A review and appraisal of the DNA damage theory of ageing

Alex A. Freitas[1,2], João Pedro de Magalhães[1]

[1] Integrative Genomics of Ageing Group, Institute of Integrative Biology,
University of Liverpool,
Biosciences Building, Crown Street, Liverpool, L69 7ZB, UK
[2] School of Computing and Centre for BioMedical Informatics,
University of Kent,
Canterbury, CT2 7NF, UK
E-mails: A.A.Freitas@kent.ac.uk, jp@senescence.info

Abstract

Given the central role of DNA in life, and how ageing can be seen as the gradual and irreversible breakdown of living systems, the idea that damage to the DNA is the crucial cause of ageing remains a powerful one. The aim of this review is to provide an overview and analysis of the evidence linking DNA damage to ageing. DNA damage and mutations clearly accumulate with age in mammalian tissues. Human progeroid syndromes resulting in what appears to be accelerated ageing have been linked to defects in DNA repair or processing, suggesting that elevated levels of DNA damage can accelerate physiological decline and the development of age-related diseases not limited to cancer. Genetic manipulations of DNA repair pathways in mice further strengthen this view with multiple strains defective in DNA repair showing progeroid symptoms. Higher DNA damage may trigger cellular signalling pathways, such as apoptosis, that result in a faster depletion of stem cells which in turn contributes to accelerated ageing. Delaying ageing by decreasing levels of DNA damage, however, has not been achieved yet, perhaps due to the complexity inherent to DNA repair and DNA damage response pathways. Another open question is whether DNA repair optimization is involved in the evolution of species longevity and we suggest that the way cells from different organisms respond to DNA damage may be crucial in species differences in ageing. Taken together, the data suggests a major role of DNA damage in the modulation of longevity, possibly through effects on cell dysfunction and loss, although understanding how to modify DNA repair systems to delay ageing remains a crucial challenge.

1 INTRODUCTION

Ageing is a widespread process, occurring in most animal species and in all human beings fortunate to live long enough to suffer the effects of ageing. A few animal species, such as Hydra, certain fish and some turtles, however, do not appear to undergo ageing, the reasons of which are far from understood (PMID: 19439974). Ageing can be defined as a progressive deterioration of physiological function, accompanied by an increase in vulnerability and mortality with age. A major motivation for ageing research is that age is the greatest risk factor for many diseases, including most types of cancer. The gradual "greying" of the world's population makes research into the mechanisms of ageing a pertinent medical, social and economic problem. Despite its importance and considerable progress in this area in the last few decades, however, ageing is still a mysterious process, whose fundamental causes are still strongly debated.

One of the reasons why the mechanism of ageing is poorly understood is the difficulty in discerning cause from effect and focusing on the underlying processes of ageing rather than effects (+ref 9). Because it is impossible to quantify ageing accurately, in spite of some efforts (PMID: 15466429), longevity is often used as a readout. Nonetheless, longevity, which can be defined as how long an organism lives and can be quantified for experimental cohorts as average or maximum longevity, can be influenced by many factors independent of ageing. Mutations that trigger specific diseases, for example, may decrease longevity without impacting on ageing. Therefore, it is crucial, even if often hard, to interpret experimental results in light of how they inform the ageing process.

Given the central role of DNA in life, and how ageing can be seen as the gradual and irreversible breakdown of living systems, it is intuitive to think that alterations to the DNA with time are a key process in ageing, perhaps even the primary, underlying cause of ageing [1]. The first suggestion that ageing could derive from mutations to the DNA was by Failla in 1958 [2] with the work of physicist Leo Szilard a year later also widely cited [3]. With technical advances permitting detection of new forms of DNA damage and mutations, the DNA damage theory of ageing has changed over the years [1]. The goal of this review is to provide an overview and analysis of the evidence suggesting DNA damage and repair play a major role in human ageing.

2 THE DNA DAMAGE THEORY OF AGEING

DNA can be subject to mutations and damage [4]. Mutations are changes in the nucleotide sequence, involving deletions, insertions, substitutions or re-arrangements of base pairs, and can lead to dysfunctional proteins. In contrast, DNA damage refers to physical or chemical alterations in the structure of the double-helix. In other words, mutations change the informational content of a DNA molecule, while damage modifies the structure of a DNA molecule. Early theories by Failla and Szilard focused on the role of mutations in ageing, yet the focus later shifted to DNA damage which can be seen as a broader theoretical framework since DNA damage can lead to mutations [1,5,6].

The DNA damage theory of ageing postulates that the main cause of the functional decline associated with ageing is the accumulation of DNA damage and ensuing cellular alterations and disruption of tissue homeostasis [7], [4]. Although damage to other kinds of molecules found in cells may also influence ageing, DNA damage is particularly important because, unlike other

cellular components which can be replaced, DNA must last the lifetime of the cell [8]. Damage to the DNA can have multiple effects, depending on the type of damage and genomic region affected [1,5,6]. In particular, DNA damage can dysregulate gene expression and cell function, impair transcription, cause cell cycle arrest and (if the damage is too serious) trigger programmed cell death (apoptosis). DNA damage can also lead to mutations when the DNA is repaired and/or replicated. Hence, the DNA damage theory of ageing can be interpreted in different ways, depending on how one interprets the relative contribution of each of those effects to the ageing process.

Although the focus of our review is on damage to the nuclear DNA (nDNA), a role of damage to mitochondrial DNA (mtDNA) in ageing has also been proposed. Variations of the DNA damage theory focusing on mtDNA damage and mutation are based on the fact that mtDNA is much more prone to damage than nDNA, since mtDNA is not protected by histone proteins and it is close to the site of reactive oxygen species (ROS) generation in the mitochondrial membrane. In addition, overall the repair of mtDNA is less efficient than the repair of nDNA. However, the relative importance of mtDNA damage for ageing is still controversial and less supported by experimental evidence than damage to nuclear DNA [9]. The mtDNA, in fact, encodes only 37 genes. As concluded by Khrapko & Vijg in a recent review of this subject [10]: "…the study of mitochondrial DNA mutations has not reached a stage at which clear, definitive conclusions can be drawn regarding causal relationships." Hence, in this review paper we focus on nDNA damage, which accounts for about 99% of cellular DNA.

Because the effects of disruption of certain DNA repair pathways in accelerating ageing are arguably the strongest evidence to date supporting the DNA damage theory of ageing this is initially reviewed herein. Major sources and types of DNA damage as well as the main DNA repair pathways associated with ageing are then described, before putting the pieces together and discussing how DNA damage may lead to cell dysfunction and loss and leading to organismal ageing.

3 PROGEROID SYNDROMES

There are many types of diseases in which patients show signs of accelerated ageing. Such diseases are called *premature ageing syndromes* [11] or *progeroid syndromes* [12]. Most of these diseases are caused by defects in DNA repair genes [13], [14], [15], supporting the idea that the balance between DNA damage and repair determines the rate of ageing.

Table 1 shows, for each of the syndromes discussed here: the genetic defect associated with the disease and the main processes affected by that defect; the mean life span of patients with that syndrome; and whether or not the syndrome is associated with an increased incidence of cancer. Only progeroid syndromes caused by single-gene defects are included because the causal mechanisms are simpler to understand. For example, Down's syndrome can be classified as a progeroid syndrome [16], but its molecular mechanisms are more poorly understood.

Table 1: Summary of major human progeroid syndromes originating in single-gene defects (adapted from [13], [4], [17])

Syndrome	Genetic defect	Mean life span (years)	Predisposition to cancer?
Werner	RecQ-like DNA helicase and exonuclease, involved in DNA	47	yes

	repair		
Hutchinson-Guilford	Lamin A, involved in nDNA replication, transcription, nuclear organisation	13	no
Trichothiodystrophy	TFIIH helicase, involved in DNA repair and transcription	10	no
Cockayne	CSA or CSB gene, involved in DNA repair and transcription	12-20	no
Ataxia telangiectasia	ATM protein kinase, involved in DNA damage response	20	yes
Rothmund-Thomson	RecQ-like DNA helicase	Normal?	yes
Xeroderma Pigmentosum	XPA – XPG genes, involved in DNA repair	Lower than normal?	yes

3.1 An overview of progeroid syndromes

3.1.1 Werner syndrome (WS)

This is usually considered the progeroid syndrome that shows the most symptoms of normal ageing and ageing-related diseases [14]. WS patients are usually normal during childhood, but stop growing during the teenage years [18]. The following ageing symptoms have been described [19], [12]: premature greying of the hair and baldness, skin and muscular atrophy, hypogonadism, poor wound healing, atherosclerosis, osteoporosis, soft-tissue calcification, juvenile cataracts, a tendency toward diabetes, and an elevated cancer frequency [4], [20]. On the other hand, WS patients show no increased tendency for neurodegeneration or Alzheimer's disease, and the immune system remains normal. The median age at death is 47 years.

WS is caused by one of a variety of mutations in a single gene (*WRN*) coding for a protein that is a member of the RecQ DNA helicase family [20]. The WRN protein is involved in several important biological processes, related to DNA replication, recombination, apoptosis and telomere metabolism, but its major function seems to be the re-initiation of stalled replication forks. The cells of WS patients show significant chromosomal abnormalities, increased frequency of deleterious mutations and accumulation of DNA double-strand breaks [21], [18]. WS fibroblasts reach the stage of replicative senescence considerably faster than normal fibroblasts, but both types of fibroblasts have been observed to have very similar transcriptional changes and gene expression patterns after senescence [20], [22].

3.1.2 Hutchinson-Gilford progeroid syndrome (HGPS)

This progeroid syndrome has an onset in childhood, much earlier than the onset of WS. HGPS patients show the following symptoms [23], [4]: premature loss of hair and subcutaneous fat (starting in the first year), postnatal growth is severely disturbed, no pre-pubertal or pubertal growth spurt, osteolysis, decreased joint mobility from the second to third year, thinning of the skin, limited sexual development and severe vascular problems in the brain and elsewhere – strokes occur at the median age of 9 years. The vast majority of patients have a normal cognitive development. The median age at death is 12 years.

HGPS is caused by a point mutation in the gene for lamin A (*LMNA*), a type of protein that forms a network of filaments beneath the inner nuclear membrane (among other possible

locations in the nucleus) [24]. A-type lamins can directly bind to DNA and to chromatin, but because they are involved in a variety of processes, the exact molecular mechanism of HGPS remains unclear. Although WS and HGPS patients have little overlap of clinical symptoms, at the cellular level both these progeroid syndromes are associated with genomic instability [24].

3.1.3 Trichothiodystrophy (TTD)

TTD patients show the following symptoms [13], [25]: neurodegeneration (including cerebellar ataxia), skeletal degeneration, impaired sexual development, cachexia, osteoporosis, cataracts, brittle hair and nails. Patients have a mean life span of just about 10 years, and show no predisposition to cancer. TTD is caused by point mutations in the *XPD* gene, which encodes one of the two core transcription factor IIH (TFIIH) helicases [13]. Different mutations in this gene can give rise to TTD, xeroderma pigmentosum or Cockayne syndrome. The helicase encoded by the *XPD* gene is involved in both DNA repair and transcription initiation [25].

3.1.4 Cockayne syndrome (CS)

CS is caused mainly by mutations in either the *CSA* or *CSB* gene. In addition, as mentioned earlier, CS can also be caused by a mutation in the *XPD* gene, whose mutation can also cause TTD or xeroderma pigmentosum (XP). CS patients show the following symptoms [13], [11]: neurodegeneration, growth retardation, cachexia, thin hair, retinal degeneration, hearing loss, and cataracts – which can be seen at birth in the most severe cases. Almost all CS patients are mentally retarded. Note that TTD and CS have several symptoms in common [25]. Despite chromosomal instability, patients show no predisposition to cancer. The average age at death for CS patients is estimated as 20 years in [13] and as 12 years in [11].

3.1.5 Ataxia telangiectasia (AT)

AT is caused by a loss-of-function mutation in the *ATM* (ataxia-telangiectasia mutated) gene. The *ATM* gene's product is a protein kinase which is involved in several signal transduction pathways, which operate both under stress and in normal physiological conditions [26]. In particular, ATM is involved in cell cycle progression and checkpoint response to DNA damage. AT can be diagnosed by a cytogenetic test that detects a high-level of chromosome breakage after ionising radiation, which reflects DNA damage repair pathways [27].

AT patients show the following main symptoms [11], [26], [28]: progressive neurodegeneration – with cerebellar ataxia becoming apparent when the patient begins to walk, telangiectases – with onset typically between 3 and 5 years of age, immunodeficiency, genomic instability, strong cancer predisposition and sensitivity to radiation, accelerated telomere loss, and growth retardation in many patients. The average life span of AT patients is about 20 years [13]. ATM-deficient mice exhibit most of the symptoms of the human disease [26]. ATM disruption in mice with short telomeres, however, results in symptoms of accelerated ageing [28].

3.1.6 Rothmund-Thomsom (RT) syndrome

RT is caused by a mutation in a gene (*RECQL4*) coding for a RecQ-like DNA helicase [26], [13]. RT patients typically exhibit the following symptoms [11]: skin changes starting in the first year of life and leading to poikiloderma, growth retardation, a variety of skeletal and ocular abnormalities, including osteoporosis and corneal/retinal atrophy, as well as juvenile cataracts. Malignancies have also been reported, and delayed or immature sexual development has been reported for about 28% of the patients. Most patients have normal intelligence. Surprisingly, RT patients seem to have a normal life span.

3.1.7 Xeroderma pigmentosum (XP)

This is a disease due to a defect in one of 7 proteins (XPA – XPG) required for nucleotide excision repair (a form of DNA repair to be reviewed later). XP victims show dramatically accelerated ageing only in areas of skin exposed to the sun and a skin cancer rate more than a thousand times greater than normal, and frequently exhibit neurodegeneration [13], [18].

3.1.8 Progeroid syndromes in mice

In addition to human progeroid syndromes, a number of mouse models have been created through genetic manipulation that exhibit evidence of accelerated or premature ageing with varying degrees of severity. As reviewed by many authors [8,9,29], a significant proportion of genes in which mutations appear to accelerate ageing in mice are also involved in DNA repair. In fact, many of the genes and pathways involved in human progeroid syndromes also result in progeroid syndromes when mutated in mice. These include *Lmna* [30] and associated laminopathy-based premature ageing syndromes [31], *Xpa* and *Xpd* [32]. Interestingly, *Wrn* mutations only result in accelerated ageing in mice with short telomeres [33].

Taken together, the results from progeroid mice confirm the observations from human progeroid syndromes and thus will not be presented in detail here (some are discussed further ahead in the context of specific DNA repair pathways). Readers are referred to one of the many excellent reviews on this topic for more detailed information [13,34]. Overall, ample evidence suggests that disruption of genes involved in DNA repair and/or DNA metabolism can result in a premature ageing phenotype.

3.2 On the relevance of progeroid syndromes to the study of human ageing

The relevance of the study of progeroid syndromes for the understanding of normal ageing is controversial [35]. A major point of criticism is that patients or animal models of progeroid syndromes show just a subset of the symptoms of normal ageing (and are called "segmental progeroid" by some authors). In addition, Miller [35] points out that it is relatively easy to make an animal have a significantly shorter life span by introducing a defect in some crucial DNA repair gene, but it is much more difficult to show that the defect is really accelerating ageing, and not all mice mutants with defective DNA-repair genes show signs of or accelerated ageing.

Counter-arguments to Miller's criticism have been provided in [36] and [20]. Hasty & Vijg [36] point out that the "segmental" nature of progeroid syndromes does not invalidate their relevance for the study of normal ageing, because every individual who undergoes normal ageing exhibits a segmental ageing phenotype. In addition, at least WS is considered to have symptoms which have a very significant overlapping with the symptoms of normal ageing [20]. Hasty & Vijg [36] also point out that deletion of some crucial DNA repair genes leads to embryonic death or cancer at an early age, so that there is no time for the ageing phenotype to appear. This shows that DNA repair is crucial for survival, but this is not incompatible with the fact that DNA repair is also important for ageing.

Our opinion is that the fact that no progeroid syndrome is a perfect phenocopy of ageing is not surprising considering the multitude of factors that can influence ageing in different tissues. The ability of a single gene disruption of DNA repair to accelerate multiple aspects of ageing, as in WS, is remarkable in itself given the complexity of the ageing process. Therefore, progeroid syndromes due to defects in DNA repair genes offer strong support to the idea that a major causal factor of ageing is the accumulation of DNA damage and mutations with time, perhaps through cell function disruption or cell loss [13].

4 SOURCES AND TYPES OF DNA DAMAGE

4.1 Major sources of DNA damage

Damage to the DNA can originate in multiple extrinsic and intrinsic sources [1,5,8]. Extrinsic sources can be grouped into chemicals and radiations, such as UV damage. Intrinsic sources include spontaneous chemical reactions and reactive oxygen species (ROS). It has been long argued that the predictable patterns of ageing (e.g., in setting a range of species-specific maximum lifespan limits) supports the importance of endogenous causes of DNA damage [1].

A common cause of DNA damage is exposure to ROS, which has long been hypothesized to be involved in ageing. ROS include superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen. Oxidized DNA can produce several kinds of DNA damage, e.g., oxidized bases, abasic sites and single- and double-strand breaks [37], [38]. Organisms have several defence mechanisms to cope with ROS, including antioxidant enzymes that eliminate ROS or convert them to less harmful molecules [39]. However, the production of ROS can be so overwhelming that those defence mechanisms are not enough, resulting in oxidative stress.

ROS can produce many different kinds of damage and mutation in DNA. For instance, the cytosine base alone can undergo oxidative damage producing at least 40 different modified species [38]. Some oxidatively modified bases block DNA replication, whilst others are mispaired and lead to base substitutions in the DNA. Interestingly, some of the progeroid syndromes caused by defective DNA repair discussed earlier – such as XP and AT – are associated with a high amount of 8-hydroxydeoxyguanosine (8-oxo-dG), a measure of oxidant-induced DNA damage [37]. First observed in rats via measurements of 8-oxo-dG [40], it is now widely accepted that oxidative damage to DNA tends to increase with age in mammalian tissues. Although normally a by-product of oxygen metabolism in mitochondria, ROS can have exogenous sources such as UV radiation [41]. Therefore, although which types of DNA damage are more important contributors to ageing is not known at present, ROS could be an important source of DNA damage in the context of ageing.

4.2 An overview of major types of DNA damage

4. 2.1 Abasic (AP) sites, depurination and depyrimidination

An abasic site, also called an "apurinic or apyrimidinic" (AP) site, is formed when a base is lost from the DNA by cleavage of a N-glycosyl bond, leaving the sugar-phosphate chain intact [39]. At normal physiological conditions, it has been estimated that 50,000-200,000 AP site lesions persist at a steady-state level in mammalian cells [42].

Abasic sites can be produced by spontaneous depurination and depyrimidination reactions. Depurination involves the loss of purine bases (adenine and guanine) from DNA. In spontaneously-occurring depurination reactions, the N-glycosyl bond to deoxyribose is broken by hydrolysis, leaving the DNA's sugar-phosphate chain intact, producing an abasic site. Depyrimidination involves the loss of pyrimidine bases (cytosine and thymine) from DNA. Depyrimidination is much less common than depurination, however, since the N-glycosyl bond between a pyrimidine base and the deoxyribose is more stable than the corresponding bond for purine bases [43]. Abasic sites can also be produced by ROS [42], [44], as well as being produced in intermediate steps of the base excision repair pathway (to be discussed later). Abasic sites are potentially mutagenic.

4.2.2 Deamination

Deamination involves the loss of amino groups from DNA bases. Almost all DNA bases undergo deamination in spontaneous reactions, with the exception of thymine – which does not have an amino group. It should be noted that most types of deaminations produce a base that does not naturally occur in DNA (the only exception is the deamination of 5-methylcytosine), and this fact facilitates the identification and excision of the deaminated base by a DNA glycosylase enzyme.

Interestingly, the fact that DNA uses T as a base, rather than the corresponding U base in RNA, provides one possible reason why the genetic code, which is thought to have been initially carried in RNA bases (A, C, G, U), was replaced by the current code carried in DNA bases [45]. In the current code, a deaminated C converted to a U can be easily recognized as damage and excised from DNA. However, if DNA used U, rather than T, as a natural base, a deanimated C converted into a U would not be so easily recognized as damage.

The most common type of deamination event in cells is deamination of cytosine into uracil. This event occurs at a rate of about 100-500 bases per cell per day in mammalian cells, in spontaneous deamination reactions [39]. In addition, cytosine can deaminate to uracil as a result of specific biological processes, such as somatic hypermutation in antibody production.

DNA can also contain the base 5-methylcytosine, which base pairs with guanine and is involved in silencing gene expression at CpG sequences. The deamination of 5-methylcytosine into thymine leads to the formation of a G-T base pair, which is potentially mutagenic. Interestingly, although only about 3% of the C bases in human DNA are methylated, GC → AT transitions at the sites of those methylated cytosines account for about one-third of the single-base mutations in inherited human diseases [46], [45].

4. 2.3 DNA strand breaks

Some strand breaks are produced in intermediate steps of natural reactions. As an example, the process of V(D)J recombination during lymphocyte development is initiated by a kind of programmed double-strand break between two recombining variable-region gene segments and their flanking sequences [47], [48]. However, some strand breaks are clearly a serious form of DNA damage and inhibit DNA replication, leading to the activation of DNA repair mechanisms. DNA strand breaks can be caused by oxidative damage to DNA [49] or by ionizing radiation [39]. Double-strand breaks can also result from the blockage or pausing of DNA replication – which can lead to replication fork collapse and free double-stranded ends [50].

It is interesting to note that disruption of pathways involved in single-strand break repair often results in neurological diseases rather than carcinogenesis or progeria. Because ROS are one of the major causes of single-strand breaks, one possible explanation is that the oxygen consumption in the nervous system makes it more susceptible to defects in single-strand break repair. Therefore, single-strand break may contribute to neurological decline [51].

Misrepaired double-strand breaks lead to genomic rearrangements, a common and serious problem in ageing organisms [52]. A considerably-increased frequency of DNA double strand breaks is observed in patients of some progeroid syndromes discussed earlier, such as WS and AT [53]. The number of single- and double-strand breaks in the neurons of rat cerebral cortex has been shown to considerably increase with age [54].

4. 2.4 Cyclobutane pyrimidine dimers (CPDs)
CPDs are characterized by covalent linkages between adjacent pyrimidines in the same DNA strand, and they are the most frequent type of photoproduct produced when DNA is exposed to UV-B [41] or to UV-C radiation [39]. The type of CPD most frequently found in DNA consists of a thymine dimer, which is known to be mutagenic in mammalian cells [55]. The formation of CPDs can also enhance the deamination of cytosine [39].

5 MAJOR DNA REPAIR PATHWAYS ASSOCIATED WITH AGEING

In this section we review three major types of DNA repair pathways, namely base excision repair (BER), nucleotide excision repair (NER) and the repair of DNA double-strand break via the non-homologous end joining (NHEJ) pathway. Out of those three pathways, BER seems the least associated with ageing, whilst the evidence for association with ageing is considerably stronger for the NER and NHEJ pathway. Note that this section does not cover some types of DNA repair that, although important, are not thought to be relevant for ageing. For instance, it does not cover mismatch repair [56], [57], since this pathway has been mainly associated with cancer [57], [58], rather than ageing. It also does not cover mechanisms that repair single-strand breaks as these have been associated with neurological disorders rather than broader aspects of ageing [51].

5.1 Base excision repair (BER)
The BER pathway corrects small alterations in a DNA strand that do not distort the overall structure of the DNA helix, such as a base altered by deamination or a missing base due to a depurination reaction. The base alterations targeted by BER may or may not block transcription and normal replication, but they frequently lead to changes in DNA sequence, being therefore potentially mutagenic [56]. BER is the main pathway to repair oxidative damage.

The BER pathway can be categorized into two sub-pathways, namely short-patch BER, where only one nucleotide is replaced; or long-patch BER, where 2-13 nucleotides are replaced [52]. The decision between performing a short-patch or long-patch repair is modulated by PARP1 and PARP2 (poly(ADP-ribose) polymerases) [59]. Some differences between these two sub-pathways are as follows [22]. First, in the short-patch pathway, DNA polymerase β (polβ) is the main gap-filling enzyme; whilst in the long-patch pathway this activity seems to be performed by polβ, polδ, polε. In addition, in the long-patch pathway, the WRN protein interacts physically and functionally with several other proteins such as PCNA and RPA, which is not the case in the short-patch pathway [22], [54].

The BER pathway is particularly important in the brain [53], [60], for at least two reasons. First, BER is the primary pathway to repair oxidative DNA damage, and this is the most likely kind of damage to occur in brain tissue, which is metabolically very active [54]. Secondly, neurons are post-mitotic (non-dividing) cells, and in principle other DNA repair pathways such as homologous recombination and mismatch repair are not important in neurons.

There is good evidence that, overall, the level of BER activity is reduced with age. In particular, the activity of polβ – an important component of the BER pathway – has been shown to be considerably reduced with age in mice in many investigations, e.g. in [54], [61], [60], [62], [63]. The activity of polγ – which performs the gap-filling step of BER in mitochondrial DNA [64] – has also been observed to decrease with age [62]. There are, however, studies reporting that some BER enzymes have an increased expression with age – see e.g. [65]. This seems

likely to be a response to increased levels of oxidative DNA damage with age, although the response is presumably not effective due to the aforementioned decrease in polβ activity.

5.2 Nucleotide excision repair (NER)

The NER pathway copes with lesions in a DNA strand that distort the DNA double helix. This kind of lesion interferes with base pairing and usually blocks transcription and normal replication [56]. NER is considered the most versatile DNA repair pathway in terms of the variety of lesions that it can recognize – it recognizes several types of bulky lesions, produced, for instance, by ultraviolet light and carcinogens.

The NER pathway is usually classified into two types, namely global genome NER (GG-NER), which occurs everywhere in the genome, and transcription-coupled NER (TC-NER), which occurs in the transcribed strand of active genes [52] [66]. In GG-NER, the first step is the recognition of the DNA damage by the XPC-HR23B complex. In contrast, in TC-NER the repair process is believed to be triggered by a stalled RNA polymerase, and initiation of the repair requires the proteins CSB and CSA [52], [56]. After the initial stage, GG-NER and TC-NER seem to proceed in an identical way. The presence of damage is verified by XPA, and if damage is absent the repair process is aborted. The XPB (ERCC3) and XPD (ERCC2) helicases in complex with the TFIIH transcription factor open the DNA helix double helix around the damage. RPA (Replication Protein A) stabilizes the open DNA by binding to the undamaged strand. The endonucleases XPF and XPG cleave the borders of the open segment in the damaged strand. The damaged segment is then removed, and the repair is completed by DNA polymerase and DNA ligase.

There has been many experiments investigating whether or not NER efficiency in repairing UV-induced damage decreases with age, with conflicting results [18]. For instance, NER efficiency was observed to decrease with age in [55], [67], [68], but observed not to decrease with age in [69]. It seems likely that these different results are due to the use of different experimental procedures and different types of damages being investigated.

As evidence for an association between NER and the ageing process, inherited defects in NER cause three major types of progeroid diseases in humans: XP, CS, and TTD. The severity of the symptoms in XP varies significantly across the different types of XP – associated with defects in different genes – and in general the more the mutation affects the NER pathway, the more severe the symptoms are [70]. Moreover, multiple mutations in NER genes have resulted in dramatically accelerated ageing phenotypes in mice [71], [72], [73], [52]. In addition, XPD-mediated NER has been observed to have a significantly role in maintaining the functional capacity of long-term reconstituting haematopoietic stem cells (LT-HSCs) with age, by helping to preserve the proliferative capacity and to prevent apoptosis under stress [74].

It should also be noted that XP – which is associated with a dramatic increase in skin cancers – is mainly caused by a defect in GG-NER; whilst the progeroid syndromes CS and TTD – which show no evidence of increased risk cancer – are caused mainly by defects in TC-NER [71]. This is because GG-NER is responsible mainly for repairing pre-mutagenic DNA lesions, preventing carcinogenesis; whilst TC-NER is responsible mainly for repairing DNA lesions that block transcription [75].

A particularly interesting gene for the study of the NER pathway is *XPD*, which encodes a helicase subunit of the transcription factor IIH complex, because different point mutations in this gene are associated with different phenotypes: cancer (XP), the TTD progeroid syndrome, or a combination of cancer and a progeroid syndrome, namely XP combined with CS (XPCS)

or XP combined with TTD (XPTTD) [73]. Hence, many mouse models have been created with mutations in the XPD gene, as follows.

First, inactivation of the *Xpd* gene led to embryonic lethality [76]. Later, the same group generated mice carrying an *Xpd* point mutation found in TTD patients, which produced mice with several symptoms of TTD, including cachexia [25]. They also crossed TTD mice with $Xpa^{-/-}$ mice, which greatly increased the NER defect. This produced mice with increased neonatal lethality and extreme cachexia. The authors proposed that the observed premature ageing of TTD mice is due to the accumulation of DNA damage, which leads to impaired transcription, apoptosis, functional decline, and depletion of cell renewal capacity.

The multiple effects of Xpd have also been exploited to create mouse models of "progeroid NER syndromes", by combining different mutant *Xpd* alleles with a $Xpa^{-/-}$ background [73]. The authors observed that such progeroid NER mice share many similarities with long-lived dwarf and calorie-restricted mice, in particular reduced postnatal growth and small size. They argued that this is likely due to an adaptive response to genomic instability during postnatal development, which involved dampening of the somatotropic GH/IGF-1 (growth hormone/insulin growth factor) axis, rather than due to the proliferative defects associated with premature cell senescence –a common explanation for this progeroid phenotype.

In another work, a mouse model was created with a mutation in the *Xpd* gene that exhibits strong signs of progeroid TTD [77], and the Xpd^{TTD} mice were also observed to have reduced body and organ weight. In this work the rate of apoptosis exceeded the rate of cell proliferation, resulting in homeostatic imbalance, and that this imbalance was associated with decreased energy metabolism and reduced IGF-1 signalling. Hence, similarly to [73], the authors [77] concluded that the reduced energy metabolism is likely an adaptive response to the increased DNA damage in those mouse mutants. It is possible, however, that decreased GH/IGF-1 signalling is a disease mechanism rather than an adaptive response targeting the ageing process.

5.3 Repair of double-strand breaks via Non-Homologous End Joining (NHEJ)

First, let us briefly discuss the difference between the NHEJ and the homologous recombination (HR) pathway, which are two basic pathways for the repair of DNA double-strand breaks. For a discussion of variants of those basic pathways, see [39], [50].

In the HR pathway the undamaged chromosome is used as the template for the repair of the broken chromosome. This type of repair involves the two sister DNA molecules that exist in each chromosome in cells that have replicated their DNA but not divided yet – i.e., in phases S and G2 of the cell cycle [56]. In contrast, the error-prone NHEJ pathway (discussed below) is mainly used in phase G1 of the cell cycle, before DNA replication, when there is no sister copy of DNA to be used in homologous recombination, although NHEJ seems to occur throughout the cell cycle [8]. The type of double-strand break repair also depends on the tissue or cell type. For instance, in non-dividing cells like neurons, it seems that homologous recombination is not an option, and double-strand breaks have to be repaired by NHEJ [78].

The NHEJ pathway simply links the ends of a double-strand break together, without using any strand as a template. This pathway is more error prone than the HR pathway, and it tends to insert deletions or insertions in DNA strands. Nonetheless, in mammalian cells this seems to be the main pathway for the repair of double-strand breaks resulting from ionizing radiation [48].

The NHEJ repair process starts with the binding of the KU heterodimer (consisting of KU70 and KU80 subunits) to the broken DNA strand ends, which recruits DNA-dependent protein kinase catalytic subunit ($DNA\text{-}PK_{cs}$) [52], [56], [79], [80]. KU is one of the most abundant

proteins in human cells [81], and it also forms a complex with the WRN, and with PARP1, suggesting that these proteins act together as "caretakers" of the genome integrity [82].

Evidence for the KU complex's role in ageing has been shown in several studies with mice knockouts, as follows. In experiments carried out around the late 1990's, $Ku80^{-/-}$ mice exhibited signs of premature ageing without significantly increased cancer [83], [84]. Surprisingly (considering that Ku70 and Ku80 form a complex), $Ku70^{-/-}$ mice exhibited instead a significant incidence of thymic lymphoma [85], [86]. However, more recently, Li et al. [87] showed that those differences were likely due to differences in genetic background and/or environment. Their experiments with three types of mice cohorts, consisting of $Ku70^{-/-}$, $Ku80^{-/-}$, and $Ku70^{-/-}$ /$Ku80^{-/-}$ double-mutant mice, showed that all these cohorts exhibit a premature ageing phenotype and lower cancer levels than previously reported for $Ku70^{-/-}$ mice.

However, a different type of phenotype is obtained when combining the deletion of Ku70 and/or Ku80 with the deletion of the well-known p53 gene, a transcription factor which, among other functions, acts as a tumor suppressor. Recently, Li et al. [88] have shown that, surprisingly, $Ku70^{-/-}/p53^{-/-}$ mice lived significantly longer than either $Ku80^{-/-}/p53^{-/-}$ mice or $Ku70^{-/-}/Ku80^{-/-}/p53^{-/-}$ triple mutant mice, due to a much lower incidence of pro-B-cell lymphoma in the former cohort.

There is also evidence that NHEJ activity is considerably reduced with age in rat cortical neurons [49], [78]. This decreased NHEJ activity cannot be trivially explained as a consequence of a reduced number of double-strand breaks, because the number of double-strand breaks in the neurons of rat cerebral cortex has been shown to considerably increase with age [54]. Also, genetic defects in the NHEJ pathway have been shown to reduce hematopoietic stem function in an age-dependent manner under conditions of stress in mice [74].

Turning to human ageing, the level of mRNA expression of KU70 was observed to decrease considerably with age in human hematopoietic stem and progenitor cells [89]. Furthermore, the levels of the KU70 and MRE11 proteins were observed to significantly decline with age [90]. In addition, KU70 expression was significantly higher in a particular community known to have the highest average lifespan in South Korea when matched to other individuals of the same age, similar life patterns and same region. Hence, the authors suggested that KU70 expression in lymphocytes may be considered a biomarker of ageing. Moreover, NHEJ has been observed to become less efficient and more error-prone in senescent human fibroblasts, possibly due to alterations in expression, availability and/or localization of KU with age [80]. A systematic analysis of DNA repair proteins associated or not with ageing – based on data mining methods – indicated that NHEJ is central to proteins associated with ageing [91].

6 From DNA damage to ageing: Networks, cells and evolution

In this section we first interpret the aforementioned relationship between DNA repair pathways and ageing in context of interactions between different molecular pathways, their effects on cellular processes and how the complexity of DNA damage response systems impacts on our understanding of the role of DNA damage in ageing. Secondly, we discuss the DNA damage theory of ageing in the context of evolution and of how differences in DNA repair could have contributed to known differences in longevity and ageing between species.

6.1 Complex interactions between molecules, pathways and cells

Human and mouse progeroid syndromes show that defects in DNA repair can accelerate the ageing phenotype, possibly by impacting to some degree on the ageing process. However, one prediction of the DNA damage theory of ageing is that improved DNA repair should lead to

slower or postponed ageing, ultimately leading to longer life span and this has not been demonstrated to date [4].

Chevanne et al. [92] compared the efficiency with which cells from young, old and centenarian subjects repair DNA strand breaks caused by sublethal concentrations of hydrogen peroxide. They observed that cells from centenarians are about as efficient in that kind of repair as the cells from young subjects, and both types of cell were considerably more efficient in that task than the cells of old subjects. They also observed that the expression level of PARP1 was significantly decreased in the cells of old subjects, but not in the cells of young and centenarian subjects. In addition, centenarians have significantly higher levels of the KU70 protein. Although these results support the hypothesis that improved DNA repair systems may lead to longer life span, they are correlative in nature and far from conclusive.

Recent studies have shown associations between human longevity and DNA repair genes. Polymorphisms in ATM are one of such case. Interestingly the ATM polymorphism associated with longevity affects ATM expression, yet it is not the variant associated with high or low ATM expression that is associated with longevity but rather the polymorphism associated with moderate expression [93]. Similarly, ERCC2 polymorphisms associated with low ERCC2 expression are associated with longevity [94].

Findings from human progeroid syndromes have been validated in mouse models through disruption of DNA repair mechanisms, but again the opposite is far from proven. One study showed that *D. melanogaster* with one or two extra copies of a DNA repair gene had a slightly extended life span [95]. There have been attempts to optimize DNA repair mechanisms in mammals through genetic manipulations but by and large these have been unsuccessful [9]. A classic example is the $p53^{+/m}$ mouse which has an activated p53 yet surprisingly displays signs of premature ageing [96]. Given the complexity of DNA repair pathways, perhaps upregulating a single DNA repair protein merely shifts the rate-limiting step to another protein and fails to have an impact on ageing.

Possibly the only mouse model hinting that improving DNA repair may delay ageing comes from cancer-resistant mice with telomerase constitutively expressed. Cancer-resistant mice with enhanced expression of p53 and other tumour suppressors, p16 and $p19^{ARF}$, have a normal ageing process [97,98]. When also overexpressing telomerase their median lifespan increases by up to 40% [99]. It is not clear whether ageing is delayed in these animals or whether DNA repair is improved but these findings do point towards some level of protection from age-related degeneration via optimization of pathways associated with cancer and DNA damage responses.

Although it is clear that DNA damage and mutations increase with age, the molecular, cellular and physiological mechanisms leading to degeneration are poorly understood. One emerging hypothesis is that alterations in DNA damage or in DNA repair pathways impact on cellular processes that either limit cell division or increase cell loss (Figure 1). In fact, a number of mutations resulting in premature ageing in humans and mice are associated with cellular phenotypes such as premature replicative senescence or increased apoptosis (reviewed in [100]). Biomarkers of DNA damage and telomere dysfunction – more precisely, human orthologs of proteins secreted from telomere-dysfunctional bone-marrow cells of late generation telomerase knockoutt mice – have also been observed in ageing humans [101].

A more specific hypothesis of the above is that DNA damage accumulating in stem cells has a particular strong contribution to ageing alterations as these will be more easily propagated in

tissues and impairs tissue regeneration. For example, disruption of ATR in adult mice results in stem cell loss and premature ageing [102]. Similarly, it has been suggested that the premature ageing observed in the aforementioned $p53^{+/m}$ mice may be caused by loss of cellularity due to stem cells undergoing premature replicative senescence [96]. In haematopoietic stem cells DNA damage has been shown to accumulate with age and contribute to functional decline [103]. Therefore, stem cell ageing caused by DNA damage remains one powerful downstream mechanisms of DNA alterations with age [100,104].

Cellular responses to DNA damage involve a large number of proteins. ATM and ATR (ataxia telangiectasia and Rad3 related) are among the key mediators of the signal transduction pathway in response to DNA damage. A large-scale proteomic analysis revealed over 700 proteins phosphorylated by ATM and ATR in response to DNA damage and painted a picture of a highly interconnected network [105]. This work exemplifies the complexity of the pathways involved and how incomplete our understanding of these networks still is.

Therefore, the path from DNA damage to ageing involves multiple interacting molecular and cellular processes (Figure 1). Given the fine-tuning necessary in DNA damage responses and during interactions between pathways associated with DNA repair, including cell cycle, perhaps it is not too surprising that researchers are yet to develop a mouse model of enhanced DNA repair that delays ageing. Likewise, the findings that it is not the highest expression of ATM or ERCC2 that is associated with human longevity need to be put in context of the hundreds of other associated proteins, as well as cellular processes, though the fact that polymorphisms in genes involved in DNA repair are associated with human longevity is noteworthy.

6.2 Species differences in ageing and DNA repair

Although ageing is observed in most animal species, and in all studied mammals, there is a great variance in the rate of ageing changes across different species [106,107]. If DNA damage is the main underlying mechanism of ageing then one hypothesis is that the optimization or enhancement of DNA repair contributed to the evolution of long-lived species [106].

Some correlations have been reported between DNA repair mechanisms and longevity in mammals [108-110]. It has been argued, however, that such correlations may be an artefact of long-lived species being on average bigger since, independently of lifespan, larger animals are expected to have higher DNA repair rates [111].

While earlier studies focused on NER, more recent studies have focused on BER, including resulting from oxidative damage, though results have been mixed [106]. One study reported a correlation between PARP1 activity and longevity of mammals [112]. To test the hypothesis that PARP1 optimization contributed to the evolution of longevity, mice with human PARP-1 ectopically expressed were generated, though it resulted in impaired DNA repair and a short lifespan [113]. Of course, because DNA repair is a complex process, it could be that ectopic expression of PARP1 disrupted the fine balance of DNA repair proteins, disrupting DNA repair.

Recent progress in large-scale sequencing methods is resulting in a growing number of sequenced genomes which can be employed for detecting genes associated with the evolution of longevity [106,114]. By employing comparative genomics to detect proteins with patterns of selection specific to long-lived lineages, we recently detected that some DNA repair proteins are associated with the evolution of longevity in mammals (Li and de Magalhaes, unpublished results). While these correlations are based on computational analyses, they provide evidence

that at least some DNA repair proteins are selected for during the evolution of long lifespans, which may be due to an optimization of DNA repair pathways.

Because DNA repair systems tend to be largely conserved evolutionary, it could also be that species differences in ageing are due to the way cells respond to DNA damage. This could involve cell decisions on how to repair the damage (i.e., which pathways to activate) or even decisions at the level of whether to repair the damage or let the damaged cell die. There are complex pathways affecting these decisions and these may be involved in species differences in ageing. We speculate that short-lived species that tend to grow faster would favour responses that optimize growth even if that leads to a build-up of damaged cells while longer-lived species could "afford" to eliminate damaged cells and grow slower, resulting in a slower build-up of damage. According to this model, trade-offs in the evolution of DNA damage responses would be important for species differences in ageing rather than DNA repair *per se*.

7 CONCLUSIONS AND PERSPECTIVES

It is undeniable that alterations to DNA can have a profound impact on cellular functions and even lead to cell death. As such, a broad array of mechanisms evolved to maintain genome integrity. These mechanisms combine processes for assuring that the DNA of each cell is maintained unchanged (including copying the DNA and repairing it) and replacing genomes damaged beyond a given threshold by cell destruction mechanisms. It is equally clear that DNA changes accumulate with age in multiple tissues and are likely to contribute to the increased cancer incidence observed with age, even though which DNA lesions are more important contributors to ageing remains unknown. One major hypothesis is that DNA damage activates signalling pathways that impact on cells and result in a depletion of stem cell stocks which then contributes to ageing. As such, the idea that DNA damage accumulation with age is the primary cause of ageing remains an intuitive and powerful one.

Human progeroid syndromes clearly show that disruption of DNA repair pathways can accelerate the ageing phenotype. Although it is not clear how representative of normal ageing the changes observed in progeroid syndromes are, the breadth of age-related changes observed prematurely in progeroid syndromes suggests that at least some aspects of ageing are the same. The fact that disruption of DNA repair can accelerate ageing is not proof by itself that DNA damage causes ageing but remains a strong argument. Recent results showing that optimization of DNA repair pathways can extend mouse lifespan, even if it remains questionable whether ageing was delayed, hint that indeed the balance between DNA damage and repair sets the pace of the ageing process, at least to some degree.

The complexity of responses to DNA damage, which involve networks of interacting processes including DNA repair mechanisms, cell cycle checkpoints and apoptotic pathways, mean that the cellular processes of DNA damage responses and DNA repair and how these impact on organismal processes like ageing remains only partly understood. Elucidating the evolution of these mechanisms and how adaptations in them could have contributed to species differences in ageing remains a promising but daunting task. Advances in data integration, modelling and high-throughput approaches have the potential to elucidate the complex interplay between DNA damage, DNA repair and their interacting networks, but there is still a long road ahead. Ultimately, however, such approaches can lead to solving ageing, one of the greatest biological riddles of our time.

ACKNOWLEDGMENTS

The work of JPM is supported by the BBSRC, the Ellison Medical Foundation and a Marie Curie International Reintegration Grant within EC-FP7. We also thank Dan Lloyd for his valuable comments about an earlier draft of this manuscript. The authors apologize to those whose work could not be cited due to lack of space.

References

[1] H.L. Gensler and H. Bernstein DNA damage as the primary cause of aging, Q Rev Biol 56 (1981) 279-303.

[2] G. Failla The aging process and cancerogenesis, Ann N Y Acad Sci 71 (1958) 1124-1140.

[3] L. Szilard On the Nature of the Aging Process, Proc Natl Acad Sci U S A 45 (1959) 30-45.

[4] R. Arking The Biology of Aging: Observations and Principles. 3rd Ed, Oxford University Press, Oxford, UK, 2006.

[5] J.H. Hoeijmakers DNA damage, aging, and cancer, N Engl J Med 361 (2009) 1475-1485.

[6] J. Vijg and M.E. Dolle Large genome rearrangements as a primary cause of aging, Mech Ageing Dev 123 (2002) 907-915.

[7] L. Szilard On the nature of the ageing process, Proceedings of the National Academy of Sciences, USA 45 (1959) 30-45.

[8] D.B. Lombard, K.F. Chua, R. Mostoslavsky, S. Franco, M. Gostissa and F.W. Alt DNA repair, genome stability and aging, Cell 120 (2005) 497-512.

[9] J.P. de Magalhaes Open-minded scepticism: inferring the causal mechanisms of human ageing from genetic perturbations, Ageing Res Rev 4 (2005) 1-22.

[10] K. Khrapko and J. Vijg Mitochondrial DNA mutations and aging: devils in the details?, Trends in Genetics 25 (2008) 91-98.

[11] K. Pesce and M.J. Rothe The premature ageing syndromes, Clinics in Dermatology 14 (1996) 161-170.

[12] G.M. Martin and H. Oshima Lessons from human progeroid syndromes, Nature 408 (2000) 263-266.

[13] P. Hasty, J. Campisi, J. Hoeijmakers, H.v. Steeg and J. Vijg Aging and genome maintenance: lessons from the mouse?, Science 299 (2003) 1355-1359.

[14] J.P.d. Magalhaes and R.G.A. Faragher Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity, BioEssays 30 (2008) 567-578.

[15] G.M. Martin Genetic modulation of senescent phenotypes in Homo sapiens, Cell 120 (2005) 523-532.

[16] G.M. Martin Genetic syndromes in man with potential relevance to the pathobiology of aging, Birth Defects Orig Artic Ser 14 (1978) 5-39.

[17] H. Lans and J.H. Hoeijmakers Ageing nucleus gets out of shape, Nature 440 (2006) 32-34.

[18] B.P. Best Nuclear DNA damage as a direct cause of ageing, Rejuvenation Research 12 (2009) 199-208.

[19] T. Davis and D. Kipling Werner Syndrome as an example of inflamm-aging: possible therapeutic opportunities for a progeroid syndrome?, Rejuvenation Research 9 (2006) 402-407.

[20] D. Kipling, T. Davis, E.L. Ostler and R.G.A. Faragher What can progeroid syndromes tell us about human aging?, Science 305 (2004) 1426-1431.

[21] K. Ariyoshi, K. Suzuki, M. Goto, M. Watanabe and S. Kodama Increased chromosome instability and accumulation of DNA double-strand breaks in Werner Syndrome Cells, Journal of Radiation Research 48 (2007) 219-231.

[22] J.W. Lee, J. Harrigan, P.L. Opresko and V.A. Bohr Pathways and functions of the Werner syndrome protein, Mechanisms of Ageing and Development 126 (2005) 79-86.
[23] R.C.M. Hennekam Hutchinson-Gilford progeria syndrome: review of the phenotype, American Journal of Medical Genetics Part A 140A (2006) 2603-2624.
[24] J.M. Bridger and I.R. Kill Aging of Hutchinson-Gilford progeria syndrome fibroblasts is characterised by hyperproliferation and increased apoptosis, Experimental Gerontology 39 (2004) 717-724.
[25] J.d. Boer, J.O. Andressoo, J.d. Wit, J. Huijmans, R.B. Beems, H.V. Steeg, G. Weeda, G.T.J.v.d. Horst, W.V. Leeuwen, A.P.N. Themmen, M. Meradji and J.H.J. Hoeijmakers Premature aging in mice deficient in DNA repair and transcription, Science 296 (2002) 1276-1279.
[26] G. Rotman and Y. Shiloh Ataxia-telangiectasia: is ATM a sensor of oxidative damage and stress?, BioEssays 19 (1997) 911-917.
[27] C.G. Woods DNA repair disorders., Archives of Diseases in Childhood 78 (1998) 178-184.
[28] K.K. Wong, R.S. Maser, R.M. Bachoo, J. Menon, D.R. Carrasco, Y. Gu, F.W. Alt and R.A.d. Pinho Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing, Nature 421 (2003) 643-647.
[29] P. Hasty, J. Campisi, J. Hoeijmakers, H. van Steeg and J. Vijg Aging and genome maintenance: lessons from the mouse?, Science 299 (2003) 1355-1359.
[30] L.C. Mounkes, S. Kozlov, L. Hernandez, T. Sullivan and C.L. Stewart A progeroid syndrome in mice is caused by defects in A-type lamins, Nature 423 (2003) 298-301.
[31] B. Liu, J. Wang, K.M. Chan, W.M. Tjia, W. Deng, X. Guan, J.D. Huang, K.M. Li, P.Y. Chau, D.J. Chen, D. Pei, A.M. Pendas, J. Cadinanos, C. Lopez-Otin, H.F. Tse, C. Hutchison, J. Chen, Y. Cao, K.S. Cheah, K. Tryggvason and Z. Zhou Genomic instability in laminopathy-based premature aging, Nat Med 11 (2005) 780-785.
[32] J. de Boer, J.O. Andressoo, J. de Wit, J. Huijmans, R.B. Beems, H. van Steeg, G. Weeda, G.T. van der Horst, W. van Leeuwen, A.P. Themmen, M. Meradji and J.H. Hoeijmakers Premature aging in mice deficient in DNA repair and transcription, Science 296 (2002) 1276-1279.
[33] S. Chang, A.S. Multani, N.G. Cabrera, M.L. Naylor, P. Laud, D. Lombard, S. Pathak, L. Guarente and R.A. DePinho Essential role of limiting telomeres in the pathogenesis of Werner syndrome, Nat Genet 36 (2004) 877-882.
[34] B. Schumacher, J.H. Hoeijmakers and G.A. Garinis Sealing the gap between nuclear DNA damage and longevity, Mol Cell Endocrinol 299 (2009) 112-117.
[35] R.A. Miller Accelerated Aging: a primrose path to insight?, Aging Cell 3 (2004) 47-51.
[36] P. Hasty and J. Vijg Rebuttal to Miller: 'Accelerated Aging': a primrose path to insight?, Aging Cell 3 (2004) 67-69.
[37] R.d. Bont and N.V. Larebeke Endogenous DNA damage in humans: a review of quantitative data, Mutagenesis 19 (2004) 169-185.
[38] A.L. Jackson and L.A. Loeb The contribution of endogenous sources of DNA damage to the multiple mutations in cancer, Mutation Research 477 (2001) 7-21.
[39] E.C. Friedberg, G.C. Walker, W. Siede, R.D. Wood, R.A. Schultz and T. Ellenberger DNA Repair and Mutagenesis. 2nd Ed, ASM Press, Washington, DC, USA, 2006.
[40] C.G. Fraga, M.K. Shigenaga, J.W. Park, P. Degan and B.N. Ames Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine, Proc Natl Acad Sci U S A 87 (1990) 4533-4537.

[41] M. Yaar and B.A. Gilchrest Photoageing: mechanism, prevention and therapy, British Journal of Dermatology 157 (2007) 874-887.
[42] J. Nakamura and J.A. Swenberg Endogenous apurinic/apyrimidinic sites in genomic DNA of mammalian tissues, Cancer Research 59 (1999) 2522-2526.
[43] T. Lindahl Instability and decay of the primary structure of DNA, Nature 362 (1993) 709-715.
[44] J. Nakamura, K.L. David and J.A. Swenberg 5'-nicked apurinic/apyrimidinic sites are resistant to ?-elimination by ?-polymerase and are persistent in human cultured cells after oxidative stress, The Journal of Biological Chemistry 275 (2000) 5323-5328.
[45] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter Molecular Biology of the Cell. 4th Ed, Garland, New York, NY, USA, 2002.
[46] D.N. Cooper and H. Youssoufian The CpG dinucleotide and human genetic disease, Human Genetics 78 (1988) 151-155.
[47] G.E. Taccioli, T.M. Gottlieb, T. Blunt, A. Priestley, J. Demengeot, R. Mizuta, A.R. Lehmann, F.W. Alt, S.P. Jackson and P.A. Jeggo Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination, Science 265 (1994) 1442-1445.
[48] J.R. Walker, R.A. Corpina and J. Goldberg Structure of the Ku heterodimer bound to DNA and its implications for double-strand break repair, Nature 412 (2001) 607-613.
[49] S. Sharma Age-related nonhomologous end joining activity in rat neurons, Brain Research Bulletin 73 (2007) 48-54.
[50] W.R. Engels, D. Johnson-Schlitz, C. Flores, L. White and C.R. Preston A third link connecting aging with double strand break repair, Cell Cycle 6 (2007) 131-135.
[51] K.W. Caldecott Single-strand break repair and genetic disease, Nat Rev Genet 9 (2008) 619-631.
[52] V. Gorbunova, A. Seluanov, Z. Mao and C. Hine Changes in DNA repair during aging, Nucleic Acids Research 35 (2007) 7466-7474.
[53] M.L. Fishel, M.R. Vasko and M.R. Kelley DNA repair in neurons: so if they don't divide what's to repair?, Mutation Research 614 (2007) 24-36.
[54] K.S. Rao DNA repair in aging rat neurons, Neuroscience 145 (2007) 1330-1340.
[55] M. Yamada, M.U. Udono, M. Hori, R. Hirose, S. Sato, T. Mori and O. Nikaido Aged human skin removes UVB-induced pyrimidine dimers from the epidermis more slowly than younger adult skin in vivo, Archives of Dermatology Research 297 (2006) 294-302.
[56] J.H. Hoeijmakers Genome maintenance mechanisms for preventing cancer, Nature 411 (2001) 366-374.
[57] G.M. Li Mechanisms and functions of DNA mismatch repair, Cell Research 18 (2008) 85-98.
[58] J. Vijg The role of DNA damage and repair in aging: new approaches to an old problem, Mechanisms of Ageing and Development 129 (2008) 498-502.
[59] S. Beneke and A. Burkle Poly(ADP-ribosyl)ation in mammalian ageing, Nucleic Acids Research 35 (2007) 7456-7465.
[60] T.H. Krishna, S. Mahipal, A. Sudhakar, H. Sugimoto, R. Kalluri and K.S. Rao Reduced DNA gap repair in aging neuronal extracts and its restoration by DNA polymerase ß and DNA-ligase, Journal of Neurochemistry 92 (2005) 818-823.
[61] D.C. Cabelof, J.J. Raffoul, Y. Ge, H.V. Remmen and A.R.H. L.H. Matherly Age-related loss of the DNA repair response following exposure to oxidative stress, Journal of Gerontology: Biological Sciences 61A (2006) 427-434.

[62] T. Kaneko, S. Tahara, M. Tanno and T. Taguchi Age-related changes in the induction of DNA polymerases in rat liver by gamma-ray irradiation, Mechanisms of Ageing and Development 123 (2002) 1521-1528.
[63] D.C. Cabelof, J.J. Raffoul, S. Yanamadala, C. Ganir, Z.M. Guo and A.R. Heydari Attenuation of DNA polymerase beta-dependent base excision repair and increased DMS-induced mutagenicity in aged mice, Mutation Research 500 (2002) 135-145.
[64] M.A. Graziewicz, M.J. Longley and W.C. Copeland DNA polymerase in mitochondrial DNA replication and repair, Chemistry Review 106 (2006) 383-405.
[65] T. Lu, Y. Pan, S.Y. Kao, C. Li, I. Kohane, J. Chan and B.A. Yankner Gene regulation and DNA damage in the ageing bring, Nature 429 (2004) 883-891.
[66] S. Moriwaki and Y. Takahashi Photoaging and DNA repair, Journal of Dermatological Science 50 (2008) 169-176.
[67] F. Hazane, S. Sauvaigo, T. Douki, A. Favier and J.C. Beani Age-dependent DNA repair and cell cycle distribution of human skin fibroblasts in response to UVA irradiation, Journal of Photochemistry and Photobiology B: Biology 82 (2006) 214-223.
[68] Y. Takahashi, S.-i. Moriwaki, Y. Sugiyama, Y. Endo, K. Yamazaki, T. Mori, M. Takigawa and S. Inoue Decreased Gene Expression Responsible for Post-Ultraviolet DNA Repair Synthesis in Aging: A Possible Mechanism of Age-Related Reduction in DNA Repair Capacity, The Journal of Investigative Dermatology 124 (2005) 435-442.
[69] T.J. Merkle, K. O'Brien, P.J. Brooks, R.E. Tarone and J.H. Robbins DNA repair in human fibroblasts, as reflected by host-cell reactivation of a transfected UV-irradiated luciferase gene, is not related to donor age, Mutation Research 554 (2004) 9-17.
[70] L. Niedernhofer Tissue-specific accelerate aging in nucleotide excision repair deficiency, Mechanisms of Ageing and Development 129 (2008) 408-415.
[71] L.J. Niedernhofer, G.A. Garinis, A. Raams, A.S. Lalai, A.R. Robinson, E. Appeldoorn, H. Odijk, R. Oostendorp, A. Ahmad, W.V. Leeuwen, A.F. Theil, W. Vermeulen, G.T.J.v.d. Horst, P. Meinecke, W.J. Kleijer, J. Vijg, N.G.J. Jaspers and J.H. Hoeijmakers A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis, Nature 444 (2006) 1038-1043.
[72] I.v.d. Pluijm, G.A. Garinis, R.M.C. Brandt, T.G.M.F. Gorgels, S.W. Wijnhoven, K.E.M. Diderich, J.d. Wit, J.R. Mitchell, C.v. Oostrom, R. Beems, L.J. Niedernhofer, S. Velasco, E.C. Friedberg, K. Tanaka, S. Harry van, J.H.J. Hoeijmakers and G.T.J.v.d. Horst Impaired Genome Maintenance Suppresses the Growth Hormone–Insulin-Like Growth Factor 1 Axis in Mice with Cockayne Syndrome, PLoS (Public Library of Science) Biology 5 (2007) 23-38.
[73] M.v.d. Ven, J.O. Andressoo, V.B. Holcomb, M.v. Lindern, W.M.C. Jong, C.I.D. Zeeuw, Y. Suh, P. Hasty, J.H.J. Hoeijmakers, G.T.J.v.d. Horst, J.R. Mitchell and P.L. Genetics Adaptive response in segmental progeria resembles long-lived dwarfism and calorie restriction in mice, PLoS (Public Library of Science) Genetics 2 (2006) 2013-2025.
[74] D.J. Rossi, D. Bryder, J. Seita, A. Nussenzweig, J. Hoeijmakers and I.L. Weissman Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age, Nature 447 (2007) 725-730.
[75] M. Ljungman and D.P. Lane Transcription - guarding the genome by sensing DNA damage, Nature Reviews - Cancer 4 (2004) 727-737.
[76] J.d. Boer, I. Donker, J.d. Wit, J.H.J. Hoeijmakers and G. Weeda Disruption of the mouse xeroderma pigmentosum group D DNA repair/basal transcription gene results in preimplantation lethality, Cancer Research 58 (1998) 89-94.

[77] J.Y. Park, M.O. Cho, S. Leonard, B. Calder, I.S. Mian, W.H. Kim, S. Winjhoven, H.V. Steeg, J. Mitchell, G.T.J.v.d. Horst, J. Hoeijmakers, P. Cohen, J. Vijg and Y. Suh Homeostatic imbalance between apoptosis and cell renewal in the liver of premature aging XpdTTD mice, PLoS (Public Library of Science) One 3 (2008) 1-10.
[78] V.N. Vyjayanti and K.S. Rao DNA double strand break repair in brain: reduced NHEJ activity in aging rat neurons, Neuroscience Letters 393 (2006) 18-22.
[79] V.B. Holcomb, F. Rodier, Y.J. Choi, R.A. Busuttil, H. Vogel, J. Vijg, J. Campisi and P. Hasty Ku80 deletion suppresses spontaneous tumors and induces a p53-mediated DNA damage response, Cancer Research 68 (2008) 9497-9502.
[80] A. Seluanov, J. Danek, N. Hause, V. Gorbunova, l. Changes in the and s. distribution of Ku proteins during cellular Changes in the level and distribution of Ku proteins during cellular senescence, DNA Repair 6 (2007) 1740-1748.
[81] W. Chai, L.P. Ford, L. Lenertz, W.E. Wright and J.W. Shay Human Ku70/80 associates physically with telomerase through interaction with hTERT, The Journal of Biological Chemistry 277 (2002) 47242-47247.
[82] B. Li, S. Navarro, N. Kasahara and L. Comai Identification and biochemical characterization of a Werner's Syndrome protein complex with Ku70/80 and Poly(ADP-ribose) Polymerase-1, The Journal of Biological Chemistry 279 (2004) 13659-13667.
[83] H. Vogel, D.S. Lim, G. Karsenty, M. Finegold and P. Hasty Deletion of Ku86 causes early onset of senescence in mice, Proceedings of the National Academy of Sciences (PNAS), USA 96 (1999) 10770-10775.
[84] C. Zhu, M.A. Bogue, D.S. Lim, P. Hasty and D.B. Roth Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates, Cell 86 (1996) 379-389.
[85] G.C. Li, H. Ouyang, X. Li, H. Nagasawa, J.B. Little, D.J. Chen, C.C. Ling, Z. Fuks and C. Cordon-Cardo Ku70: a candidate tumor suppressor gene for murine T cell lymphoma, Molecular Cell 2 (1998) 1-8.
[86] Y. Gu, K.J. Seidl, G.A. Rathbun, C. Zhu, J.P. Manis, N.V.d. Stoep, L. Davidson, H.L. Cheng, J.M. Sekiguchi, K. Frank, P. Stanhope-Baker, M.S. Schlissel, D.B. Roth and F.W. Alt Growth retardation and leaky SCID phenotype of Ku70-deficient mice, Immunity 7 (1997) 653-665.
[87] H. Li, H. Vogel, V.B. Holcomb, Y. Gu and P. Hasty Deletion of Ku70, Ku80, or both causes early aging without substantially increased cancer, Molecular and Cellular Biology 27 (2007) 8205-8214.
[88] H. Li, Y.J. Choi, M.A. Hanes, T. Marple, H. Vogel and P. Hasty Deleting Ku70 is milder than deleting Ku80 in p53-mutant mice and cells, Oncogene 28 (2009) 1875-1878.
[89] W.C. Prall, A. Czibere, M. Jager, D. Spentzos, T.A. Libermann, N. Gattermann, R. Haas and M. Aivado Age-related transcription levels of KU70, MGST1 and BIK in CD34+ hematopoietic stem and progenitor cells, Mechanisms of Ageing and Development 128 (2007) 503-510.
[90] Y.J. Ju, K.H. Lee, J.E. Park, Y.S. Yi, M.Y. Yun, Y.H. Ham, T.J. Kim, H.M. Choi, G.J. Han, J.H. Lee, J. Lee, J.S. Han, K.M. Lee and G.H. Park Decreased expression of DNA repair proteins Ku70 and Mre11 is associated with aging and may contribute to the cellular senescence, Experimental and Molecular Medicine 38 (2006) 686-693.

[91] A.A. Freitas, O. Vasieva and J.P. de Magalhaes A data mining approach for classifying DNA repair genes into ageing-related or non-ageing-related, BMC Genomics 12 (2011) 27.
[92] M. Chevanne, C. Calia, M. Zampieri, B. Cecchinelli, R. Caldini, D. Monti, L. Bucci, C. Franceschi and P. Caiafa 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 Research 10 (2007) 191-203.
[93] T. Chen, B. Dong, Z. Lu, B. Tian, J. Zhang, J. Zhou, H. Wu, Y. Zhang, J. Wu, P. Lin, H. Xu and X. Mo A functional single nucleotide polymorphism in promoter of ATM is associated with longevity, Mech Ageing Dev 131 (2010) 636-640.
[94] J. Polosak, M. Roszkowska-Gancarz, A. Kurylowicz, M. Owczarz, P. Dobosz, M. Mossakowska, A. Szybinska and M. Puzianowska-Kuznicka Decreased expression and the Lys751Gln polymorphism of the XPD gene are associated with extreme longevity, Biogerontology 11 (2010) 287-297.
[95] S. Symphorien and R.C. Woodruff Effect of DNA repair on aging of transgenic Drosophila melanogaster: I.mei-41 locus, Journal of Gerontology. A. Biological Science Medical Science 58 (2003) B782-B787.
[96] S.D. Tyner, S. Venkatachalam, J. Choi, S. Jones, N. Ghebranious, H. Igelmann, X. Lu, G. Soron, B. Cooper, C. Brayton, S. Hee Park, T. Thompson, G. Karsenty, A. Bradley and L.A. Donehower p53 mutant mice that display early ageing-associated phenotypes, Nature 415 (2002) 45-53.
[97] A. Matheu, C. Pantoja, A. Efeyan, L.M. Criado, J. Martin-Caballero, J.M. Flores, P. Klatt and M. Serrano Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging, Genes Dev 18 (2004) 2736-2746.
[98] I. Garcia-Cao, M. Garcia-Cao, J. Martin-Caballero, L.M. Criado, P. Klatt, J.M. Flores, J.C. Weill, M.A. Blasco and M. Serrano "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally, EMBO J 21 (2002) 6225-6235.
[99] A. Tomas-Loba, I. Flores, P.J. Fernandez-Marcos, M.L. Cayuela, A. Maraver, A. Tejera, C. Borras, A. Matheu, P. Klatt, J.M. Flores, J. Vina, M. Serrano and M.A. Blasco Telomerase reverse transcriptase delays aging in cancer-resistant mice, Cell 135 (2008) 609-622.
[100] J.P. de Magalhaes and R.G. Faragher Cell divisions and mammalian aging: integrative biology insights from genes that regulate longevity, Bioessays 30 (2008) 567-578.
[101] H. Jiang, E. Schiffer, Z. Song, J. Wang, P. Zurbig, K. Thedieck, S. Moes, H. Bantel, N. Saal, J. Jantos, M. Brecht, P. Jeno, M.N. Hall, K. Hager, M.P. Manns, H. Hecker, A. Ganser, K. Dohner, A. Bartke, C. Meissner, H. Mischak, Z. Ju and K.L. Rudolph Proteins induced by telomere dysfunction and DNA damage represent biomarkers of human aging and disease, Proc Natl Acad Sci U S A 105 (2008) 11299-11304.
[102] Y. Ruzankina, C. Pinzon-Guzman, A. Asare, T. Ong, L. Pontano, G. Cotsarelis, V.P. Zediak, M. Velez, A. Bhandoola and E.J. Brown Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss, Cell Stem Cell 1 (2007) 113-126.
[103] D.J. Rossi, D. Bryder, J. Seita, A. Nussenzweig, J. Hoeijmakers and I.L. Weissman Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age, Nature 447 (2007) 725-729.
[104] N.E. Sharpless and R.A. DePinho How stem cells age and why this makes us grow old, Nat Rev Mol Cell Biol 8 (2007) 703-713.

[105] S. Matsuoka, B.A. Ballif, A. Smogorzewska, E.R. McDonald, 3rd, K.E. Hurov, J. Luo, C.E. Bakalarski, Z. Zhao, N. Solimini, Y. Lerenthal, Y. Shiloh, S.P. Gygi and S.J. Elledge ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage, Science 316 (2007) 1160-1166.
[106] S.N. Austad Methusaleh's Zoo: how nature provides us with clues for extending human health span, J Comp Pathol 142 Suppl 1 (2010) S10-21.
[107] C.E. Finch Longevity, Senescence, and the Genome, The University of Chicago Press, Chicago and London, 1990.
[108] R.W. Hart and R.B. Setlow Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species, Proc Natl Acad Sci U S A 71 (1974) 2169-2173.
[109] G.A. Cortopassi and E. Wang There is substantial agreement among interspecies estimates of DNA repair activity, Mech Ageing Dev 91 (1996) 211-218.
[110] A.A. Francis, W.H. Lee and J.D. Regan The relationship of DNA excision repair of ultraviolet-induced lesions to the maximum life span of mammals, Mechanisms of Ageing & Development 16 (1981) 181-189.
[111] D.E. Promislow DNA repair and the evolution of longevity: a critical analysis, J Theor Biol 170 (1994) 291-300.
[112] K. Grube and A. Burkle Poly(ADP-ribose) polymerase activity in mononuclear leukocytes of 13 mammalian species correlates with species-specific life span, Proc Natl Acad Sci U S A 89 (1992) 11759-11763.
[113] A. Mangerich, N. Herbach, B. Hanf, A. Fischbach, O. Popp, M. Moreno-Villanueva, O.T. Bruns and A. Burkle Inflammatory and age-related pathologies in mice with ectopic expression of human PARP-1, Mech Ageing Dev 131 (2010) 389-404.
[114] J.P. de Magalhaes, C.E. Finch and G. Janssens Next-generation sequencing in aging research: emerging applications, problems, pitfalls and possible solutions, Ageing Res Rev 9 (2010) 315-323.

Figure legends

Figure 1: Overview of the DNA damage theory of ageing. A variety of intrinsic and extrinsic sources can result in DNA damage. An array of complex DNA repair mechanisms evolved to repair DNA damage, yet these are not perfect. DNA lesions in cells can lead to mutations, cell cycle arrest, blocked transcription, apoptosis and many other problems which in turn result in loss of cell function and cell death. With biological time, the accumulation of DNA damage in an increasing number of cells will lead to loss of stem cells and disruption of tissue homeostasis which causes ageing of the organism. Protein structures by the EBI (http://www.ebi.ac.uk/).

Figures

Figure 1