Current Aging Science, 2020, *13*, 31-41

REVIEW ARTICLE

The Mechanism of Programmed Aging: The Way to Create a Real Remedy for Senescence

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ARTICLE HISTORY

Received: August 08, 2019
Revised: September 17, 2019
Accepted: September 18, 2019

DOI:

10.2174/1874609812666191014111422

Abstract: ***Background*:** Accumulation of various damages is considered the primary cause of aging throughout the history of gerontology. No progress has been made in extending animal lifespan under the guidance of this concept. This concept denies the existence of longevity genes, but it has been experimentally shown that manipulating genes that affect cell division rates can increase the maximum lifespan of animals. These methods of prolonging life are unsuitable for humans because of dangerous side effects, but they undoubtedly indicate the programmed nature of aging.

***Objective*:** The objective was to understand the mechanism of programmed aging to determine how to solve the problem of longevity.

***Methods*:** Fundamental research has already explored key details relating to the mechanism of programmed aging, but they are scattered across different fields of knowledge. The way was to recognize and combine them into a uniform mechanism.

***Results*:** Only a decrease in bioenergetics is under direct genetic control. This causes many different harmful processes that serve as the execution mechanism of the aging program. The aging rate and, therefore, lifespan are determined by the rate of cell proliferation and the magnitude of the decrease in bioenergetics per cell division in critical tissues.

***Conclusion*:** The mechanism of programmed aging points the way to achieving an unlimited healthy life; it is necessary to develop a means for managing bioenergetics. It has already been substantially studied by molecular biologists and is now waiting for researchers from gerontology.

Keywords: Longevity, genetic program, natural selection, bioenergetics, mechanism of aging, aging clock, rate of aging.

1. INTRODUCTION

Hippocrates (460-377 BC) was the first person to propose a materialistic (not mystic) viewpoint on the nature of aging and lifespan limitation. He supposed that aging is caused by the gradual loss of the natural heat which is given to each organism at birth in a restricted amount. This viewpoint persisted in science until the end of the 19th century and was confirmed by accurate experiments: Max Rubner (1854-1932) performed calorimetric studies on people of different ages and reported a gradual decrease in heat production. At the same time, August Weismann, the founder of modern genetics, suggested that longevity is controlled by natural selection and is a necessary condition for the survival of the species [1]. The emergence of these two foundational concepts did not lead to the stimulation of aging research because neither knowledge about the nature of biological heat, nor knowledge in the field of genetics was then available. Some cytologists of that time believed that mitochondria are intracellular parasites, having observed their ability to move and change shape. Until the middle of the 20th century, limited research into the nature of aging was based on the assumption of the harmful effects of external factors. For example, the famous physiologist of the time, Ilya Ilyich Mechnikov believed that aging was caused by intestinal microflora and phagocytes. In turn, a radical remedy for aging was proposed: the removal of the colon.

The rapid intensification of research on aging occurred in the mid-1950s after Peter Medawar subverted Weismann's point of view. He pointed out that, in their habitat, animals never die of old age, but perish from various external causes. Thereby, natural selection cannot differentiate them according to longevity, meaning that this attribute is beyond the control of natural selection. Consequently, the genes of aging and longevity cannot exist, while aging is the result of the destructive action of harmful factors; simply put, wear and tear [2]. The "Free radical theory of aging", which supported Medawar's viewpoint, was soon put forward as the concrete mechanism of aging [3]. Soon after, George Williams offered an "Antagonistic pleiotropy theory", a new evolution-

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ary explanation for aging, which differed from Weismann's supposition [4]. Tom Kirkwood clarified the question regarding the species specificity of longevity: "Aging is not programmed but results from the accumulation of somatic damage, owing to limited investments in maintenance and repair" [5]. As molecular biology evolved, more and more new damaging agents, which confirmed the validity of Harman's mechanism of aging, were detected. Detection of Reactive Oxygen Species (ROS) generating by mitochondria became the most significant of these because it showed that the main source of damaging agents is inside the body. The mitochondrial theory of aging created on the base of this phenomenon [6] declared that ROS cause damages in mitochondrial DNA (mtDNA) and respiratory chain, which lead to a further increase in ROS production. As a result, a vicious cycle is formed, causing the progressive degradation of all body functions. The theoretical foundation of the concept of the accumulation of stochastic errors was found to be very convincing. Indeed, experiments have provided indisputable evidence that mitochondria generate ROS, which produces mutations in nuclear and mitochondrial DNA, that errors occur in the processes of DNA reduplication, transcription, and translation, that lipid peroxidation in membranes happens, and that non-enzymatic glycolysis and cross-linking between molecules takes place and more, all of which accumulates over time. The conclusion about the nature of aging is obvious. The methods of combating aging following on from this are also obvious: the prevention and repair of damage. Almost all experiments seeking to increase the maximum lifespan of animals, performed during the 20th century, were based on this concept. All possible approaches developing from this concept have been tested, but none of them has given a positive result.

In the meantime, in the last decade, convincing evidence that the aging process is under direct genetic control has been found: the mutation of the only DAF-2 gene has doubled the maximum lifespan of the *C. elegans* [7]. Some other genes with similar properties have subsequently been discovered [8-11]. To date, about 80 genes have been identified whose modification leads to an increase in the maximal lifespan [12]. All these genes have one thing in common: they are involved in metabolic pathways that ensure that the division of cells, while the damage they incur from mutations or various chemical and physical factors slows down cell proliferation. A reasonable explanation for this phenomenon has not been found to date [13]. Despite this, in the last decade, this area of practical research has increasingly moved to the forefront of the science of aging [12]. However, any anti-aging remedy suitable for human use remains elusive because the increase in longevity in current experiments is accompanied by unacceptable side effects. Takahashi and Yamanaka presented additionally strong support for the concept of programmed aging. They found four transcription factors that can convert old differentiated somatic cells into pluripotent stem ones, which can again be differentiated into specialized cells of any tissue [14]. Their functional parameters return to the high level inherent in embryonic cells. This level is maintained after their differentiation back into the original somatic cells [15, 16]. This incontestably shows that changes in the functional state of genes, rather than stochastic damages, are responsible for age-dependent attenuation of all organism functions.

The present situation is paradoxical: the conception of stochastic errors, which is well-founded and highly convincing, is unable to deliver any applied outcome. Meanwhile, proponents of the conception of programmed aging report impressive practical results, but they have no understanding of the mechanisms that underlie these phenomena. There was an objective reason for this situation emergence. Medawar's basic idea, which claimed that lifespan is not under the control of natural selection, was erroneous. In his time, his conclusion was valid, since only Darwinian individual natural selection was known. A little later on, the existence of group selection was revealed [17], whereby not only individuals but also a group of individuals [populations, species] are objects of selections. This form of natural selection is able to maintain attributes that are useful for the survival of the group, even if they are disadvantageous or harmful for individuals (for example: bravery, altruism, care for offspring). It is this form of natural selection that controls the species-specific lifespan [18-20]. This adjusts longevity according to the pressure of environmental factors in the ecological niche of the species: the more harmful the habitat, the shorter life is, and *vice versa* [18]. It is this lifespan, established by natural selection, that ensures the maximum reliability of the existence of a population (species) and its potential immortality, although it is fatal for each its individuals. This conclusion on the programmed nature of longevity was made using computational methods widely adopted in the field of ecology and evolution but is not popular in gerontology. Therefore, the Medawar's argument is considered as correct, and the Antagonistic pleiotropy theory continues to be popular.

2. THE MECHANISM OF PROGRAMMED AGING

2.1. Longevity is Under Direct Genetic Control *via* a Programmed Bioenergetics Decline

The central role of mitochondrial bioenergetics in aging was recognized at a time when the mitochondrial theory of aging was created [6]; this viewpoint has been preserved until today. In their latest review, Moh Malek and co-authors offered a modern generalized viewpoint on the role of mitochondria and bioenergetics in aging as follows: "Mitochondria are central to all basic and advanced cellular and organismal functions. In addition to the vast majority of cellular energy generated by these unique organelles, they are also essential signalling hubs and communicate with the rest of the cell through various means including reactive oxygen species" [21]. The point of view on the cause of the age-related decline in bioenergetics changed along with improvements in research methods and the accumulation of knowledge. For example, a group of researchers led by Jun-Ichi Hayashi originally confirmed the dominant notion that an age-dependent decrease in bioenergetics is caused by mutations in mitochondrial DNA [22]. Further experiments led them to the conclusion that nuclear rather than mitochondrial genome is involved in age-related mitochondrial dysfunction [23]. In this research, they also measured the level of energy production by mitochondria along the activity of cytochrome c oxidase in fibroblasts taken from 16 people at the age of 0-

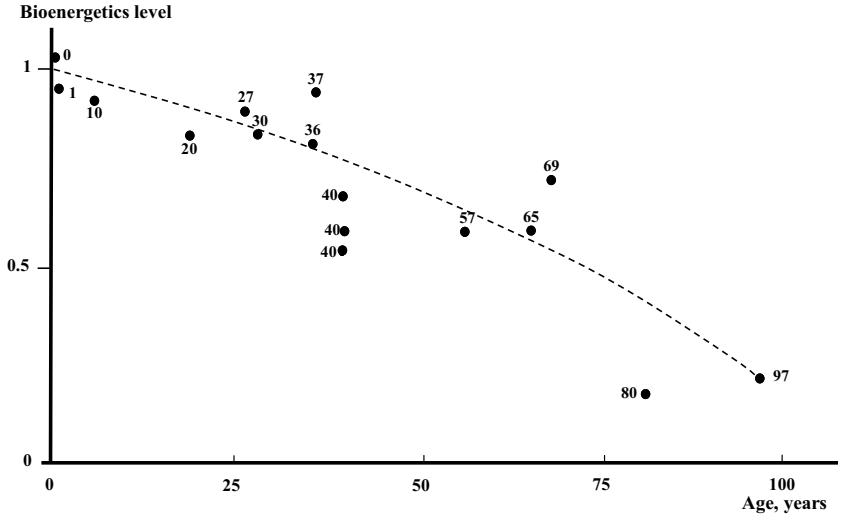

Fig. (1). Age dependence of mitochondrial energy production in human fibroblasts (according to the data of Hayashi *et al*., 1994). The bioenergetics level was measured *in vivo* according to the activity of cytochrome c oxidase. The ordinate axis shows the relative value of the bioenergetics level (bioenergetics of a new-born child is taken as the unit); the numbers near the points indicate the age of the fibroblast donor.

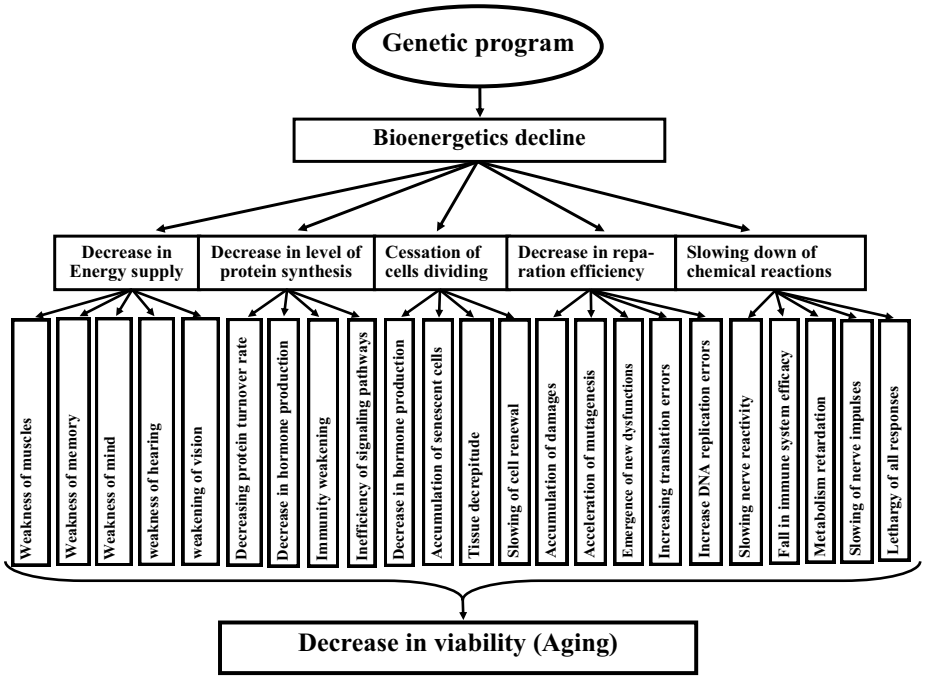

Fig. (2). Scheme of realization of the genetic program of aging. Only a decline in bioenergetics is under direct genetic control. This immediately leads to a number of detrimental processes, each of which generates a series of secondary destructive phenomena. This growing avalanche of malicious processes serves as the execution mechanism of the aging program. It causes the dysfunction of all organism functions, diseases and, ultimately, death.

97 years. Fig. (**1**) shows the curve of the age dependence of the bioenergetics level, which was constructed using the results of the mentioned experiments.

The curve indicates that the average level of mitochondrial energy production gradually decreases with aging against the background of significant individual distinctions. Recently, these authors concluded that this age bioenergetics decline is directly programmed by nuclear genomes and this phenomenon is reversible [16]. Since bioenergetics energizes all physiological, biochemical, and biophysical processes [24-27], its programmed decline inevitably leads to a weakening of all vital processes in the organism, which causes a decrease in physical and mental powers and many detrimental phenomena designated as illnesses. The programmed decline of the bioenergetics level alone is enough to cause all destructive processes accompanying the aging process. The genetic program directly triggers the bioenergetics decline only. The latter causes several harmful processes, each of which breeds, in turn, several secondary detrimental phenomena [28]. In this way, two conceptions of the nature of aging - stochastic errors accumulations and programmed - which seem to be incompatible, are combined (Fig. **2**).

Both conceptions turned out to be correct: the programmed decline in bioenergetics is the real primary cause of aging, and the avalanche of destructive processes triggered by it is the immediate mechanism of execution of the aging program. Only the components of the execution mechanism of programmed aging are revealed by researchers in experiments. In light of this, an impression arises that the prime cause of aging is multifactorial, whereas the only actual driving force remains hidden.

Two essential inferences follow from this: 1) Manipulations with any secondary destructive processes are not able to give the effect of an extension of maximum lifespan. This is confirmed by the entire history of practical research in gerontology; 2) To achieve an unlimited healthy life, it is necessary to develop a means to govern bioenergetics – this is the only way to succeed.

2.2. What a Parameter of Bioenergetics is Under Direct Genetic Control

Since the conception of stochastic errors has been dominant until now, gerontologists have not looked into the physicochemical essence of bioenergetics. Therefore, an age-related decline in bioenergetics is usually expressed by such inexplicit terms as "a decrease (decline) in energy production", "a defect of mitochondrial function", "mitochondrial dysfunction", "a defect in mitochondrial respiration", "a decline in mitochondrial function", or "dysregulated mitochondrial dynamics". A level of bioenergetics at the current time is usually measured by the amount of oxygen absorbed per unit of time. This is enough for resolving of some specific tasks but insufficient for understanding the mechanism of programmed aging and resolving the longevity problem. To achieve the main goal, it is necessary to find out what parameter of bioenergetics is directly controlled by the genetic program, what molecular mechanism performs this program and the possible means of its modification. Therefore, knowledge of the parameter of bioenergetics, which is under direct genetic control and can be measured by physicochemical methods, is absolutely necessary.

The functional state of any energetic system (mechanic, electric, aerodynamic, chemical, *etc*.) can be quantitatively described by several interdependent parameters. Two of these, an original energetic potential or a driving force (F) and the effect it causes (P), are basal. They are linked by a simple relation that is given in textbooks around the world and easily understood by everyone:

$$F = kP, \quad (1)$$

i.e., a driving force causes a direct proportional effect, or the effect is directly proportional to the driving force. In each of the energetic systems, the two have different designations, but the same sense. For example, in mechanics, these are force and work (coefficient k is friction); in electrical engineering, these are the voltage and amperage (*i.e*., Ohm's law, where k is the resistance or conductance). In chemical thermodynamics (in particular, in bioenergetics), the original energetic potential F represents a free energy change, ΔG (Gibbs energy). ΔG for macroergic (high-energy) coenzymes that function in the biological energy-generating system (ATP, NAD, NADP, GSH, *etc*.) is determined by the value of their concentrations ratio of the reduced form to the oxidized one and by the temperature. For ATP, in particular:

$$\Delta G = \Delta G^0 - RT\ln[ATP]/[ADP][[P_i], \quad (2)$$

where: ΔG^0 is the standard Gibbs energy that is measured with everything at a one-molar concentration: [ATP] = [ADP] = $[P_i]$ = 1M; R is the gas constant; T is the absolute temperature. The more negative the Gibbs energy, the higher the driving force a bioenergetics machine creates. Since ΔG^0 and R are parameters, then the [ATP]/[ADP] ratio is the only variable to determine the energy potential ΔG for warm-blooded animals (second variable, temperature, is constant for them). Consequently, for them, the formula (1) may be rewritten as:

$$[ATP]/[ADP] = kP, \quad (3)$$

where: [ATP]/[ADP] is the driving force or energy potential; P is the energy flow [effect]; k is the real-time regulator of the energy flow. The [ATP]/[ADP] ratio generated by mitochondrial bioenergetics machine predetermines the capacity of any biological system to work. It is this parameter of bioenergetics that is decreased by a genetic program to drive aging. The performance efficiency of bioenergetics depends on the ATP/ADP ratio rather than the absolute value of ATP or the number of mitochondria in cells. For example, the maximum weight that a weightlifter can lift, having a certain muscle mass, depends on the ATP/ADP ratio in his mitochondria, with the number of mitochondria in muscle cells determining how many times he can lift it. Over the years, the strength decreases, even if the muscle mass and the number of mitochondria in the muscles remain the same. The value of the ATP/ADP ratio is denoted below simply as the "bioenergetics level".

2.3. How the Aging Program Declines Viability

In a recent review, Lipsky and King denoted the central event of aging thus: "Commonly defined as the accumulation of diverse deleterious changes with time, aging exponentially increases the risk of death. By the age of 80 years, the relative risk of dying is more than 300 times greater than for someone aged 20 years. Despite the inevitability of aging and its importance to health, how and why we age remains a poorly understood aspect of human biology" [29]. Simultaneously, Sun and colleagues summarized the available information on the mitochondrial underlying this phenomenon: "A decline in mitochondrial quality and activity has been associated with the normal aging and correlate with the development of a wide range of age-related diseases" [24]. Now, we have the opportunity to clarify the question about how this decline in mitochondrial function contributes to aging and how aging exponentially increases the risk of death.

In the habitat, all organisms are under pressure from environmental factors, namely: infections, predators, cold, heat, hunger, strong stresses, typical and extreme physical and chemical influences, natural cataclysms, and many others. To survive, all organisms contrast the special protective factors against each of these challenges. The efficiency of these factors depends on the level of energy supply. If the force of the pressure of any environmental factors exceeds the effectiveness of the opposing defence factors, the organism per-

ishes. The environment pressure continuously fluctuates, occasionally reaching very large values. They are as inevitable as they are unpredictable. Because of this, there is always a risk of death for any organism at any age. Nevertheless, every death has its own viewable cause (it was this fact that Medawar noticed and used to disprove Weisman's postulation on the programmed nature of aging). The risk of death is increased during the course of life because the genetic program progressively reduces energy supply, which weakens the immune system, decreases physical and mental abilities, and dulls sensory organs, *i.e.*, viability declines. Natural death occurs when the level of energy production fails to provide even internal needs. Given the environmental pressure, a majority of a species' individuals perish long before the energy generation is reduced to a critical level of natural death. The rare lucky ones manage to avoid death from environmental factors and live the entire programmed maximum lifespan and die a natural death. Thus, it is the programmed decline in the ATP/ADP ratio, produced by a mitochondrial energy-generating machine, which causes an increased risk of death with age.

3. BIOENERGETICS AGING CLOCK AND ITS EFFECTS

3.1. The Existing Systems of Biological Timing

There are a number of terms concerning temporal phenomena in living systems, which are similar but not identical: mitotic clock, physiological clock, biological clock, epigenetic clock, ontogenetic clock, and aging clock. The aging clock here implies an internal mechanism that operates the rate of the realization of the aging program and predetermines species-specific longevity. Two molecular systems are now known to pretend this role, while another is a timer that indicates the biological age.

The notion of a mitotic aging clock currently dominates. The theory of replicative senescence is based on the doubtless fact that cells of higher eukaryotes enter a non-dividing but viable state after a certain number of duplications; this is called the Hayflick limit. It is assumed that the accumulation of non-dividing cells in tissues finally leads to organism degradation [30]. The mechanism of this phenomenon is that the ends of eukaryotic chromosomes have multiple repeating TTAGGG nucleotide sequences, called telomeres. They prevent end-to-end chromosome fusion and protect DNA from nuclease digestion. Telomeres are synthesized in embryonic cells by a special telomerase enzyme which is absent in most somatic cells. The telomeric chromosome ends of somatic cells become 50-200 nucleotides shorter with each division. As a result, after a certain number of duplications, the telomeric end is exhausted and divisions cease due to chromosome erosion [31]. This process is recognized as a mitotic clock [32], which is obvious and convincing, but now refuted by many facts: the aging cells that have the telomerase activity have been discovered and *vice versa*, plus potentially immortal tumour cells which lack telomerase activity have been found. Blasco and co-authors, who reported the most convincing data, managed to obtain mice zygotes that lacked the telomerase gene but had full-sized chromosome telomeric ends. The mice that developed from these zygotes proved to be viable and fertile. This initial length of telomeres was sufficient to support the normal viability of six generations [33]. Next, this group of researchers obtained an analogous mouse line, but with shortened telomeric ends. The mice, in this case, were only viable for four generations [34]. At present, many researchers hold the opinion that the loss of telomeric ends actually results in chromosome erosion and cell death. However, the termination of cell proliferation in the process of normal physiological aging takes place earlier than this critical moment. The telomeric apparatus cannot, therefore, serve as an aging clock, while it is an additional barrier on the path to the multiplication of malignantly transformed cells.

The neuroendocrine theory of aging developed by Vladimir Mikhailovich Dilman, under the name of the "grand biological clock", has also been referred to as the aging clock theory [35]. Just as in a technical clock, the mechanism of time reckoning in this clock is based on rhythmic oscillations which are performed by a special oscillatory circuit [36]. There are time (circadian) genes in each cell of all eukaryotic organisms. In vertebrates, they are mostly active in the suprachiasmatic hypothalamic nucleus. These genes express the proteins that inhibit their own transcription. Such systems with negative feedback produce auto-oscillations because each unit of a system reacts to the signals of other units with some lag. The greater this lag, the larger the oscillation periods. The hypothalamic oscillatory circuit is adjusted so that its rhythms are close to daytime illumination rhythms. The signals from these circadian rhythms are transmitted into the pineal gland (epiphysis), which secretes melatonin in rhythm with the oscillations, with maximum secretion during dark times of the day. The core destination of the suprachiasmatic oscillatory system is to change the melatonin production. The rhythmic fluctuations in the concentration of melatonin in the blood change the daily activity of most of the endocrine system and, subsequently, the activity of many the organism's systems. As age increases, the production of melatonin by the epiphysis decreases, and daily biological rhythms are mismatched. This aggravates the diseases that accompany aging; it can also be a direct cause of their origin [37]. Daily administration of melatonin reduces circadian rhythms disturbances in older people [38]. All empirical data indicate that the circadian clock machinery orchestrates the organism metabolism to ensure that development, survival, and reproduction are attuned to diurnal environmental variations [39], but there is no reliable information on its role in the aging process. This is a physiological clock that generates cycles one after another but does not sum the results, which is necessary to perform the role of an aging clock. Tevy *et al*. noted in a recent review devoted to links between the circadian clock, metabolic functions, and aging that: "for unknown reasons, there is a decline in circadian rhythms with age, concomitant with declines in the overall metabolic tissue homeostasis" [40], *i.e*., neither a cause of the disorder in circadian rhythms nor an interconnection between circadian oscillations and aging has been found.

Steve Horvath elaborate the method, allowing us to estimate the physiological age of tissues by the state of DNA methylation [41, 42]. This epigenetic clock is widely used in practical medicine as an age measurer, but cannot influence the rate of aging or any parameter of the senescence process.

Via this clock, the author revealed two useful facts concerning our topic. First, regardless of the age of the original tissue, the age of induced pluripotent stem cells is close to zero and equal to an embryonic one. This confirms that aging is programmed and reversible. Second, the degree of DNA methylation correlates with the number of cell doublings which have elapsed, in turn confirming the correctness core of the bioenergetics aging clock presented below.

3.2. The Bioenergetics Aging Clock

The bioenergetics aging clock follows from the bioenergetics nature of aging, as presented, and some other empirical data as a logical consequence. Aging is inherent not only to multicellular organisms but also to the cells that are cultivated *in vitro*. Hayflick and Moorhead noted, in the conclusive part of their historic work on cell cultivation, that the amount of cell divisions in culture was only determined by internal factors, *i.e*., the number of reduplications was programmed in each cell [43]. This conclusion was drawn, based on experiments in which the growth of a culture of fibroblast cells, taken from human embryos, was interrupted by freezing to -70°C for different periods. Independently of the duration of these periods and their number, the irreversible termination of proliferation took place after the summary passage of about 50 divisions.

As mentioned above, the conventional viewpoint on the mechanism of the Hayflick limit, based on the telomere shortening, is now discredited. Instead, another mechanism, which follows on from analyses of a considerable quantity of empirical data concerning the mechanism of cell reduplication, has been put forward [44]. According to this proposition, there is a specific checkpoint at the boundary between the G1 and S phases in the cycle of cell division called the restriction point. All normal dividing somatic cells make a cycle suspension here; but, after a certain number of reduplication cycles, this checkpoint becomes impassable and cells enter the non-dividing state. The mechanism of this phenomenon can be summarized briefly as follows. The cyclin-dependent kinase inhibitor p27 prevents passage through this restriction point. There is a special molecular mechanism for its removal. The efficiency of its work depends on the supply of energy. When bioenergetics levels decrease under a certain threshold, this mechanism stops inhibitor removal while cell division becomes impossible.

Together with the data reported by Hayflick and Moorhead, this leads to the conclusion that the level of cell bioenergetics, and therefore age, are strictly related to the number of duplications that have elapsed (this is also confirmed by the above-mentioned Horvath's aging clock). This provides grounds for concluding that the genetic program reduces the level of cell energetics production intermittently in the process of every mitosis. Thus, the core of the mechanism of programmed aging appears to be very simple: every cell division is followed by a slight decrease in energetics generation which in turn causes some decline in viability.

Is this aging clock applicable to the organism as a whole? The fact of the matter is that the cells in culture grow old almost synchronously, but this situation is much more complex in an organism. First, the rate of cell division varies in different tissues from zero (*e.g*., nervous cells and cardiac muscle cells) to almost permanent (*e.g*., haemopoietic and some epithelial cells). Second, each tissue represents a conglomerate of differently aged cells. This heterogeneity arises because tissues are constantly regenerated thanks to the tissue-specific stem cells which are involved in the process of division and differentiation. It has been shown that, as stem cells are divided, both *in vitro* [45] and *in vivo* [46], their proliferative potential decreases and they reach the Hayflick limit, *i.e*., stem cells also grew old [47]. After much research [48, 49], Ho *et al*. concluded: "a living organism is as old as its stem cells" [50]. Thus, the bioenergetics aging clock regulates the aging process both in cell culture and in an organism.

As can be seen from the notion of the bioenergetics aging clock, it has the opportunity setting the rate of aging by changing two parameters: 1) The rate of cell divisions and 2) The value of the ATP/ADP drop per one cell division. For brevity, the latter will be referred below as the "energy drop".

There are two aspects of longevity: the species-specific life duration and the individual lifespan. The bioenergetics aging clock completely regulates the species-specific longevity only, whereas the individual lifespan varies depending on pressure from environmental factors, *i.e*., the genetic program only provides a potential opportunity for all individuals of a species to live for a certain period, but the real (individual) lifespan is determined by this potential opportunity and the environmental conditions. For example, a mouse in its habitat lives, on average, for eight months, but, in favourable laboratory conditions, its life duration may attain three years; a human's mean length of life can now vary between 40 to more than 80 years, depending on the living standards in their country. However, the mouse and the human cannot live longer than three and 120 years, respectively, under any circumstances, even under the most favourable conditions, because the species-specific life is programmed up to these limits.

4. EMPIRICAL VERIFICATION

The experiment is the supreme judge of all theories: however compelling a theoretical presentation may be, it requires empirical verification to become correct. The correct aging clock involves explaining all phenomena associated with the problems associated with the rate of aging and longevity. As mentioned, impressive results in increasing the maximum life expectancy of animals have been obtained by followers of the concept of programmed aging. However, there is no convincing explanation for the results of these studies. The bioenergetics aging clock offers such an explanation.

4.1. Calorie Restriction

The first experiments on the impact of hunger on longevity were conducted as early as the 1930s. A paradoxical phenomenon was discovered: strict Calorie Restriction (CR), which seems to be harmful to an organism, increased mean and maximum species-specific longevity [51]. The interest in this phenomenon has not declined over time, because this manipulation remains the only method that reliably increases

the lifespan of mammals, while concurrently maintaining their health.

Later on, many different factors that cause the analogous life-extending effect, so-called CR-mimetic factors, were found. They are capable to increase the maximal lifespan of all species from yeast to mammals (repeatedly in invertebrates) and are therefore at the forefront of anti-aging researches.

Numerous hypotheses have been presented to clarify the mechanism responsible for the effect of CR including lipid biology, amino acid imbalance, the neuroendocrine system, apoptosis, systemic inflammation, and the mediation of nuclear receptor NHR-62 [52-54], but none of them has appeared to be satisfactory. The change in oxidative stress within mitochondria is predominantly recognized as a core cause of this phenomenon [55]. It is a well-known fact that a food absorbed by an organism is utilized in two ways: 1) As fuel in the mitochondrial energy-generating system, and 2) As a construction material to build the structures of oneself. A dominant explanation of the CR effect comes from the first way: the shortage of fuel inhibits mitochondrial functioning that causes a decrease in ROS production. This, in turn, gives such a beneficial effect [56]. The interpretation with the position of bioenergetics aging clock takes into account the second process: the food restriction entails a decrease in the rate of cell division due to the deficit in construction materials that are indispensable for a doubling of cell mass during cell duplication. This slows down a course of the bioenergetics aging clock, which extends the maximal lifespan.

The first backgrounds for this conclusion were obtained as far back as 1995. It was shown by experiments *in vivo* [57] and *in vitro* [58] that the rate of cell divisions in *ad libitum* diet mice is high by early middle age, but then diminishes greatly in all organs. However, such mice on CR broadly preserve cell replicative capacity by an early age and then gradually use the saved divisions at a later age. These results were confirmed repeatedly by other researchers [59]. These findings are key in understanding the nature of the life-extending effect caused by the calorie restriction. Because a decline in the level of cellular bioenergetics is mated with cell reduplications, and CR increases time intervals between divisions, retardation of the aging clock occurs, which extends life. A general rule follows from this conclusion: anything that slows (accelerates) the rate of cell division entails extending (reducing) of life duration. At the start of life, each organism has an inborn level of bioenergetics generation, which is reduced by the genetic program in small bits synchronously with cell divisions. This is a modern interpretation of the Hippocrates' idea.

Many empirical data confirm this rule. First, the most important (as construction materials) components of food are essential amino acids and essential fatty acids that cannot be synthesized in an organism and therefore must be supplied in the diet. It has been shown that increasing the content of essential amino acids, such as methionine, tryptophan, cystine, and cysteine, in the allowances of starving animals decreases the lifespan extension effect of CR and, in the case of moderate starvation, even reduces it to zero [60]. On the contrary, even if rations are complete, the shortage of essential amino acids in food increases lifespan and thus imitates the effect of CR. Solon-Biet and colleagues found incontestable evidence that the ratio of macronutrients, rather than calorie intake, influences longevity [61]. Their experiments show that lifespan can be extended in *ad libitum*-fed animals provided that the content of proteins in food is low, and *vice versa*: the calorie restriction under a condition of high-protein diets has no effect on lifespan. All these data convincingly show that the effect of CR is caused by the proliferation of cells slowing down due to a deficiency in construction materials [62].

4.2. CR-Mimetic Factors

Both CR and any other factors that slow down cell division increase lifespan. The live organism can be considered as a harmonious ensemble of physical and chemical reactions, which have been refined by evolution over many millions of years. Any mutation (*i.e.*, genetic wound) that changes the functional state of this ensemble can influence cell proliferation. If, in this case, the modified gene diminishes the rate of cell division without doing too much damage to the remaining functions, then it increases lifespan. Thus, it is evident that mutations in genes, which reduce the activity of the different signalling pathways that are necessary for the mitosis process, extend lifespan in all organisms investigated: worms, flies, and mammals [63, 64]. Clear examples are Ames dwarf mice and Snell draft mice, which live 50% longer than the wild types of mice [65-67]. They contain mutations that restrict the production of growth hormones and other regulatory compounds that affect the cell division process. A similar effect is seen when mutations reduce the activity of nutrient-sensing pathways [68]. On the contrary, the hyper-production of Insulin-like Growth Factor (IGF-1), together with Growth Hormone (GH) accelerates cell proliferation and reduces the lifespan in mammals [69].

The chemical agents that influence the rate of cell divisions are also CR-mimicking factors. For example, the Target of Rapamycin (TOR) pathway is an inducer of the cell dividing process [70-72]. Rapamycin, the chemical isolated from the bacterium *Streptomyces hygroscopicus,* inhibits this pathway and thus decelerates cell proliferation [73], resulting in lagging of the aging clock and life extension. Factors that act in a similar way to rapamycin are those that suppress the expression of such genes as GH, IGF-1, DAF-2, DAF-16, Indy, Wnt signaling, and others involved in the initiation and maintenance of cell division [74, 75]. Lithium, which suppresses protein homeostasis, has also been found to increase longevity in this way [76].

The effective CR-mimicking factor which also influences the rate of cell division is temperature. This affects the longevity of all organisms, both poikilotherms and homeotherms, highlighting a general mechanism of this phenomenon [77]. The mechanism underlying this phenomenon also remains unclear and has been investigated intensively [78]. The elementary explanation follows from the position of the bioenergetics aging clock: according to thermodynamics laws, a temperature change inevitably varies the rate of all biochemical reactions, which in turn modifies the rate of cell proliferation and thereby longevity.

The CR and the numerous CR-mimicking factors have quite different natures but the same effect of extending longevity because they all cause a decrease in the rate of cell division, which leads to a lagging of the bioenergetics aging clock.

4.3. Other Phenomena

The above only explains the phenomena associated with the influence of the rate of cell division the course of the bioenergetics aging clock. However, there are phenomena that cannot be explained in this manner. For example, a bat and a bird live 10 times longer than a ground animal of a similar size [79], and a naked mole-rat (*Heterocephalus glaber*) has impressively longer longevity in comparison with a home mouse [80]. As explained above, the reason why their life expectancy is an order of magnitude higher than that of terrestrial animals in evolutionary terms is because the environmental pressure in their ecological niches is much lower. The next question is how is this increase in longevity realized by the bioenergetics aging clock under an almost equal tempo of cell proliferation?

As mentioned, bioenergetics aging clock has, by its nature, another possibility to influence the rate of aging - by changing the value of "energy drop". It can be assumed that it is this possibility that nature uses for such a large-scale regulation. Indeed, the range of possibilities of this way longevity regulation is practically unlimited and is not subject to any external influences. If this value is big, then the generation of energy may drop to the level of senile cells over a few dividing (as, for example, in the case of *C. elegans*). Appropriately, the smaller the ATP/ADP value, which decreases under each division, the longer the lifespan. This answers the question as to why bats, birds, and naked mole-rats live longer: their cell divisions reduce bioenergetics in smaller portions. In a limiting case, if this "energy drop" tends towards zero, the course of the bioenergetics aging clock approximates to stopping. This reveals the nature of the negligible senescence of some animal species (some Mollusca, Echinoderms, freshwater Hydra and others), and also why HeLa cells and other malignant cell lines can divide continually, thus avoiding senescence.

The data considered were obtained experimentally. Besides, nature itself uses the cell proliferation rate to influence the course of the bioenergetics aging clock by all three ways possible:

1. *A Delay in the Aging Clock*: Embryonic stem cells are the precursors of all differentiated tissue. In the course of embryo development, the part of these cells suspends the divisions at the stage of tissue-specific unipotent stem cells, thereby retaining a high level of bioenergetics. This strategy allows the moribund senescent cells to be replaced throughout the life of the body until the energetic potential of the stem cells themselves has been exhausted.
2. *Temporary Stopping*: This strategy is mainly used to maintain the viability of seeds and spores of different creatures under adverse conditions. This quiescence period can either be short (for example, seasonal) or very long. Recently, a group of researchers obtained data confirming the capability of multicellular organisms for long-term cryobiosis in permafrost deposits of the Arctic. Two species of viable soil nematodes were isolated from the samples of Pleistocene permafrost deposits. The duration of natural cryopreservation of the nematodes corresponded to the age of the deposits, *i.e*., 30,000-40,000 years [81]. Furthermore, viable bacteria with an estimated age of 1.8-3.0 million years were found in samples of permafrost in the Kolyma-indigir Lowland of North-eastern Siberia [82]. These are analogues of the Hayflick and Moorhead experiment [43] made by nature itself.
3. *Complete Stopping*: In some tissues, the bioenergetics aging clock is functionally stopped entirely. It is known that all mammals' heart muscle has no stem cells. Cell divisions of this tissue occur only during embryonic development and cease immediately in the postnatal period. It is because of this shutdown of the aging clock that heart muscle cells stop aging and retain the highest activity inherent in embryonic cells. This allows them to stay young and work tirelessly throughout the life of the body. The decline in their functional ability in the later stages of life is not due to aging, but to the decrepitude of the serving tissues, mainly blood vessels. The above applies fully to nerve cells (neurocyte), which also have no stem cells. They also work tirelessly throughout the organism's lifespan, and their functional ability also decreases with age, but only because of the deterioration in blood supply and the decrepitude of the glial cells in which they are immersed.

CONCLUSION

Despite the breathtaking progress in all areas of science, especially in biology, and the emergence of powerful new technologies, gerontology has not made any progress in extending the maximum human lifespan. The primary reason for this stagnation is that the basal postulate of the dominated concept of aging states that the genes of longevity cannot exist, while age-related organism degradation is the result of the accumulation of stochastic errors. By now, it has been shown experimentally that genes of longevity exist and that their manipulation can influence the maximal lifespan. But, the obtained empirical data have no convincing substantiation.

It is time to conclude that further research in traditional direction is hopeless and we need to revive the initial ideas of Hippocrates and Weisman, which state that the aging process is programmed *via* the decline in bioenergetics. All conditions are maturated already for the realization of this way. Compared to the first half of the 20th century, genetics made enormous successes, the machinery of biological energy production has been studied substantially, and a huge amount of different fundamental knowledge has been accumulated.

The mechanism of programmed aging and its aging clock, as presented here, explains phenomena concerning the aging process and longevity. Fundamental research in molecular biology and related fields has already discovered the main details of the molecular mechanism that determine the bioenergetics level in the cell, although even the authors of

these studies are often not aware of the role of their discoveries in the mechanism of programmed aging. Take this vivid example: a group of researchers led by Bernhard Kadenbach discovered a molecular mechanism of allosteric regulation of the ATP/ADP level in mitochondria [83], which is one of the main components in the execution mechanism of programmed aging. However, they maintain that this regulation is necessary to hold down ROS production [84], private correspondence.

The molecular mechanism of execution of programmed aging and a way of its modification are to be outlined in the next article. Both genomes, nuclear and mitochondrial, and many other components realizing the aging process such as sirtuins, mobile genetic elements, and so on, find their specific roles there. While we are ready to start practical research, the most difficult task today is to shift the attention of researchers from the concept of the accumulation of stochastic errors onto programmed aging.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Weisman A. Essays upon Heredity and Kinder Biological Problems. Oxford: Clarendon Press 1891.

[2] Medawar PB. An Unsolved Problem of Biology. London: Levis 1952.

[3] Harman D. Aging: A theory based on free radical and radiation chemistry. J Gerontol 1956; 11(3): 298-300. http://dx.doi.org/10.1093/geronj/11.3.298 PMID: 13332224

[4] Williams GC. Pleiotropy, natural selection and the evolution of senescence. Evolution 1957; 11: 398-411. http://dx.doi.org/10.1111/j.1558-5646.1957.tb02911.x

[5] Kirkwood TBL. Evolution of ageing. Nature 1977; 270(5635): 301-4. http://dx.doi.org/10.1038/270301a0 PMID: 593350

[6] Miquel J, Economos AC, Fleming J, Johnson JE. Mitochondrial role in cell aging. Exp Gerontol 1980; 15(6): 575-91. http://dx.doi.org/10.1016/0531-5565(80)90010-8 PMID: 7009178

[7] Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang RAC. Elegans mutant that lives twice as long as wild type. Nature 1993; 366(6454): 461-4. http://dx.doi.org/10.1038/366461a0 PMID: 8247153

[8] Malone EA, Inoue T, Thomas JH. Genetic analysis of the roles of daf-28 and age-1 in regulating *Caenorhabditis elegans* dauer formation. Genetics 1996; 143(3): 1193-205. PMID: 8807293

[9] Tissenbaum HA, Ruvkun G. An insulin-like signaling pathway affects both longevity and reproduction in *Caenorhabditis elegans*. Genetics 1998; 148(2): 703-17. PMID: 9504918

[10] Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Müller F. Genetics: Influence of TOR kinase on lifespan in *C. elegans*. Nature 2003; 426(6967): 620. http://dx.doi.org/10.1038/426620a PMID: 14668850

[11] Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. Curr Biol 2004; 14(10): 885-90. http://dx.doi.org/10.1016/j.cub.2004.03.059 PMID: 15186745

[12] Gillespie ZE, Pickering J, Eskiw CH. Better living through Chemistry: Caloric Restriction (CR) and CR mimetics alter genome function to promote increased health and lifespan. Front Genet 2016; 7: 142. http://dx.doi.org/10.3389/fgene.2016.00142 PMID: 27588026

[13] Carmona JJ, Michan S. Biology of healthy aging and longevity. Rev Invest Clin 2016; 68(1): 7-16. PMID: 27028172

[14] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4): 663-76. http://dx.doi.org/10.1016/j.cell.2006.07.024 PMID: 16904174

[15] Choi J, Lee S, Mallard W, *et al.* A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. Nat Biotechnol 2015; 33(11): 1173-81. http://dx.doi.org/10.1038/nbt.3388 PMID: 26501951

[16] Hashizume O, Ohnishi S, Mito T, *et al.* Epigenetic regulation of the nuclear-coded GCAT and SHMT2 genes confers human age-associated mitochondrial respiration defects. Sci Rep 2015; 5: 10434. http://dx.doi.org/10.1038/srep10434 PMID: 26000717

[17] Wilson EO. Group selection and its significance for ecology. Bioscience 1973; 23: 631-8. http://dx.doi.org/10.2307/1296775

[18] Trubitsyn AG. Species-specific lifespan is under control of natural selection. Adv Gerontol 2006; 19: 13-24. PMID: 17152715

[19] Mitteldorf J, Pepper J. Senescence as an adaptation to limit the spread of disease. J Theor Biol 2009; 260(2): 186-95. http://dx.doi.org/10.1016/j.jtbi.2009.05.013 PMID: 19481552

[20] Goldsmith TC. Evolvability, population benefit, and the evolution of programmed aging in mammals. Biochemistry (Mosc) 2017; 82(12): 1423-9. http://dx.doi.org/10.1134/S0006297917120021 PMID: 29486693

[21] Malek MH, Hüttemann M, Lee I. Mitochondrial structure, function, and dynamics: The common thread across organs, disease, and aging. Oxid Med Cell Longev 2018; 2018: 1863414. http://dx.doi.org/10.1155/2018/1863414 PMID: 29576844

[22] Hayashi J, Ohta S, Kikuchi A, Takemitsu M, Goto Y, Nonaka I. Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction. Proc Natl Acad Sci USA 1991; 88(23): 10614-8. http://dx.doi.org/10.1073/pnas.88.23.10614 PMID: 1720544

[23] Hayashi J, Ohta S, Kagawa Y, *et al.* Nuclear but not mitochondrial genome involvement in human age-related mitochondrial dysfunction. Functional integrity of mitochondrial DNA from aged subjects. J Biol Chem 1994; 269(9): 6878-83. PMID: 8120050

[24] Sun N, Youle RJ, Finkel T. The mitochondrial basis of aging. Mol Cell 2016; 61(5): 654-66. http://dx.doi.org/10.1016/j.molcel.2016.01.028 PMID: 26942670

[25] Srivastava S. The mitochondrial basis of aging and age-related disorders. Genes (Basel) 2017; 8(12): 398. http://dx.doi.org/10.3390/genes8120398 PMID: 29257072

[26] Sebastián D, Palacín M, Zorzano A. Mitochondrial dynamics: Coupling mitochondrial fitness with healthy aging. Trends Mol Med 2017; 23(3): 201-15. http://dx.doi.org/10.1016/j.molmed.2017.01.003 PMID: 28188102

[27] Zhang H, Menzies KJ, Auwerx J. The role of mitochondria in stem cell fate and aging. Development 2018; 145(8): 143420. http://dx.doi.org/10.1242/dev.143420 PMID: 29654217

[28] Trubitsyn AG. Bioenergetics theory of aging. Bioenergetics. Rijeka: In Tech 2012; pp. 63-94.

[29] Lipsky MS, King M. Biological theories of aging. Dis Mon 2015; 61(11): 460-6. http://dx.doi.org/10.1016/j.disamonth.2015.09.005 PMID: 26490576

[30] Itahana K, Campisi J, Dimri GP. Mechanisms of cellular senescence in human and mouse cells. Biogerontology 2004; 5(1): 1-10. http://dx.doi.org/10.1023/B:BGEN.0000017682.96395.10 PMID: 15138376

[31] Lee HW, Blasco MA, Gottlieb GJ, Horner JW, Greider CW, De Pinho RA. Essential role of mouse telomerase in highly proliferative organs. Nature 1998; 392(6676): 569-74. http://dx.doi.org/10.1038/33345 PMID: 9560153

[32] Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM. Evidence for a mitotic clock in human hematopoietic stem cells: Loss of telomeric DNA with age. Proc Natl Acad Sci USA 1994; 91(21): 9857-60. http://dx.doi.org/10.1073/pnas.91.21.9857 PMID: 7937905

[33] Blasco MA, Lee HW, Hande MP, *et al.* Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 1997; 91(1): 25-34. http://dx.doi.org/10.1016/S0092-8674(01)80006-4 PMID: 9335332

[34] Herrera E, Samper E, Martín-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. EMBO J 1999; 18(11): 2950-60. http://dx.doi.org/10.1093/emboj/18.11.2950 PMID: 10357808

[35] Dilman VM. The Grand Biological Clock Moscow: Mir. 1998.

[36] Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 2001; 63: 647-76. http://dx.doi.org/10.1146/annurev.physiol.63.1.647 PMID: 11181971

[37] Karasek M. Melatonin, human aging, and age-related diseases. Exp Gerontol 2004; 39(11-12): 1723-9. http://dx.doi.org/10.1016/j.exger.2004.04.012 PMID: 15582288

[38] Gubin DG, Gubin GD. Daily administration of melatonin reduces circadian rhythm disturbances in older people. Curr Aging Sci 2016; 9: 5-13. http://dx.doi.org/10.2174/1874609809666151130220011 PMID: 26632428

[39] Panda S. Circadian physiology of metabolism. Science 2016; 354(6315): 1008-15. http://dx.doi.org/10.1126/science.aah4967 PMID: 27885007

[40] Tevy MF, Giebultowicz J, Pincus Z, Mazzoccoli G, Vinciguerra M. Aging signaling pathways and circadian clock-dependent metabolic derangements. Trends Endocrinol Metab 2013; 24(5): 229-37. http://dx.doi.org/10.1016/j.tem.2012.12.002 PMID: 23299029

[41] Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013; 14(10): R115. http://dx.doi.org/10.1186/gb-2013-14-10-r115 PMID: 24138928

[42] Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet 2018; 19(6): 371-84. http://dx.doi.org/10.1038/s41576-018-0004-3 PMID: 29643443

[43] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961; 25: 585-621. http://dx.doi.org/10.1016/0014-4827(61)90192-6 PMID: 13905658

[44] Trubitsyn AG. The Mechanism of phenoptosis: 2. the hayflick limit is caused by programmed decrease of the bioenergetics level. Adv Gerontol 2011; 1: 134-9. http://dx.doi.org/10.1134/S2079057011020147

[45] Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B. Aging of mesenchymal stem cell *in vitro*. BMC Cell Biol 2006; 7: 14. http://dx.doi.org/10.1186/1471-2121-7-14 PMID: 16529651

[46] Kasper G, Mao L, Geissler S, *et al.* Insights into mesenchymal stem cell aging: Involvement of antioxidant defense and actin cytoskeleton. Stem Cells 2009; 27(6): 1288-97. http://dx.doi.org/10.1002/stem.49 PMID: 19492299

[47] Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol 2007; 8(9): 703-13. http://dx.doi.org/10.1038/nrm2241 PMID: 17717515

[48] Campisi J. From cells to organisms: Can we learn about aging from cells in culture? Exp Gerontol 2001; 36(4-6): 607-18. http://dx.doi.org/10.1016/S0531-5565(00)00230-8 PMID: 11295503

[49] Hornsby PJ. Cellular senescence and tissue aging *in vivo*. J Gerontol A Biol Sci Med Sci 2002; 57(7): B251-6. http://dx.doi.org/10.1093/gerona/57.7.B251 PMID: 12084795

[50] Ho AD, Wagner W, Mahlknecht U. Stem cells and aging. EMBO Rep 2005; 6: S35-8. http://dx.doi.org/10.1038/sj.embor.7400436 PMID: 15995659

[51] McCay CM, Crowell MF, Maynard LA. The Effect of retarded growth upon the length of life span and upon the ultimate body size. J Nutr 1935; 10: 63-79. http://dx.doi.org/10.1093/jn/10.1.63

[52] Koubova J, Guarente L. How does calorie restriction work? Genes Dev 2003; 17(3): 313-21. http://dx.doi.org/10.1101/gad.1052903 PMID: 12569120

[53] Grandison RC, Piper MDW, Partridge L. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. Nature 2009; 462(7276): 1061-4. http://dx.doi.org/10.1038/nature08619 PMID: 19956092

[54] Heestand BN, Shen Y, Liu W, *et al.* Dietary restriction induced longevity is mediated by nuclear receptor NHR-62 in *Caenorhabditis elegans*. PLoS Genet 2013; 9(7): e1003651. http://dx.doi.org/10.1371/journal.pgen.1003651 PMID: 23935515

[55] Ungvari Z, Parrado-Fernandez C, Csiszar A, de Cabo R. Mechanisms underlying caloric restriction and lifespan regulation: Implications for vascular aging. Circ Res 2008; 102(5): 519-28. http://dx.doi.org/10.1161/CIRCRESAHA.107.168369 PMID: 18340017

[56] Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science 1996; 273(5271): 59-63. http://dx.doi.org/10.1126/science.273.5271.59 PMID: 8658196

[57] Wolf NS, Penn PE, Jiang D, Fei RG, Pendergrass WR. Caloric restriction: Conservation of *in vivo* cellular replicative capacity accompanies life-span extension in mice. Exp Cell Res 1995; 217(2): 317-23. http://dx.doi.org/10.1006/excr.1995.1092 PMID: 7698231

[58] Pendergrass WR, Li Y, Jiang D, Fei RG, Wolf NS. Caloric restriction: Conservation of cellular replicative capacity *in vitro* accompanies life-span extension in mice. Exp Cell Res 1995; 217(2): 309-16. http://dx.doi.org/10.1006/excr.1995.1091 PMID: 7698230

[59] Bhattacharyya TK, Jackson P, Patel MK, Thomas JR. Epidermal cell proliferation in calorie-restricted aging rats. Curr Aging Sci 2012; 5(2): 96-104. http://dx.doi.org/10.2174/1874609811205020096 PMID: 21834786

[60] Zimmerman JA, Malloy V, Krajcik R, Orentreich N. Nutritional control of aging. Exp Gerontol 2003; 38(1-2): 47-52. http://dx.doi.org/10.1016/S0531-5565(02)00149-3 PMID: 12543260

[61] Solon-Biet SM, McMahon AC, Ballard JW, *et al.* The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. Cell Metab 2014; 19(3): 418-30. http://dx.doi.org/10.1016/j.cmet.2014.02.009 PMID: 24606899

[62] Trubitsyn AG. The lag of the proliferative aging clock underlies the lifespan-extending effect of calorie restriction. Curr Aging Sci 2015; 8(3): 220-6. http://dx.doi.org/10.2174/1874609808666151002111632 PMID: 26428550

[63] Anisimov VN, Bartke A. The key role of growth hormone-insulin-IGF-1 signaling in aging and cancer. Crit Rev Oncol Hematol 2013; 87(3): 201-23. http://dx.doi.org/10.1016/j.critrevonc.2013.01.005 PMID: 23434537

[64] Lee SH, Min KJ. Caloric restriction and its mimetics. BMB Rep 2013; 46(4): 181-7. http://dx.doi.org/10.5483/BMBRep.2013.46.4.033 PMID: 23615258

[65] Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. Nature 1996; 384(6604): 33. http://dx.doi.org/10.1038/384033a0 PMID: 8900272

[66] Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. Proc Natl Acad Sci USA 2001; 98(12): 6736-41. http://dx.doi.org/10.1073/pnas.111158898 PMID: 11371619

[67] Bartke A. Can Growth Hormone (GH) accelerate aging? Evidence from GH-transgenic mice. Neuroendocrinology 2003; 78(4): 210-6. http://dx.doi.org/10.1159/000073704 PMID: 14583653

[68] Fontana L, Partridge L, Longo VD. Extending healthy life span-from yeast to humans. Science 2010; 328(5976): 321-6. http://dx.doi.org/10.1126/science.1172539 PMID: 20395504

[69] Bartke A, Quainoo N. Impact of growth hormone-related mutations on mammalian aging. Front Genet 2018; 9: 586. http://dx.doi.org/10.3389/fgene.2018.00586 PMID: 30542372

[70] Polak P, Hall MN. mTOR and the control of whole body metabolism. Curr Opin Cell Biol 2009; 21(2): 209-18.

http://dx.doi.org/10.1016/j.ceb.2009.01.024 PMID: 19261457

[71] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017; 168(6): 960-76. http://dx.doi.org/10.1016/j.cell.2017.02.004 PMID: 28283069

[72] Wipperman MF, Montrose DC, Gotto AM, Hajjar DP. Mammalian target of rapamycin: A metabolic rheostat for regulating adipose tissue function and cardiovascular health. Am J Pathol 2019; 189(3): 492-501. http://dx.doi.org/10.1016/j.ajpath.2018.11.013 PMID: 30803496

[73] Loewith R, Hall MN. Target of Rapamycin (TOR) in nutrient signaling and growth control. Genetics 2011; 189(4): 1177-201. http://dx.doi.org/10.1534/genetics.111.133363 PMID: 22174183

[74] Kenyon CJ. The genetics of ageing. Nature 2010; 464(7288): 504-12. http://dx.doi.org/10.1038/nature08980 PMID: 20336132

[75] Gruber J, Yee Z, Tolwinski NS. Developmental drift and the role of Wnt signaling in aging. Cancers (Basel) 2016; 8(8): 73. http://dx.doi.org/10.3390/cancers8080073 PMID: 27490570

[76] Lithgow G. Small molecules that suppress protein aggregation and slow aging. Abstracts of reports of 3rd international conference. Sochi, 2014.

[77] Conti B, Sanchez-Alavez M, Winsky-Sommerer R, *et al.* Transgenic mice with a reduced core body temperature have an increased life span. Science 2006; 314(5800): 825-8. http://dx.doi.org/10.1126/science.1132191 PMID: 17082459

[78] Xiao R, Zhang B, Dong Y, *et al.* A genetic program promotes *C. elegans* longevity at cold temperatures *via* a thermosensitive TRP channel. Cell 2013; 152(4): 806-17. http://dx.doi.org/10.1016/j.cell.2013.01.020 PMID: 23415228

[79] Munshi-South J, Wilkinson GS. Bats and birds: Exceptional longevity despite high metabolic rates. Ageing Res Rev 2010; 9(1): 12-9. http://dx.doi.org/10.1016/j.arr.2009.07.006 PMID: 19643206

[80] Browe BM, Vice EN, Park TJ. Naked mole-rats: Blind, naked, and feeling no pain. Anat Rec (Hoboken) 2018; 303(1): 77-88. http://dx.doi.org/10.1002/ar.23996 PMID: 30365235

[81] Shatilovich AV, Tchesunov AV, Neretina TV, *et al.* Viable nematodes from late pleistocene permafrost of the kolyma river lowland. Dokl Biol Sci 2018; 480(1): 100-2. http://dx.doi.org/10.1134/S0012496618030079 PMID: 30009350

[82] Shi T, Reeves RH, Gilichinsky DA, Friedmann EI. Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. Microb Ecol 1997; 33(3): 169-79. http://dx.doi.org/10.1007/s002489900019 PMID: 9115180

[83] Kadenbach B, Ramzan R, Wen L, Vogt S. New extension of the Mitchell Theory for oxidative phosphorylation in mitochondria of living organisms. Biochim Biophys Acta 2010; 1800(3): 205-12. http://dx.doi.org/10.1016/j.bbagen.2009.04.019 PMID: 19409964

[84] Kadenbach B, Ramzan R, Vogt S. High efficiency *versus* maximal performance- The cause of oxidative stress in eukaryotes: A hypothesis. Mitochondrion 2013; 13: 1-6.