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THE BIOLOGY AND THEORIES OF AGING

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Abstract: *Aging is a natural, time-dependent process characterized by irreversible changes in the molecules, cells, tissues, and organs. It occurs as a result of cumulative damage at different levels of the organization, in particular by damaging proteins and DNA. There is no single definition, nor a unique attitude about when and how age arises and what are its causes. The process is extremely complex and most likely a consequence of the effects of different mechanisms (not only genetic but also acquired) that lead to disrupt homeostasis, reduce stress resistance and the more frequent occurrence of the disease. There are many classifications of theories about aging and they often contradict one another. No one theory is sufficiently able to explain the process of aging. The aim of this work is to analyze the different aspects, main characteristics of the aging, individual differences in the speed of this process and theories about the mechanisms of aging.*

1. Introduction

Living organisms have many common basic properties of life, *i.e.* biological and chemical processes. A fundamental limitation of the lifetime («nothing is infinite») is also a common feature, but the differences in the rate of aging and length of life between species are evident and the result of evolution.

Aging is the internal biological process of the organism which begins with the creation of the zygote and is inevitable regardless of the environmental conditions. Better conditions of life only prolong the growing weakness and deterioration of the condition that eventually must lead to the end of life [1]. The purpose of the evolutionary study of aging is to understand why aging occurs, why species age at different rates and what mechanisms are involved in this process.

Scientific theories of aging suggest that aging and longevity are conditioned by genetic factors [2]. Living organisms have unique characteristics: they are born, grow, reproduce (and thus transmit the hereditary information to their offspring),

age and die. Previous studies on a variety of model organisms such as yeast, worm, fruit fly, mouse, have enabled the identification of genes that alter the rate of aging (which is measured by the length of life), but did not match any single gene which completely reverses the aging process [3, 4]. There is no common factor of aging for all manifestations of this biological process. Therefore, aging is considered a different kind of biological property than the morphological, physiological and behavioral. The question arises: are living organisms designed to grow old? This would indicate that aging is the function, and can be expected to have common characteristics with other evolutionary functions, including the complexity and coordination.

2. Variability of Aging

If we compare aging, or related length of life, with other characteristics of living beings, we discover that it also varies between individuals but also between different types, which indicates the inheritance is involved in that process. Comparative studies on aging show significant differences in the maximum length of life that varies from a few hours in some insects to over a hundred years for some plants such as Sequoia.

There are a number of examples of variations of aging and the associated longevity: the longest living animal species are the lake the greenland shark (*Somniosus microcephalus*) which may live up to 400 years, sturgeon (*Acipenser fulvescens*) at approximately 152 years, the big turtle (*Geochelone gigantea*) at about 152 years, and humans at about 122 years. Examples of short-lived animals can be a shrews (2 years) and salmon (3 years).

In the meaning of the phenotypic, variation of aging is in the range of those without the specific phenotype of aging (*e.g.* turtles) to the species with the most aggressive mechanisms of aging, *ie.* «biological suicide» which is usually related to the end of the reproductive period (*e.g.* Salmon). Unlike species in which gradual degradation occurs, in Salmon appears a so-called acute age and short period from the end of the spawning to the death. In this case, the accelerated aging process is more favourable for the species because the decomposition of dead Salmon provides food for offspring [5].

Cicadas are also an example of precisely programmed life cycle in which the larval stage is significantly longer than the adult since larvae live underground for up to 17 years. Water flower is a similar example of a programmed life cycle considering that adults live only a few hours or days [6].

Contrary to previous examples, there are species that do not grow old in phenotypic terms (do not exhibit characteristics of aging) because they do not show any deterioration with time, for example the ants *Pheidole dentata* [7]. The environmental influences determine the length of life. The death is caused by lack of food, disease, attacks by predators, and so on.

Aging varies between individuals, aging varies among different species, aging is in part hereditary, and are all conditions for the operation of natural selection. Longevity is associated with the amount of energy consumed in reproduction, *i.e.* it is in

line with the development and reproductive cycle of animals. For example those animal species that are rapidly developing to maturity and can proliferate rapidly have a shorter life expectancy, and those who need more time to develop and become sexually mature have a longer lifespan. Since that aging has a negative impact on the reproductive power, and therefore the adaptive value, it is expected to be «pulled out» by natural selection. The question arises: is the longevity of benefit for the species?

3. Darwin's Dilemma

Darwin believed that the limited lifetime of characteristics, determined by natural selection, was an evolutionary characteristic or adaptation. This is the kind of feature that somehow uses the species even though the limited lifetime is unfavourable from the aspect of the individuals. However, Darwin was unable to explain the mechanism by which individual characteristics that affect the weakness of individuals are not eliminated by natural selection. This has opened so-called Darwin's dilemma. Scientists have been in a dilemma between belief that aging is an adaptation or that aging is not an adaptation despite numerous experimental confirmations of the first hypothesis. Darwin's dilemma is a choice between the two points of view: the aging is the adaptation, a characteristic that has its purpose and therefore is selected (under the influence of natural selection) and aging is a feature of life as an adverse effect of a necessary process.

4. Different Theories of Aging

Aging is a complex biological process, a great enigma of life, in which the changes occur at the molecular, cellular and organic levels that are progressive and inevitable. From Weisman's time to date, many efforts have been made to explore the basic mechanisms of aging at the cellular, extracellular, genetic and molecular levels of the organism, so there are hundreds of theories of aging. An acceptable theory of aging should show agreement with Darwin's theory and at the same time explain the interspecific variation in length of life. It should combine the natural selection with the accumulation of damage.

5. Weismann Theory of Programmed Death

The first evolutionary explanation of aging, according to which it is a programmed method to limit the size of the population and avoid the spread, was given by biologist August Weismann (8; who set the theory of programmed death in 1889). According to Weismann aging is an adaptive process that facilitates change of generations and helps adaptation of organisms to environmental changes. This means that «programmed death» is the evolutionary characteristic (adaptation) developed through natural selection because it uses the species, although it has a negative effect on individual convenience. This explanation made it possible to understand the interspecific differences in the duration of life.

The main problem of the theory of adaptive evolved mechanisms is contrary to Darwin's theory of natural selection because it seems to require the evolution of

traits that are unfavourable to the adaptive value of the individual. However, many biologists believe in Darwin and Weismann's idea that aging is a feature that has a purpose because although it is disadvantageous for individual organisms it provides an evolutionary benefit for the species. Also, aging is not the only characteristic of organisms that does not fit into the classic theory of natural selection, for example the same applies to some forms of behaviour such as altruism (*e.g.* the bees).

In the mid-20th century, the nature of aging was «an unresolved problem in biology». Peter Medawar presented the scientific paper in front of the London Royal Society in which he advocated the theory of accumulation of mutation [9]. Medawar proposed a model by which the power of natural selection decreases when the organism has reached the period of sexual activity. According to him, aging is caused by random mutations that determine negative characteristics, *i.e.* degradation of molecules, cells and tissues.

With the development of genetics, the theory of accumulation of genetic damage has gathered more and more followers. Due to the accompanying changes in the morphological, physiological and behavioural characteristics observed during aging, we can treat it as a process of «biological wear» caused by the gradual accumulation of genetic damage. Aging occurs as a result of accumulation of somatic cell damage [10]. Mutations are the result of errors in the replication process, and chromosomal aberrations (which also correlate with age) are formed due to radiation or chemical damage to the genetic material.

The concept known as the mutation accumulation theory, means that a large number of genes with harmful effects that appear later are distributed in a genome with a high degree of heterozygosity in a variety of individuals. Medawar [9] suggested that aging is caused by certain genetic diseases, each of which has adverse symptoms only in distant years. Medawar rejects Weismann's idea that the programmed death is an evolutionary characteristic and points out that mutation can accumulate («in the function of time») causing death due to old age. Since Medawar's theory has linked aging to sexual maturity and reproduction it is highly appreciated and respected. According to the theory, aging can exist due to the effect of decreasing similarities that unfavourable mutations cause with age. However, the accumulation of mutations is too simple a mechanism to explain the details of the investigated processes of aging in different species. Salmon or bamboos are types of organisms that express types of deaths which are closely linked to the act of sexual reproduction. These are cases of death caused by reproduction.

Studies in experimental animals have shown that the frequency of mutations increases with aging, but it remains unclear whether they are the result of cause or consequence of aging?

6. Traditional Theories of Aging

After Weismann's theory and the theory of damage accumulation of DNA, traditional aging theories arise and suggest that aging-causing factors are genetically transmitted but not «genetically programmed». That would mean that the aging characteristics are genetically transmitted. In this way, the traditional theories of

aging combine natural selection with «accumulation of damage». Natural selection explains why animals live long enough to reproduce, and also explains why they are intensely old after reproduction.

7. Antagonistic Pleiotropic Theory

Proponents of this theory point out that aging is an inevitable side effect of reproduction. Geneticists have explained that one gene can be activated in more than one tissue and therefore damage in a single gene can affect a number of characteristics. These genes are indicated as pleiotropic genes. According to Williams [11] aging is caused by combined effect of many pleiotropic genes, each of which has a beneficial effect in a younger age, but an adverse side effect in the older age. He has assumed that species that previously reached sexual maturity and have pronounced reproductive characteristics have a shorter life expectancy. In accordance with the attitude of Medawar, adverse effects have progressively less impact on benefits as the animals age. Some believe that the theory of antagonistic pleiotropy is in conflict with modern genetics. Namely, according to this theory, the gene has a purely positive value in one stage of the life and clear negative effect in the second stage of life, and pleiotropy implies the influence of one gene on multiple characteristics at the same time. According to the theory of development, it is important that the genes are activated at the right time and in the appropriate tissues. Each gene which is activated in the wrong tissue or at the wrong stage of the life, or in the wrong circumstances, has negative consequences for the organism. The complexity of the genetic program of a species is reflected in its ability to coordinate the many different activities that have to take place at different developmental stages of an organism.

8. The Theory of Disposable Soma

This is a concept of evolution regarding the optimal level of maintenance cells. It is pointed out that organisms have a limited amount of energy which must be adequately divided between reproductive activity and maintenance of somatic, nonreproductive tissues. Despite a series of survival mechanisms, most species are not programmed well enough to allow them to survive indefinitely. Kirkwood [12] states that aging is the result of a natural degeneration process that leads to the accumulation of damage, but this damage to the organism can repair at the expense of the damage to reproductive activities. This theory combines the reduced effect of natural selection upon reaching full maturity with «accumulation of damage» and further explains the relationship between reproduction and lifetime. According to theory, the energy investment in reproduction in the younger age inevitably reduces the available energy to «repair the damage» in later years. Specifically, the cells of long-lived organisms exhibit greater capacity for repair of molecular damage and maintenance of biochemical stress than the cells of short-lived species [13].

The concept, according to which aging is an inevitable side effect of reproduction, would be acceptable if it were not for the fact that individuals who have not reproduced during their lifetime are also old.

9. Contemporary Theories of Aging

Modern approaches seek an answer to the question: is genetically programmed aging a logical extension of programmed cell death?

Skulachev [14] points out that aging is a specific biological function whose causes include shortening of telomere, change in the function of heat shock proteins and oxidation. It suggests that the function of aging is to stimulate the evolution of species through the elimination of weakened and dysfunctional individuals. This view is in accordance with the interpretation of Dobzhansky [15] that «the individuals are ephemeral, only the population survives».

Telomeres are repeatable short nucleotide sequences at the ends of chromosomes, which, as a reservoir of non-coding DNA sequence, bind proteins that prevent degradation of the chromosome and thus maintain the chromosomal integrity. It is assumed that these regions of chromosome have the role of a «biological clock» which limits the capacity of cell division. Evaluations of biological mechanisms of aging have shown that telomere shortening occurs during the successive duplication of chromosomes during the cell cycle [16]. With every cell division, the telomeres are shortening by 50-100 nucleotides due to the inability of DNA polymerase to replicate the final part of the chromosomes. Eventually, telomeres become sufficiently degraded to inhibit cell division through mitosis. Stability of telomeres is achieved by the action of enzyme telomerase [17] that is encoded by a complex of genes, among which are TEP1 with 14 chromosomes and DKC1 on X chromosome [18]. After telomere repairs this enzyme enables further cell division. High telomerase activity is present in the germ cells, stem cells, and epidermal cells of the skin as well as malignantly altered cells [19].

The findings related to diseases of aging in humans confirm that telomere shortening is the result of a decrease in activity of the enzyme telomerase. Telomeres of five year children with Progeria are as long as telomeres of the seventy-year-old men, and telomerase activity is significantly reduced.

The hypothesis that the absence of telomerase could be the cause of aging has been experimentally checked. The results of these studies show a direct connection between telomeres and the process of aging as well as the ability of telomerase to prolong life by increasing the number of cell divisions. However, a new question remains: what causes the absence of telomerase? Is it genetic changes such as the condition of other enzymopathies?

Experiments have also confirmed that free radicals can be a mechanism of aging. Functional mechanisms for repair of damage caused by oxidation prolong the process [20]. Antioxidant protective systems comprise molecules capable of interacting with free radicals and reducing the amounts of them in an organism [21]. The enzyme system of protection against free radicals consists of superoxide-dismutase, glutathione peroxidase and catalase, while non-enzymatic antioxidants are *e.g.* vitamin E, coenzyme Q10, and ascorbic acid.

It has been shown that reducing activity of the specific thermal shock factor, which is necessary for the production of proteins that cells use for repair of other damaged proteins, is correlated with the age of individuals.

These data suggest that we can think about genetically programmed aging which causes programmed weakness, programmed reduced mobility, programmed reduced sexual power, and programmed increased sensitivity to disease [22], which leads to the death but not «programmed death». This would be in line with the theory of evolution, according to which living organisms are programmed for survival, not for death.

10. Mitochondrial Theory of Aging

Cell organelles contain about four of their own circular DNA and provide about 90% of the energy required for functioning of the organism. They are more common in the cells that show a high degree of metabolic activity, such as neurons and muscle cells. Harman *et al*. [23] proposed the mitochondrial theory of aging, which was experimentally confirmed by other authors [24]. According to this theory, aging is based on the long-term accumulation of mutations in mitochondrial DNA. Mutations in mtDNA occur 10-20 times more often than mutation of nuclear DNA. Lagouge and Larsson [25] have shown that the absence of the mitochondrial polymerase, which has a role in the repairing of mtDNA mutations in knock-out mice, causes premature aging of the individuals. In contrast, there are studies that have analyzed differences in the functioning of mitochondria in the cell lines of young and old people, which has not found enough difference in mtDNA, from which it was concluded that aging would be the result of epigenetic factors, *i.e.* significant differences in the level of certain proteins. These influences of epigenetic factors on the aging process are the subject of contemporary studies [26] because it is believed that by removing these epigenetic factors it may be possible to reprogram the cells.

11. The Genetic and Environmental Basis of Aging

A large scientific challenge is to understand the influence of genetic and non-genetic factors on the aging process that require detailed theoretical studies as well as experimental verification of some hypotheses. There is numerous experimental data on small organisms on the influence of environment and the impact of the activity of genes on aging [27, 28].

In *Drosophila melanogaster* it was found that restrictive movement extends the life of these organisms. Also, changes in oxygen concentration can change the length of the insect life. The amount and caloric value of the food is an environmental factor that also affects the biological process [29].

One of the important non-genetic factors for the organism is the methylation of DNA molecules. It is an important epigenetic mechanism, influenced by various chemical agents, diet, chemicals, heavy metals, and other environmental factors, from which depend the expression of the structural and regulatory genes. While in malignantly transformed cells increased methylation of cytosine was detected, in age hypomethylation of this base is detected. Cytosine methylation catalyse the enzyme DNA methyltransferase from the group known as the DNMT (1, 2, 3L, 3A, 3V).

There are numerous findings of the genetic condition of aging. Selective breeding of flies *D. melanogaster* after 100 generations has obtained lines whose life expectancy has nearly doubled. In the nematode *Caenorhabditis elegans* a gene called *AGE1*, which slows down aging, was discovered. Also, in the genome of these organisms the gene *DAF-2* has been identified, regulated by the transcription factor DAF-16, which affects the rate of aging [30]. Kenyon [31] concludes that the induced change in the activity of this gene doubles the lifetime of this worm from 2 to 4 weeks. The facts that several genes which influence the rate of aging are related to control of energy metabolism, for example through insulin signalling, are in favour of the theory available. In yeast *Saccharomyces cerevisiae* gene *SIR 2* (Silent Information Regulator) whose activation results in increased life was detected. The nucleotide sequence of DNA (SIRh2 or sirtuin), similar to this gene has been detected in the genome of humans, but activity of this gene does not affect the aging of humans.

Studies in mice have allowed the establishment of about 20 genes which accelerate the aging process, and over 26 which slow it down.

Examples of genetic causality of aging in humans are the appearance of Werner's syndrome and some forms of progeria. Only one genetic anomaly causes the symptoms of aging in early age, so that Hutchinson-Gilford syndrome patients live up to 14 years and from Werner's up to 50 years [32].

Based on the above facts, aging can be considered prior to adaptation rather than the result of random events.

12. The Role of Gene Therapy in the Prolongation of the Aging Process

An experimentally proven link between genetic constitution and aging leads to the conclusion that the process of aging can be changed (prolonged) or even stopped. This view is advocated by various authors [14, 33]. Different approaches are proposed, from the use of the preparation for the stimulation of reparation mechanisms or antioxidants in order to reduce the rate of genetic damage to gene therapy. Vos *et al*. [33] from the Institute for Biological Studies in Salk, California, referring to previous research, states that there are so-called «embryonic genes», or cells with embryonic characteristics created by the reprogrammed cells that may eliminate the age signs. In laboratory conditions, a new type of gene therapy has been tested so far in mice cells and cells of human skin, wherein both of them showed signs of rejuvenation. After six weeks of treatment, the animals looked younger, their cardiovascular system worked better and faster and recovered more quickly from injury. Also, they lived 30% longer than untreated individuals.

Since aging has its biological function, the evolutionary implications of such procedures are uncertain.

13. Conclusions

Given the heterogeneity of aging (the variation in length of life, interspecific diversity of phenotypes of old individuals and others) it is difficult to conceive of a uni-

fied theory of aging. None of the numerous theories can be reconciled with all the scientific facts obtained in studies of biological processes during the past centuries.

However, based on new data some new conclusions can be drawn. Although aging negatively affects the suitability of individual, it provides an evolutionary advantage for species; as the behaviour of the animals as a property is structured, so aging is not subject to «orthodox» Darwinian theory of natural selection; the «phototype of aging» is a set of traits (weaker mobility, greater sensitivity to disease, weaker function of vital organs, lower sensitivity to environmental stimuli due to the dysfunction of sensory organ, etc.) and under the control of a large number of genes, whose proteins interact with one another. In addition to the complex genetic condition and the influence of non-genetic factors, the aging process is largely influenced by epigenetic factors and aging is a biological function of species that stimulates evolution.

Conflict of Interest

The authors declare that there are no conflicts of interest for the information presented in this review.

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