

REVIEW ARTICLE

An Old Question Revisited: Current Understanding of Aging Theories

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ABSTRACT “Why we age” is no longer a solely philosophical question. In parallel with the rising awareness of the social ramifications of an aging population, basic research has expanded our understanding of the intricate nature of biological aging. The present paper aims at discussing our current understanding of the molecular and cellular alterations that accompany aging. To this end, the main theories on the mechanisms of aging, error theories and program theories, will be discussed. Special focus on neuronal aging is also presented to provide illustrative examples of these aging mechanisms.

INTRODUCTION

少年易老学难成
一寸光阴不可轻

One rapidly becomes old, but learning is slow; therefore, never waste a moment.

While this ancient Chinese proverb describes the preciousness of every moment of our lifespan, it fits interestingly with the impact of aging on our society. The proportion of the aged population in the world is increasing rapidly. Taking Canada as an example, the population of over 65 is predicted to rise from the present 3.5 million to 7.7 million in the next 25 years (1). A similar trend can be found in the United States. The population of over 65- and over 85-years old is

expected to increase two and five fold respectively in the next 50 years (2). Indeed, thanks to the development of technology and medicine, our life expectancy has increased 50% in the last century (3). However, our understanding of the biological mechanisms underlying the aging process is progressing at a much slower pace. For instance, the prevalence of disabilities in the aging population has been decreasing only slowly in recent years (4). Indeed, health care spending for elders in the coming decades will become a substantial financial burden to our society (5).

This paper will explore general concepts of aging, including how we define aging and how aging occurs at the most basic molecular and cellular levels. As will become evident, both environmental and genetic factors interact through multiple different mechanisms to produce changes at the molecular and cellular levels that ultimately manifest themselves as changes that are more easily observed in tissues and organs, and in the organism as a whole. Identification of and response to these gross changes benefit from an understanding the underlying mechanisms that produced them. In the long term, a greater understanding of aging will provide solutions not only to reduce the burden caused by the aged population to the society but, more importantly, to extend the productive and healthy lifespan of the elderly in the future.

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DEFINITIONS OF AGING

Perhaps the association between aging and phenomena such as weakness, disease, and death is so strong that most definitions of aging have focused on the adverse characteristics in the last stage of life. Comfort in his book *The Biology of Senescence* has defined aging as:

“A progressive increase throughout life, or after a given stadium, in the likelihood that a given individual will die, during the next succeeding unit of time” (6).

In Comfort’s view, aging happens as a result of a progressive loss of physiological functions that culminates in death. Indeed, the progressiveness of the aging process has brought about the idea of ‘error accumulation’ throughout the lifespan. For instance, Harman has defined aging as:

“The accumulation of changes responsible for the sequential alterations that accompany advancing age and the associated progressive increases in the chance of disease and death” (7).

Recent progress in aging research has led to frequent revisions of the definition of aging. For instance, Busse has divided the aging processes into primary and secondary aging (8). In his view, primary aging is intrinsic to the organism, and the detrimental factors are determined by inherent or hereditary influences. On the other hand, secondary aging is caused by deleterious or hostile factors in the environment. However, due to the complexity of the aging process, these descriptive definitions of aging are always far from satisfactory. Taking Busse’s definition as an example, since both intrinsic and environmental factors have been shown to interact with each other during the development of various aging deficits, separating these factors into independent processes is not realistic.

While it is difficult to define aging, describing when aging begins is equally complicated. For example, even though the rat is the most commonly used mammalian model of aging, there are controversies in the definition of an aged rat. Some investigators consider any sexually mature rat to be old. Thus, 14-month-old rats have been regarded as aged rats (e.g. reference 9), whereas rats ranging from 24 to 30+ month-old are used as reference for aged rats in other studies (e.g. references 10,11). One way to define the starting point of aging is to use survival curves (12). Survival curves are constructed by plotting the percentage survival against the age of individuals in a population. Rats can be considered as entering the aging stage when their survival percentage is lower than 50%. For instance, based on survival curves of Sprague-Dawley (13), Wistar (14), Fischer (15), and Brown Norway rats (16), their 50% survival age ranged from 27 to 30 months.

While the focus here is admittedly biological, the social aspects of aging cannot be completely disregarded. For instance, in regards to what age would be considered ‘old’ for a person, the common answer would likely depend much more on social factors than on biological ones. With that said, the paper will not digress further from its stated biological focus.

MECHANISMS OF AGING

The maximum lifespan is dependent not only on the genetic heterogeneity that characterizes different species, but also on variable environmental influences (17-20). These influences of both nature and nurture give rise to various aging phenotypes that cannot be easily explained by a single hypothesis. Instead, many theories based on fundamental molecular, cellular, and systemic analyses of aging have been developed in the last few decades. These theories, which are not necessarily mutually exclusive, have been classified by the National Institute of Aging into two major categories. Program theories hold that aging is the result of the sequential switching on and off of certain genes whereas error theories emphasize that aging is the outcome of both random accumulation of error mutations and wear and tear of tissues and organs during the lifespan of an organism. The following sections summarize examples of both types of theories of aging.

ERROR THEORIES

Accumulation of mutations

Somatic mutations, including gene mutations, gene conversion, and gene amplifications, chromosomal abnormalities, and mitotic recombinations, have been widely agreed to play important roles in the development of aging (for review, see reference 21). This hypothesis stemmed from the observation that damages induced by irradiation in animals resembled phenotypic changes in aging (22). Since radiation was well known to be able to induce mutations, aging could be caused by accumulation of mutations after life-long exposure to natural levels of background radiation and other environmental agents (23,24). In fact, X-ray-induced chromosomal damages have been shown to shorten life expectancy (25). Another source of errors comes from DNA replications. It has been estimated that the error rate of DNA replication can be as high as 0.01% (26). There are systems to maintain the precision of DNA replications and, indeed, the ability to repair DNA in different organisms has been shown to correlate directly with their maximal lifespan (27,28). However, the ability to repair DNA has been shown to decrease with age (29,30). These two sources of damage, mutations by environmental factors and during DNA replication, may lead to significant levels of

chromosomal abnormalities in aged tissue (30-34). Consistent with these mechanisms, aberrations of DNA material can be found in the nucleus (35) and the mitochondria of aged cells (36,37).

One major criticism of the somatic mutation theory is that natural radiation under normal conditions may be too low to account for the overall age change (30,38,39). However, recent findings from monitoring mutation of specific genes at different time points during life showed significant levels of mutation in aged tissue. For example, studying the mutation rate of the hypoxanthine phosphoribosyl transferase (HPRT) gene revealed significantly higher mutant frequencies in aged mice (40) and humans (41). Monitoring a neutral reporter gene *lacI* during the development of transgenic mice also revealed accumulation of mutations with age (42-44). Interestingly, animals with caloric restriction, the only intervention that appears to lengthen lifespan (45,46), accumulate HPRT mutations at a much slower rate when compared with *ad libitum* fed animals (40,47). On the contrary, accumulation of HPRT mutations is higher in the senescence accelerated mouse model, which develops aging phenotypes earlier than normal mice (48). Thus, accumulation of mutations could be a major factor in determining biological aging.

Although the somatic mutation theory predicts the accumulation of mutations randomly during aging, increasing evidence shows that some genes accumulate more errors than other genes with time. For instance, human leukocyte antigen (HLA) genes have a mutation frequency two to three times higher than that of HPRT genes (49). In addition, some regions of the genome display above average mutation rates. These mutation hot spots usually contain repeat elements (50,51). One of the important examples is the telomere, which is present at the end of each DNA strand (for review, see reference 52). Accumulation of mutations in the minisatellite repeat elements of the telomere hinders the DNA synthesis machinery to replicate the very end of the lagging strand and results in chromosomal aberrations (53). Shortening of telomeres with age has been reported in different tissues (54). More importantly, reversing the shortening of telomeres has been shown to arrest the aging process. For instance, expressing telomerase, a ribonucleoprotein that is capable of correcting telomere shortening by adding TTAGGG repeats to chromosome ends, not only maintains the length of telomeres, but also extends the lifespan of human cell lines (55). Thus, even with a low overall mutation rate, the occurrence of high mutation rates in specific genomic locations in aging may result in substantial age-related functional alteration.

Accumulation of genetic errors may give rise to several cellular end points which are related to the development of aging. One of the major consequences of mutation load is cell death (56). Cell death has been shown to

contribute to various age-related functional deficits, including heart failure (57), kidney dysfunction (58), and decline of immune function (59). Indeed, apoptosis could be a mechanism for removing aged cells during aging (60-62). Cell loss resulting from removal of mutated cells could be related to the functional decline with aging. Mutation load with aging could also result in alteration of gene expression. For instance, aberrant expression of globin RNA in the liver and brain of older mice is higher than in younger controls (63). In addition, DNA methylation at CG bases, which relates to inactivation of gene expression, has been shown to decrease with age (64,65). These detrimental consequences of mutation load during aging could explain the development of functional age-related declines.

Wear and Tear

The wear and tear theory claims that with repeated use, parts in living organisms wear out and give rise to defects. These malfunctions provide the cellular substrate for the build up of physiological deficits in aging. The premise of this theory is based on the observation that the lifespan of poikilotherms is shortened by increasing the environmental temperature and prolonged by decreasing it (66,67). Indeed, active tissues with high rates of cell turnover have been shown to contain more age-related lesions. For instance, more rapid telomere loss can be found in the endothelial lining of blood vessels that were exposed to high hemodynamic stress and underwent rapid turnover (68).

Apart from damages caused by repeated usage, various exogenous or endogenous agents can damage biological macromolecules with time. Among the most common endogenous agents is the free radical (for review, see reference 69). Free radicals are reactive molecules containing one or more unpaired electrons and are produced during normal oxidative metabolism (70,71). Free radicals are able to attack other molecules indiscriminately and cause fragmentation or cross-linking of molecules (7). An increase in the level of free radicals was found in aged brains (72). The level of oxidized lipid (73) and protein (74) is also higher in aged tissue. In addition, cross linkage of both membranous structures (75) and DNA (76,77) have been shown to increase with age. Mitochondrial DNA, which lacks the protection of histones and suffers more directly from the *de novo* production of free radicals, has been shown to mutate at a much higher rate than nuclear DNA (78). Interestingly, comparative studies also reveal an inverse correlation between the level of oxidative burden and maximum life expectancy in different organisms (79).

The production of free radicals is normally controlled by various protective systems in our body. They include enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase that break down newly produced

free radicals into less reactive derivatives (for review, see reference 80). Antioxidants like vitamin E and C also play important roles in reducing active free radicals (81). While contradicting findings on the effect of these antioxidant enzymes on aging was found (e.g. see reference 82), there is evidence of insufficient antioxidant activity in aged tissue. For instance, dietary supplements of free radicals scavengers has been shown to reduce age-related declines in neuronal signal transduction and cognitive or motor behavioral deficits in aged rats (83). Indeed, some longevity genes have been shown to be related to the development of oxidative stress. For example, mutation of *mev-1*, which encodes a subunit of the enzyme succinate dehydrogenase cytochrome b, results in higher production of free radicals and shorter lifespan (84). Thus, increased free radical production and insufficient antioxidant activity in aged tissue may contribute to functional deficits with age.

PROGRAM THEORIES

The underlying assumption of program theories is the existence of genetic programs that determines the maximum lifespan for each species. Evidence to support these theories includes the observation of sequential activation and repression of genes during different stages of development. For instance, studies on rat liver cytosolic alanine aminotransferase (cAAT) have shown that the gene for A type cAAT is active in the early developmental stages and subsequently repressed. However, the expression of B type cAAT is only activated in old age (85). Similar programmed activation of some senescence markers has been reported. A liver protein named as senescence marker protein 2 is expressed maximally during prepuberty and older age (86).

Another important finding to support program theories of aging is the presence of longevity controlling genes. Mutation of some genes in the nematode *Caenorhabditis elegans* has been shown to extend life expectancy of the nematode by 40 to 100%. Examples of these genes include *age-1*, *daf-2*, and *clk-1* genes (87-89). Life expectancy of a *daf-2/clk-1* double mutant is five times longer than that of the wild type (90). While program activation of these potential longevity genes may be important in determining the lifespan of different organisms, evidence for similar candidate longevity genes in humans is lacking.

EXAMPLES OF THESE MECHANISMS IN NEURONAL AGING

Because most neurons do not replicate, one would expect that they are protected against errors arising from DNA replication. However, due to the large size and high metabolic rate of neurons, neuronal survival depends on high rate of oxidative phosphorylation (91). This may result in a large amount of free radical production and thus

confer the nervous system with a high vulnerability to oxidative stress. Indeed, this high neuronal vulnerability toward oxidative stress may be responsible for the mitochondrial DNA mutations (92,93) and nuclear mutations (77,94-96) observed in aged neuronal tissue.

Apart from inducing mutation, oxidative stress also plays important roles in the development of other age-related neuronal damage. High levels of free radicals have been related to the etiology of neurodegenerative phenotype in Alzheimer's disease (97,98) and Parkinson's disease (99,100). For instance, oxidative stress has been shown to cause the death of dopaminergic neurons in Parkinson's disease (101,102). Apoptosis can be induced by damage resulting from oxidative stress (for review, see reference 103).

While the mechanism by which free radicals damage neuronal tissue is still unknown, these reactive elements may play important roles in several age-related neuronal lesions. Oxidative stress has been suggested to cause accumulation of lipofuscin in the neurons from aged brains (104,105). Diet supplement of vitamin E can reduce the density of lipofuscin in aged neurons (106). Lipid peroxidation of neuronal membrane induced by free radicals may also play important roles in the modification of neuronal structures. For instance, long-term (10 months) removal of vitamin E from the diet results in a significant decrease in the number of synapses in the cerebellum (107). Finally, not all neuronal structures or function are equally susceptible to oxidative damage. For instance, *in vitro* studies of the effect of H₂O₂ revealed no effects on excitatory synaptic transmission, whereas reductions in transmembrane Cl⁻ gradient and inhibitory neurotransmission were found (108). The observed disappearance of inhibitory inputs may result in a rise of the level of excitatory neurotransmitters, which increase the possibility of neuronal damage due to excitotoxicity (109-111).

Findings of roles played by different molecular and cellular factors in aging have helped us understand better the age-related change at the level of organs and systems. For instance, oxidative damages have been associated with the loss of synaptic connections in aged brain (107). This age-related loss of neuronal connectivity has been related to the reduced neuronal activity in aged brains (112,113), which in turn may give rise to the development of age-related cognitive declines (114). However, translation of these cellular deficits into age-related pathologies is also difficult to understand. For example, reduction in synaptic structures in aged brain does not always result in an alteration of neuronal function. In fact, physiological compensation after the loss of synapses in aging by the neuronal circuitry has been reported in the hippocampus (115) and cerebral cortex (116). A more thorough comprehension of age-related changes in molecular, organic, and systemic

levels is therefore necessary to uncover the underlying mechanism of age-related pathologies.

SUMMARY

Aging results from a complex interplay between multiple different mechanisms. The predominant mechanism or combination of mechanisms depends on the particular tissue or cell type in question. Nonetheless, a common theme is alteration of gene products or expression. This may occur accidentally over time because of either intrinsic or extrinsic factors, or it may represent part of a predetermined sequence of events. However, it is often difficult to identify whether altered expression of a certain gene is the primary cause or if it is secondary to some other factor, and even gene mutation can represent reduced capacity for repair rather than increased rates of mutation *per se*. Another level of complexity is added to the picture when one considers how the primary changes are manifested at the level of cells, tissues, and organs, let alone the organism as a whole. In turn, developing treatments to delay or ameliorate age-related deterioration of function is a complicated task, but it will ultimately benefit from detailed understanding of the underlying changes.

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