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REVIEW

Multifactorial Nature of High Frequency of Mitochondrial DNA Mutations in Somatic Mammalian Cells

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Abstract—The high frequency of mitochondrial DNA (mtDNA) mutations in somatic mammalian cells, which is more than two orders of magnitude higher than the mutation frequency of nuclear DNA (nDNA), significantly correlates with development of a variety of mitochondrial diseases (neurodegenerative diseases, cardiomyopathies, type II diabetes mellitus, cancer, etc.). A direct cause–consequence relationship has been established between mtDNA mutations and aging phenotypes in mammals. However, the unclear nature of the high frequency of mtDNA mutations requires a comprehensive consideration of factors that contribute to this phenomenon: oxidative stress, features of structural organization and repair of the mitochondrial genome, ribonucleotide reductase activity, replication errors, mutations of nuclear genes encoding mitochondrial proteins.

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Key words: oxidative stress, frequency of mtDNA mutations, structural features, repair mechanisms, ribonucleotide reductase activity

There is now virtually no doubt that functional and pathological disorders, as well as the aging process, are associated with incomparably greater number of damages of mitochondrial DNA (mtDNA) in somatic mammalian cells than of nuclear DNA (nDNA) [1, 2].

More than 300 point mutations and deletions have been identified in the cells of patients with a variety of mitochondrial diseases, and more than 100 such mutations have been found in malignancies [3]. These damages can arise in any tissue of the body, but much more frequently they occur in the brain and in the cardiac and skeletal muscles, obviously because of significantly higher levels of metabolism and especially of oxidative phosphorylation in them than in other tissues [4]. Mitochondrial diseases are characterized by numerous clinical symptoms in different combinations (mitochondrial myopathy, ocular muscle paresis, retinitis pigmentosa, deafness, ataxia, dementia, peripheral neuropathy, cardiomyopathy, Parkinson's disease, Alzheimer's disease, etc. [1, 5, 6]). Mutations of mtDNA have been found in primary human tumors but not in the surrounding tissues [2]. Although the cause–consequence relationship of the mtDNA mutations with carcinogenesis is not yet proved directly, their contribution to this process is obvious [6, 7]. The situation is clearer when association of different mtDNA mutations with aging processes in mammals is considered. An increasing accumulation of mtDNA mutations in somatic cells of different organs of humans and laboratory animals has been shown in many works [8-10]. And finally, a direct causal relationship between mtDNA mutations and some aging phenotypes in mammals has been demonstrated in [11]. The unknown nature of the high frequency of mtDNA mutations in some diseases induced us to comprehensively consider a rather broad set of specific factors contributing to this phenomenon: oxidative stress of mitochondria, features of structural organization and repair of the mitochondrial genome, ribonucleotide reductase activity (the first link in the biosynthesis of DNA precursors), replication errors, influence of mutations in the nuclear genome

Abbreviations: ANT, adenine nucleotide translocase; AO, antioxidants; APE, apurine/apyrimidine endonuclease; BER, base excision repair; dNDP and dNTP, deoxyribonucleotide-5′-diphosphate and -triphosphate, respectively; ETC, electron transport chain; GPX, glutathione peroxidase; hMTH1, human 8-oxo-dGTPase; LPO, lipid peroxidation; MnSOD, manganese superoxide dismutase; MYH, DNA glycosylase; NTH1, endonuclease III homolog; OGG1-8-OH-dG, glycosylase; PCR, polymerase chain reaction; rNDP, ribonucleoside-5′-diphosphate; ROS, reactive oxygen species; RR, ribonucleotide reductase; TFAM, mitochondrial transcription factor A.

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genes encoding mitochondrial proteins. And what is contribution of each of these factors to mutagenesis of mtDNA? Discussion of these aspects will be favorable for better understanding of this complex situation and, possibly, facilitate searches for approaches to decrease the frequency of mtDNA mutations and, consequently, to treatment of many diseases and deceleration of aging.

Nevertheless, alongside intensive studies on damaging factors for the mitochondrial genome (and this is the subject of the present review), studies on approaches for its protection are also being conducted [9]. And just the results of the latter studies allow us to explain the integral high threshold values of mitochondrial genome mutations (45-50% for deletions and 80-85% for point mutations), which are promising for diagnosis of many mitochondrial diseases [1].

OXIDATIVE STRESS

During recent years, many researchers studying fundamental processes of energy production associated with ATP synthesis via oxidative phosphorylation began to comprehend the involvement of mitochondria also in other processes extremely important for the life of higher organisms. Oxidative stress, cell death, aging, and mitochondrial diseases occurred to be at least partially related with oxidative phosphorylation, integrity of mitochondria, and informational identity of their genetic apparatus, mtDNA [12].

Being the major intracellular provider of energy as ATP, mitochondria absorb 90% of the total oxygen consumed by the cells of higher animals. Under normal physiological conditions, 1-5% of oxygen used by mitochondria is converted into superoxide anion radicals ($O_2^{\cdot-}$), which in the presence of antioxidant enzymes change to hydrogen peroxide (H_2O_2). In the presence of bivalent iron and other metals with variable valence, H_2O_2 is converted to the highly reactive hydroxyl radical ($^{\cdot}OH$) (Fenton's reaction). All these compounds are usually called reactive oxygen species (ROS) [13, 14]. The electron transport chain (ETC) is a powerful source of ROS. The more active is ETC functioning, i.e. ATP production, the higher is the amount of ROS generated.

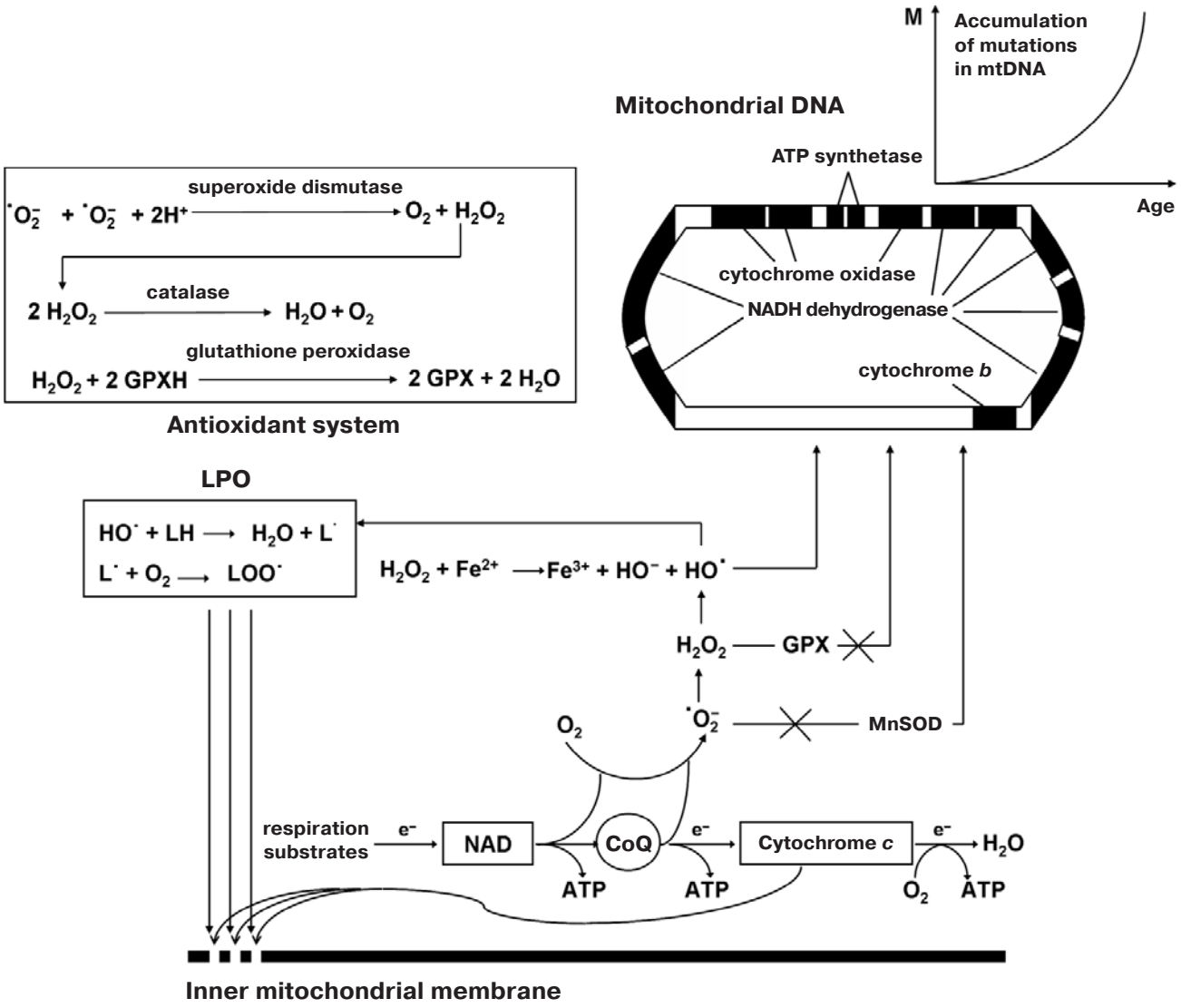
Oxidative stress can develop depending on production of each of the ROS, the rate of their relative neutralization, and their damaging effect on the cellular repair systems. Imbalance of these factors is accompanied by a serious dysfunction of mitochondria [15, 16]. Under normal physiological conditions, these harmful byproducts of respiration are usually opposed by a system of mitochondrial antioxidant enzymes: manganese superoxide dismutase (MnSOD), catalase, and glutathione peroxidase (GPX) and also a complex of endogenous low molecular weight antioxidants (AO) (Scheme). However, under both normal physiological conditions and especially under functional overloads, aging of cells and tissues, and in some pathologic states, a significant part of the ROS can "escape" antioxidant systems and induce oxidative damages of macromolecules (polysaccharides, lipids, proteins, RNA, and DNA) and their supramolecular complexes, i.e. induce oxidative stress [17, 18]. Note that the system responsible for detoxification of the most reactive radical, $^{\cdot}OH$, is unknown. Because of the abovementioned and other damages (mtDNA is much more vulnerable to exogenous chemical agents than nDNA [19]), bioenergetic functions of mitochondria decrease with age, become insufficient in patients with mitochondrial diseases, and in critical situations can be involved in programmed cell death, i.e. apoptosis [3] (Scheme).

Oxidative phosphorylation requires mtDNA. The mtDNA is attached to the inner mitochondrial membrane where the respiratory chain is uninterruptedly producing significant amounts of ROS [16]. This results in the higher sensitivity of mtDNA to oxidative damages than nDNA. The difference in their basal mutagenesis is, in particular, maintained by the absence of additional oxidative modifications of nDNA upon the stimulation of ROS production in HeLa cells by inhibition of the mitochondrial ETC. The contribution of mitochondria to the background oxidative damage of nDNA seems to be insignificant [20].

Note that the active generation of ROS and specific features of mtDNA compartmentalization on the inner mitochondrial membrane are important but not the only factors of its intense mutagenesis. For a long time, changes in mtDNA were considered to be rare genetically heritable disorders associated with encephalomyopathy. Now it is clear that somatic mutations of mtDNA occur more frequently that it was believed earlier and are an important cause for development of many diseases and aging, whereas the contribution of genetically heritable mutations of mtDNA is less significant. In particular, it seems to be associated with signs of apoptosis during oogenesis of mammals, and some authors interpret this phenomenon as adaptation of mitochondria to accumulation of harmful mutations. Oocytes with a pronounced dysfunction of mitochondria are predominantly subjected to apoptosis [21].

STRUCTURAL ORGANIZATION AND HIGH VULNERABILITY OF THE MITOCHONDRIAL GENOME

Human mtDNA is a 16,500-bp double-stranded circular molecule. Two strands, heavy (H) and light (L), called according to their gravitational density, encode only 13 proteins of the respiratory chain and a minimal set for apparatus of mitochondria translation (2 rRNA and 22 tRNA). Nevertheless, all these genes are thought to be very important for the aerobic generation of ATP that is a specific function of the respiratory chain. The



A scheme of oxidative phosphorylation, production of ROS ($O_2^{\cdot-}$, H_2O_2, $HO^{\cdot}$), enzymatic antioxidant protection (MnSOD, GPX), lipid peroxidation (LPO), structure of mitochondrial genome, mtDNA mutagenesis (its exponential growth with age), and some mitochondria-mediated apoptosis elements. Adapted from work [12] of Todorov

mtDNA has no introns and is very compact. More than 90% of mtDNA is occupied by encoding regions, whereas structural genes and their regulatory regions occupy only 1.5% of the nuclear genome. Consequently, mononucleotide polymorphism of the mitochondrial genome (mtSNPs) is more often realized in phenotypes than the nuclear SNPs [22], and this also can be a factor of the high frequency of mtDNA mutations. A similar influence of the "informational saturation" on the mutation frequency in both mtDNA and nDNA can be also detected when errors in replication of these genomes are compared considering similar accuracy of activities of mitochondrial and nuclear DNA polymerases [23].

The mtDNA contains only two noncoding regions: the D-loop and O_L. The first is about 1000 bp in length and mainly regulates transcription and replication. The second region contains 30 bases and seems to initiate L-strand replication [24].

Many somatic mutations of mtDNA in human malignant tumors are located in the D-loop and especially along the polycytidine tract (C-tract) called D310. The D310 region has a polymorphic variability in the length in different individuals and was described as a "hot zone" for somatic mutations of mtDNA in various cancers [25]. Thus, multiple mutations in the mtDNA control region were observed in human prostate tumor; therefore, it was suggested that a unique mitochondrial hypermutagenesis should exist, possibly mediated by oxidative stress [5]. A high frequency of mutations was also observed in the D-loop of mtDNA in cervical carcinoma [26]. These data on

the high level of mutagenesis in the control region of mtDNA replication seem to be an explanation of the extremely high radiation vulnerability of mtDNA biosynthesis as compared to that of nDNA of liver in rats subjected to sublethal γ-irradiation, which is really equivalent to ROS-induced damage. This effect was first described in as early as in 1969 [27].

Potential "hot spots" for oxidative modification and mutations of mtDNA are located in unusual structures of DNA, such as twisted, anti-twisted, and B-unlike ones [28]. These structural features of the structure are also responsible for mtDNA vulnerability to ROS and other free radicals.

The rate of mtDNA mutations can be more than two orders of magnitude higher than that of nRNA [29]. It has been mentioned already that ROS play the major role in damages of mtDNA. The most abundant product of nucleotide oxidation is 8-hydroxy-2′-deoxyguanosine (8-OH-dG), and this increases the frequency of the direct transversion G·C→T·A [30]. The presence of 8-OH-dG in DNA clearly indicates its oxidative damage [31].

For a long time this extraordinary mutability of mtDNA was believed to be also associated with the lack of its defense by histones and other proteins. But now this explanation does not seem obvious. Even in 1987, Miyakawa et al. [32] reported that mtDNA of *Saccharomyces cerevisiae* was tightly "packed" with a protein-like substance and has a nucleosome-like structure, which they called nucleoid. But concerning mtDNA of mammals, this concept was neglected for a long time. Only recently the existence of mtDNA nucleoid has been acknowledged by some groups of authors [33, 34]. Mitochondrial transcription factor A (TFAM) is represented just as a transcription factor by ~15 molecules per mtDNA molecule [35]. Nevertheless, the total number of human TFAM molecules bound with one mtDNA molecule is ~1000, i.e. approximately one TFAM molecule per 15 bases of mtDNA [33]. TFAM is a typical representative of a highly mobile group of proteins (HMG), which bind with DNA independently of the DNA sequence and activate transcription by changing the DNA strand structure [36]. In fact, TFAM can bind with DNA independently of its sequence despite its higher affinity for the promoter of the L- and H-strands of mtDNA. It seems that in mtDNA packing, TFAM functions similarly to histones as the main nucleoid component maintaining its structure [34].

Recently TFAM was found to be a crucial factor among those involved in maintaining mtDNA integrity, such as transcription, replication, vulnerability, and DNA repair. These functions of TFAM are closely interrelated and, along with the nucleoid formation, can maintain and protect mtDNA [37]. However, the nucleoid structure in mammals is known insufficiently, and it must be elucidated whether TFAM specifically or nonspecifically bound with mtDNA is involved in mtDNA protection against damaging factors, especially against ROS.

The number of mtDNA copies can be up to several tens of thousands per cell. The number of copies significantly depends on the energy requirements of the cell. Because of multiple repeated copies, both the wild and mutant mtDNA can coexist in the same cell. The cell usually maintains the respiratory activity of mitochondria until mtDNA mutations in the cell reach a certain level. This phenomenon is called "threshold effect". For large deletions (or point mutations in tRNA genes) the threshold is ~50%, whereas in the genes encoding proteins the fraction of point mutations can be ~80% [1]. Due to such thresholds, a situation can exist when proteins encoded by native mtDNA can for a long time compensate disorders in functions of proteins encoded by mutant mtDNA of the cell, and synthesis of new mtDNA copies on undamaged or slightly damaged mtDNA templates can be the major mechanism for maintaining the mitochondrial genome under conditions of endogenous or exogenous influences [38].

As distinguished from nDNA, mtDNA is uninterruptedly replicated even in terminally differential cells, such as neurons and cardiomyocytes [2]. Consequently, somatic mutations of mtDNA are potentially more harmful for cell functions than somatic damages of nDNA. Correspondingly, the DNA repair systems in mitochondria are more important than in nuclei, especially in nondividing cells.

FEATURES OF THE SYSTEM OF MITOCHONDRIAL DNA REPAIR

Changes in the mitochondrial genome were for a long time believed to be a result of both its high susceptibility to oxidative damage and limited repair of mtDNA as compared to nDNA. These concepts were based on data of work [39] indicating that UV-caused damage of mtDNA, i.e. pyrimidine dimers, were not repaired. Note that even systems of nDNA repair (except base excision repair) were studied insufficiently. Further studies on systems of nDNA repair revealed the complexity of these mechanisms and also promoted elucidation of specific features of mtDNA repair.

Six main pathways of nDNA repair are now known: *direct reversion* (DR), which uses repair proteins acting directly on the damaged base and restoring the correct structure without removal of the damaged nucleotide [40]; *nucleotide excision repair* (NER), which includes removal of DNA damage resulting in a structural deformation of the DNA helix [41]; *base excision repair* (BER), which corrects damage of individual bases caused, in particular, by oxidation, methylation, depurination, and deamination [42]; repair of post-replication errors mainly associated with disorders in base complementation (*mismatch repair*, MMR) [43]; *"by-passing" (translesion) synthesis* (TLS) capable of complete DNA escaping the

distorted region without production of normal hydrogen bonds [44]; *recombination repair* (RER) [23].

Initially, to study the system of mtDNA repair, homology with known proteins of nuclear repair was searched for. Oxidation of mtDNA results in a typical product – guanine oxidized in position 8 (8-OH-dG). Oxidative damage was recorded not only in the DNA strand but also in free nucleotides. 8-Oxo-dGTPase eliminates free oxidized dGTP and thus, prevents its incorporation into DNA during replication [45]. Human 8-oxo-dGTPase called hMTH1 is located in both cytosol and mitochondrial matrix [46]. And hMTH1 was the first enzyme of mtDNA repair for which the gene, protein, and activity were distinctly identified. Later all other BER enzymes necessary for repair of oxidized guanine, such as OGG1 (8-OH-dG glycosylase 1 [47]), MYH (DNA glycosylase [48]), NTH1 (endonuclease III homolog [49]), and APE (apurine/apyrimidine endonuclease [50]) were also found in mitochondria of mammals.

Studies on *in vitro* repair show that mitochondria contain all enzymes necessary for BER and that the biochemical process is virtually the same as in nuclei. A complete BER was re-created using mitochondrial extracts [51]. Different BER-encoding nuclear genes whose products are active in mitochondria of various organisms are presented in the table. Thus, *human mitochondria contain a complete system for repair of oxidized bases*. Importance of the BER system is also evidenced by a possible contribution of the decreased efficiency of the mitochondrial BER to acceleration of the organism's aging [9, 23].

The initial stage of BER is activation of glycosylase, which breaks the glycoside bond between the damaged base and sugar. Many glycosylases also have lyase activity, and they can break the main 3′-phosphodiester bond. Apurine/apyrimidine (AP) endonuclease breaks the 5′-phosphodiester bond remaining a one-nucleotide "gap", which is filled in by DNA polymerase γ [52]. This "gap" is repaired anew with a "correct" genetic material (deoxyribose-phosphate) incorporating it into the general polynucleotide chain through activation of DNA ligase III.

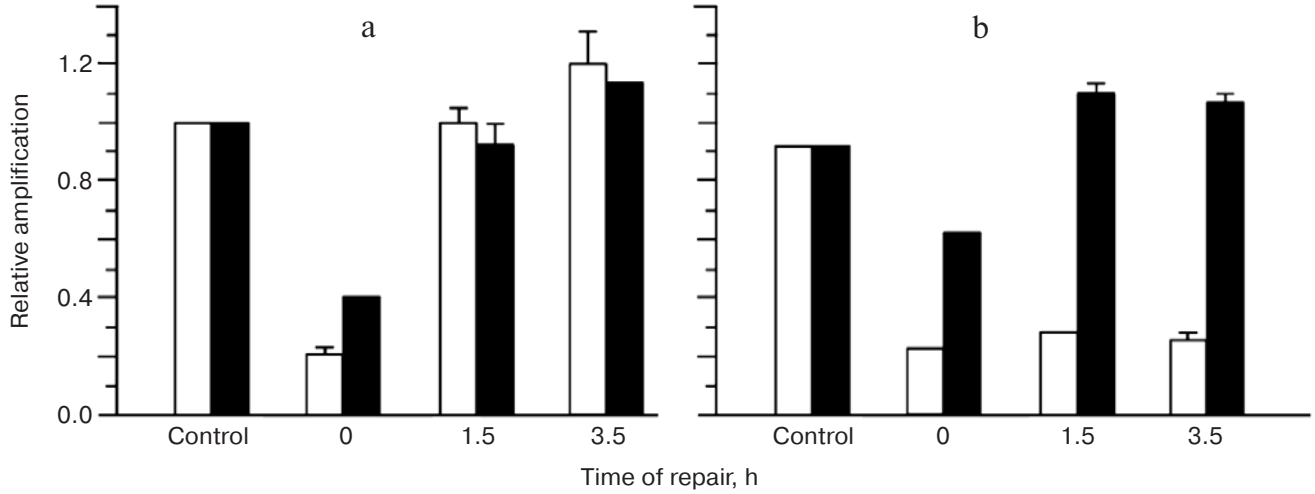
The repair of nDNA and mtDNA reveals a similarity of their BER systems, but the repair systems of nDNA include incomparably more proteins that those of mtDNA [23]. Moreover, the activities of nuclear and mitochondrial BER are different in mice given a low-calorie diet: the activity of nuclear BER increases and that of mitochondrial BER decrease that respectively, increased and decreased in mice that were given a low-calorie diet, which suggests a different regulation of BER in these two compartments of the cell [53].

In addition to BER, in mammalian mitochondria an active DR is found that efficiently removes methyl and ethyl groups, but fails to remove larger alkyl groups from the O^6 position of guanine [54]; there are also some indications of the presence of the MMR system in human mitochondria, but this activity seems to be different from the corresponding activity in the nucleus because it is not directed to break the DNA strand [55]. The RER activity also somewhat manifests itself, and this suggests that recombination can be a part of mtDNA metabolism in mammals [56]. The NER and TLS activities have not been detected in mitochondria to date. However, except for BER, data on functioning of the DR, MMR, and RER systems in mitochondria are limited [23].

All proteins of mtDNA repair are encoded in the nucleus and imported into mitochondria. The majority of the detected proteins of mtDNA repair are either identical to those of nDNA or are isoforms of nuclear proteins produced by alternative splicing [57]. Quantitative and partially qualitative differences in the repair systems of nuclei and mitochondria seem to be due to significant differences in the mutation spectra of these organelles. Without going into detail of this rather a well-studied problem [23, 58], note that BER is mainly responsible for repair of oxidative damages. And because just these damages are dominant in mitochondria, the existence of the virtually full-fledged BER system in mitochondria seems quite natural [2]. Note that the overexpression of MTH [59] or OGG1 [60] in mitochondria provides for the resistance of cells to oxidative stress, which indicates the

Genes of different organisms involved in mitochondrial BER (adapted from work [58])

Genes	Yeast	Birds	Mice	Human
Apurine/apyrimidine endonuclease (*APE*)	+	+	+	+
8-OH-dG-glycosylase 1 (*OGG1*)	+	+	+	+
8-OH-dG-glycosylase 2 (*OGG2*)	+	–	–	+
Endonuclease III homolog (*NTH1*)	+	–	+	+
DNA glycosylase homolog (*MUTY*)	–	–	–	+
Mitochondrial DNA polymerase (γPOL)	+	+	+	+
DNA ligase III	+	–	–	+
Uracil DNA glycosylase (UDG/UNG)	–	+	+	+



Activities of mtDNA and nDNA repair. Fibroblasts were exposed to 200 μM H_2O_2 during 15 (a) or 60 min (b) and were collected immediately or placed into a conditioned medium for a due time. Quantitative PCR was performed for both the mitochondrial and β-globin fragments (white and black columns, respectively). The data are presented as means ± SD (n = 3). Adapted from work [63]

importance for these organelles of the oxidative damage repair system. By contrast, the decreased activity of BER is in strict correlation with development of apoptosis in cultures of neurons [61]. Surprisingly, both activities of the respiration complexes I and IV from the liver and heart of the OGG1-deficient mice (–/–) were the same as in wild type animals; moreover, there was no sign of increased oxidative stress in mitochondria of the OGG1 (–/–) mice. This indicated the absence of a direct relationship between the increased level of oxidative damages of mtDNA and respiratory dysfunction of mitochondria [62].

Nevertheless, even the apparently full-fledged system of mitochondrial BER is less reliable than the nuclear BER. It was shown by gene-specific quantitative PCR that the treatment of human cells with H_2O_2 resulted in two to three times more damages in mtDNA than in nDNA [63]. Treatment with 200 μM H_2O_2 for 15 min caused damages in both mtDNA and nDNA that were completely repaired within 1.5 h. By contrast, 60-min treatment caused a steady damage of mtDNA, whereas damages in nDNA were virtually fully repaired (figure). What factors could contribute to this steady damage of mtDNA? Possibly, H_2O_2-induced oxidative damage could inactivate the BER enzymes in mitochondria more strongly than in nuclei. But this was not the only cause of the decrease in their activities. Thus, Stuart and colleagues [57] studied the BER system and metabolic pathways in mitochondrial and cellular lysates from mtDNA-deficient cells and suggested that an unknown activity, factor, or relationship important for BER functioning in mitochondria should exist. The activity of ribonucleotide reductase and its influence on the repair process might be such a factor, but it is still beyond the specialists' consideration.

ACTIVITY OF RIBONUCLEOTIDE REDUCTASE (RR)

As a rate-limiting enzyme in DNA synthesis, RR controls synthesis of deoxyribonucleoside-5′-diphosphates (dNDP) catalyzing replacement of the OH-group in the 2′-position of ribose residue by a hydrogen atom and producing 2′-deoxy-derivatives [64]. Incorporation of ribonucleoside-5′-diphosphates (rNDP) into a DNA molecule occurs as follows.

$$\text{rNDP} \xrightarrow{\text{RR}} \text{dNDP} \xrightarrow[\text{kinase}]{\text{NDP}} \text{dNTP} \xrightarrow[\text{polymerase}]{\text{DNA}} \text{DNA}$$

Synthesis of dNTP is strictly controlled by RR because NDP kinase does not limit the rate of this reaction. Under conditions of rest, intracellular concentrations of all four types of dNTP are extremely low and about three orders of magnitude lower than concentrations of the corresponding rNDPs. The RR-catalyzed stage is crucial for the balanced supply of the four dNTPs required for DNA synthesis. Upon inhibition of dNTP synthesis, their pools are exhausted within 15-20 sec of DNA synthesis [64] and repair [65]. The dependence on the dNTP pools is sigmoid; therefore, even a slight change in them or their imbalance cause a deep suppression of DNA synthesis and repair [64, 66].

Effective regulation of DNA synthesis by RR can be disturbed under the influence of different factors, especially ROS [67, 68]. To display the enzymatic activity, RR needs oxygen: the RR subunit (the R2 protein) contains a pair of trivalent iron ions bound by an oxygen bridge, which is a terminal acceptor of electrons in the RR molecule and is involved in the generation of tyrosyl radicals

determining the enzyme activity. During the enzymatic reaction, molecular oxygen is changed to $O_2^{\cdot-}$ and needs SOD to be neutralized; therefore, the RR activity is maintained only in the presence of this enzyme [64, 68].

More than 25 years ago RR activity was also detected in mitochondria, and activities of this enzyme and thymidine kinase correlated with synthesis of mtDNA during the regeneration of rat liver [69, 70]. The interest in RR in connection with synthesis and repair of mtDNA was recommenced only when another form of the R2 subunit was independently found in two different laboratories [71, 72]. Expression of this R2 homolog was induced by a tumor suppressor protein p53, and it was called p53R2. Biologically, the p53R2 protein is an important factor of cell survival; thus, mice deprived of this gene (*knockout*) died because of renal failure before 14 weeks of life, possibly through defects in DNA repair and increased apoptosis [73].

Importance of the p53R2-catalyzed repair of rNDP for mtDNA synthesis in non-proliferating cells was shown in [74], as well as a shift of the repair focus from p53R2 repair to synthesis of mtDNA. In fact, as differentiated from the S-phase of proliferating cells (when the dNTP level is 20-fold higher than in resting cells, because in proliferating cells deoxyribonucleotides are imported into mitochondria directly from the cytosol [75]), in non-proliferating cells the dNTP pool seems to be insufficient for provision of both repair and synthesis of mtDNA [76]. Obviously, for terminally differentiated cells it is more important to maintain synthesis of mtDNA and, consequently, the functional activity of mitochondria even at the cost of reducing repair of their mtDNA. This is an important (but not the only) factor of the mutation frequency of mtDNA in nonproliferating mammalian cells, which is associated with RR and p53R2. Under conditions of pronounced oxidative stress in mitochondria, disturbed RR and p53R2 activities also can contribute to the decrease in efficiency of mtDNA repair.

The R1 protein of the RR enzyme contains two types of sites responsible for allosteric regulation of both the total activity and substrate specificity, which determines synthesis of four dNTP types. The total activity is realized through ATP (activation) and dATP (inhibition), whereas the substrate specificity of RR is realized by the four types of dNTPs, which determine the succession of their syntheses. Consequently, the RR activity depends on synthesis of ATP [54]. However, many data suggest that oxidative stress and mtDNA mutations decrease the mitochondrial synthesis of ATP [1, 12]. Thus, a well-known situation of a "vicious circle" arises when a decrease in ATP synthesis under the influence of the abovementioned factors results in a decrease in the RR activity and imbalance of the produced dNTPs which is accompanied by a decreased efficiency of repair and increase in the frequency of mtDNA mutations which, in turn, result in a further decrease in ATP synthesis, etc.

The idea that asymmetry of the dNTP pool determines, in particular, hypermutagenesis of retroviral and mitochondrial genes, is now universally adopted [77]. Therefore, the RR activity depending on the ATP concentration may be considered as an important immediate factor of the high frequency of mtDNA mutations. Mutations of nuclear genes encoding some mitochondrial proteins are also important but an indirect factor of mtDNA mutagenesis.

MUTATIONS OF NUCLEAR GENES ENCODING MITOCHONDRIAL PROTEINS

The majority of mitochondrial diseases are caused by mutations of mtDNA [78]. Nevertheless, mutations of nDNA, which are much less frequent than those of mtDNA, can cause pronounced mitochondrial dysfunctions [79], diseases [80], and indirectly mutations of mtDNA [81]. Moreover, many proteins (>600) that are products of nuclear genes are constantly functioning in mitochondria. Upon translation in the cytosol, these proteins are transferred into mitochondria. Along with oxidative phosphorylation, they control replication and repair of mtDNA, transcription and translation (i.e. synthesis of proteins that determine maintaining of number of mtDNA copies and its integrity), and also integrity of the template and mitochondrial membranes, oxidation of substrates in the tricarboxylic acid cycle, and β-oxidation of fatty acids. These proteins partially control the urea cycle and regulation of apoptosis [1, 8]. Mutations of genes encoding the majority of the abovementioned proteins somewhat decrease the energy metabolism of mitochondria and promote their involvement into the "vicious circle" of oxidative stress and mtDNA mutagenesis. In particular, mutations in nuclear genes encoding succinate dehydrogenase [82], a component of complex I [83], and some subunits of complexes I and II [84] are directly related with the ETC, generation of ROS, mutations of mtDNA, and upon reaching the threshold effect also with mitochondrial diseases which, as distinguished from mtDNA-associated diseases, are inherited according to Mendel's laws [1].

Mutations of the adenine nucleotide translocase 1 (ANT1) gene were recorded in the skeletal and cardiac muscles of patients with progressing external ophthalmoplegia. ANT1 is involved in regulation of the nucleoside/nucleotide mitochondrial pool and can play a crucial role in maintaining the integrity of mtDNA [85]. In mice with ANT1 deficiency a moderate cardiomyopathy is developed. This enzyme is responsible for ADP import into mitochondria and for ATP export from them. Thus, ANT provides for a substrate for ATP synthase. Without phosphorylation of ADP during synthesis of ATP, the ETC virtually stops functioning. Consequently, the inactivation of the protein or mutation of the ANT gene can

inhibit the ETC, and this is accompanied by increase in $O_2^{\cdot-}$ production and induction of MnSOD, which is strongly elevated in mice with a mutant ANT1. Note that mtDNA rearrangements were noticeably accumulated in animals with mutations in the ANT1 gene [86]. Mutations in the nuclear gene of DNA polymerase γ, which is responsible for synthesis and repair of mtDNA, were found in some patients with mitochondrial diseases [87]. A similar situation was observed in cases of mutations in the thymidine phosphorylase gene [88] and in the gene of thymidine kinase-2 expressed in mitochondria [89]. But the gene of MnSOD determining antioxidant functions described above is the most important nuclear gene affecting mitochondrial functions and level of mtDNA mutations. Thus, mice deprived of the MnSOD gene died within the first 10 days of life because of acute cardiomyopathy [90] and neuronal degeneration of the basal ganglion and nerve trunk [85]. Obviously, mutations of the nuclear gene of MnSOD will also manifest themselves by mitochondrial dysfunction and development of mitochondrial diseases.

The data presented suggest that deficiencies of the abovementioned nuclear genes (the list is far from full) can induce disorders in the mitochondrial pool and ADP and ATP transfer, respectively, into and out of mitochondria, disorders in replication and repair of mtDNA, in the antioxidant status of mitochondria, functions of ETC, and ATP synthesis, i.e. all processes essential for the number of mtDNA copies and integrity and, thus, lead to mitochondrial dysfunctions and diseases.

In conclusion, note that at present we cannot accurately assess the relative contribution to mtDNA mutagenesis of every factor under consideration. But ROS generation is undoubtedly the most important. And this is the reason for the increased attention to the search for new effective antioxidants capable of reducing ROS generation in mitochondria. Moreover, structural features of mtDNA, specific features of its repair system, activity of RR, and mutations of some nuclear genes also can markedly influence mtDNA mutagenesis, but details of these influences require further comprehensive studies in the context of the search for new approaches to treatment of mitochondrial diseases and deceleration of aging.

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