The central role of DNA damage in the ageing process

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Ageing is a complex, multifaceted process leading to widespread functional decline that affects every organ and tissue, but it remains unknown whether ageing has a unifying causal mechanism or is grounded in multiple sources. Phenotypically, the ageing process is associated with a wide variety of features at the molecular, cellular and physiological level—for example, genomic and epigenomic alterations, loss of proteostasis, declining overall cellular and subcellular function and deregulation of signalling systems. However, the relative importance, mechanistic interrelationships and hierarchical order of these features of ageing have not been clarified. Here we synthesize accumulating evidence that DNA damage affects most, if not all, aspects of the ageing phenotype, making it a potentially unifying cause of ageing. Targeting DNA damage and its mechanistic links with the ageing phenotype will provide a logical rationale for developing unified interventions to counteract age-related dysfunction and disease.

There is widespread agreement that ageing in metazoa is ultimately caused by the declining force of natural selection after genes have been passed on to the next generation[1]. Mutations that have adverse effects only late in life are not eliminated by purifying selection and therefore accumulate in the germline[2]. Indeed, pleiotropic mutations that have beneficial effects before reproduction and adverse effects after reproduction may even be positively selected[3]. The consequences of accumulation of such germline mutations become evident only when lifespan is no longer curtailed by extrinsic sources of early mortality, as in modern humans or animals kept in protective environments, explaining the steep rise in multimorbidity at advanced age.

Although the evolutionary logic of ageing is clear, surprisingly little is known about its proximate causes, even though ageing is the source of most chronic diseases and the main burden for healthcare in advanced societies worldwide. A central question is whether ageing has a sheer infinite number of origins—as predicted by evolutionary theory—or whether there is one ancestral cause that was present from the beginning, which with increasing complexity of life was later joined by many secondary causes. In an attempt to better understand ageing, a number of processes that causally contribute to pathologies occurring at old age have been identified[4]. In this Review, we show how the main features of the ageing phenotype causally and mechanistically converge onto DNA damage (Fig. 1), making this a strong candidate as the primary cause of ageing.

Effects of DNA damage at the molecular level

DNA damage has a range of molecular consequences such as genome instability, telomere dysfunction, epigenetic alterations, proteostatic stress and compromised mitochondrial function.

The inherently unstable genome

Although it is the primary template encoding all genetic information, the genome is surprisingly unstable. Genome instability can be defined as the tendency of the genome to undergo mutation—that is, any permanent, transmittable alteration of the DNA sequence, such as a base substitution, a deletion or insertion, copy number variation, chromosomal aberration or retrotransposition. Mutations generally have adverse effects on function and are a major cause of cancer and genetic disease. In the germline, however, they are also the substrate of evolution.

Mutations are an inherent characteristic of both nuclear and mitochondrial genomes and a consequence of erroneous replication or repair, often starting from DNA damage. In a broader sense, genome instability can refer to the inherent characteristic of DNA to undergo chemical modification, generally termed DNA damage, that alters its structure and functional properties[5]. DNA damage has been a problem from the onset of DNA-based life owing to the ubiquity of DNA-damaging agents such as ultraviolet (UV) radiation from the sun, causing lesions that block transcription and replication. DNA damage includes spontaneous deamination and hydrolysis and many other chemical alterations, including different types of breaks, nicks, gaps, abasic sites, adducts and interstrand, intrastrand and DNA–protein crosslinks, and other subtle chemical modifications. Aberrant DNA structures, such as R-loops, G-quadruplexes and persistent single-strand regions, as well as arrested intermediates in DNA transactions such as stalled transcription, replication and recombination complexes should also be considered as DNA damage, as they compromise DNA function and trigger similar responses. DNA lesions hamper accurate replication, controlled transcription and secure storage of

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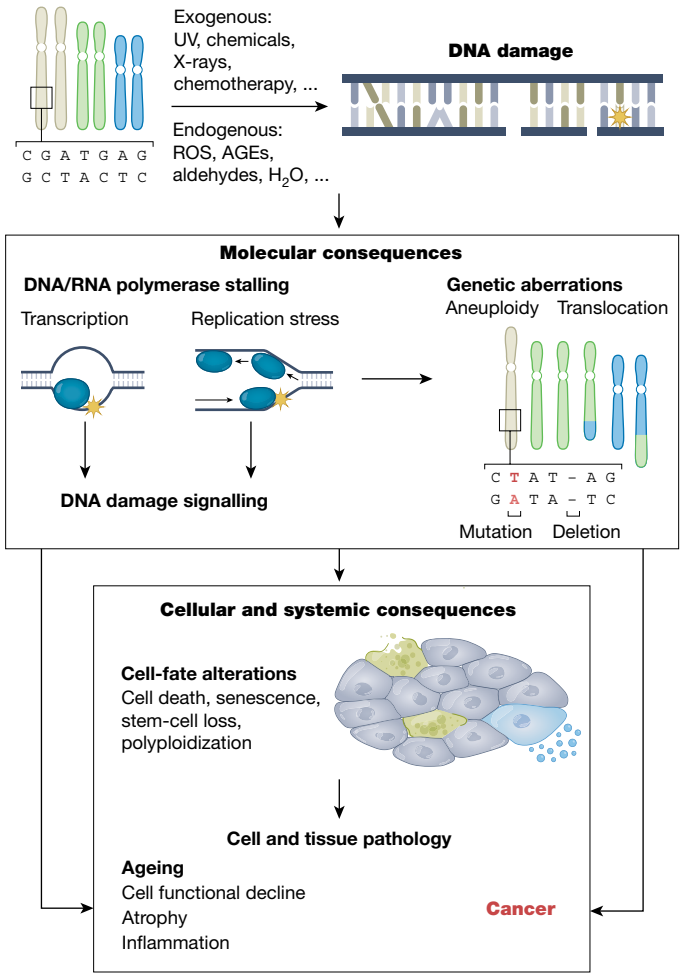

Fig. 1 | DNA damage is the driver of ageing. The nuclear and mitochondrial genomes are continuously damaged by exogenous agents (such as UV, X-rays, chemical compounds in food, water and air), endogenous sources such as reactive oxygen species (ROS), aldehydes and advanced glycation end products (AGEs) and spontaneous reactions (hydrolysis). Molecular consequences of time-dependent accumulating DNA damage are: (1) genetic aberrations, such as mutations and chromosomal instability, and (2) stalling of RNA and DNA polymerases by DNA lesions, which provokes DNA damage signalling and interferes with primary DNA functions. Cellular and tissue consequences of DNA damage include cell fate decisions such as cell death and senescence, leading to functional loss of cells and organs, cancer, atrophy and inflammation.

the genetic information. At the apex of the informational hierarchy, nuclear DNA is usually present in only two (distinct) copies and, in contrast to all other biomolecules that can be remade on the basis of instructions carried by the corresponding genes, DNA integrity is maintained only through constant repair. An elaborate network of highly sophisticated DNA repair and damage response (DDR) systems counteract the time- and exposure-dependent erosion of the genetic information. Inherited defects in these maintenance systems not only predispose cells to cancer, but also underlie numerous, segmental (that is, occurring at different rates in different tissues) forms of premature ageing in humans, indicating a tight link between genome integrity, cancer and ageing[6] (Box 1).

During normal ageing, DNA damage occurs continuously on a massive scale, owing to numerous exogenous and endogenous genotoxins. The pro-ageing effects of genotoxins are visible during photo-ageing of the skin and DNA-damaging chemotherapy[7]. Mechanical stress to tissues can also cause genome instability and may contribute to the accelerated ageing seen in Hutchinson–Gilford progeria, in which mechanical resilience of the nucleus is compromised by a mutation affecting the scaffold protein lamin A[8]. It is estimated that up to 10^5 DNA lesions occur in an active mammalian cell on a daily basis, with spontaneous hydrolysis alone resulting in about 10^4 abasic (mostly apurinic) sites[5]. Even though most of these lesions are removed efficiently, some escape detection, are irreparable, are repaired too late or are repaired in an erroneous way. In time, DNA lesions inevitably accumulate[9], making genome instability a true hallmark of ageing (Fig. 2).

Genome instability at dysfunctional telomeres

The discovery in the late 1980s that *Saccharomyces cerevisiae* ever shorter telomeres (*EST1*) mutants undergo replicative senescence[10] has popularized the concept that progressive telomere shortening drives the ageing process. In mammals, telomeres consist of thousands of TTAGGG repeats covered by the shelterin complex, which facilitates formation of a lariat-like T-loop and thereby hides the telomeric end, preventing activation of the DDR sensors[11]. Owing to incomplete lagging-strand synthesis during DNA replication, the number of telomere repeats decreases with each cell division. In the germline and in some somatic stem cells this loss is compensated by telomerase, which is silenced in most somatic cells during early development, restricting the number of cell divisions until telomeres become critically short. An unprotected telomere resembles a persistent DNA double-strand break (DSB), and triggers chronic DDR activation resulting in replicative senescence[12]. Even a single DSB is sufficient to cause full cell cycle blockade[13]. The pathogenicity of telomere shortening in ageing is an antagonistic pleiotropic effect of a trait that must have been selected for its early benefits, such as limiting unrestrainted proliferation and thus tumour formation[14].

Genetic defects in telomere maintenance cause human telomeropathies, including dyskeratosis congenita, aplastic anaemia and pulmonary and liver disease exhibiting multiple progeroid features[15]. In mice, segmental premature ageing in telomerase mutants manifests only after several generations, probably because their particularly long telomeric repeats take several generations to become critically shortened and thus dysfunctional[16]. The estimated telomere length in bulk human tissues does not suggest that telomeres become critically short in normal ageing, even at old age[17]. However, progressive telomere shortening might alter expression of specific subtelomeric genes[18] during ageing, the in vivo relevance of which is yet to be determined.

DNA damage-induced epigenetic alterations

The epigenome comprises DNA methylation and histone modifications, and is unstable over the lifetime of somatic cells. Some changes are similar among cells within a tissue and are thus probably adaptive or programmed, whereas others are progressive and/or stochastic, similar to DNA damage and mutations, contributing to intercellular heterogeneity, possibly with important functional consequences.

Chromatin modifications include phosphorylation, methylation, acetylation, ubiquitination, sumoylation, citrullination and poly ADP-ribosylation, most of which also constitute part of the DDR[19]. Age-dependent chromatin modifications include loss of histones[20] and increased 'fuzziness' of nucleosomes[21], which are linked to local and global chromatin remodelling, an imbalance of activating and repressive histone modifications, and transcriptional changes. Diverse sets of age-related alterations in DNA methylation in various tissues in humans and experimental animal models have been found to strongly correlate with chronological age and are now used as epigenetic clocks. These indicators change at similar rates in different cells, suggesting that the underlying CpG methylation status reflects adaptive changes[22].

Accumulating evidence suggests that DNA damage is a major driver of age-associated epigenetic changes. The DNA methyltransferase DNMT1 localizes to sites of DNA repair[23], and many chromatin remodellers regulate the assembly of distinct repair machineries, lesion removal and restoration of the original chromatin state, which may leave epigenetic

Box 1

DNA repair defects accelerate human ageing

Most progeroid (premature ageing-like) syndromes are caused by mutations in genes involved in maintaining genome stability. Patients with Werner syndrome often display many overt signs of ageing before they reach 30 years of age, including hair greying, type 2 diabetes, osteoporosis and cataracts. Werner syndrome, Bloom syndrome and Rothmund–Thomson syndrome are caused by mutations in RecQ helicases, which function in DNA recombination, replication, repair and telomere maintenance. Typical ageing-associated pathologies such as neurodegeneration, atherosclerosis and osteoporosis occur in Cockayne syndrome and trichothiodystrophy before the age of 10, caused by impaired transcription-coupled repair. Global-genome nucleotide-excision repair (NER) defects cause increase susceptibility to sun-induced skin cancer in patients with xeroderma pigmentosum—some of whom also suffer from accelerated neurodegeneration—by several thousand-fold.

Defects in DSB repair result in the progeroid conditions ataxia telangiectasia and Nijmegen breakage syndrome, and DNA crosslink-repair deficiencies cause Fanconi anaemia. The nuclear lamina dysfunction that underlies Hutchinson–Gilford progeria has been linked to nuclear genome instability[6].

Progeroid syndromes are segmental, as specific DNA repair defects predominantly affect specific tissues, such as haematopoiesis in ataxia telangiectasia or Fanconi anaemia. However, neurodegenerative phenotypes are widespread throughout progeroid syndromes, suggesting that neurons might be particularly sensitive to multiple defects in DNA repair[120]. Premature ageing is also found in long-term survivors of cancer who suffer from the long-lasting consequences of genotoxic chemotherapy and radiotherapy[7]. An additional category of progressive progeroid disorders affecting multiple organs is caused by mitochondrial defects[50], which probably also involve DNA damage.

Table 1 | Examples of progeroid syndromes caused by DNA repair defects

Molecular process	Progeroid syndrome	Clinical symptoms
RecQ helicases	Werner syndrome	Atrophic skin, thin grey hair, osteoporosis, type 2 diabetes, cataracts, arteriosclerosis and cancer
	Bloom syndrome	Growth retardation, immune deficiency, genomic instability and cancer
	Rothmund–Thompson syndrome	Growth deficiency, greying of hair, juvenile cataracts, skin and skeletal abnormalities, osteosarcomas and skin cancers
Transcription-coupled repair	Cockayne syndrome	Cachexia, progressive neurodegeneration, loss of retinal cells, osteoporosis, liver and kidney ageing and growth retardation
	Trichothiodystrophy	Progressive neurodegeneration, osteoporosis, cachexia, liver and kidney ageing, ichthyosis, characteristic brittle hair and nails and growth retardation
Double strand break repair	Ataxia telangiectasia	Progressive cerebellar degeneration, severe ataxia, dilated blood vessels, immunologic defects and cancer
	Nijmegen breakage syndrome	Immunodeficiency, increased cancer risk and growth retardation
Crosslink repair	Fanconi anaemia	Pancytopaenia, cancer, bone marrow failure, renal dysfunction, abnormal pigmentation and short stature
Nuclear lamina instability	Hutchinson–Gilford progeria syndrome	Alopecia, atherosclerosis, prominent scalp veins, adipose tissue storage deficiencies and high-pitched voice

marks. For example, after the repair of transcription-blocking lesions in *Caenorhabditis elegans*, H3K4me2 deposition facilitates the resumption of transcription of genes regulating protein biosynthesis and homeostasis and consequently promotes longevity[24]. The DDR in human cells leads to loss of H3K27me3, promoting cellular senescence[25]. The phosphorylated histone variant γH2AX forms foci at the site of DSBs, which accumulate in various mouse tissues with ageing[26], indicative of persistent chromatin alterations resulting from DNA damage. DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) are enriched in senescent cells. These DNA-SCARS exemplify persistent local chromatin changes due to irreparable DNA lesions[27]. It has been demonstrated in cell lines that DNA methylation patterns are altered during homologous recombination repair and are followed, weeks later, by further modification by base-excision repair-mediated transcription-associated demethylation[28]. Poly ADP-ribosylation of histones and the poly ADP-ribose polymerase 1 (PARP1) itself facilitates repair of single-strand breaks, serving as a landing platform for proteins in base-excision repair. Poly ADP-ribosylation severely decreases cellular NAD+ pools, which may trigger apoptosis or indirectly inhibit sirtuin proteins, which in turn affect genome-wide chromatin acetylation, ageing and DNA repair[29] and trigger gene expression changes that resemble those observed in ageing mouse brain[30].

It is therefore plausible that continuous induction of DNA damage and repair for tens of thousands of lesions daily leave epigenetic marks and thereby contribute to intercellular epigenetic heterogeneity in ageing, particularly as somatic cells do not have to function indefinitely and epigenetic memory is erased in the germline at the start of the next generation. Consistent with these ideas, transcription in aged cells appears to be far more variable than in young cells[31]. The DDR is therefore probably a primary cause of epigenetic changes that lead to deterioration of control of gene expression, which in turn contributes to somatic heterogeneity and time-dependent overall functional decline.

DNA damage-induced proteostatic stress

Proteostatic pathways control the synthesis, folding and degradation of proteins. Several age-related diseases are associated with protein misfolding and aggregation, including Alzheimer's disease and Parkinson's disease. Misfolded proteins can arise when structural alterations affect solubility, causing protein aggregation—for example, upon oxidative, heat, or endoplasmic reticulum stress. Multiple lines of evidence

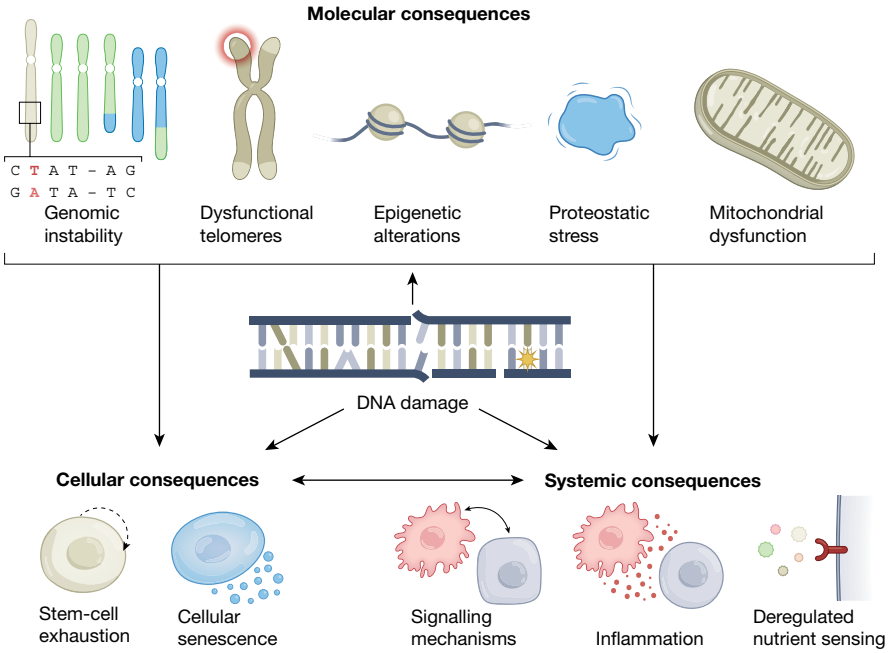

Fig. 2 | Molecular, cellular and systemic consequences of DNA damage. DNA damage and the cellular DDR can impinge on molecular processes, alter cell fate and deregulate intercellular communication. DNA damage leads to mutations or chromosomal aberrations, thus triggering genome instability. Critically shortened telomeres activate the DDR, triggering cellular senescence. DNA repair leads to chromatin remodelling, while the chromatin structure affects DNA damage susceptibility and repair access. The DDR affects autophagy and the ER-UPR, and leads to a loss of protein complex stoichiometry. Mitochondrial dysfunction is driven by NAD+ deprivation by nuclear DNA repair, DNA damage-induced mitophagy defects, and altered mtDNA polymerase expression affecting mtDNA replication. DNA damage induces dampening of nutrient-sensing pathways, which in turn affect DNA damage repair and signalling. Cellular senescence is induced in response to DNA damage, and DNA damage causes exhaustion of stem cell pools through DDR-induced apoptosis, senescence, premature differentiation and alterations of the stem cell niche. The DDR affects intercellular communication through inflammatory cytokines and dampened growth signalling.

link DNA damage to proteostatic stress. Children with the premature ageing condition Cockayne syndrome, which is caused by a defect in transcription-coupled repair, develop neurofibrillary tangles in the cerebellar cortex[32] decades earlier than in familial early-onset Alzheimer's disease. Defective transcription-coupled repair accelerates neurodegeneration in a *C. elegans* model for Cockayne syndrome, further underlining the ancestral role of DNA damage in driving age-related neuronal pathology[33]. DNA damage and altered expression and activity of DNA repair genes have been implicated in the pathogenesis of Alzheimer's disease and other dementias[34–38]—for example, reduced NER efficiency in human Parkinson's disease[39]. Several DNA repair mechanisms, particularly mismatch repair, are involved in the repeat expansion underlying Huntington's disease[40] and mutant huntingtin has been linked to defects in repairing transcription-associated DNA strand breaks[41].

DNA damage could trigger proteostatic stress, for example, through increased stalling of transcription (transcriptional stress) or mutation- or epimutation-mediated transcriptional noise. This is likely to affect assembly, stoichiometry, proper folding and functioning of proteins and protein complexes, triggering proteostatic stress and aggregation. Single-cell sequencing of human neurons has confirmed that the number of somatic mutations increases during ageing and that it does so at a higher rate in cells from patients with neurodegenerative diseases[42]. Accumulation of stochastic transcription-blocking DNA lesions in post-mitotic tissues such as neurons, which do not dilute DNA damage by replication, probably causes the genome-wide reduced expression preferentially of large genes observed during natural ageing and—in an accelerated fashion—in progeroid mice deficient in NER or transcription-coupled repair[43]. These DNA damage-driven mechanisms would explain the decoupling of transcription and protein expression[44] and loss of stoichiometry of protein complexes noted during ageing in different species[45], thus creating proteotoxic stress and protein aggregation.

Defects in chaperones, the ubiquitin proteasome system and autophagy can result in accumulation of misfolded proteins. The DDR itself can strain the proteostatic machinery[46] and endonuclease inositol-requiring protein 1α (IRE1α) and the transcription factor XBP1—both key regulators of the endoplasmic reticulum unfolded protein response (ER-UPR)—are induced in DNA repair-defective progeroid mice[43]. Autophagy is also induced by DNA damage signalling and is required for survival in the presence of persistent DNA damage[46]. When unrepaired DNA lesions drive cells into senescence, they exert a chronic senescence-associated secretory phenotype[47]; which is thought to strain the ER-UPR[48]. By contrast, calorie restriction (reduced calorie intake without malnutrition) reduces transcription stress and simultaneously alleviates the ER-UPR[43], providing a direct link between DNA damage-driven transcription stress and proteostatic stress.

Together, these observations support a central role of DNA damage, mutations and epimutations as major causes of increasing proteotoxic stress with age.

Mitochondrial dysfunction

As the organelles that regulate energy and metabolic homeostasis, mitochondria have long been associated with ageing, mostly as a major source of ROS[49], and linked with diseases associated with ageing, such as Parkinson's disease and sarcopenia[50]. The primary cause of mitochondrial dysfunction has frequently been sought in ROS-induced damage to the mitochondrial genome, which less than 17 kb in size, but is present as multiple copies in each of the thousands of organelles in a typical mammalian cell[50].

The most popular hypothesis for age-related mitochondrial dysfunction is the accumulation of somatic mutations in the mitochondrial genome as a consequence of errors occurring during replication and the lack of most of the sophisticated repair pathways that are active in the nucleus. Mice expressing a proofreading-deficient mitochondrial

DNA polymerase have highly increased numbers of mitochondrial DNA (mtDNA) mutations and display multiple symptoms of premature ageing[51,52]. Increased mtDNA mutations have been correlated to loss of cytochrome C oxidase activity in aged human skeletal muscle fibres[53,54], substantia nigra and hippocampus of normally aged human brain[55] and various other tissues[56]. However, it is unclear whether the frequency of such mtDNA mutations during natural ageing is sufficient to cause phenotypic effects[57]. Digital PCR has indicated fairly low frequencies of mtDNA deletions[58] and ultra-deep sequencing did not show an age-dependent increase of mutations in wild-type mice, instead suggesting that most somatic mtDNA mutations originate from replication errors during development[59].

The association between nuclear DNA damage and mitochondrial dysfunction indicates a role for mitophagy, the selective degradation of mitochondria by autophagy. High levels of nuclear DNA damage—for example, in cells from aged organisms or DNA repair mutants—lead to prolonged activation of PARP1, a DNA-break sensor[60] that upon activation consumes large amounts of NAD+. Inhibition of PARP1 or supplementation of NAD+ alleviated some premature ageing phenotypes associated with defects in DNA repair by restoring mitochondrial function and mitophagy[29].

Thus, although the role of mtDNA mutations in ageing remains subject to debate, several aspects have not yet been well explored, including the effect of the DNA damage itself (as opposed to mutations) on mitochondrial DNA replication and transcription, and the damage to the more than 1,000 mitochondrial genes in the nuclear genome.

DNA damage-driven cell fate decisions

DNA damage can have marked consequences on cell fate decisions, particularly when driving cells into senescence and exhausting stem cell pools.

Cellular senescence

Cellular senescence permanently arrests proliferation in response to various stresses, most of which are linked to DNA damage. Senescence was first identified as a mechanism that limits the number of population doublings in cultured human fibroblasts owing to telomere attrition, triggering DNA damage-signalled cell cycle arrest[61,62]. Senescence probably evolved as a mechanism contributing to embryogenesis, regeneration (for example, wound healing)[63] and as a cellular defence against overproliferation and thereby cancer. However, senescent cells acquire a senescence-associated secretory phenotype, secreting many pro-inflammatory cytokines, proteases and growth and angiogenesis factors that can disrupt microenvironments and compromise tissue structure and function, thereby contributing to local and systemic ageing-associated pathologies[47] and promoting cancer[64]. The pro-inflammatory mediators can promote sterile inflammation—often referred to in this context as inflammaging. Recent attention has focused on the effect of senescence in vivo, where purging p16-positive senescent cells in transgenic mice increased mean lifespan as well as aspects of healthspan[65]. Application of senolytic agents—agents that selectively eliminate senescent cells—confirms that senescent cells contribute to ageing, for example, in atherosclerotic plaques[66] and osteoarthritic lesions[67].

Cells are driven into senescence by clastogenic compounds such as bleomycin, doxorubicin or cisplatin, which frequently cause irreparable DNA damage resulting in DNA-SCARS[27]. DNA damage is also responsible for oncogene-induced senescence, which involves replication stress and subsequent DSBs as the consequence of high levels of replication associated with activated oncogenes[68]. DDR pathways, including ATR, ATM and p53, which converge on activation of the cyclin-dependent kinase inhibitors p16, p21 and p27 and hyperphosphorylation of the retinoblastoma protein, trigger the withdrawal from the cell cycle[69]. Cellular senescence can also arise as a consequence of chromosomal aneuploidy[70].

Mitochondrial-dysfunction-associated senescence[71] is not directly triggered by genotoxins. However, it is probably also driven by DNA damage, given the association with mitochondrial dysfunction described above. Cellular senescence therefore appears to be tightly integrated with the DDR or, as in mitochondrial-dysfunction-associated senescence, may be indirectly linked to DNA damage.

Stem cell exhaustion

Somatic stem cell exhaustion results in decline of stem cell number and reduced functional capacity. Different types of stem cells use distinct DDR mechanisms[72]. For instance, quiescent haematopoietic stem cells (HSCs) and hair follicle stem cells use fast, low-fidelity non-homologous end-joining, whereas cycling HSCs, intestinal stem cells and embryonic stem cells favour more accurate homologous recombination or, in the case of extensive damage, apoptosis. By contrast, irreparable damage drives melanocyte stem cells and aged hair follicle stem cells into premature differentiation, thereby clearing the stem cell pool[73]. Accumulation of DNA damage has been observed in human and mouse HSCs as well as in muscle, intestinal, mesenchymal, neural, skin and germ stem cells[72]. Various DNA repair deficiencies trigger stem cell exhaustion; muscle-forming satellite cells in progeroid *Ercc1* DNA repair mutant mice cannot follow normal proliferation and differentiation programs[74] and third-generation telomerase-deficient mouse mutants display stem cell insufficiencies in the haematopoietic system, gut, skin and testis[15].

The underlying role of DNA damage has been particularly well documented in HSCs. During ageing, HSCs expand in number but decline in pluripotency, skewing towards the myeloid lineage[75]. DNA damage increases in aged HSCs[76], probably as a result of replication stress[77]. As with most adult stem cells, HSCs reside predominantly in a quiescent state, which offers some protection from endogenous genotoxic stress, such as metabolic ROS—however, this extended quiescence leaves these cells vulnerable to accumulation of DNA lesions and their use of error-prone non-homologous end-joining increases mutagenesis[78]. Defective DNA repair limits HSC functionality in ageing and progeroid mice[79]. Thus, time-dependent accumulation of stochastic DNA damage severely hampers stem cell function, increasing mutations during human HSC ageing[80], impairing functional properties and promoting clonal expansion of positively selected somatic mutations, resulting in loss of clonal diversity[81] and increasing the potential for oncogenic transformation. Age-dependent accumulation of somatic mutations has indeed been observed in various cells types[82], such as satellite cells in humans, which acquire an average of 13 somatic mutations per year[83].

The non-cell-autonomous DDR can also compromise the stem cell niche and promote stem cell exhaustion. Genome instability in the presence of dysfunctional telomere maintenance or SIRT6 deficiency results in niche-dependent defects in HSCs[84,85]. Notch signalling by the niche regulates the level of p53 in muscle stem cells via MDM2 repression[86]. With increasing age, fading niche support drives these cells to cell death through mitotic catastrophe upon activation. In *C. elegans*, somatic niche cells regulate the DDR in germ stem cells via FGF-like signalling, and similar niche regulation of the p53-mediated DDR has been observed in mouse hair follicle stem cells[87].

Thus, accumulating DNA damage is increasingly recognized as a driver of stem cell exhaustion during ageing through a combination of apoptotis, premature differentiation, cytostatic DNA damage checkpoint signalling, accumulating mutations, and DNA damage-driven alterations in intercellular communication affecting stem cell niches.

Systemic effects of DNA damage

DNA damage systemically affects the organism trough endocrine signalling, inflammatory responses, and metabolic alterations.

Box 2

Methods to detect DNA damage

A serious challenge for linking DNA lesions to ageing has remained the methodological difficulty of accurately measuring the wide variety of chemical alterations in DNA. Key problems are the limited sensitivity and/or specificity of technologies to detect physiological levels of DNA damage and the occurrence of artefacts (such as oxidation) during DNA isolation and handling or due to interrupted DNA-metabolizing reactions (for example, topoisomerase reactions) when cells are lysed. Most lesions can only be determined in a semiquantitative or relative manner, or after exposure to unphysiologically high levels of genotoxic agents. Only some lesion types can be directly identified (but not quantified in absolute terms) through lesion-specific antibodies towards cyclobutane pyrimidine dimers, (6-4) photoproducts or 8-oxo-2′-deoxyguanosine structures or rough overall DSB and single-strand break assessment through the COMET assay, which has high variability. High performance liquid chromatography combined with advanced mass spectrometric methodologies can detect specific chemical alterations of nucleosides[121]. There are only a few examples of highly sensitive assays reporting reliable quantification of spontaneous oxidative DNA damage, most notably 8-oxo-2′-deoxyguanosine and cyclopurine lesions. Cyclopurines are endogenous transcription-blocking DNA lesions that have been shown to increase from a density of 2 to 4 per Mbp in young mice to 10–20 per Mbp in old mice[122]. Damaged DNA can be detected indirectly by long-range PCR[123], the decline in transcription of large genes resulting in a shift towards mRNAs of small genes in the ageing transcriptome of post-mitotic tissues[43], or detection of transcription-blocking lesions by strand-biased, PCR-based next-generation sequencing of DNA protected by elongating RNA polymerases[124]. Specific types of DNA lesions that are amenable to either antibody binding or enzymatic modification have been mapped by high-throughput sequencing. Third-generation sequencing technologies are rapidly advancing to detect specific DNA modifications even in low amounts of DNA[125]. The formation of DNA repair complexes such as foci formation of γH2AX, 53BP1, Rad51 and other repair or signalling proteins at DSB sites and at sites of DNA damage-induced replication stress are also useful indicators. When erroneous repair or lesion bypass during replication results in mutations, sequencing methods can be applied to detect the altered DNA sequence in single cells. Somatic mutations increase linearly during ageing in multiple tissues and species, including humans[82]. However, quantitative estimates of the total landscape of spontaneous DNA damage in humans or animals are lacking.

Signalling mechanisms influence the ageing phenotype

The importance of signal transduction mechanisms in ageing has become evident since the paradigm-shifting discovery of lifespan-extending mutations in insulin-like signalling (ILS) in *C. elegans*[88]. Consequently, several signalling systems have been shown to regulate longevity in diverse species including yeast and mammals. Interventions such as calorie restriction, at least in part, exert their anti-ageing effects by inhibiting signalling cascades such as ILS and the mammalian target of rapamycin (mTOR) pathway[89]. By contrast, inflammatory signalling is thought to promote a range of age-related pathologies.

The DDR is a potent activator of inflammatory responses. This is apparent in the response to UV-induced DNA damage in the skin, where inflammation is counteracted by systemic immunosuppression triggered by Langerhans cells migrating from the skin to the lymph nodes to activate regulatory T cells[90]. DNA damage-induced senescent cells exert complex non-cell-autonomous effects[63,64], which senolytic agents aim to curb[66,67]. DNA damage triggers innate immune responses that in *C. elegans* regulate systemic stress signalling[91]. Inflammatory responses have also been observed in DNA repair-deficient progeroid mice[92]. Simultaneously, these mice attenuate the somatotrophic (including ILS), thyrotrophic and lactotrophic hormonal axes as an anti-ageing response, similar to the effect of calorie restriction and IGF-1R mutant mice and long-lived dwarf mutant mice[85,92,93]. Unrepaired transcription-blocking lesions suppress IGF-1 signalling in mouse and human cells, resulting in increased stress resistance[94]. In *C. elegans*, ILS attenuation enhanced tissue maintenance following DNA damage accumulation through the activation of the FOXO transcription factor DAF-16[95]. The paradoxical similarity between responses triggered by DNA damage and interventions delaying ageing suggests that a systemic DDR triggers a survival response to counteract the detrimental consequences of DNA damage.

The DDR thus exerts multiple effects on age-related alterations in local and systemic communication mechanisms by affecting inflammatory and endocrine signalling components that influence the ageing process.

Anti-ageing responses affect genome stability

Nutritional interventions affect ageing and lifespan throughout the animal kingdom. Initially observed in the 1930s in rats[96], calorie restriction is the most robust universal health- and lifespan-promoting intervention in species ranging from yeast to mammals. It is thought to exert its lifespan-extending effects through specific nutrient-sensing pathways, including ILS, sirtuins and the AMP-activated protein kinase (AMPK)-regulated mTOR pathway[97]. In addition to the ILS attenuation in DNA repair-deficient progeroid mice and worms discussed above, the DDR kinase ATM phosphorylates several key proteins in the ILS–mTOR pathway after DNA damage[98].

Calorie restriction markedly delays premature ageing in DNA repair mutant mice, probably by decreasing levels of ROS and other reactive compounds, leading to reduced levels of DNA damage[43]. Longevity-promoting changes in nutrient-sensing pathways can also stimulate DNA repair itself, suggesting that some of the observed health benefits in normal ageing could be due to improved genome maintenance. In vivo inhibition of mTOR by rapamycin, which extends lifespan, increases levels of the DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT)[99]. Calorie restriction also activates SIRT1 and AMPK[4], promoting DNA damage repair and signalling as an epigenetic regulator[100] and increasing NER capacity[101], respectively. The protein kinase AKT, a central positive regulator of various nutrient-sensing pathways, negatively regulates DNA repair and inhibits key DDR factors including CHK1, TOPBP1 and p53[102]. FOXO3A, which is activated by reduced ILS, promotes the binding of TIP60 with ATM, optimizing ATM activation after DNA damage[103].

In summary, abundant evidence indicates that DNA damage affects key signalling mechanisms by impinging on ILS, sirtuins, AMPK and mTOR, which regulate lifespan and elicit anti-ageing effects of calorie restriction in model organisms.

Is DNA damage the primary cause of ageing?

DNA damage thus impinges on all major aspects of the ageing phenotype. Some of the physiological alterations caused by DNA damage in

turn boost genome instability, thereby amplifying the deterioration of homeostasis during ageing. The strong mechanistic link between DNA damage and ageing, and the role of DNA as the primary template for all cellular functions, suggest DNA damage as a candidate for the primary cause of ageing. However, there are at least three important arguments against this proposition.

First, if DNA damage is central to the ageing process, improving DNA repair would be expected to extend lifespan—however, there is little evidence for such an effect[104–106]. DNA damage comprises many distinct chemical alterations, the repair of which depends on the delicately balanced activities of at least seven core multi-enzyme pathways and many more accessory processes that encompass hundreds of genes, many of which have additional roles. Thus, the function of DNA repair in longevity cannot be generally improved by simply upregulating the activity of one or few genes. DNA repair in long-lived species has evolved over millions of years under specific selection conditions largely driven by environmental genotoxins, such as high fluxes of UV radiation or natural compounds. Moreover, as well as DNA repair, cellular systems affecting generation and outcome of DNA damage, such as metabolism, antioxidant defence, cell death, senescence and mutagenesis also have a role.

Second, reliable quantification of spontaneous DNA damage in animal or human tissues is technically extremely difficult, limiting efforts to demonstrate age-related increases to levels that impair cellular function and explain age-related pathologies (Box 2). However, DNA mutations, a consequence of erroneous DNA repair, can be determined accurately and have been shown to accumulate with age in humans and mice in a tissue-specific manner[42,107–110]. Nevertheless, while there is no doubt that accumulating mutations cause cancer and, possibly, increased cancer risk with age, it remains unknown whether their frequency is high enough to account for the loss of tissue function and increased disease risk with age. However, as well as causing mutations, accumulating DNA damage also interferes with gene expression and replication, causing replication and transcription stress, senescence, functional decline and cell death, all major drivers of ageing (Fig. 1).

A third, more recent argument against DNA damage-centric theories of ageing is the small number of DNA repair genes emerging from genome-wide association studies of ageing-related diseases or extreme longevity. However, the complexity of the genetics of ageing and longevity make the discovery of genetic associations with common variants in generally underpowered studies highly unlikely. Extreme longevity is rare and individual age-related diseases often involve genes not necessarily related to systemic ageing, such as lipoprotein genes. Nevertheless, a meta-analysis of more than 400 genome-wide association studies of five major categories of age-related diseases did identify genome-maintenance pathways[111], and genome maintenance was also the top pathway associated with the age of natural menopause[112]. Age of natural menopause is strongly linked with a wide variety of ageing pathologies, including cardiovascular disease, type 2 diabetes and osteoporosis, as well as longevity[113]. These findings are consistent with the observation in both humans and mice that the vast majority of rare genetic progeroid syndromes, in which multiple, bona fide ageing-associated diseases develop early in life, are caused by mutations in DNA repair genes[6] (Box 1).

Thus, although they are not invalid, all three arguments against a major role of DNA damage in ageing are unconvincing in view of the sheer complexity of DNA repair processes and the abundant evidence that only DNA repair dysfunction—and not defects in proteostasis, antioxidant defence, immune response or any other physiological defence system—is associated with systemic premature ageing. Collectively, the evidence indicates that DNA damage is the most likely molecular driver of ageing. DNA damage and the DDR lead to broad cellular and physiological end points that can explain the entire spectrum of ageing phenotypes, from atrophy to inflammation and cancer (Fig. 2). This understanding is not new: it has been known since the 1940s that rodents exposed to radiation exhibit multiple symptoms of premature ageing[114], and the first proposals that DNA damage was the main driver of ageing stem from the 1960s[115]. More recently, the validity of these old observations have been underscored by the notion that DNA-targeting chemotherapies and radiotherapies for cancer result in long-term accelerated, multi-organ ageing[7].

DNA damage has probably been a primary cause of ageing since the origin of life. When DNA became the genetic material, it was already far more stable than RNA, the presumed initial carrier of genetic information. The subsequent increased length of DNA templates put a premium on faithful replication and repair, which became prerequisites for rejuvenation amid the intrinsic instability of nucleic acids even during early evolution when life was not much more than compartmentalized DNA, and well before the various homeostatic alterations of ageing had evolved.

Future prospects

Time-dependent accumulation of DNA damage of endogenous and exogenous origin and its consequences progressively hamper cellular function and increase susceptibility to developing the chronic ailments of ageing. Interventions that aim to alleviate the root cause of ageing-associated multimorbidity should therefore be targeted at restoring genome integrity by reducing DNA damage and augmenting DNA repair. Reducing exogenous DNA damage—for example, through UV protection and avoidance of tobacco smoking—has already proved to lower risks of ageing-associated disease. Dietary interventions might be able to reign in some endogenous sources of DNA damage, but the majority of spontaneous lesions remain unavoidable. Augmenting DNA repair has remained a great challenge owing to the intricate complexity of the repair machineries. The highly lesion-specific photolyase repair enzymes—which are active in many species but not placental mammals—are exceptions. Ectopic expression of this enzyme is indeed sufficient to prevent UV-induced carcinogenesis in mice[116]. However, more sophisticated repair systems are needed to repair the wide range of DNA lesions that occur. Master regulators of DNA repair affecting multiple DNA repair systems have remained unknown to date, but could be discovered in future. Genetic screens using model organisms may be suitable for investigations of such mechanisms augmenting genome stability.

Since the initial proposal that DNA damage was the main cause and DNA repair was the main determinant of ageing[117,118], and the subsequent discovery that DNA repair defects can accelerate the development of a wide range of age-related pathologies[119], great strides have been made in unravelling the mechanistic links between DNA damage and nearly every aspect of the ageing process. Venturing further into the mechanisms through which DNA damage affects each of the major processes that causally contribute to pathologies occurring at old age opens perspectives to tackle the ageing process at its causal roots and thus simultaneously counteract all ageing-associated diseases.

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