

1-1-2000

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ŞÜKRAN ÇAKIR

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Recommended Citation

ÇAKIR, ŞÜKRAN (2000) "Genetics and Some Aging-Related Mechanisms," *Turkish Journal of Zoology*. Vol. 24: No. 2, Article 9. Available at: <https://journals.tubitak.gov.tr/zoology/vol24/iss2/9>

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Genetics and Some Aging-Related Mechanisms

Şükran ÇAKIR

Kırıkkale Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Kırıkkale-TURKEY

Received: 28.04.1999

Abstract: Aging can be defined as the time-dependent decline of physiological functions of an organism. Aging, a multifactorial process composed of both genetic and environmental components, is a highly complex biologic phenomenon of great importance. The details of the mechanisms leading to aging are not yet known. All changes in the process of aging have a cellular basis, and perhaps aging should be studied, fundamentally, at the cellular level under defined and controlled environmental conditions. Some genetic approaches have promise for the understanding of this multifactorial process. In this review, some causal hypotheses related to oxidative damage, caloric restriction, mitochondrial mutations, protein elongation factor (EF-1), stress proteins (Hsp70), altered gene regulation and telomere loss are discussed to clarify the mechanisms of aging. Aging must be considered as parts comprising a whole, it must be understood as the sum of its parts. Although there are some experimental results supporting these hypotheses, there is still much to learn about the genetics of aging.

Key Words: Genetics, aging, aging-related mechanisms.

Genetik ve Yaşlanma ile ilgili Bazı Mekanizmalar

Özet: Yaşlanma bir organizmanın fizyolojik fonksiyonlarındaki zamana bağlı azalma olarak tanımlanır. Genetik ve çevresel olmak üzere çok faktörlü bir proses olan yaşlanma oldukça önemli biyolojik olaylardan biridir. Yaşlanmaya neden olan mekanizmaların nedeni henüz bilinmemektedir. Yaşlanma prosesindeki bütün değişiklikler hücresel temele sahiptir ve yaşlanma belki de temel olarak tanımlanmış ve kontrollü çevre koşullarında hücresel düzeyde en iyi çalışmalıdır. Bazı genetik uygulamalar bu çok faktörlü süreci anlamayı vadetmektedir. Bu nedenle, oksidatif hasar, kalorik kısıtlama, mitokondrial mutasyonlar, protein sentez (uzatan) faktörü, değişen gen düzenlemesi, stres proteinleri ve telomer kaybı gibi yaşlanma ile ilgili sebepsel hipotezler yaşlanmanın mekanizmalarına açıklık getirebilmek için tartışıldı. Yaşlanma bir bütünün parçaları gibi göz önüne alınmalı ve parçaların toplamı gibi anlaşılmalıdır. Bu hipotezleri destekleyen bazı deneysel sonuçlar olmasına rağmen, yaşlanmanın genetiğini öğrenmek için hala daha fazlasına ihtiyacımız vardır.

Anahtar Sözcükler: Genetik, yaşlanma, yaşlanma ile ilgili mekanizmalar.

Introduction

Basic scientists have played a central role in increasing life expectancy, and are working on understanding the mechanisms of aging. The aim of science and medicine is not so much to lengthen life as to reduce the number of years that people suffer from illness or disability. The molecular causes for the aging process are multiple, involving both genetic and environmental factors. Species with short life spans have been the primary targets for studies into the genetic basis of the aging process(es). Almost all genetic studies of aging and longevity have been performed on invertebrates such as *Drosophila melanogaster* and *Caenorhabditis elegans*. The mouse, with its 2-year life span, is an exception to this rule, and several genetic studies have been performed to infer causal relationships.

Some causal hypotheses have been suggested for elucidation of the mechanisms of aging. A hypothesis ascribing one cause for aging postulates that the senescence-associated loss of functional capacity is due to the accumulation of molecular oxidative damage (1, 2, 3, 4, 5, 6). This hypothesis is based on the fact that oxygen is potentially a toxic substance, and its use by aerobes, although necessary for their immediate survival, also may be hazardous to their long-term existence. Mitochondria are the energy centers of the cell, and have their own DNA, which is more prone to damage by oxygen radicals than the DNA contained in the cell's nucleus (7). Mitochondrial DNA accumulates mutations during aging in *Drosophila* (8). Aging has been reported to be associated with dramatically decreased expression of mitochondrial RNAs. In addition, a characteristic feature of aging organisms is their loss in homeostatic functions,

including the ability of protein synthesis and turnover. In many animal species, as well as in cultured cells, age-related changes in the rate of protein synthesis have been observed (9, 10). The question of whether EF-1 α is involved in the age-related decrease of protein synthesis, therefore, still remains open (11). Some experimental findings indicate that aging may be associated with altered gene expression (12, 13). A decreased ability to survive after heat shock or stress is a characteristic of aging in *Drosophila* as well as in mammals (14, 15, 16). It has been reported that the unusual characteristic of the heat shock response in old flies is due to the presence of a higher concentration of abnormal proteins relative to those found in young flies. It is known that dietary restriction increases life span in mammals (4, 17, 18). According to Grave (19), the increase in life span caused by dietary restriction can be explained as an incidental consequence of lower reproductive effort. Eukaryotic chromosome stability by telomeres is essential for cell viability. It is a recent finding that telomeres shorten with age and play a central role in the replicative life span of eukaryotic cells (20, 21).

As mentioned above, a variety of strategies and models, such as oxidative damage, mitochondrial mutation, elongation factor, gene regulation, heat shock/stress proteins, caloric restriction, and telomere loss, have been used to understand the nature of the mechanisms underlying the phenomenon of senescence. There are many causal hypotheses to explain the mechanisms of aging. But some questions remain unanswered, such as why organisms undergo progressive and irreversible physiological decline in the latter part of their life, why life expectancy varies within and among species, and why experimental regimes with caloric restriction, antioxidant and tolerable heat shock treatment prolong the average and maximum life-span of some animals.

Some aging-related Mechanisms

Oxidative Damage

Free radicals are produced by normal enzymatic reactions and toxic chemicals. They are thought to play a role in normal aging. Oxidative damage to proteins is considered to be a result of the continuous oxidative stress to which aerobic systems are subjected. It is becoming clear that the amount of oxidized protein increases with age. This has been found to be true for insects, laboratory rodents, and humans (2, 3, 4, 5, 8, 22). The extent of oxidative damage depends upon many factors, including the rate of production of semireduced

oxygen species during aerobic metabolism, as well as the ability of the biological system to withstand oxidative stress. Oxygen metabolism produces a small percentage of semireduced oxygen species at all times, and these are the source of imposed oxidative damage potential. The basic principle of the oxidative stress hypothesis is that senescence-related loss of functions is due to the progressive and irreversible accumulation of molecular oxidative damage. Five percent or less of the total oxygen consumed forms toxic oxygen by-products. Even though a small percentage of toxic oxygen by-products are formed per unit time, this can amount to as much as 3-5 tons produced in a lifetime for a human being (9).

It has been proposed that antioxidants may positively influence the aging process, protecting the organism against free radical-induced damage (9, 23). Superoxide dismutase (SOD), catalase, vitamin E, and other antioxidants eliminate radicals or reduce their effects. Several lines of evidence support a causal connection between antioxidants and aging. Arking et al. (24) designed an experiment to understand the mechanisms regulating the aging process in *Drosophila*. Their approach was to construct an appropriate normal-lived control strain and then to derive from it by artificial selection very long-lived strains. Then they examined these strains by a variety of techniques and decided that some genetically altered processes were responsible for the extended longevity. SOD- and catalase homozygous-deficient mutants in *Drosophila* have reduced longevity (25). Orr and Sohal (26) showed that transgenic flies carrying three copies of SOD and catalase exhibited a life-span extension of up to one third. Superoxide dismutase transgene also extended life span in *Drosophila* (27). It was shown that epithalamine delays age-related changes in reproductive and immune systems and increases life span (28). This effect is in good agreement with the inhibiting effect of epithalamine in lipid peroxidation processes in fly tissues. It was also reported that the antioxidant N-acetylcysteine has a life-extending effect on *Drosophila melanogaster*, and that the dietary uptake of it results in a dose-dependent increase in life span (23).

Drosophila were examined to see if there is an increase in damage associated with free radical activity in older flies. The levels of superoxide radical and of lipid peroxides were higher in membrane samples from older flies (9). There was also a significant decrease in membrane fluidity, and an increase in ATP-dependent calcium uptake in older *Drosophila*. During their lifetime, vestigial-wing *Drosophila* displayed a greater level of free radical activity than wild-type flies and a significantly shorter life span. All of the experimental results indicate

that the level of oxidative stress is closely related to cellular damage and to life span. In conclusion, free radicals may play a central role in the aging process.

Caloric Restriction

Caloric restriction is now being increasingly used as a model regime for understanding the basic mechanisms of aging. It increases the life span in mammals (17). Experimental results have demonstrated that hsp70 mRNA levels are increased in the proximal gut of young and old rats, either with fasting or with caloric restriction. Increased expression of the cytoprotective hsp70 gene in the gut may provide a possible cellular mechanism for the beneficial effects noted with caloric restriction (17). Some age-sensitive changes such as learning, immune responses, gene expression, enzyme activities, hormonal action, glucose intolerance, DNA repair capacities, and rates of protein synthesis were examined in rodents fed a caloric restriction diet. A "delayed aging profile" was observed (6). A notable feature of the relation between caloric intake and life span is that food consumption above an optimal level progressively shortens longevity. Life-span extension by caloric restriction has also been reported in other animals, indicating a broad relation between energy intake and aging (6, 19).

Mitochondrial Mutations

Mitochondria are the main sources of energy in the cell. They contain their own DNA (mtDNA), whose genes encode components of the respiratory oxidative phosphorylation system. Mitochondria are essential for the normal functioning of all cells in the body. They are critical for the function of those tissues that are highly dependent on aerobic metabolism, such as muscle and brain. It is suggested that the accumulation of mutated mtDNA may be relevant to neurodegenerative diseases associated with aging, such as Alzheimer's, Huntington's, and Parkinson's diseases. These three disorders have been associated with impairment of mitochondrial respiratory chain function (7).

In insects there is evidence that aging is associated with structural and biochemical alterations of the mitochondria (29). The primary agent in mitochondrial senescence is probably damage by free radicals and lipid peroxides to the inner mitochondrial membrane. Oxidative damage to mtDNA has been reported to be 16 times higher than that to nuclear DNA, and to cause mutations in mitochondrial DNA (30). Damages in mtDNA increase exponentially with age in a variety of organisms (31). Mitochondrial deletions accumulate with age in *C. elegans*, but at a lower rate in long-lived *age-1* mutant

strains (32). *Drosophila melanogaster*, *Musca domestica*, *Sarcophaga bullata*, *Calliphora vicina* and *Licilia sericata* were used to determine the role of mitochondria in the aging process. The average life span potential of these species was found to be inversely correlated with the rates of mitochondrial superoxide and hydrogen peroxide production and with the level of protein carbonyls, and to be directly related to the activity of cytochrome c oxidase. These results showed that longer life span potential in these insect species is associated with relatively low levels of oxidant generation and oxidative molecular damage (8).

Protein Elongation Factor (EF-1)

Protein translation takes place in three major steps: initiation, elongation, and termination, which are promoted by soluble protein factors, known as initiation factors, elongation factors, and termination factors. Their interaction with ribosomes, mRNA, and aminoacyl-tRNAs is also important during the translation process. All of these factors may be the direct cause of impaired protein synthesis during aging. Efl-1 α is a highly conserved protein that has been the focus of intensive investigation for the last years. There are some observations suggesting that the elongation step of protein synthesis is most affected during aging. For example, in *Drosophila melanogaster*, the peptide chain elongation factor is decreased significantly with increasing age (33). It has been proposed that in senescent *Drosophila melanogaster*, the peptide synthesis elongation factor may become limiting and be responsible for the age-related decrease in protein synthesis or other homeostatic functions (11). Several reports describe a decrease in translation rate in aging *Drosophila*, which is attributed to a decrease in the expression of the mRNA encoding the translational elongation factor EF-1 α . Similar decreases in peptide elongation rate have been reported in rats and mice (35). Shepart et al. (34) generated transgenic lines to test whether overexpression of the EF-1 α gene might affect life span, and reported that transgenic lines had a significantly higher life span than controls. Subsequent analysis of these lines by other investigators, however, revealed that the transgenic flies did not overexpress EF-1 α , and that the increase in life span was due to other differences in the genetic background (11, 34). Many causal explanations of aging are related to the protein component of the cell. These include somatic mutations, transcriptional or translational errors, and protein turnover or conformational alteration. Some experimental findings support these explanations of modified proteins in a number of tissues at later ages (33).

Stress or Heat-shock Proteins (hsp)

Stress or heat shock proteins (hsp) are a family of approximately two dozen proteins found in all animals. They show a high degree of amino acid sequence homology between different species. All organisms respond to heat and certain environmental stresses by synthesizing heat-shock proteins (hsp) (9, 36, 37, 38). The types of genes induced are dependent on the nature of the stress. Although there is clearly overlap in the responses, heat stress induces one particular set of genes, and oxidative stress another. The effectiveness of these genetic responses to stress is likely to be a major factor in resistance to disease and aging.

In recent years, the effect of age on the expression of heat shock proteins, and transcriptional regulation of this system have been well studied (4, 9, 15). The induction of hsp is the most highly conserved and best understood cellular response to stress occurring in every organism (38). The expression of hsp is a perfect example of a cellular defence mechanism to protect cells and organisms from heat and other types of stress. Therefore, changes in this system have serious negative effects on the capacity of an organism to respond to changes in its environment, since aging and senescence are characterized by a reduced ability to maintain homeostasis in response to stress. Some researchers have compared the abilities of young and old cells to express hsp70. They reported that the inducibility and thermotolerance of hsp decline with age in *Drosophila*, *Caenorhabditis elegans*, and rats (38, 39). Lower levels of induction of Hsp70 mRNA and Hsp70 protein in lung and skin fibroblast cultures derived from aged rats have been observed (40, 41). Experimental results provide evidence that nitric oxide, which is highly cytotoxic, induces hsp70 expression in smooth muscle cells via heat shock transcription factor 1 activation. Induction of hsp70 could be important in protecting smooth muscle cells from injury resulting from nitric oxide stimulation (42). Studies in cultured cells have demonstrated that non-steroidal anti-inflammatory agents can potentiate heat-induced hsp70 expression. Fawcett et al. (43) investigated the influence of aspirin on hsp70 expression in intact rats subjected to heat stress. Rats were injected intraperitoneally with aspirin, and hsp70 mRNA expression was analyzed in the lung, liver and kidney. Comparison of hsp70 expression in the treatment and control groups revealed that in all tissues examined, aspirin-plus-heat treatment resulted in 3-4 fold higher levels of hsp70 mRNA. However, aspirin treatment did not alter hsp70 protein expression in the absence of heat.

Although much is being learned about the regulation of hsp gene expression, the physiological significance at the level of the whole organisms remains largely a mystery (16). Hsps and closely related proteins are also produced at normal temperatures, indicating their vital functions in normal cells.

Altered Gene Regulation

Life span is subject to genetic modification in yeasts, nematodes, fruit flies, mice, humans, and other vertebrates and invertebrates. There are many candidate genes presently under investigation regarding life span (10). Examination of gene expression and aging in adult *Drosophila* reveals that the expression of some genes is regulated by age-dependent mechanisms. Some results provide direct evidence for a link between the regulation of gene expression and life span. For example, Labuhn and Brack (12) have examined age-related changes in the mRNA levels of the gene family encoding the six *Drosophila* actin genes. Drastic changes are observed in the mRNAs coding for muscle actins, particularly the mRNAs coding for the jump and flight muscle actins. It has been reported that actin gene expression is regulated differentially in early development, and in adult and senescent flies. There is no answer yet to the question of how the actin genes are differentially regulated in flies. Transgenic flies are being used to test the effects of specific genes on aging and aging-related deterioration. Mutations of the drop-dead gene in *Drosophila melanogaster* lead to early death of the adult animal. The life span of flies mutant for the drop-dead gene is four to five times shorter than that for normal adults. In the drop-dead mutant, there is an acceleration in the temporal pattern of expression of these age-related markers (44).

Telomere Loss

Telomeres, the specialized dynamic DNA-protein structures found at the ends of all eukaryotic chromosomes, are required to stabilize chromosomes. The DNA of telomerase differs notably from other DNA sequences in both structure and function. Recent studies have shown its remarkable mode of synthesis by ribonucleoprotein reverse transcriptase, (telomerase). Moreover, telomere synthesis by telomerase has been shown to be essential for telomere maintenance and long-term viability (20, 21, 45, 46). Therefore, understanding the dynamics of telomere maintenance is central to our understanding of cell biology. This may also lead to fundamentally new insights into the complex processes of cancer and aging.

Analyses of telomere length as a function of age, either in cells from people of different ages, or as a function of cell division number in primary cultures of human fibroblasts, show that mean telomere length gradually decreases with increased age or cell division number (21). It has been suggested that the observed gradual loss of telomeric DNA could lead to chromosome instability and contribute to aging and senescence. It is known that the structure, function, and metabolism of telomeres are remarkably conserved (45). Each eukaryotic species has a characteristic telomeric repeat sequence. Limited sequence variations are found in some species. Telomeric DNA consists of simple tandem repeated sequences, characterized by clusters of G residues in one strand. Telomerase is responsible for synthesis of the G-rich strand of telomeric DNA. The central function of telomerase therefore appears to be to counterbalance this terminal DNA loss. It is thought that the ends of linear chromosomes cannot be fully replicated during each round of replication, resulting in the shortening of linear DNA molecules with each cell division. This may be the cause of cell cycle arrest in senescent cells. Harley and co-workers (21) found that telomeres shorten as a function of age in human cells in vitro and in vivo. The telomere hypothesis proposes that critically short telomeres may act as a mitotic clock to signal the arrest of the cell cycle at senescence (22).

Discussion and Conclusion

Large numbers of characters in animals vary continuously rather than discretely. Such characters can be explained by the action of not just one but of a number of different pairs of genes. This is known as multifactorial or polygenic inheritance. The individual genes may act on the character in one direction or another, but the net results of the effect of the genes is additive. Because of its complexity, it can be said that there are multiple mechanisms of aging. Cellular senescence is also determined by multiple factors, including the genetic regulation of metabolism and responses to endogenous and exogenous stresses (47, 48, 49). Neurological diseases of the elderly, a few age-related genes identified by mutations, the limited proliferative life span of human somatic cells in tissue culture, studies on the life span of the mouse, and genetic analysis of life span in shorter lived invertebrates show that genetics has been fruitfully applied to study of the aging processes (1, 3, 50). Recent findings have revealed that extended longevity is frequently associated with enhanced metabolic capacity determined by genetic factors and response to stress. As

pointed out by Johnson (50), longevity is determined by the balance between repair capability and evolutionary fitness. This means that gerontologists may find additional ways to induce the repair capabilities of the cell. This will lead not only to a longer life, but also to a richer and more healthful one.

Oxygen free radicals are generated as a by-product during normal metabolism. The defence system of the body counteracts these highly reactive chemicals and neutralizes them (51, 52). However, a small fraction of free radicals escapes, which causes damage to proteins, lipids and DNA in organisms, and hence the aging of the organism (3, 4, 5, 6). Oxidative stress can also increase cancer frequency (53). It has been hypothesized that if the free radicals are arrested or reduced, than aging can be delayed or life span could be enhanced (26). Variations in longevity among different species inversely correlate with the rates of mitochondrial generation of the superoxide anion radical (O_2^-) and hydrogen peroxide. There are many experimental observations to support the oxidative damage hypothesis of aging. The DNA within the mitochondria is more prone to damage by oxygen radicals than the DNA contained in the cell's nucleus. These damages or mutations appear to accumulate with age and lead to faulty proteins. In turn, the electron transport system and ATP synthase are disrupted and ATP production becomes less efficient. This situation is suggested to be one of the main components of aging (1, 7). The mechanisms responsible for these effects are not known. An increasing number of data suggest that oxidative damage contributes to the aging process in *Drosophila melanogaster* and other organisms (54). Results support the free radical theory of aging by suggesting that the increased resistance to oxidative stress may be among the causes of increased longevity (2, 3). Experiments have shown that oxidative damage is a candidate for such a common mechanism of aging.

Restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum life-span in mammals (4). Caloric restriction is the only widely validated method for the extension of life span and postponement of senescence and age-related diseases (e.g., cancer) in mammals (17, 19). Fruit flies that displayed extended longevity were obtained after many generations of selection for late reproduction by Jazwinski (55). The increase in life span caused by dietary restriction might be explained by retardation of growth, reduction of body fat, altered gene expression, reduction of body temperature and depression of metabolic rate, and the lessening of oxidative stress and damage (19).

A decrease in homeostatic functions, including the ability of protein synthesis and turnover, is a main characteristic of aging organisms. The relationship between protein synthesis and aging has been extensively studied. Cellular and organismal aging is accompanied by a slowing down of protein synthesis. Although it has been suggested that decreased protein synthesis may contribute to the deterioration of normal cellular machinery in senescent cells and organisms, the exact consequence of such decrease is still a mystery (11, 33, 34). EF-1 α , which has been isolated from several mammalian species, including rabbits, mice, rats, and humans (35), controls the translational rate. It is an abundant protein expressed in every cell, and encoded by more than one gene being expressed differently during development. A casual correlation has been observed between EF-1 α expression and protein synthesis with cellular and organismal aging. Nonetheless, the exact role of EF-1 α in senescence is still highly debatable.

Alterations in gene expression during aging, as reported in many different organisms, have been proposed to be an important factor causing senescence and also cancer. Examination of gene expression and aging in adult *Drosophila* reveals that the expression of some genes is regulated by age-dependent mechanisms (12). For example, transcripts for muscle actins and cytoskeletal actins decrease differently during aging in *D. melanogaster* (12, 13). Normal cells cultured in vitro lose their proliferative potential after a finite number of doublings. This process is termed replicative cellular senescence (56). The Rb and p⁵³ tumor suppressors are examples of growth inhibitors that lose the ability to be regulated and are constantly activated during senescence (57). Results provide direct evidence for a link between the regulation of gene expression and life span, and provide a direction for the genetic analysis of aging. Genes that exhibit aging-related changes in expression are now being identified. It has also been proposed that

cellular senescence results from the loss of telomeric sequence (20, 21, 45, 46). Stress, particularly oxidative stress, has a profound effect on telomere metabolism and cellular senescence. Scientists are working to explain the relationships among telomere loss, replicative senescence, immortality, and cancer. If they are successful, there may be important implications for novel drug discoveries and benefits to human health.

The heat shock protein system is a mechanism of cell defence induced by stress (42). Although their precise function or functions are not known, these proteins are generally presumed to increase the ability of cells to recover from the toxic effects of heat or other physiological stresses. It has been reported that proteins of the Hsp 70 family play diverse cellular roles. Significantly, several studies have demonstrated an accumulation of abnormal proteins as a function of age. There is a suggestion that the heat shock proteins might bind to denatured abnormal proteins produced by stress, and aid in their elimination. They have further been hypothesized to play a role in the normal assembly and disassembly of proteins, because some heat shock proteins are also present in the absence of stress (36). Advanced age is associated with a reduction in most physiological functions and in particular with a decreased ability to maintain homeostasis during stress. The amount of a heat-shock protein affects the fitness of a complex animal in the wild (3).

Aging is clearly complex and multifactorial. The factors that affect aging are genetic and environmental. Metabolic capacity, efficiency of stress responses, and dysregulation are the limiting factors for longevity. The activity, life, and death of each cell or organism are dictated by its responses to a wide variety of stimuli from its environment. If we increase our understanding of the mechanisms of aging, we may be able to prolong life and decrease the number of years that people suffer from ill health.

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