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New elements in modern biological theories of aging

ABSTRACT

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Generally and simply speaking, when human individuals think of how their bodies are aging, probably the most visible changes come first to their minds. One can notice more grey hair or the skin does not seem as smooth as it used to be. These are just external signs of a series of processes going on within our cells and bodily systems that together constitute normal aging. Aging (also called senescence) is an age-dependent decline in physiological function, demographically manifesting as decreased survival and fecundity with increasing age. Aging is also commonly defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for the increased risk of disease and death. The principal theories of aging are all specific of a distinct cause of aging, giving useful and deep insights for the understanding of age-related physiological changes.

Key words: aging, theory, lifespan, caloric restriction, oxidative damage, inflammation, longevity, evolution, genomic studies, physical exercise

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Introduction

Generally and simply speaking, when human individuals think of how their bodies are aging, probably the most visible changes come first to their minds. One can notice more grey hair, or the skin does not seem as smooth as it used to be. These are just external signs of a series of processes going on within our cells and bodily systems that together constitute normal aging. Aging (also called senescence) is an age-dependent decline in physiological function, demographically manifesting as decreased survival and fecundity with increasing age. Aging is also commonly defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for the increased risk of disease and death. The principal theories of aging are all specific of a distinct cause of aging, giving useful and deep insights for the understanding of age-related physiological changes. However, a complete view of them is necessary when discussing a process which is still obscure in some of its aspects. In this context, the search for a particular cause of aging has recently been replaced by the view of aging as an extremely complex, multi-factorial process. For this reason, the different theories of aging should be considered as complementary of others in the explanation of some or all the mechanisms of the

normal aging process. Thus far, there is no strong and convincing evidence showing the administration of existing “anti-aging” remedies can slow aging or increase longevity in humans. Nevertheless, several studies on animal models have evidenced that aging rates and life expectancy can be modified. The recent reviews confirming such idea [1–4] give an excellent overlook of the most important and generally accepted theories of aging, providing current evidence of the many scientific trials aimed at modifying the aging process.

Despite the recent advances in molecular biology and genetics, the great mysteries that control human lifespan are waiting for delineation. Many theories, which are divided into two main categories: programmed and error theories, have been proposed to explain the process of aging, but none of them is fully satisfactory. These theories often interact mutually in a complex way.

While this is certainly one of the obligatory experiences all humans have in common, scientists in the research institutes worldwide judge aging is actually one of the nature’s least understood processes.

It was suggested that the future precise research in biogerontology, such as the studies on the control of protein synthesis, validation of biomarkers of aging and understanding the biochemistry of longevity, should be conducted with the utilization of new and precise tools

of molecular and genomic technology. Molecular biologists must put their experiments into an evolutionary framework having in view the aging process. Evolutionary biologists should adopt their tools of molecular biology. The time is suitable for a synthesis of molecular biogerontology and evolutionary biology of aging.

New theories enable us to make theoretical predictions, showing the paths which can be tested in experimental laboratory and field conditions. A better understanding and further precise testing the existing and new theories may contribute to the promotion of successful aging.

Theories of aging

Several theories of aging were created to explain the process of aging, however, none of them appears to be fully satisfactory [5]. The traditional aging theories hold that aging is not an adaptation or generally genetically programmed. Modern biological theories of aging in humans can be placed within two main categories: programmed and damage (error) theories. The programmed theories imply that aging follows a biological timetable, perhaps a continuation of the one that regulates childhood growth and development. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair and defense responses. The damage or error theories emphasize environmental assaults on living organisms that induce cumulative damage at different levels of the organization of organisms as the cause of aging.

Programmed theory of aging

The programmed theory has three sub-categories: 1) Programmed Longevity. Aging is the result of a sequential switching on and off of certain genes, with senescence being defined as the time when age-associated deficits are manifested [5]. Weismann came to an idea that there is a limitation on the number of divisions that somatic cells might undergo in the course of individual life and this number, like the lifespan of individual generations of cells, is already determined in the embryonic cell [6]. Weismann contributed to the first evolutionary theory of aging which drew attention of other researchers. On the basis of theoretical inspiration alone, he rightly predicted the existence of a cell division limit [6, 7].

Endocrine theory

Biological clocks act through hormones to control the pace of aging. Recent studies confirm that aging is hormonally regulated and that insulin/IGF-1 signaling (IIS) pathway, conserved during evolution, plays a key role in the hormonal regulation of aging. Van Heemst,

in his publication, described and discussed the potential mechanism underlying interconnection of IIS and aging process [8].

Immunological theory

The immune system is programmed to decline over time, which leads to an increased vulnerability to infectious diseases and thus aging and death. It is well documented that the effectiveness of the immune system peaks at puberty and gradually declines thereafter with advance in age.

For example, as one grows older, antibodies lose their effectiveness and fewer new diseases can be combated effectively by the body, which causes cellular stress and eventual death [9]. Indeed, unregulated immune response has been linked to cardiovascular disease, inflammation, Alzheimer's disease (AD) and cancer. Although direct causal relationship has not been established for all these detrimental outcomes, the immune system has been at least indirectly implicated [10].

The damage (error) theory includes: 1) wear and tear theory and 2) rate-of-living theory. The first one implies that cells and tissues have vital parts that wear out which results in aging. Like the components of an aging car, parts of the body eventually wear out from repeated use, killing them and then the body. Even though the wear and tear theory of aging was first introduced by Weismann, a German biologist, in 1882, it sounds perfectly reasonable to many people even today, because this is what happens to most familiar things around them.

The second theory holds that the greater the organism's rate of oxygen basal metabolism, the shorter its lifespan [11]. The rate-of-living theory of aging, however helpful, is not completely adequate in explaining the maximum lifespan [12]. Rollo proposes a modified version of Pearl's rate-of-living theory emphasizing the hard-wired antagonism of growth (TOR) and stress resistance (FOXO) [13].

Cross-linking theory

The cross-linking theory of aging was proposed by Bjorksten in 1942 [14]. According to this theory, the accumulation of cross-linked proteins damages cells and tissues, slowing down bodily processes resulting in aging. Recent studies show that cross-linking reactions are involved in the age related changes in the studied proteins [15].

Free radicals theory

This theory of aging was first proposed by Gerschmann in 1954 but was precisely formulated by Harman [16] who hypothesized that a single common process, modifiable by the genetic and environmental factors, in

which the accumulation of endogenous free radicals generated in cells could be responsible for aging and death of all living beings [16, 17].

This theory was then revised in 1972 when mitochondria were identified as responsible for the initiation of the most of the free radical reactions related to the aging process. It was postulated that the lifespan is determined by the rate of free radical damage to the mitochondria [18].

The increasing age-related oxidative stress is a consequence of the imbalance between the free radical production and antioxidant defenses with a higher production of these radicals [19].

Harman [18] suggests that superoxide and other free radicals cause damage to the macromolecular components of the cell, giving rise to damaged cells, and consequently organs, which eventually stop functioning. The macromolecules such as nucleic acids, lipids, sugars, and proteins are all potential targets to free radical attack. Nucleic acids can get additional base or sugar group; break in a single and double strand fashion in the backbone and afterwards can cross link to other molecules. The body is armed in some natural antioxidants such as enzymes, which help to curb the dangerous built-up of these free radicals, without which cellular death rates would be greatly increased, and subsequent life expectancies would decrease. This theory has been bolstered by experiments in which rodents fed antioxidants achieved greater average longevity.

All organisms live in an environment that contains reactive oxygen species. Mitochondrial respiration, the basis of energy production in all eukaryotes, generates reactive oxygen species by leaking intermediates from the electron transport chain [20]. The universal nature of oxidative free radicals, and possibly of the free radical theory of aging, is suggested by the presence of superoxide dismutase in all aerobic organisms and responsible for scavenging superoxide anions [20]. Moreover, cellular oxidative damage is indiscriminate. It has been shown that oxidative modifications occur in DNA, protein and lipid molecules [21]. Elevated levels of both oxidant-damaged DNA and protein have been found in aged organisms [22, 23].

However, at present there are some experimental findings which are not consistent with this early proposal. Afanas'ev [24] suggested that reactive oxygen species (ROS) signaling is probably the most important enzyme/gene pathway responsible for the development of the of cell senescence and aging of the organism and that ROS signaling might be considered as further development of the free radical theory of aging [24].

Somatic DNA damage theory

DNA damages occur continuously in the cells of living organisms. While most of these damages are

repaired, some accumulate, as DNA polymerases and other repair mechanisms cannot correct defects as fast as they are apparently produced. In particular, there is evidence for DNA damage accumulation in non-dividing cells of mammals. Genetic mutations occur and accumulate with increasing age, causing cells to deteriorate and malfunction. Damage to mitochondrial DNA might lead to mitochondrial dysfunction. Therefore, aging results from damage to the genetic integrity of the body cells.

In the 1930s, it was found that restricting calories can extend the lifespan in laboratory animals [25]. Many studies were performed to try to elucidate the underlying mechanisms. However, our knowledge remained limited at the genetic and molecular levels until 1990 [26]. Recently, Schulz et al. have provided evidence that this effect is due to an increased formation of free radicals within the mitochondria causing a secondary induction of increased antioxidant defense capacity [27]. Shimokawa and Trindade have analyzed the new results on restricting calories-related genes or molecules in the rodent models, their importance in understanding of this process, with the particular attention on the roles of forkhead box O transcription factors, AMP-activated protein kinase, and sirtuins (specially SIRT1) in the effects of restricting calories in rodents [26].

Some neurological diseases are considered to be at high risk with increasing age, for example, AD, which is diagnosed in people over 65 years of age. The discovery of molecular basis of the processes involved in their pathology or creating and studying aging model systems may help our better understanding of the aging processing. At the early stages, the most commonly recognized symptom of AD is inability to acquire new memories. Recent studies show that endogenous neural stem cells, in the hippocampus of an adult brain, are involved in memory function [28]. Consistently, neural stem cell function in the hippocampus decreases with increased aging [4], but the reasons are still unclear.

Mitochondrial theory of aging

Studies exploring mechanisms of aging have always been particularly directed on DNA. In mammalian cells, mitochondria and the nucleus are the only organelles that possess DNA. In mammalian cells, the physiological integrity of the cell is strongly linked to the integrity of genome.

The mitochondrial DNA is present in only 1% to 3% of total genomic material in animal cells but its importance to cellular physiology is much greater than what can be estimated from its size alone. Mitochondrial DNA, in close proximity to the places of oxygen radical production and unprotected by histones that are associated with nuclear DNA, is a sensitive target for

oxygen radical attack. It was estimated that the level of oxidized bases in mitochondrial DNA is 1- to 20-fold higher than that in nuclear DNA [29, 30]. Mitochondria encode polypeptides of the electron transfer chain as well as components required for their synthesis. For this reason, any coding mutations in mitochondrial DNA will affect the entire electron transfer chain, potentially altering both the assembly and function of the products of numerous nuclear genes in electron transfer chain complexes. Finally, defects in the electron transfer chain can have pleiotropic effects because it is affecting the entire cellular energetics [31].

It was demonstrated that longevity is more strongly associated with age of maternal death than that of paternal death, suggesting that mitochondrial DNA inheritance may play an important role in determining longevity [32]. In spite of the fact that it is still not well established [33], several studies demonstrate that longevity is associated with specific mitochondrial DNA polymorphisms [34–36].

The mitochondrial DNA mutations accumulate progressively during life and are directly responsible for a measurable deficiency in cellular oxidative phosphorylation activity, leading to an enhanced reactive oxygen species production. Supporting the primary importance of mitochondria in the aging process and in determining longevity, it has been documented that several mutagenic chemicals tend to preferentially damage mitochondrial DNA. It can be hypothesized that a life-long exposure to these environmental toxins may lead to a preferential accumulation of the mitochondrial DNA damage and accelerate aging [1].

Although mild amounts of oxidative damage, such as that experienced during exercise training [37], may actually be the stimulus for physiological mitochondrial biogenesis, more severe, more extensive, or more prolonged oxidative damage is clearly toxic [38].

The telomere theory of aging

It is well-known that telomere maintenance appears to be essential for the prolonged persistence of the stem cell function in organs with extensive cell turnover [39]. However, in 1961, Hayflick revolutionized cell biology when he developed a theory relevant to telomeres known as the *Hayflick Limit*, which places the maximum potential lifespan of humans at 120 years, the time at which too many cells can no longer replicate and divide to keep things going. He theorized that the human cells ability to divide is limited to approximately 50 times, after which they simply stop dividing [40]. According to telomere theory, telomeres have experimentally been shown to shorten with each successive cell division [41]. Certain cells, such as egg and sperm cells, use telomerase to restore telomeres to the end of their chromo-

some, insuring that cells can continue to reproduce and promote the survival of the species. But most adult cells lack this capacity. When the telomeres reach a critical length, the cell stops replicating at an appreciable rate, and so, it dies off, which eventually leads to the death of the entire organism. Telomerase cannot completely prevent telomere shortening after extensive stem cell division either, providing a putative mechanism for the timely limit of the history of stem cells replication and subsequent progressive decay in the maintenance of organ homeostasis at old ages [39, 42]. A recent study shows that telomeres shorten with age in neural stem cells of the hippocampus and that telomerase-deficient mice exhibit reduced neurogenesis as well as impaired neuronal differentiation and neuritogenesis [43]. These findings indicate the link among brain aging, neural stem cells and neurological diseases [44].

Another important aspect of this problem is telomere quality, as different from telomere length. Telomeres are the weak link in DNA. They are readily damaged and must be repaired, yet they lack the repair efficiency of other DNA. This results in the accumulation of partially damaged and poorly functioning telomeres of lower quality, regardless of their length.

Inflammation hypothesis of aging

The aging process occurs because the changed energy states of biomolecules render them inactive or malfunctioning. Identical events also occur before the aging phenotype appears, but repair and replacement processes are capable of maintaining the balance in favor of the functioning molecules. After reproductive maturation, this balance slowly shifts to the one in which molecules that lose their biologically active energy states are less likely to be replaced or repaired. The diminution of repair and replacement capability is further exacerbated because the enormously complex biomolecules that compose the repair and replacement systems also suffer the same fate as their substrate biomolecules [45].

The importance of the inflammation in the aging process has been recognized only recently [46, 47]. However, inflammation has been lately more and more often considered as a cornerstone of the mechanisms underlying the aging process. Inflammation is a complex host's normal defense reaction to physiological and non-physiological stressors. Acute as well as chronic inflammatory responses are constituted by sequential phases, controlled by hormonal and cellular stimuli [48, 49].

Neuroendocrine theory of aging

A bidirectional communication between the nervous system and the immune system is generally and widely

accepted [50]. During the aging process it is possible to observe a functional decline in the immune and nervous systems, but also an impaired relationship between these two very important regulatory systems is evident, with the resulting loss of homeostasis and much higher risk of death [51, 52].

The aging is due to changes in neural and endocrine functions that are substantial for: 1) coordination and responsiveness of different systems to the external environment; 2) programming physiological responses to environmental stimuli; and 3) the maintenance of an optimal functional status for reproduction and survival.

These changes affect the neurons and hormones regulating evolutionarily important functions such as reproduction, growth and development, but also influence the regulation of survival through adaptation to stress. The lifespan regulated by “biological clocks” would undergo a continuum of sequential stages driven by nervous and endocrine signals. Changes of the biological clock would disrupt the clock and the corresponding adjustments [21, 53].

Neuroendocrine theory of aging indicates the hypothalamic-pituitary-adrenal (HPA) axis as the primary regulator, a sort of pacemaker signaling the onset and termination of each stage of life. The HPA axis controls the functional adjustments aimed at the preservation and maintenance of an internal homeostasis despite the continuing changes in the environment [21]. Therefore, aging can be estimated as the result of a decreased ability to survive stress, suggesting the close relationship between stress and longevity [21, 54]. With aging, a reduction in sympathetic responsiveness is characterized by: 1) a lower number of catecholamine receptors in peripheral target tissues; 2) a decline of heat shock proteins that increase stress resistance; and 3) a decreased capability of catecholamines to induce heat shock proteins [1].

The hormones of the adrenal cortex are glucocorticoids, mineralocorticoids and sex hormones. Among the latter is dehydroepiandrosterone, which has shown to decrease with aging. With aging and in response to chronic stress, not only feedback mechanisms may be altered, but also glucocorticoids themselves become toxic to neural cells, thus disrupting feedback control and hormonal cyclicity [21, 55, 56].

Estrogen replacement therapy represents a special case of hormone replacement therapy and deserves particular attention because of its long clinical history and apparent record of success in increasing the quality of life in postmenopausal women [1].

Caloric restriction (CR)

Caloric restriction is a non-genetic intervention that has consistently shown to slow the intrinsic rate of aging

in mammals. It is defined by the reduction in caloric intake while maintaining essential nutrient requirements.

Caloric restriction has been demonstrated through many years to extend both average and maximal lifespan in many species. Calorie intake restriction is an important nutritional cause of the increased longevity. It is unlikely that most people wish to maintain a 30–40% reduction in food intake necessary for the visible effect. The search for appropriate CR mimetic substances which are potent to alter the pathways of energy metabolism in such a way that mimics the beneficial health-promoting and anti-aging effects of CR without the necessity to reduce food intake significantly was very logical [57].

Many studies and microarray results indicate that CR acts rapidly to produce a physiological state associated with health and longevity in mammals, including humans [58, 59]. CR efficacy on humans is based on abilities to retard biological functional declines and to deter pathological processes, both of which are the major targets of oxidative stress. It modulates also the inflammatory process, a common risk factor for many chronic diseases [60].

The knowledge of CR effect was for a long time limited only to the data from the experiments on laboratory animals and was generally not profound at the genetic and molecular levels. However, the Ristow group evidenced in 2007, in an elegant way, that this effect is due to an increased formation of free radicals within the mitochondria resulting in a secondary induction of an increased antioxidant defense capacity [27]. In their publication, Shimokawa and Trindade presented and made a thorough discussion of the last results concerning the restricting calories-related genes or molecules in the rodent models. They particularly emphasized the roles of forkhead box O transcription factors, AMP-activated protein kinase, and sirtuins (particularly SIRT1) in the effects of restricting calories in rodents [26].

Concluding this very promising aspect concerning the possibility of increasing human longevity, it seems clear now that in spite of the abundant results showing evidently good health benefits and the diminishing of the aging rate by the use of caloric restriction intervention in mammalian animal models, it is nearly sure that this will be lost in the translation to human models [61].

Modern evolutionary theories of aging

The evolutionary theory indicates aging as the result of a decline in force of natural selection. This theory was first extensively formulated based on the observation of patients with Huntington’s disease, a dominant lethal mutation [62].

Prior to these findings, Darwin explained that a natural selection occurs in organisms dying primarily from predation and environmental hazards and consequently evolving a lifespan optimized for their own particular environment [21].

The process of aging increases vulnerability and ultimately leads to the death of organisms. This formula allows us to say that evolutionary theories of aging are the constructions that try to explain the developmental program established by biological evolution and the evolution of aging through the interplay between the processes of mutation and selection. They are important in estimation of the process of aging patterns and in that way form a picture which may possibly explain how longevity is connected with different species. These formulations enable us to create theoretical predictions which can be verified in experiments.

Before the theory of evolution, the process of aging was conceived in a simple way that moving parts wear out, machines break down and all parts deteriorate in the course of time. After the formulation of the theory of evolution, scientists began to wonder why evolution created such complex and well-adapted organisms. They were so perfect that it permitted them to survive from the conception to adulthood, but afterwards they fell into decay and died [2]

The evolutionary theory of aging proposes basically two models for how aging can evolve. The aforementioned models include: 1) the theory of mutation accumulation and 2) the antagonistic pleiotropy theory.

The theory of mutation accumulation originates from the ideas of Medawar, in which he argues that diminishing selection results in the accumulation of late-acting harmful genes [63]. The antagonistic pleiotropy theory created by Williams says that aging evolves due to the pleiotropic effect of genes that are beneficial early in life and then harmful at an advanced age.

The mutation accumulation theory by Medawar

The problem of how aging evolved was first widely discussed by Weismann [64] who created the theory of programmed death. It clearly suggested that aging evolved to the advantage of the species, but not the individual, and that it is an evolutionary advantage for the species possessing a limited lifespan. The evolutionary argument was utilized to propose that older individuals of a given species are expected to die of old age by specific death mechanism of natural selection, elaborated by evolution, which means the elimination of the old part of a population, so it would not compete with younger generation for food and other resources. One of the most important proposals of Weismann [65] was the necessity of successful reproduction in the world that invariably causes degradation of an individual.

In order to explain the biological mechanism of his theory of programmed death, Weismann adapted the idea that there is a specific maximal number of divisions that cells might undergo during the individual life and that this limited number, like the lifespan of the generations of cells, is already determined in the embryonic cell [53]. This surprisingly outstanding theoretical speculation concerning the existence of the cell divisions limit was confirmed experimentally in 1961 by Hayflick and Moorhead [40] who found that normal human embryonic fibroblasts undergo a finite number of cell divisions, and then they "age" and "die". In the case of fibroblasts the limit was about 50 divisions. These important results were confirmed in many other laboratories which experimented on different cell types and it is known worldwide as the *Hayflick Limit* [7].

It should be stressed that Weismann created the first evolutionary theory of aging. On the basis of theoretical inspiration alone, he precisely predicted the existence of the cell division limit.

The substantial observations that the force of natural selection is declining with age, were performed by Medawar [63]. Because all organisms once die from different causes such as diseases, accidents or other, genes beneficial early in life are favored by natural selection over genes beneficial late in life [63]. As a result, the greatest participation in creating new generations comes from young organism and only limited one from old organisms. In that way, the power of natural selection fades with age, making it possible for hazardous late acting genes to exist [66].

It was documented that if the age at death time was really determined by accumulation of late-acting deleterious mutations, one would expect this slope to become steeper with higher parental ages at death [67]. The analysis of real and accurate genealogical records on longevity in European royal and noble families indicated that regression slope for the dependence of offspring lifespan on parental lifespan increases with parental lifespan, as predicted by the mutation accumulation theory [67].

Strehler [68] determined the criteria for the aging process. It is cumulative, universal, progressive, intrinsic and detrimental. It is also relevant that the process is omnipresent. In the predictions of the mutation accumulation theory, mechanisms which limit lifespan fall into 3 categories: every aging mechanism that causes a gradual deterioration of cellular and metabolic processes with age by the appearance of cellular and molecular damage late in life (oxidative damage, somatic DNA mutation, telomere shortening, and others); other mechanisms that are linked to young adulthood (cell senescence, caloric restriction and insulin-signaling pathway regulating caloric and external mechanisms which are unavoidable consequences of old age, such

as inflammation response, caused by infection and pathogenic invasion in old age [69]. Categories 2 and 3 might be similar among the species, the first category is different among different species [2].

Charlesworth's modified mutation accumulation theory

The discovery of late-life mortality plateaus, in which cohort mortality rates appear to plateau or even decrease at later ages, challenged the validity of evolutionary explanations of aging. Therefore, Charlesworth [66] presented a modified mutation accumulation model in which mortality plateau is predicted to occur at late age if alleles affecting fitness do so for more than 1 specific age range class.

The obtained results point out that Charlesworth's modified mutation accumulation theory can both explain the origin of senescence and predict late-life mortality plateaus.

Williams' antagonistic pleiotropic theory

Williams proposed that some genes are beneficial at earlier ages but harmful at later ages. The genes with age-related opposite effects are called pleiotropic genes [70]. The theory of antagonistic pleiotropy is based on 2 assumptions. It is assumed that a particular gene may have an effect not only on one feature but on several traits of an organism (pleiotropy). The next assumption is that these pleiotropic effects may affect individual fitness in antagonistic ways. A gene that increases survival to reproductive age or reproductive output will be favored by natural selection if it decreases the chances of dying at age 10 or 20. Thus, harmful late-acting genes can remain in a population if they have a beneficial effect early in life such as increasing fitness at early ages or increasing reproductive success. Williams argues that natural selection will frequently maximize vigor in youth at the expense of vigor later and thereby produce a declining vigor during adult life later [70]. Therefore, the stipulation of the existence of pleiotropic antagonistic genes could explain the aging process. Such genes will be maintained in the population due to their positive impact on reproduction at young age and despite their negative effects at old, post reproductive age. These theoretical arguments were proved mathematically later by Charlesworth [2, 66]. The antagonistic pleiotropic theory may also explain the idea of reproductive cost or, more generally, of trade-offs between different traits of an organism in which reproduction may come with a cost for species longevity.

However, some experimental findings demonstrated that the increasing lifespan may exhibit some fitness cost only in harsh conditions, which provided a limited

support for antagonistic pleiotropic theory of aging [71, 72]. Pleiotropic antagonism forms the basis for the development of many age-related diseases. The examples of the pleiotropic antagonism mechanism include, among other, atherosclerosis, benign and malignant prostate hypertrophy, Alzheimer's disease and the reciprocal relationship between cellular senescence and cancer [2, 73].

It is also good to mention here the "paradoxical antagonistic pleiotropy" which refers to the setting of bad alleles with deleterious effect early in life but which have beneficial late effects [74]. One quoted example is the allele at the plasminogen activator inhibitor-1 locus which is found augmented in centenarians although, paradoxically, it is a predictor of recurrent myocardial infarctions in young people [75].

Antagonistic pleiotropy theory versus mutation accumulation theory

The main difference between these two theories is that in the mutation accumulation theory the genes with negative effects at old age accumulate passively from one generation to the next, while in the antagonistic pleiotropy theory these genes are actively kept in the gene pool by selection [76]. It should be noted that both theories are widely accepted as applicable. They are also not mutually exclusive and both evolutionary mechanisms may operate at the same time [2, 67].

Disposable soma theory of Kirkwood and Holliday

There were attempts to find a more proper name for the antagonistic pleiotropic theory and to specify in a more detailed way how one and the same gene could have both deleterious and beneficial effects. The disposable soma theory, which was proposed by Kirkwood and Holliday, predicts that aging occurs due to the accumulation or damage during life and that failures of defensive or repair mechanisms contribute to aging [77–79]. It postulated a special class of gene mutations with antagonistic pleiotropic effects in which hypothetical mutations save energy for reproduction (positive effect) partially disabling molecular proofreading and other accuracy promoting devices in somatic cells (negative effect). The distinction between somatic and reproductive tissues is therefore important because the reproductive cell lineage, or germ line, must be maintained at the level that preserves viability across the generations, whereas the soma needs only to support the survival of a single generation. Thus, the key feature of the disposable soma theory is its emphasis on the optimal balance between somatic maintenance and repair versus reproduction.

Evolutionary theories of aging and research on aging — existing contradiction

One of the most intriguing phenotypes in the biology of aging is that there are animals that appear not to age. In those animals (example: female turtles) both survivorship and reproductive output may increase with age [80]. These observations, which suggest some species may not age, are contradictory to the classical evolutionary models of aging, which predict that all species eventually age [81].

Rapid progress in human genomics raises the perspective of a great increase in our knowledge of the determinants of human longevity. In the intensive study in the period of the last 30 years searching for genes that affect aging in model organisms like yeast, nematode worms and fruit flies, the scientists identified many genes that alter the rate of aging. However, no gene abolishing the aging process altogether has been found [82].

Recent modifications of evolutionary theories

Recently, theoreticians have refined several aspects of the evolutionary theory [83, 84]. In contrast to Hamilton's classical analysis [81, 85], recent analysis of Baudisch shows that the strength of selection does not necessarily always increase with age; it can remain constant or even increase during adulthood [86], which might lead to either no, negligible, or "negative" aging [87]. Although the evidence for negligible or negative senescence is scant and aging might be ubiquitous [88], Baudisch's work argues that the evolution of aging is driven by changes in extrinsic mortality because such changes affect how rapidly the force of selection declines with age.

Species or populations facing high extrinsic mortality should age more rapidly than those experiencing low levels of extrinsic mortality because the strength of selection declines more rapidly in the former than in the latter, a prediction experimentally confirmed in fruit flies by Stearns and coworkers [89]. The theory proposed by Abrams indicates that in density-independent populations extrinsic mortality does not alter the rate of aging, whereas under density-dependence the effect of extrinsic mortality depends on the age-specificity of demographic changes and on whether density-dependence is mediated by changes in survival or reproduction [90–91]. The investigation suggests that in social species resource transfer across generations might select for extended post reproductive survival (e.g. menopause) and shape the evolution of aging. Finally, a model developed by Ackermann and coworkers has

addressed the origin of aging in the history of life [84]. The results demonstrate that asymmetric cell division among unicellular organisms can evolve as strategy to limit cellular damage by distributing damage unequally and that asymmetry causes the evolution of aging; aging might thus be a fundamental and inevitable property of cellular life [3].

Recent progress in theoretical aspects of the evolution of aging has been slow and much remains to be done [84, 92]. For example, there is a great need for the development of more realistic models which would study the effects of different types of age-dependent mutations on aging and how such mutations interact with the environment and other genes to influence the evolution of aging [84, 93, 94]. Mutations that affect aging might produce more complex age-dependent effects than supposed in the classical theory, with MA and AP/DS representing extremes along a continuum of possible mutational effects [3].

Evolutionary theories in the light of genomic studies

The evolutionary theory of aging offers a theoretical background helping to interpret many observations. The theory gives certain indications concerning the evolutionary mechanisms and the results indicating how the mechanism of evolution of aging is acting. However, it does not give a complete picture of the mechanism according to which the evolution of aging takes place across the particular species. Evolutionary theories of aging are useful when they give quite new opportunities for exploratory experiments by suggesting predictions which are possible to verify, but they should never be utilized to impose limitations on aging studies [67]. In fact, the evolutionary theories of aging are not complete theories, but rather a set of ideas that require further precise validation.

The research results will determine through biological approach the degree to which each theory contributes to the actual process of aging [95].

The conclusion from these experiments is that the aging process is very different in mice and humans, which indicates the need for research on human samples to resolve the aging mechanisms concerning the human longevity. A small number of age-related differences in expression are conserved across the species. These aging mechanisms may be linked to the paths in young adults or may be necessary consequences of growing old. Identifications of these pathways is substantial because they highlight specific aging mechanisms that can be dissected in model organisms to elucidate general principles of aging [69].

A very important example of research is the study of the regulation of aging by IIS in worms, flies, and mice [96–100].

Mutations in *C. elegans daf-2* and its *Drosophila* homolog *dInR* extend worm and fly lifespan, and impairing the function of both the insulin receptor and the IGF-1 receptor, promotes longevity in the mouse [96, 101–104]. Remarkably, heterozygous mutations in the IGF-1 receptor confer reduced activity in transformed lymphocytes and are significantly enriched in female centenarians of Ashkenazi Jews, suggesting that IIS might regulate longevity in humans.

The potential importance of genetic variation in IIS in affecting human longevity is also underscored by the finding that polymorphisms in FOXO3A, a human ortholog of dFOXO/DAF-16, are associated with exceptional longevity in two independent studies [105, 106].

Two excellent comparative studies were performed providing the novel insights into the problem of public versus private mechanisms of aging. McElwee et al. asked whether the downstream mechanisms whereby IIS regulates lifespan are conserved among species [107]. By comparing transcript profiles in flies, worms, and mice they found that there is little evidence for conservation at the level of orthologous or paralogous downstream genes but that two IIS regulated processes (reduced protein biosynthesis, cellular detoxification) are conserved across the species [107]. These results suggest that some of downstream targets of IIS might be lineage-specific, whereas the pathway might have conserved effects on aging at the process level [107].

Smith et al. (2008) systematically examined lifespan phenotypes of single-gene deletions of yeast orthologs of 216 known *C. elegans* aging genes and found that many of these loci are conserved, both in sequence and function [108]. In particular, among the conserved ortholog pairs, genes involved in nutrient sensing and protein translation downstream of TOR signaling were significantly enriched [108]. The development of appropriate genomic instruments and methods may permit to define well the aging process by making genome-wide scans for detection transcriptional differences between the young and the old organisms, using gene array technology assessment. Transcriptional profiles for aging contain quantitative data on age-related changes in expression, for large parts of genome. Genes that exhibit age-related differences in transcription in many species are very interesting as biomarkers of age [2].

Perspectives for research in modern biotechnology and molecular biology

Modern biotechnology noted several very important discoveries in the field of aging:

- lifespan extension in rodents by caloric restriction, provided that protein, vitamin and micronutrients are sufficiently available to prevent malnutrition;

- the occurrence of stress-resistance of long-lived mutant worm strains;
- the existence of single gene mutants which extend mouse longevity;
- the role of insulin and insulin-like signals in modulating aging rates in widely separated species and animals.

These experimental achievements have traced the path for evolutionary approaches to make an important contribution to the biology of aging and, finally, to medicine [83]. Many authors have recently underlined that a great experimental biotechnological approach in research gave very precise descriptions of the phenotypes of aging and experimental analysis of their basis [3, 83].

The important participation of biotechnology research, which may significantly help to understand the evolution of aging better, means the work on control protein synthesis, validation of biomarkers of aging, understanding the biochemistry of longevity, comparative biology studies and research in the area of geriatric pathology. It was found that both the rate of synthesis and rate of degradation of many proteins is slower in tissues of old rodents and humans, which extends time for the occurrence of different protein modifications and intra-molecular rearrangements that may ultimately lead to the creation of atypical forms and have pathological consequences. Similarly, the shortening of telomeres, the specialized nucleoprotein structures at the end of chromosomes, can induce changes in expression of genes the most closest to the telomere, known as the telomere position effect [2, 3].

Development of functional genomics tools has made it possible to define the aging process and its mechanisms by performing genome-wide scans for transcriptional difference between the young and the old using gene array technology assessment.

Conclusions

Many problems and answers, concerning basic and substantial mechanisms and paths according to which the aging process and the evolution of aging take place, are still waiting for delineation and better clarification. Evolutionary biologists have to utilize the tools of molecular biology, while molecular biologists must put their experiments into the evolutionary research framework. The present time seems to be most suitable for the integration of molecular biogerontology and the evolutionary biology of aging [3].

Finally, we should keep in our minds the important sentence of Hayflick [109] published in *Nature*: “If the main goal of our biomedical research enterprises is to resolve causes of death, then every old person

becomes a testimony to those successes. Biogerontologists have an obligation to emphasize that the goal of the research on ageing is not to increase human longevity regardless of the consequences, but to increase active longevity free from disability and functional dependence”.

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References

- Tosato M, Zamboni V, Ferrini A., Cesari M. The aging process and potential interventions to extent life expectancy. *Clin Interv Aging* 2007; 2: 401–412.
- Ljubuncic P, Reznick AZ. The evolutionary theories of aging revisited — A mini review. *Gerontology* 2009; 55: 205–216.
- Flatt T, Schmidt PS. Integrating evolutionary and molecular genetics of aging. *Biochim Biophys Acta* 2009; 1790: 951–962.
- Jin K, Minami M, Xie L, Sun Y, Mao XO, Wang Y, Simon RP, Greenberg DA. Ischemia-induced neurogenesis is preserved but reduced in the aged rodent brain. *Aging Cell* 2004; 3: 373–377.
- Davidovic M, Sevo G, Svorcan P, Milosevic DP, Despotovic N, Erceg P. Old age as privilege of the “selfish ones”. *Aging and Disease* 2010; 1: 139–146.
- Weismann A. Über Leben und Tod. Fisher, Jena 1892.
- Hayflick I. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res* 1965; 37: 614–636.
- van Heemst D. Insulin, IGF-1 and longevity. *Aging and disease* 2010; 1: 147–157.
- Cornelius E. Increased incidence of lymphomas in thymectomized mice — evidence for an immunological theory of aging. *Experientia* 1972; 28: 459.
- Rozenmuller AJ, van Gool WA, Eikelenboom P. The neuroinflammatory response in plaques and amyloid angiopathy in Alzheimer’s disease: therapeutic implications. *Curr Drug Targets CNS Neurol Disord* 2005; 4: 223–233.
- Brys K, Vanfleteren JR, Braeckman BP. Testing the rate-of-living/oxidative damage theory of aging in the nematode model *Caenorhabditis elegans*. *Exp Gerontol* 2007; 42: 845–851.
- Hulbert AJ, Pamplona R, Buffenstein R, Buttemer WA. Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol Rev* 2007; 87: 1175–1213.
- Rollo CD. Aging and the Mammalian Regulatory Triumvirate. *Aging and disease* 2010; 1: 105–138.
- Bjorksten J. The cross-linkage theory of aging. *J Am Geriatr Soc* 1968; 16: 408–427
- Bjorksten J, Tehu H. The cross-linking theory of aging — added evidence. *Exp Gerontol* 1990; 25: 91–95.
- Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1957; 11: 298–300.
- Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. *Science* 1954; 119: 623–626.
- Harman D. The clock: the mitochondria ? *Am Geriatr Soc* 1972; 20: 145–147.
- Sastre J, Pallardo FV, Garcia de la Asuncion J et al. Mitochondria, oxidative stress and aging. *Free Rad Res* 2000; 32: 189–198.
- Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; 408: 239–247.
- Weinert BT, Timiras PS. Theories of aging. *J Appl Physiol* 2003; 95: 1706–1716.
- Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Res* 1998; 78: 547–581.
- Shringarpure R, Davies KJ. Protein turnover by the proteasome in aging and disease. *Free Radic Biol Med* 2002; 32: 1084–1089.
- Afanas’ev I. Signaling and damaging functions of free radicals in aging — Free radical theory, hormesis, and TOR. *Aging and disease* 2010; 1: 75–88.
- McCay CM. Iodized salt a hundred years ago. *Science* 1935; 82: 350–351.
- Shimokawa I, Trindade LS. Dietary restriction and aging in rodents: a current view on its molecular mechanisms. *Aging and disease* 2010; 1: 89–104.
- Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* 2007; 6: 280–293.
- Shors TJ, Miesegaes G, Beylin A, Zhao M., Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 2001; 410: 372–376.
- Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci USA* 1988; 85: 6465–6467.
- Ames BN. Endogenous oxidative DNA damage, aging, and cancer. *Free Radical Res Commun* 1989; 7: 121–128.
- Alexeyev MF, LeDoux SP, Wilson GL. Mitochondrial DNA and aging. *Clin Sci (Lond)* 2004; 107: 355–364.
- Brand FN, Kiely DK, Kannel WB et al. Family patterns of coronary heart disease mortality: the Framingham Longevity Study. *J Clin Epidemiol* 1992; 45: 169–174.
- Ross OA, McCormack R, Curran MD et al. Mitochondrial DNA polymorphism: its role in longevity of the Irish population. *Exp Gerontol* 2001; 36: 1161–1178.
- Ivanova R, Lepage V, Charron D et al. Mitochondrial genotype associated with French Caucasian centenarians. *Gerontology* 1998; 44: 349.
- Tanaka M, Gong JS, Zhang J et al. Mitochondrial genotype associated with longevity. *Lancet* 1998; 351: 185–186.
- De Benedictis G, Rose G, Carrieri G et al. Mitochondrial DNA inherited variants are associated with successful aging and longevity in humans. *FASEB J* 1999; 13: 5232–1536.
- Davies KJ, Quintanilha A, Brooks GA et al. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun* 1982; 107: 1292–1299.
- Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 2000; 29: 222–230.
- Flores I, Cayuela ML, Blasco MA. Effects of telomerase and telomere length on epidermal stem cell behavior. *Science* 2005; 309: 1253–1256.
- Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961; 25: 585–621.
- Campisi J. Cancer aging and cellular senescence. *In Vivo* 2000; 14: 183–188.
- Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. *Science* 2006; 3: 1257.
- Ferron SR, Marques-Torrejon MA, Mira H, Flores I, Taylor K, Blasco MA, Farinas I. Telomere shortening in neural stem cells disrupts neuronal differentiation and neurogenesis. *J Neurosci* 2009; 29: 14394–14407.
- Taupin P. Aging and neurogenesis, a lesion from Alzheimer disease. *Aging and Disease* 2010; 1: 89–104.
- Hayflick I. Entropy explains aging, genetic determinism explains longevity, and undefined terminology explains misunderstanding both. *PLoS Genet* 2007; 3: e220.
- McGeer EG, McGeer PL. Brain inflammation in Alzheimer disease and the therapeutic implications. *Curr Pharm Des* 1999; 5: 821–836.
- Chung HY, Kim HJ, Kim JW et al. The inflammation hypothesis of aging - Molecular modulation by calorie restriction. *Ann NY Acad Sci* 2001; 928: 327–335.

48. Huerre MR, Gounon P. Inflammation patterns and new concepts. *Res Immunol* 1996; 147: 417–434.
49. Cesari M, Kritchevsky SB, Leeuwenburgh C et al. Oxidative damage and platelet activation as new predictors of mobility disability and mortality in elders. *Antioxid Redox Signal* 2005; 8: 609–619.
50. Besodovsky H, Del Rey A. Immune-neuro-endocrine interactions: facts and hypotheses. *Endocrinol Rev* 1996; 17: 64–102.
51. Fabris N. Neuroendocrine-immune interactions: a theoretical approach to ageing. *Arch Gerontol Geriatr* 1991; 12: 219–230.
52. De La Fuente M. Effects of antioxidants on immune system ageing. *Eur J Clin Nutr* 2002; 56: S5–S8.
53. Timiras PS. Biological perspectives on aging. *Am Sci* 1978; 66: 605–613.
54. Seyle H. *The stress of life*. McGraw-Hill, New York 1976.
55. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev* 1986; 7: 284–301.
56. Sapolsky RM. *Rudman Stress, the aging brain, and the mechanisms of neuron death*. MIT Press, Cambridge, MA 1992.
57. Lane MA, Roth GS, Ingram DR. Caloric restriction mimetics: a novel approach for biogerontology. In: *Biological aging: Methods and Protocols, Series: Methods in Molecular Biology* 2007; 371: 143–149.
58. Selman C, Lingard S, Chudhury A et al. Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. *FASEB J* 2008; 22: 807–818.
59. Swindell WR. Comparative analysis of microarray data identifies common responses to caloric restriction among mouse tissues. *Mechanisms of Ageing and Development*, 2008; 129: 138–153.
60. Yu BP. Why caloric restriction would work for human longevity. *Biogerontology* 2006; 7: 179–182.
61. Dirks AJ, Leeuwenburgh C. Caloric restriction in humans: potential pitfalls and health concerns. *Mech Age Dev* 2006; 127: 1–7.
62. Haldane JBS. *New paths in genetics*. Allen and Unwin, London 1941.
63. Medawar PB. *Unsolved problems of biology*. HK Levis, London 1952.
64. Weismann A. *Essays upon heredity*. Clarendon Press, Oxford 1891.
65. Weismann A. *Über die Dauer des Lebens*. Fisher, Jena 1882.
66. Charlesworth B., Fisher, Medawar Hamilton and the evolution of aging. *Genetics* 2000; 156: 927–931.
67. Gavrilov LA, Gavrilova NS. Evolutionary theories of aging and longevity. *Scientific Worlds Journal* 2002; 2: 339–356.
68. Strehler BL. Origin and comparison of the effects of time and high energy radiations on living systems. *Quart Rev Biol* 1959; 34: 117–142.
69. Kim SA. Common aging pathways in worms, flies, mice and humans. *J Exp Biol* 2007; 210: 1607–1612.
70. Williams GC. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* 1957; 11: 398–411.
71. Johnson TE, Hutchinson EW. Absence of strong heterosis for the life-span and other life history traits in *Caenorhabditis elegans*. *Genetics* 1993; 134: 465–474.
72. Walker DW, McColl G, Jenkins NL, Harris I, Lithgow GJ. Evolution of lifespan in *C. elegans*. *Nature* 2000; 405: 296–297.
73. Wick G, Berger P, Jansen Dürr P, Grubeck-Loebenstien B. A Darwinian-evolutionary concept of age-related diseases. *Exp Gerontol* 2003; 38: 13–25.
74. Martin GM. Modalities of gene action predicted by the classical evolutionary biological theory of aging. *Ann NY Acad Sci* 2007; 1100: 14–20.
75. Mannucci PM, Merati G., Peyvandi P et al. Gene polymorphisms predicting high plasma levels of coagulation and fibrinolysis proteins. *Arterioscler Thromb Vasc Buil* 1997; 17: 755–759.
76. Le Bourg É. A mini review of the evolutionary theories of aging: is it the time to accept them? *Demogr Res* 2001; 4: 1–28.
77. Kirkwood TB, Holliday R. The evolution of ageing and longevity. *Proc R Soc London, B Biol Sci* 1979; 205: 531–546.
78. Kirkwood TB, Aultad SN. Why do we age? *Nature* 2000; 408: 233–238.
79. Kirkwood TB. Evolution of ageing. *Nature* 1997; 270: 301–304.
80. Congdon JD, Nagle TD, Kinney OM, van Loben Sels RC, Quinter TR, Tinkle DW. Testing hypothesis of aging in long-lived painted turtles (*Chrysemys picta*). *Exp Gerontol* 2003; 38: 765–772.
81. Hamilton WD. The moulding of senescence by natural selection. *J Theor Biol* 1966; 12: 12–45.
82. Kirkwood TB. Evolution of Ageing. *Mech Ageing Dev* 2002; 123: 737–745.
83. Partridge L, Gems D. Beyond the theory of ageing, from functional genomics to evolvero. *Trends Ecol Evol* 2006; 21: 334–340.
84. Ackermann M and Pletcher SD. Evolutionary biology as a foundation for studying aging and aging-related disease. *Evolution in Health and Disease*. Oxford University Press, Oxford 2007: 241–252.
85. Rose MR, Rauser CL, Benford G, Matos M, Mueller JD. Hamilton's forces of natural selection after forty years. *Evolution* 2007; 61: 1265–1276.
86. Baudisch A. Hamilton's indicators of the force of selection. *Proc Natl Acad Sci USA* 2005; 102: 8263–8268.
87. Finch CE. *Longevity, senescence and genome*. University of Chicago Press, Chicago, 1990.
88. Ackermann M, Chao L, Beergstom CT, Doebeli M. On the evolutionary origin of aging. *Aging Cell* 2007; 6: 235–244.
89. Stearns SC, Ackermann M, Doebeli M, Kaiser M. Experimental evolution of aging, growth and reproduction in fruitflies. *Proc Natl Acad Sci USA* 2000; 97: 3309–3313.
90. Abrams PA. Does increased mortality favor the evolution of more and rapid senescence? *Evolution* 1993; 47: 877–887.
91. Reznick DN, Bryant MJ, Roff D, Ghlambor CK, Ghlambor DE. Effect of extrinsic mortality on the evolution of senescence in guppies. *Nature* 2004; 431: 1095–109.
92. Partridge I, Gems D. Beyond the evolutionary theory of ageing. *Trends Ecol Evol* 2006; 1: 334–340.
93. Promislow DEL, Pletcher SD. Advice to an aging scientist. *Mech Ageing Dev* 2002; 123: 841–850.
94. Promislow DEL. Protein networks, pleiotropy and the evolution of senescence. *Proc Roy Soc (London B)* 2004; 271: 1225–1234.
95. Kirkwood TB. Understanding aging from an evolutionary perspective. *J Intern Med* 2008; 263: 117–127.
96. Tatar M, Bartke A, Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 2003; 299: 1346–1351.
97. Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell* 2005; 120: 449–460.
98. Partridge L, Gems, Withers DJ. Sex and Death: What is the connection? *Cell* 2005; 120: 461–472.
99. Guarente L, Partridge L, Wallace DC. *Molecular Biology of aging*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor 2008.
100. Kenyon C, Chang J, Gensch E, Rudner A, Tabiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 1993; 366: 461–464.
101. Tatar M, Kopelman A, Epstein D, Tu MP, Yin C-M, Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine functions. *Science* 2001; 292: 107–110.
102. Holzeberger M, Dupont J, Ducos B et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003; 421: 182–187.
103. Holzeberger M, Kappeler L, De Magalhaes Filho C. IGF-1 signaling and aging. *Exp Gerontol* 2004; 39: 1761–1764.
104. Blüher M, Kahn BB., Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 2003; 299: 572–574.
105. Wilcox BJ, Donlon TA, He Q et al. FOXO3A genotype is strongly associated with human longevity. *Proc Natl Acad Sci USA* 2008; 105: 13987–13992.
106. Flachsbar F, Caliebe A, Kleindorp R et al. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA* 2009; 106: 2700–2705.
107. McElwee JJ, Schuster E, Blanc E et al. Evolutionary conservation of regulated longevity assurance mechanisms. *Genome Biol* 2007; 8: r132.
108. Smith ED, Tsuchiya M, Fox LA et al. Quantitative evidence for conserved longevity pathways between divergent eukaryotic species. *Genome Res* 2008; 18: 564–570.
109. Hayflick L. The future of ageing. *Nature* 2000; 408: 267–269.