Mitochondria and Aging

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Chapter 14
Mitochondria and Aging

Hsin-Chen Lee and Yau-Huei Wei

Abstract Aging is a degenerative process that is associated with progressive accumulation of deleterious changes with time, reduction of physiological function and increase in the chance of disease and death. Studies in several species reveal a wide spectrum of alterations in mitochondria and mitochondrial DNA (mtDNA) with aging, including (1) increased disorganization of mitochondrial structure, (2) decline in mitochondrial oxidative phosphorylation (OXPHOS) function, (3) accumulation of mtDNA mutation, (4) increased mitochondrial production of reactive oxygen species (ROS) and (5) increased extent of oxidative damage to DNA, proteins, and lipids. In this chapter, we outline the common alterations in mitochondria of the aging tissues and recent advances in understanding the role of mitochondrial H_2O_2 production and mtDNA mutation in the aging process and lifespan determination. In addition, we discuss the effect of caloric restriction on age-associated mitochondrial changes and its role in longevity. Taking these findings together, we suggest that decline in mitochondrial energy metabolism, enhanced mitochondrial oxidative stress, and accumulation of mtDNA mutations are important contributors to human aging.

Keywords Aging • mtDNA • Mitochondria • ROS • Oxidative damage • Caloric restriction

14.1 Introduction

Aging is a degenerative process that is characterized by a time-dependent decline in physiological function and an increase in the chance of disease and death. The deleterious changes with time occur in all organisms that are believed to be associated with the metabolic activity and are influenced by

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genetic and environmental factors. In the early 1950s, Harman considered factors which influenced aging and searched for factors capable of causing death that was present in every living organism, and proposed the "free radical theory of aging". He contended that free radicals, produced from normal metabolism, could be the cause of aging and aging-related degenerative diseases (Harman 1956). In 1972, he modified his theory and proposed that mitochondria are both the main source and a major target of free radicals, and the accumulation of damage with time leads to aging (Harman 1972). In 1980, Miquel and associates suggested that the progressive membrane damage in mitochondria of postmitotic cells by free radicals and lipid peroxides, as by-products of the reduction of oxygen during respiration, could cause an age-related decrease in the amount of functionally competent mitochondria with concomitant decline in cellular production of ATP and increased peroxide generation (Miquel et al. 1980). Linnane et al. (1989) further proposed that accumulation of somatic mutations in mitochondrial DNA (mtDNA) is a major contributor to human aging and degenerative diseases. The free radical theory of aging has thus been extended to the "Mitochondrial theory of aging".

Mitochondria are the main cellular energy sources that generate ATP through the process of respiration and oxidative phosphorylation (OXPHOS) in the inner membrane of mitochondria. The respiratory chain of the OXPHOS system is also the primary intracellular source of reactive oxygen species (ROS) and free radicals under normal physiological and pathological conditions. In addition, mitochondria play a central role in a variety of cellular processes, including β-oxidation of fatty acids, phospholipid biosynthesis, calcium signaling, and apoptosis.

Mitochondria contain their own DNA (mtDNA) and the mitochondrial genome is important in the maintenance of functionally competent organelle. Although the majority of the proteins involved in the OXPHOS are encoded by nuclear DNA, translated in the cytoplasm and are imported into the mitochondria, the mitochondrial genome codes for 2 rRNAs and 22 tRNAs which are required for intramitochondrial protein synthesis, and 13 polypeptides of the respiratory enzyme complexes required for normal function of the OXPHOS system. Distinct from nuclear DNA, mtDNA can be replicated independently of the cell cycle and is present in multiple copies within each cell. The actual number of mtDNA varies between cell types and is dependent on the energy demands within each cell. Any mutations in the mtDNA can co-exist with wild-type copies, a condition called heteroplasmy. The mutant mtDNAs do not exert a biochemical phenotype on a cell until the mutant load reach a certain level. The threshold of mutant/wild type can vary depending on the specific mutation and the cell type.

Mitochondrial theory of aging proposes that progressive accumulation of somatic mutations in mtDNA during an individual's lifetime causes a decline in mitochondrial bioenergetic function and is a contributory factor of aging. Under normal physiological conditions ROS are generated at very low levels during mitochondrial respiration. The complexes I and III of the respiratory chain are the main sites that electrons are leaked out to generate superoxide anions, which can be further converted to H_2O_2 and hydroxyl radicals. It has been estimated that 2–5% of oxygen consumed by mitochondria can be converted to ROS. Most of these ROS can be removed by antioxidants and free radical scavenging enzymes. However, some leakage in the antioxidant protection can damage DNA, proteins and lipids. Oxidative damage to mtDNA may lead to strand breaks of DNA and the occurrence of mutations in mtDNA. Accumulation of these mtDNA mutations could result in dysfunction of the respiratory chain, leading to increased ROS production in mitochondria and subsequent accumulation of more mtDNA mutations. This vicious cycle has been proposed to account for an increase in oxidative damage during aging, which leads to the progressive decline of cellular and tissue functions as a result of insufficient supply of energy and increased susceptibility to apoptosis (Linnane et al. 1989; Wei 1992; Lee and Wei 2007).

In the past decades, these aging theories have been extensively tested by many approaches and substantial supportive evidence has been gained from molecular and cellular biological studies. Studies from aging humans and animals have shown good correlations between aging, mitochondrial

function decline, and accumulation of mtDNA mutations. However, although it is clear that oxidative damage increases during aging, the role of mitochondrial oxidative stress in the aging process remains controversial. This chapter reviews recent advances in the understanding of the roles that alterations of mitochondria and mtDNA may play in aging. In addition, the roles of ROS in aging-associated mitochondrial function decline and somatic mtDNA mutation, and the effect of caloric restriction on mitochondria in aging are discussed.

14.2 Changes of Mitochondrial Morphology with Age

Evidence based on electron microscopic studies has shown that mitochondrial disorganization accumulated with age in a variety of tissues, especially postmitotic cells such as neurons and muscle cells. Age-related changes in the muscle mitochondria of insects (e.g., flies) have been described, including a decrease in the number of mitochondria accompanied by an enlargement and more irregular structure of the remaining mitochondria, a loss of the stacked arrays of mitochondrial cristae, and a disruption of crystal packing with expansion of the intracristal space (Miquel et al. 1980). Similar age-related changes were also observed in the myocardium, the skeletal muscle, liver, kidney, and the cerebral cortex of mammals (e.g., mice, rats, and human subjects). The mitochondrial structural changes in these old organisms included a decrease in the total number of mitochondria, an increase in their size, and a decrease in the number of mitochondrial cristae (Miquel et al. 1980).

Recent work has shown that mitochondria are very dynamic organelles and maintain a constant remodeling of mitochondrial architecture, allowing morphological transitions from individual structures to complex tubular networks through fusion and fission (Chen and Chan 2009). It has been established that this dynamic structure is regulated by proteins controlling fission, such as hFis1 and Drp1, and fusion, such as mitofusin 1 and 2 (MFN1 and 2) and OPA1. These proteins appear to be critical for normal mitochondrial function and abnormalities in mitochondrial dynamics play an important role in the pathophysiology of neurodegenerative diseases (Chen and Chan 2009). Evidence reveals that the fission-related protein hFis1 has been associated with the process of senescence in mammalian cells (Lee et al. 2007). Using RNA interference to reduce hFis1 in mammalian cells, it was found that mitochondria become enlarged and flattened and these morphological changes are correlated with increased β-galactosidase activity, a marker of cell senescence, and decreased mitochondrial membrane potential, increased ROS generation and DNA damage. This suggests that hFis1 plays an important role in cell senescence and both the structure and dynamics of mitochondrial architecture are important factors involved in the cell senescence program. However, the role of proteins that regulate fission/fusion in aging warrants further investigation.

14.3 Mitochondrial OXPHOS Function Declines with Age

Bioenergetic studies of the human and animals have suggested that respiratory function of mitochondria declines in aging post-mitotic tissues. Immunohistochemical staining showed that cytochrome *c* oxidase (COX)-negative cardiomyocytes and muscle fibers are present in the heart, limb, diaphragm and extraocular muscle of normal elderly subjects, and that the number of COX-negative muscle fibers is increased with age in the human subjects (Müller-Höcker 1989, 1990; Müller-Höcker et al. 1993). Moreover, a decrease of COX proteins was observed in aged human brain (Ojaimi et al. 1999a). An age-related increase in the number of COX-negative muscle fibers was also found in fruit flies, *Drosophila melanogaster* (DiMauro et al. 2002).

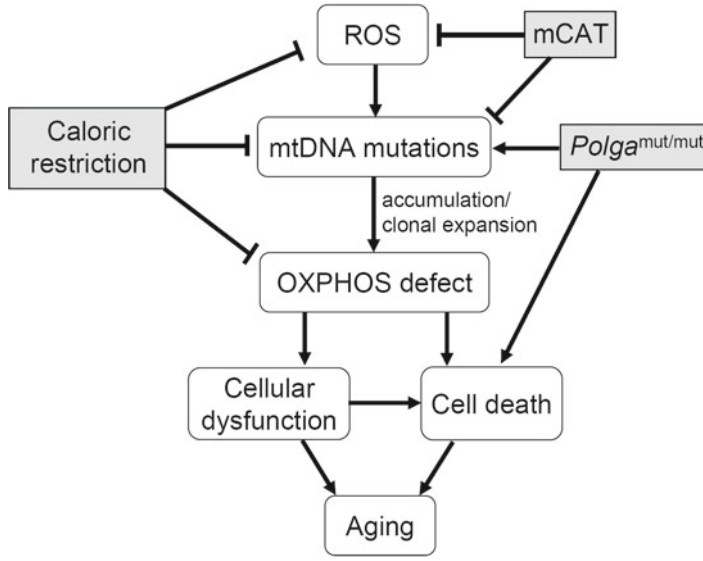

Fig. 14.1 Mitochondrial role in the aging process. There is increasing amount of evidence to suggest that accumulation of mitochondrial DNA (*mtDNA*) mutations with age plays a role in cellular dysfunction in many tissues. MtDNA mutations could be caused by increased oxidative stress. If a mutated mtDNA molecule is replicated and clonally expanded within a cell, this cell may become deficient in respiration and oxidative phosphorylation (*OXPHOS*). The compromised energy production could lead to cellular dysfunction and/or cell death, which may result in tissue dysfunction and onset of aging. Recent studies revealed that overexpressing human catalase localized to mitochondria (*mCAT*) can reduce mtDNA mutations in tissues during aging and increase the lifespan of transgenic mice. Mitochondrial DNA polymerase γ-mutated mice (*Polga*$^{mut/mut}$) can accumulate high level of mtDNA mutations and increased apoptosis in tissues and develop premature aging phenotype. In addition, caloric restriction has been demonstrated to be able to delay the onset of age-associated phenotypes and extend lifespan in diverse living organisms. It has also been found that caloric restriction is able to reduce the rate of mitochondrial ROS production, mtDNA mutations, and the abnormalities in the mitochondrial OXPHOS system. These lines of evidence have supported the concept that the decline in energy metabolism, ROS overproduction and accumulation of mtDNA mutations in tissue cells are important factors that contribute to the aging process

Numerous biochemical studies on isolated mitochondria revealed that the electron transport activities of respiratory enzyme complexes gradually decline with age in the brain, skeletal muscle, liver and skin fibroblasts of normal human subjects (Trounce et al. 1989; Yen et al. 1989; Cooper et al. 1992; Hsieh et al. 1994; Boffoli et al. 1994; Ojaimi et al. 1999b; Greco et al. 2003; Short et al. 2005). Similar age-related decline in the activities of respiratory enzymes was also demonstrated in the flight muscle of flies (Bulos et al. 1972, 1975), as well as in skeletal muscle, heart, liver of rats (Torii et al. 1992a, b; Takasawa et al. 1993; Sugiyama et al. 1993; Lesnefsky and Hoppel 2006) and dogs (Sugiyama et al. 1993), and in brain of rhesus monkeys (Bowling et al. 1993).

Age-related decrease in ADP-stimulated (State 3) respiration and respiratory control of isolated mitochondria were reported in flies (Martinez and McDaniel 1979) and in the myocardium of old rats (Murfitt and Sanadi 1978). The respiratory control ratio, OXPHOS efficiency, the rates of resting and ADP-stimulated respiration and ATP synthesis of isolated mitochondria were all found to decline, although with different degrees, with age in the skeletal muscle and liver (Trounce et al. 1989; Yen et al. 1989) and in human skin fibroblasts (Greco et al. 2003). The age-associated decrease in mitochondrial membrane potential, the driving force for OXPHOS and the increase in proton leakage of the respiratory chain were found to correlate with reduced ATP synthesis in tissues of old animals (Pieri et al. 1993; Hagen et al. 1997; Harper et al. 1998) and in skin fibroblasts from elderly subjects (Greco et al. 2003). A study further confirmed that the activities of mitochondrial respiratory enzymes

and that mitochondrial ATP production rate are declined with age in muscle biopsies from 146 healthy subjects (Short et al. 2005).

One study assessed *in vivo* rates of mitochondrial oxidative metabolism by ^{13}C nuclear magnetic resonance (NMR) spectroscopy and phosphorylation activity by ^{31}P NMR and found approximately 40% reduction in oxidative phosphorylation activity of mitochondria in skeletal muscle of healthy elderly subjects as compared with that of the young controls (Petersen et al. 2003).

The age-associated decreases in mitochondrial respiratory proteins and OXPHOS function could be resulted from the decline in the rates of mtDNA transcription and mitochondrial protein synthesis. Many lines of evidence revealed that steady-state levels of mitochondrial RNA transcripts are decreased in old *D. melanogaster* (Calleja et al. 1993) and in various aging tissues of the human and animals (Gadaleta et al. 1990; Fernandez-Silva et al. 1991; Barrientos et al. 1997a, b). Moreover, an age-dependent decline in the rate of protein synthesis was observed in *D. melanogaster*, mouse liver and kidney (Marcus et al. 1982; Bailey and Webster 1984), and human skeletal muscle (Rooyackers et al. 1996) as well as skin fibroblasts (Greco et al. 2003). The age-related decrease in mitochondrial gene transcripts and proteins of human skeletal muscle was found to closely relate to the reduction of mtDNA content and the ATP production rate of mitochondria (Short et al. 2005).

Different lines of evidence from immunohistochemical staining, biochemical studies and *in vivo* investigations have supported the central idea that the decline in bioenergetic function of mitochondria plays an important role in aging (Fig. 14.1). The decline in mitochondrial respiratory function can lead to lower ATP production and more ROS formation in aged tissues.

14.4 MtDNA Mutations Accumulate with Age

Numerous molecular studies using polymerase chain reaction (PCR) or quantitative PCR techniques demonstrated that somatic mutations in mtDNA progressively accumulate with age in a variety of tissues of humans (Yen et al. 1991; Torii et al. 1992a, b; Lee et al. 1994a), rhesus monkeys (Schwarze et al. 1995) and rodents (Khaidakov et al. 2003). These age-related mtDNA mutations include large-scale deletions (Yen et al. 1991; Zhang et al. 1992; Lee et al. 1994a), tandem duplications (Lee et al. 1994b; Wei et al. 1996) and point mutations (Zhang et al. 1993; Münscher et al. 1993; Michikawa et al. 1999; Wang et al. 2001; Khaidakov et al. 2003). Many lines of studies have revealed that multiple deletions of mtDNA occur in the skeletal muscle, heart, and brain of aged human subjects and mice, and suggest that a broad spectrum of mtDNA deletions is accumulated with age. Many types of tandem duplications in the D-loop region of mtDNA were identified in a variety of tissues of elderly subjects (Lee et al. 1994b; Wei et al. 1996). Some pathogenic point mutations in the tRNA genes of mtDNA (Münscher et al. 1993; Zhang et al. 1993) and high levels of point mutations in the D-loop region of mtDNA (Michikawa et al. 1999; Wang et al. 2001) have also been found to accumulate with age in human tissues and cultured human skin fibroblasts.

It has been observed that terminally differentiated tissues with active oxidative metabolism, such as skeletal muscle, heart, and brain, accumulate relatively higher levels of mutant mtDNA during the aging process. Many of these mtDNA mutations start to occur after mid-1930s and accumulate with age in post-mitotic tissues of humans (Yen et al. 1991; Lee et al. 1994a). It is noteworthy that occurrence of mtDNA deletions was correlated with the reduction of mitochondrial respiratory chain enzyme activities in aging human skeletal muscles (Hsieh et al. 1994; Lezza et al. 1994).

Initially, most studies showed that the overall proportion of a specific mutant mtDNA is lower than a level of approximately 0.1% of total mtDNA molecules in any somatic tissue examined (Wei and Lee 2002). The levels are far lower than a threshold of mtDNA mutation (~80–100% of the mtDNA) required for the expression of a defective electron transport system in patients with mitochondrial myopathies (Shoubridge et al. 1990; Schon et al. 1994; He et al. 2002). However, it could be because most investigators

screened for mtDNA mutations in the whole tissue rather than individual cells. The mutated mtDNA molecules may be unevenly distributed and can accumulate clonally in certain cells, causing a mosaic pattern of respiratory chain deficiency in somatic tissues during aging. Besides, the unevenly distributed mutant mtDNA molecules were largely diluted by wild-type mtDNA when the whole tissue was used to screen for mtDNA mutations. The idea was supported by some evidence that the proportion of mtDNA with a large-scale deletion was well correlated with that of COX-negative fibers in the same subjects (Kovalenko et al. 1997; Pesce et al. 2001). Using a single fiber PCR method or the laser-capture microdissection technique (Kopsidas et al. 1998; Cao et al. 2001; Wanagat et al. 2001), it was further demonstrated that COX-negative fibers in the skeletal muscle of normal elderly subjects and aged rats contain reduced levels of full-length mtDNA and high levels of mtDNA deletions.

Using both histological and polymerase chain reaction (PCR) analyses, it was shown that mtDNA deletions clonally accumulate to high levels within COX^{-}/SDH^{+} regions (>90% of total mtDNA) in vastus lateralis muscle of human subjects aged 49–93 years (Bua et al. 2006). Moreover, the amplitude of decline in the levels of the deletion-containing mtDNA in the transition regions and in mitochondrial electron transport chain-normal regions immediately adjacent to the transition regions suggest that the accumulation of mtDNA deletions precedes the electron transport chain deficiency (Bua et al. 2006). The high levels of accumulated mtDNA deletion were also observed to link to enzymatic and morphological abnormalities of mitochondria such as fiber splitting, atrophy, and breakage in muscle fibers from aged rats (Herbst et al. 2007). These observations strongly support the notion that mtDNA deletions play a causal role in mitochondrial dysfunction of skeletal-muscle fibers with age, a process that ultimately leads to fiber loss.

Studies on the dissected substantia nigra of post-mortem human brains further revealed that very high levels of mtDNA deletions are present in dopaminergic neurons from very old individuals (Bender et al. 2006; Kraytsberg et al. 2006). Importantly, the proportion of mtDNA with deletions increased significantly with age and neurons containing over 60% of deleted mtDNA molecules were associated with a striking loss of COX activity. These findings suggest that mtDNA mosaic seems to parallel the occurrence of the bioenergy mosaic, and mtDNA mutations can reach rather high levels in the cells of elderly subjects. The accumulation of mtDNA mutations with age in tissues of human and rodents can cause adverse effects and may play a causal role in aging (Fig. 14.1).

Although actual mechanism for the formation of mtDNA deletions has remained unclear, oxidative damage-associated single- or double-strand DNA breaks might be involved in their formation. It has been shown that the proportion of mtDNA with deletions correlates with the level of oxidative modification (8-OHdG content) of mtDNA (Hayakawa et al. 1992). It has also been demonstrated that treatment of human skin fibroblasts with sublethal dose of oxidative stress results in the formation and accumulation of the common 4,977 bp deletion in mtDNA (Dumont et al. 2000). In addition to normal aging, environmental insults, such as UV irradiation (Pang et al. 1994; Yang et al. 1995; Berneburg et al. 1999), cigarette smoking (Fahn et al. 1998; Lee et al. 1999), and betel quid chewing (Lee et al. 2001), have been demonstrated to increase the levels of mtDNA with large-scale deletion. These studies suggest that oxidative damage to mtDNA is a major cause of instability and mutations (point mutation and deletion) of the mitochondrial genome in the tissues of elderly subjects (Linnane et al. 1989; Beckman and Ames 1998). These findings provided evidence to support the notion that ROS and free radicals play an important role in the mechanism underlying aging-associated somatic mutation of mtDNA.

14.5 Mitochondrial ROS Production Increases with Age

A majority of intracellular ROS are thought to be generated as byproducts of oxidation-reduction reactions in mitochondrial respiratory chain (Beckman and Ames 1998). Knowledge of how mitochondria produce ROS is based on the evidence from isolated mitochondria. During respiration,

superoxide anions ($O_2^{\cdot -}$) and organic free radicals (e.g., ubisemiquinone and flavosemiquinone) are generated from mitochondria, mainly through Complex I and Complex III in the respiratory chain (Chance et al. 1979; Wei et al. 1981). Although it is still not clear about the actual rate of mitochondrial ROS production *in vivo*, it was estimated that about 2% of the oxygen consumed by isolated mitochondria from mammalian tissues is converted to ROS, including superoxide anions, hydrogen peroxide (H_2O_2), and hydroxyl radicals (Chance et al. 1979; Beckman and Ames 1998).

Evidence supporting the link of mitochondrial generation of superoxide anions and H_2O_2 to aging is provided by studies on isolated mitochondria of tissues from insects and mammals (Ku et al. 1993; Sohal et al. 1995). It was reported that the average lifespan of different species of flies is inversely correlated with the rate of mitochondrial superoxide anions and H_2O_2 generation (Sohal et al. 1995). Similar correlations were also observed in the comparison among different mammalian species, including mouse, hamster, rat, guinea pig, rabbits, pig, and cow (Ku et al. 1993). Moreover, the rates of ROS production from mitochondria are increased with age in the brain, heart, and kidney of mice (Sohal et al. 1994).

It has become clear that under resting conditions in which mitochondria are not actively making ATP, mitochondria exhibit a low rate of oxygen consumption, high membrane potential across the inner membrane, and high proportion of reduced electron carriers (NADH/NAD^+ ratio and $CoQH_2$/CoQ ratio), the rates of ROS production from isolated mitochondria are high (Murphy 2009). This suggests that *in vivo* factors leading to these conditions will favor ROS production. However, the extent to which these situations arise *in vivo* with age is not clear.

14.6 Oxidative Damages Accumulate with Age

Several antioxidant enzymes and small-molecular-weight antioxidants within the cell and mitochondrion can cope with and dispose of most of ROS generated by aerobic metabolism under normal physiological conditions. The important antioxidant enzymes include manganese superoxide dismutase (MnSOD), copper/zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GPx) and catalase. Superoxide anions can be dismutated to H_2O_2 by MnSOD and Cu/ZnSOD. MnSOD is located in the mitochondrial matrix and CuZnSOD is localized in the mitochondrial intermembrane space and cytosol (Okado-Matsumoto and Fridovich 2001; O'Brien et al. 2004; Inarrea et al. 2007). H_2O_2 can be scavenged by either GPx in the mitochondrial matrix or catalase in the cytosol. Although several studies on age-related changes in antioxidant enzymes have generated conflicting results (Sohal et al. 1994; Beckman and Ames 1998; Lu et al. 1999), it is generally accepted that a leakage of ROS from mitochondria and an excess production of ROS that overwhelms the antioxidant defense system can cause oxidative damage to cellular constituents, including nucleic acids, lipids, and proteins. As the major intracellular source of ROS, mitochondria are subjected to direct attack by ROS in animal and human cells. Moreover, superoxide anions in the intermembrane space might be released into the cytosol through the voltage-dependent anion channel (VDAC) (Han et al. 2003), and H_2O_2 can move across mitochondrial membranes into the cytosol to damage extra-mitochondrial constituents.

Many lines of evidence suggest that oxidative damage to nuclear DNA and mtDNA of tissue cells are increased with age of the mammals (Beckman and Ames 1998; Hamilton et al. 2001a, b). Some characteristics of mammalian mtDNA were thought to render it to be highly susceptible to oxidative damage, including its close proximity to the sites of ROS production from the respiratory chain, lack of protection by histones, and limited capacity for repair of DNA damage. It has been documented that the steady-state levels of oxidative modifications observed in mtDNA, especially 8-OH-dG, are several-fold higher than those in nuclear DNA (Richter et al. 1988; Ames et al. 1993; Barja and Herrero 2000; Hamilton et al. 2001a, b). The levels of oxidative modifications in mtDNA of the diaphragm and heart muscle were found to increase with the age of human

subjects (Hayakawa et al. 1991, 1992). The age-associated oxidative damage to mtDNA was shown to correlate with mitochondrial glutathione oxidation in the liver, kidney and brain of rats and mice (de la Asuncion et al. 1996). In addition to modifications of the purine and pyrimidine bases, oxidative damage to DNA can lead to abasic sites, single- and double-strand breaks as well as cross-links to other DNA molecules. DNA damage accumulation can block the progression of DNA polymerase resulting in decreased amplification of the target sequence. Studies using a gene-specific DNA damage assay based on quantitative PCR revealed that mtDNA is more susceptible to oxidative damage than is nuclear DNA (Yakes and van Houten 1997), and that the mtDNA damage accumulates with age in post-mitotic tissues (Lin et al. 2003).

The hydroxyl radical is one of the most potent inducers of lipid peroxidation. It has been shown that the content of lipid peroxides in mitochondria is increased with age (Hruszkewycz 1992). The extent of lipid peroxidation was also correlated with alterations in mitochondrial respiration and OXPHOS activity, inner membrane barrier properties, maintenance of mitochondrial membrane potential, and mitochondrial Ca^{2+} buffering capacity (Britton et al. 1990; Albano et al. 1991; Bacon et al. 1993). Cardiolipin resides primarily in the inner membrane of mitochondria and the highly unsaturated nature of the fatty acyl chains in cardiolipin is required for optimal function of many of the proteins involved in mitochondrial respiratory chain. It has been shown that increased ROS production from mitochondria may result in oxidation and depletion of cardiolipin, as well as inhibition of COX activity (Paradies et al. 2000). Peroxidation of cardiolipin can impair the barrier function of the inner membrane and facilitate the detachment of cytochrome *c* from mitochondrial respiratory chain and contribute to apoptosis (Petrosillo et al. 2001).

On the other hand, it has been shown that the amounts of proteins with oxidative modifications, such as oxidation of the sulfhydryl groups of proteins or the formation of protein carbonyls, in mitochondria are increased with age (Sohal et al. 1994; Agarwal and Sohal 1995; Sohal 2002). The age-related increase in the level of oxidized proteins was also observed in human skin fibroblasts and the mitochondrial fraction accumulated higher levels of protein carbonyl than the whole-cell lysate of these skin fibroblasts (Miyoshi et al. 2006). This increase in the level of proteins with oxidative modification correlated with a decline in the intracellular ATP level (Miyoshi et al. 2006). Aconitase and adenine nucleotide translocase (ANT) have been found to be the preferred targets of oxidative damage to mitochondrial proteins during aging of the animals (Gardner et al. 1994; Yan et al. 1997; Yan and Sohal 1998). In addition, DNA polymerase γ was shown as one of the major targets of oxidative damage in mitochondrial matrix, which may contribute to a reduction in mtDNA replication and DNA repair activities in mitochondria (Graziewicz et al. 2002). Oxidative modification of proteins can alter protein structure and/or result in a loss of their normal function, which might lead to further ROS production from mitochondria.

14.7 Role of Mitochondrial ROS in Aging

The evidence that mitochondrial ROS production and accumulation of oxidative damage increase with age corroborates the possibility that mitochondrial ROS cause adverse effects in aging. To investigate the importance of mitochondrial ROS in aging, several genetics approaches have been used.

Studies of animals deficient in MnSOD have provided evidence to substantiate the role of mitochondrial superoxide anions in aging. Mice deficient in the MnSOD exhibited neonatal lethality in association with dilated cardiomyopathy and lipid accumulation in the liver (Li et al. 1995; Melov et al. 1999). These mice also displayed severe mitochondrial dysfunction and enhanced oxidative damage to mitochondria (Li et al. 1995; Melov et al. 1999). The $Sod2^{+/-}$ mice showed partially reduced (30–80%) scavenging activity for superoxide anions in all tissues throughout life and had an increased oxidative damage to mitochondria as compared with the $Sod2^{+/+}$ mice (Williams et al. 1998; Kokoszka et al. 2001; Van Remmen et al. 2003; Mansouri et al. 2006). However, the activities of

respiratory enzyme Complex I and ATP synthase were not significantly reduced in old $Sod2^{+/-}$ mice compared with age-matched wild-type mice (Mansouri et al. 2006). In addition, several aging biomarkers, such as cataract formation, defective immune response, and formation of glycoxidation products such as carboxymethyl lysine and pentosidine in skin collagen, change with age in the similar extent in both wild-type mice and the $Sod2^{+/-}$ mice (van Remmen et al. 2003). Importantly, the life spans of the $Sod2^{+/-}$ mice were not shorter than those of wild-type mice, which suggest that increased mitochondrial superoxide anions production is not sufficient for acceleration of the aging process (van Remmen et al. 2003).

On the other hand, it was shown that transgenic mice that over-expressed a mitochondrially localized version of the human catalase (mCAT) exhibit prolonged median and maximum lifespan (Schriner et al. 2005). The extension of lifespan was associated with a specifically increased H_2O_2-scavenging activity in mitochondria and attenuated oxidative damage to mtDNA, as well as reduced accumulations of deletion and point mutation of mtDNA in heart and muscle tissues (Schriner et al. 2005; Vermulst et al. 2007, 2008). The results suggest that mitochondrial H_2O_2 plays an important role in aging and determination of lifespan of the animals (Fig. 14.1).

14.8 Consequences of Somatic mtDNA Mutations in Aging

Human mitochondrial genome codes for 13 polypeptides, which are crucial components of the OXPHOS system, and two rRNAs (12S and 16S) and 22 tRNAs essential for protein synthesis in mitochondria. Because of the crucial role of mitochondrial genome in the OXPHOS function, accumulation of mtDNA mutations can result in energy crisis, oxidative stress or cell death, which may contribute to aging phenotype.

The causal role of mtDNA mutations in mammalian aging is supported by the studies using mtDNA mutator mice harboring homozygous genetic defects in the proofreading exonuclease activity of mitochondrial DNA polymerase γ (Polg A) (Trifunovic et al. 2004; Kujoth et al. 2005). These mtDNA mutator mice were found to accumulate elevated levels of mtDNA mutations, including point mutations and deletions. There was a mosaic pattern with COX deficiency, an accumulation of abnormal mitochondria, and a progressive reduction of respiratory chain enzyme activities and mitochondrial ATP production rate in the hearts of the mtDNA mutator mice (Trifunovic et al. 2004). Importantly, the mtDNA mutator mice had a reduced lifespan and exhibited accelerated onset of aging-related phenotypes such as weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spine), loss of muscle mass (sarcopenia), osteoporosis, anemia, thymic involution, testicular atrophy associated with the depletion spermatogonia, reduced fertility, loss of intestinal crypts, heart enlargement and hearing loss (Trifunovic et al. 2004; Kujoth et al. 2005). These findings have provided strong evidence to support the notion that accelerating the mtDNA mutation rate can result in premature aging.

According to the mitochondrial theory of aging, these accumulated mtDNA mutations may compromise the integrity of the electron transport chain and increase the formation of ROS, creating a vicious cycle of mutagenesis that continuously amplifies the production of cytotoxic oxygen free radicals. However, it was found that despite increased mutational load in mtDNA, mitochondria from these mtDNA mutator mice did not show increased oxidative stress (Kujoth et al. 2005). Levels of H_2O_2, markers of oxidative damage to DNA (8-OH-dG), proteins (protein carbonyls) and lipids (F2-isoprostanes) were not significantly different in the somatic tissues between mutant and wild-type mice. In addition, mouse embryonic fibroblasts from mtDNA mutator mice exhibited normal rate of ROS production and did not show increased sensitivity to oxidative stress (Trifunovic et al. 2005). There were no significant changes in the levels of antioxidant enzymes, protein carbonyls, and aconitase enzyme activity in tissues from the mtDNA mutator mice. These findings indicate that there was no or only minor oxidative stress in tissues from these mice (Trifunovic et al. 2005).

Therefore, although the mtDNA mutator mice accumulated mtDNA mutations and exhibited severe respiratory chain dysfunction, accelerated development of aging phenotype through mtDNA mutations can occur in the absence of increased ROS production or oxidative stress. However, this does not rule out the possibility that mtDNA mutation is downstream of mitochondrial ROS generation.

Evidence from mouse embryonic fibroblasts derived from the mtDNA mutator mice revealed that accelerated aging in the mice is not associated with an intrinsic defect in cellular proliferation or accelerated cellular senescence (Kujoth et al. 2005). Instead, the tissue dysfunction and pathological changes in the mtDNA mutator mice were found to associate with the extent of apoptosis (Kujoth et al. 2005). The levels of apoptotic markers (e.g., caspase 3 activation) were found to increase more rapidly during aging in the mutator mice as compared with wild-type mice. These results suggest that age-related accumulation of mtDNA mutations in normal or Plog A mutant mice promote apoptosis and contribute to the decline of physiological function, which may be an important mechanism of aging in mammals (Fig. 14.1).

The mtDNA mutator mice have high levels of point mutations and deletions of mtDNA. Recent studies debated whether point mutations or deletions of mtDNA are the driving force behind premature aging in the mtDNA mutator mice (Vermulst et al. 2007, 2008; Edgar et al. 2009). Distinct from the mtDNA mutator mice with homozygous defects in the proofreading-deficient *Polga* allele ($Polga^{mut/mut}$), heterozygous mice ($Polga^{+/mut}$) did not show the features of accelerated aging and a significant reduction in the mean lifespan (Trifunovic et al. 2004; Kujoth et al. 2005). Using a more sensitive assay, Vermulst et al. (2007) found that heterozygous mice ($Polga^{+/mut}$) displayed more than 100-fold increase in the point mutation frequency without manifesting features of premature aging and thus suggested mtDNA point mutations do not limit the lifespan of normal mice. The same group further reported that mtDNA deletions are associated with a number of age-related pathologies of the mtDNA mutator mice, which suggest that mtDNA deletions drive premature aging in the mtDNA mutator mice (Vermulst et al. 2008). On the other hand, Edgar et al. (2009) reported that mitochondrial protein synthesis is unimpaired but the stability of several respiratory chain complexes is severely impaired in the mtDNA mutator mice. They suggested that random point mutations in mtDNA are the driving force behind premature aging in mtDNA mutator mice through causing amino acid substitutions in mtDNA-encoded respiratory chain subunits, which leads to decreased stability of the respiratory enzyme complexes and progressive decline of respiratory chain function (Edgar et al. 2009). In addition, it was found that the mtDNA mutator mice harbor linear mtDNAs (Trifunovic et al. 2004). Bailey et al. (2009) provided evidence that the linear mtDNAs were derived from replication intermediates and suggested that the mtDNA mutator mice display elevated replication pausing and chromosomal breakage at fragile sites of mtDNA and the perturbed mtDNA replication is likely to contribute to the pathophysiologic features of the mtDNA mutator mice.

The mtDNA mutator mice provided genetic evidence linking mtDNA mutations to aging phenotypes and shortened lifespan. However, the mechanism by which accelerated mtDNA mutation rate results in premature aging has remained unclear. It is an open question whether certain types of mtDNA mutations are particularly important for human aging and whether the functional impact of mtDNA mutations in mice is the same as those observed in the mammals and humans (Khrapko and Vijg 2009).

14.9 Effect of Caloric Restriction on Mitochondria in Aging

Caloric restriction, a reduction in caloric intake of 20–40% without malnutrition, is the only experimental manipulation that could delay the onset of age-associated phenotypes and extend the average and maximum lifespan in several living organisms ranging from yeast and nematodes to rodents and monkeys (Bordone and Guarente 2005). Caloric restriction could also reduce the incidence of

age-related diseases including diabetes, cancer, cardiovascular disease, brain atrophy, immune deficiencies and mortality in rhesus monkeys (Roth et al. 2004; Colman et al. 2009).

Studies using oligonucleotide-based microarrays to analyze age-associated transcriptional changes in different species revealed that genes involved in mitochondrial energy metabolism showed similar age-related changes (Park and Prolla 2005; Zahn et al. 2007). A meta-analysis of age-related gene expression profiles using 27 datasets from mice, rats, and the human has identified several common signatures of aging, including increased expression of genes involved in inflammation and immune responses, and reduced expression of genes associated with mitochondrial energy metabolism (de Magalhães et al. 2009). Importantly, most of the age-associated alterations in gene expression can be completely or partially prevented by caloric restriction (Park and Prolla 2005). These studies in diverse species suggest that dysregulation of mitochondrial energy metabolism is a hallmark of aging, and that caloric restriction-induced metabolic reprogramming plays a critical role in its retardation of the aging process.

It has been proposed that caloric restriction increased longevity by metabolic changes linking to reducing ROS production and decreasing cellular damage (Sohal and Weindruch 1996; Gredilla and Barja 2005). It was reported that mitochondria isolated from caloric-restricted rodents exhibited a reduction in ROS production and a lower steady-state levels of oxidative stress as compared with animals fed on the ad libitum diet (Sohal et al. 1994; Gredilla et al. 2001; López-Torres et al. 2002; Sanz et al. 2005; Hagopian et al. 2005). Aging-related increases in the levels of oxidative damage to mtDNA, proteins and lipids in mitochondria were attenuated or prevented in caloric-restricted animals (Lass et al. 1998; Gredilla et al. 2001; López-Torres et al. 2002; Sanz et al. 2005).

In addition, caloric restriction significantly reduced the age-dependent decline of mitochondrial respiratory capacity (Sohal et al. 1994; Hagopian et al. 2005). It was demonstrated that caloric restriction attenuated the abnormalities of mitochondrial electron transport chain, but did not affect the length or associated fiber atrophy of the muscle with mitochondrial abnormalities (Bua et al. 2004). This suggests that calorie restriction affects the onset but not the progression of electron transport chain abnormalities, which thus limits a process that ultimately results in fiber breakage and fiber loss. Moreover, it was reported that caloric restriction reduces age-related accumulation of mtDNA deletions in skeletal muscle and liver of caloric-restricted rats (Lee et al. 1998; Cassano et al. 2004).

Based on the above-mentioned studies in several species, we suggest that caloric restriction changes energy metabolism, and this altered metabolic state can reduce mitochondrial ROS production and oxidative damage, prevent the formation of mtDNA mutation, and reduce abnormalities in mitochondrial electron transport chain as well as ATP synthesis, which retard the aging process and extend life span (Fig. 14.1). It awaits further study to elucidate the underlying mechanism by which caloric restriction leads to the reprogramming of energy metabolism.

14.10 Concluding Remarks

In this chapter, we have outlined several common mitochondrial changes in the aging process, which include increased disorganization of mitochondrial structure, progressive decline in mitochondrial OXPHOS function, accumulation of mtDNA mutations, as well as increased mitochondrial ROS production and oxidative damage. These age-associated changes in mitochondria are well correlated with the deteriorative processes of tissues in aging. Convincing evidence from several genetic approaches supports the causal role of mitochondrial H_2O_2 production and mtDNA mutation in the aging process and determination of lifespan of animals. In addition, it has been shown that caloric restriction without malnutrition can modulate energy metabolism of mitochondria and prevents or attenuates most of these age-associated mitochondrial changes, and thereby slow down the aging process and extends lifespan in diverse organisms.

Although abundant experimental data have been gathered in the past decade to support the concept that decline in mitochondrial energy metabolism, ROS overproduction and accumulation of mtDNA mutations in tissue cells are important contributors to human aging, the detailed mechanisms by which these biochemical events cause human aging have remained to be established. Gaining insights into the execution and regulation of these key events in aging will help us to better understand the age-related alterations in the structure and function of mitochondria as well as dysregulation of mtDNA metabolism in the aging process. It is also crucial to unravel the changes in the signaling pathways between the nucleus and mitochondria in aging tissue cells in order to elucidate the molecular mechanism of aging and to better manage aging and age-related diseases in the human.

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