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Mitochondrial biogenesis and mitochondrial DNA maintenance of mammalian cells under oxidative stress

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Abstract

Mitochondrial biogenesis and mitochondrial DNA (mtDNA) maintenance depend on coordinated expression of genes in the nucleus and mitochondria. A variety of intracellular and extracellular signals transmitted by hormones and second messengers have to be integrated to provide mammalian cells with a suitable abundance of mitochondria and mtDNA to meet their energy demand. It has been proposed that reactive oxygen species (ROS) and free radicals generated from respiratory chain are involved in the signaling from mitochondria to the nucleus. Increased oxidative stress may contribute to alterations in the abundance of mitochondria as well as the copy number and integrity of mtDNA in human cells in pathological conditions and in aging process. Within a certain level, ROS may induce stress responses by altering expression of specific nuclear genes to uphold the energy metabolism to rescue the cell. Once beyond the threshold, ROS may cause oxidative damage to mtDNA and other components of the affected cells and to elicit apoptosis by induction of mitochondrial membrane permeability transition and release of pro-apoptotic proteins such as cytochrome *c*. On the basis of recent findings gathered from this and other laboratories, we review the alterations in the abundance of mitochondria and mtDNA copy number of mammalian cells in response to oxidative stress and the signaling pathways that are involved.
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Keywords: Mitochondria; Biogenesis; Mitochondrial DNA; Oxidative stress; Somatic mutation; Copy number

Abbreviations: AP, apurine/apyrimidine; BER, base excision repair; CAMK, Ca^{2+}/calmodulin-dependent protein kinase; CREB, cyclic AMP response element-binding protein; CuZnSOD, copper/zinc superoxide dismutase; COX, cytochrome *c* oxidase; eNOS, endothelial nitric oxide synthase; GPx, glutathione peroxidase; hOGG1, human 8-oxoguanine DNA glycosylase; JNK, *c*-Jun N-terminal kinase; LPS, lipopolysaccharides; mtDNA, mitochondrial DNA; mtSSB, mitochondrial single-strand DNA binding protein; mtTFA, mitochondrial transcription factor A; MnSOD, manganese superoxide dismutase; nDNA, nuclear DNA; MPT, mitochondrial permeability transition; NER, nucleotide excision repair; NO, nitric oxide; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; PGC-1, peroxisome proliferators-activated receptor γ coactivator-1; PI3K, phosphatidylinositol 3′-kinase; PKC, protein kinase C; POLG, DNA polymerase γ; ROS, reactive oxygen species; TCA, tricarboxylic acid

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1. Introduction

Mitochondria are the intracellular organelles responsible for biological oxidation in mammalian cells. They contain double membranes and several hundreds of proteins and 2–10 copies of mitochondrial DNA (mtDNA) in the matrix enclosed by mitochondrial inner membrane. Although mitochondria have their own genome, most of the proteins and enzymes that reside in mitochondrial membranes are nuclear gene products. Their principal function is to synthesize ATP through electron transport and oxidative phosphorylation (OXPHOS) in conjunction with the oxidation of metabolites by tricarboxylic acid (TCA) cycle and catabolism of fatty acids by β-oxidation. Part of the reactions of the biosynthesis of pyrimidines and hemes as well as the transcription, translation, and replication of mtDNA are also carried out in mitochondria. In addition to the production of energy, mitochondria are also the main intracellular source and immediate target of reactive oxygen species (ROS), which are continually generated as byproducts of aerobic metabolism in mammalian cells. The other important function of mitochondria is to act as an arbitrator in the initiation and execution of apoptosis. Mitochondria, thus, play a pivotal role in the determination of life and death of the mammalian cell (Lee & Wei, 2000).

Each mammalian cell contains several hundreds to more than a thousand mitochondria. The size, shape, and abundance of mitochondria vary dramatically in different cell types and may change under different energy demand and different physiological or environmental conditions. The abundance of mitochondria in a cell is determined by the biogenesis and division of the organelles (Attardi & Schatz, 1988). The abundance of mitochondria per cell is tightly controlled by the activation of specific transcription factors and signaling pathways (Attardi & Schatz, 1988; Moyes & Hood, 2003).

In this article, we review the main features of mitochondrial biogenesis, mtDNA maintenance, and the nuclear genes controlling these processes. Alterations in these processes in response to oxidative stress and the signaling pathways involved are also discussed.

2. Mitochondrial biogenesis and mtDNA maintenance

Mitochondria import most of their phospholipids from the cytoplasm to form and maintain their membranes (Moyes & Hood, 2003). Cardiolipin is an acidic and hydrophobic phospholipid required for the function of many mitochondrial proteins such as cytochrome *c* oxidase. The inner membranes of mitochondria are involved in the biosynthesis of cardiolipin and are rich in this phospholipid. The amount of cardiolipin in mitochondrial inner membrane is changed in response to the level of thyroid hormones, chronic contractile activity of muscle, and aging in the human (Paradies & Ruggiero, 1990; Takahashi & Hood, 1993; Paradies, Petrosillo, & Ruggiero, 1997).

Biosynthesis of mitochondrial proteins requires contributions from mitochondria and the nucleus, but most of them are encoded by nuclear genes and synthesized outside of the mitochondria. The proteins are imported into mitochondria by complex multiple mechanisms. On the other hand, 13 polypeptides including seven subunits of NADH dehydrogenase (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6), three subunits of cytochrome *c* oxidase (COI, COII, and COIII), two subunits of F_0F_1 ATPase (ATPase 6 and ATPase 8), and cytochrome *b* are encoded by mtDNA and synthesized in the organelle (Attardi & Schatz, 1988). Mammalian mtDNA also codes for two rRNAs and a set of 22 tRNAs that are essential for protein synthesis in mitochondria. The assembly and functioning of the respiratory enzyme complexes in mammalian cells require coordinated expression and interaction between gene products of the mitochondrial and nuclear genomes (Poyton & McEwen, 1996). The gene expressions in mitochondria and the nucleus responds in a complex manner to a variety of physiological and developmental signals including growth activation (Luciakova, Li, & Nelson, 1992), neoplastic transformation (Shmookler & Goldstein, 1983; Torroni, Stepien, Hodge, & Wallace, 1990), muscle contraction (Williams, Salmons, Newsholme, Kaufman, & Mellor, 1986), cell differentiation, and hormone action (Wiesner, Kurowski, & Zak, 1992).

In mammalian cells, each mitochondrion harbors 2–10 copies of mtDNA, which is a circular double-strand DNA molecule (Robin & Wong, 1988). The replication of mtDNA occurs predominantly in the

late S and G_2 phases of the cell cycle, but may occur throughout the cell cycle (Bogenhagen & Clayton, 1977). In addition, it has been shown that the mtDNA replication does not occur concurrently with the growth and division of the organelles (Shadel & Clayton, 1997). Thus, mtDNA replication may not be coupled with mitochondrial proliferation. In the human, the copy number of mtDNA varies with the cell type of the tissues and is usually maintained within a range (Moraes, 2001). Under normal physiological conditions, mtDNA molecules double in every cell cycle, which is required if each daughter cell is to maintain a constant amount of mtDNA. When physiological conditions are changed, mtDNA copy number can be modulated according to the energy need of the cell. It has been shown that the copy number of mtDNA in tissue cells are changed during cell growth and differentiation, and after hormone treatment and exercise (Williams et al., 1986; Renis, Cantatore, Loguercio Polosa, Fracasso, Gadaleta, 1989; Shay, Pierce, & Werbin, 1990; Wiesner et al., 1992). However, it remains unclear as to how the copy number of mtDNA and the abundance of mitochondria are regulated under different physiological and developmental conditions.

3. Nuclear genes controlling mitochondrial biogenesis and mtDNA maintenance

Controlling the biogenesis of mitochondria and the maintenance of mtDNA is a complex biological process. Nuclear genes encode all the proteins and enzymes that are involved in mtDNA replication, transcription, and translation in mitochondria (Shadel & Clayton, 1997). In the past decade, it has been demonstrated that a number of protein factors encoded by nuclear genes are involved in the biogenesis of mitochondria and maintenance of mtDNA copy number (Scarpulla, 1997).

Nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) are transcriptional regulators that act on the nuclear genes coding for constituent subunits of the OXPHOS system. In addition, they also regulate the expression of many other genes involved in mtDNA replication via binding to the consensus sequences in the promoters of the OXPHOS genes in the nucleus (Evans & Scarpulla, 1990; Virbasius, Virbasius, & Scarpulla, 1993a; Virbasius, Virbasius, & Scarpulla, 1993b). Mitochondrial transcription factor A (mtTFA) is a transcription factor that acts on the promoters within the D-loop region of mtDNA and regulates the replication and transcription of the mitochondrial genome (Virbasius & Scarpulla, 1994). It has been established that the mtTFA gene contains consensus-binding sites for both NRF-1 and NRF-2, which provide a unique mechanism for the cell to integrate the expression of nuclear DNA-encoded proteins with the transcription of genes encoded by mtDNA (Virbasius & Scarpulla, 1994).

There is growing evidence to suggest that peroxisome proliferators-activated receptor gamma coactivator-1α (PGC-1α) is a major regulator of mitochondrial biogenesis (Puigserver & Spiegelman, 2003). In conjunction with a network of transcription factors, PGC-1α helps to coordinate the expression of genes involved in aerobic metabolism. Many nuclear genes coding for mitochondrial enzymes are responsive to PGC-1α, which stimulates the expression of other transcription factors involved in the coordinated expression of mitochondrial genes, such as NRF-1 and NRF-2, which trigger the expression of nuclear genes coding for polypeptides of the respiratory chain and proteins involved in transcription and replication of mtDNA (Wu et al., 1999). A novel PGC-1-related coactivator, PGC-1β, has recently been identified to bear biochemical properties similar to those of PGC-1α (Andersson & Scarpulla, 2001; Lin, Puigserver, Donovan, Tarr, & Spiegelman, 2002a). It has been shown that an increase in the mRNA and the protein levels of PGC-1α accompanies mitochondrial proliferation during adaptive thermogenesis (Lowell & Spiegelman, 2000) and muscle regeneration (Duguez, Feasson, Denis, & Freyssenet, 2002; Lin et al., 2002b). Overexpression of PGC-1α leads to mitochondrial proliferation in the heart (Lehman et al., 2000), adipocytes (Lowell & Spiegelman, 2000), and myoblasts (Wu et al., 1999) of the transgenic mice. These observations suggest that PGC-1α plays a key role in the control of mitochondrial biogenesis and mtDNA maintenance and possibly serves as a link between external stimuli and mitochondrial biogenesis.

The factors involved in the replication of mtDNA include the *cis*-regulatory element in the non-coding (D-loop) region of mtDNA and several nuclear DNA-encoded *trans*-acting factors. These *trans*-acting factors include mitochondrial DNA polymerase γ (POLG), mitochondrial RNA polymerase, mtTFA, en-

donuclease G, mtDNA single-stranded binding protein (mtSSB), and other potential factors that regulate the replication and transcription of mtDNA and the processing of mtRNA (Shadel & Clayton, 1997; Moraes, 2001). Since the initiation of mtDNA replication at the heavy strand origin (O_H) of the D-loop region requires transcription of a RNA primer, mtTFA and mitochondrial RNA polymerase are required for synthesis of the primer and for the maintenance of mtDNA (Shadel & Clayton, 1997). It was found that endonuclease G is involved in the processing of the RNA primer (Cote & Ruiz-Carrillo, 1993). In vertebrates, POLG is responsible for the biosynthesis of mtDNA (Shadel & Clayton, 1997). In addition, it has been shown that mtSSB is required for mtDNA replication, and that the expression of mtSSB is strictly regulated to control the copy number of mtDNA (Schultz et al., 1998). Helicases and topoisomerases are likely involved in mtDNA replication but the mechanisms of their action are still poorly understood.

Recently, it was shown that POLG is expressed in a stable form and at normal levels in cells lacking mtDNA, which suggests that POLG level is not responsive to the abundance of mtDNA in a cell (Davis, Ropp, Clayton, & Copeland, 1996). The amount of mtDNA varies in accordance with the amount of mtTFA in a cell (Poulton et al., 1994; Shadel & Clayton, 1997). However, it remains unclear whether the observed change in the copy number of mtDNA is the consequence of an alteration in the rate of replication of mtDNA, which is determined by the level of mtTFA in the cell.

4. Mitochondrial signals affecting nuclear respiratory genes

Mitochondria are the major source of ROS and calcium store of mammalian cells. An alteration in the redox state or calcium homeostasis induced by changes in mitochondrial function may be involved in the communication between mitochondria and the nucleus.

It has been well established that NADH dehydrogenase and the protonmotive Q cycle in the electron transport chain are the major sites that continually generate ROS such as superoxide anions and H_2O_2 (Boveris & Chance, 1973). It was reported that 1–5% of the oxygen consumed by mitochondria in tissue cells is converted to ROS under normal physiological condition (Chance, Sies, & Boveris, 1979). Moreover, production of superoxide anions by mitochondria is increased by defects in the respiratory chain, such as those seen in the affected tissues of patients with mitochondrial diseases (Wei & Lee, 2003) or aged individuals (Wei & Lee, 2002). It was observed that human cells respond to defective respiratory function by promoting expression of nuclear and mitochondrial genes through an H_2O_2-dependent signaling pathway (Suzuki, Kumagai, Goto, & Sugiura, 1998). It was also reported that the content of mtDNA in the HeLa S3 cells is increased about two-fold after treatment with 0.1 μM rotenone, which inhibits the activity of Complex I (Miyako, Kai, Irie, Takeshige, & Kang, 1997). A study using mtDNA-less ρ^o cells showed a concurrent increase of intracellular ROS and elevated expression of NRF-1 and mtTFA (Miranda, Foncea, Guerrero, & Leighton, 1999). Moreover, after treatment of human MRC-5 lung cells with sub-lethal concentrations of antimycin A, the elevation of intracellular ROS elicited an increase in the mitochondrial mass of the cells (Lee, Yin, Lu, Chi, & Wei, 2000). These results support the notion that ROS play an important role in the signaling from mitochondria to the nucleus (Poyton & McEwen, 1996).

On the other hand, it was observed that depletion of mtDNA below a certain threshold or treatment of mammalian cells with respiratory inhibitors increased steady-state levels of cytosolic Ca^{2+} (Biswas et al., 1999). This increase of cytosolic Ca^{2+} may alter activities of Ca^{2+}-dependent transcription factors. The genetic and metabolic stress of mitochondria causes activation of calcineurin, NFAT, ATF2, and NFκB/Rel factors, which collectively alter the expression of nuclear OXPHOS genes, including the gene-encoding subunit Vb (*COX Vb*) of cytochrome *c* oxidase (Biswas et al., 1999). Moreover, it was found that mitochondrial stress-induced activation of NFκB/Rel factors involves inactivation of IκBβ through calcineurin-mediated dephosphorylation (Biswas, Anandatheerthavarada, Zaidi, & Avadhani, 2003). These features of mitochondrial stress-induced NFκB/Rel activation, which is independent of IκBα and IκBβ kinases, affect gene targets that are different from those of cytokine- and TNFα-induced signaling. Thus, it was proposed that both mtDNA mutation/depletion and metabolic stress in mammalian cells are transmitted as a retrograde Ca^{2+} signal, which,

in turn, alters the expression of nuclear OXPHOS genes and mitochondrial biogenesis (Biswas et al., 1999).

5. Mitochondrial antioxidant and DNA repair systems in mtDNA maintenance

In the mammalian cell, mtDNA maintenance requires not only faithful mtDNA replication, but also repair and recombination of mtDNA (Shadel & Clayton, 1997). It has been proposed that a mutated sequence in the D-loop of mtDNA could affect its binding affinity to *trans*-acting factors encoded by nuclear genes (Shadel & Clayton, 1997). Therefore, the integrity of mtDNA may affect the maintenance of mtDNA in a cell. Human mtDNA is more susceptible to oxidative damage and consequently acquires mutations at a higher rate than does nuclear DNA (Richter, Park, & Ames, 1988; Ames, Shigenaga, & Hagen, 1993) due to exposure to high levels of ROS generated during respiration, lack of protective histones, and limited capacity for repair of DNA damage.

To cope with the ROS continually produced by aerobic metabolism, human cells have developed antioxidant enzymes including mitochondrial manganese superoxide dismutase (MnSOD), copper/zinc superoxide dismutase (Cu/ZnSOD), glutathione peroxidase (GPx), and catalase (Chance et al., 1979; Ames et al., 1993). A large number of other factors contribute to the defense against ROS, including Vitamins A, C, and E, and glutathione. Mitochondrial MnSOD and cytosolic Cu/ZnSOD convert superoxide anions to H_2O_2, which is then transformed to water by GPx or to water and oxygen by catalase. Although these enzymes together with other antioxidants can dispose of ROS and free radicals, a fraction may escape the defense mechanism and cause damage to cellular constituents including nucleic acids, proteins, and lipids. An excess of ROS is harmful to cells, and thus, any signal or stimulus that leads to over-production of ROS may cause a redox catastrophe culminating in cell death.

Damage to DNA by oxidative stress comprises oxidative damage to bases and sugar phosphates as well as single- or double-strand breaks in DNA. The adjacent single-strand breaks in the opposite strands may be converted to double-strand breaks upon replication. Unless the damages are repaired or removed, both single- and double-strand breaks in mtDNA will result in poor quality of the DNA template for its replication (Souza-Pinto, Croteau, Hudson, Hansford, & Bohr, 1999). Accumulated base damages in DNA may be mutagenic and/or inhibit the replication of mtDNA.

The base excision repair (BER) is an important mechanism for the removal of oxidative DNA damage. Recent studies revealed that mitochondria contain the BER system such as uracil- or 8-OHdG DNA glycosylase (Grollman & Moriya, 1993; Takao, Zhang, Yonei, & Yasui, 1999), apurinic/apyrimidinic (AP) endonucleases (Tomkinson, Bonk, & Linn, 1988; Croteau et al., 1997; Souza-Pinto et al., 1999), and 8-OHdGTPase (Kang et al., 1995). It was reported that the level of 8-oxo-dGTPase in heart mitochondria was increased in mice with myocardial infarction, which suggests that the enzyme bears important function in the protection against oxidative damage to mtDNA (Tsutsui et al., 2001). Moreover, it has been shown that the recombinational DNA repair system exists in mammalian mitochondria (LeDoux et al., 1992; Thyagarajan, Padua, & Campbell, 1996). However, pyrimidine dimers induced by UV radiation cannot be repaired in mitochondria, because the nucleotide excision repair (NER) system exists only in the nucleus and is absent in mitochondria (Clayton, Doda, & Friedberg, 1974; Sancer, 1996).

6. Mitochondrial biogenesis and mtDNA maintenance under oxidative stress

Alteration in intracellular level of ROS is associated with changes in mitochondrial abundance, mtDNA copy number, and the expression of respiratory genes. It is observed that increase in the mtDNA copy number per cell is associated with elevated oxidative stress in the aging tissues, including the tissues of the old rat (Gadaleta et al., 1992), and the brain (Barrientos et al., 1997a), lung (Lee, Lu, Fahn, & Wei, 1998), and skeletal muscle (Barrientos et al., 1997b; Pesce et al., 2001) of aged individuals. These findings are consistent with the observations that the mtDNA copy number is increased at the late passage of diploid human cells (Shmookler & Goldstein, 1983). The increase of mtDNA copy number in aging tissue cells is suggested as a result of the feedback response that compensates for defective mitochondria bearing impaired respiratory chain or mutated mtDNA (Lee & Wei, 2000).

Moreover, the abundance of mitochondria in human lung fibroblast cell line MRC-5 was found to increase with replicative senescence, which occurred concurrently with higher intracellular levels of ROS (Lee, Yin, Chi, & Wei, 2002). Therefore, increase in oxidative stress plays a critical role in the increase of mitochondrial abundance and mtDNA content in tissue cells during the aging process.

In addition, oxidative stress contributes to accumulation of somatic mutation and oxidative damage to mtDNA in human and animal tissues in aging (Wei & Lee, 2002). Moreover, ROS and free radicals generated by the environmental insults (e.g., UV radiation, cigarette smoke, and air pollutants) and xenobiotics (e.g., drugs and betal quid), and alcohol drinking may induce the accumulation of mtDNA mutations in the human tissues during the aging process (Yang, Lee, & Wei, 1995; Mansouri et al., 1997; Fahn et al., 1998; Lee et al., 2001). It was recently demonstrated that the D-loop region was highly susceptible to attack by electrophilic compounds and inflicted with more oxidative damage when compared with the other regions of mtDNA (Mambo et al., 2003). Somatic mutations in the D-loop region are associated with a decrease in mtDNA copy number in hepatocellular carcinoma (Lee et al., 2004). Thus, persistent oxidative stress in mitochondria is not only a contributory factor to the somatic mtDNA mutations but also alters the rate of mtDNA replication, and thereby leads to a decline in mitochondrial respiratory function.

It was recently demonstrated that non-lethal concentration of H_2O_2 and buthionine sulphoximine, which depletes intracellular glutathione, induce an increase in the mitochondrial mass and the mtDNA copy number of human cells (Lee et al., 2000). The mRNA levels of the nuclear genes involved in mitochondrial biogenesis, especially PGC-1 and NRF-1, were increased upon H_2O_2 treatment and replicative senescence of human cells (Lee et al., 2002). Moreover, the expressions of mtTFA and NRF-1, and their DNA-binding activities were increased in human cells with impaired respiratory function (Suzuki et al., 1998) and in the skeletal muscle of elderly subjects (Lezza et al., 2001). Oxidative stress induced by injection of lipopolysaccharides (LPS) increased NRF-1 activity and mtTFA gene expression and stimulated mtDNA replication and cell proliferation in rat liver (Suliman, Carraway, Welty-Wolf, Whorton, & Piantadosi, 2003). Taken together, these observations clearly suggest that endogenous and exogenous oxidative stress is one of the factors involved in the increase of mitochondrial abundance, and mtDNA copy number of the human and animal cells.

The increase of mitochondrial abundance in response to oxidative stress appears to depend on mitochondrial membrane potential and protein synthesis in cytoplasm (Lee et al., 2002). A study on the increase in mitochondrial mass of mtDNA-less ρ^0 cells in response to oxidative stress suggests that the proteins encoded by mtDNA appear not to be involved in the increase of mitochondrial mass under oxidative stress. Moreover, increase of mitochondrial respiratory enzymes and over-proliferation of mitochondria are often manifested as the so-called “ragged-red fibers” in the skeletal muscle of patients with mitochondrial myopathies and even in the muscle of patients with mtDNA depletion (Wallace, 1992; Wei & Lee, 2003). The increase in mitochondrial biogenesis in the skeletal muscle of patients with mtDNA depletion is not accompanied by an increase in mtDNA copy number. These findings together suggest that the proteins encoded by nuclear genes are the major determinant of the increase in the abundance of mitochondria and mtDNA copy number of human cells in response to oxidative stress.

It was also reported that the abundance of mitochondria was increased in some tumor cells under stress conditions by treatment with aphidicolin (Camilleri-Broet, Vanderwerff, Caldwell, & Hockenbery, 1998), doxorubicin (Kluza et al., 2004), etoposide (Reipert, Berry, Hughes, Hickman, & Allen, 1995), genistein (Pagliacci et al., 1993), herbimycin A (Mancini et al., 1997), leflunomide (Spodnik et al., 2002), mitoxantrone (Kluza et al., 2004), and taxol (Karbowski et al., 2001), respectively. The elevated production of ROS may play a role in the increase of mitochondrial abundance in some of the drug-treated cells. However, it is difficult to correlate the increase in the number of mitochondria with changes in a common function or the activity of a specific mitochondrial enzyme. Recently, it was reported that taxol causes an increase in mitochondrial abundance and an increase in intracellular level of acetylated α-tubulin, while nocodazole and colchicines, which depolymerize microtubules, could not induce the proliferation of mitochondria, suggesting a possible role of microtubules in the biogenesis of mitochondria (Karbowski et al., 2001).

7. Consequences of oxidative stress-induced increase in mitochondria and mtDNA

In aging or affected tissues of patients with neuromuscular diseases, oxidative stress-induced increase in mitochondria and mtDNA molecules may compensate for the decline of mitochondrial respiratory function. At the same time, ROS would also be generated from the increased mitochondria in these cells and thus cause much more oxidative damage to mitochondria and other intracellular constituents including DNA, RNA, proteins, and lipids, and consequently lead the cell to enter the process of senescence or apoptosis (Bladier, Wolvetang, Hutchinson, de Haan, & Kola, 1997; Chen et al., 1998).

This scenario of oxidative stress response plays two roles in the affected cells. On one hand, it stimulates mitochondrial proliferation to supply energy to meet the need for cell survival, including repair of damage and synthesis of new proteins. On the other hand, it causes excess ROS production and further oxidative damage, and thereby initiating the aging process or cell death. The outcome of the events leading to the increase in mitochondrial abundance and mtDNA copy number is dependent on the level of oxidative stress, the capacity of intracellular antioxidants system, and the quality of mitochondria and mtDNA. When cells have higher antioxidant capacity and good quality of parental mitochondria and mtDNA, the response to mild oxidative stress will lead to an increase in the abundance of mitochondria and mtDNA molecule. This may result in an increase in energy supply by the increased mitochondria. However, when the capacity of antioxidant system is compromised, the exposure of tissue cells to higher oxidative stress will result in an increase of defective mitochondria and mutated mtDNA, and thus, a cyclic increase in ROS production and oxidative damage. Once beyond a threshold, mitochondria could drive the cell into an irreversible cell death process. Excess production of peroxides oxidizes the glutathione pool and may also allow the formation of critical protein dithiols on the mitochondrial permeability transition (MPT) pore, which triggers pore opening (Chernyak & Bernardi, 1996). Induction of the opening of MPT pore may be involved in disruption of the mitochondrial membrane potential and releasing of cytochrome *c* and proapoptotic molecules (e.g., apoptosis-inducing factor) from the intermembrane space of mitochondria to the cytosol (Kroemer, Dallaporta, & Resche-Rigon, 1998). Moreover, increased ROS may induce the peroxidation of cardiolipin in the mitochondrial inner membrane, which triggers dissociation of cytochrome *c* from cardiolipin (Imai & Nakagawa, 2003). Once cytochrome *c* is released, the cell is committed to die by either apoptosis involving activation of the apoptotic caspase cascade and nucleic DNA fragmentation, or necrosis due to collapse of the respiratory function, resulting from over-production of ROS and insufficient supply of ATP. The bioenergetic and redox catastrophe together with the activation of caspases and nucleases drive the cells to death (Kroemer et al., 1998). This scenario underscores the increase of mitochondrial abundance and mtDNA copy number in the affected tissues of the elderly subjects or patients with chronic diseases including mitochondrial myopathy, arrythmia, and atrial fibrillation (Lin, Lee, Su, & Wei, 2003). Understanding of the oxidative stress-elicited alterations in mitochondrial abundance and mtDNA copy number is thus important for the development of novel drugs for prevention and treatment of aging-related chronic degenerative diseases.

8. Signaling pathways involved in the regulation of mitochondrial biogenesis

Although significant advances have been made in the understanding of the role of transcription factors and co-activators in mediating the transcriptional control of respiratory gene expression, the mechanisms by which the expression of these proteins is regulated remain largely unknown. Many links between Ca^{2+} and respiratory gene expression mediated by Ca^{2+}-dependent regulatory enzymes, Ca^{2+}/calmodulin-dependent protein kinase (CAMK) and protein kinase C (PKC) have been implicated in the control of expression of respiratory genes in the skeletal muscle. It was reported that treatment of muscle cells with the Ca^{2+} ionophore A23187 to increase the intracellular Ca^{2+} concentrations resulted in an increases of the cytochrome *c* gene expression through a Ca^{2+}-sensitive and PKC-dependent pathway (Freyssenet, DiCarlo, & Hood, 1999). Studies on transgenic mice with a skeletal muscle-specific constitutively active CAMKIV showed increased mitochondrial biogenesis in the skeletal muscle of the mice, which suggests

that CREB mediates the Ca^{2+}-dependent regulation of mitochondrial gene expression (Wu et al., 2002). Moreover, it was demonstrated that increase in the PGC-1α level and the mitochondrial abundance are dependent on the activities of CAMKIV (Wu et al., 2002) and AMP kinases (Zong et al., 2002), which are regulated by changes in intracellular Ca^{2+} concentrations and the ATP/AMP ratio, respectively. These results suggest that the gene expression and activity of PGC-1α and other factors, which are involved in the regulation of mitochondrial abundance, are further regulated by effector proteins that sense changes in the intracellular Ca^{2+} level and energy status of the target cell. The link between oxidative stress, activities of Ca^{2+}-dependent regulatory enzymes and respiratory genes expression remains to be elucidated.

Many signaling pathways have been shown to be responsive to oxidative stress elicited by excess production of ROS. Both AP-1 and NF-κB are the transcription factors long established to respond to oxidative stress (Lee & Wei, 2000). It was demonstrated that electrical stimulation of cardiomyocytes induces the expressions of the nuclear-encoded subunit Va of cytochrome *c* oxidase and cytochrome *c* through effects on AP-1 signaling via *c*-Jun N-terminal kinase (JNK) and NRF-1 (Xia, Buja, Scarpulla, & McMillin, 1997). However, the link between electrical stimulation and increase in respiratory gene expression is probably not mediated by ROS. Very few direct links have been established between the transcriptional activity of AP-1 or NF-κB and expression of either respiratory genes or their transcription regulators. It was reported that PGC-1 can be activated by a p38 MAPK-sensitive pathway, and that PPARα gene expression is dependent on p38 MAPK activation (Puigserver et al., 2001). Regulation of the expression of respiratory genes via p38 MAPK-dependent pathways may be important in the induction of mitochondrial proliferation by extrinsic factors such as cytokines. However, the involvement of p38 MAPK-dependent pathway in the oxidative stress-induced increase in mitochondrial mass remains to be established. Recently, activation of the pro-survival phosphatidylinositol 3′-kinase (PI3K)–Akt pathway was linked to the ROS signaling that leads to increased biogenesis of hepatic mitochondria after LPS-induced liver damage in the rat (Suliman et al., 2003). It was found that Akt expression is upregulated by oxidants and its activity is increased in the rats treated with *tert*-butyl hydroperoxide, which is mediated by activation of the PI3K pathway (Suliman et al., 2003). Moreover, it was found that inhibition of PI3K and Akt phosphorylation resulted in the suppression of NRF-1 binding activity, which clearly suggests the involvement of PI3K/Akt pathway in the activation of NRF-1 (Suliman et al., 2003). Thus, activation of PI3K–Akt may play an important role in the stimulation of mitochondrial biogenesis of mammalian cells by oxidative stress (Fig. 1).

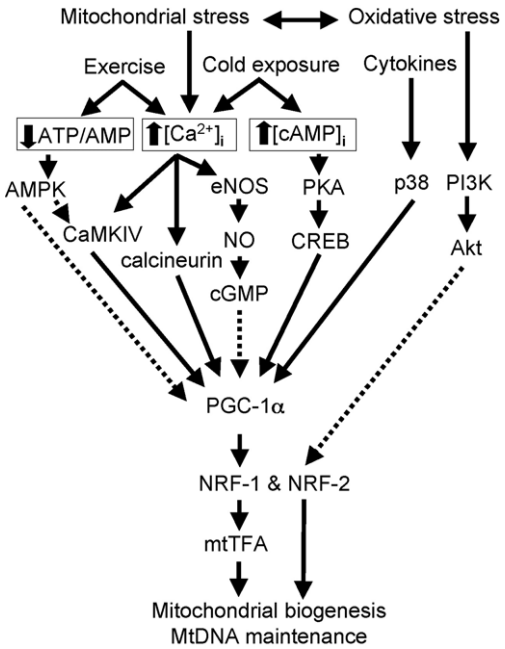

Fig. 1. Signal transduction pathways involved in mitochondrial biogenesis in mammalian cells. Intracellular levels of Ca^{2+}, cAMP, NO, and the ATP/AMP ratio are modulated by exercise, cold exposure, cytokines treatment, and mitochondrial stress. These signaling molecules activate specific proteins and/or enzymes, which, in turn, activate diverse signaling pathways and affect the expression and/or the activity of PGC-1α. Increased expression and activity of PGC-1α stimulate mitochondrial biogenesis by activating relevant transcription factors (e.g., NRF-1, NRF-2, and mtTFA). Oxidative stress has been shown to induce changes in mitochondrial mass and mtDNA copy number. However, it remains unclear as to whether different signaling pathways are involved in regulating PGC-1 expression and mitochondrial biogenesis under oxidative stress. Solid lines denote established signaling pathways; dashed lines represent putative signaling cascades. Abbreviations used: i, intracellular; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; AMPK, AMP-dependent protein kinase; CAMKIV, Ca^{2+}/calmodulin-dependent protein kinase IV; CREB, cyclic AMP response element-binding protein; PKA, protein kinase A; p38, p38 MAP kinase; PI3K, phosphatidylinositol 3′-kinase; Akt, protein kinase B.

Recently, it was reported that elevated levels of nitric oxide (NO) stimulate mitochondrial biogenesis by upregulation of the expression of PGC-1α through a soluble guanylate cyclase-sensitive, cGMP-dependent signaling pathway in a number of unrelated cell lines (Nisoli et al., 2003). In addition, targeted disruption of the endothelial nitric oxide synthase (eNOS) gene in vivo resulted in significant reduction in the level of mitochondrial mass (Nisoli et al., 2003). These results suggest that the signaling pathway may represent a conserved mechanism by which many types of mammalian cells regulate the number of mitochondria. However, it warrants further investigation as to whether this signaling cascade represents a general mechanism by which mammalian cells regulate mitochondrial biogenesis in response to oxidative stress (Fig. 1).

9. Concluding remarks

Mitochondria are the major supplier of energy in the form of ATP in mammalian cells. When the cells sense a deficiency in mitochondrial respiratory function during aging or upon exposure to the environmental stress, mitochondria are motivated to provide energy to meet the need of the cells. In order to increase energy supply for repairing and eliminating the damage of cellular components, some signals (e.g., H_2O_2) are transmitted to the nucleus, by which the cell may stop growth and concurrently induce mitochondrial proliferation and mtDNA amplification to produce more functional mitochondria. After the damage is effectively repaired or eliminated, the cell may re-enter the cell cycle and resume normal growth. However, once the damage persists too long or is too serious to be repaired, the mitochondria could sense and integrate the extracellular or extra-mitochondrial stress and signals to drive the cell into an irreversible cell death process.

Several degenerative diseases and aging have been established to be associated with chronic exposure to ROS, which leads to mitochondrial function decline and increased production of free radicals and mtDNA damage. Long-term exposure of a human cell to ROS may initiate a vicious cycle to result in a decrease of the capacity of stress response, decrease in ATP synthesis, and further increase of ROS production of the cell. This, in turn, may elicit more serious consequences of enhanced oxidative damage and cell death in affected tissues, when ROS reaches a threshold level. Experimental data from this and other laboratories have supported the notion that mutation and oxidative damage to mtDNA and mitochondrial respiratory function decline are important contributors to human aging (Wei & Lee, 2002). On the other hand, exposure to environmental insult and metabolism of drugs can enhance ROS production in affected cells. Mild oxidative stress may stimulate increase in the abundance of mitochondria and mtDNA content. However, long-term exposure to oxidative stress may result in severe oxidative damage and aggravate the aging- or disease-related oxidative damage and perturb the stress response of the target cells (Wei & Lee, 2002). While the classical role of mitochondria in generation of ATP by aerobic metabolism has been established for more than half a century, the other face of mitochondria in carrying out apoptosis has just been recognized less than 10 years ago. The role of mitochondrial biogenesis and mtDNA maintenance in the determination of life and death of the mammalian cell under oxidative stress has been increasingly appreciated in recent years. Elucidation of the changes in the number and function of mitochondria and mtDNA in the process of stress response to render a cell to survive or to die should be of prime importance for a better understanding of mitochondrial function in mammalian cells.

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