. The success story of such preventive measures ranges from the reduction in scrotal cancers in chimney sweepers to the decline in tobacco smoking and hopefully will soon result in the further mitigation of industrial and traffic emissions. Boosting DNA repair activity, however, has proven far more complex. Overexpression of single DNA repair genes has shown ambiguous results likely because of the disturbance of stoichiometries of the multisubunit complexes of DNA repair (8). By contrast, the deacetylase Sirt6 has been suggested to augment DSB repair via its regulatory role in nonhomologous end-joining (NHEJ) and homologous recombination (HR) (9). Much more headway has been made on the reverse, the inhibition of DNA repair to trigger the demise of cancer cells that, due to their specific genetic composition, critically depend on a particular DNA repair mechanism (10). Here, the inhibition of PARP has been clinically pursued and shown to be effective in cancer types that are defective in HR such as BRCA1 mutant tumor cells. Interestingly, PARP inhibition has also been reported to alleviate the cytotoxic effects of DNA repair defects. For instance, a mutation in XRCC1 leads to a single-strand break (SSB) repair defect, triggering cerebellar ataxia due to motor neuron loss (11). The persistence of SSBs could lead to excessive PARylation that deprives the cellular NAD^+ pools, and PARP inhibition could prevent this. Such types of NAD^+ deprivation have also been reported in other DNA repair deficiencies linked to neuronal loss, such as ataxia telangiectasia (AT) or nucleotide excision repair (NER) defects (12). Here, supplementation with NAD^+ precursors could alleviate the pathological consequences. These examples demonstrate the importance of the DNA damage response (DDR) mechanisms that determine the consequence of DNA damage. Thus, it is not necessarily DNA damage itself but how a cell responds to the damage that dictates the pathological outcome.

The degree of genome maintenance varies according to cell type. Generally, major distinctions between somatic and germ cells are highly instructive for understanding such differences. Mutations in germ cells can have far greater consequences as they may affect descendants throughout generations. In fact, all genetic diversity that exists in nature originated from mutations in heritable genomes. Although such germline mutations are prerequisite for genome evolution and evolutionary radiation, they can be detrimental for the maintenance of a species and fertility. The DNA repair and DDR mechanisms in germ cells must therefore be particularly well controlled in order to achieve the low germline mutation rates. In dioecious species, the sex-specific gametogenesis programs entail distinct constraints on genome maintenance and usage of particular DNA

repair mechanisms. In humans, oogenesis occurs during early development, and the meiotically arrested oocytes are stored up to decades before fertilization, requiring long-term genome maintenance. Oocytes are in fact highly sensitive to DNA damage. In mice, a small number (3 to 4) of DSBs suffice to trigger apoptosis (13). In humans, that apoptotic threshold is higher, but apoptotic counterselection against genomically compromised oocytes forms an important pillar for mitigating inheritance of germline mutations in many species. In contrast to the female germline's small contribution to heritable mutations, the male germline accounts for 80% of germline mutations in humans (14). Here, spermatogenesis is maintained throughout adult life. Recent germline sequencing studies found that only a minority of humans show hypermutations in germ cells, and this could be linked to either a genetic defect in a DNA repair mechanism, such as NER, or genotoxic chemotherapy close to the time of conception (15). DNA repair mechanisms and the apoptotic elimination of immature sperm function well during spermatogenesis, again indicating a combination of repair and selection. However, mature sperm are extremely vulnerable to DNA damage because their highly compacted chromatin structure is inaccessible to DNA repair machineries (16).

The conferral of germline-like DNA repair mechanisms to somatic cells is challenging because of the range of involved DNA repair systems but also because of the importance of selection. Such selection is also used in somatic cells and governed by the DDR. Here, the apoptotic removal of damaged cells as well as the cessation of proliferative activity via cellular senescence comprise important mechanisms to avoid negative consequences of genomically compromised cells. By contrast, during aging, some mutationally altered cells can also gain a selective advantage and outcompete other cells. This occurs, for instance, during clonal expansion during hematopoiesis, where single clones carrying precancerous mutations might expand, giving them a proliferative advantage. Such an expansion is consistent with aging being the single greatest risk factor for cancer.

Here, we discuss the recent advances in understanding the role of DNA repair mechanisms in the aging process. We first provide an overview of how a functional genome is maintained during an individual's lifespan. We discuss how DNA damage impacts somatic and reproductive aging. Then, we explore how, by contrast, germ cells can maintain their genomes indefinitely and how a lack of control of germline DNA damage can have transgenerational effects and lead to genetic diseases. Finally, we provide an outlook on how therapeutic strategies could improve genome maintenance and thus target the aging process and age-related diseases at their root cause.

2. ROLE OF GENOME INSTABILITY IN SOMATIC AGING

2.1. Mutation Accumulation During Somatic Aging

Mutations of all types, including small deletions, base substitutions, and genome arrangements, constantly accumulate in somatic cells because of errors in DNA repair or, in cycling cells, due to the replication of damaged DNA. In normal, noncancerous tissues, these mutations have been difficult to detect by traditional bulk tissue sequencing approaches because they are present in only a small fraction of cells and, thus, occur at low allelic frequency. In recent years, however, new genome sequencing techniques of single cells or clones derived from a single cell, together with bioinformatic advances to filter out analysis artefacts, have revolutionized our ability to trace the mutation history of individual cells in normally aging tissues (17, 18), demonstrating that the somatic mutation burden increases with age in both cycling and postmitotic human cells, at least with regard to single-nucleotide variants (SNVs) (19–21). Comparative sequencing analysis across many mammalian species also showed that average SNV rates within tissues are inversely correlated with lifespan, with long-lived species accruing mutations at a slower rate than short-lived species (2). These data suggest a direct connection between genome instability and aging.

A major limitation of exome sequencing studies carried out to date is that while they are able to reliably detect SNVs and small insertions and deletions, they are largely blind to genome structural variations (SVs) such as large-scale deletions, inversions, translocations, and mobile element insertions, which are predicted to have much more profound functional effects than SNVs (18). Indirect, low-throughput assays indicate that SV frequency increases with age in the mouse heart and liver as well as in human and mouse lymphocytes. However, an accurate estimation of SV frequency during aging based on single-cell sequencing remains missing.

Although genome sequencing data suggest a connection between genome instability and aging, clear causal links and relative contributions of somatic mutations to aging-associated phenotypes have yet to be established. For decades, discussions on the role of somatic mutations in aging have focused on their potentially negative effects on gene expression and protein fidelity, which may in turn trigger general cellular dysfunction and induce cell death or senescence (18, 22). It is plausible that this is true for SVs, which usually affect large genomic areas that cover not only coding sequences but also gene regulatory elements (18). The contribution of SNVs to the aging process is less well understood (reviewed in 18). Mammalian cells are tolerant of large increases in SNV burden because most SNVs, even if they occur in coding genes, are not sufficient to trigger cellular dysfunction or cell death (23, 24). This explains why cells can acquire thousands of point mutations without obvious functional decline (25, 26). In addition, individuals with germline mutations in genes encoding DNA polymerase ε and δ subunits (*POLE/POLD1*) that diminish proofreading activity during DNA replication display highly elevated somatic mutation rates and a higher colorectal and endometrial cancer incidence yet do not exhibit any other significant premature aging phenotypes (27). Similar results were obtained from individuals carrying mutations in *MUTYH*, which encodes a DNA glycosylase that is active in base excision repair (BER) and whose inactivation also causes a hypermutator phenotype (28). In addition, Lynch syndrome patients, who carry defects in the mismatch repair (MMR) pathway, have an extremely high risk of developing colorectal cancer but do not suffer from any premature aging-related phenotypes (29). These examples are often used to argue against a causal role for somatic SNVs in aging, at least in the context of highly proliferating tissues, such as colon, which rely on high replication fidelity and the repair of bases that were not properly incorporated during replication. However, these data do not rule out that somatic SNVs, nevertheless, may contribute to the aging of postmitotic tissues, such as brain, where they are induced largely by oxidative stress rather than DNA replication (30).

An extension of the classic model of how somatic mutations, both SNVs and SVs, may contribute to the aging process postulates that a subset of mutations, those that favor proliferation over homeostasis and functional integrity, are positively selected during cell proliferation and, as driver mutations, promote the clonal expansion of functionally altered cells within aging tissues (2). This aging model is analogous to what is known about the genetic events during carcinogenesis, where driver mutations in transforming precancerous cells are positively selected to convey a growth advantage at the expense of the organism (31). Evidence for the accumulation of clonal cell populations containing positively selected somatic mutations has been found in many human tissue types including blood (32, 33), esophagus (26), and skin (25). Interestingly, possible links between clonal expansion in aging tissues and disease etiology were shown in the context of clonal hematopoiesis and cardiovascular disease (32) as well as insulin resistance and chronic liver disease (34). In addition, somatic mutations in the genes encoding PIK3CA and subunits of the cerebral cavernous malformation (CCM) complex can drive the clonal growth of CCMs in the brain, leading to seizures and strokes at a young age (35). However, we are only beginning the process of mapping aging driver mutations, and significantly more work is needed in this emerging area of aging research to understand how clonal cell populations promote aging-associated phenotypes and diseases other than cancer.

Interestingly, long-lived animals appear to have developed more efficient DNA repair pathways compared to short-lived species (9, 36), possibly explaining the negative correlation between somatic mutation rate and lifespan. For example, naked mole rats, which live more than 30 years, have more efficient repair pathways to deal with bulky DNA lesions and base damage compared to mice, which live only up to 3 years (37). However, whether the age-associated increase in mutational burden is directly driven by a concomitant decrease in DNA repair fidelity is unclear. Numerous studies reported an age-associated decline in DNA repair fidelity (reviewed in 38), albeit using indirect detection methods (i.e., comet assays or γH2AX levels) or targeting only a subset of DNA lesions (38, 39). Germline mutations in genes encoding DNA repair factors can cause segmental aging phenotypes (see Section 2.3), also supporting a relationship between DNA repair capacity and aging.

In addition, enhancing aspects of the DDR can positively affect lifespan in mice. For example, activation of ataxia-telangiectasia mutated (ATM) with low doses of chloroquine in a mouse model of Hutchinson-Gilford progeria syndrome (HGPS) reduces progeroid features and extends lifespan (40), and overexpression of SIRT6, which promotes DNA repair but is also associated with other cellular pathways, protects genomic stability and increases longevity (9). Nevertheless, whether there is also a correlation between lifespan and DNA repair efficiency in humans has yet to be clarified. Although there is some evidence that variants of select DNA repair genes are enriched in certain populations of long-lived people, variants that enhance the activity of DDR pathways so far do not appear to be generally associated with longevity (41, 42). In addition, while the total burden of somatic mutations increases with age, mutation rates are remarkably constant in normal cells (43, 44), also arguing against a model in which somatic mutations accelerate aging by eroding DNA repair pathways. Given these conflicting pieces of evidence, substantially more work is needed to understand how the activity of individual DNA repair pathways affects lifespan, whether DNA repair decline occurs in a general or tissue-specific manner, and whether it affects aging globally or in the context of distinct aging-related phenotypes.

2.2. Consequences of DNA Damage During Somatic Aging

Somatic mutations and their downstream effects on gene expression and protein fidelity and on the clonal expansion of dysfunctional cells are not the only way that genome instability contributes to the aging process. Cells accumulate unresolved DNA lesions during aging (6, 7). It is becoming increasingly clear that this DNA damage and the DDR it triggers are important contributors to cellular dysfunction during aging because they can directly interfere with essential DNA metabolic processes, such as transcription and replication; remodel the epigenome; disrupt cellular energy and protein homeostasis; alter cell fate decisions; and cause stem cell exhaustion (45) (**Figure 2**).

2.2.1. DNA lesions are obstacles to DNA transcription and replication. Certain types of DNA lesions, especially DNA breaks and bulky, helix-distorting lesions, are steric obstacles to RNA and DNA polymerases trying to read through DNA. Thus, the most immediate consequence of persistent DNA damage is the dysregulation of DNA metabolic reactions such as transcription and replication. Progeroid mice that are deficient in NER, which is the pathway that removes transcription-blocking bulky lesions, experience high levels of transcriptional stress coupled with alterations in gene expression in postmitotic cells (46). These gene expression profiles are similar to the reduced expression of long genes and the elevated expression of short genes observed during normal aging (47). Because DNA damage occurs stochastically, gene expression is particularly reduced in the context of long genes likely because the length of the open reading frame correlates with the propensity of incurring DNA lesions. Of note, RNA polymerase stalling is not the only possible explanation for the age-dependent decline in transcription output. Other, not mutually

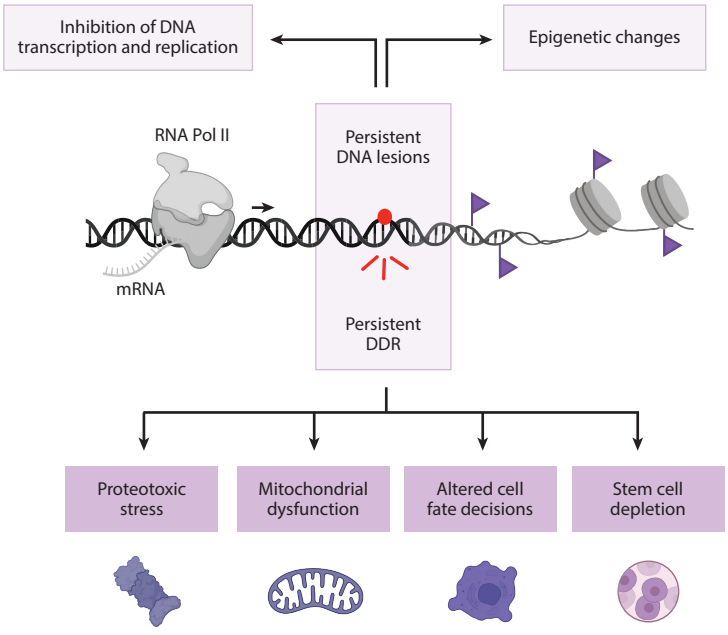

Figure 2

Consequences of persistent DNA lesions and DNA damage signaling during somatic aging. Unrepaired DNA lesions are steric obstacles to the transcriptional (*shown*) and replicative machineries (*not shown*). As a consequence, they can directly impact gene expression and interfere with DNA replication. In addition, unrepaired DNA damage and also DNA repair or persistent DNA damage signaling cause changes in DNA methylation and histone modification patterns (*purple flags*), which in turn affect chromatin structure and alter transcriptional output. Persistent activation of DNA damage signaling also contributes to cellular aging by promoting proteotoxic stress and mitochondrial dysfunction; inducing apoptosis or senescence, thereby altering cell fate; and depleting stem cell pools. Abbreviations: DDR, DNA damage response; RNA Pol II, RNA polymerase II. Figure adapted from images created with BioRender.com.

exclusive explanations may include defects in the recovery of gene expression, disturbed transcription factor binding or altered regulation of RNA polymerase II elongation, epigenetic effects on the open reading frames, or aberrant mRNA processing. Intriguingly, the single most effective lifespan extension treatment, calorie restriction, can alleviate the changes in length-dependent gene expression, suggesting a fundamental contribution of transcriptional decline to the aging process (47).

In cycling cells, DNA DSBs and interstrand crosslinks (ICLs) pose potentially serious challenges to replication fidelity. For example, hematopoietic stem cells (HSCs) self-renew throughout life and accumulate extensive, unrepaired DNA damage with age (48). Aged HSCs display delayed entry into S phase, high levels of replication fork stalling, and decreased replication fork speed, which have been attributed to age-related changes in *MCM* gene expression but which likely are also exacerbated by the presence of unrepairable DNA damage (49). The net result of age-associated replication stress in HSCs is exhaustion of the stem cell pool by differentiation or induction of senescence (see Section 2.2.5). In liver and kidney cells, unresolved replication stress can induce defects in chromosome segregation and polyploidization (50).

2.2.2. Persistently activated DNA damage responses trigger changes in the epigenetic landscape. DNA repair relies extensively on the action of chromatin remodelers and other

DNA- and chromatin-modifying enzymes, which dramatically alter the epigenetic landscape around DNA lesions to render damaged chromatin permissive for DNA damage signaling and repair reactions. One of the earliest events following induction of DNA breaks is the rapid phosphorylation of the histone variant H2AX, which occurs in the order of a megabase surrounding the DNA lesion and is generally used as a marker for DDR activation (51). This modification, also termed γH2AX, accumulates in aging tissues, indicative of persistent DNA damage–induced chromatin changes (6). Numerous other histone modifications are differentially regulated in the context of different DDR pathways, many of which, particularly those related to histone acetylation and methylation, are linked to aging or age-related diseases (52). In addition, DNA repair is often accompanied by the deposition of histone variants such as macroH2A1.2 and H2A.Z, which have been shown to increase during aging (53, 54).

Even if DNA repair is completed and DDR pathways are turned off, the original epigenetic state is not necessarily completely reset, resulting in the persistence of damage-induced epigenetic alterations following lesion removal. For example, in *Caenorhabditis elegans*, repair of transcription-blocking DNA lesions is concluded by the post-repair deposition of H3K4me2, which facilitates the recovery of protein biosynthesis and homeostasis (55). In addition, HR-mediated repair of DNA DSBs induces de novo methylation of the repaired DNA segment (56). Of note, DNA DSBs trigger long-term alterations in the histone methylations H3K4me2/3 and H3K9me2/3, which not only are transmitted to daughter cells but can be further altered by BER-mediated transcriptional demethylation 7 days after the initial DNA damage has occurred (57). These mechanisms likely contribute to the heterogeneity in DNA methylation profiles seen among distinct cell populations during aging (58).

The potential consequences of epigenetic and chromatin changes induced by persistent DDR activation are wide ranging. The redistribution of transcriptionally activating and repressive chromatin marks is likely a contributor to the increase in cell-to-cell transcriptional heterogeneity (also called transcriptional noise) that is observed in many aging tissues (59, 60) and sufficient for an accurate prediction of age (61). It likely not only drives non-cell-type-specific gene expression patterns but also promotes proteostatic stress (see Section 2.2.4) and causes loss of cell-to-cell communication. DDR-induced chromatin changes can also lead to massive alterations in local and global chromatin structure, which in turn affect transcriptional output and may render DNA susceptible to additional damage.

2.2.3. DNA damage promotes mitochondrial dysfunction. Mitochondrial dysfunction is a primary hallmark of aging (62). Mouse models for progeroid genome instability syndromes, including Cockayne syndrome (CS, mutations in *CSA* and *CSB*), xeroderma pigmentosum (XP, mutation in *XPA* to *XPG*), and AT (mutations in *ATM*), display mitochondrial dysfunction phenotypes such as increased membrane potential and oxygen consumption rates as well as accumulation of damaged mitochondria, indicating direct links between nuclear DDR pathways and mitochondrial dysfunction during aging (reviewed in 63). ATM, in particular, appears to have a central role in connecting nuclear DNA damage levels to mitophagy and apoptosis via NEMO, JNK, and BID signaling pathways (64): At low DNA damage levels, ATM ensures mitochondrial homeostasis by promoting mitophagy. However, at high, persistent DNA levels, ATM instead triggers apoptotic death. Individuals with AT clinically resemble patients suffering from mitochondrial diseases (65), underscoring the importance of mitochondrial dysfunction in the etiology of this syndrome.

Another DDR protein that directly connects nuclear damage to mitochondrial dysfunction is PARP1, which catalyzes the NAD^+-dependent formation of poly-ADP ribose (PAR) chains at DNA damage sites (66). It is activated in response to DNA SSBs and DSBs but also bulky lesions, base mismatches, and replication stress. It is essential for multiple aspects of DDR signaling and

repair, including lesion detection, recruitment and regulation of repair factors, local phase separation and chromatin remodeling (66, 67). PARP1 is a major consumer of NAD^+, which is the precursor for its PAR chains. The excessive activation of PARP1, for example, in the context of persistent DNA damage, leads to severe depletion of NAD^+ from various other fundamental metabolic processes, most notably mitochondrial respiration. A main consequence of this is the inhibition of cellular ATP production, which causes a cellular energy crisis that feeds back to every other aspect of cellular metabolism and function (11, 68). NAD^+ is also a rate-limiting cofactor of sirtuin deacetylases, which regulate many acetylation-dependent processes in mitochondria, the cytosol, and the nucleus. Accordingly, the DNA damage–dependent hyperactivation of PARP1 causes indirect inhibition of these sirtuins, leading to profound mitochondrial dysfunction and decreased mitophagy (12, 69) but also triggering changes in chromatin structure and gene expression (70) and reducing DNA repair efficiency (71, 72). Prolonged hyperactivation of PARP1 when DNA damage cannot be repaired, NAD^+ depletion, and PARP1-dependent free PAR chains trigger a programmed cell death pathway called parthanatos, which is affiliated with many aging-associated diseases ranging from diabetes to cardiovascular disease, neurodegenerative diseases, and others (reviewed in 73). This pathway is mediated by the mitochondrial release of apoptosis-inducing factor (AIF) but is independent of canonical apoptosis, necrosis, or autophagy. PARP1 hyperactivation is observed in many aging-associated neurodegenerative diseases (11, 74), while NAD^+ levels are reduced in aged mice and *C. elegans* (69). Conversely, inhibition of PARP1 or supplementation of NAD^+ boosts mitochondrial function, reduces parthanatos, and partially alleviates aging-associated phenotypes, also in the context of DNA repair deficiency (12, 73, 75, 76).

2.2.4. DNA damage causes proteotoxic stress. Persistent DNA lesions and their associated responses are tightly connected to the onset of proteotoxic stress during aging. Indeed, cells from individuals carrying mutations in genes that encode DDR proteins such as ATM (77, 78), WRN (79), and XPA (80) accumulate significantly higher levels of protein aggregates compared to wild-type cells. In addition, DNA repair–defective progeroid mice hyperactivate the endoplasmic reticulum unfolded protein response, suggesting that the inability to repair DNA damage causes the accumulation of misfolded proteins (46). Several mechanisms contribute to DNA damage–related proteotoxic stress. For example, a major consequence of DNA damage–dependent transcriptional stalling and increased transcriptional noise is the generation of stoichiometric imbalances in the assembly of macromolecular protein complexes (81). In addition, dysfunctional transcription of genes encoding chaperones or components of the ubiquitin-proteasome system (UPS) and autophagy system will directly lead to cytotoxic protein misfolding and aggregation (82).

Furthermore, the DDR modulates the activity of the UPS, for example, by adding a plethora of posttranslational modifications to proteasomal subunits (83). This is important to facilitate the scheduled degradation of chromatin and repair factors to promote DNA repair (84). Cells from individuals carrying mutations in *XPA*, which is essential for NER, exhibit lower proteasome activity and accumulate protein aggregates (80), and *XPA*-deficient *C. elegans* show increased levels of polyubiquitylated proteins (85), indicating that persistent DNA damage can cause proteotoxic stress by overwhelming the UPS.

DDR pathways also crosstalk directly with the autophagy system, which is essential for the clearance of misfolded proteins, abnormal macromolecular complexes, and damaged or supernumerous organelles. For example, ATM and ATR (ataxia-telangiectasia and Rad3-related), which are apical kinases in DNA damage signaling, stimulate autophagy after DNA DSB induction and in the context of oxidative stress (via ATM) or after treatment with UV and alkylating reagents (via ATR) (86, 87). The damage-dependent activation of autophagy is conserved from yeast to humans and has been coined genotoxin-induced targeted autophagy to distinguish it from

rapamycin-induced autophagy (88). High levels of persistent DNA damage disturb autophagic flux and protein turnover, which is especially relevant in neurons because they are particularly sensitive to proteotoxic stress and critically rely on proficient autophagic responses (89, 90).

2.2.5. Persistent DNA damage alters cell fate decisions. If DNA damage cannot be repaired in a timely manner, for example, when the DNA damage load is too high for the repair machinery to cope with, cells will initiate cell death or enter senescence. During aging, this has profound systemic consequences, as it increases inflammation, drives stem cell exhaustion, and decreases tissue connectivity and homeostasis.

2.2.5.1. Apoptosis. Apoptosis is a programmed form of cell death that is essential for countless biological processes during development and adulthood. The main executors of the apoptotic program are caspases, which are activated through several extrinsic and intrinsic pathways (91). Many types of cytotoxic stressors can initiate apoptosis, one of which is persistent DNA damage. In this case, the transcription factor p53 promotes the expression of proapoptotic factors and of a signaling cascade whose endpoint is caspase activation and cell demolition. Tissues from numerous organs display increased apoptotic activity during aging (92–95), although it is not well understood to what degree DNA damage, as opposed to other types of cellular stress signals, contributes to this increase.

2.2.5.2. Senescence. Senescence is a cellular state of stress-induced cell cycle arrest, in which damaged cells are not able to proliferate nor to die (96). The metabolic landscape of senescent cells is heterogeneous but highly divergent from that of normal cells. It is particularly characterized by the production and secretion of a plethora of factors that include proinflammatory cytokines and chemokines termed the senescence-associated secretory phenotype (SASP), which promotes the clearance of damaged cells by the immune system. A negative side effect of this immunoclearance mechanism is, however, the induction of local chronic inflammation and tissue damage (96). Senescent cells can be observed in many aging tissues (97, 98), although an accurate estimation of senescence burden during aging is currently not known. Nevertheless, data from many studies suggest that senescence-induced tissue inflammation contributes to multiple age-related diseases, including atherosclerosis (99), cancer (100), and neurodegeneration (101). The cellular stresses causing age-related senescence can be manifold, ranging from mitochondrial to proteotoxic but also genotoxic stress (96). A direct relationship between persistent DNA damage and induction of senescence has been described in the context of mammalian cell cultures and in many aging mouse and human tissue types (102–104). Senescent cells also display so-called DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS), which are subnuclear foci enriched for DDR markers that likely reflect persistent chromatin changes induced by unrepairable DNA damage and promote the maintenance of senescence-associated growth arrest and the secretion of IL-6, a SASP component (105, 106). Interestingly, persistent DNA lesions in senescent cells are particularly enriched at telomeres, likely because DDR mechanisms are suppressed at these loci to ensure telomere integrity (102, 104).

Of note, not all instances of induced inflammation during aging occur through senescence. For example, during aging of the skin, repeated UV exposure causes oxidative stress in dermis cells that triggers the innate immunity's complement system and the infiltration of macrophages into the dermis. Release of reactive oxygen species (ROS) and proinflammatory cytokines then causes chronic inflammation (107).

2.2.5.3. Stem cell functionality. DNA damage accumulates not only in differentiated tissues but also in stem cells (reviewed in 48). Depending on the stem cell niche, persistent DNA damage can reduce either proliferative or differentiation capacity, cause premature differentiation, or

trigger apoptosis or senescence (48). In addition, mutations may clonally expand, leading to the accumulation of functionally altered stem cell populations (108). The net outcome is functional decline of the stem cell niche over time and, ultimately, stem cell exhaustion and loss of tissue homeostasis. For example, HSCs, which are critical for the maintenance of the hematopoietic system, have been shown to decline in number and function with age (49, 109, 110). Progeroid mouse models deficient in telomere maintenance or various DNA repair pathways loose a functional HSC niche in bone marrow (109, 111, 112). HSCs are particularly sensitive to the loss of ERCC1-XPF, which is a key factor in NER but also in ICL and DSB repair because they fail to renew and differentiate in the absence of this DNA repair protein. Accordingly, ERCC1-XPF deficiency leads to the decline of the stromal cell population in bone marrow (113). Together, these data suggest that persistent DNA damage can be detrimental to stem cell homeostasis and promote stem cell aging.

2.3. Cell and Tissue Specificity of Somatic Genome Instability

The spectrum of somatic mutations and the total mutational burden depend on the age of the donor and vary between cells and tissues (18). For example, bile ductal cells acquire on average 9 mutations per year, whereas intestinal crypts accumulate as many as 49 mutations within the same time frame (20). In addition, rapidly proliferating cells of the small intestine accumulate mainly SNVs, whereas largely postmitotic organs such as the brain additionally show evidence of much harder to detect SVs (43, 114, 115). These differences reflect the fact that tissues are differentially exposed to endogenous and exogenous genotoxic stimuli, causing different types of DNA damage.

Germline mutations in components of DNA repair pathways cause devastating human diseases collectively referred to as genome instability syndromes (reviewed in 116). The phenotypes imparted by these monogenic syndromes are heterogeneous but can be categorized broadly into developmental defects; congenital defects in various tissues and organs; increased cancer incidence; and, in many cases, accelerated onset of aging phenotypes. Progeroid genome instability syndromes do not fully recapitulate normal aging. Most of them display instead segmental aging phenotypes, the nature of which depend on the DNA repair pathway that is affected in the disease (116).

For example, defects in DNA SSB repair factors such as APTX, TDP1, and XRCC1 cause premature aging primarily in the cerebellum, likely because this tissue experiences comparatively high levels of oxidative stress and ROS, which induce SSBs. By contrast, mutations in NHEJ repair factors such as ATM, NBS, and LIG4 are profoundly immunodeficient and have a high risk to develop early-onset lymphoma and leukemia because NHEJ-mediated DSB repair is essential for successful V(D)J and class switch recombination during the maturation of the immune system (117). In addition, ATM mutations also cause neurodegeneration, which primarily affects Purkinje cells in the cerebellum. Why Purkinje cells are particularly sensitive to ATM deficiency is only partially understood (118).

Another case in point is a syndrome, XP, which is caused by mutations in a number of genes (*XPA*, *XPB*, *XPC*, *XPD*, *XPE*, *XPF*, *XPG*, and *XPV*) encoding central players of the NER pathway (119). XP patients suffer from pigmentation abnormalities, skin atrophy, and a several-thousandfold increased skin cancer susceptibility. These pathologies are all linked to the failure of skin cells to remove UV irradiation–induced cyclobutane pyrimidine dimers (CPDs) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PPs), which are bulky, helix-distorting DNA lesions requiring removal by the NER pathway. These lesions are highly mutagenic when left unrepaired, thus triggering skin cancer development.

A progeroid genome instability syndrome that affects multiple organ systems is Bloom syndrome (BS). It is caused by mutations in the gene encoding the RecQ helicase BLM, which is a

critical regulator of recombination and also DNA end resection in multiple repair processes, most notably the replication stress response, the DNA DSB response, and telomere maintenance (120). Recently, BLM has also been implicated in the processing of ultrafine anaphase bridges during mitosis and of DNA secondary structures such as G-quadruplexes as well as in the regulation of RNA::DNA hybrids (121). BS patients have an average lifespan of 26 years and suffer from a wide spectrum of aging-associated diseases such as chronic obstructive pulmonary disease, diabetes, and especially early-onset cancer, which is the principal cause of death for these patients (120). What sets BS apart from most other genome instability syndromes is that cancer development is not restricted to certain tissues or organs. Instead, the distribution of cancers with regard to type and anatomic location is similar to the general population, with the main difference being that in BS patients, cancers develop much earlier in life. One possible explanation is that BLM can act as a powerful tumor suppressor because of its multiple functions in various DNA repair pathways but in particular because it restricts excessive DNA recombination. Indeed, BLM deficiency causes an extremely high frequency of sister chromatid exchanges and high rates of heterozygosity, which then affect cycling cells across all organ systems (reviewed in 121).

Together, progeroid genome instability syndromes are important model systems to dissect how distinct types of DNA lesions and their corresponding repair pathways contribute to the various phenotypic aspects of aging.

3. ROLE OF GENOME INSTABILITY DURING REPRODUCTIVE AGING

3.1. The Distinct Mechanisms of Female and Male Gametogenesis

Oocytes generally provide, in addition to the maternal genome, most of the cytosolic components, including most organelles to the zygote. Therefore, a significant amount of resources is deposited into oocytes, making their maintenance an important factor that impacts the maintenance mechanisms of maternally inherited genomes even though the detailed mechanism of oogenesis can vary among different species (122). In most mammals, oogenesis is initiated during fetal development and arrested at the germinal vesicle (GV) stage of the first meiotic prophase at around the time of birth. Females are born with a limited number of GV oocytes, which are encapsulated with a single layer of somatic cells called granulosa cells. These oocytes with surrounding granulosa cells form a structure named the primordial follicle, and they may reside within the ovary for as long as 50 years before developing to mature oocytes. Upon puberty, an endogenous luteinizing hormone surge stimulates the maturation and ovulation of primordial follicles. During this maturation, an oocyte resumes meiosis I, and this metaphase I (MI) oocyte divides into two daughter cells: a haploid secondary oocyte and an extruded polar body. The secondary oocyte arrests again at the metaphase II (MII) stage and resumes meiosis until successful fertilization. After fertilization, meiosis is completed with the extrusion of the second polar body (122) (**Figure 3**).

Spermatogenesis produces mature sperm from primordial germ cells (123). Spermatozoa provide few resources, such as the centrioles, and are usually much smaller than oocytes, requiring a high compaction of the paternal genome, which has a profound impact on the genome structure and its maintenance. Spermatogenesis can be divided into three phases: proliferative, spermatogonial, and spermiogenesis. In the first phase, the primordial germ cells, called spermatogonia cells, undergo several rounds of DNA replications and mitosis to maintain the pool of stem cells. At each round of proliferation, the spermatogonia can either renew themselves or differentiate into a new cell type. The self-renewal capacity is retained in the spermatogonia until the generation of type B spermatogonia, which are the precursors of the spermatocytes. Type B spermatogonia enter the last round of mitosis and produce primary spermatocytes—the cells that initiate meiosis. In the second phase, the primary spermatocytes will complete meiosis I and II and eventually

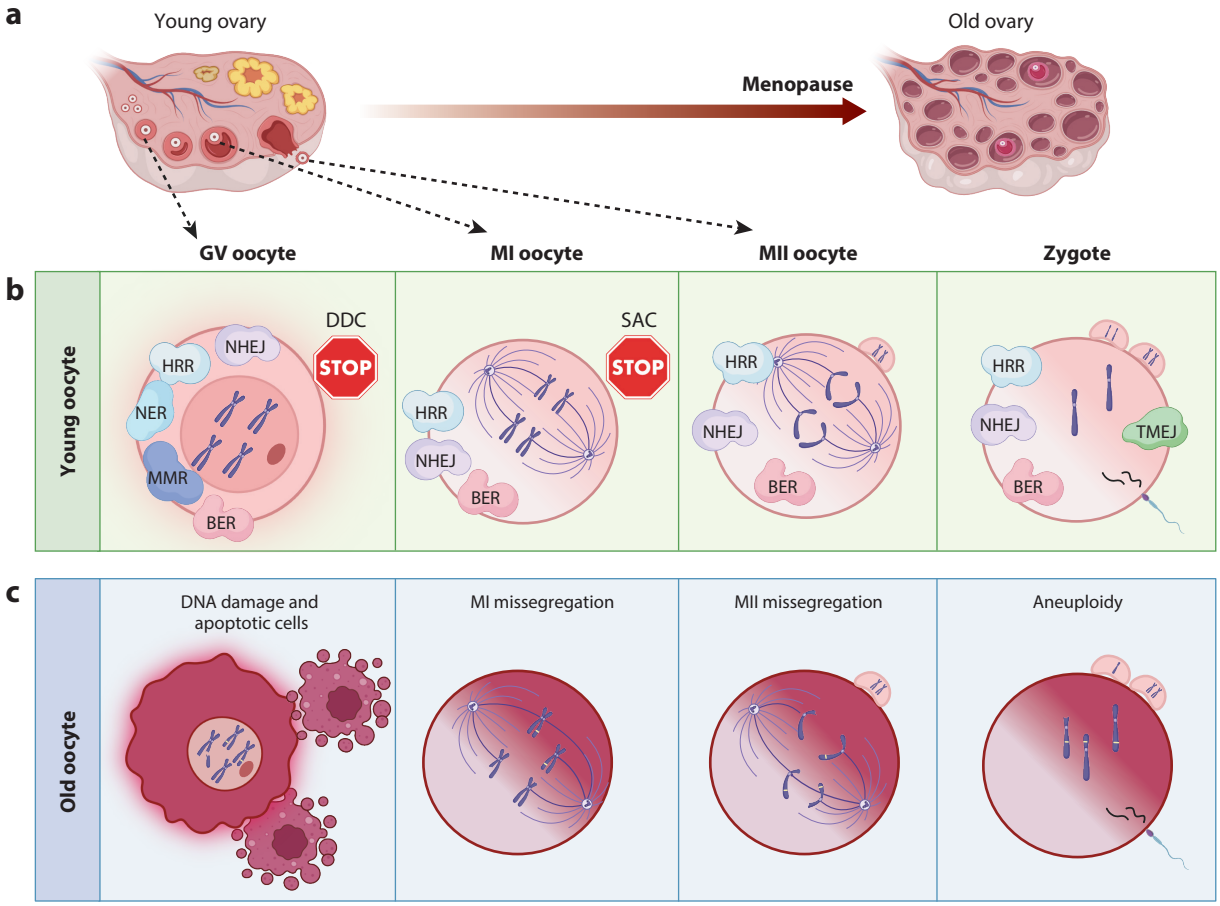

Figure 3

Genome instability and female reproductive aging. (*a*) Schematic representation of ovarian aging. The young ovary exhibits normal oogenesis (as shown in panel *b*), which displays different stages of the oocyte. However, advanced maternal age leads to the depletion of primordial follicles and degeneration of ovary morphology and structure, ultimately culminating in menopause and the end of the female reproductive lifespan. (*b*) At young maternal age, different stages of the oocyte employ distinct DNA repair mechanisms. The repair processes include HRR, NHEJ, MMR, NER, BER, and TMEJ. The DDC can be triggered in GV-stage oocytes but is lost in later stages of oogenesis. After GVBD, MI oocytes may arrest due to the activation of an ATM/ATR-independent SAC. (*c*) At advanced maternal age, more GV-stage oocytes undergo apoptosis. Increased DNA DSBs, shortened telomeres, and elevated DNA mutations are observed in all oocyte stages, accompanied by decreased expression of DNA repair proteins. Chromosome missegregation rates are significantly increased during MI and MII mitosis, resulting in embryonic aneuploidy. Abbreviations: ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; BER, base excision repair; DDC, DNA damage checkpoint; DSB, double-strand break; GV, germinal vesicle; GVBD, germinal vesicle breakdown; HRR, homologous recombination repair; MI, metaphase I; MII, metaphase II; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; SAC, spindle assembly checkpoint; TMEJ, theta-mediated end joining. Figure adapted from images created with BioRender.com.

generate haploid spermatids. From phase I to phase II, the cells gradually move further from the basement membrane of the seminiferous tubule and closer to the lumen. In the last phase of spermatogenesis, the spermatids mature and differentiate into spermatozoa, which contain acrosome and flagellum that allow spermatozoa to meet and fertilize eggs (**Figure 4**).

A major change during the last phase is the exchange of histone with protamine, which is a relatively small protein containing mainly arginine (124). Protamines replace 90–95% of histones in

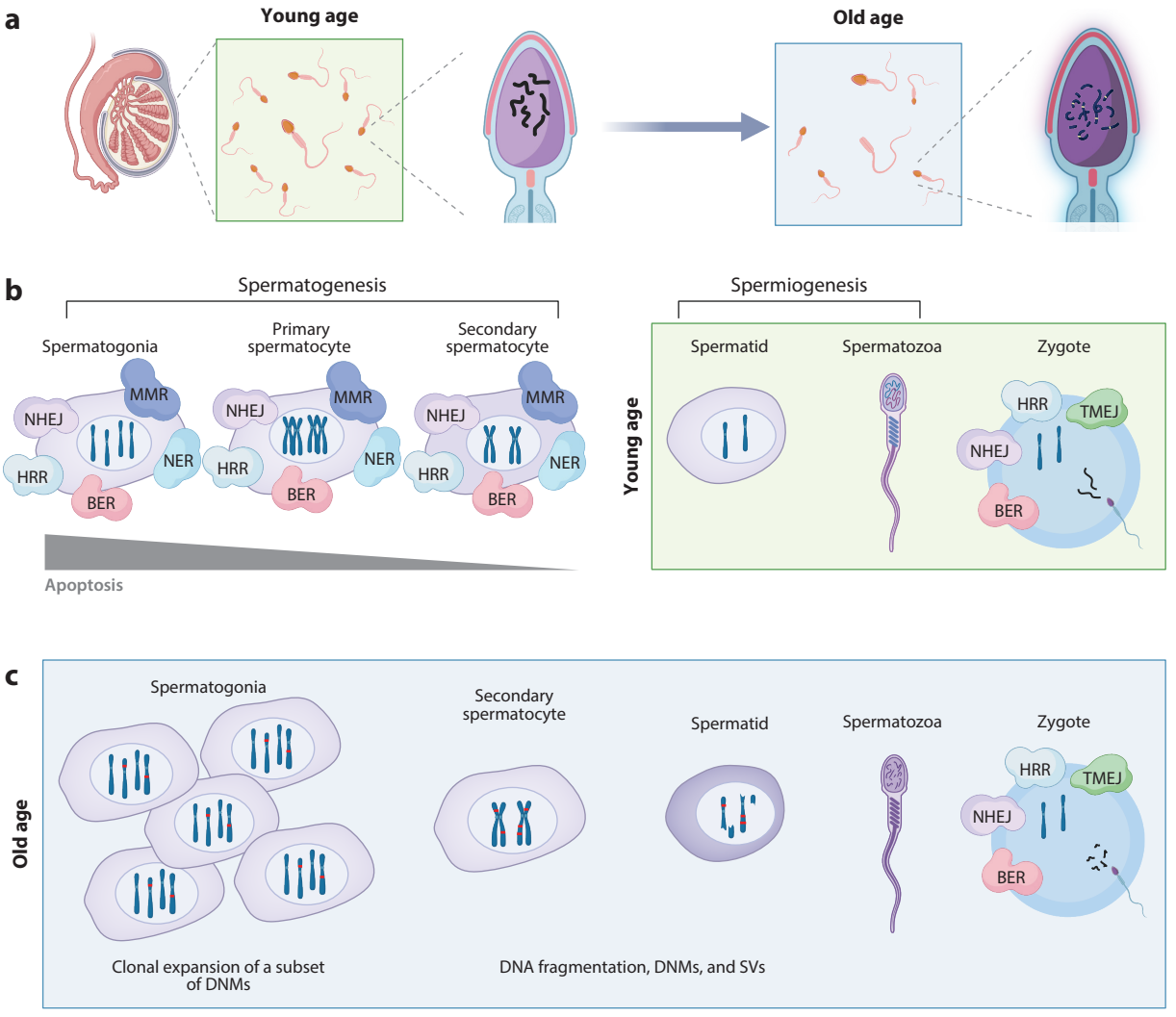

Figure 4

Genome instability and male reproductive aging. (*a*) Illustration of the effects of advanced paternal age on sperm quantity and quality. With increasing paternal age, there is a decline in both the quantity and quality of sperm. This decline is associated with an increase in the DNA fragmentation index, DNMs, and SVs. (*b*) Depiction of the process of spermatogenesis, which includes the mitotic spermatogonia and meiotic spermatocyte stages, and spermiogenesis, which is the maturation of spermatozoa. Spermatogonia and spermatocytes are highly resistant to DNA damage due to their full engagement of several DNA repair mechanisms, including HRR, NHEJ, MMR, NER, and BER. By contrast, spermatids and mature spermatozoa have limited DNA repair capacity, and any persistent DNA damage may be repaired by the oocyte after fertilization via HRR, NHEJ, BER, and TMEJ. Unrepaired DNA damage during spermatogenesis can trigger apoptosis, which is lost during spermiogenesis. (*c*) A selective expansion of a small subset of DNMs (*red marks*) detected in spermatogonia of males with advanced age. Additionally, DNMs and SVs (*red marks*) are increased in sperm due to continuous cell divisions. The increased DNA fragmentation in spermatozoa associated with advanced paternal age may contribute to embryonic aneuploidy after fertilization due to the engagement of error-prone repair machinery. This figure speculates that DNA damage hypersensitivity during spermiogenesis causes sperm DNA fragmentation. Abbreviations: BER, base excision repair; DNM, de novo mutation; HRR, homologous recombination repair; MMR, mismatch repair; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; SV, structural variant; TMEJ, theta-mediated end joining. Figure adapted from images created with BioRender.com.

mature spermatozoa via a multistep process that requires the loosening of the chromatin structure by histone hyperacetylation and the introduction and—following histone replacement—ligation of DNA breaks by topoisomerase II. Failure to ligate the breaks can lead to DNA fragmentation, resulting in poor embryo quality.

Genome stability in germ cells is essential for maintaining germ cell quantity and quality. Importantly, DNA damage in germ cells may result in heritable mutations that can lead to genetic disorders. Recently, transgenerational effects impacting the genome integrity of offspring have also been observed. Here, we discuss the DNA repair mechanisms in oocytes and sperm and how they impact reproductive aging. In addition to nuclear DNA, mitochondrial DNA is also subject to genotoxic insults. We summarize the role of mitochondrial genome instability during reproductive aging in the **Supplemental Text**.

Supplemental Material >

3.2. Maintenance of Genome Stability in Oocytes

Females are born with a limited number of oocytes, and the long-lasting arrest of primordial follicles causes oocytes to be subjected to various endogenous and exogenous genotoxic sources that threaten their genome stability. Therefore, maintaining genome stability during the extended periods of oocyte arrest is crucial for preserving their function.

Oocytes employ several strategies to mitigate the detrimental effects of DNA damage (**Figure 3**). For example, TAp63-mediated apoptosis in primordial follicles safeguards oocytes from propagating harmful mutations to offspring (13, 125). However, this apoptosis quality-control mechanism is only present in oocytes from primordial to primary follicles but absent from fully grown oocytes (125). One would anticipate that other DNA damage responses may provide protection for the oocyte's genome integrity. Moreover, blocking the apoptosis pathway in female mice still gives rise to healthy offspring (13), implying that oocytes can rapidly detect and repair damaged DNA. However, unlike somatic cells, oocytes lack a robust G2/M DNA damage checkpoint (126). Thus, provided the genome damage is not too severe, oocytes can proceed to germinal vesicle breakdown (GVBD) but will arrest at the MI stage through the engagement of an ATM/ATR-independent spindle assembly checkpoint (SAC) (127). Oocytes can engage a large spectrum of DNA repair pathways, as HR, NHEJ, BER, MMR, and NER components are expressed throughout all developmental stages of oocytes (128).

DSBs in oocytes are considered a major threat to genome integrity. DNA DSBs are commonly induced and accumulate in oocytes as a consequence of meiotic recombination, endogenous oxidative stress, exogenous stressors, and maternal aging (129, 130). The DSBs in oocytes are mainly repaired by two pathways, HR and NHEJ. Due to the presence of the sister chromatid in meiotic prophase oocytes, HR is considered to be the predominant repair pathway in arrested primordial follicles (131). Blocking apoptosis in primordial follicles can activate HR to remove DSBs efficiently (132), and key components of HR, such as RAD51 and BRCA2, are highly expressed in primordial follicle oocytes (133). Reducing RAD51 in oocytes induces cell death due to the inability to remove DNA lesions (133). In contrast to HR, NHEJ can be error prone, since the DSBs are directly ligated without using homologous templates. NHEJ is involved in DNA DSB repair in the MI stage oocytes and promotes the meiotic progression to MII via mediating the SAC, as inhibiting NHEJ leads to increased MI arrest (134). Taken together, in oocytes, HR and NHEJ coordinate to repair the DSBs during meiosis, with each pathway having a distinctive role along different stages of oocyte maturation.

Metabolic activity in primordial follicle oocytes triggers not only DNA DSBs but also SSBs, which are repaired mainly by BER. The BER components PARP1 and XRCC1 are readily detectable in oocytes (128). An oocyte deficient in PARP1 exhibits incomplete homologous

chromosome synapsis and persistent DNA damage (135). Both PARP1 and PARP2 are required for oocyte maintenance, as their double knockout causes depletion of the ovarian reserve. In addition, the XRCC1-binding partner, polymerase β (Polβ), which is crucial for ligation efficiency during BER, shows decreased expression in aged mouse oocytes (136). Heterozygosity of Polβ causes the depletion of ovarian reserve, while overexpression of Polβ can increase oocyte survival (136). Together, these data suggest a central role of BER in maintaining oocyte genome stability.

Additional DNA repair pathways, such as MMR and NER, also play important roles in genome maintenance in oocytes. For example, mutations in MMR genes lead to a defect in the completion of meiosis upon fertilization in mice (137), and mice carrying mutations in the NER genes *Ercc1* and *Xpd* show decreased fertility (138).

3.3. Genome Instability and Female Reproductive Aging

Due to social and/or economic reasons, an increasing number of women tend to delay having children until they reach an advanced age. It is well documented that advanced maternal age results in a decrease in fertility and an increase in chromosomal aberrations and miscarriage. Increasing maternal age is associated with the loss of genome stability in oocytes. Understanding the role of genome instability in female reproductive aging is important for developing potential therapeutic approaches to prevent the adverse consequences of reproductive aging.

3.3.1. Genome instability and age of natural menopause. Menopause marks the end of the female reproductive lifespan in humans, characterized by the permanent cessation of ovarian function. The age of natural menopause (ANM) ranges from 40 to as late as 62 years. Early or late ANM is not only linked with female fertility (139) but also associated with the risk of breast cancer (140), osteoporosis (141), and cardiovascular disease (142). The variation in the ANM illustrates the variability of ovarian aging, and the multiple factors that are involved in the regulation of ANM. Genetic factors were suggested to account for about 42% of the variation in ANM (143). Genome-wide association studies (GWASs) of ANM have so far identified genetic loci involved in DDR, immune function, and mitochondria biogenesis (144). Mechanistically, DDR genes were indeed shown in mice to impact the pace of the age-dependent loss of the ovarian reserve.

The role of DDR and genome stability in ANM was first reported by two large GWASs (145, 146) that both identified a DNA damage response gene, *MCM8*. The MCM protein family is a key component of the prereplication complex and regulates DNA replication (147). In addition, MCM8, together with MCM9, can form a complex at the DSB site and recruit the HR protein RAD51 to promote error-free repair (148). Mutations in *MCM8* are associated with a delay in ANM (each allele of *MCM8* could postpone the ANM by 1 year) and ovarian failure (149), indicating a crucial role of DSB repair in maintaining normal ovarian function. So far, around 300 genomic loci associated with ANM have been identified, and broader involvement of DDR in ANM was confirmed by several independent GWASs, particularly genes involved in HR (144). Specifically, BRCA1 and its associated proteins, which are implicated in most genetic studies of ANM, are key components of HR and related to breast and ovarian cancer (150). This finding could partially explain the clinical correlation between the early onset of menopause and the risk of breast cancer (140). In addition, the MMR genes *MSH5* and *MSH6* have been identified in two independent GWASs (151, 152). Mutations of *MSH5* and *MSH6* cause a shortening of reproductive lifespan and early ANM. The ANM might also have a hereditary effect, as a recent study suggested that women who have an earlier ANM tend to pass down more de novo mutations to their offspring (153). Genome stability is thus an important determinant of ANM and reproductive aging.

3.3.2. Genome instability and oocyte aging. Maternal age is associated with an increased incidence of chromosomal abnormalities in oocytes (154), indicating an age-dependent decline of genome stability in oocytes. In aging human and rodent primordial follicles, expression of the DSB repair genes *BRCA1* and *RAD51* declines and is correlated with increased levels of γH2AX, indicative of DSBs (155). The accumulation of DNA damage and reduced DNA repair were also observed in the granulosa cells that are essential for follicle growth and development. Aging granulosa cells in rhesus monkeys displayed increased γH2AX and decreased BRCA1 (156), suggesting declining DNA repair capacities. Mutations in *BRCA1* indeed reduced primordial follicle numbers and perturbed the reproductive capacity (157). Although the expression of BRCA2, another protein involved in DSB repair, does not show a change during oocyte aging, women carrying mutations in *BRCA2* show primary amenorrhea with reduced recruitment of RAD51 to DSB sites (158). In addition to DSB repair proteins, one study reported that the expression of XPD (or ERCC2) is decreased in aged rat primordial follicles (159). Together, these findings indicate that oocyte aging is associated with reduced DNA repair capacity and increased DNA damage. The decline of DNA repair capacity also suggests that older women are more sensitive to DNA damage agents, such as chemotherapy or radiotherapy. Indeed, there is an observation that the risk of acute ovarian failure increased significantly with the age of receiving cancer treatment (160).

The shelterin complex at the telomeres protects the end of chromosomes from being detected as DSBs, and their attrition also impacts genome stability in oocytes. Due to the high guanine content of the repetitive telomeric sequence, telomeres are susceptible to oxidative stress and ROS, which could induce DNA SSBs at telomeres and lead to telomere shortening (161). An analysis of women undergoing in vitro fertilization at an advanced age showed that they have a tendency to have shorter telomere length compared to young women (162). In addition to maternal age, pathological conditions that increase ROS levels, such as obesity, could also shorten telomeres and accelerate reproductive aging (163). The length of telomeres can be extended by telomerase activity or the telomerase-independent alternative lengthening of telomeres (ALT) mechanism. Older female mice exhibit a decline in the expression of TERT, a catalytic enzyme of telomerase, and telomeric activity in oocytes compared with young mice (164). Mutations in telomerase in rodents and humans can lead to progressive reduction in telomeric length over generations and result in sterility due to the chromosomal misalignments in oocytes (165). The ALT pathway is utilized in oocytes after fertilization (166), although it remains unclear whether it is involved in oocyte aging. In *C. elegans*, mutation of *rfs-1*, a paralog of a human DNA repair gene *RAD51D*, leads to characteristics of ALT and causes transgenerational sterility, which is exacerbated by maternal age (167). Telomeric diseases also impact the oocyte quantity and quality. For example, dyskeratosis congenita is a telomeropathy, which is attributed to mutations in telomerase-associated factors such as *DKC1*. Patients suffering from dyskeratosis congenita have telomere shortening in oocytes and embryos and poor responses to ovarian stimulation (168). The shortening of telomeres in oocytes can negatively affect oocyte quality and female fertility regardless of maternal age.

3.4. Genome Instability and Male Reproductive Aging

Unlike women, men do not experience a rapid, drastic decline in fertility with age. Male reproductive aging occurs gradually with increased sperm DNA fragmentation and mutations. Importantly, increasing paternal age correlates with congenital disease in their offspring. This highlights the importance of understanding the genomic stability of sperm DNA during male reproductive aging.

3.4.1. Maintenance of genome stability in sperm. Sperm DNA damage can be triggered by either endogenous or exogenous sources (**Figure 4**). Endogenous sources include replication

error, ROS, histone replacement, and abortive apoptosis (cells destined to be eliminated that escape apoptosis and are released in the ejaculate). Exogenous sources can be chemo- or radiotherapy, smoking, alcohol abuse, and air pollution. Spermatogonia are efficient in DNA surveillance and repair until the spermatozoa are transmitted and stored in the epididymis. Mature sperm, so-called spermatozoa, have a limited ability to repair DNA damage. Interestingly, spermatozoa harboring DNA damage show no difference in fertilization rate compared to undamaged sperm (169). In this case, the oocyte repair machinery is responsible for repairing sperm DNA damage.

Spermatogonia stem cells are highly resistant to DNA damage, as they are equipped with most DNA repair mechanisms. The main source of DNA damage in early spermatogenesis is linked to the proliferation of spermatogonia. This replication-related DNA damage is repaired by the efficient proofreading function of DNA polymerases along with MMR. Male mice deficient in the MMR proteins MLH3 and MLH1 suffer from infertility, as spermatocytes fail to proceed beyond the pachytene stage of meiosis (137, 170). Sperm DNA is also prone to oxidative damage due to the activity of mitochondria, which is repaired by BER (171). However, once the spermatogonia differentiate into spermatozoa, the BER pathway becomes truncated and cannot exert the full repair capacity (172). In this case, the persistent DNA damage will only be repaired in the fertilized oocytes. Furthermore, NER is also active during spermatogenesis, removing helix-distorting lesion types. DNA DSBs can also be efficiently removed during spermatogenesis, primarily by HR and NHEJ (173). Mutations in NHEJ and HR lead to increased apoptosis, chromosomal abnormalities, and embryonic lethality (174). In addition, alternative end joining and single-strand annealing contribute to the repair of DSBs, particularly in the absence of NHEJ (175).

Unrepaired DNA damage in spermatogonia can trigger apoptosis. However, once the spermatogonia transform into differentiated spermatozoa, they gradually lose their capacity to undergo apoptosis since these cells are transcriptionally and translationally silenced (176). Sometimes incomplete apoptosis, or abortive apoptosis, in spermatozoa leads to DNA fragmentation and impaired embryogenesis.

The highly compacted chromatin structure prevents the engagement of DNA repair mechanisms in mature sperm. Thus, DNA damage occurring in mature sperm is only repaired after fertilization by maternal DNA repair proteins, and here the zygotic repair fidelity of paternal DNA is surprisingly low. Chromosomal SVs in embryos such as reciprocal translocations are primarily of male descent (177). Sperm treated with mutagenic agents, such as ethyl methanesulfonate (EMS) and radiation, result in chromosomal aberrations in zygotes (16, 178). This chromosomal aberration indicates the engagement of error-prone repair machineries in the zygotic stage. After fertilization, the paternal chromatin is decondensed by topoisomerase II, resulting in transient DNA DSBs (179). Maternal NHEJ is active during the replacement of sperm protamine immediately after fertilization (180). The disruption of both HR and NHEJ in mouse oocytes can significantly increase the sperm-derived chromosomal abnormalities in zygotes. Here, the chromosomal abnormality in maternal NHEJ-deficient zygotes is much higher compared to that of zygotes with maternal HR depletion (181). We recently found that in *C. elegans*, DNA damage occurring in mature sperm is predominantly repaired by maternal theta-mediated end joining (TMEJ) (16). These observations indicate that sperm DNA damage can be repaired after fertilization by maternally provided repair machineries. However, these repair machineries are typically error prone, thus leading to paternally derived chromosomal aberrations, which may influence the viability and health of the offspring. Similarly to the SVs that are generated by maternal TMEJ of DSBs introduced by damaged mature sperm, SVs in humans are predominantly introduced through the paternal genome and carry to a significant degree the TMEJ signature (16). These observations suggest that the maternal repair machinery, by acting on paternal DNA, greatly influences genome evolution.

3.4.2. Genome instability and sperm aging. Compared to that of women, the age-associated decline of male fertility is less pronounced and less well understood. Epidemiologic studies found an association between paternal age and the increased risk of several psychiatric disorders, including autism spectrum disorder (182) and schizophrenia (183). Moreover, this correlation is also found in congenital heart disease (184), epilepsy (185), and intellectual disability (186). The increased risk of these diseases is attributed to the accumulation of paternal germline mutations and epigenetic alteration with age (187). Here, we discuss the relationship between paternal germline mutation and male reproductive aging.

Although the male germline is well protected by the DNA repair pathways, these pathways are not perfect; thus, de novo mutations (DNMs) occur in every generation. Males contribute 75–80% of DNMs to the gene pool (14, 188), and the mutation rate is positively correlated with paternal age (14). On average, each additional year in paternal age at conception results in two additional DNMs in offspring (14). Germline DNMs are the driving force for evolution, but they may also contribute to congenital diseases. The most likely cause of the paternal age effect on DNMs is the number of genome replications that accumulate with paternal age. The replication number in a 20-year-old male has been estimated to be 150, and this increases to 610 replications in a 40-year-old male (189). Although there are no replication-associated mutation signatures enriched in the paternal germline with advanced age (190), the locations of these mutations are significantly enriched in the early-replicating, genic region (191). Whole-genome sequencing revealed the presence of mutation clusters, which correspond to the observation that multiple DNMs are located in close vicinity (191, 192), indicating the existence of mutational hot spots. However, the cause of these paternal age–associated mutation hot spots is still unclear.

Advanced paternal age can also lead to a selective expansion of a small subset of DNMs in the testis (193). Mutations that promote symmetric rather than asymmetric cell divisions are consequently clonally expanded through the self-renewal of spermatogonial stem cells (194). As a result, such so-called selfish mutations accumulate with advanced age (193) and might cause several developmental disorders in the offspring (195). The incidence of those developmental diseases increases with paternal age at conception (196). The selfish behavior of mutations not only occurs in testis but also in somatic events driving tumorigenesis (195).

3.5. Transgenerational Effects of Germline Genome Instability

Whether and how maternal and paternal exposure to various environmental factors can affect the risk for diseases in subsequent generations have been subject to intense investigation and debate. Transgenerational effects have mostly been suggested to be mediated through epigenetic alterations such as DNA methylation and noncoding RNAs in the parental germline. In contrast to those transiently occurring epigenetic alterations, permanent alterations of the genome by DNA damage or mutations in the exposed parental germline could also be involved. Indeed, DNA damage in the germline induced by radiation can lead to transgenerational effects in both invertebrates and vertebrates (197). The epidemiological and genetic studies in humans, however, have remained controversial (198).

Similar to the germline DNMs, transgenerational effects in offspring mainly originate from paternal DNA damage (199). Studies in mice have shown that paternal radiation exposure results in an increased incidence of cancer in the offspring (200). An elevation of DNMs in expanded tandem repeat loci was detected in the nonexposed first-generation offspring, and the increased mutation rate is attributed to the persistence of DNA DSBs (199). Paternal exposure to radiation 6–7 weeks before conception leads to embryonic cell proliferation defects, suggesting that type B spermatogonia are responsible (201). Paternal radiation exposure can also increase chromosomal aberrations in the F1 generation (200, 202). The chromosomal aberrations and increased mutation

load in the offspring of irradiated males indicate that the stability of the F1 genome is compromised, as the amount of endogenous DNA damage is significantly elevated in the nonexposed offspring of irradiated male mice (199). In *C. elegans*, DNA damage in mature sperm results in genome instability in the nonexposed F1 generation. This effect is attributed to the engagement of the error-prone TMEJ in the paternal DNA damage in the zygote (16). These findings together provide plausible explanations for the increased incidence of cancer in the offspring of irradiated male mice. Apart from the radiation-induced germline mutations, chemotherapy can also damage the paternal germline genome and in humans is associated with hypermutation in offspring (15).

Induction of DNA damage in germ cells via smoking, exposure to environmental contaminants, and diet might also transmit developmental defects and metabolic disturbances to the next generation (203, 204). Paternal smoking is a predictor of DNA damage levels in the umbilical cord blood of the F1 generation, while maternal passive smoke exposure cannot predict DNA damage in cord blood, indicating this effect is transmitted via the spermatozoan genome (203). Obese parents tend to give birth to children who are predisposed to obesity (205). DNA damage in the sperm of obese males was suggested to occur due to an increase in oxidative stress (206). Polycyclic aromatic hydrocarbon exposure is significantly associated with increased DNA adducts in sperm and related to childhood cancer (207, 208). Pesticide use in agricultural areas has been correlated with congenital diseases in children, which is attributed to high DNA damage and ROS levels in semen (209). Taken together, the germline DNA damage–induced transgenerational effect is mainly of paternal origin, indicating that the DNA integrity of sperm can significantly influence the genome stability of the offspring.

4. OUTLOOK AND THERAPEUTIC APPROACHES TO PREVENT SOMATIC AND REPRODUCTIVE AGING

The causal role of DNA damage in the aging process, both in the various somatic cell types and in germ cells, mandates the development of intervention strategies targeting genome stability as a root cause mechanism. The complexity of the distinct DNA repair systems, each comprising multiprotein machineries, has thus far precluded significant inroads to such interventions. Prevention of exogenous genotoxic stress, by contrast, has already had a significant impact, including reducing tobacco smoking and UV-induced carcinogenesis and mitigating the risk of genotoxins in work environments. Single repair systems are also applicable to specific lesions, such as photolyase enzymes and T4 endonuclease V, which are single repair enzymes that repair UV-induced CPDs and in mice could effectively prevent UV-induced skin carcinogenesis (210). Overexpression of single DNA repair enzymes, however, appears to have heterogenous effects likely due to a disruptive effect on the stoichiometry of repair complexes (8). Modifications of enzymes involved in regulating repair processes, such as Sirt6 or the supplement of recombinant Rad51, have been reported to improve repair (9, 133, 211). Species that have adapted to ecological niches with high levels of radiation are particularly protected from DNA DSBs. *Deinococcus radiodurans* and tardigrades are examples of bacteria and animals, respectively, that are highly radiation resistant. The Dsup protein of tardigrades was initially reported to confer elevated radiation protection to human cells (212); however, an adverse effect on DNA damage in neurons was recently reported (213). Thus, it might not be a simple undertaking to introduce higher levels of genome protection from other species to humans.

A conceptually different strategy is to consider how the highly effective DNA repair mechanisms of germ cells could be transferred for somatic maintenance. We recently discovered in *C. elegans* that the DREAM complex, which is assembled in somatic cell lineages, represses the transcription of a wide array of DNA repair genes operating in all of the distinct genome maintenance pathways (214). Mutations in the various DREAM components lead to derepression of

DNA repair genes, subsequently augmenting DNA repair kinetics and conferring resistance to essentially any type of DNA damage. The DREAM complex is highly conserved and also represses DNA repair gene expression in human cells. Indeed, pharmacological inhibition of the DREAM assembly conferred DNA damage protection in quiescent human cells and prevented DNA damage accumulation and photoreceptor loss in vivo in progeroid mice. Therefore, the conferral of germline-like DNA repair capacities to somatic tissues might provide a therapeutic strategy to extend genome maintenance and potentially reduce the risk of aging-associated diseases and cancer.

In germ cells, targeting the TAp63-induced apoptosis pathway may retain the primordial follicle pool following cisplatin and radiation exposure (13, 215), making it a potential approach to prevent the aging-induced loss of primordial follicles. Advanced paternal age is associated with increased DNA damage–induced mutation rate, and the specific role of TMEJ in generating SVs in paternal genomes (16) suggests that interventions targeting TMEJ might prevent the negative heritable effects of male reproductive aging.

Taken together, the causal role of genome instability in driving the aging process has been increasingly recognized. The mechanistic understanding of the regulation of DNA repair mechanisms in somatic and germ cells has already provided fundamental conceptual insights into the role of genome maintenance in somatic and reproductive aging, its associated diseases, and how genome evolution is shaped by distinct DNA repair mechanisms.

DISCLOSURE STATEMENT

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