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# Is There an Unambigous Answer to the Question: Whether the Aging Program Exists or Not?

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**Abstract**—The article discusses up-to-date viewpoints on the reasons of aging in respect to their compliance with the concepts of programmed or stochastic aging.

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*I salute in my mind to the wisdom of the dear nature–*
*It puts labels with its skillful hand.*
*Sasha Chernyj, "Harmony," 1908*

## Classification of Theories of Aging

The modern gerontology has not yet made an essential breakthrough in understanding of aging and its mechanisms. In his work devoted to classification of aging theories, Medvedev [1] mentioned more than 300 theories, but no one of then, in this opinion, possesses qualities of a real theory and only a few can be considered as hypotheses. The table below lists the most known aging theories which are traditionally classified as stochastic (probabilistic) and programmed aging ones [2]. This classification is fairly arbitrary. When studying the reasons and mechanisms of aging, one faces interpenetration of both different and similar processes interpretable in terms of both the stochastic and programmed aging theories. We consider it reasonable to consider aging as an integral process underlaid by both ontogenetic and cumulative mechanisms which, in their turn, are affected by genetic and ecological factors [3].

Rattan [4] notes that the traditional division of aging theories into stochastic and programmed aging ones has actually become out-of-date and points to principles which can form a basis of a unified aging theory. This is an evolutionary approach including molecular, genetic, post-genetic, and epigenetic (not directly gene-mediated) aging factors, as well as homeostasis or homeodynamics of biological systems.

Yin and Chen [5] in their review reported a classification of the modern theories of aging, based on the integration level: organismal, organ, cellular, and molecular. The authors of this classification note that 24 of 28 their mentioned theories treat aging as a consequence of exposure to a variety of external factors that affect the life process, i.e. define aging as a passive accumulation of damage in a human body. Among the four aging theories mentioned as programmed aging ones, the authors focused on the theory of cellular senescence, based on data on cellular mitosis and telomere shortening. This theory radically differs from theories that consider aging at an organismal level. As stressed in [5], the term "nondividing cells" does not imply old cells, since post-mitotic neurons and most myocites, while not proliferating after differentiation in the embryogenesis period, may stay undamaged in the body over the whole life. Moreover, the slowdown in functioning the whole organism, caused by aging, is difficult to explain in terms of telomere shortening.

Kordyum [6] mentions that almost all definitions of the aging process include quite general phrases like "decay of vital forces…" or "decline of functional activity… ." The only clear definition that takes account of reality and abilty to measure it takes for the final reference point of life its cessation, i.e. death, and treats aging by life expectancy. A process in this definition is in fact substituted by its result. According to the author, such treatment allows many things to be quantitatively assessed not only at a statistical, but also at a populational level. At the latter level, the reasons

Theories of stochastic and programmed aging ([2], with supplements)

| Theory (reason of aging) | Basic postulates | Modern state |
|---|---|---|
| | Stochastic theories | |
| Somatic mutations | Somatic mutations disturb genetic information and reduce cell function | Supported by present evidence |
| Error catastrophe | Errors in transcription and/or translation reduce cell function | Original theory denied, modification are developed |
| DNA damage, DNA repair | DNA damage is constantly repaired by various mechanisms. Effective repair positively correlates with lifespan and gets less effective with age | Supported by present evidence |
| Prortein damage | Conformational damage of proteins and enzymes (cross-linking) damage cell function | The same |
| Cross-links | Chemical cross-linking of physiologically significant macromolecules (for instance, collagen) damages cell and tissue function | The same |
| Wear out | Accumulation of cell and tissue damage impairs bodily functions | Quite possible reason of aging |
| | Theories of programmed aging | |
| Genetic theories | Aging is caused by programmed changes in gene expression or by expression of specific proteins | Supported by present evidence |
| Death genes | Cell death genes exist | The same |
| Selective death | Cell death is caused by specific membrane receptors | The same |
| Telomere shortening | Age-related telomere shortening *in vitro* and *in vivo* leads to chromosomal instability and cell death | The same |
| Differentiation disorders | Errors in gene activation-repression mechanisms results in the synthesis of excessive, insignificant, or unnecessary proteins | Quite possible reason of aging |
| "Waste" accumulation | Accumulation of metabolism wastes adversely affects cell vitality | Some evidence is available |
| Neuroendocrine theories | Failure of thy nervous and endocrine systems to maintain homeostasis. Loss of homeostasis leads to aging and death | Evidence in respect of the female reproductive system and certain specific situations was obtained |
| Immunologic theory | Certain alleles can favor or unfavor longevity | Supported by present evidence |
| Metabolic theories | Longevity is inversely proportional to metabolism rate | Denied |
| Free-radical theories | Longevity is inversely related to the degree of free-radical damage of cell macromolecules and directly related to the efficiency of antioxidant systems | Supported by present evidence |
| Aging clocks | Aging and death are predetermined by a biologic plan | The same |
| Evolutionary theories | Natural selection gets rid of individuals after they have reproduced | Some evidence is available |

for deaths are actually quite different, but each induvual dies for its own unique reasons. Kordyum stresses that even though one excludes all these particular reasons and accidents, eradicates leading pathologies, creates an ideal ecology, and provides favorable life and social conditions, i.e. excludes all external unfavorable factors and diseases, the unevoidable nature of senescence, specifically complete consenescence of the organism, when existence becomes incompatible with the most favorable conditions, will prevail. This "dry residue" is taken to be senescence. And this is its mechanisms and fundamental principles that in the limit come down to two reasons: stochastic (senescence as a result of a

combination of unfavorable random conditions) and programmed (senescence as an act envisaged by the nature and evolution).

The general postulates of the programmed death concept in essence read as follows: Old organisms occupy the ecological niche which has quite a definite volume and thus prevent young generation from entering to the life. Therefore, death is envisaged by a program, since it is "useful for the species." All supportive examples, for example, death of butterflies after procreation or plants after fruiting, are, in Kordyum's opinion [6], fully irrelevant to the problem. Here the mechanism of self-killing of a young organism, rather than the mechanism of senescence is operative.

Let us consider the most recognized theories of aging.

### Free-Radical Theory of Aging: Basic Postulates

The theory of aging the most fruitfully developing over the past years is the free-radical theory which was almost simultaneously advanced by D. Harman in 1956 [7] and Emanuel' in 1958 [8]. This fundamental theory explains not only the mechanism of aging, but also a wide range its associated pathological processes (cardiovascular diseases, aging-associated immune depression and brain dysfunction, cataract, cancer, etc.).

According to this theory, the reactive oxygen species (ROS), such as superoxide radical ion $O_2^{\bullet-}$, $H_2O_2$, hydroxyl radical $HO^{\bullet}$, and, probably, singlet oxygen $^1O_2$, produced primarily in mitochondria damage cellular macromolecules. It is suggested that ROS damage membranes, collagen, DNA, chromatin, structural proteins, and lipids are involved in regulation of intracellular calcium levels, etc. [7, 9–12]. Aging and aging-associated diseases are derived from the accumulation with age of oxidative damage to DNA, proteins, lipids, and other vitally important macromolecules.

The source of free radicals in a human body are mitochondrial electron-transport chains; microsomal oxidation of xenobiotics and physiologically active compounds; xantinoxidase and other cytosolic oxidases; phagocytising cells (NADPH oxidase); oxidation of hemoglobin and myoglobin; autooxidation of reduced molecules; UV radiation and photochemical reactions; and ionizing radiations.

The cellular targets of free radicals are plasmatic and intracellular membranes (lipids and proteins), endoplasmatic reticulum, cytosolic proteins, mitochondrial and nuclear DNA, and the extracellular targets are lipoproteids and conjunctive tissue.

### Mitochondrial Theory of Aging

Over the past years the mitochondrial theory of aging has received recognition. This theory is based on two following suggestions [13, 14].

First, it is suggested that mutations of mitochondrial DNA (mtDNA) accumulate with age and may attain a considerable frequency. However, this suggestion alone cannot explain the role of mtDNA in aging, since each cell contains several mitochondrial genomes. Random mutagenesis will form a mixture of different types of mutant molecules and wild-type molecules. A special mechanism is required for phenotypically significant mutants to form in concentrations sufficient for exceeding the phenotypic threshold [15, 16].

Second, it is suggested that mtDNA mutations distribute so that each cell contains one mutation or, primarily, one type of mutations. Therewith, mutation can realize to a full measure its potential ability to disturb cell physiology and thus be actively involved in the aging process. Even though there is still little direct evidence to show that mtDNA mutations contribute much to senescence, except, perhaps, for sarcopenia (degenerative loss of muscle mass), recent research gave unequivocal evidence for both the above suggestions and thus further evidence for the mitochondrial mutation theory of aging [14, 16, 17]. Extrapolation of the exponential mtDNA damage accumulation (both point mutations and deletions) to 100% in human heart cells gives an estimate of 129 years. The damage to mtDNA disturbs mitochondrial respiration, which enhances ROS formation, thereby causing further mtDNA damage.

Dufour et al. [18] obtained convincing evidence showing that the aging of the filamentous fungus *Podospora anserina* is associated with mtDNA instabilities, namely, accumulation of a circular multidimensional DNA molecule called senDNA. The authors established that inactivation of the nuclear *COX5* gene encoding subunit V of the cytochrome *c* oxidase complex leads to the exclusive use of the alternative respiratory pathway and to a decrease in production of ROS, which essentially increases the longevity associated with stabilization of the mitochondrial chromosome. Moreover, this mutation prevents accumulation of senDNA in mitochondria.

The advocates of the mitochondrial theory of aging suggest that the aging process is underlaid by progressive loss of mitochondrial function in different body tissues [12, 14, 19]. This viewpoint is supported by the following facts:

– accumulation of big deletions and point mutations in tissue mtDNA in aged individuals and depletion of mtDNA copy number;

– age-related decrease in the activity of enzymes responsible for electon transport in the respiratory chain, lymphocytes, skeletal muscles, and cardiomycites;

– as a consequence of these processes, enhanced ROS generation and vigorous peroxide oxidation of lipids and membrane proteins;

– changes in mitochondrial morphology and decrease of the mitochondrial membrane potential which provides energy of ATP synthesis;

– rapid cellular senescence in young rats and susceptibiliy of their cells to degeneration on microinjections of fibroblast mitochondria from old rats;

– inverse correlation between mitochondrial hydrogen peroxide production and maximum longevity of a species.

Ames [20] found that cellular mitochondria of old rats produce a lot of oxidation products. The author discusses the role of deficiency of micronutrients, in particular those incorporated in vitamins, in the development of mitochondrial decay and degenerative diseases of aging.

Maternally inherited mtDNA replicates over the whole life both in mitotic and post-mitotic cells, which eventually results in that mutations in mtDNA occur much more frequently than in nuclear DNA. This is largely defined by the inefficiency of the reparation systems of mtDNA and its closeness to the mitochondrial membrabe when ROS are generated. Age-related disruptions of mitochondrial respiration were revealed not only in normal tissues, but also in people with neuroegenerative diseases (Alzheimer disease, Parkinson disease, Huntington chorea, astasia, skeletal and cardiac myopathy, etc.) [21]. These diseases, too, feature a high frequency of mtDNA mutations. A correlation of apoptosis and mtDNA fragmentation has been reported.

The inheritable polymorphism of mtDNA is associated with aging and longevity: The J haplotype has been much more frequently observed in 100-year-old men in Nothern Italy than in young [22]. It is interesting that the same mitochondrial haplotype is quite frequently expressed in many complex diseases, exemplifying the antagonistic pleiotropy, i.e. differential action of a gene or genes which reveal themselves unfavorably in a young age and favorable in an old age, thus ensuring "happy" aging. Moreover, there is some information that the frequencies of mtDNA polymorphisms in elderly persons in Italy, Ireland, and Japan are quite different [23].

Trubitsyn [19] suggests that the leading reason of aging is the decay of cellular bioenergetic processes, which is directly coded by nuclear genome. It his opinion, the superoxide radical cation generated by the respiratory chain is neutralized by the antioxidant system in two stages: Its reaction catalyzed by superoxide dismutases (SOD) forms hydrogen peroxide which reacts with glutathion peroxidase to give water and oxygen. The other reaction that occurs concurrently with the glutathion peroxidase reaction is the Fenton's reaction leading to hydroxyl radical. The programmed decay of the mitochonrial bioenergetic function inhibits glutathion peroxidase, which increases the concentration of hydrogen peroxide in tissues and enhances its flow through the Fenton's reaction system. Thus, aging and enhanced generation of aggressive oxygen species are two consequences of programmed decay of cellular bioenergetics (for details, see the paper of Trubitsyn entitled "Aging as a Result of Realization of the Genetic Phenoptosis Program" in this issue).

Andziak et al. [24] reported the observations which can be considered as one of the most serious challenges to the more and more critisized theory of oxidative stress. The most commonly cited evidence for this theory is the results of measument of the contents of oxidants and/or antioxidants in tissues of animals with different lifespans depending on genetic and environmental changes. A more direct approach is direct measurement of oxidative damages in tissues. The authors of [24] made use of sensitive techniques to measure oxidative damages in tissues of laboratory mice and their far-related long-living rodents, viz. naked mole rats (NMR). The latter are of the same size as normal mice, they usually live more than 20 years (a maximum of 28.3 years). Tissues of young adult NMR were compared with those of young (3 months) and old (2 years) mice. The oxidative damage in both the latter cases was much stronger than in NMR (from 1.5 to 10 times depending on the tissue and analytical

technique). It was also found that NMR tissues contain lower levels of reduced glutathion and other antioxidants and their cellular environment is less prone to oxidation. Even though reduced oxidative damage to key tissues may well be suffice to prolong the lifespan of certain species like *D. melanogaster* [25], the results of research on NMR gave evidence to show that this is not necessary.

According to Austad [26], no evidence, even though abundant, is able to shake confidence of the scientific community in that oxidative stress is a widespread mechanism of aging, notwithstanding the fact that this mechanism is known to underlie certain specific neurodegenerative diseases. However, some evidence is available against the involvement of oxidative damage, say, in NMR tissues, to the aging process. Thus, Van Remmen et al. [27] showed that mice with a reduced MnSOD activity, as would be expected, feature increased oxidative damage to tissues but are not short-living, whereas overexpression of the key antioxidant enzyme CuZnSOD does not increase the lifespan in mice [28]. However, these challenges to the oxidative damage hypothesis do not rule out this hypothesis but suggest that it should be revised and refined.

## Evolutionary Theories of Aging. Disposable Soma Theory

The phenomenon of aging is a mistery of evolution. Since long-living individuals can give more descendants than short-living, natural selection should favor longer life. In this connection a question arises: Why the lifespan of most species is fairly short (less than 1 year) [29]? At least three hypothesis have been advanced to explain aging from an evolutionary point of view.

Medawar [30] suggested a theory of mutation accumulation, according to which the force of natural selection in an organism in the postreproductive age rapidly declines to the point of being no longer able to effectively resist destructive mutations. Mutation accumulation leads to physical disorders in the organism.

According to William's antagonistic pleiotropy theory [31], aging develops since natural selection gives preference to allelic genes favorable in a young age, even though they are unfavorable in an old age. The force of selection favoring such alleles is maximal in the first reproduction period, after which aging commences.

Based on the classical views of Weismann [32], Medawar [30], and Williams [31, 33] on the role of natural selection associated with the accumulation of unfavorable mutations, Kirkwood [34, 35] developed an evolutionary theory of aging, the key point of which is the concept of disposable soma. In his opinion, evolutionary theories of aging allow certain predictions as to the role of genome factors in the aging process:

(1) specific aging genes are unlikely to exist;

(2) genes the most essential for aging and longevity are most likely to control soma maintaining and desposal;

(3) other genetically defined trade-off factors responsible for the preference of young organisms over old;

(4) numerous mutations are possible, whose delayed effects contribute to the aged phenotype.

Obviously, the aged phenotype is determined by a multitude of genes, and much effort if needed to find out what are their total number and categories and what of them actually essentially contribute to aging. The evolutionary genetics of aging has not yet received adequate recognition, but its significance will increase with time [36, 37]. Of great interest are attempts to compare age-related trends in the expression of genes controlling certain physiological functions on aging of different animal species (nematodes, fruit flies, mice, monkeys, and humans) [38].

Human longevity is largely dependent on genetic factors, but nongenetic factors, such as nutrition and life style, too, are of great importance. Therefore, it is quite important to identify genes contributing to human life expectancy and longevity, as well as development of age-related diseases, but of no less importance is to reveal interrelatioships between genes and environmental factors. Life style and social economic factors have a major impact on human longevity. In the oldest age groups, death rates no longer decelerate exponentially with age [39]. The most probable explanation of this phenomenon consists not in the deceleration of aging as such, but in that this deceleration reflects the heterogeneity of the population, for example, frail people die earlier that robust. That intriguing fact that decreased death rates and even the "plateau effect" in the oldest age groups are characteristic not only of humans, but also of certain insects, raises a number of questions of

principle, which still have to be answered satisfactorily [35, 39].

Of interest is the bioeschatology concept of Akif'ev and Potapenko [40], focused on search for molecular genetic mechanisms undelying, on the one hand, senescence and death of individuals and, on the other, extinction of species [40]. Studying features of radiation-induced lifespan shortening in drosofila, the researches found out that aging occurs in a hidden form over the whole life in adult flies, but its outcome, i.e. death, takes place after a certain interval. Moreover, the final phase of aging is triggered by disorders in functioning tissiue-specific genes of nerve cells. Popov [41], having leveled criticism at this concept, suggests that organisms tend to replicate themselves but are unable to make absolutely identical copies for an indefinitely long time. Therefore, a species changes from generation to generation even though it has already fairly well adapted to the environment. Therewith, since organisms change only in definite directions in force of their characteristic physical and chemical limitations, the species, too, changes in definite directions, even though the already formed directions are irrational and lead to extinction. Makrushin [42] suggests that extinction of species is aimed at eliminating carriers of a concervative genetic information which interferes with biosphere development.

The above-described evolutionary theories predict that the rate of aging should correlate with the rate of intrinsic mortality rise. When intrinsic mortality is low, individuals can live very long, and there will be enough time for natural selection. In its turn, the process of natural selection will slow down aging and extend life expectancy due to natural selection which acts in favor of anti-aging mechanisms and against mutations and genes finctioning to shorten life and having the properties of antagonistic pleiotropy. By contrast, at a high extrinsic mortality, not so many individuals will live long under the above natural selection conditions. In this case, the evolution process will accelerate aging and shorten life expectancy. Surely, natural selection will have impact on an individual by making its less vulnerable to extrinsic mortality factors. However, since Darwin's "hostile forces of nature" are not infrequently stochastic (for example, bad weather or lack of food) or coevolutionary (predators, competitors, diseases), external reasons of death cannot be eliminated completely.

A large body of evidence has been accumulated, showing that aging and maximum life expectancy correlate with extrinsic mortality, as predicted by the evolutionary hypothesis. For example, bats whose ability to fly save them from predators, can live three times longer than terrestial animals comparing with them in size and metabolism rate [43]. Birds, too, hunt on terrestial animals which compare with them in size, and in captivity birds can live much longer than terrestial animals of the same size, even though their metabolism rate is higher [43]. Opossums (*Didelphis virginianis*) inhabitating on islands where no predators have lived over many generations live double as long as continental opossums. Naked mole rats (*Heterocephalus glader*), socially highly organized animals living in big underground colonies and well protected from predators both by a tight ground and by collective protection, are extremely long-living animals (longer than 20 years) [24]. The "queen" in socially organized insects (ants, bees, and termites), who resides in the most safe and protected place, can live 10–100 times longer than worker species in captivity.

Life expectancy is directly related to the early commencement of reporoduction and its intensity in drosofilas, guppy, and, probably, humans [44, 45]. Predictions of the evolutionary theories of aging are supported by certain empirical observations. The mechanisms underlying slow aging of long-living and well protected species can operate at the cellular level. Thus, in mammals a positive correlation between cellular stress resistance and maximum lifespan [46]. The contents of ROS in tissues of long-living mammals and drosofilas is higher compared with short-living species [47]. Blanco and Sherman [48] have analyzed lifespan data for 1193 fish, reptile, and amphibia species to reveal a clear correlation between maximum lifespan and body size for each phylogenetic group [48]. Species living in safe conditions have a longer lifespan than those living in unsafe conditions. These data provide convincing evidence for an important role of such factor as protection from predators in the evolution of aging.

Developing the aging initiation and evolution concept, Boiko [49] mentioned that aging is not an unnecessary attribute of multicelled organisms, it is only characteristic of certain phylogenetic groups. First multicelled organisms on the Earth have inherited from single-celled eucariots cellular senescence mechanisms and programmed cell death (apoptosis), which pose virtually no limit on the lifespan of individuals. Senescence and death by senescence are, according to Boiko, are later evolutionary acquirements emerged

with the development of unitary organization of multicelled organisms. Boiko suggests that senescence (age-related self-destruction by intrinsic reasons in multicelled organisms) develops on the background of primary immortality). In his opinion, senescence and death by senescence in not a necessary but desirable attribute of multicelled syngenetic organisms, since reproduction facilitates species-formation processes at the species formation phase, thereby driving expansion of phylogenetic groups. In other words, the existence of an intrinsic self-destruction mechanism gives certain evolutionary advantages, specifically acceleration of the evolution process and replacement of one species by others, which creates prerequisutes for evolutionary progress [49].

Interesting, while quite controversial, concepts of the nature of aging have been developed by Kordyum [6]. Suggesting that life is a form of existence of information which serves for self-sustaining and self-existence, he stresses that life does not struggle against aging. Life exists beyond aging and eliminates even "embryonic plasma" at the approaches to aging, since everything creating obstacles on the way to aging already potentially "does not fit." This is the basic principle: not a struggle against aging (which is conceptually impossible by the basic laws of existence and condemned in advance) but existence beyond aging.

According to Kordyum, the line of "embryonic plasma" passes through a vicious sieve of all kinds of fit selection. A real standard is selected due to elimination of all information carriers where this strandard is not fitted completely. And all individuals present for life as a phenomenon are nothing but a means to provide this continuity. Already over 4 billion years life has sacrificed individuals to sustain its existence beyond aging [6]. As the author states further, every-thing else in the biosphere, incluing all individuals and their groups, i.e. species, become, after they have fulfilled their task (or have attempted not to fulfill it), nothing but a consumed material or wastes. Aging is a form and phenotypical mechanism of their elimination. Individual that have fulfilled the "quantum" task of life to exist beyond are no longer needed.

### Aging as a Slow Phenoptosis

Over the past years, Academician Skulachev has developed the concept of phenoptosis as a mechanism of aging and programmed death [50–52]. Phenoptosis is defined as the process of biochemical self-destruction at the organismal level. Mitoptosis, apoptosis, and collective apoptosis or organoprosis are the mechanisms of programmed self-destruction at the mitochondrial, cellular, and supercellular levels, respectively. It is suggested that any sufficiently complex biological system has a program oa self-destruction of its contituent parts. Such program is realized if a given constituent part proves to be harmful (or, sometimes, simply unnecessary) for the system as a whole [51].

The author exemplifies the phenoptosis of once-reproduced multicellular organisms by *Imago Mayfly* whose lifespan is a little longer than a day, the mite *Adactilidium* whose youngs hatch inside the mother's body and eat their way out, thereby killing their mother, certain squids whose males dye immediately after they have released their spermatophors under a female's skin, female octopuses who lose appetite and dye from starvation after their youngs have hatched from eggs; bamboo which can live 15–20 years reproducing by vegetation but dry after flowering at the height of summer immediately after seed ripening.

A well-known example of programmed death is provided by the salmon *Oncorhynchus* which dyes exhibiting signs of accelerated senescence shortly after spawning. Therewith, the senescence process switches on when the salmon has left ocean and is ascending a river for spawning. During this period the blood levels of glucose, fatty acids, and cholesterol in salmon sharply increase (up to 25.9 mM or 1000 mg % during spawning), the adrenal function enhances, and thymus atrophy and adiposis develop. Salmon dyes of multiple myocardial, brain, kidney, and lung infarctions [53]. Senescence and death do not happen in salmons with removed adrenals or sex glands [54].

Skulachev [50–52] suggests that aging in animals, including mammals, is a slow phenoptosis. The biological meaning of this phenomenon is likely to consist in its accelerative effect on the evolutionary process. To certain limits, a shorter life may favor evolution by accelerating change of generations. These mechanisms will favor evolution if environmental situation will get worse. When the ebvironmental conditions get betted, evolution slows down, which is evidenced by decreased prolificacy and increaseв lifespan. After birds and mammals had occupied the air ocean, they became much less proficient and live several times longer than terrestial animals [43].

The biochemical mechanism of phenoptosis in eukariots involves ROS generation by mitochondrial

cells and cell apoptosis. Some evidence is available showing that intramitochondrial ROS have an important function in these processes: They attack primarily mtDNA and play a specific role in the regulatory cascade leading to aging of yeast, nematodes, drosophilas, and mammals [10, 51, 55, 56]. Trifunovic et al. [57] found that the expression of mutant mtDNA polymerase which is still able to synthesize DNA but no longer able to control this synthesis results in a considerable increase of the mtDNA mutations rate, especially in the cytochrome *b* region, development of many signs of aging, as well as reduction of lifespan. Similar data were obtained by other researchers on $PolgA^{D257A/D257A}$ mice carrying mutation in the same locus of mtDNA polymerase. [58]. Introduction of cyclosporin A, a potent inhibitor of the inner mitochondrial membrane, prevented mtDNA mutations in mice in which DNA polymerase modifications were restricted exclusively by the heart muscle [59].

As mentioned above, mitochondria of birds produce much less hydrogen peroxide than mitochondria of mammals of the same weight [12]. The same difference is observed between bat and shrew: The lifespan of the latter is 17 times shorter than the lifespan of the former. These data provide supportive evidence for the viewpoint that the "rheostat" that contols the lifespan is the rate of intramitochondrial ROS generation.

## Marginotomy Theory

In 1971, Olovnikov was the first to mention the problem of incomplete terminal DNA replication and formulated its essence in terms of the so-called theory of marginotomy [60]. He suggested that the cyclic form of procaryotic genome is a means to protect the latter from the incomplete terminal DNA replication effect. In a series of his theoretical works, Olovnikov also predicted the fact of shortening chromosome DNA ends on doubling normal somatic cells, which allowed to explain the reason for the Hayflick limit of cell division [61]. Olovnikov suggested that in germinative (sex) cells such form of DNA polymerase should be expressed that is responsible for maintaining a stable telomere length at the chromosome ends of these cells.

Excellent evidence for this hypothesis was obtained in 1985, when the predicted polymerase was identified as a telomerase [62]. At the same time Olovnikov predicted that the same DNA polymerase (i.e. telomerase) which is coded in the genome specifically for the sake of germinative cells can be expressed in cancer cells and it is this polymerase that endows cancer cells with potential immortality, i.e. unlimited cell division. An important supplement to the theory of marginotomy was an indication that the shortening of telomere DNA in certain species can be compensated for in one more way, specifically by adding to the chromosome end of a ready DNA fragment from the outside. As shown, the telomere DNA in sex cells of drosophilas, shortened as a result of imcomplete replication, can be lengthened by the transposition mechanism operating to transfer the DNA fragment to telomerase end. And, finally, it was suggested [63] that in normally divided somatic cells shortening of the buffer telomere DNA entails a loss of informationally significant sequences and thus initiates cell aging (the telomere theory of cell aging).

Olovnikov also predicted existence of telomerase as an enzyme compensating replicative telomere shortening. Apart from a purely scientific value, Olovnikov's predictions created prerequisites for search for telomerase inhibitors for anticancer therapy applications.

Later in 2000, Olovnikov advanced a theory of genome function regulation (fountain theory), which was based on the concept of the regulatory function of ionic channels in the inner nuclear membrane [64]. Disturbances in their function may result in accumulation of chromosome damage and certain epigenetic effects, for example, such as changes in gene position, which may contribute to organism aging. The author suggests that the primary mechanism of aging is a "quantitative traits disease" whose course is affected by the nature of ionic modulation of transcriptional gene productivity. As telomeres in aging cells get shorter, ionic channels dependent on so-called fountain RNAs may prove inaccessible for subtelomere genes, which may affect the productivity of the corresponding genes and serve as a factor of cell aging.

These views are, to a certain extent, consistent with viewpont of Makrushin [65], who noted that the senescence of cells capable of division is not a reason of death of senecsence, since death occurs before the replicative limit is ehxausted. Analysing invertebrate senescence processess, the author concludes that the destruction of vitally important organs in invertebrates which reside at the base of the phylogenetic tree of the Metazoa forms a part of the normal ontogenesis process. This process is accompanied by adaptation to

environment deterioration and by sexless reproduction. Over the course of evolution, the reversibility of these destructive processes was lost, and their value changed. One of them, namely involution, became to cause death of senescence.

### Neuroendocrinologic (Elevation) Theory of Aging and Development of Age-related Pathology

The elevation theory of aging and development of age-related pathology in higher organisms belongs to the most bright and thoroughly developed concepts in gerontology [3, 66, 67].

In early 1950s, Dil'man, based on his research on the age-associated dynamics in women of such parameters as the state of reproductive function, presence of hot flashes, and excess body weight and cholesterol level, advanced and substantiated an idea that there exists a common regulatory mechanism responsible for the onset and development in an organism during its ontogenesis of various homeostatic systems. In his opinion, this mechanism is the age-related attenuation of hypotalamus sensitivity to regulatory homeostatic signals. Experimental research and clinical observations showed that just this process causes age-related switching-on and switching-off the reproductive function in a woman's organism, age-related changes in the hypothalamic–pituitary–adrenal (HPA) system providing the tonic level of blood glucocorticoid hormones, and their circadian cycle and stress-induced secretion enhancement, which gives rise to hyperadaptosis. Analogous age-related changes in the metabolic homeostat system which controls appetite and energy supply, include increase of body fat content, attenuation of tissue sensitivity to insuline (prediabetes), and development of atherosclerosis

The elevation theory discovers the role of age-related changes in the three main superhomeostats (reproductive, adaptative, and metabolic) in forming phenomena of key importance in terms of life expectancy, such as metabolic immune depression and cancrophilia, i.e. in creating conditions favoring malignant tumor development [3]. Developing and deepening his concept for over 40 years, Dil'man came to a conclusion that senescence and senescence-related diseases are not programmed events but are nothing but by-products of the implementation of a genetic program, and, therefore, senscence are natural results of the genetic program [3, 67]. The Dil'man's ontogenetic model of the development of age-related pathology opened-up new approaches to prevention of untimely aging and age-related diseases most commonly leading to human death or, according to Dil'man, principal diseases. These diseases include heart diseases, malignant neoplasms, cerebrovascular pathology, metabolic immune depression, atherosclerosis, old-age diabetes and obesity, mental depression, autoimmune diseases, hyperadaptosis, and some others. The ontogenetic theory of disease development suggests that the development of such diseases can be slowed down by stabilizing the homeostasis state at a level reached by the end of organism development. Dil'man suggests that the human lifespan can be prolonged by slowing down the senescence process.

More than 40 years have passed since Dil'man published *Lancet* [66] his paper with basic postulates of the elevation theory. How today we can evaluate his contribution to the development of modern concepts of the mechanism of aging? Let us consider this question on three examples.

At present sufficient evidence has been accumulated in favor of the Dil'man's hypothesis on the role of primary changes in the hypotalamic regulation of the reproductive function in its switching-off mechanism. Thus, evidence showing that the level of gonadotropins (sex hormones) in women's blood increases with age [68]. The basic reason for this age-related changes lies, in Dil'man's opinin, in gradual increase of the hypotalamus sensitivity threshold to homeostatic inhibition by sex hormones. Look at what the prominent German researcher Rossmanith writes in [69]: "The age-related dynamics of gonadotropin secretion in post-menopause women shows that these changes are a function of aging. As a result, primary changes in the hypotalamus rather than in hypophyse are responsible for aging in women. Moreover, aging can also disturb the sensitivity of the hypotalamus to ovary sex hormones by the negative feedback mechanism, and, therewith, disturbances in the regulation of central neurotransmitters occur in parallel with that." Dil'man formulated the mechanism of age-related switching-off of the reproductive function just in the same way and supported this hypothesis by the results of clinical and experimental research performed at his laboratory [70]. Wise et al. [71] established that in middle-age rats the hypotalamic structures reposponsible for the ovulatory release of the gonadotropin-releasing hormone become less sensitive to estrogen, and this is the main reason for switching-off the reproductive function. Unfortunately, these two

comprehensive reviews have not even mentioned the earlier priority research of Dil'man and co-workers.

The unevoidable age-related build-up of body weight and fat content is considered by Dil'man as a consequence of genetically programmed increase of the sensitivity threshold of the hypotalamic saturation center to "glucose and insulin signals", and, therewith, he attached to insuline particular significance in the development of metabolic disorders [70].

Facchini et al. [72] postulated that hyperinsulinemia can favor oxidative stress, therewith favoring, irrespective of hyperglycemia, aging and age-associated diseases, such as diabetes, atherosclerosis, hypertonia, and cancer. Hyperinsulinemia develops as a secondary disease in connection with the disturbed ability of insulin to stimulate glucose metabolism in skeletal muscles (resistance to insulin develops). The authors suggest that their hypothesis is confirmed by the observation of an essentially prolonged lifespan of the nematode *C. elegans* with mutations inhibiting insuline signal transmission [73] or prolonged lifespan on restriction of calorie intake and the attendant decrease of glucose and insuline blood levels and oxidative stress [74].

Decreased levels of the growth hormone, insuline, and insuline-like growth factor 1 (IGF-1) are the key factors serving to prolong the life of Ames dwarf mice [75]. Female mice with a partially knocked-out IGF-1 receptor gene ($Igf1r^{+/-}$) exhibited a 33% longer lifespan compared with wild female mice ($p < 0.001$) and no dwarfism. The basic metabolism, body temperature, food consumption, physical activity, and fertility in them were the same as in control mice. The spontaneous malignancy rate, too, did not differ from control. At the same time, the $Igf1r^{+/-}$ mice features a higher resistance to oxidative stress than control wild mice ($Igf1r^{+/+}$) [76]. Fat-specific insulin receptor knock-out (FIRKO) mice showed decreased body fat contents and prolonged (by 18%) average and maxium lifespans [77].

It was found that people 100 and more years old are much less frequently resistant to insulin and more frequently preserve the function of β cells of the insular apparatus than younger people [78]. Facchini et al. [72] consider the resistance to insulin and hyperinsulinemia as new inportant carcinogenic factors.

Note that certain pharmaceuticals, in particular antidiabetic biguanides, which enhance the sensitivity of tissues to insulin and their tolerance to carbohydrates, decrease the level of lipids, and eliminate metabolic immune depression, could prolong the life of mice and rats and decrease the rate of spontaneous and chemically or radiation-induced malignant tumors [74].

Decreased hypothalamic threshold of sensitivity to inhibition by glucocorticoids is observed not only on aging, but also on accelerated development of a series of age-related diseases, including decrease of cognitive function [67, 79]. It was established that this process is contributed to a certain extent by a decrease in the quantity and effectiveness of glucocorticoid receptors in hyppocamp and dysregulation of the HPA axis [80]. *In vitro* experiments showed than an age-related decrease in the sensitivity of corticotropes to glucocorticoids suggesting that there is a direct, pituitary-mediated dysregulation of the HPA axis in rat starts as early as middle age [81]. It was established that one of the first units in the chain of organism disorders induced by genotoxic stress is enhanced expression of the oncosuppressor and antiapoptotic gene bcl-2, which decreases the ability of dexametasone to suppress expression of propiomelanocortin and secretion of the adrenocorticotropic hormone by pituitary cortitrophs [82].

Further evidence for the Dil'man's view of the role of the age-related cholesterol accumulation in immune-competent cell membranes in the mechanisms of metabolic immune depression and its contribution in aging and development of age-related pathologies was reported in [83].

Thus, more and more evidence is being accumulated in favor of the Dil'man's hypothesis that the main age-related pathologies develop as a result of the realization of a general development program related simultaneosly to all systems in an organism. Golubovskii [84] noted that 20–25 years is a "normal" term for advanced discoveries to gain recognition. However, it should be mentioned that the Dil'man's theory fails to explain aging in species which do not have the hypotalamus.

## CONCLUSIONS

According to programmed aging theories, various modifications of genes controlling the aging process, should whether accelerate or decelerate aging. Numerous examples of such modifications in laboratory mice are known, prividing evidence to show that the aging process involves quite a wide spectrum

of genes [83]. Many of these genes control the stability of the genome (for example, DNA repair genes), while other are responsible for maintaining proliferative tissue homeostasis (telomerase gene, apopotosis and intercellular junction genes, etc.), for carbohydrate bioenergetics and metabolism (genes controlling signaling in the growth hormone–insulin–IGF-1–glucose system), and for the onset and development of age-related diseases, for example, tumors. Mutations or knock-out of certain circadian rhytm genes (*Per-2, Bmal1, clock*) cause accelerated aging syndromes, each having its specific features [83]. The very fact that there is a great variety of genes whose modifications can really prolong or shorten life casts doubts in the existence of a universal aging program.

The same conclusion follows from the consideration of data on the pharmacological modification of aging [83, 85]. Quite different pharmaceuticals acting on quite different targets in an organism are able to prolong the lifespan essentially.

Let us now come back to the question in the title of this paper: "Is There an Unambigous Answer on the Question: Whether the Aging Program Exists or Not?" In our opinion, this question is impossible to answer now. It is also impossible to give an unambiguous definition of aging or choose unambiguous criteria for differentiating between the "normal" aging and age-related diseases [83].

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