Chapter 1
An Unsolved Problem in Gerontology Yet: Molecular Mechanisms of Biological Aging—A Historical and Critical Overview

Sataro Goto

Abstract I discuss the historical background of the original proposals and modern versions of the selected theories of the molecular mechanisms of biological aging, i.e., the mutation or genome instability theory, the free radical or oxidative stress theory, the mitochondrial theory, the error catastrophe theory, the altered protein or protein homeostasis or proteostasis theory, the dysdifferentiation or epigenetic theory, and the hyperfunction theory, adding a brief comment on a recent popular theory of "epigenetic clock" in this revised version of my previous overview (Goto, Aging mechanisms. Longevity, metabolism and brain aging, Springer, Berlin, 2015). I have involved the development of some of the theories, which are therefore described in more detail than others. A discussion on the definition of aging and general comments on the aging theory are described. A most popular theory of aging, the free radical or oxidative theory, was proposed more than half a century ago but has recently faced severe criticisms to which I shall refer. So far, no single theory has been able to successfully explain the mechanism of biological aging. We are thus awaiting emergence of a new paradigm or an integration of the existing theories for better understanding of the mechanism.

This article is a revised version of my previous contribution to the Springer book (Goto 2015).

S. Goto (✉)

Institute of Health and Sports Science & Medicine, Juntendo University Graduate School, Chiba, Japan

Department of Aging Neuroscience, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
e-mail: sgotou@juntendo.ac.jp; gotosataro@sakura.juntendo.ac.jp

1.1   Introduction

The average human life span in developed countries has increased by more than 20 years in the past several decades. Our major concern has shifted from an increase in the life span to an extension of the health span by retarding the progress of frailty due to lowered physical activities and inadequate nutrition in elderly people, thus reducing risks of potentially fatal diseases such as cancer, cardiovascular disease, stroke, kidney disorder, type 2 diabetes mellitus, etc. Currently, elderly people are more concerned of maintaining high quality of life by delaying frailty that results from the decline of physiological functions such as sarcopenia and osteoporosis, even when such conditions are not directly fatal by themselves. However, it is often stated that the major risk factor for developing the geriatric diseases mentioned above is old age, or rather, biological aging itself. This means that the biological mechanisms of aging are likely to underlie the etiologies and progress of age-related diseases, although aging itself is not technically a disease.

Since Peter Medawar stated in 1952 that aging is *an unsolved problem of biology* (Medawar 1952), the mechanisms of aging have been the subject of intensive research interest, and a large number of papers have been published on the mechanisms of aging. Half a century after Medawar's statement, leading scientists of biogerontology claimed that aging i*s* no longer an unsolved problem in biology (Holliday 2006; Hayflick 2007). Robin Holliday wrote that recently published major books on aging agree that the biological reasons for aging in mammals are now well understood and that the mechanism of biological aging is therefore no longer an unsolved problem. It is true that there appears to be similar, apparently common or conserved, senescent phenotypes in different species of animals in which longevity differs by several 100-fold (see Fig. 1.1 and Table 1.1); however, the very basic problems of the mechanism behind such species differences in longevity are not clear nor have been studied deeply enough.

In this chapter, I provide an overview of selected theories of the mechanisms of biological aging. The overview includes theories of historical interest that are not necessarily widely accepted currently and/or theories that have since been transformed into modern versions. The latter group is presented under the same sections as the original theories from which they are derived.

1.2   The Definition of Aging

There are two words with somewhat similar meanings that are commonly used in gerontology but are often confused, i.e., *aging* and *senescence*. Caeb Finch writes in his influential book that the term *aging* is mainly used to describe any changes that occur during the passage of physical time, during which there need be not common mechanisms, such as the aging of collagen, the aging of diploid cells in culture or of erythrocytes in circulation, the aging of populations or societies, or the aging of
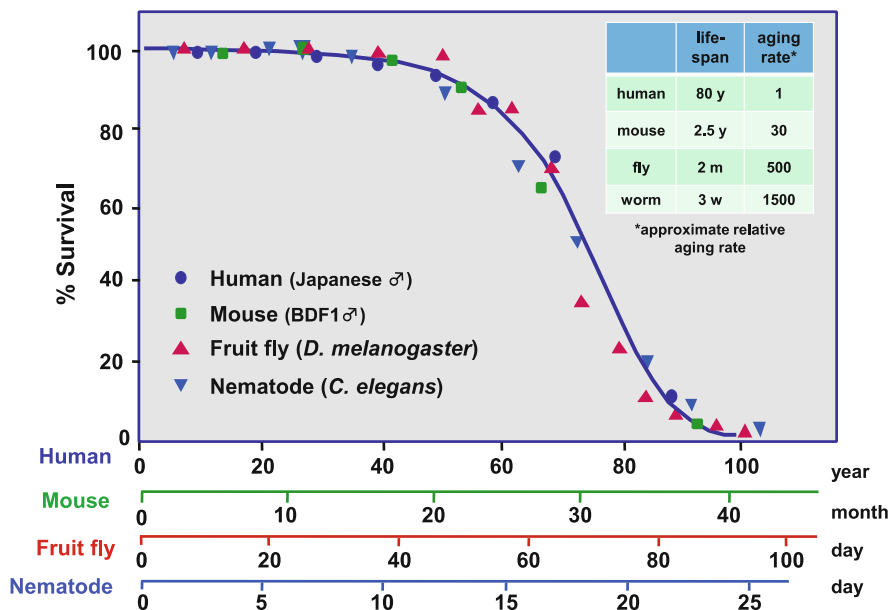
Fig. 1.1 Survival curves of human, mouse, fruit fly, and nematode. (Adapted and modified from Goto S (2002) Saibo kogaku 21: 704–708 (in Japanese))

genes and species during evolution. In contrast, the term *senescence* is used to describe age-related changes in an organism that adversely affect its vitality and functions and, most importantly, increase its mortality rate as a function of time (Finch 1990). Robert Arking states that "the terms *aging* and *senescence* seem to overlap considerably, and the difference between them may be one of emphasis rather than fundamentals" (Arking 1998). Because the term *aging* is often used to convey what he describes as *senescence* in most current gerontology writing, I use the term *aging* to discuss the mechanisms of aging (senescence) in this chapter.

To cite a few examples of the definition of *aging* (*senescence*) by leading scientists in biomedical gerontology books, Medawar wrote, as cited by Bernard Strehler in his book (Strehler 1977), "*Senescence* may be defined as that change of the bodily faculties and sensibilities and energies which accompanies aging, and which renders the individual progressively more likely to die from accidental causes of random incidence." Strehler himself defines it as "the changes which occur (1) generally in the postreproductive period and (2) which result in a decreased survival capacity of the part of the individual organism." He further notes that "different evolutionary lines might very well decline in their survival capacities for entirely different immediate reasons. It may also be, however, that there are one or more dominant mechanisms of aging, common to all higher forms of life." Alex Comfort defines *senescence* (*aging*) as a decrease in viability (leading to an increasing probability of death) with increasing chronological age and an increase in

Table 1.1 Common and uncommon aging phenotypes in human and model animals (Adapted from Vijg and Campisi (2008) and modified by Goto (2015))

Phenotype	Human	Mouse	Fly	Nematode
Decreased cardiac function	Yes	Yes	Yes	NA
Apoptosis, cellular senescence	Yes	Yes	Yes	?
Cancer, hyperplasia	Yes	Yes	No	No
Genome instability	Yes	Yes	Yes	Yes
Macromolecular aggregates	Yes	Yes	Yes	Yes
Reduced memory & learning	Yes	Yes	Yes	NA
Decline in GH, DHEA, testosterone, IGF	Yes	Yes	?	?
Increase in gonadotropins, insulin	Yes	Yes	?	?
Decreased thyroid function	Yes	Yes	NA	NA
Decrease in innate immunity	Yes	Yes	Yes	Yes
Increase in inflammation	Yes	Yes	No	No
Skin morphology changes	Yes	Yes	?	Yes
Decreased mitochondrial function	Yes	Yes	Yes	Yes
Sarcopenia	Yes	Yes	Yes	Yes
Osteoporosis	Yes	Yes	NA	NA
Abnormal sleep	Yes	Yes	Yes	?
Decrease in vision	Yes	Yes	?	NA
Demyelination	Yes	Yes	?	No
Decreased fitness	Yes	Yes	Yes	Yes
Arteriosclerosis	Yes	No	NA	NA
Changes in fat	Yes	Yes	?	?

Note: Highlights by yellow are common aging phenotypes in listed animals. *NA* not applicable

vulnerability (Comfort 1964). The term vulnerability may be rephrased as frailty, a term more commonly used in geriatric medicine in recent years.

Surveying the definition of aging in gerontology literatures, I note that aging can be defined as a progressive functional decline with advancing age that occurs in every individual, sooner or later, within a population of a species, beginning around the time of reproductive maturity and leading to an increased probability of death over time. Theories of the mechanisms of aging that can fit with this definition will be examined in this chapter.

1.3 Aging Theories

In 1990, Zhores Medvedev wrote that more than 300 theories about the biological mechanisms of aging could be found in the literature (Medvedev 1990). Among the theories cited in his review, some are still popular, and some have disappeared or have been transformed, while other new theories have emerged and are currently

being tested for validity. Theories of aging are mixed in that there are different levels of aging phenomena at the molecular, cellular, tissue, organ, or systemic levels.

George Martin has proposed a classification of the mechanisms of aging into two categories: public and private mechanisms (Martin et al. 1996a). The public mechanisms of aging are those that could potentially be applied to the aging of different animals and tissues or cells, while the private mechanisms of aging are those that appear to be only true in specific species, cells, tissues, or organs. For example, the immunological theory can only be true in animals such as mammals with appropriate immune system but may not be true in nematodes or insect models which lack in acquired immunity seen in mammals. When thinking about the aging that occurs in any somatic cells of different species of animals, it is more appropriate to focus on "public" mechanisms rather than "private" mechanisms for the purposes of our discussion. See discussion on "public" and "private" mechanisms of aging in a literature (Partridge and Gems 2002).

In this chapter, I therefore discuss the mechanisms of aging that can mainly, although not exclusively, be viewed as public. The private mechanisms of aging, however, are by no means unimportant. Indeed, they are useful by themselves to explain particular etiologies or the progress of individual age-related diseases. It should be noted that private mechanisms often involve public mechanisms. For instance, endocrinological decline with age, a private mechanism of aging, can be caused by public mechanisms, such as oxidative stress or protein alteration. It should be noted that each theory is naturally not mutually exclusive or incompatible each other, but may instead be regarded as a part of other theories.

Figure 1.1 illustrates age-related changes in the mortality rate of different animal species, with life span difference of more than 1000-fold (e.g., between human and nematode). The apparent similarity of the survival curves may suggest that the underlying mechanisms of aging are common among the shown animal species. In fact, many aging phenotypes are conserved in model animals and human, as shown in Table 1.1 (Vijg and Campisi 2008). It should be noted, however, that no overall correlation of age regulation was found in the gene expression database, at least between mice and humans, for example, and therefore, aging processes in mice and humans may be fundamentally different, despite certain commonalities in the observed transcriptional profiles in the genes, for example, of electron transport chain for aging mouse, human, fly, and nematode (Zahn et al. 2007). In the following sections, I examine selected public mechanisms of aging.

1.4 Mutation Theory of Aging/Genome Instability Theory of Aging

This theory predicts that mutations accumulating in the genome are responsible for aging, i.e., physiological decline with advancing age. One of the early proponents of the theory was Leo Szilard. As a nuclear physicist, he proposed that somatic cell

mutations induced by ionizing radiation generated in reactions such as the nuclear fission and fusion would accelerate aging (Szilard 1959). Ionizing radiation in fact shortened the life span of mice and rats, shifting the survival curves to the left, with similar shapes as unirradiated controls, apparently being reminiscent of an acceleration of normal aging (Lindop and Rotblat 1961). It was later shown, however, that the major cause of the observed life span shortening was an increased rate of carcinogenesis rather than an acceleration of physiological aging in general. Irradiated rodents have therefore not been used as models of accelerated aging. In the meantime, it has been reported that the DNA repair activity of skin fibroblasts in cultures irradiated with ultraviolet light depends on an animal's maximum life span (Hart and Setlow 1974). The activity of cells from long-lived animals, such as human, elephant, and cow, was nearly five times higher than that in short-lived animals such as rat and mouse. Although the repair capacity and life span were not proportional, it was thought that long-lived species may have a more active repair system that could therefore play a role in deceleration of aging rate. More recently, it was reported that base excision repair activity declines with age in mice in the brain, liver, spleen, and testes (Cabelof et al. 2002). To study the mutation frequency in vivo, selectable markers, such as hypoxanthine phosphoribosyltransferase (HPRT) of purine metabolism, have been used to detect 6-thioguanine-resistant cells that are defective in the HPRT gene. Using this method, it was reported that the mutation frequency increased with age (from 2 to 94 years of age) in cultured human kidney tubular epithelial cells (Martin et al. 1996b). To overcome the limitation that the cells to be assayed must proliferate in vitro in the assay, transgenic mice with reporter genes, such as the bacterial lacZ gene, have been developed. The DNA recovered from the transgenic mouse tissues, including the brain and heart, consisting of mainly postmitotic cells, was screened for mutations in the integrated shuttle vector in a bacterial host (Dollé et al. 2000). Significant age-related mutant frequency was found to increase from 10×10^{-5} (3 months old) to 25×10^{-5} (33 months old) in the small intestine and from 5 to 10×10^{-5} in the heart of mice. However, no change was observed in the brain (5×10^{-5}) between the young and old animals. It is noted that the increase was linear from young to old ages, with no larger changes at older ages.

Because functional decline with age is apparently more significant in the brain and heart than in the intestine and because the frequency of mutation is not high enough to account for the level of decline, it appears to be difficult to ascribe a cause of aging to the age-related accumulation of mutations. In fact, the serious proponents of this theory recognize one important question about this theory, stating that "it is not known whether the frequency of the random changes is sufficient to cause the phenotypic effects generally associated with aging" as cited from the abstract of a paper by Vijg and Suh (2013). The readers are advised to also refer to a recent general view on this theory (Moskalev et al. 2012).

1.5 Free Radical Theory of Aging/Oxidative Stress Theory of Aging

The free radical theory of aging is one of the most well-known and popular theories of aging proposed so far. The theory has currently been transformed into the "oxidative stress theory of aging" because oxidative stress most frequently involves reactive oxygen species (ROSs) and because the causative agents of the stress are not only free radicals but also include non-radical ROS such as hydrogen peroxide (Martin et al. 1996a). The principle of the theory was originally proposed by Denham Harman more than half a century ago (Harman 1956). The history of the theory and the inside story of how the idea came to him are found in an interview with him (Harman and Harman 2003). He was originally a chemist specializing in free radicals who later became interested in aging and established himself as a medical scientist. In the beginning, the theory apparently did not attract as much interest from scientists working on aging as other theories, such as the mutation theory and the protein cross-linking theory. This is likely because radicals were not familiar to biologically oriented scientists, and the theory appeared to be too simple and straight forward to explain the complex aging phenomena. However, after superoxide dismutase (SOD), which catalyzes dismutation of superoxide radical forming hydrogen peroxide, was reported to be widely distributed in mammalian tissues (McCord and Fridovich 1969), more researchers became interested in the capacity of free radicals to damage a variety of cellular constituents, potentially leading to aging. The major targets of free radical damage were believed to be membrane lipids, which contain many unsaturated fatty acids that are easily attacked by radicals to produce lipid peroxides. Lipid peroxides were thought to be components of the lipofuscin age pigment, a then well-known histological marker of aged cells that consume substantial amounts of oxygen, such as neurons and kidney cells. Lipid peroxidation has readily been measured as thiobarbituric acid reactive substances (TBARS), although the method to measure TBARS may be problematic in specificity and, recently, such substances as isoprostanes have been used to evaluate the oxidation. DNA was another molecule of interest for oxygen radical attack. It can form 8-hydroxy-2-deoxyguanosine (8-oxodG), which is relevant to cancers that increase with age (Fraga et al. 1990).

Oxidatively modified proteins have attracted the least interest mainly because of limitations in the methods to detect them despite the fact that the catalytic activities of enzymes have long been known to decrease with age (Stadtman 1988) and therefore can drive aging. Earl Stadtman and his collaborators established a convenient method to detect oxidatively modified proteins in which reactive carbonyl moieties are generated as oxidation products in amino acid residues such as lysine, arginine, and proline that can be measured by spectrophotometric or immunological methods after the reaction of proteins with 2,4-dinitrophenylhydrazine to derivatize the carbonyls to the hydrazones.

All cellular components (e.g., membrane phospholipids, nucleic acids, and proteins) have been reported to be oxidatively damaged with age, which could

potentially cause the physiological decline of the organisms (Cutler and Rodriguez 2003). The free radical theory of aging has prompted researchers to study radical scavengers and antioxidants to see if such chemicals can extend the life span of animals. Harman himself showed in his early studies that the synthetic antioxidants 2-mercaptoethylamine and butylated hydroxytoluene can extend the life span of mice (Harman 1968). Numerous studies have been conducted since then to try to extend the life span of experimental animals or to ameliorate age-related diseases in humans that are possibly caused by ROS, mostly using antioxidant vitamins, such as vitamins C and E, or natural products such as polyphenols and carotenes. The results, however, have been rather disappointing in human clinical trials attempting to reduce the risks of age-related diseases, although antioxidant supplements had been reported to be promising in experimental animals (Sadowska-Bartosz and Bartosz 2014). In human studies, it has been reported in a systematic review and meta-analysis of randomized trials with a total of 232,606 participants that antioxidant supplements (β-carotene, vitamins A and E) can even significantly increase all-cause mortality (Bjelakovic et al. 2007). In animal studies, for example, the popular "anti-aging" polyphenol resveratrol, which is not necessarily supposed to act as an antioxidant, has been shown to not extend the life span of genetically heterogeneous mouse strains that mimic human population in multiple laboratories (Strong et al. 2013).

The free radical theory of aging appeared to explain the rate of living theory of aging, which was first proposed many years ago (Pearl 1928), suggesting that there is an inverse relationship between the metabolic rate and longevity in different animal species. However, it turned out that this does not apply to mammals. The opposite was even true intraspecifically when energy expenditure and the life span of individual mice were studied, in that the higher the energy expenditure (indicating a larger consumption of oxygen), the longer the life span, contrary to what is expected from the free radical theory of aging (Speakman et al. 2004). Based on studies of genetically modified mice showing under- or overexpression of genes of antioxidant enzymes (e.g., cytoplasmic and mitochondrial superoxide dismutases, catalase, glutathione peroxidase), it was concluded that all of the antioxidant enzymes studied separately or in combination do not significantly influence the life span in mice (Pérez et al. 2009). On the other hand, it is true that oxidative damage in lipids, DNA, and proteins increases with age, as described above, suggesting an involvement of free radicals in aging. Additionally, a variety of mutant animals with longer life spans show increased resistance to oxidative damage (Brown-Borg 2006; Pickering et al. 2017). Thus, potential roles of ROS in driving aging should not be underestimated, although they may not play a crucial role in life span determination.

It has often been stated that the major source of ROS generation is mitochondria, as discussed later in the mitochondrial theory of aging. However, apart from ROS generated in the mitochondria as byproducts, oxidants can be generated as normal products in multiple enzyme reactions catalyzed by oxidases, such as NADPH oxidase, xanthine oxidase, and monoamine oxidase, contributing to overall cellular oxidative stress. Such oxidants can damage cellular molecules and also play important roles as signaling factor (Finkel 2011). Although the involvement of ROSs in

signal transduction have attracted more interest in recent years than their potential detrimental role in aging, I do not discuss details of this topic as it is beyond the scope of this overview.

I instead discuss the hormetic roles of ROSs that are relevant to aging. Hormesis is a dose-response relationship that exhibits stimulation at low doses and inhibition at higher doses, although whether a response is beneficial or harmful can be complex and is often not immediately obvious (Calabrese and Mattson 2011). Exposure to a variety of stressors, such as toxins, heat, ROS, and radiation, can induce an adaptive response if they are not too strong, making an organism more resistant to subsequent stronger challenges (Gems and Partridge 2008). Nematodes pretreated with hyperbaric oxygen became more resistant to semilethal oxygen exposure (Cypser and Johnson 2002). Interestingly, an oxidative stressor (juglone) could induce substantial resistance to a lethal challenge. The life span of the pretreated worms was increased compared to naive counterparts. We have shown that regular moderate exercise in old rats can reduce oxidative stress, as measured by protein and DNA oxidation, by upregulating anti-oxidation systems, including the glutathione, proteasome, and DNA repair enzymes (Goto and Radák 2009; Nakamoto et al. 2007; Radák et al. 2001). Other investigators have also demonstrated that exercise induces antioxidant enzymes (Gomez-Cabrera et al. 2008) and that antioxidant vitamins C and E ameliorate the beneficial effects of exercise (Ristow et al. 2009). Exercise hormesis is well recognized, as the ROS induced by moderate exercise constitutes a significant mechanism of beneficial effects of the regimen (Gomez-Cabrera et al. 2008; Radák et al. 2005). See also the discussion on mitohormesis in the mitochondrial theory of aging section.

Thus, ROSs have two sides, making this theory somewhat complex. On the one hand, ROSs are believed to have detrimental effects, as proposed in the original theory. On the other hand, they are also thought to have beneficial effects as signaling factors and factors that can protect an organism against stresses that they may encounter in life.

1.6 The Mitochondrial Theory of Aging

Mitochondria have long been known to be the power station of eukaryotic cells, generating the majority of ATP and therefore being vital to life. After the proposal of the free radical theory of aging, these organelles have attracted increased interest in the other side of life, as they use most of oxygen taken up by cells that could potentially be converted to damaging reactive oxygen species (ROSs) in the respiratory chain. Harman was the first to suggest that mitochondria can be a major source of free radicals and also a principal target of the damage that drives aging as an obvious extension of the free radical theory of aging (Harman 1972). In fact, mitochondrial DNA (mtDNA) and proteins are more vulnerable to oxidation than cytoplasmic or nuclear proteins and nucleic acids, likely due to their proximity to the electron transport chain, the lack of histones to protect the DNA, and their low repair

activities. Later, Jaime Miquel expanded the mitochondrial theory of aging (Miquel et al. 1980). A number of papers in support of the theory have been published. It has often been cited that ROSs (such as hydrogen peroxide) generated in the mitochondria account for 1–2% of the total oxygen uptake (Chance et al. 1979). Even higher values of 4–5% have been reported (Luft and Landau 1995). However, later studies have criticized these reports, and the current estimation for these values is as low as 0.15% (St-Pierre et al. 2002).

Point mutations that may occur due to oxygen radicals accumulate in mtDNA with aging, possibly also due to mtDNA polymerase errors, suggesting that this process may cause the age-related functional decline of cells and tissues (Michikawa et al. 1999). For this reason, mice with defective mtDNA polymerase have been constructed as a model of premature aging to prove or disprove this theory (Trifunovic et al. 2004). Studies of these mice demonstrated that the animals with a homozygous mutation (mtDNA mutator mouse) expressing proofreading-deficient mtDNA polymerase γ show reduced life span. They also show phenotypes of accelerated aging at 6–9 months of age, such as hair loss and graying, sarcopenia, osteoporosis, heart enlargement, and reduced subcutaneous fat, all of which are features that are typical of human aging (Trifunovic et al. 2004). Despite these premature aging phenotypes and the accumulation of mtDNA mutations, no increase in hydrogen peroxide production and oxidative stress markers (protein carbonyl, 8-OHdG, and F2-isoprostane) has been observed in isolated mitochondria and tissues of the mice. Thus, these findings did not support the idea that mtDNA mutations cause increased ROS production that might drive aging. One criticism of this research is that these mice may not represent natural human aging because the levels of mtDNA mutations in human tissues are an order of magnitude lower than in the mutator mice (Khrapko et al. 2006). It should, however, be noted that a recent report on the mtDNA mutator mice showed that the hydrogen peroxide levels in the aged animals were increased relative to the young mutator or wild type mice, suggesting that prolonged exposure to higher concentrations of ROSs could contribute to accelerated aging (Logan et al. 2014). Thus, the possible contribution of ROSs to aging in the mtDNA mutator mice remains controversial. Interestingly, however, 5 months of endurance exercise can rescue premature mortality in the mutator mice by inducing mitochondrial biogenesis, thereby mitigating the development of sarcopenia, brain atrophy, cardiac hypertrophy, and other age-related pathologies (Safdar et al. 2011). Endurance exercise rescued mtDNA depletion in multiple tissues and reduced the frequency of point mutations in the mutant mice. These data support the view that lifestyle can improve the systemic deterioration of mitochondrial function that could increase morbidity and mortality with aging.

Supporting evidence for the mitochondrial theory of aging has been obtained in transgenic mice overexpressing human catalase in the mitochondria, which exhibit increased life spans with reduced cardiac pathologies and cataract severity (Schriner et al. 2005). These mice exhibited higher aconitase activity, a marker of antioxidant capacity, in the heart and lower 8-OHdG in the DNA of the skeletal muscle, suggesting that oxidative stress can be ameliorated by the overexpression of catalase targeted to mitochondria.

In view of the controversy regarding the contribution of mitochondrial ROS in aging, it is worthy of referring to the concept of mitochondrial hormesis (or mitohormesis) (Schulz et al. 2007; Ristow 2014). It was found that nematodes treated with 2-deoxyglucose (2DG), an inhibitor of glycolysis, exhibited a prolongation of their life span with a compensatory increase in mitochondrial respiration, which is associated with increases in the level of ROS, followed by increased expression of catalase, which scavenges hydrogen peroxide (Schulz et al. 2007). When the worms were pretreated with VC, VE, or other antioxidants, the elevation of catalase was abolished, and the extension of life span of the worms treated with 2DG was blocked. It thus appears that mitochondrial oxidants induced an increased defense against oxidative stress as a hormetic response because excess oxidants are obviously detrimental.

The mitochondrial theory of aging has thus developed into a theory evaluating the roles of ROS generated from the organelle as signals for cellular homeostasis rather than simply as damaging chemicals, as originally suggested. Also, I should add that results incompatible with this theory are reported (Lapointe and Hekimi 2010).

1.7 The Error Catastrophe Theory of Aging

This theory was most prominently advanced by Leslie Orgel (1963) in accordance with the development of molecular biology of the gene expression in the 1960s, such as the research on the mechanisms of replication, transcription, and translation. This theory predicted that nucleic acids and proteins inevitably contain errors when they are synthesized because the information transfer in each step of gene expression and maintenance is not perfectly accurate and the synthesizing machineries consisting of error-containing molecules would make further errors, thus forming a vicious cycle of error propagation that could result in the gradual loss of cellular function, i.e., catastrophe, with age. Although this theory is usually regarded as being advocated by Orgel, it should be noted that Zhores Medvediev presented a similar idea independently (Medvediev 1962). This theory has attracted particular attention from scientists interested in the molecular mechanisms of aging because it suggests a hypothesis that is experimentally testable by means of emerging theoretical and technological developments of research in gene expression.

Possible detrimental consequences of the propagation of errors are likely more serious in nondividing cells than in dividing cells because error-containing dividing cells can be eliminated and replaced by new cells or can be diluted by cell division, while error-containing molecules may be repaired or replaced by metabolic turnover in nondividing and/or slowly dividing cells.

Of the types of errors in information transfer, translational errors had been most extensively studied. These errors can occur in two independent steps of translation:

(1) The charging of individual tRNAs by cognate amino acids and (2) the decoding of codon of mRNA. The former step is catalyzed by aminoacyl tRNA

synthetases that may mischarge amino acids to tRNAs by imperfect enzymes. The latter step occurs on ribosomes by matching codons with anticodons of charged tRNA. A number of studies on the rate of mistranslation (error frequency) in aging had been conducted mainly using young and senescent cells in culture. For example, the error frequency of actin synthesis was studied in human fibroblasts at different replicative ages (Harley et al. 1980): Histidinol, an analogue of histidine, was added to the culture medium and thereby blocked the charging of tRNA for histidine. The decrease in the histidine-charged tRNA concentration induces an incorporation of glutamine into actin in the place of histidine because the codons for glutamine (CAA or CAG) are similar to those for histidine (CAU or CAC) so that errors of translation can occur due to codon-anticodon mispairing at the third position. Late-passage cells from fetal, young, and old donors cultured in vitro showed similar or lower error frequencies than the corresponding early-passage cells, suggesting that error propagation does not occur and thus fails to support the error catastrophe theory of aging. In another study, age-related changes in the charging error were examined in vivo by the incorporation of ^{14}C-methionine and ^{3}H-ethionine, an analogue of methionine, into proteins of young and old mouse livers (Ogrodnik et al. 1975). It was expected that ethionine could be mischarged to tRNA in place of methionine by methionyl tRNA synthase if the fidelity of the enzyme would be decreased with age. The misincorporation of ethionine in the place of methionine was 10–50% higher in ribosomal proteins of old animals, indicating that the charging fidelity indeed declines in older animals, although it was not clear if the error rate propagates with age.

As for the recognition of natural amino acids in young and old animals, we have studied the age-related changes in the fidelity of aminoacylation by tyrosyl-tRNA synthetase isolated from the liver of rats (Takahashi and Goto 1988). The enzymes were purified from the livers of young (4–7-month-old) and old (27–29-month-old) rats, such that no detectable phenylalanyl-tRNA synthetase was contaminated to study the misrecognition of phenylalanine as tyrosine by the enzyme. The error frequency of the tyrosyl-tRNA synthetase (on the order of 10^{-8}) from the older animals was slightly lower than that from the younger animals, but this difference was not statistically significant. Thus, the fidelity of aminoacyl tRNA synthetase did not appear to decline significantly in old age, again suggesting that errors in translation would not increase with aging at the stage of tRNA charging with amino acid in translation.

The fidelity of decoding on ribosomes from young and old animals had been mostly studied by assessing the misincorporation of non-cognate amino acids using synthetic mRNA of homopolymers, such as poly(U) which codes for phenylalanine polymers. The misincorporation of leucine into the poly(U)-dependent synthesis of polyphenylalanine using ribosomes of tissues did not differ significantly between young and old mice (Mori et al. 1979). We have, instead, studied codon recognition fidelity using a unique group of natural mRNAs that code for limited species of amino acids. Protamines are highly basic nuclear proteins from fish sperm consisting of 33 amino acid residues.

They contain only seven different amino acid species, of which approximately two-thirds are arginine. It was therefore possible to study the incorporation of radioactive amino acids in vitro that are not coded in the mRNAs. The fidelity of the decoding of the mRNAs on ribosomes from the livers of mice between 2 and 29 months of age was found to not change significantly (Mori et al. 1983). Thus, these findings are not consistent with the error catastrophe theory of aging in terms of the predicted age-related changes in translational fidelity. This is probably because the proofreading mechanisms (Hopfield 1974; Fersht 1980) of translation are maintained throughout life, keeping the fidelity high enough, such that propagation of error would not occur.

More recently, the high fidelity of translation has been discussed from evolutionary perspectives as it can be important for survival by avoiding protein misfolding (Drummond and Wilke 2009) (see also Sect. 1.8). Another possibility that error-containing proteins do not increase with age is that such proteins may be preferentially degraded and replaced by intact molecules by metabolic turnover as discussed in the next session (Sect. 1.8).

Other steps of information transfer in which error catastrophe could occur are DNA replication and transcription. No age-dependent differences have been found between the fidelity of nuclear DNA polymerase-α and nuclear DNA polymerase-β that were partially purified from the regenerating livers of young (6-month-old) and old (28-month-old) mice when the enzymes were tested for copying bacteriophage φX174 DNA (Silber et al. 1985). The same group of investigators showed that the fidelity of highly error-prone DNA polymerase-β in the brain of young and old mice was not significantly different when copying the same bacteriophage DNA (Subba Rao et al. 1985). Thus, although available reports on the possibility of age-related changes in the fidelity of DNA polymerases are limited, it appears that the error catastrophe theory of aging is not supported by the information transfer in nuclear DNA replication. Although Orgel implied that transcription errors can lead to the catastrophe (Orgel 1963), I am not aware of a published paper on age-related changes in the fidelity of nuclear gene expression or of RNA polymerases in the nucleus (Imashimizu et al. 2013). The integrity of RNA coded in mitochondrial DNA has been studied in the brain of young (1-month-old) and older (18-month-old) mice (Wang et al. 2014). The transcriptional error of the mitochondrial RNA polymerase is site-specific and varied greatly among different genes. The error levels in two age groups, however, were not significantly different, suggesting that error propagation does not occur during aging. It is noted that transcriptional errors were independent of the DNA mutation frequency and were up to 200-fold more frequent than replication errors. The authors therefore conclude that the mitochondrial transcription fidelity limits the impact of mitochondrial DNA mutation.

Thus, the error catastrophe theory of aging, which was once a popular hypothesis, is not supported by the current experimental evidence. This theory thus seems to have been largely forgotten, but it should be noted that pathologist George Martin has argued that "it may have been given a premature death certificate" because drifts in gene expression may be responsible for the "quasi-stochastic" distribution of

lesions in geriatric pathologies, such as Alzheimer's disease and atherosclerosis and that errors in information transfer could feasibly contribute to this process (Martin 2012).

Although it is unlikely that error catastrophe occurs in genetic information transfer, it should be noted that errors in protein synthesis can occur as the misfolding of higher structures during translation. In fact, the rate of folding errors can be as high as 30% of newly synthesized proteins, even though misfolding may be mostly prevented by chaperons (Schubert et al. 2000) (see also: Sect. 1.8).

1.8 The Altered Protein Theory of Aging/Protein Homeostasis or Proteostasis Theory of Aging

The origin of this theory may be traced back to Friz Verzár, who reported an age-related increase in collagen cross-linking in rat tail tendons (see Nagy 1986). A large number of studies have confirmed that changes in collagen occur with age in various tissues and animals (Robert 2006). However, because collagen is an extra-cellular protein and its relevance to cellular metabolisms is limited, researchers interested in aging and inspired by the findings became more concerned about the age-related changes of enzymes and other proteins involved more directly in intra-cellular functions. In the meantime, studies on the error catastrophe theory of aging have failed to support the predicted propagation of errors in translation as described above and instead suggested the presence of altered forms of enzymes in aged cells and tissues. Thus, altered enzymes were interpreted to be formed not by translational errors but by posttranslational modifications.

Altered forms of enzymes in old cells and animal tissues have been detected by various means. They have been shown to have low specific activity (by between 30 and 70%) per unit weight of purified enzyme (Rothstein 1981). One problem with finding altered forms of an enzyme through purification is that altered enzymes with reduced activity are often lost during the purification process, as purification protocol usually depends on enzymatic activity. Altered enzymes have been detected in crude extracts without purification that depends on enzyme activity, since antibodies against an enzyme molecule can react with enzymes with no or reduced activity that remain immunologically cross-reactive as the native enzyme (Gershon and Gershon 1970). Another frequently used method was to examine the heat-stability of an enzyme in cell or tissue extracts. An enzyme likely becomes heat-labile if it is altered such that the mixture of native and altered enzymes has a biphasic or quasi-biphasic heat-inactivation kinetic curves for the activity so that the percentage of the altered form of an enzyme could be evaluated for the extent of alteration (Houben et al. 1984). Thus, many altered proteins, mainly enzymes, have been reported to increase in cells and tissues with aging, suggesting that they may be responsible for the age-related decline of physiological functions.

The causes of these alterations have been suggested to be posttranslational modifications, such as oxidation or nitrosylation by ROSs or RNSs (reactive nitrogen species) and glycation by glucose. In some cases, reactive aldehydes derived from lipid peroxides are responsible for the modifications. We and other investigators have shown that the heat-labile enzymes described above are generated by a reaction with ROSs in vitro (Takahashi and Goto 1990). The chemistry of modifications has been studied extensively, proving that the side chains of specific amino acid residues, such as lysine, arginine, and proline, are modified (Stadtman 1993). Notably, carbonyl moieties generated by oxidation have most frequently been used to evaluate oxidative stress on proteins by biochemical or immunochemical methods (Levine et al. 1990; Nakamura and Goto 1996), although this method is not without problems (Fedorova et al. 2014; Goto and Nakamura 1997). In addition to a correlative relationship between the oxidative modification of proteins and aging, a causal relationship between age-related increases in oxidative stress and functional decline has been suggested (Martin et al. 1996a; Martin and Grotewiel 2006). However, despite numerous reports on the possible involvement of protein oxidation in aging, it is hard to decide its major contribution, as multiple effects of oxidative stress on other molecules, such as DNA and membrane phospholipids, do occur in parallel.

The glycation caused by nonenzymatic chemical reactions of proteins with glucose is another well-recognized posttranslational modification that increases with age in long-lived proteins, such as collagens and elastin, as well as lens crystallins. The glycation of proteins ends up in generating a variety of products collectively called AGEs (advanced glycation end products). Because proteins exposed to a high concentration of glucose in the blood for a long period of time are susceptible to this modification, it accumulates frequently in extracellular matrix proteins and proteins with very low turnover rates. Glycation appears to be less involved in the age-related functional decline of cells as a general cause than other posttranslational modifications that occur more frequently inside cells. Nevertheless, there is no question that glycation is involved in age-related diseases of endothelial cells, such as in atherosclerosis, cardiovascular pathologies, and renal disorders, in which tissue microvessel dysfunction is involved.

More recently, apart from the posttranslational modifications described above, specific altered proteins with abnormal conformational structures in age-related neurodegenerative diseases, such as Alzheimer's disease (amyloidβ and tau tangles), Parkinson's disease (mutant α-synuclein), Huntington's disease (mutant huntingtin), and amyotrophic lateral sclerosis (misfolded SOD1), have been studied extensively (Stefani 2004; Labbadia and Morimoto 2015). More generally, amyloid diseases that impair the functions of different organs are also protein conformation diseases that increase with age. There are many other examples of protein misfolding and aggregation causing age-related diseases (Chiti and Dobson, 2017; Klaips et al. 2018). While numerous cases, especially in neurodegenerative diseases, have been reported in which protein alterations produce age-related pathologies, it is not clear whether such changes also contribute to the functional decline of cells and tissues in physiological aging. It is possible that minor alterations of individual proteins cause

undetected changes, yet result in significant physiological deterioration in a long period of aging.

The accumulation of altered proteins with age can be driven by either increases in the formation or the decline of degradation, or both processes. While the mechanisms involved in the formation of such proteins have been extensively studied, the decrease in degradation or elimination has attracted less interest. Rudolf Schoenheimer described for the first time the dynamic state of body constituents, such as lipids and proteins, as early as the late 1930s, when the stable isotope technique became available to label cellular and extracellular components for chasing the fate of the labeled materials, thereby highlighting the importance of metabolic turnover as a homeostatic life maintenance mechanism. Due to the difficulty of the access to the historical book *The Dynamic State of Body Constituents* (Harvard University Press, Cambridge, MA, 1949) written by him, I cite instead an excellent overview on this topic (Kennedy 2001). Schoenheimer's view, however, was challenged by Jacques Monod (*Nobel Prize* laureate for the operon theory) and collaborators, who studied the turnover of β-galactosidase in growing *E. coli* and concluded that most proteins in the cells are static rather than in a dynamic state (Hogness et al. 1955). They further suggested that the proteins in mammalian tissues would also be stable because the apparent dynamic state in these cells may be interpreted as some proteins being secreted or lost by cell death. However, it was shown that proteins in rabbit macrophages, nondividing cells, actually turnover, thus not supporting Monod's hypothesis (Harris and Watts 1958). Even so, protein degradation has not attracted the same intense research interest as other more positive biological processes such as protein and nucleic acid synthesis.

The degradation of intracellular proteins was originally thought to be mainly dependent on lysosomes, which were found to contain multiple proteolytic enzymes (cathepsins) with different specificities at acidic pH values (de Duve 1983). While lysosomal proteolysis is thought to be nonspecific with regard to the protein substrates degraded, the half-life of different proteins was reported to vary considerably. This fact facilitated studies on non-lysosomal protein degradation that were first performed in rabbit reticulocytes that do not have lysosomes. The extensive research on non-lysosomal protein degradation has established the mechanisms of the ubiquitin-proteasome system of proteolysis, showing that substrate proteins are marked with ubiquitin for degradation and digested by proteasomes (in the case of 26S proteasome, see below) (Ciechanover 2005). The proteasome is a multi-catalytic protease complex that exists in two forms, 26S and 20S, that differ in subunit composition but share a common catalytic specificity. The 26S proteasome degrades proteins tagged with ubiquitin chains and ATP dependently, while the 20S proteasome degrades non-ubiquitinated proteins without using ATP.

On the other hand, the lysosomal pathway of proteolysis has developed into the elucidation of autophagy-lysosome systems, in which protein aggregates and damaged organelles are specifically recognized and destroyed, contrary to what was originally believed to be nonspecific (Koga et al. 2011). Both systems of protein degradation have profound impacts on aging and age-related diseases, particularly in

neurodegenerative diseases (Rubinsztein et al. 2011; Saez and Vilchez 2014; Klaips et al. 2018).

The altered protein theory of aging prompted studies on protein turnover in aging (Van Remmen et al. 1995; Goto et al. 2001). For example, it was demonstrated that the half-lives of enolase in nematodes and aldolase in mice are extended in old animals compared with their younger counterparts, as determined by pulse-chase experiments. We found that the half-life of the various proteins introduced into mouse hepatocytes in primary culture were extended by 40–60% in the cells from old animals (Ishigami and Goto 1990; Goto et al. 2001). It was also shown in vivo that prematurely terminated puromycinyl peptides, as a model of altered proteins, are much more slowly degraded in the livers of old mice than in those of younger animals (Lavie et al. 1982). Thus, the degradation of normal and abnormal proteins was shown to be impaired in old animals, and these findings were comparable with the age-related accumulation of altered proteins in different tissues. In the meantime, it was firmly established that the ubiquitin-proteasome system and the autophagy-lysosome system are responsible for intracellular protein degradation as described above. Many studies have demonstrated that proteasome activity declines with age (Saez and Vilchez 2014; Shibatani et al. 1996). We have shown that the activities of both the 20S and 26S forms of the liver proteasome decline similarly with aging in three age groups of rats of from 8–10 to 25–28 months of age (Hayashi and Goto 1998). Despite the decline in the enzyme activities, the amount of catalytic subunits measured by immunoblot did not change with age, suggesting that posttranslational modifications or subunit replacement are responsible for the decreased activities. In fact, other investigators have reported that the subunit composition of the proteasome is altered in aged tissues. Furthermore, a subunit of the proteasome is sensitive to oxidative modification (Ishii et al. 2005), suggesting that oxidative stress can accelerate the accumulation of oxidized proteins in aging by reducing the efficiency of damaged proteins. It is interesting to note that the 20S proteasome degrades oxidatively modified proteins selectively (Davies 2001) and that the 26S proteasome can be reversibly dissociated to produce the 20S proteasome by removing 19S regulators upon oxidative challenge, thereby facilitating adaptation to stress (Grune et al. 2011). It should be mentioned that the Lon protease plays an important role in the degradation of oxidized mitochondrial proteins, the activity of which declines with age and contributes to the accumulation of damaged proteins in the organelle (Ngo et al. 2013).

When the damage to proteins is extensive, forming insoluble cross-linked aggregates that are not degraded by proteasomes, the autophagy-lysosome system degrades them in addition to removing the damaged organelles (Wong and Cuervo 2010). The autophagy-lysosome system is considered to act via microautophagy, macroautophagy, and chaperon-mediated autophagy, and the latter two systems are the predominant mechanisms of autophagy in animals. Macroautophagy refers to the digestion of contents of cytoplasmic regions engulfed in membrane vesicles, which then fuse with lysosomes for degradation. Chaperon-mediated autophagy is the digestion of substrates bound to the chaperon heat-shock cognate protein (hsc70), which is recognized by lysosomes via an interaction with the receptor protein on the

surface. Substrates translocated across the lysosomal membrane are then digested. The activities of these autophagic processes decline with aging (Rubinsztein et al. 2011). The age-associated decline in the chaperon-mediated autophagy can be caused by decreased content of the substrate receptor (lysosome-associated membrane protein type 2a) (Cuervo and Dice 2000) and the age-associated impairment of lysosomal function (Kurz et al. 2008).

A number of studies have established the extensive involvement of altered protein conformation in age-associated neurodegenerative diseases. These are mainly due to the impaired functions of ubiquitin-proteasomes and/or autophagy-lysosome systems and the chaperon dysfunctions described in many excellent reviews (Takalo et al. 2013; Hipp et al. 2019). However, I do not go into the details of these studies as this subject is of little relevance to the scope of this overview, although it is conceivable that these mechanisms are also involved in the general age-related functional decline of housekeeping proteins.

Thus, the original idea that accumulation of altered proteins causes a variety of aging phenotypes has expanded to include different aspects of life processes. The altered protein theory of aging/proteostasis theory of aging has now become one of the most widely accepted theories to explain the basic mechanisms of aging.

1.9 Dysdifferentiation Theory of Aging/Epigenetic Theory of Aging

Richard Cutler suggested that differentiated cells can undergo changes in transcription during aging, such that the strict pattern of gene expression is gradually relaxed, leading to the deterioration of the functions of cells and tissues (Cutler 1991). This idea, called the dysdifferentiation theory of aging, was based on the finding that the expression of globin or its related mRNA and murine leukemia virus RNA is increased in the brains and livers of aged mice compared to their younger counterparts (Ono and Cutler 1978). More recently, it has been shown that gene expression becomes gradually heterogeneous in the tissues of individuals with advancing age, including the cerebral cortex and hippocampus (Somel et al. 2006). These findings are compatible with the dysdifferentiation theory of aging.

This theory had never been popular, but has been recently revived as the epigenetic theory of aging. Epigenetics is a phenomenon in which a fixed pattern of gene expression in a cell, or an organism is inherited from one generation to the next without changes in the genomic nucleotide sequence. This definition has been broadened to include the long-term stable control of gene expression in differentiated cells in a body without changes in the nucleotide sequence, as manifested in various physiological and pathological situations, including aging and age-related diseases. The epigenetic regulation of long-term cell-specific gene expression is determined by a variety of mechanisms, including DNA methylation, histone modifications, and microRNA expression (Brunet and Berger 2014; Raj and Horvath

2020). These epigenetic mechanisms of gene modulation are influenced throughout life by both internal and external stimuli, such as energy metabolism, nutrition, and exercise, and can therefore impact on the physiological aging and the incidence of age-related diseases (Lopez-Otin et al. 2013; Goto et al. 2015).

It has been shown in twin studies that there are far more differences in the patterns of DNA methylation and histone acetylation in the circulating lymphocytes of older (50 years of age) monozygotic twins compared with younger (3 years of age) twins (Fraga et al. 2005). Interestingly and consistently with the findings, the differences in the gene expression between the older pairs were much greater than those in the younger pairs. These findings suggest that an identical genome in early life could undergo different epigenetic modifications throughout life, potentially resulting in differences in the aging rates and/or in their vulnerability to diseases. This type of variable epigenetic modifications may partly explain the relatively low contribution (approximately 30%) that genes have on longevity compared with environmental factors (Ljungquist et al. 1998; Dato et al. 2017).

Frailty is a common manifestation of physiological aging. It has been reported that a worsening frailty status, as measured by the loss of body weight, the development of sarcopenia and muscle weakness, and the reduction in physical activity, is associated with decreased global DNA methylation in the peripheral blood cells of individuals aged 65–105 years old over a 7-year follow-up period (Bellizzi et al. 2012). Aging is often associated with reduced levels of global DNA methylation (hypomethylation), mostly in cytosine base of CpG sequences, but its physiological implications remain mostly unclear. However, it should be mentioned that the age-related hypermethylation can occur in some cases of cancer such as promoter regions of tumor suppressor genes increasing the risk of carcinogenesis with age (Kulis and Esteller 2010).

In recent years, epigenetic modifications have attracted a particular interest following Steve Horvath published an influential paper on DNA methylation (DNAm) and aging covering a variety of tissues of different organisms in normal and pathological situations, coining a term "epigenetic clock" that appears to predict biological age rather than chronological age (Horvath 2013; Levine et al. 2018; Ryan 2021). I do not discuss this emerging topic in detail as many review articles have been published (see, e.g., an article by Jylhava et al. (2017) for a comparison among potential age predictors such as telomere attrition including DNAm age). It should, however, be noted that it is not clear whether DNAm is simply a marker of aging or has a causal or mechanistic relationship with changes of gene expression that should be relevant to physiological decline of cells and tissues with age, i.e., biological aging. In fact, Horvath admits that "I do not find that age effects on DNAm levels affect gene" and "the relationship between DNAm levels and expression levels is complex" (Horvath 2013). In a recent systematic survey of the epigenetic clock, Oblak et al. (2021) state that a majority of parameters potentially related to the epigenetic clock is age-related diseases such as cancer, cardiovascular disease, lung disease including air pollution caused disorders, diabetes mellitus and mental disorders, etc. but so far apparently not clearly relevant to physiological decline in normal aging. Notably, the authors describe that frailty, a hallmark of human biological

aging, does not have any significant effects. Therefore, I would think that DNAm age could not predict biological or physiological age but possibly can predict the remaining time of life or health span as DNA methylation being predictive of susceptibility to some kinds of age-related diseases.

Changes in the posttranslational modification of histones also occur with age, which can lead to reduced gene expression, as decreased acetylation allows the chromatin to more tightly condense by increasing the interactions with DNA. As an example, we have shown that acetylation of lysine 9 in histone H3 is reduced in aged rat livers compared to younger counterparts, suggesting a possible mechanism of decrease in the expression of certain genes with age (Kawakami et al. 2009). Memory impairment is a common feature of old animals and a serious problem for elderly people. It has been reported that the acetylation of specific lysine residues in histone H3 and H4 are transiently increased in the hippocampus of young (3-month-old) mice subjected to contextual fear conditioning but not in their older (16-month-old) counterparts (Peleg et al. 2010). These findings suggest that memory impairment in old animals is correlated with defects in learning-induced histone acetylation. Intriguingly, the administration of histone deacetylase inhibitors, such as sodium butylate, to old mice prior to the memory conditioning increased the acetylation significantly in the coding regions of learning-regulated genes. These findings suggest that the dysregulation of histone acetylation is causally related to age-associated memory impairment, raising a possible mechanism for the treatment of this disorder.

MicroRNAs (miRNA) are another epigenetic modifier of aging that have been widely studied in recent years (Bushati and Cohen 2007; Grasso et al. 2014). The RNAs are short, noncoding RNAs coded in the nuclear genome affecting transcription or mRNA stability and thus can influence gene expression in aging and diseases. Different kinds of miRNA have been reported to change with age in invertebrate models such as nematode and fruit fly as well as normal tissues (brain, skeletal muscle, heart, etc.) of mice and rats (Kinser and Pincus 2020). miRNAs secreted from cells and tissues exist in the circulation and thus have been studied for a possible biomarker of aging. It should be mentioned that functional roles of miRNA and regulation of its gene expression have remained to be defined, and therefore appeared to have limited significance at present to explain the mechanisms of aging.

1.10   The Hyperfunction Theory of Aging

This recently proposed new theory of aging that is apparently against the traditional view of aging deserves mentioning, as it particularly opposes the influential free radical theory of aging and may open up a new door to explain the mechanisms of aging. In most of the aging theories described so far, aging is believed to be due to an accumulation of detrimental molecular changes in protein and nucleic acid that is induced by ROSs and other chemicals or by errors in critical life maintenance

processes. Mikhail Blagosklonny proposed that aging is instead caused by the hyperfunction of growth, such as hypertrophy and hyperplasia, rather than an increase in the damage that occurs later in life, leading to age-related pathologies (Blagosklonny 2008). His claim is based on reports that contradict the ideas that aging is caused by an accumulation of molecular damage. According to such ideas, the molecular damage is mainly due to ROS. The reduced translation activity due to the deletion of ribosomal S6 protein kinase 1, a component of the target of rapamycin (TOR) pathway, is believed to lead to an increased life span and resistance to age-related pathologies (Selman et al. 2009). TOR is an evolutionarily conserved protein kinase that regulates growth and metabolism and is involved in the modulation of aging (Kapahi et al. 2010). Blagosklonny admits that damage accumulation can cause the deterioration of cellular functions over time but also predicts that an organism could not live long enough to accumulate a lethal level of damage (Blagosklonny 2008). It is possible, however, that damage accumulation would increase the probability of death when exposed to internal and external stress, thus constituting a mechanism of aging. He stresses the role of the TOR pathway by placing it in the center of the hyperfunction theory of aging because most factors that appear to reduce the activity of TOR retard aging and extend the life span of model organisms (Blagosklonny 2012). Gems and Partridge support the idea of hyperfunction as a mechanism of aging but state that it remains unclear how the pathway controls the rate of aging and life span (Gems and Partridge 2013). This theory predicts a form of antagonistic pleiotropy (Austad and Hoffman 2018) in which hyperfunction increases fitness early in life but can be harmful in old age. The identity of the intrinsic or extrinsic factors that maintain hyperfunction in the face of declining metabolic activity with age remains unknown. It should be noted that a recent report describes that rapamycin extends the life span of mice but ameliorates few aging phenotypes, such that its effects are not due to a modulation of aging but are instead related to aging-independent drug effects (Neff et al. 2013).

1.11   Summary and Perspectives

Despite extensive efforts to solve *an unsolved problem of biology* for nearly three quarter of a century since Medawar wrote a book with this title, no single theory has yet fully explained the mechanism of aging. As all animals are considered to be the products of evolution, it is assumed that there are conserved aging mechanisms even between species with remarkably different life spans, such as humans, mice, fruit flies, and nematodes (see Fig. 1.1 and Table 1.1). Although there appear to be conserved pathways that potentially drive aging (Kenyon 2010), it is not known how these very basic molecular mechanisms result in such great life span variation. The mechanism has remained as *an unsolved problem in gerontology*. The leading theories that have so far been proposed are apparently acceptable at least in part, but not without objections, and different theories interrelate with each other by one theory being a part of the others, suggesting that each one can contribute partly to be

integrated into the whole process of aging. In addition, it has been proposed that chance or stochasticity in addition to genes and environments can play a role in aging regardless of the mechanisms in both humans and model organisms (Kirkwood and Finch 2002; Vaupel et al. 1998). Nevertheless, no one would think that a lucky mouse can live for 100 years and an unlucky normal human would die of aging in 3 or 4 years, showing that the gene undoubtedly play a definitive role for the rate of aging and life span determination. But nobody knows which gene or genes are responsible, and a little effort has appeared to be made so far to identify one.

A major target of future studies of aging will be how to integrate the different theories to understand the mechanisms of varied aging rates in different animal species and individual differences of the aging rate within a species.

We are perhaps in the stage of awaiting a new paradigm or an integration of the existing theories to provide us with an improved understanding of the mechanism of aging.

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