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OXIDATIVE STRESS AND AGING: A COMPARISON BETWEEN VERTEBRATES AND INVERTEBRATES

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12.1 INTRODUCTION

Aging and death, the two ubiquitous phenomena, have always been an enigma for mankind. Except for certain primitive organisms such as bacteria that propagate through simple division, all organisms including humans must age and subsequently face death, yet their life span can vary. Among major model organisms, the worm *Caenorhabditis elegans* lives for 2 weeks, the fly *Drosophila melanogaster* lives for 2 months, the mouse *Mus musculus* lives for 2 years, and humans live for approximately 80 years [1].

Old age in most species is associated with impaired adaptive and homeostatic mechanisms leading to susceptibility to environmental/internal stresses with increasing rates of disease and death [2]. Since ancient times humans have pondered over the mysterious question: How do we age? How do different organism/species show different life spans, and what are the factors that determine longevity? Hippocrates (460–377 B.C.) defined aging as an irreversible and actual event dictated by the gradual loss of heat. Darwin established the scientific background to understand the process of aging; he explained aging as the loss of excitability over time.

The term "aging" refers to the biological process of growing older in a deleterious sense, which some authors call "senescence" [3, 4]. It is a unique feature of the life cycle of all organisms. In scientific terms, aging may be defined as an inevitable process of accumulation of molecular, cellular, and organ damage, leading to loss of function and increased vulnerability to disease and death [5].

12.2 THEORIES OF AGING

Aging is a multifactorial process caused by damage by a variety of cellular components, followed by their accumulation. It is characterized by progressive deterioration of physiological functions and metabolic processes [6, 7]. Within an organism, manifestation of aging occurs at genetic, molecular, cellular, organ, and system levels [8]. Long-term studies on numerous animal models (in vivo and in vitro) have generated a number of theories that attempt to elucidate the cause/mechanism(s) of aging, since it is doubtful that a single theory can explain all the mechanism of aging. Presently more than 300 theories of aging are in existence [9].

The evolutionary theory of aging states that aging occurs because the force of natural selection declines with age in populations, making it possible for hazardous late-acting genes to exist [10, 11]. The life history principle describes aging as an emergent phenomenon that takes place primarily in the protected environment and that allows survival beyond the natural life span that would occur in the wild. The natural or essential life span (ELS) of a species is the time required to fulfill the Darwinian purpose of life, that is, successful reproduction. The period of extended survival beyond the ELS is defined as the period of aging [12]. The mutation accumulation theory argues that detrimental, late-acting mutations

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may accumulate in the population and ultimately lead to pathology and aging [13]. According to the energy consumption hypothesis, animals are born with a limited amount of potential energy or physiological capacity, the faster they use it, the faster they will die [14]. Later this hypothesis became the rate of living theory: The faster the metabolic rate, the faster the biochemical activity, the faster an organism will age. The protein error theory also tried to explain the mechanism of aging. According to this theory an induction and increase in protein errors can accelerate aging in human cells and bacteria [15–17]. Similarly, the accuracy of protein synthesis can slow aging and increase the life span in fungi [18, 19].

The cellular senescence/telomere theory explains that cells have a limited proliferative potential. After a finite number of divisions, cells enter into a state of senescence, and this process of cell senescence limits the number of cell divisions [20]. The number of divisions a cell can undergo in culture is known as the Hayflick limit and has been postulated to determine the maximum life span of an organism [21, 22]. Furthermore, it was elaborated that this specific type of cellular senescence results from the loss of a small amount of DNA at the end of the chromosome, resulting in ever shorter telomeres. This limit in the replicative capacity results in terminally arrested cells with altered cellular physiology that might contribute to aging and cancer through secondary effects on neighboring cells in tissue [23, 24].

The role of genes in regulation of longevity has also been put forward; this theory postulates that aging results from changes in gene expression [25–27]. The free radical theory of aging, developed by Denham Harman in 1956, is one of the most influential theories in describing the aging mechanism. According to this theory free radicals, specifically hydroxyl and hydroperoxyl, are formed endogenously from normal oxygen-utilizing metabolic processes as by-products that play a essential role in aging and age-related processes. Mitochondria play a central role in generation of free radicals through impaired function of the electron transport chain, and these free radicals elicit damaging properties. Aging results in accumulation of free radical damages as a function of time [28–31]. Support for the free radical theory of aging has increased progressively over the years, and growing numbers of studies implicate free radical reactions in aging and the pathogenesis of specific diseases such as diabetes, cancer, and heart diseases [32–34].

12.3 FREE RADICAL/OXIDATIVE STRESS THEORY OF AGING

Among several theories that attempt to explain the aging mechanism, the free radical/oxidative stress theory of aging offers the best mechanistic elucidation of the aging process and other age-related events [28, 35].

Interest in the free radical theory was at first very limited because of persistent doubt about the existence of oxygen free radicals in biological systems despite the reports by Gerschman et al. [36] and the detection of radicals by Commoner and co-workers [37]. The discovery of superoxide dismutase (SOD) by McCord and Fridovich [38] and the demonstration of the existence of H_2O_2 in vivo by Chance [39] gave credibility to and raised the profile of the hypothesis. In 1972, Harman modified his free radical theory, ascribing a central role to mitochondria [29] because mitochondria generate a disproportionately large amount of free radicals/reactive oxygen species (ROS) in cells [39]. Later on, correlative evidence supporting the free radical theory of aging was published. Later on, several reports substantiated Harman's hypothesis that free radical oxidative damage increases during aging [40–42].

In the last few decades, Harman's hypothesis has been refined; it is now accepted that not only free radicals but also other forms of activated oxygen such as peroxides and aldehydes (which are not technically free radicals) play a role in oxidative damage in cells. A collective term, "reactive oxygen species (ROS)," has been introduced to define these oxidants including free radicals. This realization led to a modification of the free radical theory, that is, the oxidative stress theory of aging [43].

Apart from respiratory chain in mitochondria, there are other endogenous sources of ROS including immune reactions, enzymes such as xanthine oxidase and nitric oxide synthase, and transition metal-mediated oxidation [44]. A diverse range of exogenous sources of ROS are reported, which incorporate ionizing and nonionizing radiations, pollutants, natural toxic gases such as ozone, drugs, and toxins including oxidizing disinfectants [34, 45]. ROS production and accumulation is a common denominator in many diseases and environmental insults and can lead to severe cellular damage resulting physiological dysfunction and cell death in virtually all aerobes. When oxidative stress occurs, cells function to counteract the oxidant effects and to restore redox balance by resetting critical homeostatic parameters. Such cellular activity leads to activation or silencing of genes encoding defensive enzymes, transcription factors, and structural proteins [34, 46]. According to the free radical theory of aging, oxidative stress increases with increasing age; this condition leads to accumulation of oxidation products of lipids, nucleic acids, proteins, sugars, and sterols ultimately causing cellular dysfunction and making the body prone to external deleterious agents (Fig. 12.1). In agreement with the free radical/oxidative stress theory of aging, it was investigated that mtDNA deletions are induced by oxidative stress and dramatically accumulate with age in organisms ranging from worms to humans [40, 47].

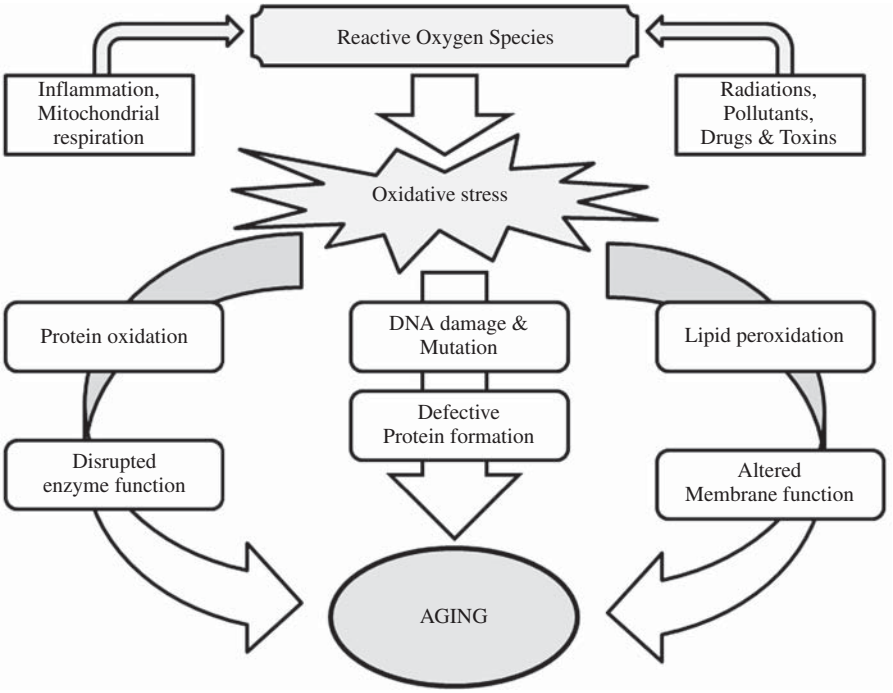

Fig. 12.1 Reactive oxygen species generated by endogenous as well as exogenous sources cause oxidative damage and accumulation of proteins, lipids and DNAs, when defensive mechanisms of body become weak. These disturbances cause organelle damage, changes in gene expression followed by altered cellular responses which ultimately results into aging.

12.4 AGING IN INVERTEBRATES: ROLE OF OXIDATIVE STRESS

The role of oxidative stress in aging is clearly seen in *Podospora anserina*, a fungus that belongs to the ascomycete family of fungi. The growth of hyphae in *P. anserina* is not indeterminate; however, its growth arrests on reaching a certain length and hyphae eventually wither and die. The period to onset of the condition of senescence is referred to as the life span of *P. anserina*. Tudzynski and Esser in 1979 stated the central involvement of mitochondria in determining the timing of senescence [48]. Recently, Dufour et al.[49] proved the involvement of ROS in life span limitation of *P. anserina* and demonstrated that elimination of the mitochondrial electron transport chain extended the hyphal life span of *Podospora* by at least threefold. It has been proposed that oxidative damage to mitochondrial DNA by the mitochondrial ROS triggers senescence [40, 50, 51].

Saccharomyces cerevisiae, the common yeast, is the other invertebrate whose life span is directly determined by oxidative stress/ROS. *S. cerevisiae* also belongs to the ascomycete family. This single-cell budding yeast is a good organism for studying the aging process. For multiplication *S. cerevisiae* uses asymmetric cell division, which results in a small daughter cell; the process is known as budding. Interestingly, a yeast cell (mother cell) can only bud a finite number of times, after which it loses its capacity to multiply and becomes sterile. This budding period of the yeast cell is called the clonal life span [40].

Studies have revealed oxidative damage accumulation during clonal life span. Accumulation of oxidatively damaged proteins has been reported in yeast mother cells during this period [52]. The attack by ROS against proteins modifies amino acid residues generating carbonyl moieties, which has been identified as an early marker for protein oxidation and is used as a measure of protein damage [53]. In yeast cells, protein carbonyls have been shown to accumulate during chronological aging, in a manner dependent on the rate of mitochondrial ROS production [54]. Besides overexpression of the methionine oxidation repair enzyme, MsrB and MnSOD have been unambiguously shown to increase chronological life span of yeast cells [40, 55]. Collectively, these data suggest that ROS limit life span during aging in *S. cerevisiae*; however, the role of oxidative stress in determining the clonal and chronological life span in yeast cells is still not fully understood.

The nematode *Caenorhabditis elegans* is one of the most frequently used invertebrate models for studying the aging process because of its many interesting characteristic features ideally required to study mechanism(s) of aging. Large numbers of offspring, short generation

time, and the ability to be stored frozen have attracted the attention of biogerontologists. Unlike the unicellular yeast, it allows studies of different cell types and organs, such as the nervous or digestive systems; it is also more closely related to mammals [5]. During the past few years, a large number of long-lived mutants of *C. elegans* have been identified that are now frequently used as models in aging research. The commonly used mutants of *C. elegans* are *daf-2* and *age-1* [56, 57]. The daf-2 gene encodes a protein with structural similarity to the mammalian insulin and the insulin-like growth factor receptors, while the age-1 gene encodes a catalytic subunit of phosphatidylinositol-2-OH kinase, which is involved in a conserved signal transduction pathway downstream of the insulin-like receptor [56]. Studies on *daf-2* reveal that these mutants show resistance to oxidative stress, elevated antioxidative enzymes, and expression of some antioxidant genes; all these strongly support the oxidative stress hypothesis of aging [58, 59]. Several recent studies report that there is indeed slower age-related accumulation of protein carbonyl groups in long-lived *C. elegans* strains compared to wild type [60, 61] and faster accumulation in a short-lived strain [61].

In support of the free radical theory in the aging process, Lithgow and co-workers showed that catalytic antioxidants like catalase (CAT) and SOD markedly extend the life span of *C. elegans* [62]. Although some reports do contradict the oxidative stress theory in *C. elegans* aging, there is enough evidence for oxidative stress as a life span determinant in *C. elegans* [40].

The free radical theory of aging has also been extensively studied in the fruit fly (*Drosophila melanogaster)*. The fly is important for establishing evolutionary conservation of mechanisms and for studying events in different tissues that are more numerous and differentiated than in *C. elegans*. Early experiments have indicated that there is a quasi-linear, inverse relationship between life span and oxygen tension in *Drosophila*, and it was seen that increase in oxygen tension above 21% shortened the life span [63, 64]. In 2004, Landis et al. [65] in their microarray study compared gene expression patterns in old and young flies by treating young flies with 100% oxygen. The results were interesting: Young flies treated with 100% oxygen exhibited many gene expression changes resembling those in old flies. This indicated that oxidative injury plays a prominent role in normal fly aging and suggests that the life span shortening under mild hyperoxia (40% O_2) may be true accelerated aging [40].

Genetic manipulation of the SOD activity in *Drosophila* emphasizes the direct association between oxidative stress and life span. Increasing the activity of different forms of SOD by two- to fourfold suggests that elevated antioxidant defense is necessary for extended longevity in *Drosophila* [66]. In 1995, Sohal et al. reported that concomitant overexpression of SOD and CAT increased both average and maximum life span of *D. melanogaster* [67]. Parkes et al. also targeted the overexpression of CuZnSOD in *Drosophila* and reported that overexpression of CuZnSOD in motor neurons resulted in an increase in life span as well as an increase in resistance to paraquat and γ-irradiation [68]. Phillips and co-workers in 2000 reported that MnSOD overexpression increased life span in *Drosophila* [69].

Some recent transgenic studies provide more evidence for the oxidative stress theory of aging in *Drosophila sp*. Overexpression of the protein oxidative damage repair enzyme peptide-*S*-methionine sulfoxide reductase (MsrA) was found to increase average life span in several independent insertion lines [70]. Many contradictory reports have been published that argue the role of oxidative stress in the aging process of *Drosophila sp*. Nevertheless, there is a fair amount of evidence to show the involvement of ROS as a limiting factor in determining the life span of *D. melanogaster* [40].

In addition to the above-discussed invertebrate models there are many other invertebrate species such as helminths in which the direct influence of oxygen radicals in longevity determination has been reported [40].

12.5 AGING IN VERTEBRATES: ROLE OF OXIDATIVE STRESS

Vertebrates are an evolved group of animals that include mammals and humans. Invertebrate model organisms have been used for the discovery of genes and mechanisms involved in extension of life span, but the mouse is the most practical mammal for establishing whether homologous genes and processes can extend healthy life span [5]. In vertebrate models caloric restriction has been shown to increase life span. Caloric restriction also protects against age-related decline in function and disease in rodents and monkeys, and in humans it reduces risk factors for diabetes, cardiovascular disease, and cancer [5]. The most accepted explanation behind caloric restriction life extension is based on the free radical theory of aging: The rate of mitochondrial free radical production is directly proportional to the oxidative damage. A decreased rate of operation of mitochondrial electron transport chain leads to a lower oxidative burden and a higher life span [71].

Studies on transgenic mice have been carried out to understand the relation between free radical generation, oxidative stress, and longevity. Takagi and co-workers in 1999 reported an increase in life span of mice after approximately threefold overexpression of human thioredoxin 1 (*hTrx1*) in different tissues [72]. An increased

resistance to cerebral ischemia and to UV-induced oxidative stress was also demonstrated in *hTrx1* mice. Mitsui et al. in 2002 documented that hTrx1 mice lived about 35% longer than littermate control mice. In addition, a 22% increase in maximal life span was also observed [73]. In continuation, transgenic mice over-expressing metallothionein have been shown to have an increased mean life span of about 15% relative to control mice. Metallothionein is a low-molecular-weight protein rich in cysteine residues; it exhibits heavy metal detoxification and free radical scavenging abilities [74].

Transgenic mice that overexpress human catalase in peroxisomes, nucleus, or mitochondria have also been tested [76]. The mitochondrial overexpression of catalase (MCAT) in mice caused significant delay in the development of age-related cataracts. This was an interesting finding in support of the free radical theory of aging since the development of age-related cataracts has been shown to be inversely related to life span in humans, and its incidence is reduced in mice that exhibit extended longevity [40, 75]. It was seen that Ames dwarf mice have an increased life span compared to their wild-type littermates [76]. Further investigations revealed that the dwarf mice have increased levels of CAT and SOD [77, 78]. The Ames dwarf mice also showed reduced levels of DNA and protein oxidation in liver [78]. Embryonic fibroblasts from these mice are resistant to UV radiation, heat stress, paraquat, hydrogen peroxide, and cadmium [56, 79].

In addition, interspecies comparisons of the life span in vertebrates show a very clear role of oxidative stress in determining longevity. In 1999, Kapahi et al. performed comparative experiments to measure the resistance of fibroblasts and lymphocytes from hamster, rat, marmoset, rabbit, sheep, pig, cow, and human to various oxidative stresses such as paraquat, sodium arsenite, hydrogen peroxide, and *tert*-butyl hydro-peroxide (tBHP). Interestingly, they observed that cells from species with a longer life span had increased resistance to oxidative stress and proposed that cellular defenses that help cells to survive acute stresses are associated with longevity [80]. These observations are consistent with the free radical/oxidative stress theory of aging.

In humans there is an array of experimental evidence in agreement with the free radical/oxidative stress theory of aging [16, 34]. Over the past 10 years, major developments have been made in the assay of oxidative damage to lipids, DNA, and protein to measure specific types of oxidative damage in specific types of cells/tissues during aging. Several reports document the age-dependent increase in lipid peroxidation, protein oxidation, and nucleic acid damage in humans, and there exists a significant negative correlation with antioxidant capacity of plasma [34, 81–83]. Overexpression of antioxidative enzymes/defense mechanisms during aging in humans has also been reported in recent studies that support the oxidative stress theory of aging [83–85].

12.6 CONCLUSION

The phenotype and the rate of progression of aging are highly variable in different species and in organisms within a species. Although there is no doubt that aging is a multifactorial process that is governed by more than one factor, it is becoming increasingly clear that oxidative stress and the resultant damage to biomolecules play a vital role in several age-related pathologies. Despite some contradictory reports, there is sufficient experimental evidence to conclude that oxidative stress, if not alone, is one of the main limiting factors for aging and life span in invertebrates and vertebrates under normal atmospheric conditions.

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