Published in final edited form as:

Mol Cell. 2016 September 1; 63(5): 729–738. doi:10.1016/j.molcel.2016.08.004.

Do DNA double-strand breaks drive aging?

Ryan R White[1,*,#] and **Jan Vijg**[2,*]

[1]Laboratory of Genome Maintenance, The Rockefeller University, 1230 York Ave, New York, NY 10065

[2]Department of Genetics, Albert Einstein College of Medicine, 1301 Morris Park Ave, Bronx, NY 10461

Abstract

DNA double-strand breaks (DSBs) are rare but highly toxic lesions, requiring orchestrated and conserved machinery to prevent adverse consequences, such as cell death and cancer-causing genome structural mutations. DSBs trigger the DNA damage response (DDR) that directs a cell to repair the break, undergo apoptosis or become senescent. There is increasing evidence that the various endpoints of DSB processing by different cells and tissues are part of the aging phenotype, with each stage of the DDR associated with specific aging pathologies. In this perspective we discuss the possibility that DSBs are major drivers of intrinsic aging, highlighting the dynamics of spontaneous DSBs in relation to aging, the distinct age-related pathologies induced by DSBs, and the segmental progeroid phenotypes in humans and mice with genetic defects in DSB repair. A model is presented as to how DSBs could drive some of the basic mechanisms underlying age-related functional decline and death.

Aging can be defined as a series of time-related, degenerative processes beginning in adulthood that eventually ends life. Aging, often referred to as organismal senescence, is universal among mammals and occurs in most organisms, with some exceptions in species such as *Hydra*. Aging leads to loss of function and a steeply increasing risk of diseases, such as cancer, heart disease, and diabetes. While multifactorial in nature, it is conceivable that aging is ultimately caused by a combination of toxic by-products of normal metabolism, such as reactive oxygen species (ROS) and imperfections in the systems that normally are capable of repairing cellular damage. Because of its central role in all living systems, DNA has long been considered as a major target of age-related cellular damage and a potential universal cause of aging (Gorbunova and Seluanov, 2016; Vijg, 2007). Indeed, the challenge that such damage posed to even the earliest living systems necessitated the emergence of highly advanced, conserved pathways of genome maintenance. Without such evolved pathways cells could not survive the high influx of spontaneous DNA damage, which is

[*]To whom correspondence should be addressed: Ryan R White, PhD, The Rockefeller University, 1230 York Ave, New York, New York 10065, rwhite@rockefeller.edu, Jan Vijg, PhD, Albert Einstein College of Medicine, 1301 Morris Park Ave, Bronx, NY 10461, jan.vijg@einstein.yu.edu.

[#]Lead contact

estimated at ~2×10^5 lesions per day in a typical mammalian cell (Barnes and Lindahl, 2004). The majority of this damage is repaired swiftly and therefore unlikely to drive the aging process; however, some highly toxic lesions persisting in a very small quantity can pose a considerable threat to the cell and the organism. The best known of such lesions is the DNA double-strand break (DSB).

DNA DSBs occur in any given cell in the order of 10 to 50 per cell per day, depending on cell cycle and tissue (Vilenchik and Knudson, 2003). DSBs, as well as other lesions affecting both DNA strands, such as interstrand cross-links (ICLs), are difficult to remove and often lead to cell death, cellular senescence, or mutations (most notably genome structural variation). DSBs can be induced spontaneously, for example by ROS, but also through exposure to ionizing radiation, treatment with chemotherapeutic agents, or even through ingestion of nutritional supplements, such as bioflavonoids. In this perspective we will review current evidence that DNA DSBs are key lesions driving some of the specific molecular and cellular end points that characterize mammalian aging and age-related disease.

DSBs, DSB processing and aging

Once a DSB occurs within a cell, there is a rapid, concerted signaling cascade to process the damage and prevent adverse consequences that could impact cellular function and survival. Collectively this is known as the DNA damage response (DDR), which has been extensively reviewed, both for DNA damage in general and specifically for DSBs (Ceccaldi et al., 2016; Ciccia and Elledge, 2010; Jackson and Bartek, 2009). Here, the DDR in response to DSBs will be discussed in terms of its possible relationship with aging and longevity.

DSBs are detected by sensor proteins, most notably the MRE11/RAD50/NBS1 (MRN) complex and the Ku70/Ku80 heterodimer, which through many possible mediators and effectors signal the processes that lead to a number of possible outcomes, almost all of which are relevant to the aging process. The pathway choice that determines the outcome depends on a number of factors, including the type and/or severity of the damage, the cell type (e.g., mitotically active or not, stem cell or normal somatic cell), cell cycle stage, chromatin status and, possibly, age of the organism (Ceccaldi et al., 2016).

The best possible outcome of DSB processing is actual repair and restoration of an intact double helix. However, even if repair is successfully completed it may have been erroneous resulting in DNA mutations. Other pathway choices with possible adverse outcomes relevant to aging are apoptosis and cellular senescence.

At early age, under normal physiological conditions, adverse outcomes of DSB processing are rare and not immediately harmful to the organism. Indeed, they are beneficial since rapid repair promotes cell survival, even if this occasionally goes at the expense of mutations. The same applies to apoptosis, generally an efficient way to cleanse the soma of irreversibly damaged cells. Likewise, cellular senescence is now established as a key developmental process (Munoz-Espin et al., 2013) as well as a trigger of tissue remodeling upon damage (Demaria et al., 2014). However, at old age, the cumulative effects of all these events may

well be a cause of some of the well-documented phenotypes of aging, such as atrophy, inflammation, immuno-senescence, and cancer.

One of the first questions arising in relation to DSBs and aging is whether the frequency of such events increases with age. Spontaneous DNA damage levels in human and animal tissues are normally very low in spite of their high influx, due to the enormous efficiency of genome maintenance, making it difficult to study these events during normal aging in mammals. This is especially true for the highly toxic DSBs. However, snapshot estimates of DSB levels can be inferred by detecting DSB protein aggregates as microscopically visible, subnuclear foci. Most commonly used is phosphorylated histone H2AX (γH2AX), which often co-localizes with ATM, 53BP1, and RAD51. γH2AX foci are often used as a surrogate for DSBs based on their quantitative correlation with increasing doses of ionizing radiation (Rothkamm et al., 2015). Interestingly, while most foci quickly disappear after radiation, some remain for days to months (Siddiqui et al., 2015). γH2AX foci were especially shown to be highly persistent in close proximity to telomeres (Fumagalli et al., 2012), which may reflect uncapped telomeres. Such persistent γH2AX foci have also been observed spontaneously, and increase with age, in human and animal cells and tissues, ranging from hematopoietic stem cells (HSCs) and dermal fibroblasts to liver, brain and ovarian primordial follicles (Hewitt et al., 2012; Rube et al., 2011; Sedelnikova et al., 2004; Titus et al., 2013; Wang et al., 2009). While such persistent γH2AX foci could reflect insufficient H2AX dephosphorylation (Mamouni et al., 2014), which may get worse with age, it is tempting to speculate that instead they mark DSBs that are difficult to repair, such as uncapped telomeres, which could explain their accumulation over time. Since persistency of γH2AX foci appears to depend on genomic location, it would be important to analyze DSBs with more direct assays capable of mapping these events genome-wide at the basepair level (Crosetto et al., 2013). Of note, the observed accumulation of persistent γH2AX foci is in keeping with a wealth of evidence that increased macromolecular damage, possibly as a consequence of imperfect maintenance systems is a hallmark of the aging process (Lopez-Otin et al., 2013).

The presence of persistent DSBs has been shown to directly affect the functionality of the cell, specifically within the stem cell compartment. For example, when quiescent, HSCs are not affected by DSBs; however, when prompted to replicate in response to stress, HSCs from aged mice were found to divide more slowly or die (Flach et al., 2014; Moehrle et al., 2015; Rossi et al., 2007; Walter et al., 2015). Indeed, replication stress has been identified as a potent driver of functional decline of old HSCs (Flach et al., 2014). Proliferating stem cells are very sensitive to radiation-induced apoptosis, which then forces quiescent stem cells to proliferate to replace lost stem cells. Interestingly, when HSCs exit from quiescence virtually all DNA repair pathways are stimulated to repair damage before the cells continue cycling, even in aged HSC populations. The latter were found to exhibit a larger amount of SSBs and DSBs than cells from young animals (Beerman et al., 2014). Hence, at least in the hematopoietic system, DSBs limit cell renewal capacity and accumulation of DSBs during aging could adversely affect regeneration.

In the neuronal stem cell compartment the effects of DSBs are different. Here, when neuronal stem cells encounter damage, they are forced to undergo premature senescence or

terminally differentiate into astroglial cells (Schneider et al., 2013). This forced senescence intrinsically diminishes the proliferative potential of neuronal stem cells and over time may contribute to the neurodegeneration and cognitive impairment often seen in aging.

Taken together, there is consensus that γH2AX foci increase with age in multiple tissue types as well as in senescent cells. It is unknown if this increase reflects the accumulation of unrepaired DSBs, progressively delayed repair events, possibly as a consequence of an inherently limited capacity to process DSBs, or simply increasingly ineffective dephosphorylation. The latter seems unlikely because age-related accumulation of 53BP1 foci, an independent marker for DSB repair, has also been observed (White et al., 2015). While the data pointing to possible physiological effects of γH2AX foci accumulating with age, specifically in HSCs, still remain inconsistent and needs further study, it is reasonable to hypothesize that DSB accumulation during aging is a major factor in the impairment of stem cell proliferative capacity.

DSB processing and repair as a function of age

Based on the evidence that unrepaired DSBs accumulate with age and are associated with a major age-related cellular phenotype, i.e., cellular senescence, it is important to study various aspects of the DDR in relation to aging. Interestingly, levels of ATM and its phosphorylation after ionizing radiation, which determines the kinase activity, were found to decrease in aged mice. This decreased ATM function was suggested to underlie the observed decline in p53 response to ionizing radiation in old animals (Feng et al., 2007). Also, the increased level of persistent γH2AX foci in ovarian mouse and human primordial follicles mentioned above was found to be accompanied by a reduced expression level of genes involved in DSB processing, including ATM, MRE11, RAD51 and BRCA1, but not BRCA2 (Oktay et al., 2015; Titus et al., 2013), which may account for the decline of oocyte reserves in aging mammals.

More information is available on the capacity to repair DSBs in relation to age. DSB repair consists of two main pathways: homologous recombination (HR) and non-homologous end joining (NHEJ) (Aparicio et al., 2014; Chapman et al., 2012). In mammals NHEJ is the most commonly used pathway in all phases of the cell cycle. It does not require homologous ends and signals DSBs through the Ku70/Ku80 heterodimer and DNA-PK_{CS}. After minimal processing by nucleases, the two ends of broken DNA are joined by the LIGIV/XRCC4/XLF complex. NHEJ is error-prone because end processing can lead to small basepair deletions and translocations (Ghezraoui et al., 2014; Lieber, 2010). In addition to classical NHEJ, alternate pathways, such as Alt-NHEJ or microhomology-mediated end joining (MMEJ), can operate in mammalian cells. Although distinct mechanisms between Alt-NHEJ and MMEJ are still an active area of research, in general both are kinetically slower than NHEJ, rely on microhomologies at DNA ends, and can give rise to larger basepair deletions than classical NHEJ as well as translocations. Indeed, these alternative pathways are now considered a major source of genomic instability, which makes them highly relevant as DSB-induced mechanisms of aging and senescence. The alternative end joining pathways also involve different proteins; they can be initiated by PARP1, and can

also involve factors that function in HR, such as BRCA1 (Iliakis et al., 2015; Sfeir and Symington, 2015).

The other main pathway for DSB repair, HR, is evolutionarily highly conserved and, in contrast to NHEJ, is typically considered error-free. It operates during S and G_2 phases using sister chromatids as template for repair after extensive end resection. In mammalian cells, even in cycling cells, HR is much less important than NHEJ. Indeed, it seems that DSBs caused by replication stress are the primary substrates for HR in mitotic cells. HR is initiated by signaling from ATM or ATR, with many factors involved in end resection, including the MRN complex, BRCA1, CtIP and members of the RECQ family of helicases. While generally error-free, HR can still give rise to genome rearrangements when recombination involves homologous sequences on heterologous chromosomes or repeat elements (Hu et al., 2013; Morales et al., 2015; Richardson and Jasin, 2000). For example, in yeast unequal homologous recombination at the rDNA locus leads to the production of extrachromosomal DNA, which is likely to be the cause of yeast replicative aging (Sinclair and Guarente, 1997), but can also occur in humans (Warmerdam et al., 2016).

While rapid joining of DSB ends is obviously beneficial for the survival of the cell, the mutations associated with DSB repair are potential causal factors in aging and age-related diseases, most notably cancer. Interestingly, based on the known frequency of cytogenetically detectable chromosome breaks in human cells it has been estimated that over the lifetime of an average individual each typical somatic cell would have an estimated 2,300 imprecise repair sites, distributed throughout the genome, as a consequence of errors associated with end joining (Lieber and Karanjawala, 2004). To confirm this estimate it would be necessary to analyze the somatic genome for all possible genome structural variations, including deletions varying from a few to many thousands of basepairs. Since such events would be different from cell to cell such an analysis has not been possible. However, genome structural variations, including deletions and translocations, have been demonstrated to occur in mouse somatic tissues and increase with age (Dolle et al., 1997; Dolle et al., 2000). These results were based on breakpoints detected in a genomically-integrated selectable reporter gene rather than genome-wide sequence analysis. By extrapolating from the 3000 bp reporter locus to the genome overall, genome rearrangements in the mouse heart were estimated to increase with age to about 40 rearrangements per cardiomyocyte (Dolle and Vijg, 2002). More recently, quantitative assays have been developed to comprehensively analyze genome structural variation by next-generation sequencing (Chiarle et al., 2011; Maslov et al., 2015; Quispe-Tintaya et al., 2016). Using these and other assays, which hopefully will emerge in the near future, it should be able to empirically confirm the impact of erroneous DSB repair on the aging process.

Apart from the mutational consequences of DSB repair, the possibility has been considered that a decline in repair capacity with age would lead to reduced cell survival and therefore cell depletion. The first evidence of decreased NHEJ efficiency in aging was observed in senescent human diploid fibroblasts, where NHEJ events were scored using a GFP reporter plasmid co-transfected with I-SceI, a restriction enzyme, to induce DSBs within the construct (Seluanov et al., 2004). However, cellular senescence, i.e., *in vitro* aging, is not the same as organismal aging. Nevertheless, these findings were consistent with results showing

that the capacity of cell extracts prepared from isolated neurons from rat cerebral cortex to join a linearized plasmid declines with age (Vyjayanti and Rao, 2006). Using a similar approach, another group found that NHEJ capacity declines with age in rat lung, liver, and kidney. Interestingly, they also found that dietary restriction, a life span extension intervention, prevented the age-dependent decline in NHEJ, albeit the extent of compensation was variable among tissues (Lee et al., 2011).

More recently, an age-related decline in NHEJ was confirmed using a transgenic mouse model with a GFP-based NHEJ reporter cassette knocked into the ROSA26 locus. In this model, DSBs are generated by the site-specific endonuclease, I-SceI, with the reconstitution of a functional GFP gene a measure of NHEJ activity. NHEJ was shown to significantly decline with age in fibroblasts from heart, lung, kidney, and skin, as well as in astrocytes, with skin showing the most drastic decline (Vaidya et al., 2014). Skin fibroblasts also have the highest rate of senescence in aged mammals (Jeyapalan et al., 2007).

Many studies have shown that HR repair efficacy declines with age in mammals. The first evidence of this was for interchromosomal HR, where DSBs were created at I-SceI restriction sites on two heterologous chromosomes but between homologous nonfunctional GFP reporters, in both young and old mice. Of the tissues analyzed, the most drastic age-related decline in HR was observed in pancreas, lung, and thymus, while more modest declines were observed in spleen and kidney (White et al., 2013). Similar results were obtained for intrachromosomal HR, using a non-functional GFP reporter to measure spontaneous HR in mouse tissue (Sukup-Jackson et al., 2014). In these mice it was shown that recombinant cells accumulate with age in the colon (Sukup-Jackson et al., 2014). Since HR is more dependent on progressing through S and G_2 phases of the cell cycle, due to reliance of an available sister chromatid, observations of reduced repair efficacy may be more indicative of reduced cellular proliferation in aging tissues, for example, due to the increase in senescent cells across tissues (Wang et al., 2009). Indeed, Mao et al. showed that senescent fibroblasts displayed a suppression of HR greater than that observed in early replicating fibroblasts, obviously due to the G1 arrest of the cells. However, HR could be stimulated in both replicating and senescent fibroblasts by the overexpression of SIRT6, a mono-ADP ribosyltransferase previously associated with the control of longevity and the regulation of DNA repair, most likely through the activation of PARP1 and CtIP (Mao et al., 2011; Mao et al., 2012).

An intriguing recent report highlighted that knocking down of BRCA1 in the mouse dentate gyrus increased DSBs and led to cognitive deficits in learning and memory. BRCA1 was also found to be reduced in the brains of AD patients and human amyloid precursor protein (hAPP) transgenic mice (Suberbielle et al., 2015). Since HR is not the pathway of choice in post-mitotic cells these results are difficult to interpret. However, it should be noted that BRCA1 also participates in Alt-NHEJ (see above).

In summary, there is ample evidence for age-related changes in DSB processing and repair, including observed declines in NHEJ and HR, the two main pathways for repairing DSBs. Still unknown are the mechanisms that underlie these age-related changes. However, somewhat ironically, it is conceivable that the accumulation of DNA damage itself,

including DSBs, contributes to the age-related functional loss in DNA damage responses, either directly by affecting gene transcription or though the genome-wide accumulation of mutations (Gorbunova and Seluanov, 2016; Lieber and Karanjawala, 2004). Irrespective of the mechanisms underlying the observed age-related loss of DSB repair capacity and increased numbers of DSBs with age, it is important to test if reduced DSB repair can lead to aging phenotypes. This can be studied by testing the effects of heritable defects in DSB repair pathways.

Do defects in DSB repair cause premature aging?

One approach to gain more insight into the basic mechanisms of aging is the use of segmental progeroid syndromes, i.e., diseases in which multiple phenotypes generally associated with normal aging appear prematurely. This approach is somewhat controversial in the field. In contrast to most areas in biology, e.g., development, cancer, many investigators in aging research do not accept mutants with compromised function as informative, but require improved function and lifespan extension as the way to reveal mechanisms underlying aging. Nevertheless, important new findings based on such models have provided new insights into possible causes of aging (Hasty et al., 2003). Indeed, it now appears that most, if not all, human premature aging diseases are caused by heritable mutations in genes directly or indirectly affecting genome maintenance. Moreover, mutations targeted to genes involved in genome maintenance, usually in mice, have been demonstrated to cause multiple symptoms of premature aging. The unique association of premature aging with defects in genome maintenance provides strong evidence for DNA damage as a driver of aging (Hasty et al., 2003; Hoeijmakers, 2009), with several of the key proteins involved in DSB processing and repair implicated in aging and aging-related disease (Ciccia and Elledge, 2010). However, while progeroid syndromes and mouse models provide evidence for some involvement of DSB repair defects in premature aging, the picture is far from clear.

The best example of a human progeroid syndrome is Werner Syndrome (WS), a rare autosomal recessive disorder caused by heritable mutations in the *WRN* gene, a RecQ helicase (Monnat, 2010). RecQ helicases are highly conserved proteins with roles in NHEJ and HR (Bernstein et al., 2010). WS patients undergo normal development, but then prematurely exhibit many clinical signs of aging, including graying-hair, sarcopenia, skin atrophy, telangiectasia, osteoporosis, increased incidence of cancer (characteristically of mesenchymal origin), atherosclerosis, and shortened lifespan (Goto, 1997). Intriguingly, $Wrn^{-/-}$ mice do not display any of the clinical phenotypes associated with WS (Lombard et al., 2000), but when crossed to mice lacking *Terc* (telomerase RNA-component) most clinical phenotypes of WS were recapitulated (Chang et al., 2004). This is most likely due to the presence of unusually long telomeres in laboratory mice. There are multiple examples of differences between mice and humans in genome maintenance and these results obtained with WRN underscore that sometimes-additional genetic factors are needed to recapitulate a human phenotype in the mouse.

Syndromes caused by defects in other RecQ helicases, such as Bloom syndrome and Rothmund-Thomson syndrome, caused by mutations in the *BLM* and *RECQL4* genes,

respectively, are much less convincing as segmental progeroid syndromes for reasons unknown. Indeed, at least BLM is also involved in HR, with the syndrome mainly displaying increased cancer.

Another human disease often considered a segmental progeroid syndrome is Ataxia-Telangiectasia (A–T), a rare autosomal recessive disorder caused by truncating mutations in *ATM*. Its most prevalent phenotypes are progressive cerebellar ataxia, dilated blood vessels (telangiectasia), immunodeficiency, increased susceptibility to lymphoid cancers, and premature graying of hair (Shiloh and Ziv, 2013). Cells isolated from patients display increased DSBs, sensitivity to ionizing radiation, and also show a lack of p53 activation in response to DNA damage as well as increased telomere shortening (Metcalfe et al., 1996; Meyn, 1995) with premature senescence of cells in culture. Thus, while intimately involved in the response to DSBs. A–T is a much less convincing accelerated aging syndrome than WS.

Mice with homozygously disrupted *Atm* mimic the clinical human A–T phenotype, including neurological dysfunction albeit without ataxia (Barlow et al., 1996). Interestingly, similar to *Wrn*-null mice, when *Atm*-null mice were bred into a *Terc*-deficient background symptoms of premature aging were greatly increased, varying from kyphosis to reduced muscle and fat mass (Wong et al., 2003). Hence, in both Wrn- and Atm-deficient mice some form of premature aging is greatly accelerated when accompanied by a telomere defect. The explanation for this probably resides in increased genomic instability, such as uncapped telomeres, or apoptosis resulting in diminished stem cell reserve (Sharpless and DePinho, 2007). This is supported by the observation that conditional deletion of *Atr* results in multiple symptoms of premature aging, such as graying hair, alopecia, kyphosis, and osteoporosis (Ruzankina et al., 2007). Hence, replicative stress could be a major mechanism of stem cell aging, possibly through the production of DSBs (Flach et al., 2014), although more in depth studies should be performed.

Mice harboring mutations in genes involved in NHEJ also show signs and symptoms of premature aging. The first such report was in mice deficient in Ku80 (Vogel et al., 1999). Ku80-deficient mice have a significantly shortened life span and exhibit early onset of common aging pathologies, such as osteoporosis, kyphosis, multiple liver pathologies, alopecia, and cancer (Vogel et al., 1999). Moreover, these mice display severe combined immunodeficiency, due to the defect in immunoglobulin class switch and V(D)J recombination (Zhu et al., 1996). Similar progeroid phenotypes were observed in mice deficient for Ku70 or DNA-PK_{cs} (Espejel et al., 2004; Li et al., 2007; Reiling et al., 2014). However, Ku heterodimers do not act solely in NHEJ, but has other functions, most notably in telomere maintenance (Downs and Jackson, 2004). Telomere erosion is considered a major pro-aging phenotype and can lead to DSBs, but it cannot *a priori* be assumed that premature aging in Ku-deficient mice is due to defective DSB repair. DNA Ligase IV, the necessary ligase in NHEJ, was found to be embryonically lethal, most likely due to a large amount of unresolved DSBs occurring during neuronal development (Frank et al., 2000), and thus its relationship to aging remains elusive.

Yet more insight into aging is derived from BRCA1 and BRCA2 mutants. Although a complete understanding of the role these proteins play is still lacking, they both function in HR with loss of either BRCA1 or BRCA2 resulting in a reduction in HR (Moynahan and Jasin, 2010) and both are implicated in hereditary breast and ovarian cancer susceptibility. Mice with homozygous deletions of either *Brca1* or *Brca2* are embryonically lethal, which is generally attributed to the high levels of cellular senescence and apoptosis during development (Sharan et al., 1997; Xu et al., 2001). Interestingly, when $Brca1^{+/-}$ mice were crossed into a $p53^{+/-}$ background, homozygous embryonic lethality was rescued and these mice displayed multiple premature aging phenotypes, such as osteoporosis, atrophy, kyphosis, decreased body weight, and increased tumor incidence (Cao et al., 2003).

Yet another genome maintenance gene, that if inactivated, showing symptoms of premature aging is *ERCC1*. The ERCC1–XPF heterodimer is a 5'-3' structure-specific endonuclease that plays an important role in nucleotide excision repair, ICL repair, and DSB repair. Cells deficient in ERCC1–XPF are sensitive to DSBs and show reduced activity in the single strand annealing process of HR (Ahmad et al., 2008; Niedernhofer et al., 2004). Homozygous knockouts for *Ercc1* display extreme growth retardation and a severely shortened lifespan, within 4 weeks of birth. These mice also have a host of age-related pathologies ranging from osteoporosis, neurodegeneration, and sarcopenia, as well as liver and renal failure (Garinis et al., 2008). In the $Ercc1^{-/\Delta 7}$ mutant mice, the presence one truncated allele is able to delay the progression of the segmental progeroid features and increase life expectancy to ~ 20 weeks (Dolle et al., 2011). The only reported human patient corresponding to the *Ercc1*-deficient mice had a mutation in *XPF* (Niedernhofer et al., 2006).

Together, human progeroid syndromes and mouse models deficient in components of DSB repair underscore a possible role for DSBs as drivers of aging. In this respect, they confirm the potential importance of the observed age-related decline in various aspects of DSB repair for the emergence of aging phenotypes. Importantly, distinctive phenotypic trends can be inferred from the commonalities shared between the human syndromes and their respective mouse models. These include osteoporosis, general atrophy, neurological deficits, as well as the obvious immunodeficiency and increased predisposition to malignancy. However, the situation is far from clear, as illustrated by the fact that disruption of a major player in DSB processing, i.e., ATM, shows a very limited premature aging phenotype. Also other disorders with DSB repair defects (DNA ligase IV Syndrome, Artemis deficiency, XLF deficiency) do not show multiple progeroid phenotypes. To some extent this could be due to differences between mice and humans, with more distinct progeroid syndromes appearing when also deficiencies in other genes are present. However, the main reason for the apparent inconsistency between DSB repair mutants and premature aging is likely the difficulty of mimicking natural aging through only one or few gene mutants. For example, defects in ATM may not result in premature aging precisely because signals to elicit cell death are now prevented. Conversely, gene defects found associated with *bona fide* aging symptoms may not act by impairing just DSB repair, which could explain why WS patients show marked progeroid features but their DSB repair capacity is nearly normal. Hence, what would be needed is conclusive evidence that DSBs themselves, either endogenous or exogenous, are capable of inducing aging phenotypes.

Can DSBs themselves cause aging?

To definitively show that DNA DSBs can, at least in part, cause aging, approaches that allow induction of these highly toxic lesions alone, without pleiotropic side effects, are essential. Early studies revealed that radiation exposure, known to induce DSBs, appeared to show early signs of aging in rodents as well as humans (Henshaw et al., 1947; Russ and Scott, 1939; Warren, 1971).

More recently, it was shown that many adult survivors of juvenile cancer develop symptoms of premature aging later in life, up to 30 years after treatment with high doses of anticancer agents, such as radiation and chemotherapeutic drugs, most of which induce DSBs not only in the tumor cells but also in adjacent normal cells (Oeffinger et al., 2006). Symptoms include increased risk for heart failure, severe cognitive decline, coronary heart disease, secondary neoplasms and advanced frailty (Inskip et al., 2009; Ness et al., 2013; Oeffinger et al., 2006). It is tempting to speculate that premature accumulation of DSBs and the effects of their processing are the cause of premature aging in these childhood cancer survivors.

However, it remains difficult to rule out the possibility that premature aging under these circumstances is a consequence of the pleotropic effects caused by clastogenic compounds or DNA repair defects. Indeed, agents that damage DNA often also cause damage to proteins or other macromolecules and genes involved in DNA repair may have other functions as well. Hence, it is important to analyze the effects of DSBs only. Two mouse models were recently developed to address this critical gap (Figure 1). The first such model involves an adenoviral-based delivery system capable of inducing “clean” DSBs, through a tetracycline-controlled SacI restriction enzyme, in mouse liver after tail vein injection (White et al., 2015). Applying this system to induce DSBs in the liver of young mice revealed that at 1 and 2 months after treatment patterns of liver-characteristic aging pathology were increased, similar to those seen in naturally aged mice, e.g., karyomegaly, inflammatory infiltrates, megamitochondria, and markers of cellular senescence. Additionally, alterations in liver gene expression profiles were found to overlap significantly with those of normally aged mice, indicating significant increases in inflammation pathways and decreases in liver function (White et al., 2015). However, some typical age-related pathologies, such as lipofuscin accumulation, were not found to be increased. Also cancer was not observed at this early age level, but may still occur at a high frequency at later ages.

The above studies were restricted to mouse liver; however, another mouse model was developed recently harboring a transgene capable of ubiquitous expression of the I-PpoI restriction enzyme to induce DSBs (Kim et al., 2015). Although as of yet this model has not been reported to show symptoms of premature aging (David Sinclair, personal communication).

While initial results obtained with one of these mouse models suggest that DSBs alone are indeed capable of inducing multiple aging phenotypes in parallel in an accelerated manner, follow-up studies at later time points are required to test the effects of DSBs on life span and health span. Importantly, these same models could prove useful in testing the effect of

putative interventions in reducing the adverse consequences of DSBs, such as senescence, cell death, and inflammation.

Future prospects

There is now a growing body of evidence that toxic DNA damage, most notably DNA DSBs, may be capable of driving multiple age-related phenotypes. As we have seen, normal DSB repair responses show a tendency to decline with age while DSBs and the consequences of their erroneous repair, i.e., genome structural variation, increase in tissues of aging mammals. Moreover, defects in DSB repair are sometimes associated with premature aging in both humans and mice, while the long-term effects of agents known to induce DSBs include mild symptoms of progeria. Indeed, new mouse models demonstrating that induction of DSBs alone can recapitulate multiple premature aging phenotypes very early in life are the closest we have come to establishing a cause and effect relationship. However, a direct link of DSBs to normal aging remains unproven due to at least two important gaps in our knowledge.

First, we do not know if DSBs and their accumulating adverse effects ever rise to a high enough level to explain the observed functional loss and increased disease risk that are hallmarks of aging. To begin studying this it will be necessary to define the landscape of DSBs and their consequences in detail. In turn, this means that we need quantitative methods to study persistent DSBs, genome structural variations and other mutations at the base pair level, as well as accurate measures for senescent cells and loss of normal cells in aging organisms. While significant improvements are being made, we do not yet have the necessary information to begin generating the quantitative cause and effect models.

Second, while inactivation of genes involved in DSB processing and repair can cause some forms of accelerated aging, the overall pattern is still murky, with many DSB repair deficiencies showing a limited number of premature aging pathologies. While such findings are by themselves not surprising, since it is highly unlikely that any single gene defect can produce a complete phenocopy of the normal aging process, it nevertheless calls for an alternative approach to test a role for DSBs in aging. One way would be to use the DSB induction models in a controlled manner, testing if the severity of the various aging phenotypes increases with the amount of DSBs. However, a secondary approach would be to generate mouse models engineered to improve DSB processing and repair and/or prevent some of their adverse effects. Indeed, a demonstration that interventions known to extend life span in multiple organisms, such as dietary restriction (Fontana and Partridge, 2015), do so by diminishing DSBs and/or their consequences would be necessary. Recent efforts have focused attention on several potential ‘master’ regulators of organismal longevity, which also appear to affect DSB repair, such as the sirtuin family of mono-ADP-ribosyltransferases or deacetylases, and the FOXO family of transcription factors. FOXO3A has been genetically associated with extreme longevity in humans (Flachsbart et al., 2009) and SIRT1 and SIRT6 have been mechanistically linked to increased life span in animal models (Kanfi et al., 2012; Mostoslavsky et al., 2006). All three genes have also been implicated in DSB repair (Mao et al., 2012; Mostoslavsky et al., 2006; Oberdoerffer et al., 2008; Tran et al., 2002). A demonstration that increased life span of mouse models engineered to have

improved sirtuin or FOXO function also reduces spontaneous DSBs and their molecular and cellular end points would provide critical evidence for a causal role of DSBs in the aging process.

Collectively, the data presented above on the effects of age on DSBs and DSB repair, the occurrence of premature aging in humans and mice with genetic defects in DSB repair, and the pro-aging effects of DSBs induced by radiation, chemotherapeutic agents or genetic constructs harboring restriction enzymes, suggest some causal role for DSBs as one of the main culprits driving aging. We have illustrated this in a model, in which DSBs promote aging through a variety of molecular and cellular end points, including genome structural variations, cellular senescence, and apoptosis (Figure 2). Together these end points, which are likely to differ across tissues, can contribute to some well-documented hallmarks of aging, including neurodegeneration, cancer, loss of regenerative capacity and inflammation. However, before the true role of DSBs in the process of age-related tissue degeneration, functional loss and organismal decline can be understood, a much more rigorous, quantitative examination of the mechanisms underlying the sequence of events from DSBs to the aging phenotype is essential.

Acknowledgments

The research in the author's laboratory is supported by NIH grants AG017242, AG047200, CA180126, and by the Glenn Foundation (JV). RW is supported in part by funds from a Merck Postdoctoral Fellowship at The Rockefeller University.

REFERENCES

Ahmad A, Robinson AR, Duensing A, van Drunen E, Beverloo HB, Weisberg DB, Hasty P, Hoeijmakers JH, Niedernhofer LJ. ERCC1-XPF endonuclease facilitates DNA double-strand break repair. Molecular and cellular biology. 2008; 28:5082–5092. [PubMed: 18541667]

Aparicio T, Baer R, Gautier J. DNA double-strand break repair pathway choice and cancer. DNA Repair (Amst). 2014; 19:169–175. [PubMed: 24746645]

Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D, et al. Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell. 1996; 86:159–171. [PubMed: 8689683]

Barnes DE, Lindahl T. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu Rev Genet. 2004; 38:445–476. [PubMed: 15568983]

Beerman I, Seita J, Inlay MA, Weissman IL, Rossi DJ. Quiescent hematopoietic stem cells accumulate DNA damage during aging that is repaired upon entry into cell cycle. Cell Stem Cell. 2014; 15:37–50. [PubMed: 24813857]

Bernstein KA, Gangloff S, Rothstein R. The RecQ DNA helicases in DNA repair. Annu Rev Genet. 2010; 44:393–417. [PubMed: 21047263]

Cao L, Li W, Kim S, Brodie SG, Deng CX. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. Genes & development. 2003; 17:201–213. [PubMed: 12533509]

Ceccaldi R, Rondinelli B, D'Andrea AD. Repair Pathway Choices and Consequences at the Double-Strand Break. Trends in cell biology. 2016; 26:52–64. [PubMed: 26437586]

Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, Pathak S, Guarente L, DePinho RA. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. Nature genetics. 2004; 36:877–882. [PubMed: 15235603]

Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. Mol Cell. 2012; 47:497–510. [PubMed: 22920291]

Chiarle R, Zhang Y, Frock RL, Lewis SM, Molinie B, Ho YJ, Myers DR, Choi VW, Compagno M, Malkin DJ, et al. Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. Cell. 2011; 147:107–119. [PubMed: 21962511]

Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell. 2010; 40:179–204. [PubMed: 20965415]

Crosetto N, Mitra A, Silva MJ, Bienko M, Dojer N, Wang Q, Karaca E, Chiarle R, Skrzypczak M, Ginalski K, et al. Nucleotide-resolution DNA double-strand break mapping by next-generation sequencing. Nature methods. 2013; 10:361–365. [PubMed: 23503052]

Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Laberge RM, Vijg J, Van Steeg H, Dolle ME, et al. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell. 2014; 31:722–733. [PubMed: 25499914]

Dolle ME, Giese H, Hopkins CL, Martus HJ, Hausdorff JM, Vijg J. Rapid accumulation of genome rearrangements in liver but not in brain of old mice. Nature genetics. 1997; 17:431–434. [PubMed: 9398844]

Dolle ME, Kuiper RV, Roodbergen M, Robinson J, de Vlugt S, Wijnhoven SW, Beems RB, de la Fonteyne L, de With P, van der Pluijm I, et al. Broad segmental progeroid changes in short-lived Ercc1(-/Delta7) mice. Pathobiology of aging & age related diseases. 2011; 1

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 U S A. 2000; 97:8403–8408. [PubMed: 10900004]

Dolle ME, Vijg J. Genome dynamics in aging mice. Genome research. 2002; 12:1732–1738. [PubMed: 12421760]

Downs JA, Jackson SP. A means to a DNA end: the many roles of Ku. Nature reviews. Molecular cell biology. 2004; 5:367–378. [PubMed: 15122350]

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; 5:503–509. [PubMed: 15105825]

Feng Z, Hu W, Teresky AK, Hernando E, Cordon-Cardo C, Levine AJ. Declining p53 function in the aging process: a possible mechanism for the increased tumor incidence in older populations. Proc Natl Acad Sci U S A. 2007; 104:16633–16638. [PubMed: 17921246]

Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, Reynaud D, Alvarez S, Diolaiti ME, Ugarte F, Forsberg EC, et al. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature. 2014; 512:198–202. [PubMed: 25079315]

Flachsbart F, Caliebe A, Kleindorp R, Blanche H, von Eller-Eberstein H, Nikolaus S, Schreiber S, Nebel A. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc Natl Acad Sci U S A. 2009; 106:2700–2705. [PubMed: 19196970]

Fontana L, Partridge L. Promoting health and longevity through diet: from model organisms to humans. Cell. 2015; 161:106–118. [PubMed: 25815989]

Frank KM, Sharpless NE, Gao Y, Sekiguchi JM, Ferguson DO, Zhu C, Manis JP, Horner J, DePinho RA, Alt FW. DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. Mol Cell. 2000; 5:993–1002. [PubMed: 10911993]

Fumagalli M, Rossiello F, Clerici M, Barozzi S, Cittaro D, Kaplunov JM, Bucci G, Dobreva M, Matti V, Beausejour CM, et al. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nature cell biology. 2012; 14:355–365. [PubMed: 22426077]

Garinis GA, van der Horst GT, Vijg J, Hoeijmakers JH. DNA damage and ageing: new-age ideas for an age-old problem. Nature cell biology. 2008; 10:1241–1247. [PubMed: 18978832]

Ghezraoui H, Piganeau M, Renouf B, Renaud JB, Sallmyr A, Ruis B, Oh S, Tomkinson AE, Hendrickson EA, Giovannangeli C, et al. Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining. Mol Cell. 2014; 55:829–842. [PubMed: 25201414]

Gorbunova V, Seluanov A. DNA double strand break repair, aging and the chromatin connection. Mutation research. 2016

Goto M. Hierarchical deterioration of body systems in Werner's syndrome: implications for normal ageing. Mechanisms of ageing and development. 1997; 98:239–254. [PubMed: 9352493]

Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. Aging and genome maintenance: lessons from the mouse? Science. 2003; 299:1355–1359. [PubMed: 12610296]

Henshaw PS, Riley EF, Stapleton GE. The biologic effects of pile radiations. Radiology. 1947; 49:349–360. [PubMed: 20266009]

Hewitt G, Jurk D, Marques FD, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF. Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. Nature communications. 2012; 3:708.

Hoeijmakers JH. DNA damage, aging, and cancer. The New England journal of medicine. 2009; 361:1475–1485. [PubMed: 19812404]

Hu L, Kim TM, Son MY, Kim SA, Holland CL, Tateishi S, Kim DH, Yew PR, Montagna C, Dumitrache LC, et al. Two replication fork maintenance pathways fuse inverted repeats to rearrange chromosomes. Nature. 2013; 501:569–572. [PubMed: 24013173]

Iliakis G, Murmann T, Soni A. Alternative end-joining repair pathways are the ultimate backup for abrogated classical non-homologous end-joining and homologous recombination repair: Implications for the formation of chromosome translocations. Mutat Res Genet Toxicol Environ Mutagen. 2015; 793:166–175. [PubMed: 26520387]

Inskip PD, Robison LL, Stovall M, Smith SA, Hammond S, Mertens AC, Whitton JA, Diller L, Kenney L, Donaldson SS, et al. Radiation dose and breast cancer risk in the childhood cancer survivor study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2009; 27:3901–3907. [PubMed: 19620485]

Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009; 461:1071–1078. [PubMed: 19847258]

Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U. Accumulation of senescent cells in mitotic tissue of aging primates. Mechanisms of ageing and development. 2007; 128:36–44. [PubMed: 17116315]

Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, Bar-Joseph Z, Cohen HY. The sirtuin SIRT6 regulates lifespan in male mice. Nature. 2012; 483:218–221. [PubMed: 22367546]

Kim J, Sturgill D, Tran AD, Sinclair DA, Oberdoerffer P. Controlled DNA double-strand break induction in mice reveals post-damage transcriptome stability. Nucleic acids research. 2015

Lee JE, Heo JI, Park SH, Kim JH, Kho YJ, Kang HJ, Chung HY, Yoon JL, Lee JY. Calorie restriction (CR) reduces age-dependent decline of non-homologous end joining (NHEJ) activity in rat tissues. Experimental gerontology. 2011; 46:891–896. [PubMed: 21821112]

Li H, Vogel H, Holcomb VB, Gu Y, Hasty P. Deletion of Ku70, Ku80, or both causes early aging without substantially increased cancer. Molecular and cellular biology. 2007; 27:8205–8214. [PubMed: 17875923]

Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annual review of biochemistry. 2010; 79:181–211.

Lieber MR, Karanjawala ZE. Ageing, repetitive genomes and DNA damage. Nature reviews. Molecular cell biology. 2004; 5:69–75. [PubMed: 14708011]

Lombard DB, Beard C, Johnson B, Marciniak RA, Dausman J, Bronson R, Buhlmann JE, Lipman R, Curry R, Sharpe A, et al. Mutations in the WRN gene in mice accelerate mortality in a p53-null background. Molecular and cellular biology. 2000; 20:3286–3291. [PubMed: 10757812]

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013; 153:1194–1217. [PubMed: 23746838]

Mamouni K, Cristini A, Guirouilh-Barbat J, Monferran S, Lemarie A, Faye JC, Lopez BS, Favre G, Sordet O. RhoB promotes gammaH2AX dephosphorylation and DNA double-strand break repair. Molecular and cellular biology. 2014; 34:3144–3155. [PubMed: 24912678]

Mao Z, Hine C, Tian X, Van Meter M, Au M, Vaidya A, Seluanov A, Gorbunova V. SIRT6 promotes DNA repair under stress by activating PARP1. Science. 2011; 332:1443–1446. [PubMed: 21680843]

Mao Z, Tian X, Van Meter M, Ke Z, Gorbunova V, Seluanov A. Sirtuin 6 (SIRT6) rescues the decline of homologous recombination repair during replicative senescence. Proc Natl Acad Sci U S A. 2012; 109:11800–11805. [PubMed: 22753495]

Maslov AY, Quispe-Tintaya W, Gorbacheva T, White RR, Vijg J. High-throughput sequencing in mutation detection: A new generation of genotoxicity tests? Mutation research. 2015; 776:136–143. [PubMed: 25934519]

Metcalfe JA, Parkhill J, Campbell L, Stacey M, Biggs P, Byrd PJ, Taylor AM. Accelerated telomere shortening in ataxia telangiectasia. Nature genetics. 1996; 13:350–353. [PubMed: 8673136]

Meyn MS. Ataxia-telangiectasia and cellular responses to DNA damage. Cancer research. 1995; 55:5991–6001. [PubMed: 8521380]

Moehrle BM, Nattamai K, Brown A, Florian MC, Ryan M, Vogel M, Bliederhaeuser C, Soller K, Prows DR, Abdollahi A, et al. Stem Cell-Specific Mechanisms Ensure Genomic Fidelity within HSCs and upon Aging of HSCs. Cell Rep. 2015; 13:2412–2424. [PubMed: 26686632]

Monnat RJ Jr. Human RECQ helicases: roles in DNA metabolism, mutagenesis and cancer biology. Semin Cancer Biol. 2010; 20:329–339. [PubMed: 20934517]

Morales ME, White TB, Streva VA, DeFreece CB, Hedges DJ, Deininger PL. The contribution of alu elements to mutagenic DNA double-strand break repair. PLoS genetics. 2015; 11:e1005016. [PubMed: 25761216]

Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell. 2006; 124:315–329. [PubMed: 16439206]

Moynahan ME, Jasin M. Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. Nature reviews. Molecular cell biology. 2010; 11:196–207. [PubMed: 20177395]

Munoz-Espin D, Canamero M, Maraver A, Gomez-Lopez G, Contreras J, Murillo-Cuesta S, Rodriguez-Baeza A, Varela-Nieto I, Ruberte J, Collado M, et al. Programmed cell senescence during mammalian embryonic development. Cell. 2013; 155:1104–1118. [PubMed: 24238962]

Ness KK, Krull KR, Jones KE, Mulrooney DA, Armstrong GT, Green DM, Chemaitilly W, Smith WA, Wilson CL, Sklar CA, et al. Physiologic frailty as a sign of accelerated aging among adult survivors of childhood cancer: a report from the st jude lifetime cohort study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013; 31:4496–4503. [PubMed: 24248696]

Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, Appeldoorn E, Odijk H, Oostendorp R, Ahmad A, van Leeuwen W, et al. A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature. 2006; 444:1038–1043. [PubMed: 17183314]

Niedernhofer LJ, Odijk H, Budzowska M, van Drunen E, Maas A, Theil AF, de Wit J, Jaspers NG, Beverloo HB, Hoeijmakers JH, et al. The structure-specific endonuclease Ercc1-Xpf is required to resolve DNA interstrand cross-link-induced double-strand breaks. Molecular and cellular biology. 2004; 24:5776–5787. [PubMed: 15199134]

Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, Park SK, Hartlerode A, Stegmuller J, Hafner A, Loerch P, et al. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell. 2008; 135:907–918. [PubMed: 19041753]

Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, Friedman DL, Marina N, Hobbie W, Kadan-Lottick NS, et al. Chronic health conditions in adult survivors of childhood cancer. The New England journal of medicine. 2006; 355:1572–1582. [PubMed: 17035650]

Oktay K, Turan V, Titus S, Stobezki R, Liu L. BRCA Mutations, DNA Repair Deficiency, and Ovarian Aging. Biol Reprod. 2015; 93:67. [PubMed: 26224004]

Quispe-Tintaya W, Gorbacheva T, Lee M, Makhortov S, Popov VN, Vijg J, Maslov AY. Quantitative detection of low-abundance somatic structural variants in normal cells by high-throughput sequencing. Nature methods. 2016

Reiling E, Dolle ME, Youssef SA, Lee M, Nagarajah B, Roodbergen M, de With P, de Bruin A, Hoeijmakers JH, Vijg J, et al. The progeroid phenotype of Ku80 deficiency is dominant over DNA-PKCS deficiency. PloS one. 2014; 9:e93568. [PubMed: 24740260]

Richardson C, Jasin M. Frequent chromosomal translocations induced by DNA double-strand breaks. Nature. 2000; 405:697–700. [PubMed: 10864328]

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; 447:725–729. [PubMed: 17554309]

Rothkamm K, Barnard S, Moquet J, Ellender M, Rana Z, Burdak-Rothkamm S. DNA damage foci: Meaning and significance. Environ Mol Mutagen. 2015; 56:491–504. [PubMed: 25773265]

Rube CE, Fricke A, Widmann TA, Furst T, Madry H, Pfreundschuh M, Rube C. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. PloS one. 2011; 6:e17487. [PubMed: 21408175]

Russ S, Scott GM. Biological Effects of Gamma Irradiation (Series II). The British Journal of Radiology. 1939; 12:440–441.

Ruzankina Y, Pinzon-Guzman C, Asare A, Ong T, Pontano L, Cotsarelis G, Zediak VP, Velez M, Bhandoola A, Brown EJ. Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. Cell Stem Cell. 2007; 1:113–126. [PubMed: 18371340]

Schneider L, Pellegatta S, Favaro R, Pisati F, Roncaglia P, Testa G, Nicolis SK, Finocchiaro G, d'Adda di Fagagna F. DNA damage in mammalian neural stem cells leads to astrocytic differentiation mediated by BMP2 signaling through JAK-STAT. Stem Cell Reports. 2013; 1:123–138. [PubMed: 24052948]

Sedelnikova OA, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, Barrett JC. Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nature cell biology. 2004; 6:168–170. [PubMed: 14755273]

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 U S A. 2004; 101:7624–7629. [PubMed: 15123826]

Sfeir A, Symington LS. Microhomology-Mediated End Joining: A Back-up Survival Mechanism or Dedicated Pathway? Trends Biochem Sci. 2015; 40:701–714. [PubMed: 26439531]

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P, Bradley A. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature. 1997; 386:804–810. [PubMed: 9126738]

Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nature reviews. Molecular cell biology. 2007; 8:703–713. [PubMed: 17717515]

Shiloh Y, Ziv Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nature reviews. Molecular cell biology. 2013; 14:197–210.

Siddiqui MS, Francois M, Fenech MF, Leifert WR. Persistent gammaH2AX: A promising molecular marker of DNA damage and aging. Mutat Res Rev Mutat Res. 2015; 766:1–19. [PubMed: 26596544]

Sinclair DA, Guarente L. Extrachromosomal rDNA circles--a cause of aging in yeast. Cell. 1997; 91:1033–1042. [PubMed: 9428525]

Suberbielle E, Djukic B, Evans M, Kim DH, Taneja P, Wang X, Finucane M, Knox J, Ho K, Devidze N, et al. DNA repair factor BRCA1 depletion occurs in Alzheimer brains and impairs cognitive function in mice. Nature communications. 2015; 6:8897.

Sukup-Jackson MR, Kiraly O, Kay JE, Na L, Rowland EA, Winther KE, Chow DN, Kimoto T, Matsuguchi T, Jonnalagadda VS, et al. Rosa26-GFP direct repeat (RaDR-GFP) mice reveal tissue- and age-dependence of homologous recombination in mammals in vivo. PLoS genetics. 2014; 10:e1004299. [PubMed: 24901438]

Titus S, Li F, Stobezki R, Akula K, Unsal E, Jeong K, Dickler M, Robson M, Moy F, Goswami S, et al. Impairment of BRCA1-related DNA double-strand break repair leads to ovarian aging in mice and humans. Science translational medicine. 2013; 5:172ra121.

Tran H, Brunet A, Grenier JM, Datta SR, Fornace AJ Jr, DiStefano PS, Chiang LW, Greenberg ME. DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science. 2002; 296:530–534. [PubMed: 11964479]

Vaidya A, Mao Z, Tian X, Spencer B, Seluanov A, Gorbunova V. Knock-in reporter mice demonstrate that DNA repair by non-homologous end joining declines with age. PLoS genetics. 2014; 10:e1004511. [PubMed: 25033455]

Vijg, J. Aging of the genome : the dual role of the DNA in life and death. Oxford; New York: Oxford University Press; 2007.

Vilenchik MM, Knudson AG. Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. Proc Natl Acad Sci U S A. 2003; 100:12871–12876. [PubMed: 14566050]

Vogel H, Lim DS, Karsenty G, Finegold M, Hasty P. Deletion of Ku86 causes early onset of senescence in mice. Proc Natl Acad Sci U S A. 1999; 96:10770–10775. [PubMed: 10485901]

Vyjayanti VN, Rao KS. DNA double strand break repair in brain: reduced NHEJ activity in aging rat neurons. Neurosci Lett. 2006; 393:18–22. [PubMed: 16226837]

Walter D, Lier A, Geiselhart A, Thalheimer FB, Huntscha S, Sobotta MC, Moehrle B, Brocks D, Bayindir I, Kaschutnig P, et al. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature. 2015; 520:549–552. [PubMed: 25707806]

Wang C, Jurk D, Maddick M, Nelson G, Martin-Ruiz C, von Zglinicki T. DNA damage response and cellular senescence in tissues of aging mice. Aging cell. 2009; 8:311–323. [PubMed: 19627270]

Warmerdam DO, van den Berg J, Medema RH. Breaks in the 45S rDNA Lead to Recombination-Mediated Loss of Repeats. Cell Rep. 2016; 14:2519–2527. [PubMed: 26972008]

Warren S. Radiation and aging. Bulletin of the New York Academy of Medicine. 1971; 47:1355–1360. [PubMed: 5292251]

White RR, Milholland B, de Bruin A, Curran S, Laberge RM, van Steeg H, Campisi J, Maslov AY, Vijg J. Controlled induction of DNA double-strand breaks in the mouse liver induces features of tissue ageing. Nature communications. 2015; 6:6790.

White RR, Sung P, Vestal CG, Benedetto G, Cornelio N, Richardson C. Double-strand break repair by interchromosomal recombination: an in vivo repair mechanism utilized by multiple somatic tissues in mammals. PloS one. 2013; 8:e84379. [PubMed: 24349572]

Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, Gu Y, Alt FW, DePinho RA. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. Nature. 2003; 421:643–648. [PubMed: 12540856]

Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, Harris CC, Deng CX. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. Nature genetics. 2001; 28:266–271. [PubMed: 11431698]

Zhu C, Bogue MA, Lim DS, Hasty P, Roth DB. Ku86-deficient mice exhibit severe combined immunodeficiency and defective processing of V(D)J recombination intermediates. Cell. 1996; 86:379–389. [PubMed: 8756720]

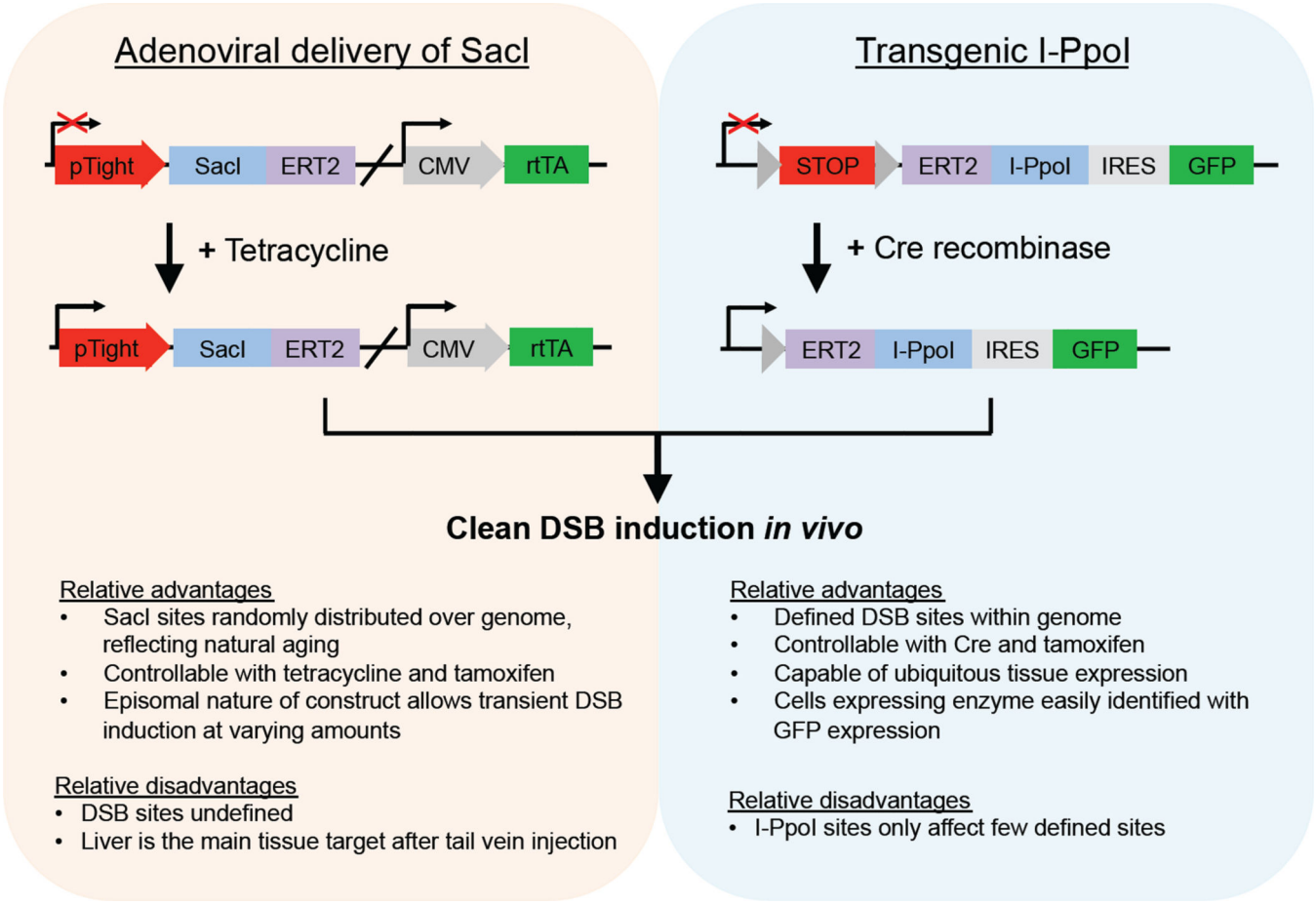

Figure 1. Models to study DSB-induced aging

Two methods to induce clean DSBs provide tools to dissect the age-related consequences stemming from DSBs alone. The first method induces DSBs via adenoviral delivery of a restriction enzyme, SacI, which is under control of tetracycline and tamoxifen (White et al., 2015). The second method, a transgenic mouse model, induces DSBs conditionally using the I-PpoI restriction enzyme fused to GFP under control of tamoxifen, and in a tissue-specific manner using Cre recombinase (Kim et al., 2015).

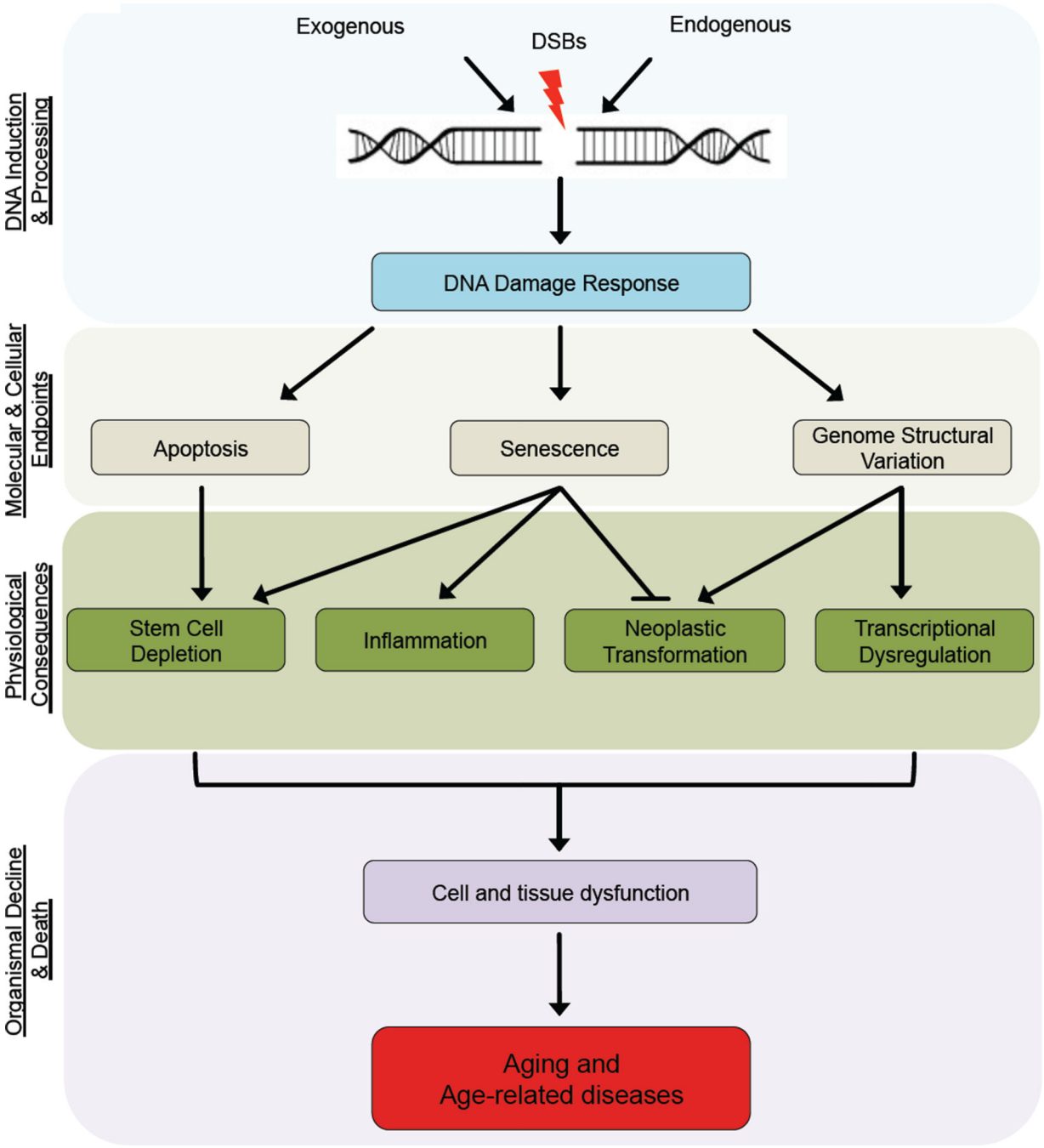

Figure 2. DNA double-strand breaks (DSBs), cellular consequences, and the pathways leading to aging

DSBs, arising endogenously or exogenously, activate the DDR that directs cells to repair the break, enter apoptosis, or undergo cellular senescence. The resulting molecular and cellular end points can cause the physiologically detrimental consequences over time leading to the overall decline of an organism, ultimately resulting in disease and death.