Chapter 29

Genomic Instability and Aging: Causes and Consequences

C. Sidler
ETH Zürich, Zürich, Switzerland

Chapter Outline

1. Introduction 511
2. Age-Related Accumulation of DNA Damage and Genomic Instability 512
2.1 Accumulation of Point Mutations, Insertions, and Deletions 512
2.2 Accumulation of Large Chromosomal Aberrations 512
3. Causes of Age-Dependent Accumulation of Genomic Instability 512
3.1 Oxidative Stress 512
3.2 Depurination, Depyrimidination, and Deamination 513
3.3 Replication Errors and Replication Stress 513
3.3.1 Werner Syndrome 514
3.3.2 Bloom Syndrome 515
3.3.3 Rothmund–Thomson Syndrome 515
3.4 Deterioration of Genome-Maintenance Mechanisms 515
3.4.1 Mismatch Repair 515
3.4.2 Base Excision Repair 516
3.4.3 Nucleotide Excision Repair 516
3.4.4 Double-Strand Break Repair 516
3.5 Altered Nuclear Architecture 517
3.6 Selection 518
4. Genomic Regions With Various Susceptibility to Genomic Instability 518
4.1 Nuclear DNA 518
4.1.1 Telomeric DNA 519
4.1.2 Ribosomal DNA 519
4.1.3 DNA Repeats 520
4.2 Mitochondrial DNA 520
5. Role of Genomic Instability in Aging? 520
5.1 Effect of Genomic Instability on the Gene Expression Profile 521
5.2 Physiological Consequences 521
6. Conclusion 521
Glossary 522
List of Abbreviations 522
References 523

1. INTRODUCTION

Aging is associated with the progressive functional decline of the body's tissues and organs, resulting in an increasing chance of death at any time point. As such it is a major risk factor for developing age-related pathologies including cancer, cardiovascular diseases, autoimmune diseases, and neurodegenerative diseases. The increasing fraction of the elderly within human populations has made aging a primary health concern. Studies on aging in various model systems, from unicellular organisms (such as yeast) to mammals (such as mice) have revealed several common molecular traits that are associated with aging. These include altered epigenetic profile, mitochondrial dysfunction, altered protein homeostasis, cellular senescence, reduced stem cell function, changes in inter- and intracellular signaling, genomic instability, and telomere shortening [1].

While there is evidence that supports roles for all of these in aging, there is also extensive interplay between the different processes. For instance, mitochondrial dysfunction is characterized by reduced mitochondrial biogenesis, increased respiratory rates, or dysfunction of the electron transport chain, resulting in increased generation of mitochondrial reactive oxygen species (ROS) with age. ROS can in turn cause oxidative damage to the macromolecules within the cell [2].

The "somatic mutation accumulation theory of aging" postulates that accumulation of mutations with age results in functional decline and ultimately leads to an increasing chance of death at any given time point [3,4], and was later modified

Genome Stability. http://dx.doi.org/10.1016/B978-0-12-803309-8.00029-X

into the "DNA-damage accumulation theory of aging." As mentioned previously, organismal aging is caused by a complex interplay of different molecular changes affecting the various tissues within the organism and resulting in their functional deterioration. While keeping this in mind, there is extensive evidence that indicates a central role of DNA-damage accumulation and resulting genomic instability in aging.

2. AGE-RELATED ACCUMULATION OF DNA DAMAGE AND GENOMIC INSTABILITY

The "somatic mutation accumulation theory of aging" was originally put forward by Failla to explain the increasing death rate within the male population of New York with increasing age [3], and by Szilard as a testable theory to explain why organisms age [4]. The central idea of these theories is that the genetic material acquires mutations at a steady rate. While these mutations are random, the risk to accumulate an amount of mutations that is no longer compatible with survival increases with age. According to this theory, aging would result in increased cell death due to mutation accumulation and would thereby promote functional decline. As the role of DNA as genetic material became better understood, this theory evolved into the "DNA-damage theory of aging," which considers the role of DNA damage and its molecular and cellular consequences in the process of aging. In agreement with this theory, different types of DNA damage including DNA double-strand breaks (DSBs) and oxidative DNA damage accumulate with age in various model organisms [5].

When considering the amount of DNA lesions that are detected in a cell at a given time point, it is important to keep in mind that this represents a reflection of the steady state. While DNA damage is reversible, products of faulty DNA repair and replication are irreversible and promote genomic instability. Genomic instability is commonly triggered at sites of single-stranded DNA gaps and DSBs and frequently results in point mutations, microsatellite contractions or expansions, copy number variation, loss of heterozygosity, or large genome rearrangements. However, their detection in the context of a whole genome is not entirely straightforward, as these events are rare at the scale of a whole tissue.

2.1 Accumulation of Point Mutations, Insertions, and Deletions

Most of the evidence for the accumulation of smaller somatic mutations, such as point mutations, small insertions, and deletions, was obtained by using reporter assays. These are commonly based on altered phenotypes caused by mutation of either an endogenous gene (such as the hypoxanthine phosphoribosyl transferase (*HPRT*) locus [6]) or a transgene (such as a *lacZ* reporter [7]). While scoring mutation frequencies using an endogenous reporter system relies on the suitability of the cells under study for cultivation, transgene-based scoring of mutation frequencies can be performed by excising the transgene and determining the mutation frequency in *Escherichia coli*. Results from such studies have provided evidence for increasing mutation rates with age [8].

2.2 Accumulation of Large Chromosomal Aberrations

Due to their easier detection, large chromosomal abnormalities have been observed in aging cells quite early on. Large chromosomal abnormalities accumulate in proliferating as well as in postmitotic cells with increasing age [9]. For instance, human brain cells accumulate high levels of aneuploidy with increasing age [10].

3. CAUSES OF AGE-DEPENDENT ACCUMULATION OF GENOMIC INSTABILITY

The accumulation of mutations with age is the result of a balance between lifelong exposure to DNA-damaging agents and subsequent repair of the lesions. DNA is exposed to various intrinsic and extrinsic sources of DNA damage, including chemicals, radiation, pathogens, ROS, hydrolysis, and DNA replication and repair errors. The predictability of patterns of aging symptoms points to a central role of intrinsic factors in the process. Therefore, intrinsic factors that affect the level of genomic instability in a cell will be discussed in the following sections, while keeping in mind that extrinsic damaging factors may accelerate the accumulation of DNA damage additionally.

3.1 Oxidative Stress

Among the intrinsic damaging agents, ROS are considered central contributors to human aging as formulated in the "free radical theory of aging" [11]. This theory is based on the observation that metabolic activity negatively correlates with life span, which may be mediated by increased ROS production and consequent oxidative damage.

The most prominent ROS are superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, which are mainly produced during oxidative phosphorylation in the inner mitochondrial membrane.

Exposure of DNA to ROS can result in the oxidation of bases, and formation of abasic sites, DNA single-strand breaks (SSBs), and DSBs. Unlike damaged proteins or lipids, damaged DNA cannot simply be replaced. Therefore, the cell needs efficient repair mechanisms; otherwise, these lesions can cause replication stress or result in point mutations through repair errors. For instance, 8-oxo-guanine, which is one of the most frequent types of oxidative damage, can result in GC→TA transversions through mispairing during DNA replication [12].

Under normal physiological conditions, cells employ various strategies for detoxification of ROS, including enzymes, such as catalases, glutathione peroxidases, and sodium dismutases, and molecules with antioxidant properties, such as vitamins and glutathione.

Nonetheless, several lines of evidence support a role of oxidative damage in the aging process. First, several studies reported an accumulation of oxidative DNA damage with age [5]. Second, mitochondrial ROS generation increases with age, due to increased respiratory rates or dysfunction of the electron transport chain [2]. On the other hand, studies on the effects of mutations affecting antioxidant enzymes on life span have yielded contradictory results.

As previously mentioned, many tissues in different species have been shown to accumulate oxidative DNA damage with increasing age. An analysis of urinary excretion rates of oxidized nucleotides has shown decreasing rates of excretion with increasing age, suggesting that the rate of oxidation decreases with the decreasing metabolic rate during aging [13]. However, cells in the aging organism still accumulate oxidative DNA damage at steady rates, indicating reduced capacity for repair of oxidative damage with increasing age. In line with this, the repair of oxidative damage is more efficient in cells from young when compared to aged subjects [14] (see Section 3.4).

Although the exact role of oxidative damage in aging is not completely clear, it is unarguably an important internal source of DNA damage contributing to the age-associated accumulation of DNA damage and genomic instability.

3.2 Depurination, Depyrimidination, and Deamination

In addition to ROS, DNA is abundantly exposed to water, which can induce spontaneous hydrolysis of the glycosylic bond, resulting in abasic sites. This occurs at up to 10,000 sites per cell per day [15]. If not repaired before DNA replication, these sites can be subject to mispairing and are therefore potentially mutagenic. However, whether this contributes significantly to age-associated genomic instability remains to be tested.

While not that frequent, deamination also contributes to the accumulation of point mutations. In particular, deamination of 5-methylcytosine to thymine creates potentially mutagenic G-T base pairs. Since deamination of 5-methylcytosine creates thymine, which is a normal nucleotide, it is not as easily detected as damage. Therefore, 5-methylcytosine deamination constitutes a significant source of mutation [16]. Due to the important role of CpG methylation in epigenetic regulation, such deamination may not only affect the DNA sequence per se, but also its regulation. While a role of this mutagenicity in cancer is well established, no significant accumulation of C→T transitions has been detected in mouse livers with increasing age [17]. This is likely due to the activity of a thymine glycosylase (MBD4) that specifically recognizes and repairs G-T mispairs that are preferably located in a CpG sequence context [18].

Thus, while both spontaneous hydrolysis of glycosylic bonds and deamination of bases are internal sources of DNA damage, their role in human aging is unclear.

3.3 Replication Errors and Replication Stress

In general, genomic DNA is most vulnerable during DNA replication and is subject to replication errors and replication stress that can cause secondary damage.

While DNA polymerases replicate DNA with very high fidelity, faulty incorporation of nucleotides occurs in about 1 in 100,000 bases, of which 99% are fixed by proofreading. The remaining mismatches rely on repair by mismatch repair (MMR) [19]. In addition to mismatches, replication slippage contributes to length variations of repetitive sequences, which is a result of misalignment of the template and the newly synthesized DNA strand, thus introducing either small insertions or deletions [20].

While the fidelity of DNA replication seems to be unaltered with age [21], changes in the activity of MMR may potentially contribute to the accumulation of mutations caused by replication errors (see Section 3.4).

In addition, several conditions can result in slowing or stalling of replication forks, which is termed replication stress. These include reduced nucleotide pool, unrepaired DNA damage, frequency of initiation of DNA replication, impaired de novo nucleosome assembly, and mutations in genes required for replication [22]. Upon prolonged fork stalling, the

replication fork can collapse or regress, resulting in the formation of mutagenic Holliday junctions [23], and can induce several types of genomic instability, including SSBs and DSBs. The major repair mechanism to process DSBs generated at stalled replication forks is homologous recombination (HR), but in the absence of HR, nonhomologous end joining (NHEJ) can repair the lesion, potentially resulting in genomic instability in the form of large genome rearrangements [24]. Processing of breaks generated at stalled replication forks can further result in sister chromatid exchange, and chromosome loss or fragility. Thus, this process is very tightly coordinated with cell cycle regulation and damage checkpoints.

Mice with defects in Mcm2 exhibit reduced licensing of DNA-replication origins, severe problems in proliferative cells, such as stem cells, and have a drastically reduced life span [25]. In addition, several human premature aging syndromes, including Werner syndrome (WS), Bloom syndrome (BS), and Rothmund–Thomson syndrome (RTS), are caused by mutations in DNA helicases of the RECQ family, which play a role in stabilizing stalled replication forks, checkpoint activation, and preventing and resolving mutagenic intermediate structures at stalled replication forks [26]. Taken together, this indicates that DNA-replication stress may contribute to aging by contributing to genomic instability, particularly in proliferating cells.

3.3.1 Werner Syndrome

WS is the human premature aging syndrome that recapitulates the most traits that are also associated with normal human aging, including increased risk for age-related diseases, such as atherosclerosis, osteoporosis, diabetes, and cancer, as well as other symptoms of aging, such as hair loss and cataracts [27] (Table 29.1). It is caused by a mutation in RECQ-like DNA helicase and exonuclease, which is involved in various processes including DNA replication and recombination, with a major role in the reinitiation of stalled replication forks [28]. In line with this, cells from WS patients accumulate DNA

TABLE 29.1 Human Premature Aging Syndromes and Associated Impairments in Genome Maintenance

Syndrome	Mutation	Role in Genome Maintenance	Traits of Aging	References
Trichothiodystrophy (TTD)	*TFIIH*, *XPB*, *XPD*	TC-NER, transcription	Neurologic and skeletal degeneration, osteoporosis, ichthyosis, early graying of hair, infertility, and brittle hair and nails	[46]
Cockayne syndrome (CS)	*CSA* or *CSB*	TC-NER	Cachexia, neuronal degeneration, loss of retinal cells, poor growth, cataracts, photosensitivity, atherosclerosis, diabetes, hypertension	[46]
Xeroderma pigmentosum (XP)	*XPA-XPG*	NER	CS symptoms and in addition: hypersensitivity to UV exposure, pigment alterations, and high incidence of skin cancer	[46]
Ataxia telangiectasia (AT)	*ATM*	DDR	Progressive cerebellar degeneration, severe ataxia, growth retardation, dilated blood vessels, immunologic defects, and cancer	[51]
Rothmund–Thomson syndrome (RTS)	*RECQL4*	DNA repair	Growth deficiency, gray hair, cataracts, poikiloderma, osteosarcomas, and skin cancers	[28]
Werner syndrome (WS)	*WRN*	Telomere maintenance, DNA recombination and repair	Atrophic skin, thin gray hair, osteoporosis, type II diabetes, autoimmunity, skin and muscle atrophy, poor wound healing, cataracts, atherosclerosis, hypogonadism, and cancer	[27,28]
Bloom syndrome (BS)	*BLM*	Mitotic recombination	Growth retardation, sun sensitivity, immune deficiency, genomic instability, cancer, and diabetes	[28]
Hutchinson–Gilford progeria syndrome (HGPS)	*LMNA*	Nuclear lamina function	Alopecia, sarcopenia, atherosclerosis, osteolysis, prominent scalp veins, loss of subcutaneous fat, vascular problems, limited sexual development, and high-pitched voice	[59]
Dyskeratosis congenita (DC)	*DKC1*	Telomere maintenance	Growth retardation, microcephaly, cerebellar hypoplasia, mental retardation, progressive combined immune deficiency, and aplastic anemia	[72]

damage, such as DSBs, and genomic instability in the form of instability of repetitive loci, chromosomal aberrations and mutations, and telomere instability [29].

3.3.2 Bloom Syndrome

BS is caused by mutations in *BLM*, which also belongs to the family of RECQ helicases (Table 29.1). BLM helicase resolves Holliday junction-like recombination intermediates, blunt-ended DNA duplexes with internal bubbles, and G-quadruplexes that are prevalent within telomeric DNA [28]. BS cells are characterized by the accumulation of chromosomal aberrations, including sister chromatid exchanges, polycentric chromosomes, breaks, and translocations [30].

3.3.3 Rothmund–Thomson Syndrome

RTS is triggered by a mutation in a RecQ-like helicase *RECQL4* [31]. The molecular functions of RECQL4 are less understood, but it has been associated with function in DSB repair, DNA replication, and telomere maintenance [28]. Further, RTS cells also exhibit chromosomal aberrations that mainly include large chromosomal rearrangements and isochromosome formation [32]. However, although patients present with signs of premature aging, most patients seem to have a normal life span (Table 29.1).

3.4 Deterioration of Genome-Maintenance Mechanisms

In addition to mechanisms that generate DNA damage, there is evidence that DNA-repair pathways deteriorate with increasing age, mainly due to the reduced expression and/or activity of several key enzymes. However, the role of deteriorating genome-maintenance mechanisms in aging is still controversial. The observation that polymorphisms in DNA-repair genes, such as *ATM* and *XPD*, are associated with longevity in human populations [33,34] supports a role for genome maintenance in preventing functional deterioration.

On the other hand, experimental reduction of DNA damage does not consistently result in life span extension. Therefore, defects in single DNA-repair pathways may have tissue-specific effects rather than affecting organismal aging.

3.4.1 Mismatch Repair

MMR mainly repairs DNA lesions caused by faulty DNA replication or repair, resulting in mismatches or small insertion and deletion loops, or deamination of 5-methylcytosine. Failure of MMR is often associated with point mutations or microsatellite instability (Fig. 29.1). Both elevated rates of microsatellite instability with increasing age [35,36], and the reduced capacity of cell extracts from old donors to repair induced mismatches [37], indicate an age-dependent decline in MMR activity.

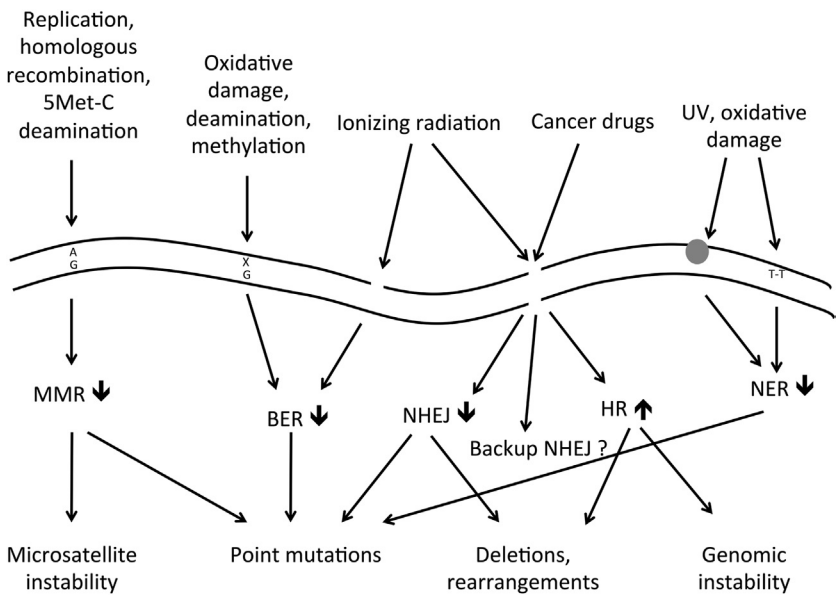

FIGURE 29.1 DNA damage and age-related changes in DNA repair. Different DNA-damaging agents that cause different types of DNA damage are listed on top, the repair pathways responsible to fix them in the middle and possible consequences of their dysfunction on the bottom. *Arrows next to the repair pathways* indicate functional decline with age. The *gray circle* indicates DNA damage in the form of bulky adducts. *Modified from Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411(6835):366–74.*

Further, mutations in MMR genes in humans are associated with cancer susceptibility [38]. This may indicate a role for age-dependent decline in MMR in the increasing cancer susceptibility with increasing age.

3.4.2 Base Excision Repair

Base excision repair (BER) is responsible for fixing damaged DNA bases, such as products of oxidative damage, deamination, and SSBs. Failure to repair these types of lesions can result in point mutations (Fig. 29.1). BER activity decreases with age in several organs, including the brain, likely due to the reduced activity of several DNA glycosylases and DNA polymerase β [39].

Assessing the effect of mutations in BER genes on life span in mammalian model organisms is difficult, since most of them are embryonic lethal. Milder defects in BER genes, on the other hand, result in elevated cancer incidence—for instance, in mice with haploinsufficiency of DNA polymerase β [40]. Although, when tested in yeast, mutations in BER genes result in life span shortening and this effect is cumulative [41], suggesting that functional BER is required to prevent aging.

Since BER is an important repair pathway to repair oxidative damage, cells with high metabolic activity and ROS generation may be particularly sensitive to declining BER activity with age. In line with this, BER deficiency has been detected in brains of Alzheimer's disease (AD) patients [42], and mutations in AP endonucleases were associated with amyotrophic lateral sclerosis (ALS) [43]. Thus, increased oxidative stress paralleled with impaired repair of oxidative damage seems to contribute to age-related neurodegeneration and neurodegenerative disorders.

3.4.3 Nucleotide Excision Repair

Nucleotide excision repair (NER) excises and repairs nucleotides that are modified by bulkier adducts, including oxidative damage and cyclobutane pyrimidine dimers caused by UV exposure. Defective NER contributes to the accumulation of point mutations (Fig. 29.1).

There is evidence that NER activity decreases with age, based on the observation that the repair of UV-induced damage in normal skin fibroblasts declines and becomes more mutagenic with increasing donor age [44]. A possible reason may be the reduced expression of NER genes in older individuals [45].

Mutations in several NER genes cause premature aging syndromes in humans, indicating an important role of NER in preventing age-dependent functional decline. These include trichothiodystrophy (TTD), which is caused by a mutation in the TFIIH helicase that is involved in NER, Cockayne syndrome (CS), which is caused by mutations in the *CSA* or *CSB* genes that function in the transcription-coupled NER pathway, and xeroderma pigmentosum (XP), which is caused by mutations in the *XPA-G* genes that are also involved in the NER pathway (Table 29.1) (Chapter 25) (for review see Ref. [46]). CS cells exhibit extensive chromosomal instability, however this does not make patients more prone to developing cancer, possibly due to their higher propensity to undergo apoptosis in response to UV damage [47]. In contrast, XP cells also exhibit defects in global genome NER, thus resulting in the accumulation of genome-wide mutations. XP patients show dramatically increased the rate of skin cancer and accelerated aging limited to areas of the body that are exposed to the sun [48]. Further, the effect of mutations in NER genes on aging is cumulative, indicating that functional NER is required to prevent functional decline.

3.4.4 Double-Strand Break Repair

DSBs are particularly toxic lesions that can be generated as a result of exposure to ionizing radiation, oxidative damage, or during replication, and if left unrepaired, they may result in loss of chromosomal segments. Errors in repair of DSBs can also be detrimental as they can result in translocations, ring chromosomes, and end fusions.

There are two major pathways involved in DSB repair: homologous recombination (HR) and NHEJ. HR uses stretches of extensive sequence homology as templates for repair and is therefore considered a precise repair mechanism. NHEJ, on contrary, rejoins the ends at a DSB with little or no consideration for homology and is therefore considered error prone.

Relative pathway usage depends on cell-cycle stage and sequence context. HR is mostly limited to S and G2 phases of the cell cycle; therefore, NHEJ is the main pathway for DSB repair during G1 phase including in nonproliferating and senescent cells. HR frequency increases if DSBs occur in close proximity to repetitive sequences [49]. Overall, the use of NHEJ exceeds the use of HR by orders of magnitude.

The initial step in both mechanisms involves DNA-damage signaling and rapid recruitment of ataxia telangiectasia mutated (ATM) [50], which induces downstream repair. The importance of this in maintaining genome integrity and preventing aging is accentuated by the fact that loss of function of ATM causes ataxia telangiectasia (AT), which shares

several features of normal aging. For instance, cells from AT patients have unstable telomeres and enter premature cellular senescence. This may be due to the function of ATM in the formation of the telomeric T-loop that protects chromosome ends [51].

In addition to the DNA-damage response, the repair mechanisms that repair DSBs are also affected by aging, as is discussed in the following sections.

3.4.4.1 Nonhomologous End Joining

NHEJ ligates two broken ends together, thereby frequently resulting in small insertions and deletions. However, postmitotic cells rely on NHEJ for repairing DSBs. Failure to faithfully repair DSBs can result in point mutations, deletions, and large genome rearrangements (Fig. 29.1). However, NHEJ activity and fidelity decline with age [52], which may be in part due to altered expression levels, activity, and distribution of key repair enzymes [53]. Further, mutations in NHEJ genes including *Ku70* and *Ku80* have been associated with shortened life spans in mice [54]. In addition, defects in DNA-PKcs resulted in impaired telomere maintenance and shortened life span in mice [55]. Taken together, these lines of evidence suggest that NHEJ plays an important role in preventing age-related increase in genomic instability and functional decline.

3.4.4.2 Homologous Recombination

HR is a precise mechanism of DSB repair that makes use of homologous sequences to repair the lesion. However, the recombination of misaligned sequences can result in chromosome rearrangement and copy number variants. In fact, the frequency of such nonallelic recombination resulting in increasing frequency of genomic rearrangements increases with age [56]. This increase in HR seemed to be tissue specific, due to limited capacity of cells that underwent rearrangements to clonally expand, for instance, in skin [57].

In summary, most genome-maintenance mechanisms have been shown to become less active and/or less precise with increasing age, contributing to age-associated genomic instability. Moreover, genetic impairments in DNA-repair pathways are often associated with shortened life span and increased cancer susceptibility, further indicating that faithful and efficient DNA repair is crucial in preventing age-related disease and functional decline.

3.5 Altered Nuclear Architecture

The role of the nuclear architecture and organization in aging has only become appreciated in recent years. By segregating genomic DNA into regions of euchromatin and heterochromatin, active transcription and repression, and sites of active DNA repair, the overall nuclear organization has profound effects on gene expression patterns, but also on DNA stability.

Perturbation of the nuclear architecture caused by a mutation in the lamin A (*LMNA*) gene is a hallmark of the Hutchinson–Gilford progeria syndrome (HGPS). The most common *LMNA* mutation results in missplicing of the *LMNA* transcript, resulting in the accumulation of a truncated protein, which is called progerin [58]. Nuclei from HGPS patients are characterized by the loss of interaction of heterochromatin with the nuclear lamina as well as general perturbation of heterochromatin, which results in induction of transcription of pericentric satellites III. HGPS cells further exhibit impairments in the DNA-damage response, recruitment of repair proteins to DNA-damage sites, and DNA repair, which is reflected in elevated genomic instability. The *LMNA* splicing defect also leads to inappropriate localization of telomeres within the nucleus, resulting in a loss of heterochromatin at telomeres, telomere shortening and genomic instability, thereby contributing to the establishment of premature cellular senescence (for review see Ref. [59]). Taken together these observations indicate that the integrity of the nuclear membrane and its role in nuclear organization are crucial to maintaining genome integrity and preventing aging.

Changes in nuclear architecture have also been observed during normal human aging. For instance, progerin also accumulates in human cells during normal ageing [60] and is associated with similar changes to nuclear organization as in HGPS cells. In addition, senescing cells accumulate senescence-associated heterochromatin foci (SAHF), which are formed de novo for instance at E2F-target promoters and silence proliferative genes, thereby promoting cell-cycle exit [61]. SAHF formation is promoted by senescence-dependent reorganization of the nuclear lamina and associated chromatin [62]. This is paralleled with heterochromatin relaxation at perinuclear repetitive DNA sequences, which may promote instability of these regions.

Further, since the DNA-damage response promotes alterations in chromatin that can extend up to megabases around the break site [63], the accumulation of unrepaired DNA damage during aging may also contribute to age-related changes in chromatin structure. This can affect expression patterns of genes around the damaged site. For instance, persistent

oxidative damage in promoter regions is associated with gene repression in the cortex of the human brain [64], suggesting that break-induced changes in gene expression may contribute to age-related alterations of the gene-expression profile and thus contribute to functional changes in cells and tissues.

In summary, it seems that altered nuclear organization and chromatin structure play a crucial role in maintaining genome integrity and preventing age-associated changes, while, on the other hand, accumulation of DNA damage affects chromatin structure, potentially resulting in age-associated changes in gene-expression profiles. A better mechanistic understanding of the establishment and role of altered nuclear organization in aging will clarify the significance of epigenetic changes in genomic instability during the aging process.

3.6 Selection

Evidence presented in the previous paragraphs indicates that the accumulation of unrepaired and misrepaired DNA damage and resulting genomic instability is promoted by several active mechanisms that either generate or fail to repair DNA lesions. In addition, passive accumulation through a shift in selection that limits survival of cells in the presence of DNA damage may occur as well.

Selection requires a quality check system, which in the case of proliferating cells is given by cell-cycle regulation and checkpoints that monitor genomic stability and ensure that a cell only propagates in the absence of DNA damage.

Early in life, when a large amount of cell divisions is required to form tissues and organs of the growing organism, DNA damage also accumulates [65]. Unrepaired DNA damage leads to checkpoint activation and cells with erroneous genomes either repair the damage or get eliminated, which can be detrimental to organismal survival. This is evidenced by the observation that the majority of spontaneously aborted embryos carry chromosomal abnormalities [66]. On the other hand, in the older organism many tissues contain large number of postmitotic cells that do not undergo the same quality control any longer and therefore passively accumulate unrepaired damage and genomic instability [67]. Thus, while the accumulation of DNA damage early in life can be detrimental, the accumulation later in life is more tolerated and interferes with tissue function rather than with organismal survival.

In summary, numerous internal sources of DNA damage, in particular oxidative damage, along with lifelong exposure to external sources of DNA damage, contribute to the accumulation of DNA damage throughout life. While several genome-maintenance mechanisms are in place to cope with the damage, these deteriorate with increasing age. In addition, alterations in nuclear architecture affect chromatin organization, which in turn influences the appropriate localization, stability, and regulation of genome regions. Lastly, nonproliferative cells with unrepaired DNA damage may accumulate in tissues of older organisms due to the lack of selective mechanisms that eliminate them. Taken together, this can explain the observed accumulation of point mutations and larger chromosomal abnormalities observed in tissues of aging organisms.

4. GENOMIC REGIONS WITH VARIOUS SUSCEPTIBILITY TO GENOMIC INSTABILITY

When considering the consequences such accumulation of DNA damage and resulting genomic instability may have, this depends on the affected sequence and the type of instability. In addition to protein-coding sequences, the genome consists of a large fraction of repetitive sequences, which are inherently more difficult to replicate and repair, and are therefore common targets for genomic instability.

Moreover, genomic DNA consists of nuclear DNA, which encodes the large majority of all protein-coding genes and exists in one diploid copy per cell, and mitochondrial DNA (mtDNA), which encodes 37 genes that mostly encode mitochondrial components and exists as two to five copies of circular, supercoiled DNA in hundreds to thousands of mitochondria per cell. The unique challenges these different sequence contexts present to the maintenance of genome integrity and their consequences for the aging process are discussed in the following sections.

4.1 Nuclear DNA

Nuclear DNA encodes the majority of genes that are required for life. The nuclear DNA consists of protein-coding sequences, which account for 2% of the total DNA, and noncoding sequences that include RNA-coding sequences, structural components, regulatory sequences, but also extensive repetitive DNA sequences. While a point mutation affecting a protein-coding gene or its regulatory elements may affect the function of this particular protein and the molecular network it plays a role in, larger aberrations like rearrangements may affect the functionality of a larger

number of genes and thereby have further reaching physiological consequences. If a mutation is introduced at a specific locus in a specific cell within a tissue, this may not have a significant effect on the tissue. However, clonal expansion of cells that carry mutations can lead to an amplification of the phenotypic outcome and affect tissue function to a greater extent [68].

On the other hand, about 30% of the nuclear DNA consists of highly repetitive sequences, such as telomeres, ribosomal DNA (rDNA), microsatellites, and minisatellites that are difficult to replicate and repair. Thus, they are more susceptible to the accumulation of genomic instability and seem to play more specific roles in cellular senescence and aging, as will be discussed in the following sections.

4.1.1 Telomeric DNA

Human telomeres consist of TTAGGG repeats that can extend more than 10 kilobases at the linear ends of DNA. Telomeric DNA is protected by the shelterin complex to prevent nucleolytic degradation, constant activation of DNA-damage signaling, and unscheduled DNA repair at the unprotected ends, which could otherwise result in end-to-end fusions [69].

However, during the replication of linear DNA, 50 to 200 base pairs are lost from the ends in each cycle. Thus, in the absence of mechanisms that resynthesize them, telomeres shorten with every cell division. When telomeres become critically short and exposed, DNA-damage signaling is activated and triggers growth arrest [70].

In addition, due to their G-rich sequence, telomeres form G-quadruplex structures, which can interfere with normal DNA-replication fork progression [71] and contribute to replication stress and genomic instability.

While a role of telomere shortening in cellular senescence is widely accepted, there is also ample support for a role of telomere shortening in organismal aging. For instance, mutations that result in compromised telomere maintenance are associated with human premature aging syndromes, such as WS, HGPS, and dyskeratosis congenita (DC). DC patients carry mutations in *DKC1*, which is a structural part of RNP complexes including telomerase [72] (Table 29.1).

Further, telomere shortening also occurs during normal aging. For instance, leukocyte telomere length decreases over time in most people [73] and reduced telomere length correlates with the development of several age-related deficiencies, such as atherosclerosis and risk for development of cardiovascular disease [74].

As telomeres shorten with each cell division, cells that are highly proliferative cells, such as stem cells and immune cells, are particularly sensitive to telomere shortening [75]. Thus, telomere shortening may play a role in age-associated functional decline through inducing senescence in highly proliferative tissues.

4.1.2 Ribosomal DNA

rDNA constitutes another large region of repetitive DNA sequence within nuclear DNA. Due to its repetitive nature, rDNA is susceptible to recombination and as a consequence to deletion/insertions, which makes rDNA one of the largest fragile sites of the genome. In addition, rDNA constitutes a common site for replication fork arrest due to the presence of multiple replication fork barriers [76], which serve to prevent collisions between DNA replication and transcription.

The role of rDNA instability in aging is well characterized in yeast (for review see Ref. [77]). Yeast senescence is associated with increased recombination within rDNA, resulting in rDNA circle excision, which is likely caused by the relaxation of heterochromatin in this region. However, mutant yeast that exhibit rDNA instability without accumulating rDNA circles have a shorter life span than wildtype, suggesting that rDNA instability is sufficient to promote aging. In line with this, rDNA instability was identified as one of the major reasons that affected life span in genetically diverse yeast strains. Based on this, Kobayashi has put forward the "rDNA theory of aging," which postulates that rDNA is the region within the genome that is most sensitive to age-dependent accumulation of DNA damage and thereby may act as a DNA-damage sensor within the genome [78].

In contrast to yeast cells, human somatic cells do not express telomerase for the maintenance of telomeres. Therefore, telomere instability seems to play a more prominent role in human cellular senescence and aging than rDNA instability. However, some evidence for increased rDNA instability with age in humans exists. For instance, nondividing cells such as nerve, heart, and skeletal muscle tissues exhibit extensive loss of rDNA with increasing age [79]. Similarly, the accumulation of extrachromosomal rDNA with age has also been described in normal human cells [80]. In addition, cells from BS patients show extensive rDNA instability due to aberrant recombination [81], which may contribute to the increased cancer susceptibility. However, while rDNA also exhibits instability during human aging and in cells from patients with premature aging disorders, its role in human aging is still unclear.

4.1.3 DNA Repeats

Other DNA repeats, such as retrotransposons, micro- and minisatellites, are also prone to genomic instability. Such repetitive sequences are prone to random expansions and deletions due to faulty replication or repair, or in the case of retrotransposons to mutagenesis by excision and integration.

Transposable elements make a considerable fraction of the total genomic DNA sequence of humans. There are three major families of retrotransposons—L1, Alu, and SVA, and they make up for about 50% of the human genome. These sequences are usually silenced by heterochromatin; however, it was shown that, for instance, during differentiation of brain cells, retrotransposons can be activated and integrate into protein-coding genes, thereby modulating their expression [82]. Further, as cells become senescent, heterochromatin in regions of constitutive heterochromatin is increasingly reduced and results in the expression of transposable elements and ultimately in retrotransposition [83]. In addition to modifying expression levels, retrotransposons have also been shown to contribute to genomic instability by causing the DSBs [84], and due to their high frequency within the genome, they can also provide substrates for unequal recombination, resulting in sequence loss, inversions, or duplication [85].

Although less abundant, micro- and minisatellites and satellite DNA sequences constitute about 3% of the genomic DNA sequence in humans. Microsatellites are tandemly repeated sequences, which consist of units that are 1–6 base pairs long. Repeats of longer units are classified as microsatellites or satellites, such as centromeric tandem repeats. It was shown that the mutation rate within microsatellite regions increases with age in humans [86]. Mutation of microsatellite sequences is often in the form of small expansions or deletions, which are the result of DNA-replication slippage. Increased microsatellite instability was also detected in hematopoietic stem and progenitor cells and T-cell clones from human subjects with increasing age and correlated with reduced expression of MMR gene *MLH1* [36], and may contribute to replicative senescence and tumorigenesis.

4.2 Mitochondrial DNA

In contrast to nuclear DNA, mtDNA is not associated with histones and is therefore much more accessible to DNA damage. Due to the close proximity to the respiratory chain, mtDNA is also highly exposed to oxidative damage. This is countered by a much less efficient repair system than that in place at the nuclear DNA, as mtDNA repair is limited to BER [87] and MMR [88]. Similar to nuclear BER, mitochondrial BER activity also declines with age [89], and mtDNA has also been shown to accumulate small deletions with increasing age. In addition, replication errors have also been determined as a significant source of point mutations in mtDNA, and mitochondrial deletions are thought to occur in a replication-dependent manner through mispairing between direct repeats within mtDNA. However, DSBs may also promote mtDNA deletions by unknown molecular mechanisms (for review see Ref. [90]).

On the other hand, every cell contains several hundreds to thousands of mitochondrial genomes, possibly allowing for complementation [91]. Also, every single mitochondrial genome can tolerate a certain extent of mutations and deletions before inducing mitochondrial dysfunction. Thus, while being more prone to the accumulation of genomic instability, mitochondrial genomes also seem to be very resistant to its consequences.

However, several specific point mutations reach very high copy numbers in individuals of increasing age. Clonally expanded mitochondrial mutations of the *COX* gene accumulate in single muscle fibers with age, leading to functional deterioration, and in neurons within the substantia nigra, resulting in impaired respiration within the affected neurons [90]. The absence of a human progeroid syndrome that is characterized by mtDNA instability suggests that mtDNA instability is not a central cause of human aging. However, since clonally expanded mtDNA mutations affect cells within tissues with high respiratory requirements by resulting in respiratory impairments, they may contribute to the functional decline of these tissues.

5. ROLE OF GENOMIC INSTABILITY IN AGING?

As discussed thus far, there is ample evidence for DNA-damage accumulation with increasing age, which is a result of lifelong exposure to DNA-damaging agents, including intrinsic exposure to oxidative stress, and deteriorating genome-maintenance mechanisms coupled with altered selection. If this constitutes a driving force to human aging, this must translate into functional deterioration of tissues and organs in a somewhat predictable way.

Since the function of cells, tissues, organs, and organisms relies on complex regulatory networks, mutations that affect any point of these networks are expected to impede the appropriate function of the entire network. Therefore, many different mutations may result in a similar phenotype. Such mutations occurring in highly differentiated postmitotic tissues are very likely to negatively affect cell function and thereby contribute to the functional decline of the tissue. On the other

hand, genomic instability also interferes with cell physiology; for instance, the accumulation of unrepaired DNA damage can result in cell death or cellular senescence and interfere with tissue homeostasis.

5.1 Effect of Genomic Instability on the Gene Expression Profile

Aging is characterized by extensive changes to the gene expression profile. Some of these changes have been linked to oxidative damage–induced repression of affected promoters. For instance, DNA damage–induced epigenetic silencing of promoters of genes required for cognitive function may play a role in cognitive decline associated with aging [64]. Such DNA damage–induced silencing is usually reversed upon faithful repair of the lesion; however, in a fraction of cells the lesion remains unrepaired resulting in permanent silencing of the locus [92].

5.2 Physiological Consequences

Further, in cells that divide actively, unrepaired DNA damage or telomere dysfunction triggers DNA-damage signaling and activation of DNA-damage checkpoints. Depending on the severity of the damage, cells can enter a terminal cell–cycle arrest or undergo apoptosis, both of which can interfere with tissue homeostasis and contribute to the functional decline.

While apoptosis contributes to the loss of functional cells, the consequences of cells entering senescence likely depend on the cells or cell types undergoing senescence. While senescence is a feature of the differentiated cells in most organs, senescence of the stem cells that are responsible for tissue renewal and repair is likely detrimental to the function of the tissue. In line with this, hematopoietic stem cells were shown to accumulate DNA damage with increasing age, which contributes to dysfunction of stem cells and the functional decline of the hematopoietic tissue [93]. However, while a decreasing functionality of stem cells with age is observed, it is still unclear whether this is due to stem cell aging or whether it is a result of the changing environment for stem cells within the aging tissue. A 2014 study supports an active role of stem cell aging in the age-dependent functional decline, by showing that adult quiescent muscle stem cells switch to irreversible senescence in muscles of geriatric mice [94].

The accumulation of senescent cells in tissues can result in altered microenvironment through altered secretion profiles [95], which can also disrupt tissue homeostasis and even stimulate proliferation of premalignant cells [96], or interfere with tissue regeneration [97]. Thus, the altered microenvironment within aging tissues may contribute both to the functional decline of the tissue as well as to the increased cancer incidence with increasing age.

On the other hand, immune cells are differentiated cells that rely on proliferation in order to perform their function. After several rounds of clonal selection, T-cells can become replicatively senescent in vitro, and T-cells with senescence-like features are also found in vivo [98]. This is thought to contribute to immunosenescence—the age-related deterioration of the immune system.

6. CONCLUSION

Genomic instability has long been considered to be a central driving force in aging, and extensive evidence has accumulated over the years supporting DNA-damage accumulation with age and its role in genomic instability. The findings that compromised genome-maintenance results in shortened life spans of model organisms or in human premature-aging syndromes suggest that genome maintenance is indeed a central antiaging mechanism.

However, aging of an organism is a very complex process, during which different tissues gradually functionally deteriorate. Since each tissue has its own characteristics and challenges, it is likely that their functional deterioration also follows individual patterns. Vijg and Dolle have put forward a model, according to which aging is not a clonal phenomenon, but rather arises from increasing heterogeneity of the cells in a tissue [99]. At the level of cells, accumulation of random mutations can contribute to this heterogeneity. At the level of tissues, different challenges to genomic integrity may promote aging. For instance, neural cells, which have high respiratory requirements, may be more susceptible to the accumulation of oxidative damage and cell death, whereas T cells that rely on continued proliferation may be more susceptible to senescence caused by telomere shortening.

If genomic instability is considered as a mechanism driving this age-associated mosaicism by contributing accumulation of mutations and promoting downstream outcomes such as altered gene expression, cell-cycle arrest, or cell death (Fig. 29.2), a better mechanistic understanding of what triggers age-related deterioration of genome-maintenance mechanisms or changes in nuclear architecture may provide valuable insights into the role of genomic instability in aging. In addition, a better understanding of interactions between cells and tissues in the aging organism will help in determining a hierarchy of age-related changes.

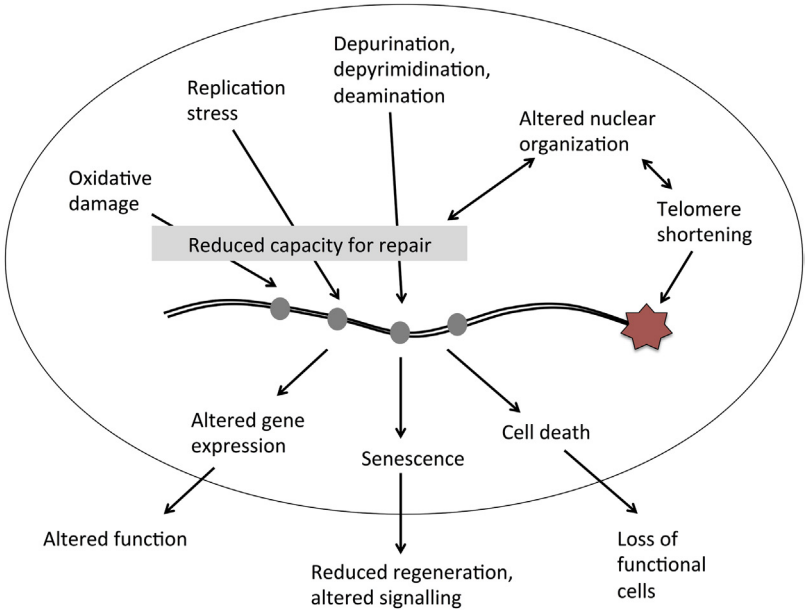

FIGURE 29.2 Mechanisms contributing to genomic instability and their role during aging. *Gray bubbles* represent DNA lesions, *arrows* indicate contributions of processes to the given outcomes, and *two-directional arrows* indicate interplay between two processes.

GLOSSARY

Cellular senescence Terminal cell–cycle arrest of normal diploid cells.
Chromosomal abnormalities Missing, additional, or irregular DNA sequence within chromosomes, including aberrations in chromosome number.
G-quadruplex Four-stranded structure in DNA or RNA formed through hydrogen bonds between four guanines.
Haploinsufficiency The presence of only one functional copy of a gene is not sufficient to produce the wild-type phenotype.
Isochromosome Chromosome with two identical arms, either two short arms or two long arms.
Large chromosomal rearrangements Large structural changes to chromosomes including duplications, deletions, inversions, and translocations.
Microsatellites Short sequence of tandem repeats of a 2–5 base pair–long motif.
Minisatellites Repetitive sequence consisting of repeats of a 10–60 base pair–long motif.
Replication origin licensing Assembly of required factors that allows replication origin to start DNA replication.
Replication stress Slowing or stalling of replication forks caused by several conditions including unrepaired DNA damage.
T-loop Structure formed at the telomere ends by looping back of single-stranded overhangs and annealing with double-stranded telomeric sequence.

LIST OF ABBREVIATIONS

AD Alzheimer's disease
ALS Amyotrophic lateral sclerosis
AT Ataxia telangiectasia
ATM Ataxia telangiectasia mutated
BER Base excision repair
BS Bloom syndrome
CS Cockayne syndrome
DKC Dyskeratosis congenita
DSB Double-strand break
HGPS Hutchinson–Gilford progeria syndrome
HR Homologous recombination
MMR Mismatch repair
nDNA Nuclear DNA
NER Nucleotide excision repair
NHEJ Nonhomologous end joining
PD Parkinson's disease
rDNA Ribosomal DNA
ROS Reactive oxygen species
RTS Rothmund–Thomson syndrome

SAHF Senescence-associated heterochromatin foci
SSB Single-strand break
TTD Trichothiodystrophy
WS Werner syndrome
XP Xeroderma pigmentosum

REFERENCES

[1] Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153(6):1194–217.
[2] Lee HC, Wei YH. Mitochondria and aging. Adv Exp Med Biol 2012;942:311–27.
[3] Failla G. The aging process and cancerogenesis. Ann N Y Acad Sci 1958;71(6):1124–40.
[4] Szilard L. On the nature of the aging process. Proc Natl Acad Sci USA 1959;45(1):30–45.
[5] Moskalev AA, Shaposhnikov MV, Plyusnina EN, Zhavoronkov A, Budovsky A, Yanai H, et al. The role of DNA damage and repair in aging through the prism of Koch-like criteria. Ageing Res Rev 2013;12(2):661–84.
[6] Albertini RJ. HPRT mutations in humans: biomarkers for mechanistic studies. Mutat Res 2001;489(1):1–16.
[7] Gossen JA, Vijg J. A selective system for lacZ- phage using a galactose-sensitive *E. coli* host. Biotechniques 1993;14(3):326, 330.
[8] Dolle ME, Snyder WK, Gossen JA, Lohman PH, Vijg J. Distinct spectra of somatic mutations accumulated with age in mouse heart and small intestine. Proc Natl Acad Sci USA 2000;97(15):8403–8.
[9] Jacobs PA, Court Brown WM, Doll R. Distribution of human chromosome counts in relation to age. Nature 1961;191:1178–80.
[10] Rehen SK, Yung YC, McCreight MP, Kaushal D, Yang AH, Almeida BS, et al. Constitutional aneuploidy in the normal human brain. J Neurosci 2005;25(9):2176–80.
[11] Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956;11(3):298–300.
[12] Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature 1991;349(6308):431–4.
[13] Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med (Berl) 1996;74(6):297–312.
[14] Chevanne M, Calia C, Zampieri M, Cecchinelli B, Caldini R, Monti D, et al. Oxidative DNA damage repair and parp 1 and parp 2 expression in Epstein-Barr virus-immortalized B lymphocyte cells from young subjects, old subjects, and centenarians. Rejuvenation Res 2007;10(2):191–204.
[15] Lindahl T, Nyberg B. Rate of depurination of native deoxyribonucleic acid. Biochemistry 1972;11(19):3610–8.
[16] Cooper DN, Youssoufian H. The CpG dinucleotide and human genetic disease. Hum Genet 1988;78(2):151–5.
[17] Singhal RP, Mays-Hoopes LL, Eichhorn GL. DNA methylation in aging of mice. Mech Ageing Dev 1987;41(3):199–210.
[18] Hendrich B, Hardeland U, Ng HH, Jiricny J, Bird A. The thymine glycosylase MBD4 can bind to the product of deamination at methylated CpG sites. Nature 1999;401(6750):301–4.
[19] Kunkel TA. Evolving views of DNA replication (in)fidelity. Cold Spring Harb Symp Quant Biol 2009;74:91–101.
[20] Streisinger G, Okada Y, Emrich J, Newton J, Tsugita A, Terzaghi E, et al. Frameshift mutations and the genetic code. This paper is dedicated to Professor Theodosius Dobzhansky on the occasion of his 66th birthday. Cold Spring Harb Symp Quant Biol 1966;31:77–84.
[21] Silber JR, Fry M, Martin GM, Loeb LA. Fidelity of DNA polymerases isolated from regenerating liver chromatin of aging *Mus musculus*. J Biol Chem 1985;260(2):1304–10.
[22] Burhans WC, Weinberger M. DNA replication stress, genome instability and aging. Nucleic Acids Res 2007;35(22):7545–56.
[23] Sogo JM, Lopes M, Foiani M. Fork reversal and ssDNA accumulation at stalled replication forks owing to checkpoint defects. Science 2002;297(5581):599–602.
[24] Aguilera A, Garcia-Muse T. Causes of genome instability. Annu Rev Genet 2013;47:1–32.
[25] Pruitt SC, Bailey KJ, Freeland A. Reduced Mcm2 expression results in severe stem/progenitor cell deficiency and cancer. Stem Cells 2007;25(12):3121–32.
[26] Bennett RJ, Keck JL. Structure and function of RecQ DNA helicases. Crit Rev Biochem Mol Biol 2004;39(2):79–97.
[27] Goto M. Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. Mech Ageing Dev 1997;98(3):239–54.
[28] Croteau DL, Popuri V, Opresko PL, Bohr VA. Human RecQ helicases in DNA repair, recombination, and replication. Annu Rev Biochem 2014;83:519–52.
[29] Ariyoshi K, Suzuki K, Goto M, Watanabe M, Kodama S. Increased chromosome instability and accumulation of DNA double-strand breaks in Werner syndrome cells. J Radiat Res 2007;48(3):219–31.
[30] German J, Archibald R, Bloom D. Chromosomal breakage in a rare and probably genetically determined syndrome of man. Science 1965;148(3669):506–7.
[31] Kitao S, Shimamoto A, Goto M, Miller RW, Smithson WA, Lindor NM, et al. Mutations in RECQL4 cause a subset of cases of Rothmund–Thomson syndrome. Nat Genet 1999;22(1):82–4.
[32] Lindor NM, Devries EM, Michels VV, Schad CR, Jalal SM, Donovan KM, et al. Rothmund–Thomson syndrome in siblings: evidence for acquired in vivo mosaicism. Clin Genet 1996;49(3):124–9.
[33] Chen T, Dong B, Lu Z, Tian B, Zhang J, Zhou J, et al. A functional single nucleotide polymorphism in promoter of ATM is associated with longevity. Mech Ageing Dev 2010;131(10):636–40.
[34] Polosak J, Roszkowska-Gancarz M, Kurylowicz A, Owczarz M, Dobosz P, Mossakowska M, et al. Decreased expression and the Lys751Gln polymorphism of the XPD gene are associated with extreme longevity. Biogerontology 2010;11(3):287–97.

[35] Krichevsky S, Pawelec G, Gural A, Effros RB, Globerson A, Yehuda DB, et al. Age related microsatellite instability in T cells from healthy individuals. Exp Gerontol 2004;39(4):507–15.
[36] Neri S, Gardini A, Facchini A, Olivieri F, Franceschi C, Ravaglia G, et al. Mismatch repair system and aging: microsatellite instability in peripheral blood cells from differently aged participants. J Gerontol A Biol Sci Med Sci 2005;60(3):285–92.
[37] Annett K, Duggan O, Freeburn R, Hyland P, Pawelec G, Barnett Y. An investigation of DNA mismatch repair capacity under normal culture conditions and under conditions of supra-physiological challenge in human CD4+T cell clones from donors of different ages. Exp Gerontol 2005;40(12):976–81.
[38] Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993;75(5):1027–38.
[39] Cabelof DC, Raffoul JJ, Yanamadala S, Ganir C, Guo Z, Heydari AR. Attenuation of DNA polymerase beta-dependent base excision repair and increased DMS-induced mutagenicity in aged mice. Mutat Res 2002;500(1–2):135–45.
[40] Cabelof DC, Ikeno Y, Nyska A, Busuttil RA, Anyangwe N, Vijg J, et al. Haploinsufficiency in DNA polymerase beta increases cancer risk with age and alters mortality rate. Cancer Res 2006;66(15):7460–5.
[41] Maclean MJ, Aamodt R, Harris N, Alseth I, Seeberg E, Bjoras M, et al. Base excision repair activities required for yeast to attain a full chronological life span. Aging Cell 2003;2(2):93–104.
[42] Weissman L, Jo DG, Sorensen MM, de Souza-Pinto NC, Markesbery WR, Mattson MP, et al. Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res 2007;35(16):5545–55.
[43] Olkowski ZL. Mutant AP endonuclease in patients with amyotrophic lateral sclerosis. Neuroreport 1998;9(2):239–42.
[44] Moriwaki S, Ray S, Tarone RE, Kraemer KH, Grossman L. The effect of donor age on the processing of UV-damaged DNA by cultured human cells: reduced DNA repair capacity and increased DNA mutability. Mutat Res 1996;364(2):117–23.
[45] Goukassian D, Gad F, Yaar M, Eller MS, Nehal US, Gilchrest BA. Mechanisms and implications of the age-associated decrease in DNA repair capacity. FASEB J 2000;14(10):1325–34.
[46] Lehmann AR. DNA repair-deficient diseases, xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. Biochimie 2003;85(11):1101–11.
[47] Ljungman M, Zhang F. Blockage of RNA polymerase as a possible trigger for UV light-induced apoptosis. Oncogene 1996;13(4):823–31.
[48] Kraemer KH, Lee MM, Scotto J. Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. Arch Dermatol 1987;123(2):241–50.
[49] Siebert R, Puchta H. Efficient repair of genomic double-strand breaks by homologous recombination between directly repeated sequences in the plant genome. Plant Cell 2002;14(5):1121–31.
[50] Andegeko Y, Moyal L, Mittelman L, Tsarfaty I, Shiloh Y, Rotman G. Nuclear retention of ATM at sites of DNA double strand breaks. J Biol Chem 2001;276(41):38224–30.
[51] Di Domenico EG, Romano E, Del Porto P, Ascenzioni F. Multifunctional role of ATM/Tel1 kinase in genome stability: from the DNA damage response to telomere maintenance. Biomed Res Int 2014;2014:787404.
[52] Seluanov A, Mittelman D, Pereira-Smith OM, Wilson JH, Gorbunova V. DNA end joining becomes less efficient and more error-prone during cellular senescence. Proc Natl Acad Sci USA 2004;101(20):7624–9.
[53] Seluanov A, Danek J, Hause N, Gorbunova V. Changes in the level and distribution of Ku proteins during cellular senescence. DNA Repair (Amst) 2007;6(12):1740–8.
[54] Li H, Vogel H, Holcomb VB, Gu Y, Hasty P. Deletion of Ku70, Ku80, or both causes early aging without substantially increased cancer. Mol Cell Biol 2007;27(23):8205–14.
[55] Espejel S, Martin M, Klatt P, Martin-Caballero J, Flores JM, Blasco MA. Shorter telomeres, accelerated ageing and increased lymphoma in DNA-PKcs-deficient mice. EMBO Rep 2004;5(5):503–9.
[56] Flores M, Morales L, Gonzaga-Jauregui C, Dominguez-Vidana R, Zepeda C, Yanez O, et al. Recurrent DNA inversion rearrangements in the human genome. Proc Natl Acad Sci USA 2007;104(15):6099–106.
[57] Wiktor-Brown DM, Olipitz W, Hendricks CA, Rugo RE, Engelward BP. Tissue-specific differences in the accumulation of sequence rearrangements with age. DNA Repair (Amst) 2008;7(5):694–703.
[58] De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, et al. Lamin a truncation in Hutchinson–Gilford progeria. Science 2003;300(5628):2055.
[59] Gonzalo S, Kreienkamp R. DNA repair defects and genome instability in Hutchinson–Gilford Progeria Syndrome. Curr Opin Cell Biol 2015;34:75–83.
[60] Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. Science 2006;312(5776):1059–63.
[61] Narita M, Nunez S, Heard E, Lin AW, Hearn SA, Spector DL, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 2003;113(6):703–16.
[62] Sadaie M, Salama R, Carroll T, Tomimatsu K, Chandra T, Young AR, et al. Redistribution of the Lamin B1 genomic binding profile affects rearrangement of heterochromatic domains and SAHF formation during senescence. Genes Dev 2013;27(16):1800–8.
[63] Rogakou EP, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol 1999;146(5):905–16.
[64] Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, et al. Gene regulation and DNA damage in the ageing human brain. Nature 2004;429(6994):883–91.
[65] Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med 2009;15(5):577–83.
[66] Lebedev IN, Ostroverkhova NV, Nikitina TV, Sukhanova NN, Nazarenko SA. Features of chromosomal abnormalities in spontaneous abortion cell culture failures detected by interphase FISH analysis. Eur J Hum Genet 2004;12(7):513–20.

[67] Rehen SK, McConnell MJ, Kaushal D, Kingsbury MA, Yang AH, Chun J. Chromosomal variation in neurons of the developing and adult mammalian nervous system. Proc Natl Acad Sci USA 2001;98(23):13361–6.
[68] Piotrowski A, Bruder CE, Andersson R, Diaz de Stahl T, Menzel U, Sandgren J, et al. Somatic mosaicism for copy number variation in differentiated human tissues. Hum Mutat 2008;29(9):1118–24.
[69] Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. Science 2012;336(6081):593–7.
[70] Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell 2004;14(4):501–13.
[71] Edwards DN, Machwe A, Wang Z, Orren DK. Intramolecular telomeric G-quadruplexes dramatically inhibit DNA synthesis by replicative and translesion polymerases, revealing their potential to lead to genetic change. PLoS One 2014;9(1):e80664.
[72] Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. Nature 1999;402(6761):551–5.
[73] Chen W, Kimura M, Kim S, Cao X, Srinivasan SR, Berenson GS, et al. Longitudinal versus cross-sectional evaluations of leukocyte telomere length dynamics: age-dependent telomere shortening is the rule. J Gerontol A Biol Sci Med Sci 2011;66(3):312–9.
[74] Willeit P, Willeit J, Brandstatter A, Ehrlenbach S, Mayr A, Gasperi A, et al. Cellular aging reflected by leukocyte telomere length predicts advanced atherosclerosis and cardiovascular disease risk. Arterioscler Thromb Vasc Biol 2010;30(8):1649–56.
[75] Lee HW, Blasco MA, Gottlieb GJ, Horner 2nd JW, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. Nature 1998;392(6676):569–74.
[76] Little RD, Platt TH, Schildkraut CL. Initiation and termination of DNA replication in human rRNA genes. Mol Cell Biol 1993;13(10):6600–13.
[77] Ganley AR, Kobayashi T. Ribosomal DNA and cellular senescence: new evidence supporting the connection between rDNA and aging. FEMS Yeast Res 2014;14(1):49–59.
[78] Kobayashi T. A new role of the rDNA and nucleolus in the nucleus–rDNA instability maintains genome integrity. Bioessays 2008;30(3):267–72.
[79] Johnson R, Strehler BL. Loss of genes coding for ribosomal RNA in ageing brain cells. Nature 1972;240(5381):412–4.
[80] Kunisada T, Yamagishi H, Ogita Z, Kirakawa T, Mitsui Y. Appearance of extrachromosomal circular DNAs during in vivo and in vitro ageing of mammalian cells. Mech Ageing Dev 1985;29(1):89–99.
[81] Killen MW, Stults DM, Adachi N, Hanakahi L, Pierce AJ. Loss of Bloom syndrome protein destabilizes human gene cluster architecture. Hum Mol Genet 2009;18(18):3417–28.
[82] Baillie JK, Barnett MW, Upton KR, Gerhardt DJ, Richmond TA, De Sapio F, et al. Somatic retrotransposition alters the genetic landscape of the human brain. Nature 2011;479(7374):534–7.
[83] De Cecco M, Criscione SW, Peckham EJ, Hillenmeyer S, Hamm EA, Manivannan J, et al. Genomes of replicatively senescent cells undergo global epigenetic changes leading to gene silencing and activation of transposable elements. Aging Cell 2013;12(2):247–56.
[84] Gasior SL, Wakeman TP, Xu B, Deininger PL. The human LINE-1 retrotransposon creates DNA double-strand breaks. J Mol Biol 2006;357(5):1383–93.
[85] Fitch DH, Bailey WJ, Tagle DA, Goodman M, Sieu L, Slightom JL. Duplication of the gamma-globin gene mediated by L1 long interspersed repetitive elements in an early ancestor of simian primates. Proc Natl Acad Sci USA 1991;88(16):7396–400.
[86] Coolbaugh-Murphy MI, Xu J, Ramagli LS, Brown BW, Siciliano MJ. Microsatellite instability (MSI) increases with age in normal somatic cells. Mech Ageing Dev 2005;126(10):1051–9.
[87] LeDoux SP, Wilson GL, Beecham EJ, Stevnsner T, Wassermann K, Bohr VA. Repair of mitochondrial DNA after various types of DNA damage in Chinese hamster ovary cells. Carcinogenesis 1992;13(11):1967–73.
[88] de Souza-Pinto NC, Mason PA, Hashiguchi K, Weissman L, Tian J, Guay D, et al. Novel DNA mismatch-repair activity involving YB-1 in human mitochondria. DNA Repair (Amst) 2009;8(6):704–19.
[89] Imam SZ, Karahalil B, Hogue BA, Souza-Pinto NC, Bohr VA. Mitochondrial and nuclear DNA-repair capacity of various brain regions in mouse is altered in an age-dependent manner. Neurobiol Aging 2006;27(8):1129–36.
[90] Kujoth GC, Bradshaw PC, Haroon S, Prolla TA. The role of mitochondrial DNA mutations in mammalian aging. PLoS Genet 2007;3(2):e24.
[91] Ono T, Isobe K, Nakada K, Hayashi JI. Human cells are protected from mitochondrial dysfunction by complementation of DNA products in fused mitochondria. Nat Genet 2001;28(3):272–5.
[92] O'Hagan HM, Mohammad HP, Baylin SB. Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. PLoS Genet 2008;4(8):e1000155.
[93] Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 2007;447(7145):725–9.
[94] Sousa-Victor P, Gutarra S, Garcia-Prat L, Rodriguez-Ubreva J, Ortet L, Ruiz-Bonilla V, et al. Geriatric muscle stem cells switch reversible quiescence into senescence. Nature 2014;506(7488):316–21.
[95] Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 2008;6(12):2853–68.
[96] Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci USA 2001;98(21):12072–7.
[97] Conboy IM, Conboy MJ, Smythe GM, Rando TA. Notch-mediated restoration of regenerative potential to aged muscle. Science 2003;302(5650):1575–7.
[98] Effros RB. From Hayflick to Walford: the role of T cell replicative senescence in human aging. Exp Gerontol 2004;39(6):885–90.
[99] Vijg J, Dolle ME. Large genome rearrangements as a primary cause of aging. Mech Ageing Dev 2002;123(8):907–15.
[100] Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411(6835):366–74.