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Forum Review

Maintaining Genetic Integrity in Aging: A Zero Sum Game

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ABSTRACT

Aging of somatic cells can be defined as the gradual loss of the information embedded in the global and local properties of complex macromolecular networks. This loss of information may reflect the dynamic interplay between stochastic factors, such as the accumulation of unrepaired somatic damage, and gene-encoded programmatic responses. This would ultimately result in loss of function, impaired response to environmental challenge, and a progressively increased incidence of disease. Here the authors present the case for aging as a continuous battle between maintaining genomic integrity and ensuring sufficient cell functional mass. Focusing on aging of the liver in rodents, evidence is presented that normal aging is associated with a gradual accumulation of random alterations in the DNA of the genome as a consequence of imperfect DNA repair and a decrease in the rate of DNA damage-induced apoptosis. Apoptosis is the cell's genome maintenance mechanism of last resort and an imbalance towards apoptosis can contribute to manifestations of aging-related phenotypes, as exemplified by mouse models of premature aging due to genetic defects in genome maintenance. Prospects to reset the clock in this zero sum game between survival and the maintenance of phenotypic integrity will be discussed. *Antioxid. Redox Signal.* 8, 559–571.

INTRODUCTION

TIME-DEPENDENT ALTERATIONS in complex networks of macromolecular interactions, such as protein–protein, protein–DNA, and protein–RNA interactions, could disrupt the cell's organizational principles, resulting in degeneration, disease, and death. A long-term goal of the science of aging is to understand the basic mechanisms underlying the decline of the many complex, functionally interacting, subsystems in the somatic cells of metazoa after the period of first reproduction.

Whereas the ultimate cause of aging is now generally sought in the greater relative weight placed by natural selection on early survival and reproduction than on maintaining vigor at later ages (49), our understanding of its proximal causes is still very limited. Thus far, the facets of aging that have been most deeply explored involve sets of functional responses occurring over time through the coordinated action of the products of multiple genes. While such gene-encoded programmatic responses are important and can explain a fair number of physiological changes observed in aging organisms, the proximal causes of aging should most likely be sought in stochastic effects of damaging agents, from both endogenous and environmental sources. Indeed, the evolutionary theory of aging essentially rules out a genetic program to drive the aging process (49).

The stochastic component of aging is often conveniently ignored, since it cannot be as easily studied as its programmatic component. In spite of the early realization of the single cell as the universal foundation of all living organisms, biological processes have been studied almost exclusively in populations of cells or tissues. The focus of attention was invariably on responses common to all or most cells in the tissue. This is exemplified by current gene expression profiling of aging using microarrays, which has resulted in the identification of several organ and tissue-specific functional signa-

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tures of aging in multiple organisms, such as increased stress responses (68). Although individual patterns of aging with a role of chance events, interacting with genes and the environment, are increasingly appreciated (30), the interplay between stochastic mechanisms of aging and its programmatic components is virtually unexplored. In the absence of a genetic program to drive the aging process, it is not clear how consistent patterns of gene expressional changes with age, observed in many species, can explain the aging phenotype.

An example as to how stochastic factors can interact with programmatic responses in causing aging involves the interplay between DNA damage and programmed cell death (apoptosis). Of all the damage inflicted on macromolecular complexes, DNA damage is likely to be the most critical. Indeed, DNA damage gives rise to genome sequence alterations, for example, through erroneous DNA repair. In contrast to damage to proteins or lipids, genome sequence alterations are irreversible, except when the genetically altered cell is eliminated (i.e., through apoptosis). The latter represents a programmatic response to an inherently stochastic problem and serves to maintain phenotypic integrity. However, it cannot but lead to cell depletion, which on the long term may greatly reduce organ function. This continuous balance between maintaining phenotypic integrity and ensuring cell functional mass is essentially a zero sum game. We first discuss the evidence for increased genomic instability with age, and the mechanism through which this could lead to age-related functional alterations and disease. We will then provide evidence for apoptosis as a critical factor in determining the balance of the organism's phenotypical integrity and cell functional mass.

SOMATIC MUTATIONS AS A CAUSE OF AGING

Somatic mutagenesis, a stochastic process *par excellence*, has since long been considered as a major causal factor in age-related cellular degeneration and death (29, 79, 86). Mutations (i.e., irreversible changes in DNA sequence organization) are inextricably linked to the evolution of different life forms by providing the substrate of natural selection. However, too many mutations, without a mechanism to escape their adverse effects, can be harmful to a cell population. In this respect, mutagenesis is a two-edged sword. Indeed, as has been demonstrated in unicellular organisms, increased mutation loads decrease fitness and, at least in the absence of sex or recombination, can lead to senescence-like phenomena and population extinction (3, 4, 48). The effect of random mutations on the fitness of a cell population is well illustrated by the work of Elena and Lenski, who compared average fitness (i.e., growth rate) for three groups of E. coli strains: harboring one, two or three randomly introduced insertion mutations. Average fitness was found to decline with the number of mutations (28).

Populations of *Escherichia coli* cells are obviously not the same as organs and tissues of mammals, and a similar experiment as done by Elena and Lenski is difficult to carry out with mammalian cells in view of their much larger genomes and different genomic organization. Nevertheless, similar to small populations of unicellular organisms without the opportunity for sexual reproduction, the somatic cells of multicellular organisms are also vulnerable to deleterious mutations. It is conceivable that genome-wide mutation accumulation can cause deleterious changes in cells which may ultimately result in organ dysfunction. In contrast to unicellular organisms, which represent both germ line and soma, somatic cells of metazoa do not need to maintain genetic variation to provide for evolutionary change. Therefore, in theory, maximization of genome maintenance mechanisms, only for their somatic cells, could prevent any significant mutation accumulation in such organisms. However, evolutionary theory would not predict a further optimization of cellular maintenance and repair than strictly necessary to reach the age of first reproduction (50). Hence, it is likely that already at reproductive maturity the maximum number of somatic mutations compatible with optimal fitness will have been reached. Even slight increases of the mutation load above this threshold may then already begin to adversely impact on the structure and function of an organism.

A most likely cause for the majority of somatic mutations arising in aerobic organisms is oxidative DNA damage. Denham Harman was the first to recognize the role of free radicals as a most logical explanation for the various forms of cell and tissue damage observed to occur during aging (36). As by-products of oxidative phosphorylation and other biological and physiological processes, oxygen radicals can induce a variety of damages into cellular DNA and other biological macromolecules like proteins and lipids. The lesions induced in DNA by free radicals are diverse and include a variety of adducts, as well as abasic sites, cross links, and DNA single and double strand breaks. While virtually all of such lesions are repaired by the cell's intricate complex of genome maintenance systems (38), some result in mutations (i.e., irreversible changes in the DNA sequence). Mutagenesis can occur, for example, through errors in replicating or repairing a damaged template. Hence, while most DNA damage is temporary, mutations represent an irreversible loss of genome integrity, sometimes with phenotypic consequences.

DETECTING GENOMIC MUTATIONS IN THE AGING MOUSE

In order to begin testing the hypothesis that mutation accumulation affects fitness in mammals, methods are needed to quantify and characterize spontaneous genomic mutations in different tissues and organs. Mutations can vary from point mutations, involving single or very few base pairs to large deletions, insertions, duplications, and inversions. In organisms with multiple chromosomes, DNA from one chromosome can be joined to another and the actual chromosome number can be affected. Other types of permanent stochastic changes that can impact on normal patterns of gene expression involve epigenetic changes, such as alterations in methylation patterns and changes in the histone code (81). Because genomic mutational events are rare and do not affect DNA chemical structure, they are not easy to detect and the

total number of mutations in the average cell of an aged tissue cannot be quantified. The same is true for most epigenetic alterations. Hence, while we know that DNA damage induces a variety of cellular responses, the possible impact of its stochastic endpoint, that is, the spectrum of alterations in the genome's information content, on the aging process is unknown.

In the past, mutation detection *in vivo* has been limited to cytogenetic analysis of actively proliferating cells, such as lymphocytes, for the occurrence of chromosomal aberrations. Later, also smaller mutations, such as point mutations, could be detected in such cells, using selectable marker genes, the most popular being the HPRT locus test (1). Both cytogenetic tests and the HPRT assay have indicated the accumulation of mutations with age in lymphocytes from both humans and mice (20, 41, 82). However, these results have been interpreted with caution in view of the fact that the assays used could only be applied to actively proliferating cells. This may offer a poor reflection of the *in vivo* system where the majority of adult human and animal cells only rarely undergo cell division. In order to extend these studies to the *in vivo* situation, transgenic mouse models have been developed harboring chromosomally integrated bacterial mutation reporter genes, which can be recovered from their integrated state, transferred to *E. coli* and then analyzed for mutations (34). One of these models, based on chromosomally integrated plasmids containing the lacZ reporter gene (Fig. 1), has made it possible to quantify and characterize a wide range of somatic mutations (including large genome rearrangements) at a neutral, nonexpressed marker locus in various mouse organs and tissues as the animals age (5). Using these mice it has been demonstrated that mutations at the lacZ locus accumulate with age in most organs and tissues, albeit at greatly different rates (23, 24).

It is possible that ROS, considered as a major cause of aging (42), are the main proximate cause of such increasing genomic instability. Apart from certain signature mutations, most notably GC to TA transversion mutations, ROS is also known to induce large genomic mutations, possibly through erroneous repair of double-strand breaks. Especially the liver is highly vulnerable to oxidative damage in view of its high oxygen metabolism. Indeed, in mice with an engineered genetic defect in the removal of the major oxidative base, 8-oxoG, this oxidative modification was found greatly elevated in liver (where it accumulated with age), but not in other organs (65). Liver is also the main target organ in mice with a CuZnSOD deficiency, which rapidly develop hepatocellular carcinomas (27).

We have demonstrated a significant increase of mutations at the lacZ locus in liver of normal mice during aging (23). A sizable fraction of the mutations found to accumulate appeared to be large genome rearrangements, the relative frequency of which is strongly accelerated at the end of life (Fig. 2). Such large genomic mutations can be detected through restriction enzyme digestion of the lacZ-plasmid recovered from a mouse tissue and scored as a mutation on the selective plate (Fig. 1). Mutations that do not alter the restriction pattern are point mutations, that is, single base changes or small deletions or insertions. Most of the mutations that cause changes in the restriction pattern are deletions and other types of rearrangements, with one breakpoint in a lacZ gene of the plasmid cluster and the other breakpoint elsewhere in the mouse genome. Physical characterization of a number of such events in liver and other organs of old mice, indicated intrachromosomal deletions or inversions, varying from smaller than 100 kb to 66 Mb, as well as translocation events (33). At the time, we considered a reduced apoptosis rate as a possible explanation for the sharp increase in genome rearrangements in the liver at the end of life. Indeed, these mutational events likely result from erroneous repair of DNA double-strand breaks, a highly toxic type of lesion that can be induced by ROS. DNA double-strand breaks and the resulting increased genomic instability likely leads to increased apoptosis, which would result in organ dysfunction (see below).

Increased genomic instability with age is likely to contribute to the exponential increase in cancer incidence with age. However, apart from inducing cellular responses, such as apoptosis, random DNA damage and mutations can also be expected to contribute to aging-related organ dysfunction by causing changes in genes or in their expression levels. In the past, such a role was considered unlikely in view of the presumed extremely low frequency of mutational events and the relatively small fraction of the genome that was considered functionally relevant. Indeed, almost immediately after the somatic mutation hypothesis was first presented (79), Maynard-Smith argued against it on this basis (58). However, at that time mutations were mainly seen as gene inactivating events. Since protein-coding genes make up only slightly more than 1% of the genome sequence, direct gene inactivation through random mutagenesis is unlikely to occur at high frequency.

It is now estimated that up to 50% of the mammalian genome is transcribed (72). It is assumed that this noncoding transcribed part of the genome has a regulatory role, which is greatly facilitated by the unique single-stranded nature of RNA. Therefore, while only few spontaneous mutations are likely to hit protein-coding genes, at least half of the spontaneous mutation load of an aging mammalian cell would be expected to occur in gene regulatory regions. Even a relatively small number of mutations could therefore exert some adverse effect on the cell. This is especially true for large mutational events, such as chromosomal aberrations and aneuploidy. Such random mutations could influence patterns of gene regulation through gene dose effects or position effects of regulatory sequence interactions (87). As mentioned above, a substantial fraction of the spontaneous mutations at the lacZ locus, found to accumulate in liver during aging are genome rearrangements (Fig. 2) (e.g., deletions, inversions, and translocations). Their total number per diploid genome has been estimated through extrapolation from their frequency at the lacZ transgene locus to the genome overall. In liver, they increase from an average of 9 in young animals to 27 per diploid genome in old mice (25). They can be expected to result in partial haploidization of genomic regions or the loss of spatial interactions between regulatory sequences and promoter regions. This could lead to variations in gene expression.

Due to the large size of many rearrangements, even at low absolute numbers, the effects of such mutations would far ex-

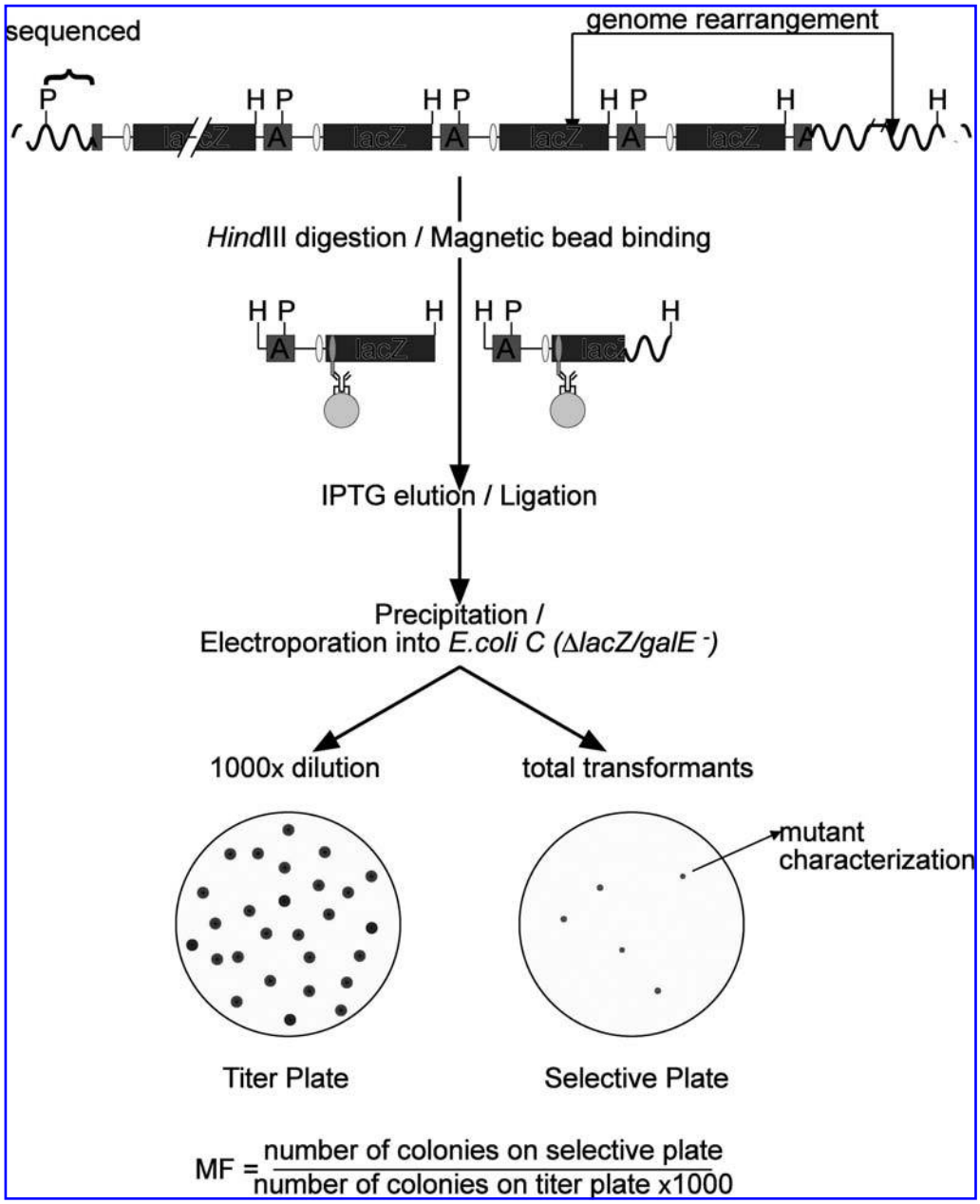

FIG. 1. Schematic depiction of the LacZ-plasmid mouse model for mutation analysis *in vivo*. In this system, plasmids are excised from genomic DNA with HindIII, followed by their separation from the mouse genomic DNA using magnetic beads, precoated with a lacI repressor protein. The plasmids are then ligated and transferred to *Escherichia coli* C (ΔlacZ, galE⁻) using electrotransformation. A small amount of transformants is plated in medium with X-gal to determine the total number of plasmids rescued. The remainder is plated on medium containing the lactose analogue p-gal. In the presence of p-gal, the mutation in its galE gene prevents *E. coli* cells with a wild-type lacZ gene to grow. Hence, only the cells harboring a lacZ mutant plasmid survive. The mutation frequency is the ratio of the colonies on the selective plate versus the colonies on the titer plate (times the dilution factor).

ceed the effects that can be expected from single base mutations. The perturbed transcriptomes resulting from these large genomic events would greatly alter cellular networks controlling a variety of subtle functions. Moreover, any functional effect of such a rearrangement on the activity of a gene would be enormously enhanced through epistatic effects. For example, it has recently been demonstrated that haploinsufficiency of the Nkx3.1 locus in the mouse increases the probability of stochastic activation or inactivation of its target genes (56). The results of this study, which involved over 50 Nkx3.1 target genes in the mouse prostate, revealed a spectrum of dosage sensitivity, varying from relative insensitivity to

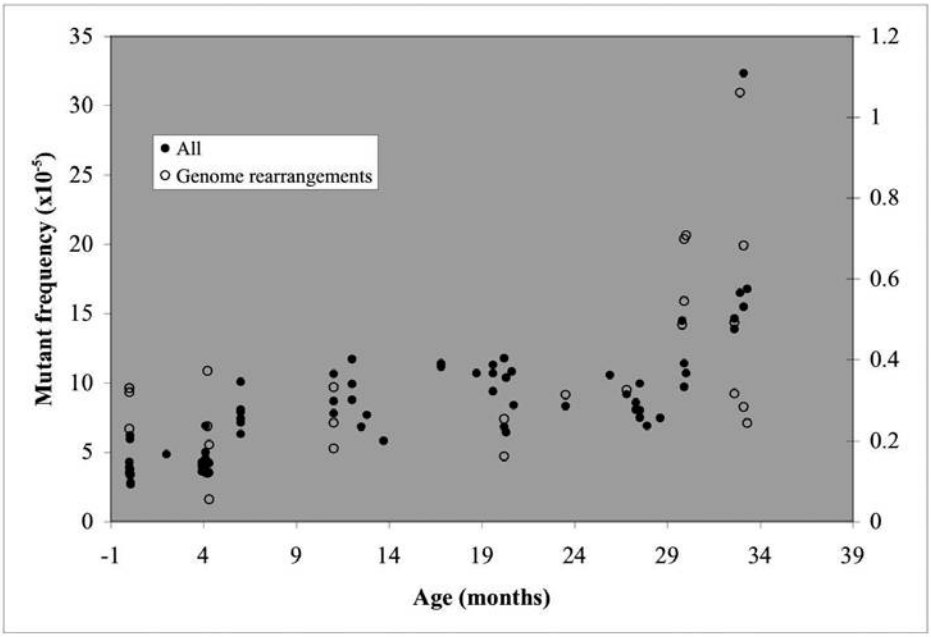

FIG. 2. Increased mutation frequencies at the lacZ reporter locus in liver of C57Bl/5 mice with age. After an initial increase, the frequencies of all mutations (*closed symbols*) reach a plateau, until they rise further at late age. This is especially true for large genome rearrangements (*open symbols*), that is, mutations with one breakpoint in a lacZ gene and the other elsewhere in the mouse genome (*see text*), which increase very rapidly at old age. Each determination point represents the liver of one animal.

Nkx3.1 dosage to complete loss of expression even in the Nkx3.1 heterozygotes.

Hence, the gradual accumulation of large genomic alterations in aging organs and tissues may affect patterns of gene regulation in a stochastic manner, resulting in a mosaic of cells, varying from cells that escaped significant damage, to cells with severe dysfunctions, transformed cells, and cells that are dying. Such a process of slow genomic breakdown would be greatly accelerated by declining DNA repair activities with age. However, it would occur even without such a decline. Indeed, as mentioned above, genome maintenance is imperfect and not designed for periods far beyond the reproductive age. Hence, some DNA damage will inevitably escape the scrutiny of the repair systems and persist over time or will be converted into mutations, including large genome rearrangements. However, there is an ultimate cleansing mechanism in the form of apoptosis, to which we will now turn.

CELL SURVIVAL VERSUS CELL DEATH IN NORMAL AGING

The recognition that tumor development involves an imbalance between cell proliferation and apoptotic cell death is a relatively recent concept in cancer biology. In the steady-state condition of the organism, cell division must be counterbalanced by cell death (70). The term apoptosis was originally introduced by Kerr to describe a form of hepatocellular cell death occurring in ischemic liver disease (45). Hepatocytes in adult rodents have a long lifespan and rarely divide under normal conditions. However, in certain situations of pathophysiological stress, such as partial hepatectomy, viral infection, or chemical injury, these cells are able to divide in order to restore the loss of liver mass (80). For example, after partial hepatectomy, remaining hepatocytes proliferate to restore the mass of the organ within days to weeks, showing a tremendous capacity to proliferate (21, 61). Rodent liver has offered one of the best models to study effects of environmental or endogenous variables on growth control, apoptosis, and carcinogenesis (43, 60). It has been shown that spontaneous apoptosis increases in the liver of rodents subjected to caloric restriction, a condition known to extend lifespan and retard the incidence of spontaneous and chemically induced tumor formation (9, 35, 39, 40). This suggests that an efficient apoptotic response to endogenous damage plays a protective role against cancer, a major aging-related disease.

A direct correlation between apoptosis rates and cancer susceptibility has been demonstrated using the rat as a model organism after treatment with the organ-specific carcinogenic agent, methyl methanesulfonate (MMS) (76). MMS is a strong inducer of brain tumors, whereas it is a weak hepatocarcinogen, even in the regenerating liver of rats (78). The tissue specificity of the carcinogenic effect of MMS is of particular interest because MMS does not require metabolic activation and should yield equal amounts of DNA damage in different tissues. Indeed, the initial amount of DNA damage caused by MMS was shown to be similar in the liver and brain of rats following intraperitoneal injection (90). Therefore, the cellular responses induced by damaged DNA, such as apoptosis or activation of signaling systems, not the DNA damage itself, are likely to determine the tissue-specificity of tumor formation by MMS. Indeed, MMS induces apoptosis in the

nontarget tissue liver but not in the target tissue brain of rats, demonstrating a strong correlation between the ability of a tissue to undergo apoptosis and its resistance to carcinogenesis (76). These results suggested that induction of apoptosis is involved in the protective response in the liver to prevent tumor development by eliminating alkylation damaged cells after exposure to MMS. This may explain why MMS does not induce tumors in the liver even during regeneration after partial hepatectomy (13).

The robust induction of apoptosis in the liver after MMS injection provided a unique opportunity to investigate whether deregulation of apoptotic responses to genotoxic stress occurs *in vivo* with age. To investigate if the capacity to remove damaged cells is maintained throughout life, apoptotic potential in the liver of young versus old rats was assessed after treatment with MMS. The results indicated that the apoptotic potential is dramatically reduced in the liver of old rats after MMS treatment (Fig. 3) (77), suggesting that the livers of old rats are resistant to apoptosis in response to a moderate dose of genotoxic stress compared to their young counterparts. Since young and old animals differ significantly in body composition and general metabolism, it is conceivable that the downregulation of apoptosis in the liver of old rats is really due to a lower or delayed induction of DNA damage. However, this possibility is unlikely since the initial amount of DNA damage induced by simple alkylating agents is not lower and persists longer in the liver of old as compared to young animals (31). Therefore, the observed age-dependent decline in apoptosis most likely indicates a genuine decline in apoptotic response during aging.

Alkylation damage, such as induced by MMS, is a major form of spontaneous DNA damage (51). Therefore, the observed age-related defect in apoptotic response to MMS-induced DNA damage could have important implications for the capacity of aged individuals to withstand genotoxic stress from both endogenous and exogenous sources. In this respect, it is tempting to speculate that decreased apoptotic potential contributes to the increased incidence of liver cancer in old rodents (8). Moreover, this age-related down-regulation of apoptosis provides a potential mechanism for the aforementioned accelerated increase of genome rearrangements at the lacZ reporter gene in old mice (23). However, it should be emphasized that this result could well be tissue-specific, possibly being characteristic of mitotic tissues, but not postmitotic tissues. Indeed, in contrast to liver, no MMS-induced apoptotic response has been observed in the brain, either at young or old age ((76); Suh, unpublished results). Moreover, in contrast to liver, genome rearrangements in brain are infrequent at both young and old age (23).

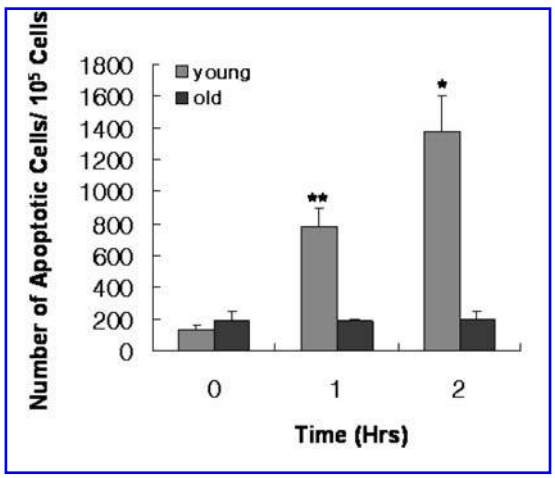

FIG. 3. Induction of apoptosis in the liver of young but not in old rats after MMS treatment (77). Female Fischer 344 rats of 2 months (young) and 26 months (old) were treated with 1.5 mmol/kg body weight MMS. The numbers of apoptotic cells for each animal were microscopically determined after *in situ* labeling of nuclear DNA fragmentation (TUNEL). Each determination point is the average of three animals. *Bars* indicate the standard deviations. The increase in the rate of apoptosis in young rats was significant by *t*-test (**$p < 0.001$, *$p < 0.05$).

Little is known about the possible molecular signaling defect(s) that might be responsible for the age-related dysfunction of apoptosis in response to genotoxic damage. Among the major pathways involved in regulating cellular responses to genotoxic stress, we have focused on the mitogen-activated protein kinase (MAPK) signaling pathways. MAPKs play a critical role in the regulation of cell proliferation, differentiation, and apoptosis. MAPKs comprise a ubiquitous family of tyrosine/threonine kinases and include extracellular signal-regulated kinases (ERKs), c-Jun NH_2-terminal kinases (JNKs) and p38 MAPKs (12, 63, 67). The JNKs and p38 MAPKs are collectively termed the stress-activated protein kinases (SAPKs) because they are activated by similar stress-related stimuli (44, 67). DNA damaging agents such as ultraviolet light, ionizing radiation, 1-β-D-arabinofuranosylcytosine (araC), *cis*-platinum, mitomycin C, chemotherapeutic drugs, and alkylating agents are known to activate MAPKs in cultured mammalian cell lines (46, 47, 52, 64, 66, 71). Activation of ERKs has been primarily implicated in cell proliferation and survival, whereas activation of SAPKs is involved in growth arrest and apoptosis (12, 67).

Recently, we have shown no significant changes in activation of ERKs after MMS treatment in the liver of young animals, whereas MMS strongly induces activation of both ERK isoforms in the liver of old animals (Suh *et al.*, unpublished results). We suggest that activation of ERKs in the liver of old but not young rats after MMS treatment may play a role in downregulation of apoptosis and increased carcinogenesis at old age by inducing survival and proliferation of cells with MMS-damaged DNA. Because aging did not significantly alter JNK signaling pathways in the liver (74), we hypothesized that the balance between survival (ERK) and death (JNK and p38) signaling pathways may be critical in determining cellular fate, and that MMS-induced differential activation of each MAPK pathway ultimately determines the functional outcome (74, 75) (Fig. 4). The age-related changes in MMS-induced activation of MAPKs may shift the balance toward survival and proliferation. Maintaining apoptosis rates in an organ as the liver, exposed to progressively increasing ROS levels, would protect genome integrity, but they would undoubtedly do so at the cost of an acute loss of organ capacity. While an age-related increase in liver cancer has been documented in rodents (8), it is not a major cause of death,

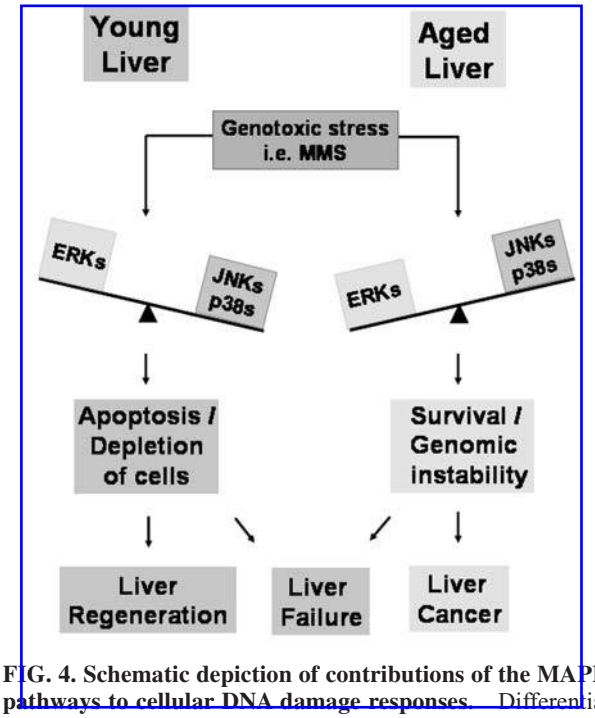

FIG. 4. Schematic depiction of contributions of the MAPK pathways to cellular DNA damage responses. Differential activation of MAPKs was observed in the liver of young and old animals after MMS treatment. The balance between the survival (ERK) and the death (SAPK) signaling pathways is critical in determining cellular fate after stressful stimuli.

unless some upstream defense mechanism, such as DNA repair or antioxidant defense are defective (see below). It is therefore expected that a more general decline in organ capacity due to the progressive increase of cells harboring a structurally compromised genome is preferred over a more acute loss of organ capacity.

With respect to aging, apoptosis should be considered as a double-edged sword, acting in a cell type-specific manner. While diminished responses to cell death signaling in proliferative tissues would lead to accumulation of damaged cells that could be a significant factor in the development of cancer, increased rates of apoptosis in postmitotic cells, such as neurons, could contribute to functional decline of organs due to excessive cell loss (75). Indeed, increased apoptosis is known to contribute to age-related pathologies in postmitotic tissues, such as brain (57). High levels of apoptosis in an organ as the liver would protect against cancer, but possibly at the cost of dramatic losses of liver capacity. In this zero sum game, it is ironic that increased apoptosis may provide increased protection against cancer, a major age-related disease, but only at the cost of accelerating many other signs and symptoms of aging. For the organism to survive, lost cells must be replaced. The rate of cell loss and the rate of renewal will determine the normal tissue function. When loss exceeds replacement, it will lead to a decline in organ function and ultimately organ failure. Therefore, adult mammals require extensive proliferation and tissue replacement to survive over longer periods of time. Recent evidence has identified significant capacities for repair and regeneration even in organs once thought to be postmitotic such as the pancreatic islets and the brain (73).

CANCER AND AGING: IMBALANCE BETWEEN CELL SURVIVAL AND CELL DEATH

Recently a number of mutant mice harboring specific defects in one or more DNA repair or genome maintenance pathways has been shown to display multiple phenotypes of accelerated aging (37, 55). There is evidence that in most of these models there is an imbalance between genome maintenance, which is diminished, and apoptosis or cellular senescence, which are accelerated. We will discuss two of the best described models to illustrate how the zero sum game of aging, maintaining the balance between genomic integrity and cell functional mass, can quickly acquire new standards upon interference in the species' genetic make-up. The first model is a mouse harboring a hypomorphic mutation in a gene participating in both transcription and repair. The second involves a mouse with a defect in double-strand break repair.

DNA repair and transcription

Transcription of genes occurs regardless of the cell's proliferative status and during all phases of the cell cycle, except during mitosis when transcription is suppressed due to the condensation of chromatin. Compared to replication, which takes place only in proliferating cells and only during S-phase of the cell cycle, the transcription machinery renders itself easy access to scan DNA integrity and to monitor the levels of DNA damage. Therefore, transcription is increasingly recognized as a damage dosimeter, where the severity of damage, the ability to remove the lesions and the kinetics of mRNA synthesis recovery determine whether the cell lives or dies (53, 59).

DNA lesions blocking transcription can have severe consequences for the cell through the induction of apoptosis. Transcription-coupled repair (TCR) is a specialized form of repair, removing transcription blocking lesions in currently active genes. Cells from patients with defects in TCR have defects not only in the recovery of RNA synthesis following UV-irradiation, but also in general transcription (6, 53). Exposure studies have indicated the link between the deficiency of TCR and the efficiency of apoptosis. For example, human fibroblasts that are deficient in the removal of transcription blocking lesions were shown to be more susceptible to induction of p53 and apoptosis after treatment with DNA damaging agents, compared to their TCR-proficient counterparts (2, 84). Moreover, the triggering mechanism for p53 accumulation and apoptosis in response to genotoxic stress was dependent upon persistent lesions specifically in the transcribed strand of active genes (26, 54, 92). These results suggest that blockage of transcription might be involved in p53 signaling and in the induction of apoptosis. Similarly, in mouse models with defects in TCR, the development of sunburn as a result of apoptosis occurs at much lower doses of UV light than in

wild type littermates (7, 14–17), although the level of spontaneous apoptosis in these mice has not been determined.

The XPD gene encodes the 5′ to 3′ DNA helicase subunit of basal transcription factor TFIIH, which is involved in TCR, but also in global genome nucleotide excision repair (GG-NER), which mainly removes helix-distorting types of DNA lesions from the genome overall (38). Complete inactivation of the XPD helicase is not viable in the mouse or in cells. Mutations in this gene in humans can cause trichothiodystrophy, a segmental progeroid syndrome, resembling Cockayne syndrome, which is much better known. Mice carrying a TTD type of mutation (Arg722Trp) in the XPD gene revealed a striking correspondence with the complex pleiotropic human phenotype (17). This includes the hallmark of the disorder, reduction of hair-specific cysteine-rich matrix proteins resulting in brittle hair, as well as growth delay, reduced fertility and lifespan, skin abnormalities, UV sensitivity, and a partial NER deficiency. At the level of DNA repair the XPD^{TTD} mutation causes a partial defect in both GG-NER and TCR. In contrast to human TTD, Xpd^{TTD} animals display a mild UV-induced skin cancer predisposition, which may be attributed to the inability of rodents to repair certain types of UV-induced DNA lesions by global genome repair.

A systematic life span study of XPD^{TTD} animals, in comparison with littermate controls, revealed a host of premature aging phenotypes, on top of the TTD features (14). Due to the defect in GG-NER, the XPD^{TTD} cells can be expected to suffer from increased DNA damage in their global genome, which can lead to increased genomic instability and mutation, a risk factor of malignant transformation. On the other hand, due to the defect in TCR and general transcription, the XPD^{TTD} cells are prone to apoptosis through signals mediated by the stalled RNA polymerase II at the lesion (69, 85), which may counterbalance the effect on increased mutation loads by removing damaged cells. Indeed, it is conceivable that while the about 30% remaining GG-NER activity would be able to sufficiently suppress mutations, the combined defect in TCR and transcription may shift the balance towards apoptosis, a condition promoting aging.

We investigated the impact of the Xpd mutation on the physiology of liver, the central metabolic organ of the body and a major target organ of oxidative DNA damage, and demonstrated a strong induction of apoptotic cells as compared to the wild type controls (Table 1; Suh *et al.*, unpublished observations). It is possible that the increased apoptosis underlies some forms of age-related phenotypes observed in the XPD^{TTD} mouse (i.e. reduced body weight and the reduced incidence of cancer). Indeed, after crossing these mice with the lacZ-reporter mice no evidence for increased genomic instability was observed (93). This was in contrast to a mouse model with a completely inactivated nucleotide excision repair system, that is, Xpa null mice (19), in which mutations were found to accumulate substantially more rapidly than in the controls (32). This increase in mutation accumulation involved only point mutations; genome rearrangements were not or hardly affected (93). The increased mutation accumulation in liver of the Xpa mutants was accompanied by a slight increase in apoptosis, but much less severe than in the Xpd animals (Table 1) (Suh *et al.*, unpublished results). Taken together, these results suggest that the tumor suppression facilitated by a strong increase in the apoptotic response outweighs the contribution of a repair defect to genomic instability in the XPD^{TTD} mice. Clearly, the imbalance between cell survival and elimination caused by the genetic defect alters the rules of the zero sum game and is the most likely cause of the premature aging phenotype in these animals.

DNA repair of DNA double-strand breaks

Double-strand breaks in DNA are highly toxic lesions that can be created through a variety of mechanisms, including effects of reactive oxygen species. Double-strand breaks are repaired by either of two mechanistically distinct DNA repair pathways, homologous recombination (HR) or nonhomologous end-joining (NHEJ) (83). A key factor of DSB repair by NHEJ is the DNA end-binding Ku70/Ku80 heterodimer. Mice harboring a null mutation in the Ku80 gene have a significantly shorter life span and display a range of premature aging phenotypes (89), many of which overlap with the Xpd mutant mice discussed above.

While in the XPD^{TTD} mice the dramatic premature aging phenotype is mainly caused by the transcription defects (the nucleotide excision repair process itself cannot be very important in aging in view of the lack of a premature aging phenotype of the Xpa null mice), the importance of the Ku80 mice lies in the critical process of NHEJ. Indeed, in view of the importance of ROS as a main causal factor in aging, double-strand breaks are expected to be prominent among the DNA lesions naturally accumulating with age. Also in this

TABLE 1. GENOMIC INSTABILITY, APOPTOSIS, CANCER, AND AGING

Mouse model	*Defective function*	*Genomic instability*	*Apoptosis*	*Cancer predisposition*	*Premature aging*
$Xpd^{TTD/TTD}$	TCR, Transcription	−	+++	↓	↑↑↑
$Ku80^{-/-}$	NHEJ of DSBs	+	+/−	↓	↑
$Xpa^{-/-}$	NER	+	+	↑	↑↑
$Sod^{-/-}$	Antioxidant defense	+++	+	↑↑↑	↑

DSB, double strand break; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; TCR, transcription coupled repair.

mutant mouse we observed an increased apoptosis rate in liver, but substantially less dramatic as in the XPD^{TTD} mouse (Table 1). However, in this case also an increase in genomic instability at the lacZ reporter locus was observed (Table 1). In contrast to the Xpa null mice, in which mainly point mutations were found to increase with age, the genomic instability in the Ku80 mutant exclusively involved genome rearrangements (22) (Busuttil *et al.*, unpublished results). Moreover, in contrast to the XPD^{TTD} mice, cells from Ku80 mutant mice also display increased cellular senescence (89). Hence, in this case accelerated aging is also a consequence of an imbalance between survival and apoptosis, but caused by the genomic instability itself rather than interference in transcription.

Assuming that ROS are the main driving force of the increased DNA damage that becomes fatal to these mutants due to their defects in DNA repair, genetic defects in antioxidant defense could likely result in a similar premature aging phenotype. This does not appear to be the case. For example, mice that are deficient in CuZn superoxide dismutase undergo persistent and widespread oxidative DNA damage, have a reduced life span and undergo hepatocarcinogenesis later in life (27). Mild symptoms of premature aging have been observed in this mouse model (Huang, personal communication).

Interestingly, after crossing the SOD1-defective mice with the lacZ reporter mice, a highly elevated mutation frequency in liver as compared to control mice, was already observed as early as 2 months of age (Table 1) (94). Like in the Xpa mutant mice, which also show an increased incidence of liver cancer at old age (18), most of these mutations were point mutations. In the SOD1 null mouse virtually all point mutations were GC to AT transitions and GC to TA transversions, signature mutations of oxidative damage. Apoptosis rate in liver was slightly increased (Table 1) (94).

A general conclusion that can be drawn from the examples presented is that imbalance towards apoptosis leads to premature aging, especially if another DNA damage response, cellular senescence, is also elevated. This can happen without any observable increase in mutation rate, but it can also be driven by increased genome rearrangements, as in the Ku80 mouse model. In fact, other DNA repair defective mouse models, including the BRCA1 defective hypomorph (10) and the combined Atm/Terc and Wrn/Terc deficient mice, also display accelerated aging in the presence of increased apoptosis and gross chromosomal alterations (11, 91). These examples demonstrate that re-writing the rules of the zero sum game of aging, by altering the balance between cell survival and cell elimination, can greatly accelerate senescent changes in organs and tissues.

GENERAL DISCUSSION AND FUTURE PROSPECTS

In the presence of proficient genome maintenance systems, aging depends on a delicate balance between cell survival and cell elimination. Since genome maintenance is never perfect and damage in biological macromolecules accumulates at relentless pace, cell elimination is an attractive option, especially at young age, to prevent cancer depending on the cellular context of organ or tissue. High levels of apoptosis in an organ as the liver would protect against cancer, but possibly at the cost of dramatic losses of liver capacity. To maintain biological stability, redundancy has possibly emerged as a major mechanism during evolution. Indeed, although an age-related increase in liver cancer has been documented in rodents (8), it certainly is not a major cause of death. At old age, the balance may shift towards cell survival, which could be important to maintain liver function at the cost of increased cancer risk. In the zero sum game of aging, whichever option will be selected, one necessarily goes at the cost of the other with organ dysfunction as the inevitable result. We have schematically depicted this quandary of the aging cell in Figure 5.

As we have shown, genetic defects in a cell's repertoire of genome maintenance systems often result in premature aging phenotypes and a reduced lifespan. In practically all these cases the accelerated aging phenotypes appear to be due to premature organ dysfunction as a consequence of increased cellular responses to the genetic defects, which may involve accelerated accumulation of DNA damage. This imbalance is indicated in Figure 5. In these DNA repair-deficient mouse models, accelerated aging is likely to result from a shift in the zero sum game of aging towards cell elimination. Indeed, in these mouse models p53 is often constitutively activated, a situation that is now considered as a pro-aging state (95). An accelerated loss of functional cells leading to organ dysfunction in these mouse models is a likely explanation for the observed premature aging symptoms, such as osteoporosis, atrophic skin, hypogonadism, muscle atrophy, and anemia (96). It should be noted that organ dysfunction during normal aging could be due to an increased number of cells harboring functional defects; while in the accelerated aging mice it is likely the loss of functional cells that causes the problem. The difference would be due to a change in the rules of the zero sum game of aging caused by altering the genetic make-up of the organism.

Is it possible to intervene in a manner so as to reset the entire balance between cell survival and cell death in a way that delays aging of a species? There are obviously large differences in natural lifespan between species, which are likely due to differences in somatic maintenance. However, interventions in the processes that interface at the cell's eventual decision level between survival and death are likely to cause side effects, the consequences of which are difficult to predict. Still, evidence has emerged that lifespan—within a species—is more flexible than previously thought. Genetic mutations downregulating activities of growth, reproduction, or nutrient sensing have now been demonstrated to significantly increase longevity in a wide range of multicellular organisms, from nematodes to mice (88). There is evidence that the mechanism of such life extension involves interference in the generation of somatic damage, the upregulation of mechanisms that protect against such damage, or both. Some potential master regulators of the seemingly orchestrated upregulation of cellular defense have been identified, including the forkhead/winged helix transcription factor FOXO (DAF-16 in nematodes) and the NAD-dependent histone deacetylase SIRT1 (Sir2 in yeast, in which it was originally identified)

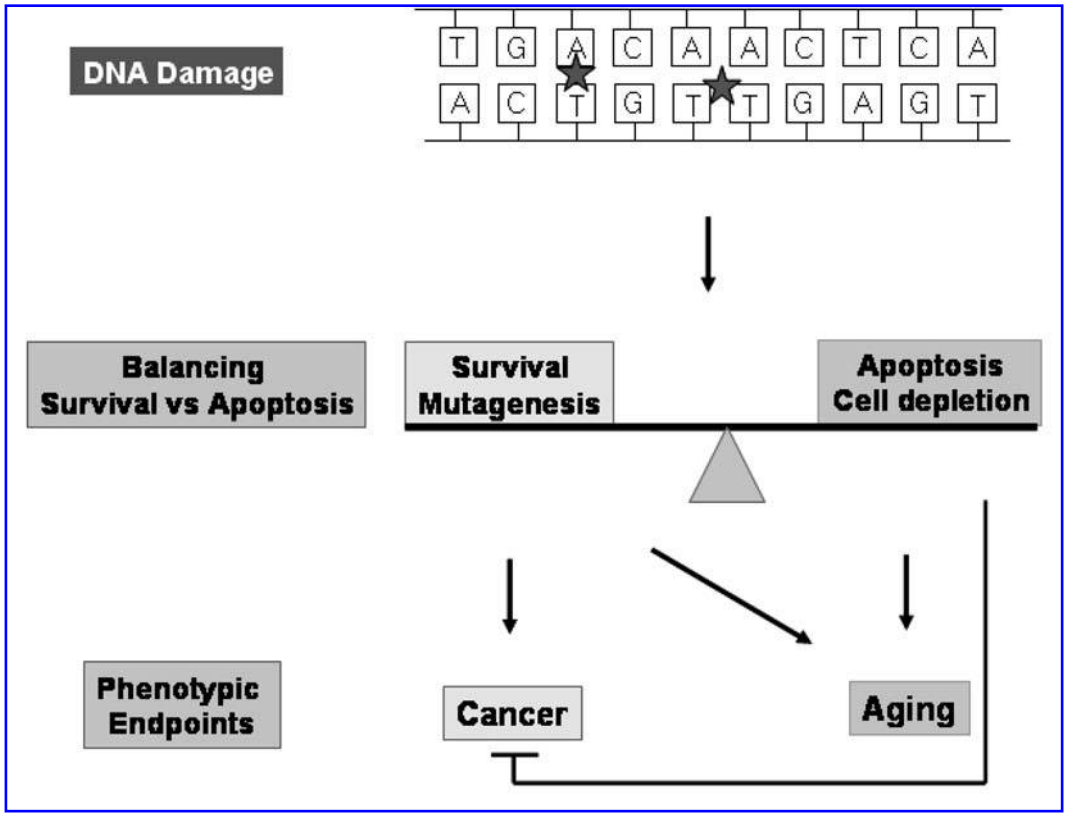

FIG. 5. Consequences of imbalance between cell survival and death: cancer or aging. Cellular responses are triggered by DNA damage, including DNA repair, cell cycle checkpoints, and apoptosis. Defects in DNA repair and cell cycle checkpoints result in genomics instability, a condition promoting cancer. DNA damage signaling induces apoptosis when DNA damage is too severe or in response to cytotoxic lesions (i.e., replication- or transcription-blocking lesions), leading to the death of damaged cells, a condition promoting organ-degenerative aspects of aging. Any changes in homeostatic balance between cell survival and death will manifest as phenotypic consequences. The continuous balance between maintaining phenotypic integrity and cell functional mass during aging is essentially a zero sum game.

(55). It has been demonstrated that SIRT1 is capable of deacetylating FOXO, increase its ability to induce cell cycle arrest, raise its resistance to oxidative stress, but inhibit its ability to induce cell death (62). This suggests that there may be pathways that can downregulate cellular responses against somatic damage without uprooting the cell's genome integrity. Interestingly, we recently demonstrated decreased genomic instability at the lacZ reporter locus in Ames dwarf mice, harboring a mutation that greatly reduces growth hormone levels and downregulates insulin signaling, similar to the longevity mutants in worms and flies (Garcia *et al.*, unpublished observations). These animals live 40% longer, possibly also due to a general upregulation of cellular defense systems. Hence, based on our increased insight in such mechanisms we may once succeed in beating the zero sum game of aging, by resetting the balance to the advantage of cell survival at no cost for genomic integrity.

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ABBREVIATIONS

CS, Cockayne syndrome; DSB, double strand break; ERK, extracellular signal-regulated kinase; GG-NER, global genome nucleotide excision repair; JNK, c-Jun NH_2-terminal kinase; MAPK, mitogen-activated protein kinase; NER, nucleotide excision repair; NHEJ, non-homologous end joining; MMS, methyl methanesulfonate; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; TCR, transcription coupled repair; TTD, trichothiodystrophy.

REFERENCES

1. Albertini RJ. HPRT mutations in humans: biomarkers for mechanistic studies. *Mutat Res* 489: 1–16, 2001.
2. Andera L and Wasylyk B. Transcription abnormalities potentiate apoptosis of normal human fibroblasts. *Mol Med* 3: 852–863, 1997.

3. Andersson DI and Hughes D. Muller's ratchet decreases fitness of a DNA-based microbe. *Proc Natl Acad Sci USA* 93: 906–907, 1996.
4. Bell G. *Sex and Death in Protozoa.* Cambridge, UK: Cambridge University Press, 1988.
5. Boerrigter ME, Dollé ME, Martus HJ, Gossen JA, and Vijg J. Plasmid-based transgenic mouse model for studying in vivo mutations. *Nature* 377: 657–659, 1995.
6. Bohr VA, Sander M, and Kraemer KH. Rare diseases provide rare insights into DNA repair pathways, TFIIH, aging and cancer center. *DNA Repair (Amst)* 4: 293–302, 2005.
7. Brash DE, Wikonkal NM, Remenyik E, van der Horst GT, Friedberg EC, Cheo DL, van Steeg H, Westerman A, and van Kranen HJ. The DNA damage signal for Mdm2 regulation, Trp53 induction, and sunburn cell formation *in vivo* originates from actively transcribed genes. *J Invest Dermatol* 117: 1234–1240, 2001.
8. Bronson RT. Rate of occurrence of lesions in 20 inbred and hybrid genotypes of rats and mice sacrificed at 6 month intervals during the first years of life. In: *Genetics of Aging,* II. Caldwell, New Jersey: Telford Press, 1990.
9. Bronson RT and Lipman RD. Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. *Growth Dev Aging* 55: 169–184, 1991.
10. Cao L, Li W, Kim S, Brodie SG, and Deng CX. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Brca1 full-length isoform. *Genes Dev* 17: 201–213, 2003.
11. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, Pathak S, Guarente L, and DePinho RA. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet* 36: 877–882, 2004.
12. Cobb MH. MAP kinase pathways. *Prog Biophys Mol Biol* 71: 479–500, 1999.
13. Craddock VM and Henderson AR. Effect of a single treatment with the alkylating carcinogens demethylnitrosamine and methyl methanesulphonate on liver regenerating after partial hepatectomy. IV. Effect on methylase-mediated methylation of DNA. *Chem Biol Interact* 27: 81–89, 1979.
14. de Boer J, Andressoo JO, de Wit J, Huijmans J, Beems RB, van Steeg H, Weeda G, van der Horst GT, van Leeuwen W, Themmen AP, Meradji M, and Hoeijmakers JH. Premature aging in mice deficient in DNA repair and transcription. *Science* 296: 1276–1279, 2002.
15. de Boer J, de Wit J, van Steeg H, Berg RJ, Morreau H, Visser P, Lehmann AR, Duran M, Hoeijmakers JH, and Weeda G. A mouse model for the basal transcription/DNA repair syndrome trichothiodystrophy. *Mol Cell* 1: 981–990, 1998.
16. de Boer J and Hoeijmakers JH. Cancer from the outside, aging from the inside: mouse models to study the consequences of defective nucleotide excision repair. *Biochimie* 81: 127–137, 1999.
17. de Boer J, van Steeg H, Berg RJ, Garssen J, de Wit J, van Oostrum CT, Beems RB, van der Horst GT, van Kreijl CF, de Gruijl FR, Bootsma D, Hoeijmakers JH, and Weeda G. Mouse model for the DNA repair/basal transcription disorder trichothiodystrophy reveals cancer predisposition. *Cancer Res* 59: 3489–3494, 1999.
18. de Vries A, van Oostrom CT, Dortant PM, Beems RB, van Kreijl CF, Capel PJ, and van Steeg H. Spontaneous liver tumors and benzo[a]pyrene-induced lymphomas in XPA-deficient mice. *Mol Carcinog* 19: 46–53, 1997.
19. de Vries A, van Oostrom CT, Hofhuis FM, Dortant PM, Berg RJ, de Gruijl FR, Wester PW, van Kreijl CF, Capel PJ, van Steeg H, *et al.* Increased susceptibility to ultraviolet-B and carcinogens of mice lacking the DNA excision repair gene XPA. *Nature* 377: 169–173, 1995.
20. Dempsey JL, Pfeiffer M, and Morley AA. Effect of dietary restriction on in vivo somatic mutation in mice. *Mutat Res* 291: 141–145, 1993.
21. Diehl A and Rai RM. Liver regeneration 3: Regulation of signal transduction during liver regeneration. *FASEB J* 10: 215–227, 1996.
22. Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, Max EE, Ried T, and Nussenzweig A. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 404: 510–514, 2000.
23. Dollé ME, Giese H, Hopkins CL, Martus HJ, Hausdorff JM, and Vijg J. Rapid accumulation of genome rearrangements in liver but not in brain of old mice. *Nat Genet* 17: 431–434, 1997.
24. Dollé ME, Snyder WK, Gossen JA, Lohman PH, and Vijg J. Distinct spectra of somatic mutations accumulated with age in mouse heart and small intestine. *Proc Natl Acad Sci USA* 97: 8403–8408, 2000.
25. Dollé ME and Vijg J. Genome dynamics in aging mice. *Genome Res* 12: 1732–1738, 2002.
26. Dumaz N, Duthu A, Ehrhart JC, Drougard C, Appella E, Anderson CW, May P, Sarasin A, and Daya-Grosjean L. Prolonged p53 protein accumulation in trichothiodystrophy fibroblasts dependent on unrepaired pyrimidine dimers on the transcribed strands of cellular genes. *Mol Carcinog* 20: 340–347, 1997.
27. Elchuri S, Oberley TD, Qi W, Eisenstein RS, Jackson Roberts L, Van Remmen H, Epstein CJ, and Huang TT. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene* 24: 367–380, 2005.
28. Elena SF and Lenski RE. Test of synergistic interactions among deleterious mutations in bacteria. *Nature* 390: 395–398, 1997.
29. Failla G. The aging process and carcinogenesis. *Ann NY Acad Sci* 71: 1124–1135, 1958.
30. Finch CE and Kirkwood TB. *Chance, Development, and Aging*. Oxford, 1999.
31. Gaubatz JW and Tan BH. Introduction, distribution, and removal of 7-methylguanine in different liver chromatin fractions of young and old mice. *Mutat Res* 375: 25–35, 1997.
32. Giese H, Dollé ME, Hezel A, van Steeg H, and Vijg J. Accelerated accumulation of somatic mutations in mice deficient in the nucleotide excision repair gene XPA. *Oncogene* 18: 1257–1260, 1999.
33. Giese H, Snyder WK, van Oostrom C, van Steeg H, Dollé ME, and Vijg J. Age-related mutation accumulation at a lacZ reporter locus in normal and tumor tissues of Trp53-deficient mice. *Mutat Res* 514: 153–163, 2002.
34. Gossen JA, de Leeuw WJ, Tan CH, Zwarthoff EC, Berends F, Lohman PH, Knook DL, and Vijg J. Efficient rescue of integrated shuttle vectors from transgenic mice: A model

for studying mutations in vivo. *Proc Natl Acad Sci USA* 86: 7971–7975, 1989.
35. Grasl-Kraupp B, Bursch, W, Ruttkay-Nedecky B, Wagner A, Lauer B, and Schulte-Hermann R. Food restriction eliminates preneoplastic cells through apoptosis and antagonizes carcinogenesis in rat liver. *Proc Natl Acad Sci USA* 91: 9995–9999, 1994.
36. Harman D. Free radical theory of aging. *Mutat Res* 275: 257–266, 1992.
37. Hasty P, Campisi J, Hoeijmakers J, van Steeg H, and Vijg J. Aging and genome maintenance: lessons from the mouse? *Science* 299: 1355–1359, 2003.
38. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature* 411: 366–374, 2001.
39. Hursting SD, Perkins SN, and Phang JM. Calorie restriction delays spontaneous tumorigenesis in p53-knockout transgenic mice. *Proc Natl Acad Sci USA* 91: 7036–7040, 1994.
40. James SJ and Muskhelishvili L. Rates of apoptosis and proliferation vary with caloric intake and may influence incidence of spontaneous hepatoma in C57BL/6 x C3H F1 mice. *Cancer Res* 54: 5508–5510, 1994.
41. Jones IM, Thomas CB, Tucker B, Thompson CL, Pleshanov P, Vorobtsova I, and Moore DH, 2nd. Impact of age and environment on somatic mutation at the hprt gene of T lymphocytes in humans. *Mutat Res* 338: 129–139, 1995.
42. Judge S, Jang YM, Smith A, Hagen T, and Leeuwenburgh C. Age-associated increases in oxidative stress and antioxidant enzyme activities in cardiac interfibrillar mitochondria: Implications for the mitochondrial theory of aging. *FASEB J* 19: 419–421, 2005.
43. Kanzler S and Galle PR. Apoptosis and the liver. *Semin Cancer Biol* 10: 173–184, 2000.
44. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 270: 16483–16486, 1995.
45. Kerr J. Shrinkage necrosis: A distinct mode of cellular death. *J Pathol* 105: 13–20, 1971.
46. Kharbanda S, Pandey P, Ren R, Mayer B, Zon L, and Kufe D. c-Abl activation regulates induction of the SEK1/stress-activated protein kinase pathway in the cellular response to 1-beta-D-arabinofurnosylcytosine. *J Biol Chem* 270: 30278–30281, 1995.
47. Kharbanda S, Ren R, Pandey P, Shafman TD, Feller SM, Weichselbaum RR, and Kufe D. Activation of the c-Abl tyrosine kinase in the stress response to DNA damaging agents. *Nature* 376: 785–788, 1995.
48. Kibota TT and Lynch M. Estimate of the genomic mutation rate deleterious to overall fitness in *E. coli*. *Nature* 381: 694–696, 1996.
49. Kirkwood TB. Understanding the odd science of aging. *Cell* 120: 437–447, 2005.
50. Kirkwood TB, Feder M, Finch CE, Franceschi C, Globerson A, Klingenberg CP, LaMarco K, Omholt S, and Westendorp RG. What accounts for the wide variation in life span of genetically identical organisms reared in a constant environment? *Mech Ageing Dev* 126: 439–443, 2005.
51. Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 362: 709–715, 1993.
52. Liu Y, Gorospe M, Yang C, and Holbrook NJ. Role of mitogen-activated protein kinase phosphatase during the cellular response to genotoxic stress. Inhibition of c-Jun N-terminal kinase activity and AP-1-dependent gene activation. *J Biol Chem* 270: 8377–8380, 1995.
53. Ljungman M and Lane DP. Transcription—guarding the genome by sensing DNA damage. *Nat Rev Cancer* 4: 727–737, 2004.
54. Ljungman M and Zhang F. Blockage of RNA polymerase as a possible trigger for u.v. light-induced apoptosis. *Oncogene* 13: 823–831, 1996.
55. Lombard DB, Chua KF, Mostoslavsky R, Franco S, Gostissa M, and Alt FW. DNA repair, genome stability, and aging. *Cell* 120: 497–512, 2005.
56. Magee JA, Abdulkadir SA, and Milbrandt J. Haploinsufficiency at the Nkx3.1 locus. A paradigm for stochastic, dosage-sensitive gene regulation during tumor initiation. *Cancer Cell* 3: 273–283, 2003.
57. Mattson M, Duan W, Pedersen WA, and Culmsee C. Neurodegenerative disorders and ischemic brain diseases. *Apoptosis* 6: 69–81, 2001.
58. Maynard Smith J. A theory of ageing. *Nature* 184: 956–957, 1959.
59. McKay BC, Stubbert LJ, Fowler CC, Smith JM, Cardamore RA, and Spronck JC. Regulation of ultraviolet light-induced gene expression by gene size. *Proc Natl Acad Sci USA* 101: 6582–6586, 2004.
60. Michalopoulos G. Liver regeneration and growth factors: old puzzles and new perspectives. *Lab Invest* 67: 413–415, 1992.
61. Michalopoulos G and DeFrances MC. Liver regeneration. *Science* 276: 60–66, 1997.
62. Motta MC, Divecha N, Lemieux M, Kamel C, Chen D, Gu W, Bultsma Y, McBurney M, and Guarente L. Mammalian SIRT1 represses forkhead transcription factors. *Cell* 116: 551–563, 2004.
63. Ono K and Han, J. The p38 signal transduction pathway: activation and function. *Cell Signal* 12: 1–13, 2000.
64. Osborn MT and Chambers TC. Role of stress-activated/c-Jun NH_2-terminal protein kinase pathway in the cellular response to adriamycin and other chemotherapeutic drugs. *J Biol Chem* 271: 30950–30955, 1996.
65. Osterod M, Hollenbach S, Hengstler JG, Barnes DE, Lindahl T, and Epe B. Age-related and tissue-specific accumulation of oxidative DNA base damage in 7,8-dihydro-8-oxoguanine-DNA glycosylase (Ogg1) deficient mice. *Carcinogenesis* 22: 1459–1463, 2001.
66. Pandney P, Raingeaud J, Kanek M, Weichselbaum R, Davis RJ, Kufe D, and Kharbanda S. Activation of p38 mitogen-activated protein kinase by c-Abl-dependent and -independent mechanisms. *J Biol Chem* 271: 23775–23779, 1996.
67. Paul A, Wilson S, Belham CM, Robinson KJM, Scott PH, Gould GW, and Plevin R. Stress-activated protein kinases: activation, regulation, and function. *Cell Signal* 9: 403–410, 1997.
68. Prolla TA. Multiple roads to the aging phenotype: insights from the molecular dissection of progerias through DNA microarray analysis. *Mech Ageing Dev* 126: 461–465, 2005.

69. Queille S, Drougard C, Sarasin A, and Daya-Grosjean L. Effects of XPD mutations on ultraviolet-induced apoptosis in relation to skin cancer-proneness in repair-deficient syndromes. *J Invest Dermatol* 117: 1162–1170, 2001.
70. Raff M. Social controls on cell survival and cell death. *Nature* 356: 397–400, 1992.
71. Sanchez-Perez I, Murguia JR, and Perona R. Cisplatin induces a persistent activation of JNK that is related to cell death. *Oncogene* 16: 533–540, 1998.
72. Shabalina SA and Spiridonov NA. The mammalian transcriptome and the function of non-coding DNA sequences. *Genome Biol* 5: 105, 2004.
73. Sharpless NE and DePinho RA. Telomeres, stem cells, senescence, and cancer. *J Clin Invest* 113: 160–168, 2004.
74. Suh Y. Age-specific changes in expression, activity, and activation of the c-Jun NH_2-terminal kinase and p38 mitogen-activated protein kinases by methyl methanesulfonate in rats. *Mech Ageing Dev* 122: 1791–1811, 2001.
75. Suh Y. Cell signaling in aging and apoptosis. *Mech Ageing Dev* 123: 881–890, 2002.
76. Suh Y, Kang UG, Kim YS, Kim WH, Park SC, and Park JB. Differential activation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinases by methyl methanesulfonate in the liver and brain of rats: implication for organ-specific carcinogenesis. *Cancer Res* 60: 5067–5073, 2000.
77. Suh Y, Lee KA, Kim WH, Han BG, Vijg J, and Park SC. Aging alters the apoptotic response to genotoxic stress. *Nat Med* 8: 3–4, 2002.
78. Swann PF and Magee PN. Induction of rat kidney tumours by ethyl methanesulphonate and nervous tissue tumours by methyl methanesulphonate and ethyl methanesulphonate. *Nature* 223: 947–949, 1969.
79. Szilard L. On the nature of the aging process. *Proc Natl Acad Sci USA* 45: 30–45, 1959.
80. Talarmin H, Rescan C, Cariou S, Glaise D, Zanninelli G, Bilodeau M, Loyer P, Guguen-Guillouzo C, and Baffet G. The mitogen-activated protein kinase kinase/extracellular signal-regulated kinase cascade activation is a key signaling pathway involved in the regulation of G(1) phase progression in proliferating hepatocytes. *Mol Cell Biol* 19: 6003–6011, 1999.
81. Tra J, Kondo T, Lu Q, Kuick R, Hanash S, and Richardson B. Infrequent occurrence of age-dependent changes in CpG island methylation as detected by restriction landmark genome scanning. *Mech Ageing Dev* 123: 1487–1503, 2002.
82. Tucker JD, Spruill MD, Ramsey MJ, Director AD, and Nath J. Frequency of spontaneous chromosome aberrations in mice: effects of age. *Mutat Res* 425: 135–141, 1999.
83. van Gent DC, Hoeijmakers JH, and Kanaar R. Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet* 2: 196–206, 2001.
84. van Gijssel HE, Mullenders LH, van Oosterwijk MF, and Meerman JH. Blockage of transcription as a trigger for p53 accumulation by 2-acetylaminofluorene DNA-adducts. *Life Sci* 73: 1759–1771, 2003.
85. van Oosten M, Rebel H, Friedberg EC, van Steeg H, van der Horst GT, van Kranen HJ, Westerman A, van Zeeland AA, Mullenders LH, and de Gruijl FR. Differential role of transcription-coupled repair in UVB-induced G2 arrest and apoptosis in mouse epidermis. *Proc Natl Acad Sci USA* 97: 11268–11273, 2000.
86. Vijg J. Somatic mutations and aging: a re-evaluation. *Mutat Res* 447: 117–135, 2000.
87. Vijg J. Impact of genome instability on transcription regulation of aging and senescence. *Mech Ageing Dev* 125: 747–753, 2004.
88. Vijg J and Suh Y. Genetics of longevity and aging. *Annu Rev Med* 56: 193–212, 2005.
89. Vogel H, Lim DS, Karsenty G, Finegold M, and Hasty P. Deletion of Ku86 causes early onset of senescence in mice. *Proc Natl Acad Sci USA* 96: 10770–10775, 1999.
90. Wani AA and D'Ambrosio SM. Specific DNA alkylation damage and its repair in carcinogen-treated rat liver and brain. *Arch Biochem Biophys* 246: 690–698, 1986.
91. Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, Gu Y, Alt FW, and DePinho RA. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. *Nature* 421: 643–648, 2003.
92. Yamaizumi M and Sugano T. U.V.-induced nuclear accumulation of p53 is evoked through DNA damage of actively transcribed genes independent of the cell cycle. *Oncogene* 9: 2775–2784, 1994.
93. Dolle ME, Busuttil RA, Garcia AM, Wijnhoven S, van Drunen E, Niedernhofer LJ, van der Horst G, Hoeijmakers JH, van Steeg H, and Vijg J. Increased genomic instability is not a prerequisite for shortened lifespan in DNA repair deficient mice. *Mutat Res* Feb 9: 2006 [Epub ahead of print]
94. Busuttil RA, Garcia AM, Cabrera C, Rodriguez A, Suh Y, Kim WH, Huang TT, and Vijg J. Organ-specific increase in mutation accumulation and apoptosis rate in CuZn-superoxide dismutase-deficient mice. Cancer Res 65: 11271–11275, 2005.
95. Vijg J and Hasty P. Aging and p53: getting it straight. A commentary on a recent paper by Gentry and Venkatachalam. *Aging Cell* 4: 331–333, 2005.
96. Hasty P and Vijg J. Accelerating aging by mouse reverse genetics: a rational approach to understanding longevity. *Aging Cell* 3: 55–65, 2004.

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2. E. Zi#tkiewicz, A. Wojda, M. Witt. 2009. Cytogenetic perspective of ageing and longevity in men and women. *Journal of Applied Genetics* **50**:3, 261-273. [CrossRef]
3. Bibliography 417-599. [CrossRef]
4. Christiaan Leeuwenburgh , Tomas A. Prolla . 2006. Genetics, Redox Signaling, Oxidative Stress, and Apoptosis in Mammalian Aging. *Antioxidants & Redox Signaling* **8**:3-4, 503-505. [Citation] [Full Text PDF] [Full Text PDF with Links]