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REVIEW

Multiple Roles of Mitochondria in Aging Processes

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Summary

Aging is a multifactorial process influenced by genetic factors, nutrition, and lifestyle. According to mitochondrial theory of aging, mitochondrial dysfunction is widely considered a major contributor to age-related processes. Mitochondria are both the main source and targets of detrimental reactions initiated in association with age-dependent deterioration of the cellular functions. Reactions leading to increased reactive oxygen species generation, mtDNA mutations, and oxidation of mitochondrial proteins result in subsequent induction of apoptotic events, impaired oxidative phosphorylation capacity, mitochondrial dynamics, biogenesis and autophagy. This review summarizes the major changes of mitochondria related to aging, with emphasis on mitochondrial DNA mutations, the role of the reactive oxygen species, and structural and functional changes of mitochondria.

Key words

Mitochondria • Aging • mtDNA • Oxidative stress

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Introduction

Progressive aging of the elderly population is an increasingly important feature of societies in the developed countries (Olshansky 2013). In biology, aging is defined as an age-dependent or age-progressive decline in the intrinsic physiological function, leading to an increase in age-specific mortality rate and a decrease in age-specific reproductive rate (Anton *et al*. 2015, Basaria 2013, Fulop *et al.* 2010). Human aging starts after the third decade and is commonly associated with the accumulation of physical, psychological, and social changes leading to general decline in wellness, reduction in mobility (which is critical factor impacting the quality of life) and the onset of many age-related diseases including atherosclerosis, heart disease, hypertension, cancer, arthritis, cataract, Alzheimer's disease, and type 2 diabetes mellitus (Baumgartner *et al*. 1998, Liu 2014). In spite of intense research, the mechanisms that underlie age-dependent changes are still largely unknown (Barja 2013).

Mitochondrial dysfunction has long been considered a major contributor to aging and age-related diseases (for a review, see Chistiakov *et al*. 2014). With advanced age, mitochondrial dynamics, biogenesis, and oxidative phosphorylation capacity decrease, whereas mitochondrial DNA (mtDNA) damage, production of reactive oxygen species (ROS), induction of apoptotic events, and oxidation of numerous mitochondrial proteins leading to formation of protein-protein cross-linkages and protein fragmentation progressively increase (reviewed in Chistiakov *et al*. 2014, Gonzalez-Freire *et al*. 2015). Accumulation of ROS and oxidative damage have been linked to multiple pathologies, including neurodegenerative diseases, diabetes, cancer, and

premature aging. In 1956, Harman postulated free radical theory of aging stating that aging process depends on ROS production leading to oxidative damage and subsequent malfunction of nucleic acids, proteins and lipids (Harman 1956). This theory was later extended to mitochondrial theory of aging suggesting that both the main source and target of oxygen and nitrogen radicals produced in association with aging processes are mitochondria (Payne and Chinnery 2015, Ziegler *et al*. 2015). Dysfunction of mitochondrial electron-transporting system leads to increased production of ROS, which results in mtDNA damage followed by mutations causing compromised mitochondrial protein function and further increase in production of oxygen and nitrogen radicals. Although this theory was challenged by some experimental observations documenting that at least in some species, reduced oxidative damage may not invariably prolong life span, excessive production of ROS related to mitochondrial malfunction is still considered an important factor contributing to accelerated aging process (Avantaggiato *et al*. 2015, López-Lluch *et al.* 2015). In addition, mitochondrial health and aging may be affected by subject parameters like physical activity history (Barrientos *et al*. 1996, Gnaiger 2009, Lanza *et al.* 2008), age (Bua *et al.* 2006), caloric restriction (Schiff *et al*. 2011), drugs (Heller *et al*. 2012), and various comorbidities including obesity (Boudina and Graham 2014), sarcopenia (Figueiredo *et al.* 2008, Marzetti *et al*. 2010, Moore *et al*. 2010), hypertriglyceridemia, dyslipoproteinemia, insulin resistance, abnormal glucose tolerance, and hypertension (Bonomini *et al*. 2015).

In this review, we briefly revise the major changes of mitochondria related to aging, with emphasis on: i) structural and functional changes of mitochondria, ii) mitochondrial DNA mutations, and iii) the role of ROS.

Overview of mitochondria – structure and functions

About 1.5 billion years ago, mitochondria (from Greek *mitos* (thread-like) and *khondros* (grain or granule)) and eukaryotic cells established a symbiotic relationship. Mitochondria are subcellular, self-autonomous, highly dynamic and pleomorphic organelles surrounded by double-membrane system. They contain their own genetic system and sophisticated enzymatic machinery enabling many distinct metabolic processes (Wang *et al*. 2010). They are found in almost all human cells with exception of mature erythrocytes (Logan 2006, Zhang *et al*. 2012).

The primary function of mitochondria is to produce adenosine triphosphate (ATP) by the process of oxidative phosphorylation through the respiratory system (they synthesize more than 95 % ATP for cellular utilization). General view of the mitochondrial respiratory system is shown in Figure 1A. This system is composed of four respiratory complexes (I-IV) localized in the inner mitochondrial membrane, two mobile carriers (coenzyme Q and cytochrome *c*), and ATP synthase. On complexes I and II, reduced coenzymes, nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) are oxidized, respectively. Reduced coenzymes are generated by various dehydrogenases particularly in tricarboxylic acid cycle. The electrons released by the oxidation of reduced coenzymes are transferred through the respiratory complexes to the molecular oxygen providing energy to pump protons across the inner mitochondrial membrane (complexes I, III and IV). Created electrochemical potential (proton-motive force) is then used by ATP synthase to form ATP from ADP and P_i (Benard *et al*. 2006, Zeviani and Di Donato 2004).

In addition to energy, mitochondria also generate ROS and represent the major source of their cellular production. Leakage of electrons, mainly from complexes I and III, leads to one-electron reduction of oxygen to form superoxide anion that is the precursor of most ROS and a mediator in oxidative chain reactions. Superoxide anion then undergoes dismutation catalyzed by superoxide dismutase thus producing longer-lived and membrane permeant hydrogen peroxide. This molecule can be fully or partially reduced to water or hydroxyl radical, respectively (Turrens 2003, Murphy 2009). Under normal conditions, ROS are maintained at physiological levels by several endogenous systems of antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidases and glutathione reductase (Dai *et al*. 2014). Schematic view of ROS production is shown in Figure 2.

Besides this, mitochondria also play an essential role in amino acid and lipid metabolism, calcium homeostasis, regulation of apoptosis, cell cycle regulation, and thermogenesis (Friedman and Nunnari 2014).

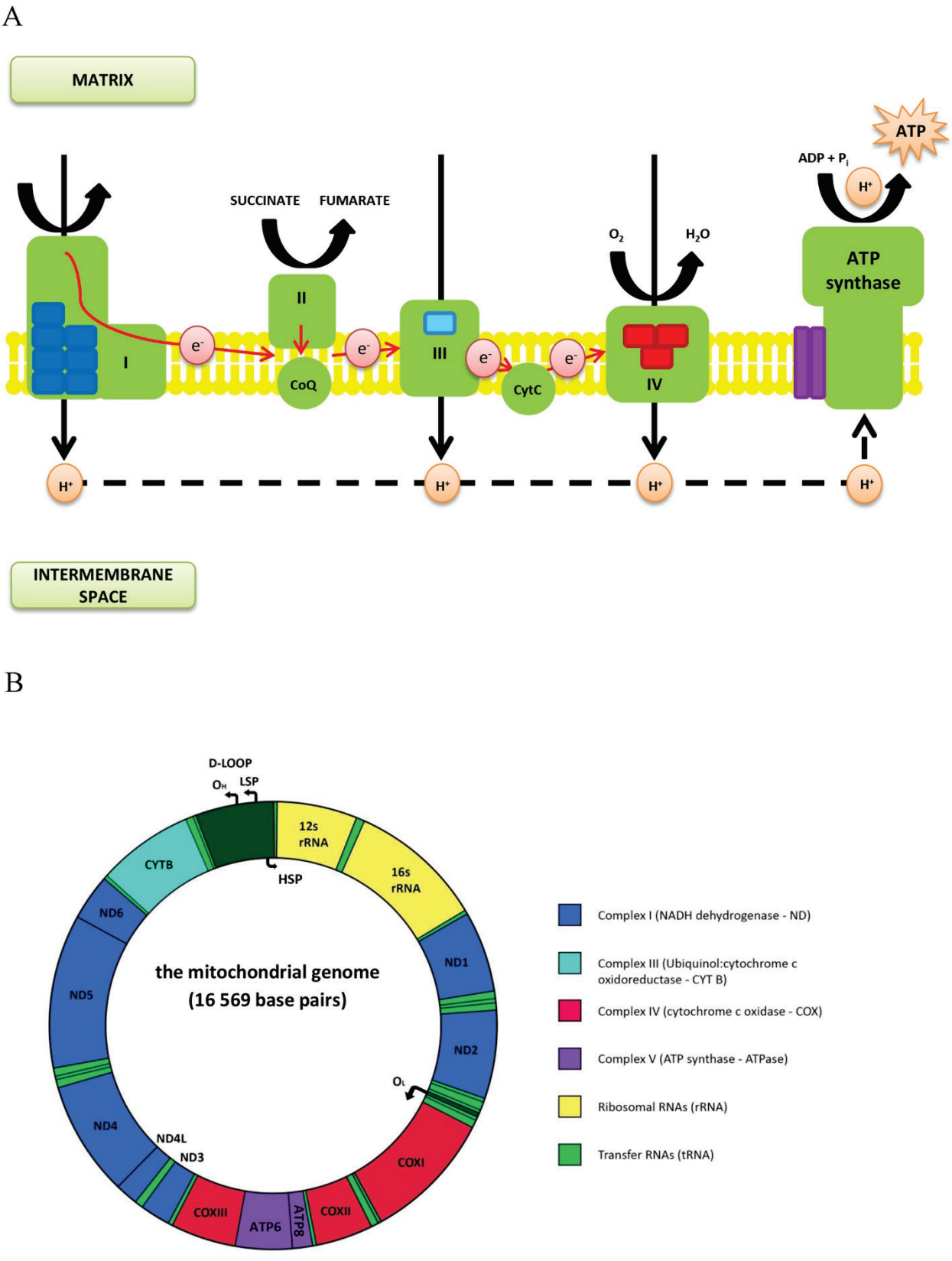

Fig. 1. General view of the mitochondrial respiratory system and genome. **A**. The respiratory system consists of four complexes (I – NADH dehydrogenase, II – succinate dehydrogenase, III – cytochrome *c* reductase and VI – cytochrome *c* oxidase) and ATP synthase. Subunits of complexes encoded by mtDNA are shown in same colors as in part B. CoQ – coenzyme Q, CytC – cytochrome *c*, e^- – electron. **B**. mtDNA is schematized together with the encoded genes. The D-loop contains the promoters for transcription of the H and L strand (HSP, LSP) as well as the origin of replication of the leading strand of mtDNA (O_H). The color indicates to which part of the respiratory system the subunit-encoding gene belongs to.

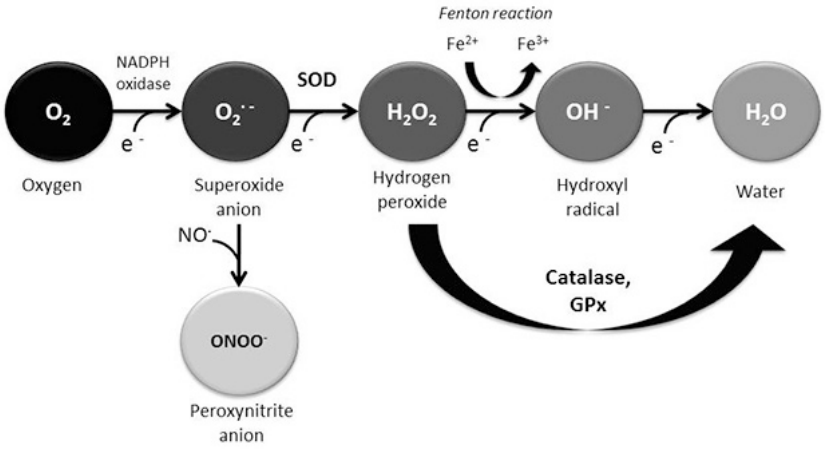

Fig. 2. Schematic representation of ROS production. In the course of metabolic processes are generated reactive oxygen and nitrogen species and this scheme shows the process of their transformation. e^- – electron, O_2 – oxygen, $O_2^{\bullet-}$ – superoxide anion, H_2O_2 – hydrogen peroxide, OH^- – hydroxyl radical, H_2O – water, NO – nitric oxide, $ONOO^-$ – peroxynitrite anion, SOD – superoxide dismutase, GPx – glutathione peroxidase.

Structural and functional changes of mitochondria associated with aging

Mitochondria typically form a reticular network radiating from the nucleus. This network is regulated through the complex coordination of fission, fusion and distribution events and is essential to maintain normal mitochondrial functions (Palmer *et al*. 2011, Roy *et al.* 2015). Fusion processes are associated with the optimization of mitochondrial function by mixing of intramitochondrial components to dilute any damaged mtDNA and proteins, whereas fission events are associated with the removal of damaged mitochondria by selective mitochondrial autophagy (Papáčková and Cahová 2014). Once this balance is lost, various mitochondrial and cellular functions are changed as well (Terman *et al.* 2010).

A number of studies have demonstrated that aging is accompanied by a decrease in mitochondrial dynamics that leads to compromised function and formation of morphological alterations. In the skeletal muscle and brain, aging is associated with loss of mitochondria that are abnormally enlarged and more rounded in shape (Palomera-Avalos *et al.* 2016, Crane *et al.* 2010, Shigenaga *et al.* 1994, Terman *et al*. 2010). Beregi *et al.* (1988) showed that in the mitochondria of human and mouse skeletal muscle, in addition to abnormal size, inner membrane cristae were irregularly spaced, disrupted and replaced by lamellar, myelin-like structures with advancing age. On the other hand, Poggi *et al.* (1987) reported a decrease in mitochondrial size and mitochondrial percentage per fibre area when analyzed human *m. vastus lateralis* (Poggi *et al*. 1987). Changes in mitochondrial volume, shape or length seem to be a general feature of the aging process as they have been described not only in muscle or neuron, but also in the liver, bone, fibroblasts and mesenchymal stem cells derived from adipose tissue (Allen *et al*. 2015, Mahmoud and Hegazy 2016, Marycz *et al*. 2016, Shum *et al*. 2016, Yen *et al*. 1989).

Besides morphological changes, significant functional alterations were demonstrated in mitochondria during aging. An age-dependent mitochondrial dysfunction may comprise decreased electron transfer rates, increased H^+ permeability of the inner membrane, and impairment of the H^+-driven ATP synthesis (Navarro and Boveris 2007, Porter *et al*. 2015). In humans, the maximum ATP production rate in the skeletal muscle appears to decline by 8 % per decade due to a combination of reduced mitochondrial content and compromised function of the existing mitochondrial population (Short *et al*. 2005). Similarly, Conley *et al.* (2000) reported nearly 50 % reduction in oxidative capacity of the *vastus lateralis* muscle in humans aged 65-80 compared to subjects ranging in age from 25 to 48 years (Conley *et al*. 2000). Also measurements of mitochondrial oxidative/phosphorylation activity based on $^{13}C/^{31}P$ nuclear magnetic resonance spectroscopy found approximately 40 % reduction in mitochondrial oxidative and phosphorylation activity in elderly compared to young people (Petersen *et al*. 2003). Detailed analysis showed that activities of complexes I and IV were reduced, whereas activities of complexes II, III and V were mostly unaffected (Benzi *et al*. 1992, Boffoli *et al*. 1994, Chabi *et al*. 2005, Crane *et al*. 2010, Fattoretti *et al*. 2001, Kerner *et al.* 2001, Kumaran *et al*. 2005, Larsson 2010, Pastoris *et al*. 2000, Tonkonogi *et al*. 2003). The likely reason is that the complexes I and IV are largely encoded by mtDNA that is more vulnerable and more susceptible to damage and mutation (Benzi *et al*. 1992, Boffoli *et al*. 1994, Chabi *et al*. 2005, Cooper *et al*. 1992, Crane *et al*. 2010, Kumaran *et al*. 2005, Larsson

2010, Tonkonogi *et al*. 2003). The activities of citric acid cycle enzymes (isocitrate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase) were also found to be decreased in skeletal muscle of aged rats (Kumaran *et al*. 2005). In contrast, preserved muscle oxidative capacity was rarely reported in the human and mouse skeletal muscle (Kumaran *et al*. 2005, Larsson 2010).

Reduced capacity of the respiratory chain was reported not only in the cardiac and skeletal muscle, but also in the liver, brain, kidney, platelets and lung (Acuña-Castroviejo *et al*. 2012, Choksi *et al.* 2011, Ojaimi *et al.* 1999, Shi *et al*. 2010, Short *et al*. 2005). Ojaimi *et al.* (1999) documented an age-related fall in cytochrome *c* oxidase (complex IV) activity in the frontal cortex, superior temporal cortex, cerebellum and putamen (Ojaimi *et al*. 1999). Yen *et al*. (1989) analyzed State 3 and State 4 respiration rates, respiratory control ratio, and ADP/O ratio in human liver; their results showed decreasing respiratory control and ADP/O ratios with the advancing age (Yen *et al*. 1989). Lenaz (1998) focused on the function of mitochondria in human platelets during aging and suggested the titre for half-inhibition of complex I by rotenone as a suitable biomarker of aging and age-related diseases (Lenaz 1998).

Some of the morphological and functional changes described above might be secondary as mitochondrial biogenesis and energy production are controlled at many different levels, e.g. by hormones. Receptors for glucocorticoids, oestrogens, androgens, and thyroid hormones have been detected in mitochondria of various cell types. Age-related decrease in the levels of hormones affecting directly or indirectly mitochondria may at least partly contribute to the overall decline in mitochondrial dynamics (Chen *et al*. 2009, Knuever *et al*. 2012, Psarra and Sekeris 2008, Scheller and Sekeris 2003, Weitzel *et al*. 2003). E.g. Knuever *et al.* (2012) identified thyrotropin-releasing hormone (TRH) as a potent novel neuroendocrine stimulator of mitochondrial activity and biogenesis in human: TRH significantly enhanced mitochondrial complex I and IV activities thereby increasing the oxygen consumption (Knuever *et al*. 2012).

Mitochondrial DNA mutations in aging

Typical metazoan mitochondrial genome (mtDNA) is a closed double-stranded circular molecule that varies in size among different species ranging from 11.4 kb to 32.1 kb (Gissi *et al.* 2008). Structure and gene organization of mtDNA is highly conserved among mammals.

Human mitochondrial DNA spanning 16 569 base pairs (Anderson *et al*. 1981) contains 37 genes coding for 2 rRNAs, 22 tRNAs and 13 polypeptides (that all are components of the oxidative phosphorylation system, Castellana *et al*. 2011). Individual strands of mtDNA differ in the base composition: the heavy strand is rich in guanines and encodes majority of mitochondrial genes (12 polypeptides, 2 rRNAs and 14 tRNAs), the light strand is rich in cytosines and carries genes for 1 polypeptide and 8 tRNAs (Clayton 2000). General view of the mitochondrial DNA structure is shown in Figure 1B. Because of the small size of mtDNA, there are only small portions of non-coding sequences like D-loop (playing crucial role in replication and transcription); genes are lacking introns and may overlap (Taanman 1999). Recently, novel non-coding transcripts originating from mitochondrial genome were discovered, but their function is yet to be elucidated (Rackham *et al*. 2011, Ro *et al*. 2013).

Mammalian cells, depending on their metabolic and functional activity, can contain hundreds to ten thousands of mtDNA molecules, with multiple copies per one mitochondria (Chinnery and Hudson 2013). Replication of mtDNA is independent of the cell replication and is performed by the DNA polymerase gamma (PolG), encoded by the nuclear gene POLG. PolG is a heterotrimeric complex of one catalytic and two accessory subunits (Kaguni 2004), which, in cooperation with DNA helicase Twinkle and single strand binding protein mtSSB, is sufficient to replicate mtDNA even *in vitro* (Korhonen *et al*. 2004).

MtDNA is organized, similarly to bacterial genomes, into nucleoids containing one to several copies of mtDNA molecules associated with DNA-binding proteins like mitochondrial transcription factor TFAM and others (Bogenhagen 2012). Association with proteins increases the protection of mtDNA against harmful agents, particularly ROS and reactive nitrogen molecules produced by mitochondrial metabolism (Gaziev *et al.* 2014), similarly to the protection of nuclear DNA by histone proteins.

Mitochondrial function is largely dependent on the supply of proteins coded by nuclear genome. Around 1500 proteins are estimated to be associated with mitochondria, depending on organism and tissue (Lotz *et al*. 2014). All proteins coded by mtDNA form crucial

components for formation of electron transport complexes; in mtDNA, 7 subunits of complex I, 1 subunit of complex III, 3 subunits of complex IV and 2 subunits of complex V are encoded (Schon *et al*. 2012).

Changes in the mitochondrial genome in the form of point mutations as well as larger genome rearrangements like deletions arise over the lifespan of the organism. Important aspect influencing genesis of mtDNA mutations is the multicopy nature of mtDNA. Usually, only a portion of mtDNA molecules is mutated, creating the mtDNA heteroplasmy that can refer to both germinal and somatic mutations (Wallace and Chalkia 2013). Pathological manifestation of the mutation depends on so-called threshold effect – situation, where the accumulation of dysfunctional product reaches certain level and the mitochondria are further unable to perform essential functions (Rossignol *et al*. 2003).

Mutations in the mtDNA are accumulated over the lifespan of the organism faster than in the case of nuclear genome (Gaziev *et al*. 2014, Haag-Liautard *et al.* 2008). Factors contributing to the increased incidence of mtDNA mutations include: i) the lack of histones that are believed to reduce DNA damage in the nucleus; ii) chemical modification of mtDNA caused by intrinsic alkylation agents, ROS, reactive nitrogen species, some hormones and extrinsic toxic substances like ethanol, chemotherapeutic drugs and many others; iii) close proximity of mtDNA to the site of ROS production, i.e. the system of mitochondrial respiratory complexes; iv) number of available DNA repair systems that are much more limited in mitochondria than in the nucleus; v) deficiencies in 5'-3' proofreading capacity of PolG during the mtDNA replication that seem to be the major cause of mtDNA mutations (Alexeyev *et al.* 2013). This fact is supported by the observation that the mice strain mutated in PolG shows mutator phenotype with the rapid accumulation of mtDNA mutations and shortened lifespan (Kujoth 2005) and that in the older age, mutations caused by PolG mistakes rather than those induced by ROS are present (Kennedy *et al*. 2013).

Investigation of the direct effect of particular mtDNA mutation on aging is very difficult. Even in the old individuals, the total combined rate of mutations is very low, not reaching the estimated threshold for the manifestation of the mutation (Pinto and Moraes 2015). Most commonly studied tissues with respect to mtDNA mutagenesis are brain, cardiac and skeletal muscle or colonic crypt stem cells, where different mutations are formed and fixed. Even if the total level of mutated mtDNA is low to affect the whole tissue, mutations can form "weak spots" that are able to paralyze the tissue function (Khrapko and Turnbull 2014).

Reactive oxygen species and aging

There are many sources of intracellular ROS in mammals, such as NADPH oxidases, xanthine oxidase, and monoamine oxidase; however, the mitochondrial electron transport system has been recognized as their major cellular generator (almost 90 % of the total ROS produced in the cell) (Bratic and Trifunovic 2010, Dai *et al.* 2014, Liu *et al*. 2002). ROS are highly reactive molecules having potential to cause oxidative deterioration of proteins, lipids and DNA. ROS generate a variety of DNA lesions, such as oxidized DNA bases, abasic sites, oxidative DNA adducts, and DNA strand breaks (Cui *et al*. 2012).

In many studies dealing with aging processes, ROS have been suggested to be major mediators of age-associated cellular damage in addition to be likely involved in the pathogenesis of some age-related neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Bowling and Beal 1995, Good *et al.* 1996, Liu *et al*. 2002). Many theories of aging based on putative detrimental effects of ROS have been proposed, including the free radical and mitochondrial theories of aging (Chistiakov *et al*. 2014, Indo *et al*. 2015, Mikhed *et al*. 2015, Valerio and Nisoli 2015, Yan 2014). These theories speculate that cumulative damage to mitochondria caused by reactive radicals is a primary driving force of aging and major determinant of the lifespan. According to these hypotheses, mtDNA mutations caused by ROS alter the expression of oxidative phosphorylation complexes, which leads to limited mitochondrial respiratory enzymes activities, decrease in mitochondrial membrane potential and compromised production of ATP. Defective transport of electrons and their leak prior to the reduction of oxygen to water at cytochrome *c* oxidase causes the production of superoxide. Two major sites for ROS generation are believed to be complexes I and III. Complex I is probably the most affected by ROS because seven of its subunits are encoded by mtDNA. Complex I activity is significantly affected by aging in rat brain and liver mitochondria as well as in human platelets (Genova *et al*. 2004). In turn, increased production of superoxide and related radicals leads to further oxidation stress-related changes in mitochondrial structure (accumulating

vacuoles, cristae abnormalities), distribution, and dynamics that can result in critical mitochondrial dysfunction with ROS-accelerated ROS generation and even faster (Bratic and Trifunovic 2010).

Hypothesis of contribution of ROS to the processes of aging has been supported by genetic experiments. One of the master regulators of ROS production and oxidative stress is the isoform of the growth factor adapter (p66shc). The $p66^{shc}$ knockout mice, one of the best characterized genetic models of longevity, showed a considerable increase in the life span (Migliaccio *et al.* 1999). Also caloric restriction, through the modulation of IGF-1, TOR, sirtuins, and AMP kinase signaling pathways, has a beneficial impact on mitochondrial function and causes a significant reduction of mitochondrial ROS production. Downregulation of IGF-1 pathway is followed by the activation of FoxO transcription factors, which induces the transcription of antioxidant genes such as genes for antioxidant enzymes (Enns *et al.* 2008, Treuting *et al.* 2002, Vendelbo and Nair 2011).

Despite a large body of evidence supporting the role of ROS in aging, the free radical and mitochondrial theories of aging face some challenges as several experimental rodent and mice models of antioxidant manipulation have failed to affect the lifespan. Interestingly, a paradoxical increase in longevity was observed in mitochondrial respiration mutants of *Caenorhabditis elegans* at elevated levels of ROS (Cui *et al*. 2012, Yang and Hekimi 2010). Some studies focusing on experiments with long-lived rodents did not find any convincing correlation between the level of oxidative damage and aging (Panieri *et al*. 2013, Sena and Chandel 2012, Wu and Bratton 2013). In addition, although overexpression of mitochondrial Mn-superoxide dismutase (Mn-SOD) in *Drosophila melanogaster* extended the lifespan, murine overexpression of SOD1 did not affect it (Andziak *et al*. 2006, Cui *et al*. 2012, Dai *et al*. 2014, Huang *et al*. 2000). Manipulation with murine catalase targeted to mitochondria showed increased longevity while mice lacking Mn-SOD exhibited a 30 % decrease in life expectancy and died from premature death associated with severe mitochondrial dysfunction and neurodegeneration (Cui *et al*. 2012, Lebovitz *et al.* 1996, Schriner *et al*. 2005, Sun *et al*. 2002).

Besides their detrimental roles, ROS seem to be more important as signaling molecules in regulation of the cell cycle progression, apoptosis, necrosis, growth arrest or senescence (Panieri *et al*. 2013, Sena and Chandel 2012, Wu and Bratton 2013). They were shown to activate transcription factor – hypoxia-induced factor-1 (HIF-1), which is associated with prolonged lifespan or to activate some tyrosine kinases, mitogen activated protein kinases or RAS proteins (Bratic and Larsson 2013, Cui *et al*. 2012). Based on these new findings, the gradual ROS response hypothesis of aging has been postulated (Hekimi *et al*. 2011), stating that ROS-induced stress response is essential for the maintenance of tissue homeostasis. In aging process, more stress is generated, leading to the increased production of ROS. When the certain threshold of ROS is reached, they start to add additional damage to the tissue, potentiating the tissue dysfunction and aging.

Taken together, more investigations are required to clarify the precise role that free radicals play in aging.

Conclusions

The role of mitochondria in the aging processes seems to be ambiguous – they initiate multiple processes leading to age-associated deterioration of the cellular functions and become targets of reactions leading to increased ROS generation, mtDNA mutations, oxidation of mitochondrial proteins with subsequent induction of apoptotic events, impaired oxidative phosphorylation capacity, mitochondrial dynamics, biogenesis and autophagy (Fig. 3). Studies using transgenic or knockout animals revealed many mitochondria-related factors potentially affecting longevity – uncoupling proteins, sirtuins, antioxidant enzymes, FOXO group of transcription factors, AMP-activated protein kinase etc. (Jang and Van Remmen 2009, Vendelbo and Nair 2011). Recently, various mouse models associated with severe mitochondrial dysfunction and premature aging have been reviewed (Grimm *et al.* 2016). In addition, genes having potential pro-longevity effects have been screened in *Caenorhabditis elegans* after mild mitochondrial stress (Maglioni *et al.* 2016). More work is needed to clarify their precise role in the process of natural aging and putative beneficial effects of their activators/inhibitors.

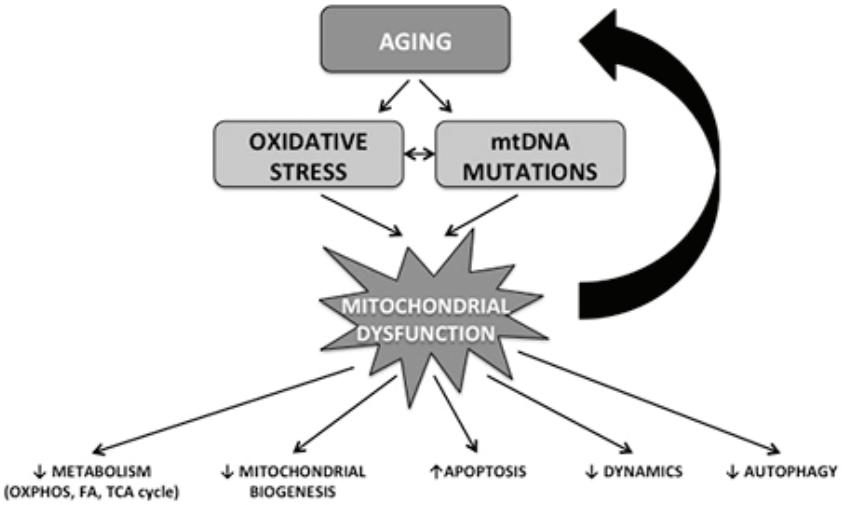

Fig. 3. Schematic view on effects of aging on mitochondrial dysfunction. OXPHOS – oxidative phosphorylation, FA – fatty acids, TCA cycle – tricarboxylic acid cycle.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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