Aging is a universal, lifelong and inevitable phenomenon, the product of an interaction between genetic, environmental and lifestyle factors. The biological changes that occur during the aging process affect virtually all components of living systems, from the molecular up to that of the whole organism. These changes lead to a breakdown in the normal homoeostatic mechanisms, and so the functional capacity of the body and its ability to respond to a wide variety of extrinsic and intrinsic agents are often decreased. The result of this is the degradation of structural elements within the cells, tissues and organs of the body, leading eventually to the onset of age-related pathologies and ultimately death. The purpose of this paper is to present the evidence that implicates somatic mutations in the aetiology of the aging process.

**Theories of aging**

Many theories have been proposed in an attempt to account for the aging process [1]. Aging theories can be broadly divided into two categories.

**Programmed or genetic theories**

The basis of these theories is that the process of aging follows some type of biological timetable, perhaps a continuation of the one that regulates childhood growth and development, and which results from the temporal switching on/or off of a number of genes.

**Damage-accumulation (stochastic) theories**

The damage-accumulation theories propose that intrinsic and extrinsic insults to cellular biomolecules (carbohydrates, lipids, proteins and the nucleic acids) occur throughout life. This results in cumulative damage to these biomolecules, which will gradually cause alterations in their biological function and so result in physiological changes leading to pathology and/or death.

**DNA damage and mutations**

Random DNA damage can be caused by a wide variety of extrinsic and intrinsic agents, to which we are constantly exposed [2,3]. The rate of DNA damage as well as the persistence of lesions within DNA depends on the activity and efficacy of a wide variety of molecular defence systems. These defence systems exist to reduce the incidence of, remove and/or repair DNA damage and include antioxidants, DNA-repair mechanisms, heat shock proteins and other stress proteins. Imperfections in the defence systems that protect against the fixation of DNA damage may lead to an accumulation of mutations. Mutations may result in the synthesis of aberrant proteins with altered/no biological function, alterations to the transcriptional and translational machinery of a cell or deregulation of gene control. The phenotypic effect to a cell of the accumulation of such mutations may be to lead to alterations in cellular function and so cause alterations to normal physiological processes.

**DNA damage and mutation as a function of age**

Much experimental evidence has been published that details increases in DNA damage and mutation with age in a wide variety of cell types from a number of different organisms, including humans. There are reports of the accumulation of deletional mutations within gene sequences containing tandem repeats, including those within ribosomal DNA genes, as a function of age [4]. Deletions and point mutations of the mitochondrial genome accumulate in aging human tissues [5]. The frequency of mutations at various genetic loci increase with age [6–12].

In a recent study within this laboratory, the mutant frequency at the hypoxanthine–guanine phosphoribosyltransferase (hpgr) gene locus was assessed in lymphocytes isolated from healthy male volunteers from three age groups (35–39, 50–54 and 65–69 years). The results showed that the mean mutant frequency in the 65–69-year-olds was about twice that in the 35–39 and 50–54-year-olds (1.95 ± 1.02/10⁶ cells, 0.98 ± 0.52/10⁶ cells and 0.95 ± 0.46/10⁶ cells respectively) increasing by about 1.33% per year, after 54 years. In addition, there was an increased frequency of chromosomal aberrations in the 65–69-year-old group, compared with the other two age groups [13].
Several studies have also shown that with age there is an increase in single-strand DNA breaks and/or alkali-labile sites in a variety of tissues [14-17]. Results from this laboratory showed an age-related increase in basal levels of DNA damage within lymphocytes from the male subjects [18].

Changes in DNA bases and deoxyribose sugars may also occur with age, some of which have the potential to be mutagenic [19,20]. A loss of 5-methylcytosine from DNA may also occur and this may lead to the dedifferentiation of cells.

**Mechanisms leading to increases in DNA damage and mutations**

The observed increase in DNA damage and/or mutations with age could be due to a constant rate of accumulation of damage. This would imply that the rate of DNA damage or DNA-repair capacity does not change with age. Alternatively, it could also be the consequence of an accumulation of mutations at an increasing rate. This may be due to increasing DNA susceptibility to damage or to decreases in the efficacy of the defence systems against DNA damage with age. In this laboratory we assessed in vitro antioxidant status [by measuring blood levels of superoxide dismutase (EC 1.15.1.1), glutathione peroxidase (EC 1.11.1.9), catalase (EC 1.11.1.6), caeruloplasmin, uric acid and bilirubin] and the DNA-repair capacity of lymphocytes, within our study subjects. The results did not show any statistically significant differences in the mean levels of the antioxidants between the three different age groups. To investigate DNA repair after DNA damage induced by reactive oxygen species, an ELISA was used to quantify DNA damage at various times after treatment of lymphocytes with H$_2$O$_2$. No significant differences were found in H$_2$O$_2$ susceptibility with age immediately after treatment but there was an age-related increase in DNA damage remaining 90 min after H$_2$O$_2$ treatment. These results suggested that the age-related increase in mutant frequency at the hgprt locus was not contributed to by alterations of in vitro antioxidant status with age but by a decreased efficacy of the repair of DNA damage induced by reactive oxygen species with age [18].

**Mutation — a cause of pathology and aging?**

Although experimental evidence clearly shows an age-related increase in somatic mutations, this increase does not necessarily lead to the various physiological and pathological changes that occur during aging.

The physiological alterations with age proceed at different rates in different individuals and include: immune system decline, slowing of metabolism, decline in kidney and liver function, loss of elasticity in blood vessels, decreased bone mass, sensory impairment, drying of the epidermis, thinning of the dermis and loss of 20% of brain weight. A number of age-related pathologies may develop as a consequence of these and other physiological changes. The most common age-related pathologies are: atherosclerosis, arthritis, osteoporosis, cataracts, chronic renal failure, diabetes, senile dementia and most types of cancer.

What role do mutations play in the physiological and pathological changes associated with the aging process and, by inference, the aging process itself? For verification of the somatic mutation theory of aging, conditions that decrease or increase somatic mutations would be expected to increase or decrease respectively lifespan. There is some evidence to support this.

1. Woodruff and Nikitin [21] reported that the lifespan of *Drosophila melanogaster* males that contained 17 somatically active P elements (added in artificially in suitable inserts) had a lifespan of 15 days, compared with 40 days for their male sibs with a similar genetic background but with no active P elements. Such an effect on lifespan is probably due to induced somatic genetic damage when P elements are active. Similarly, in *Drosophila simulans*, movement of the mariner transposable element significantly decreased lifespan [22]. Although these findings do not confirm that DNA element movement is a cause of natural senescence, they do suggest a close relationship between somatic genetic damage and aging.

2. McCay et al. [23] reported that by feeding rats and mice diets severely deficient in calories (approx. 35% of *ad libitum*-fed animals, after the initial period of growth) the aging of their body tissues was retarded, the development of disease and tumours was inhibited and the lifespans of the animals were significantly prolonged. In such conditions of caloric restriction there is a substantial decrease in the age-related increase in mutations [24]. The exact mechanisms leading to this decrease in mutations are not completely understood but may involve modulation of free-radical metabolism [25], and/
or the reduced hormone excretion that occurs in dietary-restricted animals may lower whole-body metabolism and so result in less 'wear and tear' to body organs and tissues [26].

(3) Treatment of whole organisms with agents known to induce mutations have been shown to decrease lifespan, e.g. X-rays shorten lifespan in Drosophila [27] and mice [28,29], and UV irradiation shortens lifespan in Paramecium [30]. High and low oxygen tension respectively decreases or increases lifespan in Caenorhabditis [31].

(4) Elucidation of the mechanism(s) leading to the formation of a cancerous cell has shown that non-hereditary forms of the disease result from an accumulation of mutations (three to seven) in critical genetic targets (usually those controlling cell growth or cell death) [32]. Since cumulative risk of cancer increases with age, this suggests an importance of mutation for both the processes of carcinogenesis and aging.

(5) Recently the genetic defect that predisposes individuals to the development of the premature aging disease, Werner’s syndrome, has been elucidated [33]. Individuals with this disease carry two copies of a mutant gene that codes for a helicase enzyme. In the light of the biological function of helicases (to split apart or unwind the two strands of the DNA double helix), the proposed reason for the premature aging in Werner’s individuals is that the defective helicase prevents DNA-repair enzymes from removing background DNA damage. This persistent damage would thus be fixed as mutations, with consequence deleterious effects on cellular function. The molecular defects underlying other human disorders of premature aging, e.g. Cockayne’s syndrome, which exhibits a selective deficiency in the preferential repair of active genes [34], or Hutchinson–Gilford progeria, which also has an associated repair defect [35], tend to suggest an involvement of genomic instability or DNA damage as the cause of the symptoms associated with these diseases.

(6) A deficiency in the mitochondrial enzyme complex I has been suggested to be the likeliest effect of random mitochondrial mutation [5]. In Parkinson’s syndrome the dopaminergic cells of the substantia nigra undergo early degeneration and death. It may be that this cell death is the result of cumulative mutations within mitochondrial DNA, leading to complex I deficiency and an increased generation of superoxide, which can be toxic to cells [36].

It is quite possible that somatic mutations could occur without substantial deleterious effects if, for example, the cells containing the mutations were selected against in terms of decreased survival or if the cells in which the mutations arose were subjected to a high rate of replacement in vivo. Many tissues in the adult are steady replaced, and thus any mutations occurring within them may not have a phenotypic effect. However, if mutations become fixed within the stem cells, then there is the potential for physiological effects within the progeny cells.

Addressing the question posed in the title, mutations within nuclear and mitochondrial DNA do arise as a consequence of damage by intrinsic and/or extrinsic agents and/or imperfections in the defence systems against DNA damage. Over time, mutations will accumulate within cells. These mutations may affect genes controlling cellular defence systems, which may exacerbate the subsequent accumulation of mutations. Provided that they are not lethal, the mutations may cause alterations to cell function. Such changes in cell function may result in the onset of pathology and so eventually to death. Thus somatic mutations are partially the cause and partially the effect of aging.

DNA Damage and Mutagenesis


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Gene regulation by low-dose ionizing radiation in a normal human lung epithelial cell line

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Introduction

Ionizing radiation is an environmental mutagen and carcinogen and is widely used in cancer therapy. Although the effects of high doses have been extensively reported (reviewed in [1]), little is known about the induction of cellular signaling events and specific gene expression after low-dose radiation exposure. Understanding these signaling events, especially at low doses, may provide insights into the mechanism of low-dose-ionizing-radiation-induced carcinogenesis after environmental exposure.

There have been several reports suggesting an adaptive response of cells and organisms to low-dose radiation exposure. Hypersensitivity of maize plants has been demonstrated at doses below 0.5 Gy of γ-rays [2,3], and an inflexion in their survival curve at this dose indicated induced radioresistance at higher doses. Induction of radioresistance has also been observed with increasing dose for the lepidopteran insect line TN-368 [4-6]. Several other reports have also described the protective effects, measured at the survival level, of pretreating cells with a small conditioning dose of radiation. This induced resistance has been reported in Chlamydomonas [7], fern sporelings [8] and yeast [9], and others [10] have observed the effect by assessing chromosomal aberrations in human lymphocytes. Similar results were obtained by measuring the mutation frequency at the hprt locus of V79 hamster cells [11].

Abbreviations used: Cu-SOD, Cu2+ superoxide dismutase; GADD, growth arrest and DNA damage inducible; Mn-SOD, Mn2+ superoxide dismutase; PKC, protein kinase C; Poly(A)+, polyadenylated; TNF, tumour necrosis factor.