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SURVEY AND SUMMARY
Changes in DNA repair during aging

Vera Gorbunova*, Andrei Seluanov, Zhiyong Mao and Christpher Hine

Department of Biology, University of Rochester, Rochester, NY 14627, USA

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ABSTRACT

DNA is a precious molecule. It encodes vital information about cellular content and function. There are only two copies of each chromosome in the cell, and once the sequence is lost no replacement is possible. The irreplaceable nature of the DNA sets it apart from other cellular molecules, and makes it a critical target for age-related deterioration. To prevent DNA damage cells have evolved elaborate DNA repair machinery. Paradoxically, DNA repair can itself be subject to age-related changes and deterioration. In this review we will discuss the changes in efficiency of mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER) and double-strand break (DSB) repair systems during aging, and potential changes in DSB repair pathway usage that occur with age. Mutations in DNA repair genes and premature aging phenotypes they cause have been reviewed extensively elsewhere, therefore the focus of this review is on the comparison of DNA repair mechanisms in young versus old.

EVIDENCE FOR AGE-RELATED CHANGES IN DNA REPAIR FROM THE STUDIES OF SOMATIC MUTATIONS

The prevailing view regarding causes of aging is that aging results from accumulation of somatic damage. Damage to DNA can lead to cell cycle arrest, cell death or mutation. The majority of mutations do not kill the cell, but when accumulated in sufficient numbers may lead to deregulation of transcription patterns (1), reduced fitness and ultimately the aging phenotype.

Accumulation of mutations with age has been studied extensively in mice and humans. The early studies of mutations in the HPRT locus in cultured lymphocytes from young and old individuals have shown accumulation of mutations with age in both humans and mice (2–6). The studies using transgenic mouse models allowed the measurement of the mutation frequency in other mammalian tissues and loci. These assays measure mutation frequency in chromosomally integrated LacZ (7) or LacI (8–10) transgenes, which are rescued in *Escherichia coli* and analyzed for mutations using beta-galactosidase assay. Using these mice, it was demonstrated that point mutations accumulate with age (9–13) and furthermore, the mutation rate is higher in old animals (10). Not only did mutations accumulate but a characteristic type of mutations, genomic rearrangements, appear in old individuals (11,14–18).

Why does the rate of mutations increase with age and genomic rearrangements appear? Multiple studies have shown a higher load of DNA damage in old organisms (19–23). But why is there more damage? It is tempting to suggest these changes are caused by DNA repair machinery becoming less efficient and more error-prone with age. We will now discuss the studies, which directly measured DNA repair efficiency in young and old.

AGE-RELATED CHANGES IN MISMATCH REPAIR (MMR)

MMR removes mispaired bases resulting from replication errors, recombination between imperfectly matched sequences and deamination of 5-methyl-cytosine. DNA replication past a mismatched base pair would result in a point mutation. The MMR system is also thought to play a role in repair of oxidative damage by mechanisms that are not well understood (24). Several lines of evidence indicate the importance of the MMR system to the aging process. MMR is essential for maintenance of repeated sequences, as mutations in MMR genes are associated with a substantial destabilization of microsatellites (25), and microsatellite instability increases with aging in humans (26–28). The rate of MMR has been analyzed in *in vitro* aging human T cell clones (29). Cells at different passages were treated with mismatch-inducing agent and mismatch frequency was determined using a modification of the alkaline comet assay. Results showed a decline in MMR with increasing *in vitro* age. Thus, there is evidence of age-related alterations in MMR; however, more studies

*To whom correspondence should be addressed. Tel: +1 585 275 7740; Fax: +1 585 275 2070; Email: vgorbuno@mail.rochester.edu

are needed which would directly measure MMR capacity in young and old individuals.

AGE-RELATED CHANGES IN BASE EXCISION REPAIR (BER)

Excision repair removes lesions that affect only one DNA strand, which permits excision of the lesion and subsequent use of the complementary strand to fill the gap. BER corrects small DNA alterations that do not distort the overall structure of DNA helix, such as oxidized bases, or incorporation of uracil. Excision repair is critically important for repairing base damage induced by reactive oxygen species. BER is classified into two sub-pathways: short-patch BER; a mechanism whereby only 1 nucleotide is replaced or long-patch BER; a mechanism whereby 2–13 nucleotides are replaced. BER is initiated by DNA glycosylases, which cleave N-glycosylic bond of damaged bases leaving apurinic/apyrimidinic site (AP site). The abasic site is then processed by AP endonuclease (APE1) leaving a single-stranded gap. The gap is filled by DNA polymerase β and ligated by DNA ligase (30,31).

Age-related changes in BER have been intensively studied using several experimental approaches. Measuring the levels and kinetics of AP sites following DNA damage in nuclear DNA (32) showed that senescent human fibroblasts as well as leukocytes from old donors have higher basal level of AP sites than young cells. Upon treatment with H_2O_2 or MMS the level of AP sites rose faster in the young cells than in the old cells suggesting a deficiency in DNA glycosylase activity (32).

Measurement of oxidized guanine (8-oxoG) kinetics in genomic DNA of young and old mice after exposure to γ-irradiation did not reveal a difference in the rate of 8-oxoG removal; however, the tissues of old animals appeared to accumulate higher levels of 8-oxoG after irradiation (23). Another way of measuring BER is by comparing *in vitro* incision activity in tissue extracts from young and old animals. Protein extracts are incubated with radiolabeled oligonucleotides containing single base lesions and the appearance of cleavage products is analyzed on a gel (33). A significant decrease in the mitochondrial incision activity of 8-oxoG DNA glycosylase, uracil DNA glycosylase and the endonuclease III homolog was found in the brains of old mice, whereas smaller changes were observed in nuclear incision activity (30,33,34). Similarly, a decline in cleavage activity was observed in mitochondrial, and to a lesser extent nuclear extracts from senescent human fibroblasts (35).

A different *in vitro* assay measuring the repair of a synthetic DNA substrate containing a single G:U mismatch showed a strong decline in BER activity in brain, liver and germ cell nuclear extracts of old mice (36). Germ cell extracts from old mice were found to contain reduced levels of APE1, and supplementation with purified recombinant enzyme restored the activity to the level of young animals (37). Reduced abundance of DNA polymerase β in brain extracts from mice and rats has been reported (36,38,39).

In addition to altered enzyme activity, the mechanism for age-related decline of BER may lie in altered response to DNA damage. For example, the expression of DNA polymerase β and AP endonuclease was induced by DNA damage in young mice, while aged mice showed a lack of inducibility (40). Furthermore, old mice and senescent human fibroblasts were deficient in the translocation of oxoguanine DNA glycosylase and AP endonuclease into both their nuclei and mitochondria (41,42). An intriguing finding showed that the efficiency of BER may be sequence-specific with the promoters of the genes whose expression is downregulated with age being repaired poorly compared to genes that are not affected by age (43). In summary, multiple evidence indicates that BER undergoes age-related changes, which are likely to contribute to the accumulation of oxidative DNA lesions and mutations with aging.

AGE-RELATED CHANGES IN NUCLEOTIDE EXCISION REPAIR (NER)

NER removes short DNA oligonucleotides containing a damaged base (44). NER recognizes bulky lesions caused by carcinogenic compounds, and covalent linkages between adjacent pyrimidines resulting from UV exposure. NER is further classified into global genome repair (GG–NER) that occurs everywhere in the genome, and transcription-coupled repair (TCR), which removes lesions in the transcribed strand of active genes. NER is a multistep process involving multiple proteins (44). In GG–NER DNA damage is recognized by XPC–HR23B complex, followed by damage verification by XPA. The DNA is unwound by XPB and XPD (ERCC3 and ERCC2) helicases in complex with TFIIH basal transcription initiation factor, and the incision is made in the damaged strand by XPF and XPG. The damaged strand is removed, and repair is completed by DNA polymerase and DNA ligase. In the TCR pathway stalled RNA–PolII on the damaged DNA template is believed to initiate the repair process, which requires the TCR-specific proteins CSB and CSA.

The relationship between NER and aging has long been studied by either evaluating repair during *in vitro* senescence of human fibroblasts and lymphocytes, or in cells or tissues derived from donors of different age. Studies using *in vitro* senescence yielded conflicting results. Hart and Setlow (45) reported a reduction in unscheduled DNA synthesis following DNA damage in late passage WI-38 human fibroblasts compared to early passage cells. While no differences in the repair replication were found using the same cell line (46). A more recent study (47) measured the kinetics of the disappearance of cyclobutane pyrimidine dimers (CPDs) from genomic DNA in human fibroblasts and trabecular osteoblasts aged *in vitro*. In this method (48) cells are treated with UV, genomic DNA is extracted and incubated with T4 endonuclease V, which cleaves the DNA at pyrimidine dimers. Then the DNA is cleaved with restriction enzymes, separated on an alkaline gel and the intensity of the bands corresponding to specific genes is determined by Southern hybridization. Both

actively transcribed and inactive genes were analyzed, and no clear differences in the rate of CPD removal were found (47). Removal of CPDs was also examined using *in situ* assay with CPD antibodies (49). This study showed reduced UV-induced CPD removal in senescent compared to young fibroblasts (49).

Contrasting results between different studies may be explained by the sensitivity of cells to culture conditions such as serum concentration, and differences in cell cycle distribution during treatment (50). Furthermore, replicative aging may vary between cell lines, depending on the cell donor. For example, a decline in NER with increasing passages was documented for lymphocytes derived from two different donors but not in the lymphocytes from the third donor (51).

Studies of NER in cells or tissues from young and old individuals consistently showed a decline of NER capacity with increasing age. Earlier works used unscheduled DNA synthesis (52) or a plasmid reactivation assay as a measure of NER (53–55). In the plasmid reactivation assay a plasmid containing chloramphenicol acetyltransferase (CAT) is irradiated with UV to introduce DNA damage, plasmids are transfected into host cells and percentage of CAT activity relative to undamaged control plasmid is measured. A large study using human peripheral blood lymphocytes from 135 individuals aged 20 to 60 showed a decline of UV damage repair (54,55). The rate of decline was calculated as 0.63% per year, which amounts to ~25% decrease over a 40-year period (54,55). This analysis was further extended to show similar decline in NER in skin fibroblasts (53). In addition to reduced plasmid reactivation, cells from older donors introduced an increased number of mutations in the transfected plasmid (53). This suggests that not only the repair becomes less efficient with age, but it also makes more errors. A disadvantage of plasmid reactivation assay is that it does not differentiate between different types of repair, and types of photoproducts.

Other assays measured the rate of removal of specific photoproducts. A study using T4 endonuclease V for detection of CPDs in hepatocytes of 6 and 24 months old rats showed that removal of CPDs from two nontranscribed genes was ~40% lower for cells isolated from old rats than for cells isolated from young animals (56). In contrast, the age-related decline of CPD removal was less apparent in a transcribed gene, where only the rate of CPD removal was slower in old animals, but no difference in the number of CPDs was found after 24 h (56). By a similar assay, repair efficiency of telomeric DNA was reported to be lower in fibroblasts isolated from older human donors (57). Thus, it appears that aging has a greater effect on repair of nontranscribed genes.

Another method of detection of CPDs and (6–4) pyrimidone photoproducts (PP) became available with development of CPD and (6–4) PP-specific antibodies (58). DNA is isolated from cells after UV-irradiation and hybridized with antibodies. In agreement with earlier results this method showed a decrease in removal of CPDs and (6–4) PPs in dermal fibroblasts of older human donors (59). Decline in repair of (6-4) PPs was also shown in round spermatids of 14 months old compared to 2 months old mice (60). The method was further developed to use PP-specific antibodies for immunohistochemistry, which allowed studying the removal of CPDs *in situ* in human epidermis (61). Skin at the upper arm of young and old volunteers was exposed to UVB, and biopsied at different time points after irradiation. The biopsy material was either used for DNA extraction and probed with CPD antibodies or analyzed by immunohistochemistry. CPDs were removed from epidermis at 4 days after irradiation in the young subjects, and between 7 and 14 days in older subjects (61). Since the process of CPD removal is a combination of NER and epidermal turnover, the CPDs are likely to be removed from aged skin after 7–14 days by turnover of the epidermis. Thus, *in situ* studies further suggest that NER declines with age. Another *in situ* study with human volunteers using a postlabeling method based on quantification of photoproducts by HPLC found that photoproducts in UV-irradiated skin are induced at a higher frequency in old individuals (62). The latter study, however, did not detect age-related differences in NER due to large individual variations in the study population (63).

In summary, there is compelling evidence that NER declines with age. Little is known about the mechanism responsible for this decline. Reduced constitutive protein levels of ERCC3, PCNA, RPA, XPA and p53 that participate in NER were reported in older humans (59). Interestingly, treatment with short oligonucleotides that mimic DNA damage signal, stimulated NER in fibroblasts from old donors (64). The oligonucleotide treatment also upregulated the levels of NER proteins (64). Thus, the decline may be caused by lower levels of NER enzymes or by altered induction of DNA damage response.

Recent studies have shown that multiple mutations in the NER genes result in dramatically accelerated aging phenotypes (65–67). The progeroid phenotypes caused by NER defects were associated with characteristic changes of the global transcription patterns. Similar changes were seen in wild type mice in response to stress and during aging. It is tempting to speculate that the decline of NER function that occurs in normal individuals contributes to the onset of aging.

AGE-RELATED CHANGES IN DOUBLE-STRAND BREAK (DSB) REPAIR

A DSB is the most lethal of all DNA lesions. If unrepaired, a DSB leads to loss of chromosome segments and threatens the survival of the cell. Equally detrimental to the organism are misrepaired DSBs that destabilize the genome and lead to genomic rearrangements. Genomic rearrangements become common in aging organisms (11,14–18) ultimately leading to deregulation of transcription (1) and malignancies.

DSBs in DNA are repaired by two major mechanisms: homologous recombination (HR) and nonhomologous end joining (NHEJ). During HR-mediated repair of DSB, the sister chromatid is used as a template to copy the missing information into the broken locus. Repair by HR is mediated by Rad51 protein with the help of other members of Rad52 epistasis group (68,69). Since sister

chromatids are identical to each other, DNA damage can be repaired faithfully with no genetic consequence. In contrast, the NHEJ pathway simply fuses two broken ends with little or no regard for sequence homology. NHEJ starts with binding of Ku70/Ku80 heterodimer to the broken DNA ends (70). Ku facilitates recruitment of Artemis-DNA–PKcs complex, which processes the ends to prepare them for ligation (71). Next, the gaps are filled by DNA polymerase of polX family, and covalently joined by XRCC4-DNA ligase IV complex (71). NHEJ is rarely error-free leading to deletions or insertions of filler DNA (72–74). DSBs situated between two direct repeats can also be repaired by single-strand annealing (SSA), a highly mutagenic recombinational mechanism in which the sequence between the repeats is deleted.

The first indication of age-related changes in DSB repair is the exponential increase in the incidence of cancer with age (75), since cancer is associated with genome rearrangements and loss of heterozygosity (LOH). Sequence analysis of genomic rearrangements in the LacZ locus in aged transgenic mice (11) and also in the endogenous HPRT locus in human lymphocytes (76) did not reveal regions of extended homology at the break-points, suggesting that the rearrangements may have resulted from erroneous NHEJ.

Despite the tremendous impact of DSB repair on aging process, age-related changes in DSB repair are currently understudied. Early studies using variations of the comet assay documented age-related decline of the efficiency of rejoining of X-ray-induced DNA breaks in normal human lymphocytes (77,78). The methodology, however, did not allow addressing a specific repair mechanism.

Our laboratory has designed a sensitive assay for NHEJ (73). The assay is based on a reporter cassette containing GFP gene interrupted by an intron, containing recognition sites for the rare cutting endonuclease I-SceI. The GFP gene in the original construct is inactive, however, induction of DSBs by I-SceI and subsequent repair restores GFP activity. Since NHEJ takes place within intronic sequences, GFP activity is reconstituted even if repair is accompanied by deletions or insertions. The number of GFP positive cells serves as a measure of NHEJ efficiency, and can be quantified by FACS. In addition the fidelity of repair can be determined by sequencing the repair products. We used this assay to study senescence-related changes of NHEJ in normal human fibroblasts. The efficiency of NHEJ was reduced up to 4.5-fold in presenescent and senescent relative to young cells. Strikingly, we observed that end joining in old cells was associated with extended deletions. These results indicate that end joining becomes less efficient and more error-prone in senescent cells (73).

NHEJ in the brain of young and old rats has been analyzed using *in vitro* plasmid rejoining assay. In this assay linearized plasmids are incubated with nuclear extract, and plasmid concatamers resulting from NHEJ are quantified on a gel. This assay showed that NHEJ efficiency is reduced in the brains of old rats (79,80). NHEJ is also decreased in the brains of Alzheimer's disease patients compared to normal controls (81).

What is the molecular mechanism responsible for age-related decline of NHEJ? The level of Ku, which is a protein responsible for recognition and binding to DSBs, has been shown to decline markedly in the testis of aging rats (82), and lymphocytes from human donors of increasing age (83). We observed a 50% reduction in Ku70 and Ku80 protein levels in senescent human fibroblasts (84). Furthermore, intracellular distribution of Ku differed between young and senescent cells. In young cells, Ku was distributed throughout the cytoplasm and the nucleus, and translocated to the nucleus in response to γ-irradiation. We hypothesize that the cytoplasm of young cells contains Ku that serves as a reserve that is recruited to the nucleus upon DNA damage. In contrast, in senescent cells, all Ku was localized in the nucleus, so no additional Ku can be brought into the nucleus to repair DNA damage. Moreover, our results suggest that nuclear Ku in senescent cells is unavailable for repair of new lesions (84). Ku may be trapped by unrepairable DNA damage foci (21,22) that accumulate in the nuclei of senescent cells. Ku heterodimer forms a ring that is loaded onto the DNA strand (85), and it is unknown what takes Ku off the DNA. The release of Ku from DNA may be impaired in senescent cells. Alternatively, Ku may remain at the damage sites and shortened telomeres, which cannot be repaired by DNA repair machinery in senescent cells. Impaired nuclear targeting and DNA binding activities of Ku have been reported in peripheral blood mononuclear cells of aged humans (86,87). Thus, decline of NHEJ with age may be caused by reduced availability and altered regulation of Ku.

Disruption of genes involved in DSB repair often leads to premature aging phenotypes with notable examples being Werner syndrome, Ataxia telangiectasia, and mice deficient for Ku80, DNA–PKcs and ERCC1 (88). The exact mechanism by which these mutations cause progeroid phenotype is unknown, and likely involves a combination of disregulation of transcription and an increase of cellular senescence and apoptosis. As DSB repair becomes less efficient during normal aging the same pathways may contribute in a more subtle way into the onset of aged phenotype.

RELATIVE USAGE OF DSB REPAIR PATHWAYS WITH INCREASING AGE

As we discussed above DSBs can be repaired by three distinct pathways: mutagenic NHEJ and SSA, and potentially nonmutagenic HR. Does the usage of DSB repair pathways change with age? This question was addressed in *Drosophila* male germline (89). The males carried a reporter cassette where DSBs were generated by constitutively expressed I-SceI endonuclease. The cassette allowed to differentiate repair events as NHEJ, SSA or HR between two homologous chromosomes. HR in male germline has increased from 14% in young flies to 60% in old ones, with a corresponding decrease in SSA and NHEJ (89).

Can this finding be generalized to mammalian DNA repair? We did observe 4.5-fold decrease in NHEJ in

senescent human fibroblasts (73), however, this was clearly not due to an increase in HR, since HR is virtually absent in replicatively senescent cells (Seluanov,A. and Gorbunova,V., in preparation). Lack of HR in G0-arrested cells is not unexpected, as HR is restricted to G2/M phase of cell cycle when a sister chromatid is available (90,91). Potentially, a homologous chromosome can be used as a template in nondividing cells, however, repair using a homolog is three orders of magnitude less frequent than the use of a sister chromatid in mammalian cells (92,93). This preference is explained by sister chromatids being in close proximity after DNA replication, in contrast to homologs that are not paired in mitosis and may be harder to bring together. Expression of Rad51 protein is cell cycle-regulated (94,95), and Rad51 becomes undetectable when cells enter replicative senescence (Seluanov,A. and Gorbunova,V., unpublished data). Therefore, the major pathway for repair of DSBs in mammalian G1/G0 cells is NHEJ (90,91,96).

While senescent cells do not use HR, the situation is less clear in an aging organism, which still contains dividing cells. However, several studies show that HR is more active in ES cells (97) and in early embryonic development (98), compared to late developmental stages and somatic cells. As the body ages, fewer cells divide, cell cycle slows down and more cells are in G1/G0 stage, therefore we predict that the usage of HR will further decrease. This prediction, of course, needs to be tested in aging mammals.

Another important point is that HR tested by Preston *et al*. (89) is HR between homologous chromosomes. In mammalian cells mitotic recombination is rare and potentially dangerous process, as it is associated with LOH and cancer. Specific mechanisms controlled by Bloom protein (99), the MMR system (100) and genetic divergence between homologs (101) suppress mitotic recombination in mammalian cells. LOH takes place in precancerous cells and is a major contributor to the tumorogenic process (102). However, LOH and cancer do occur with increased frequency with advancing age (75,103). Thus, the finding of increased HR between homologs in aging flies may be compared to increased frequency of abnormal mitotic recombination events in aging mammals. It is possible that mechanisms suppressing mitotic recombination start to fail in aging cells, or that old individuals contain more premalignant cells that tend to be more recombinogenic. In summary, changes in DSB repair pathway usage may have profound implications for human aging and cancer. Age-related changes in DSB repair pathways have not been studied in mammals, and more research is clearly needed in this area.

CONCLUSIONS

There is sufficient evidence that all pathways of DNA repair, MMR, excision repair and DSB repair, become less efficient with age leading to accumulation of mutations (Figure 1). Much less is known about the causes of this deterioration. Several studies point out age-related

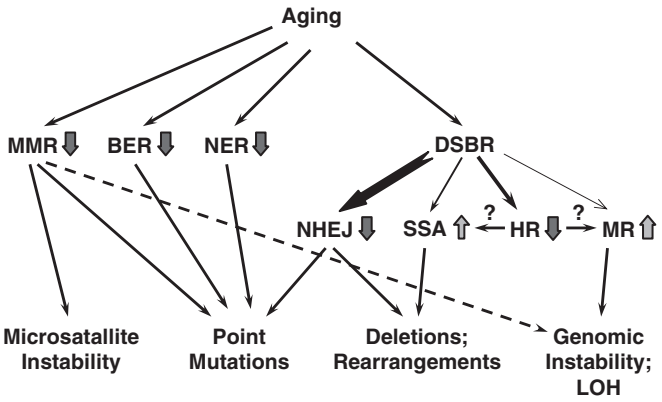

Figure 1. Age-related changes in DNA repair and their consequences. Inefficient MMR leads to microsatellite instability, point mutations and potentially, to increased frequency of LOH. Decline in efficiency and fidelity of BER and NER leads to point mutations. Less efficient and more error-prone NHEJ results in point mutations and genomic rearrangements. As fewer cells are in G2 stage, the usage of sub-pathways of DSB repair (DSBR) may also change, where precise HR between sister chromatids declines, giving way to more mutagenic SSA and mitotic recombination (MR) with homologous chromosome leading to genomic instability and LOH.

decrease in expression of DNA repair enzymes or their activities (36–39,59,82,84). Why do levels of DNA repair enzymes decline with age, and why are all the DNA repair systems affected? Since DNA repair and DNA damage response are tightly controlled processes it is tempting to speculate that DNA damage response becomes less efficient or somewhat disregulated with age. Indeed, alterations in the response of DNA repair proteins to DNA damage have also been reported (40–42,64,84,86,87). Apoptosis, which is another process triggered by DNA damage, was shown to become down-regulated in aging (104) and senescence (105), which was further connected to altered p53 activity (105). Even beyond DNA damage response, it is a general notion that old organisms become more sensitive to stress, hence stress responses may be altered. Recently a connection was made between the stress response elicited by DNA damage and insulin/IGF1 signaling (65,106). As stress signaling becomes disregulated with age it may in turn affect DNA repair.

Do the observed changes in DNA repair activities with age contribute to the onset of aging? It may be difficult to infer cause–effect relationship from the studies of normal aging, however, mouse knockout studies can provide answers. Defects in DNA maintenance may lead to accelerated aging. Notable examples are mice deficient in NHEJ or mice deficient in TCR (107,108). It has to be mentioned that not all DNA repair mutants show progeroid phenotypes, and it appears that only mutations in particular pathways affect lifespan. However, as most premature aging syndromes are caused by mutations in DNA repair genes it is reasonable to assume therefore that normal aging is caused, in part by the decline in DNA repair capacity.

The overall sequence of events can be as follows (Figure 2): spontaneous mutations and rearrangements

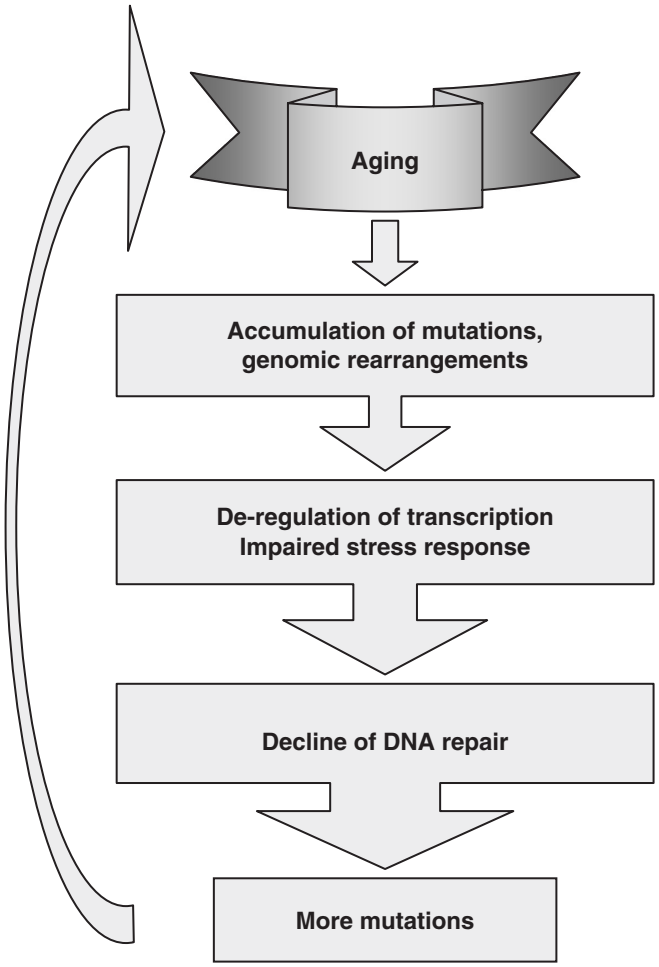

Figure 2. The vicious cycle of aging and genomic instability. Spontaneous mutations and rearrangements accumulate with time leading deregulation of transcription, impaired stress response and diminished function of DNA repair genes. Decline of DNA repair efficiency and fidelity leads to more mutations, and further exacerbates the age-related functional decline.

gradually impair the function of genes involved in stress response and DNA repair. DNA repair becomes less efficient and more error-prone leading to cascading accumulation of DNA damage and mutations, which further exacerbate age-related physiological decline.

Accumulation of mutations may contribute to aging by affecting essential genes and disturbing transcription patterns (1). In addition, unrepaired DNA damage may contribute to aging by increasing apoptosis. A shift in the balance between cell death and cell renewal may lead to exhaustion of the stem cell pool, loss of tissue cellularity and functional decline. Accelerated aging due to loss of stem cells has been observed in mice with overactive p53 (109). Mice with mutated DNA polymerase γ lacking the proofreading activity exhibit accelerated aging due to increased apoptosis (109), and remarkably several NER mutants undergo premature aging due to increased apoptosis, which is not associated with an increase in mutation frequency (110). Furthermore, DNA damage and mutation may perturb chromatin structure and cause epigenetic changes.

Why is DNA repair not perfect? Mutations provide material for natural selection and adaptation. Furthermore, organisms in the wild do not live forever and often succumb to predation or accidents long before they accumulate enough mutations to show manifestations of aging. Therefore, there was no advantage in investing into maintenance of perfect DNA repair system. However, tremendous differences in lifespan between animal species, suggest that there is a wide spectrum of how 'imperfect' maintenance mechanisms can be. Thus, there is no reason why DNA repair cannot be improved. That said, overexpression of downstream DNA repair enzymes often resulted in toxicity (111), which underscores the importance of coordinated expression of DNA repair factors. However, changes in upstream regulators may be a more promising approach to improve DNA repair. In conclusion, more research is needed to understand the nature and mechanisms of age-related changes in DNA repair. This knowledge may potentially allow treatments to upregulate DNA repair and delay aging and cancer.

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