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Review

Mitochondrial DNA maintenance and bioenergetics

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Abstract

Oxidative phosphorylation requires assembly of the protein products of both mitochondrial and of nuclear genomes into functional respiratory complexes. Cellular respiration can be compromised when mitochondrial DNA (mtDNA) sequences are corrupted. Oxidative damage resulting from reactive oxygen species (ROS) produced during respiration is probably a major source of mitochondrial genomic instability leading to respiratory dysfunction. Here, we review mechanisms of mitochondrial ROS production, mtDNA damage and its relationship to mitochondrial dysfunction. We focus particular attention on the roles of mtDNA repair enzymes and processes by which the integrity of the mitochondrial genome is maintained and dysfunction prevented.
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1. Introduction

The mitochondrial proteome has been estimated to contain approximately 2000 distinct proteins, though proteomic analyses typically only identify a subset of 600–750 of them [1–3]. The vast majority of these mitochondrial proteins is encoded by nuclear genes and imported into the organelle through protein import complexes [4]. Human mitochondrial DNA (mtDNA) encodes just thirteen proteins, all of which are subunits of the large multimeric respiratory complexes. Despite this low proportional contribution to the organelle's protein complement, mtDNA-encoded proteins are essential for the assembly of competent respiratory chains. mtDNA-less (ρ^0) cells do not respire [5]. Thus, the mitochondrial genome is essential for normal cell bioenergetics and physiology.

Oxidative damage and mutations are common in mtDNA [6], and because mtDNA encodes respiratory complex subunits, they can manifest directly as dysfunctional respiration [7]. Elevated levels of mtDNA mutations also appear to promote apoptotic cell death, cardiomyopathies and premature aging [7–10]. In all eukaryotic cells, mitochondria contain enzymes that recognize and repair oxidative and other nucleotide and base modifications present in DNA. These enzymes intervene to prevent mutation following events that damage mtDNA. This is particularly important because mtDNA contains relatively high levels of pre-mutagenic modifications [11] and, even in post-mitotic tissues, continues to replicate throughout the lifespan of an organism. Mitochondrial genomic maintenance may therefore play a pivotal role in preventing cellular dysfunction and degeneration in disease and aging [12].

In this review, we discuss mechanisms and characteristics of mitochondrial reactive oxygen species (ROS) production and the effects of ROS on mitochondrial genomic stability. We review the important enzymes and pathways responsible for maintaining the integrity of mtDNA in the face of oxidative damage, particularly the DNA base excision repair (BER) pathway. We discuss the physiological regulation of BER and the contribution it and other repair pathways make to the maintenance of mitochondrial and cellular functions.

2. Respiration and ROS production

Superoxide is produced as a byproduct of mitochondrial respiration (reviewed in [13]), at complexes I and III, via single electron reduction of oxygen. Superoxide is also produced by

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the electron transfer flavoprotein during fatty acid oxidation [14], by α-ketoglutarate dehydrogenase complex [15], by complex II [16] and by glycerol-3-phosphate dehydrogenase [17]. The quantitatively most important site(s) have not been agreed upon and may differ in specific tissues, species or physiological contexts.

There is also as of yet no consensus on the magnitude of mitochondrial superoxide production under baseline physiological conditions in vivo. However, contexts in which elements of the respiratory chain are highly reduced are typically associated with enhanced superoxide production (reviewed in [18]). Thus, superoxide production is particularly high under non-phosphorylating conditions [19], and is reduced in the presence of uncouplers [20]. The rate of superoxide formation is also proportional to oxygen tension [21]. Superoxide production is enhanced by redox cycling drugs such as paraquat and doxorubicin (adriamycin) [21].

Mitochondrial superoxide production has been estimated to be as high as 2% of all molecular oxygen reduced during respiration [19]. However, this is likely to be an overestimate, as the measurements from which they are derived have been made under conditions in which oxygen tensions were at least an order of magnitude higher than those found within most animal cells in vivo [22]. As mitochondrial production of superoxide increases proportionately with oxygen tension, the true rate of production is likely to be at least 10-fold lower than these estimates. In addition, many studies have employed respiratory chain inhibitors to accentuate the rate of superoxide production during respiration and thus facilitate its measurement [23]. In the absence of inhibitors of respiratory complexes, superoxide production of isolated rat liver mitochondria is less than 0.15% of total electron flux [14]. Thus, the rate at which mitochondria produce superoxide under normal conditions in vivo is probably less than 0.1% of the respiratory rate. Nonetheless, given the vast throughput of electrons through mitochondrial respiration, even this fraction of a percent could be biologically significant.

A long-standing hypothesis suggests high levels of mtDNA mutations may increase mitochondrial ROS production leading to cell degeneration or death (e.g., [12]). Some mtDNA mutations are indeed associated with elevated ROS production. For example, human fibroblasts from patients with complex I deficiencies produce superoxide at greater rates than those from healthy individuals [24]. Similarly, *Caenorhabditis elegans* harbouring a mutation in a subunit of succinate dehydrogenase cytochrome b show evidence of oxidative stress [25]. Another strain of *C. elegans*, with a complex I mutation, is also short-lived and hypersensitive to elevated oxygen levels, suggesting a connection between mtDNA mutations and ROS production [26]. However, in the two strains of mtDNA mutator mice recently produced [7,8], no increase in mitochondrial ROS production was observed in cultured fibroblasts [27] or isolated tissue mitochondria [8], despite accelerated rates of aging in these mice [7,8]. Additionally, no evidence for elevated levels of oxidative damage was found in heart or liver tissue [8]. Thus, while ROS mediates mtDNA oxidative damage and mutagenesis, relatively high loads of randomly distributed mtDNA mutations do not appear to propagate a 'viscious cycle' of ROS overproduction. While these mtDNA mutations interfere with mitochondrial and cellular functions, the specific mechanisms by which this occurs remain to be elucidated.

3. Oxidative DNA modifications

Oxidative damage to DNA can result in base or sugar adducts, single and double strand breaks, as well as cross-links to other molecules [28,29]. Many of these DNA modifications are mutagenic, and thought to contribute to cancer, aging and neurodegenerative diseases [28]. ROS induces single and double strand breaks in the DNA backbone and crosslinks with other macromolecules. In the event that $HO^{\cdot}$ abstracts a proton from the deoxyribose, strand breaks may occur due to the production of a sugar radical which promotes the release of the affected DNA base [30]. When whole cells or isolated chromatin are exposed to ionizing radiation, cross-links can occur between DNA bases and amino acid residues in nuclear proteins [31,32].

More than 20 base lesions have been identified as resulting from hydroxyl radical attack on the double bonds of the DNA bases and/or the abstraction of a proton from the methyl group of thymine and each of the C − H bonds of the sugar backbone. Chemical modifications of both pyrimidine and purine bases have been characterized in detail (as reviewed in [33]). The most common pyrimidine lesion, thymine glycol [34] has been determined to block replication and transcription rather than cause mutagenesis [35]. Generally, $HO^{\cdot}$ radicals add to the C4, C5 and C8 position of purines, thereby generating $HO^{\cdot}$ adduct radicals. By far the most common and extensively studied purine modification is 8-oxodeoxyguanine (8-oxodG). 8-oxodG is formed by the addition of $HO^{\cdot}$ to C-8 of guanine. In the *syn* conformation 8-oxodG has been found to cause GC → TA transversions [36]. The mitochondrial polymerase γ also introduces this signature mutation at high frequency when replicating past 8-oxodG [37]. 8-oxodG in mtDNA also results from mis-incorporation of 8-oxodGMP opposite adenine, and this may be a quantitatively more significant mechanism [38].

Damage to mtDNA has been suggested to have a greater impact on cellular function than damage to nuclear DNA, despite the presence of multiple copies of mtDNA per mitochondrion and per cell [39,40]. Levels of 8-oxodG in mtDNA are several times higher than in nuclear DNA [11]. It is likely that other oxidative lesions are also higher in mtDNA than in nuclear DNA. Elevated levels of mtDNA oxidative damage have been identified in aging, neurodegenerative diseases and several other pathophysiological conditions (summarized in [41]), and may contribute to mitochondrial dysfunction.

4. Mitochondrial respiratory dysfunction and mitochondrial genomic instability

Mitochondrial genomic instability can affect respiration. ρ^0 cells without mtDNA do not respire [5]. Specific mutations in mtDNA can abolish or severely compromise the activities of respiratory complexes when they are present in a sufficiently

high proportion of mtDNA molecules (see [42] for overview). Surprisingly, given the presence of multiple copies, the random accumulation of mutations in mtDNA due to the targeted disruption of polymerase γ exonuclease (proofreading) activity also causes respiratory dysfunction [7].

A number of studies have shown a rapid onset of mtDNA instability following oxidative stress. Cultured cells exposed to chemical oxidants [43] or ionizing radiation [44] rapidly accumulate mtDNA mutations. Respiratory poisons like adriamycin, which generates matrix superoxide via interaction with complex I, also cause mtDNA mutagenesis in *S. cerevisiae* [45,46]. Similarly, farnesol stimulates ROS production by interference with respiration and mtDNA mutagenesis [47]. These latter studies appear to demonstrate the ability of respiration-derived ROS to compromise mitochondrial genomes. The enhancement of this process in mitochondrial SOD null cells [46] also suggests that this is specifically a ROS-mediated effect. However, ROS-independent mechanisms cannot be completely excluded. Interference with respiration may alter the import or synthesis of nucleotides needed for mtDNA replication [48,49]. Faithful mtDNA replication is dependent upon the maintenance of these pools [50,51]. Also, dysregulation of matrix ATP levels may affect the integrity of ATP-consuming mtDNA maintenance processes. Thus, functional mitochondrial respiration, including ADP phosphorylation, membrane potential and relatively low rates of ROS production, may all be necessary to maintain the stability of the mitochondrial genome.

5. Repair and maintenance of mtDNA

Given the potential for oxidative mtDNA damage to compromise function, processes that remove and/or repair damage are potentially capable of preventing catastrophic declines in respiratory competence and/or cellular degeneration. A number of DNA repair activities and pathways have been identified and characterized in both vertebrate and yeast mitochondria. Perhaps, the most studied DNA repair pathway in mitochondria is the base excision repair (BER) pathway, present in all eukaryotic cells studied to date. However, evidence for mismatch and recombinational repair of mtDNA in *S. cerevisiae* is strong. Some recent studies have suggested that both pathways could operate also in vertebrate mitochondria, though this is much more equivocal.

A mitochondrial uracil DNA glycosylase was identified in mitochondrial fractions of human cells in 1980 [52], but failure of mammalian mitochondria to remove UV-induced pyrimidine dimers [53] and certain other adducts [54,55] suggested that no active DNA repair was present in mammalian mitochondria. Subsequently, however, enzymatic removal of O6-ethylguanine from rat mitochondria was documented [56], and a mitochondrial endonuclease specific for apurinic/apyrimidinc sites was identified and characterized [57]. This was followed by the identification of other DNA glycosylases, including Ogg1 which excises 8-oxodG [58] and Nth1 which excises oxidized pyrimidines [59,60]. A mtDNA ligase [61,62] has been identified in mitochondria. One of the first studies indicating that mammalian mitochondria exhibit a BER capacity involved studying the removal of alkali-labile sites in rat insulinoma cells following treatment with streptozotocin, an alkylating antibiotic [63]. Complete repair of abasic sites has been demonstrated in vitro with enzymes isolated from mitochondria of the frog *Xenopus laevis* [64]. Complete repair and resynthesis of a uracil-containing oligonucleotide by mammalian mitochondrial lysates can be accomplished in vitro [65]. It has thus become widely accepted that mitochondria are capable of base excision repair (BER). Indeed, repair of some forms of oxidative DNA damage occurs more efficiently in mitochondria than in the nucleus of cultured hamster cells [66], suggesting that the mitochondria capacity for BER is comparable to that of the nucleus.

The complete mitochondrial BER pathway requires at least four enzymes: a DNA glycosylase, an AP-endonuclease (or alternative mechanism for processing abasic sites), DNA polymerase γ and DNA ligase. During the first step of this pathway, a DNA glycosylase removes the damaged base leaving an abasic site. AP-endonuclease recognizes this abasic site and processes it, producing a 3′OH and 5′ phosphate DNA break. From this point there are two sub-pathways, short patch and long patch BER that can occur in the nucleus. However, only the short patch pathway, which replaces a single damaged base, has been demonstrated in mitochondria [28]. The identities of BER enzymes in mitochondria are not yet all known. For example, two AP endonucleases have been localized to mammalian mitochondria: APE1 [67–69] and APE2 [70]. Also, AP endonuclease-independent mechanisms of BER have been identified in the nucleus [71] and could be present in mitochondria, though they have not yet been identified. Thus, more work is necessary to characterize the nature of the BER pathway in mammalian mitochondria.

In addition to BER, several other DNA repair mechanisms have been demonstrated in mammalian mitochondria. For example, removal of 8-oxodGTP, an oxidized form of dGTP, from the mitochondrial matrix is catalyzed by a mitochondrially localized human MutT homolog (MTH1) [72], which possesses both 8-oxo-dGTPase and 8-oxodATPase activities [73]. The former activity helps to prevent the accumulation of 8-oxodG in DNA due to the misincorporation of 8-oxo-dGTP [74]. An isoform of MutY homologue (MYH) has also been identified in mammalian mitochondria [75]. This activity removes adenine mispaired with 8-oxodG in DNA, thus providing another opportunity for correct pairing of cytosine with 8-oxodG. Other recent evidence suggests that mammalian mitochondria have a capacity for mismatch repair. Mason et al. [76] demonstrated removal of GT and GG mismatches by rat mitochondrial lysates. There are contradictory results as to whether this and/or other mismatch repair activities are catalyzed by the protein MSH2 [76,77]. Also, Msh5 and Mlh1, mammalian MutS and MutL homologues respectively, have been identified in a proteomic analysis of mouse mitochondria [2]. Together, these results suggest that mitochondria possess one or more mismatch repair activities that have yet to be identified conclusively.

While mammalian mitochondria have the ability to efficiently remove sugar and base lesions through the BER pathway, more complex damage, such as DNA cross-links and double strand breaks, require recombination repair mechanisms. However, the existence of recombination repair in mammalian mitochondria also remains controversial [78–80]. Initial indications that homologous repair may be present in mitochondria stem from investigations involving the repair of cisplatin interstrand crosslinks, which are efficiently removed from hamster cells [81]. Further support for the presence of homologous repair was gained through demonstration of recombination of plasmids by human mitochondrial protein extracts, thereby proving that mammalian mitochondria possess the enzymes required to catalyze homologous repair [82]. Indeed, double strand break repair protein Mre11A has been identified in a proteomic analysis of mouse liver mitochondrial inner membrane [83], and numerous other proteins involved in recombination have been reported in mouse mitochondria (see [84]). In vivo tests for the existence of mtDNA recombination in mammalian cells have yielded disparate results however, ranging from absence of recombination [85], to rare occurrence of recombination [86], to a relatively common recombination of mtDNA molecules [87,88]. Thus, mtDNA recombination capacity may be tissue specific or selective recruitment of the necessary proteins may occur only in some physiological contexts. Further study seems to be required to determine the extent and importance of mtDNA recombination repair in the mitochondria of mammalian cells.

mtDNA repair has been extensively studied in the yeast *S. cerevisiae*, which has provided an excellent model system. Yeast mitochondria also have a BER pathway that participates in repair of oxidative DNA damage (reviewed in [89]). Uracil DNA glycosylase (Ung1p), oxoguanine DNA glycosylase (Ogg1p) and the oxidized pyrimidine glycosylase (Ntg1p) have all been identified in yeast mitochondria. The apurinic/apyrimidinic endonuclease Apn1 is the major AP endonuclease activity in yeast mitochondria [90]. Yeast mitochondria also possess a robust mismatch repair activity catalyzed by Msh1p, which repairs G:A mispairs generated by replication past 8-oxodG, as well as other mismatches [91]. Recombinational repair appears to be better developed in yeast than mammalian mitochondria. Mrh1p, involved in recombinational repair, plays a significant role in maintenance of mitochondrial function in the face of oxidative damage [92]. The helicase Pif1p is also involved in repair of oxidative damage in mtDNA [93]. In addition, several proteins of unknown function have also been identified as participating in the repair of oxidative mtDNA damage in yeast. For example, Mgm101p, a DNA binding component of the mitochondrial nucleoid is important in repair of oxidative mtDNA damage [94].

6. Physiological regulation of mtDNA BER

By far, the most extensively studied and most conclusively demonstrated pathway for mtDNA repair is BER. The first step in the repair of mtDNA base modifications is the recognition and removal of the oxidized base. Removal of the common oxidative lesion 8-oxodG is catalyzed by Ogg1. Ogg1 is encoded in the nuclear genome and mitochondrial- and nuclear-directed transcripts are produced from the same gene [95]. Thus, regulation of Ogg1 expression in mitochondria and the nucleus will share some common features. The Ogg1 gene promoter lacks TATA and CAAT boxes and, in HeLa cells, its transcription is invariant throughout the cell cycle [96]. However, a binding site for nuclear respiratory factor 2 (Nrf2) is also present [97], suggesting expression is sensitive to oxidative stress [98].

Experimental evidence for induction of Ogg1 expression by oxidative stress is contradictory. Typically, Ogg1 expression has been found to be relatively unresponsive to oxidative stress in experiments with cultured cells. Neither H_2O_2 nor lipopolysaccharide (LPS) stimulate Ogg1 expression in HeLa cells [96], and similar results were found for other cell lines [99]. Low doses of ionizing radiation also fail to stimulate Ogg1 expression in human lymphoblastoid cells [100]. Similarly, Ogg1 levels by Western blot change relatively little in ρ^0 143B osteosarcoma cells [67], despite the much reduced ROS generation in these cells. However, during in vivo experiments, physiological conditions associated with intracellular oxidative stress do elevate Ogg1 protein levels and activity. Ogg1 protein levels appear to increase up to 60% (although not statistically significant) in humans subjected to long-term hypoxia at high altitude [101]. Ogg1 protein levels are elevated 2-fold in brain tissue of mice following an episode of ischemia–reperfusion, presumably due to intracellular oxidative stress induced by this treatment [102]. Ogg1 protein levels are also elevated in the pancreatic islet cells of humans suffering from type II diabetes [103], another disease associated with intracellular oxidative stress. Interestingly, a recent study has shown that, although cultured human HCT116 cells do not upregulate Ogg1 protein levels in response to 100 μM H_2O_2, the alkylating agent methylmethane sulfonate (MMS) elicits an up to 4-fold increase [97]. Two interpretations of these disparate results are that: (1) regulation of Ogg1 expression in rapidly dividing cells (e.g., in culture) is controlled primarily by factors other than ROS, whereas in post-mitotic cells in vivo ROS plays a more important role in regulation, and (2) that H_2O_2 is not a physiological regulator of Ogg1 expression, though some other ROS may be. However, taken together the results to date suggest the importance of developing in vivo animal models for investigating physiological regulation of mitochondrial BER.

While overall cellular expression levels of Ogg1 are likely to be important in regulating its activity in mitochondria, the import and specific activity of Ogg1 are also effective means for regulating its activity within this compartment. Mitochondrial Ogg1 activity increases with aging, independent of nuclear Ogg1 activity ([104]; but see also [105]). One possible explanation of this observation is altered subcellular distribution of Ogg1 in older animals. Szczesny et al. [106] demonstrated defective Ogg1 import into mitochondria in aging mice and also in senescent human fibroblasts. This would effectively lower the intramitochondrial activity of the enzyme, despite

apparently unchanged protein levels. Ogg1 is also post-translationally modified by reversible phosphorylation of serine/threonine residues, and this modification alters its specific activity [107]. However, no information is yet available on the relationship between Ogg1 phosphorylation state and its mitochondrial activity. Ogg1 activity is also modulated by its interaction with nitric oxide (NO) via direct nitrosylation of the enzyme [108]. As NO is produced within the mitochondrial matrix ([109]; but see also [110]), this could also represent a mechanism for regulating Ogg1 activity. In conclusion, various physiologically relevant effectors might regulate mitochondrial Ogg1 activity independently of transcriptional regulators, thus allowing specific control of its activity in mitochondria. However, much work is required to determine whether such regulatory mechanisms are active and physiologically relevant in mitochondria.

While little information is available regarding the regulation of other DNA glycosylase activities in mitochondria, more is known about the regulation of subsequent steps in the BER pathway. The second step in the pathway is believed to be catalyzed by an apurinic/apyrimidinic endonuclease (APE) which may be APE1, though this has not been definitively demonstrated. APE1 is strongly regulated by intracellular redox [111]. Its translocation to mitochondria is altered in ρ^0 cells, which have a substantially decreased APE activity and lowered APE1 protein levels by Western blot [67]. Exposure of ρ^0 cells to H_2O_2 increases APE activity in both cellular and mitochondrial lysates to normal (wild type ρ^+) levels. Also, in wildtype cells oxidative stress induces translocation of APE1 into mitochondria [67]. However, the significance of upregulating mitochondrial APE1 in response to oxidative stress is not known. Addition of pure recombinant APE1 to an in vitro assay of BER in ρ^0 mitochondria does not stimulate repair of a uracil-containing oligonucleotide [63]. This probably indicates that mitochondrial APE activity is in great excess and has little control over total BER activity. Most of the control over BER pathway activity probably lies with polymerase γ.

Polymerase γ has two activities relevant to BER. In addition to its polymerase activity, the catalytic subunit of the enzyme has a 5′-deoxyribose phosphate lyase (dRPase) activity that may be 'rate-limiting' for the BER pathway [112,113], as has been suggested for polymerase β in nuclear BER [114]. Therefore, overall BER pathway activity may be largely controlled by polymerase γ. Polymerase γ expression is strongly regulated by factors that increase intracellular oxidative stress. For example, administration of adriamycin, an anti-cancer drug that is sequestered in mitochondria where it interacts with complex I to produce superoxide, elicits an increase in polymerase γ activity in rat hearts [115]. Similarly, γ-ray irradiation of rats increases polymerase γ activity 3-fold [116]. Injection of LPS, which generates intracellular oxidative stress via tumour necrosis factor (TNF)-stimulated pathways, induces polymerase γ expression in rat heart and liver [117,118]. Thus, polymerase γ expression and activity is upregulated by oxidative stress. Presumably, this involves an upregulation of polymerase γ protein levels in mitochondria, such that dRPase activity would also be increased. A likely model for regulation of BER pathway activity therefore involves the ROS-mediated regulation of dRPase activity. This would make mtDNA BER responsive to oxidative stress but, as polymerase γ is both the replicative and repair polymerase in mitochondria, it may simultaneously stimulate mtDNA replication. It is not clear whether the replication and repair of mtDNA can be differentially regulated. In replicating cultured cells, the enzymes of BER and replication can be isolated in the same insoluble fraction [119], and may in fact be physically associated with each other. Thus, interactions amongst BER proteins and other mitochondrial components could allow a differential regulation of repair and replication. In summary, there is evidence for pre- and post-translational modulation of the expression levels and activities of mtDNA BER enzymes (Fig. 1). The physiological importance of these various mechanisms of regulatory control remain to be elucidated.

7. Organization of mtDNA repair

Two kinds of evidence suggest that mtDNA repair occurs in a complex associated with the mitochondrial inner-membrane: immunohistochemical localization using electron microscopy shows Ogg1 and MYH particles at the inner membrane (reviewed in [103]); also, subfractionation of isolated mitochondria identifies all of the major BER activities in an insoluble particulate fraction that contains inner membrane proteins [119]. With the exception of AP endonuclease activity,

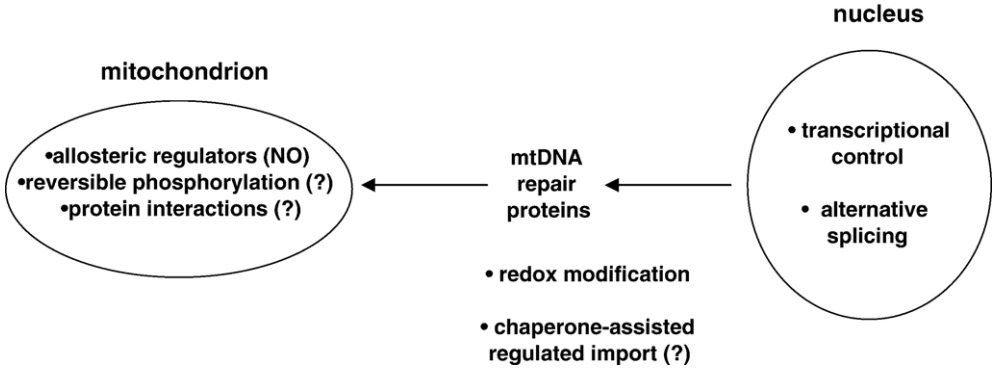

Fig. 1. Physiological regulation of mtDNA repair activities. Shown are various sites of pre- and post-translational events shown or thought (=?) to participate in the regulation of DNA repair activities in mitochondria.

the majority of each individual BER activity was recovered in the particulate fraction. Complete repair and resynthesis of a uracil-containing oligonucleotide was also demonstrated in this fraction. Further, the association of BER proteins with the inner membrane-containing fraction was dependent upon interactions with mtDNA, as it occurred also in ρ^0 cells but could be disrupted by low salt concentrations. In the same fraction, other proteins involved in mtDNA transactions, including mtTFA and endonuclease G, were also identified. Together, these data are consistent with the presence of an inner membrane-associated mtDNA repair complex [120]. The nature of this hypothetical complex remains to be elucidated, but it could be a component of the mitochondrial nucleoid. Nucleoids are inner membrane-associated structures dispersed in mitochondria containing multiple copies of mtDNA as well as polymerase γ and numerous other proteins involved in mtDNA maintenance and replication [121,122].

Nucleoids have been best studied in the yeast, *S. cerevisiae* ([123] for review). Yeast nucleoids contain multiple copies of mtDNA and various proteins associated with replication, maintenance and transcription. They appear to be associated with the proteins Mdm10p, Mdm12p and Mmm1p, which form a double membrane-spanning structure that interacts with the cytoskeleton [124]. Interestingly, the yeast mitochondrial polymerase Mip1p cosediments with an inner membrane-containing particulate fraction [125], as polymerase γ does in human mitochondria [119]. Indeed, Mip1p has been shown to be present in nucleoids [126]. Thus, it is possible that BER proteins may also associate with nucleoids. If this is true, reversible associations/dissociations and interactions may provide an additional means of regulating BER functions. Further study will clarify whether this model is correct.

8. Biological significance of mtDNA repair

Various experimental models have been used to study the physiological significance of mtDNA repair, including overexpression and underexpression. Careful experiments with HeLa cells have demonstrated the ability of enhanced mitochondrial Ogg1 activity to preserve cell viability under conditions of oxidative stress [127]. In these experiments, the nuclear Ogg1 isoform was expressed as a chimera with the MnSOD mitochondrial targeting sequence. Mitochondrial Ogg1 activity was enhanced approximately 10-fold. Viability of cells exposed to micromolar concentrations of menadione, which generates superoxide in the matrix, was significantly enhanced by overexpression of Ogg1. Similar results have been obtained with pulmonary artery endothelial cells [128], and under a conditional expression regime [129]. The enhanced resistance to cellular oxidative stress is not restricted to Ogg1 overexpression. The *Escherichia coli* endonucleases III and VIII, which remove oxidized pyrimidines from DNA, confer a similar resistance to oxidative stress when expressed in cultured HeLa cells [130]. Taken together, these studies indicate that enhanced mtDNA BER confers resistance to cell death caused by oxidative stress in cultured cells. However, because all three enzymes are bifunctional, with both glycosylase and AP lyase activities, it is not clear specifically which activity is responsible for the enhanced stress resistance. As the authors suggest [130], experiments with a recombinant lyase activity alone will resolve this question.

Despite this apparent ability of glycosylase overexpression to promote the preservation of mitochondrial function, various strains of mice null for DNA glycosylases which are known to operate in mitochondria do not appear to be adversely affected, and show no signs of mitochondrial dysfunction. For example, Ogg1$^{-/-}$ mice, which accumulate 8-oxodG in mtDNA to levels 10- to 20-fold higher than wildtype controls [131] develop, grow and age normally ([132]; but see also [133]) and show no evidence of mitochondrial dysfunction [41]. Similarly UDG$^{-/-}$ mice, which lack mitochondrial uracil incision activity, are apparently unaffected by this deficit under normal conditions [134]. Data on mitochondrial bioenergetic function in these mice has not been presented. In yeast (*S. cerevisiae*) in which mitochondrial UDG activity has been specifically targeted by an inhibitor [135] there is no evidence of increased mutagenesis of mtDNA.

Interestingly however, UDG$^{-/-}$ mice are susceptible to neuronal injury following ischemia–reperfusion [134]. Cultured fibroblasts and primary cortical neurons from UDG$^{-/-}$ mice also have decreased resistance to nitrative stress caused by exposure to nitroprusside, which produces nitric oxide. Although the UDG$^{-/-}$ mice are deficient in both mitochondrial and nuclear activities, the authors suggest that these phenotypes are due to mtDNA damage, on the basis of apparent mitochondrial dysfunction and the observation that mitochondrial (but not nuclear) UDG activity is dramatically upregulated following exposure. A plausible interpretation of these studies together is that the DNA glycosylases are not essential for preservation of mitochondrial function under normal conditions, but play an important role in enhancing cell survival under conditions of extreme oxidative stress. It will be interesting to test this hypothesis, for example, by investigating the susceptibility of Ogg1$^{-/-}$ mice to ischemia–reperfusion injury and treatments that augment intramitochondrial ROS production.

What is the mechanism by which mitochondrial glycosylase activities rescue cells from death? Overexpression of Ogg1 in mitochondria provides protection against cell death occurring within 24 h of exposure to an oxidative stress [127]. While this is consistent with a mutation accumulation mechanism for mitochondrial dysfunction, it may also result from effects on transcription. The T7 RNA polymerase, which bears substantial sequence similarity to the mitochondrial RNA polymerase [136], is inhibited by oxidative lesions including 8-oxodG [137]. Lesions can either block transcription or introduce mutations into transcripts, and both effects can be substantial. For example, adenine is misincorporated opposite 8-oxodG with 70% frequency. Oxidative damage has been shown to decrease the level of a mitochondrial transcript encoding subunits of the ATP synthase and cytochrome oxidase [138]. It will be interesting to investigate the effect of mtDNA BER deficiency in the immediate and short-term following oxidative stress.

Table 1
Mitochondrial genomic instability caused by deficiencies of BER and other proteins involved in mtDNA repair and maintenance

mtDNA repair deficiency	Fold-increase in mtDNA point mutations	Increased incidence of petite colonies	Reference
ogg1Δ	10	+	[145]
ntg1Δ	2	+	[93]
ung1Δ	3	+	[143]
apn1Δ	1	n.d.	[144]
msh1/MSH1	7	+++	[146]
pif1Δ	29	++	[93]
pif1Δntg1Δ	56	+++	[93]
abf2Δntg1Δ	50	++	[93]
mgm101Δ	n.d.	+++	[94]

'+' denotes qualitatively the increase in petite colony formation due to gene deletions. n.d. = no data.

The direct impact of deficient mtDNA repair is more readily addressed in yeast, where the frequency with which specific mutations occur can be measured using antibiotic selection assays (see 139 for review). For example, point mutations at specific nucleotide positions in mtDNA can confer resistance to the antibiotics erythromycin [139] and chloramphenicol [140], or to the ATP synthase inhibitor oligomycin [141,142]. This approach has been used to quantify the incidence of mtDNA point mutations due to Ogg1p [91], Ung1p [143], Ntg1p [93,46] and Apn1p [144] deficiencies in yeast grown on glycerol medium, which requires respiration. Individual BER enzyme deficiencies increase the incidence of specific mtDNA point mutations by up to 10-fold (Table 1). Ogg1p and Ung1p deficiencies also lead to approximately 3-fold increases in the rate of generation of petite colonies that cannot respire [143,145].

Analyses of the frequency of mtDNA loss or mutation are also useful for investigating the relative importance of specific mtDNA repair activities. For example, despite being relatively tolerant to deficiencies of BER glycosylases, yeast mitochondria rapidly lose respiratory competence in the absence of mismatch repair protein Msh1p [91,146], or the mtDNA helicase Pif1p [93,46], suggesting that the absence of individual DNA glycosylases may be less debilitating than deficiencies in mismatch or recombinational repair in yeast mitochondria.

What is the significance of these mtDNA repair deficiencies in terms of mitochondrial respiratory function? Petite colonies represent an extreme endpoint of complete respiratory incompetence, and antibiotic resistance assays measure the rate of occurrence of a small subset of specific mutations. On the other hand, oxidative mtDNA damage is likely to be randomly distributed and lead to many different kinds of lesions distributed throughout mitochondrial genomes. This enhanced accumulation of random mutations would be expected to partially compromise oxidative phosphorylation (as is observed in mtDNA mutator mice; [7]), or perhaps increase ROS production (though this has not been observed in mtDNA mutator mice [8]). Direct measurements of oxidative phosphorylation and ROS production in yeast mitochondria from mtDNA repair-deficient strains are required to assess the quantitative relationships between mutation load and mitochondrial function in yeast.

9. Conclusions and prospectus

Whereas many aspects of mtDNA repair, particularly in mammalian mitochondria, remain poorly characterized or contentious, it is now widely accepted that mitochondria possess a relatively robust BER pathway that participates in the removal of oxidative and other forms of damage. However, the organization, physiological regulation, and biological significance of mitochondrial BER are only beginning to be characterized. Given the recent demonstration of a causal link between mtDNA hypermutability, mitochondrial dysfunction, and cellular degeneration/death [7–10], it will be important to understand the mechanisms of BER in mitochondria and measure the contribution that the pathway makes to maintaining mitochondrial genomic stability in health and disease.

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References

[1] S.W. Taylor, E. Fahy, B. Zhang, G.M. Glenn, D.E. Warnock, S. Wiley, A. N. Murphy, S.P. Gaucher, R.A. Capaldi, B.W. Gibson, S.S. Ghosh, Characterization of the human heart mitochondrial proteome, Nature Biotech. 21 (2003) 281–286.
[2] V.K. Mootha, J. Bunkenborg, J.V. Olsen, M. Hjerrild, J.R. Wisniewski, E. Stahl, M.S. Bolouri, H.N. Ray, S. Sihag, M. Kamal, N. Patterson, E.S. Lander, M. Mann, Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria, Cell 115 (2003) 629–640.
[3] A. Sickmann, J. Reinders, Y. Wagner, C. Joppich, R. Zahedi, H.E. Meyer, B. Schonfisch, I. Perschil, A. Chacinska, B. Guiard, P. Rehling, N. Pfanner, C. Malsinger, The proteome of *Saccharomyces cerevisiae* mitochondria, Proc. Natl. Acad. Sci. U. S. A. 23 (2003) 13207–13212.
[4] A. Chacinska, P. Rehling, Moving proteins from the cytosol into mitochondria, Biochem. Soc. Trans. 32 (2004) 774–776.
[5] J. Shen, N. Khan, L.D. Lewis, R. Armand, O. Grinberg, E. Demidenko, H. Swartz, Oxygen consumption rates and oxygen concentration in molt-4 cells and their mtDNA depleted (rho0) mutants, Biophys. J. 84 (2003) 1291–1298.
[6] D. Kang, N. Hamasaki, Alterations of mitochondrial DNA in common diseases and disease states: aging, neurodegeneration, heart failure, diabetes, and cancer, Curr. Med. Chem. 12 (2005) 429–441.
[7] A. Trifunovic, A. Wredenberg, M. Falkenberg, J.N. Spelbrink, A.T. Rovio, C.E. Bruder, Y.M. Bohlooy, S. Gidlof, A. Oldfors, R. Wibom, J. Tornell, H.T. Jacobs, N.G. Larsson, Premature aging in mice expressing defective mitochondrial DNA polymerase, Nature 429 (2004) 417–423.
[8] G.C. Kujoth, A. Hiona, T.D. Pugh, S. Someya, K. Panzer, S.E. Wohlgemuth, T. Hofer, A.Y. Seo, R. Sullivan, W.A. Jobling, J.D. Morrow, H. Van Remmen, J.M. Sedivy, T. Yamasoba, M. Tanokura, R. Weindruch, C. Leeuwenburgh, Mitochondrial DNA mutations, oxidative stress and apoptosis in mammalian aging, Science 309 (2005) 481–484.
[9] D. Zhang, J.L. Mott, P. Farrar, J.S. Ryerse, S.-W. Chang, M. Stevens, G. Denniger, H.P. Zassenhaus, Mitochondrial DNA mutations activate the mitochondrial apoptotic pathway and cause dilated cardiomyopathy, Cardiovasc. Res. 57 (2003) 147–157.
[10] J.L. Mott, D. Zhang, J.C. Freeman, P. Mikolajczak, S.W. Chang, H.P. Zassenhaus, Cardiac disease due to random mitochondrial DNA mutations is prevented by cyclosporine A, Biochem. Biophys. Res. Commun. 319 (2004) 1210–1215.
[11] M.L. Hamilton, H. Van Remmen, J.A. Drake, H. Yang, Z.M. Guo, K.

Kewitt, C.A. Walter, A. Richardson, Does oxidative damage to DNA increase with age? Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10469–10474.
[12] E. Dufour, N.-G. Larsson, Understanding aging: revealing order out of chaos, Biochim. Biophys. Acta 1658 (2004) 122–132.
[13] M.D. Brand, C. Affourtit, T.C. Esteves, K. Green, A.J. Lambert, S. Miwa, J.L. Pakay, N. Parker, Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins, Free Radic. Biol. Med. 37 (2004) 755–767.
[14] J. St. Pierre, J. Buckingham, S.J. Roebuck, M.D. Brand, Topology of superoxide production from different sites in the mitochondrial electron transport chain, J. Biol. Chem. 277 (2002) 44784–44790.
[15] A.A. Starkov, G. Fiskum, C. Chinopoulos, B. Lorenzo, S.E. Browne, M.S. Patel, M.F. Beal, Mitochondrial α-ketoglutarate dehydrogenase complex generates reactive oxygen species, J. Neurosci. 24 (2004) 7779–7788.
[16] H.R. McLennan, M. Degli Esposti, The contribution of mitochondrial respiratory complexes to the production of reactive oxygen species, J. Bioenerg. Biomembr. 32 (2000) 153–162.
[17] S. Miwa, J. St-Pierre, L. Partridge, M.D. Brand, Superoxide and hydrogen peroxide production by *Drosophila* mitochondria, Free Radic. Biol. Med. 35 (2003) 938–948.
[18] P.S. Brookes, Mitochondrial H(+) leak and ROS generation: an odd couple, Free Radic. Biol. Med. 38 (2005) 12–23.
[19] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs, Physiol. Rev. 59 (1979) 527–605.
[20] S.S. Korshunov, V.P. Skulachev, A.A. Starkov, High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria, FEBS Lett. 416 (1997) 15–18.
[21] J.F. Turrens, Mitochondrial formation of reactive oxygen species, J. Physiol. 552 (2003) 335–344.
[22] O.P. Habler, K.F.W. Messmer, The physiology of oxygen transport, Transfus. Sci. 18 (1997) 425–435.
[23] G. Barja, The quantitative measurement of H_2O_2 generation in isolated mitochondria, J. Bioenerg. Biomembranes 34 (2002) 227–233.
[24] B.H. Robinson, Human complex deficiency: clinical spectrum and involvement of oxygen free radicals in the pathogenicity of the defect, Biochim. Biophys. Acta 1364 (1998) 271–286.
[25] N. Ishii, M. Fujii, P.S. Hartman, M. Tsuda, K. Yasuda, N. Senoo-Matsuda, S. Yanase, D. Ayusawa, K. Suzuki, A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and aging in nematodes, Nature 394 (1998) 694–697.
[26] L.I. Grad, B.D. Lemire, Mitochondrial complex I mutation in *Caenorhabditis elegans* produce cytochrome oxidase deficiency, oxidative stress and vitamin-responsive lactic acidosis, Hum. Mol. Genet. 13 (2004) 303–314.
[27] N.-G. Larsson, The role of somatic mtDNA mutations in mammalian aging. Presented at: Mitochondrial DNA transactions in health and disease, Howard Hughes Medical Institute, September (2005).
[28] V.A. Bohr, Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells, Free Radic. Biol. Med. 32 (2002) 804–812.
[29] B.S. Berlett, E.R. Stadtman, Protein oxidation in aging, disease and oxidative stress, J. Biol. Chem. 272 (1997) 20313–20316.
[30] B. Halliwell, O.I. Aruoma, DNA damage by oxygen-derived species, FEBS 281 (1991) 9–19.
[31] L.K. Mee, S.J. Adelstein, Predominance of core histones in formation of DNA–protein crosslinks in γ-irradiated chromatin, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 2194–2198.
[32] E. Gajewski, M. Dizdaroglu, Hydroxyl radical induced cross-linking of cytosine and tyrosine in nucleohistone, Biochemistry 29 (1990) 977–980.
[33] M.S. Cooke, M.D. Evans, M. Dizdaroglu, J. Lunec, Oxidative DNA damage: mechanisms, mutation and disease, FASEB J. 17 (2003) 1195–1214.
[34] E.C. Friedberg, G.C. Walker, W. Siede, DNA Repair and Mutagenesis, ASM Press, Washington, DC, 1995.
[35] H. Ide, Y.W. Kow, S.S. Wallace, Thymine glycols and urea residues in M13 DNA constitute replicative blocks in vitro, Nucleic Acids Res. 13 (1985) 8035–8052.
[36] A.P. Grollman, M. Moriya, Mutagenesis by 8-oxoguanine: an enemy within, Trends Genet. 9 (1993) 246–249.
[37] K.G. Pinz, S. Shibutani, D.F. Bogenhagen, Action of mitochondrial DNA polymerase at sites of base loss or oxidative damage, J. Biol. Chem. 270 (1995) 9202–9206.
[38] K. Miyako, C. Takamatsu, S. Umeda, T. Tajiri, M. Furuichi, Y. Nakabeppu, M. Sekiguchi, N. Hamasaki, K. Takeshige, D. Kang, Accumulation of adenine DNA glycosylase-sensitive sites in human mitochondrial DNA, J. Biol. Chem. 275 (2000) 12326–12330.
[39] S.P. LeDoux, W.J. Driggers, B.S. Hollensworth, G.L. Wilson, Repair of alkylation and oxidative damage in mitochondrial DNA, Mutat. Res. 434 (1999) 149–159.
[40] V.A. Bohr, DNA damage and its processing. Relation to human disease, J. Inherit. Metab. Dis. 25 (2002) 215–222.
[41] J.A. Stuart, B.M. Bourque, N.C. Souza-Pinto, V.A. Bohr, No evidence of mitochondrial respiratory dysfunction in OGG1-null mice deficient in removal of 8-oxodeoxyguanine from mitochondrial DNA, Free Radic. Biol. Med. 38 (2005) 737–745.
[42] D.C. Wallace, Animal models for mitochondrial disease, Methods Mol. Biol. 197 (2002) 3–54.
[43] E. Mambo, X. Gao, Y. Cohen, Z. Guo, P. Talalay, D. Sidransky, Elecrophile and oxidant damage to mitochondrial DNA leading to rapid evolution of homoplasmic mutations, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1838–1843.
[44] S. Prithivirajsingh, M.D. Story, S.A. Bergh, F.B. Geara, K.K. Ang, S.S. Ismail, C.W. Stevens, T.A. Buchholz, W.A. Brock, Accumulation of the common mitochondrial DNA deletion induced by ionizing radiation, FEBS Lett. 571 (2004) 227–232.
[45] G. Kim, H. Sikder, K. Singh, A colony color method identifies the vulnerability of mitochondria to oxidative damage, Mutagenesis 17 (2002) 375–381.
[46] N.A. Doudican, B. Song, G.S. Shadel, P.W. Doetsch, Oxidative DNA damage causes mitochondrial genomic instability in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 25 (2005) 5196–5204.
[47] K. Machida, T. Tanaka, K. Fujita, M. Taniguchi, Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial electron transport chain in the yeast *Saccharomyces cerevisiae*, J. Bacteriol. 180 (1998) 4460–4465.
[48] M. Loffler, J. Jockel, G. Schuster, C. Becker, Dihydroorotat-ubiquinone oxidoreductase links mitochondria in the biosynthesis of pyrimidine nucleotides, Mol. Cell. Biochem. 174 (1997) 125–129.
[49] N. Gattermann, M. Dadak, G. Hofhaus, M. Wulfert, M. Berneburg, M.L. Loffler, H.A. Simmonds, Sever impairment of nucleotide synthesis through inhibition of mitochondrial respiration, Nucleosides Nucleotides Nucleic Acids 23 (2004) 1275–1279.
[50] S. Song, L.J. Wheeler, C.K. Mathews, Deoxyribonucleotide pool imbalance stimulates deletions in HeLa cell mitochondrial DNA, J. Biol. Chem. 278 (2003) 43893–43896.
[51] S. Song, Z.F. Pursell, W.C. Copeland, M.J. Longley, T.A. Kunkel, C.K. Mathews, DNA precursor asymmetries in mammalian tissue mitochondria and possible contribution to mutagenesis through reduced replication fidelity, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 4990–4995.
[52] C.T.M. Anderson, E.C. Friedberg, The presence of nuclear and mitochondrial uracil-DNA glycosylase in extract of human KB cells, Nucleic Acids Res. 8 (1980) 875–888.
[53] D.A. Clayton, J.N. Doda, E.C. Friedberg, The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 2777–2781.
[54] B.G. Niranjan, N.K. Bhat, N.G. Avadhani, Preferential attack of mitochondrial DNA by aflotoxin B1 during hepatocarcinogenesis, Science 215 (1982) 73–75.
[55] M. Miyaki, K. Yayagai, T. Ono, Strand breaks of mammalian mitochondrial DNA induced by carcinogens, Chem. Biol. Interact. 17 (1977) 321–329.
[56] M.S. Satoh, N. Huh, M.F. Rajewsky, T. Kuroki, Enzymatic removal of

O6-ehtlylguanine from mitochondrial DNA in rat tissues exposed to N-ethyl-N-nitrosourea in vivo, J. Biol. Chem. 263 (1988) 6854–6856.
[57] A.E. Tomkinson, R.T. Bonk, S. Linn, Mitochondrial endonuclease activities specific for apurinic/apyrimidinic sites in DNA from mouse cells, J. Biol. Chem. 263 (1988) 12532–12537.
[58] T.A. Rosenquist, D.O. Zharkov, A.P. Grollman, Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 7429–7434.
[59] M. Takao, H. Aburatani, K. Kobayashi, A. Yasui, Mitochondrial targeting of human DNA glycosylases for repair of oxidative DNA damage, Nucleic Acids Res. 26 (1998) 2917–2922.
[60] B. Karahalil, N.C. Souza-Pinto, J.L. Parsons, R.H. Elder, V.A. Bohr, Compromised incision of oxidized pyrimidines in liver mitochondria of mice deficient in NTH1 and OGG1 glycosylases, J. Biol. Chem. 278 (2003) 33701–33707.
[61] C.J. Levin, S.B. Zimmerman, A DNA ligase from mitochondria of rat liver, Biochem. Biophys. Res. Commun. 69 (1976) 514–520.
[62] U. Lakshminpathy, C. Campbell, The human DNA ligase III gene encodes nuclear and mitochondrial proteins, Mol. Cell. Biol. 19 (1999) 3869–3876.
[63] C.C. Pettepher, S.P. LeDoux, V.A. Bohr, G.L. Wilson, Repair of alkali-labile sites within the mitochondrial DNA of RINr 38 cells after exposure to the nitrosourea streptozotocin, J. Biol. Chem. 266 (1991) 3113–3117.
[64] K.G. Pinz, D.F. Bogenhagen, Efficient repair of abasic sites in DNA by mitochondrial enzymes, Mol. Cell. Biol. 18 (1998) 1257–1265.
[65] S.G. Nyaga, V.A. Bohr, Characterization of specialized mtDNA glycosylases, Methods Mol. Biol. 197 (2002) 227–244.
[66] T. Thorslund, M. Sunesen, V.A. Bohr, T. Stevnsner, Repair of 8-oxoG is slower in endogenous nuclear genes than in mitochondrial DNA and is without strand bias, DNA Repair 1 (2002) 261–273.
[67] B. Frossi, G. Tell, P. Spessotto, A. Colombatti, G. Vitale, C. Pucillo, H_2O_2 induces translocation of APE/Ref-1 to mitochondria in the Raji B-cell line, J. Cell. Physiol. 193 (2002) 180–186.
[68] J.A. Stuart, K. Hashiguchi, D.M. Wilson III, W.C. Copeland, N.C. Souza-Pinto, V.A. Bohr, DNA base excision repair activities and pathway function in mitochondrial and cellular lysates from cells lacking mitochondrial DNA, Nucleic Acids Res. 32 (2004) 2181–2192.
[69] B. Szczesny, S. Mitra, Effect of aging on intracellular distribution of abasic (AP) endonuclease 1 in the mouse liver, Mech. Ageing Dev. 9 (2005 June) (Electronic publication).
[70] D. Tsuchimoto, Y. Sakai, K. Sakumi, K. Nishioka, M. Sasaki, T. Fujiwara, Y. Nakabeppu, Human APE2 protein is mostly localized in the nuclei and to some extent in the mitochondria, while nuclear APE2 is partly associated with proliferating cell nuclear antigen, Nucleic Acids Res. 29 (2001) 2349–2360.
[71] L. Wiederhold, J.B. Leppard, P. Kedar, F. Karimi-Busheri, A. Rasouli-Nia, M. Weinfeld, A.E. Tomkinson, T. Izumi, R. Prasad, S.H. Wilson, S. Mitra, T.K. Hazra, AP endonuclease-independent DNA base excision repair in human cells, Mol. Cell 15 (2004) 209–220.
[72] D. Kang, J. Nishida, A. Iyama, Y. Nakabeppu, M. Furuichi, T. Fujiwara, M. Sekiguchi, K. Takeshige, Intracellular localization of 8-oxodGTPase in human cells, with special reference to the role of the enzyme in mitochondria, J. Biol. Chem. 270 (1995) 14659–14665.
[73] Y. Nakabeppu, Regulation of intracellular localization of human MTH1, OGG1, and MYH proteins for repair of oxidative DNA damage, Prog. Nucleic Acids Res. Mol. Biol. 68 (2001) 75–94.
[74] T. Tajiri, H. Maki, M. Sckiguchi, Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in *Escherichia coli*, Mutat. Res. 336 (1995) 257–267.
[75] T. Ohtsubo, K. Nishioka, Y. Imaiso, S. Iwai, H. Shimokawa, H. Oda, T. Fujiwara, Y. Nakabeppu, Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria, Nucleic Acids Res. 28 (2000) 1355–1364.
[76] P.A. Mason, E.C. Matheson, A.G. Hall, R.N. Lightowlers, Mismatch repair activity in mammalian mitochondria, Nucleic Acids Res. 31 (2003) 1052–1058.
[77] Z.Y. Chen, R. Felsheim, P. Wong, L.B. Augustin, R. Metz, B.T. Kren, C.J. Steer, Mitochondria isolated from liver contain the essential factors required for RNA/DNA oligonucleotide-targeted gene repair, Biochem. Biophys. Res. Commun. 285 (2001) 188–194.
[78] A. Eyre-Walker, P. Awadalla, Does human mtDNA recombine? J. Mol. Evol. 53 (2001) 430–435.
[79] B. Pakendorf, M. Stoneking, Mitochondrial DNA and human evolution, Annu. Rev. Genomics Hum. Genet. 6 (2005) 165–183.
[80] N.B. Larsen, M. Rasmussen, L.J. Rasmussen, Nuclear and mitochondrial DNA repair: similar pathways? Mitochondrion 5 (2005) 89–108.
[81] S.P. LeDoux, G.L. Wilson, E.J. Beecham, T. Stevnsner, K. Wassermann, V.A. Bohr, Repair of mitochondrial DNA after various types of DNA damage in Chinese hamster ovary cells, Carcinogenesis 13 (1992) 1967–1973.
[82] B. Thyagarajan, R.A. Padua, C. Campbell, Mammalian mitochondria possess homologous DNA recombination activity, J. Biol. Chem. 271 (1996) 27536–27543.
[83] S. Da Cruz, I. Xenarios, J. Langridge, F. Vilbois, P.A. Parone, J.C. Martinou, Proteomic analysis of the mouse liver mitochondrial inner membrane, J. Biol. Chem. 278 (2003) 41566–41574.
[84] H. Prokisch, C. Andreoli, U. Ahting, K. Heiss, A. Ruepp, C. Scharfe, T. Meitinger, MitoP2: the mitochondrial proteome database—Now including mouse data, Nucleic Acids Res. 34 (.2006) D705–D711.
[85] J. Poulton, Mitochondrial DNA and genetic disease, Dev. Med. Child Neurol. 35 (1993) 833–840.
[86] A. Sato, K. Nakada, M. Akimoto, K. Ishikawa, T. Ono, H. Shitara, H. Yonekawa, J.-I. Hayashi, Rare creation of recombinant mtDNA haplotypes in mammalian tissues, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6057–6062.
[87] M. D'Aurelio, C.D. Gajewski, M.T. Lin, W.M. Mauck, L.Z. Shao, G. Lenaz, C.T. Moraes, G. Manfredi, Heterologous mitochondrial DNA recombination in human cells, Hum. Mol. Genet. 13 (2004) 3171–3179.
[88] G. Zsurka, Y. Kraytsberg, T. Kudina, C. Kornblum, C.E. Elger, K. Khrapko, W.S. Kunz, Recombination of mitochondrial DNA in skeletal muscle of individuals with multiple mitochondrial DNA heteroplasmy, Nat. Genet. 37 (2005) 873–876.
[89] F. Foury, J. Hu, S. Vanderstraeten, Mitochondrial DNA mutators, CMLS cell, Mol. Life Sci. 61 (2004) 2799–2811.
[90] D. Ramotar, C. Kim, R. Lillis, B. Demple, Intracellular localization of the Apn1 DNA repair enzyme of *Saccharomyces cerevisiae*. Nuclear transport signals and biological role, J. Biol. Chem. 268 (1993) 20533–20539.
[91] P. Dzierzbicku, P. Koprowski, M.U. Fikus, E. Malc, Z. Ciesla, Repair of oxidative damage in mitochondrial DNA of *Saccharomyces cerevisiae*: involvement of the MSH1-dependent pathway, DNA Repair 3 (2004) 403–411.
[92] F.H. Ling, E. Morioka, E. Ohtsuka, T. Shibata, A role for MHR1, a gene required for mitochondrial genetic recombination, in the repair of damage spontaneously introduced in yeast mtDNA, Nucleic Acids Res. 28 (2000) 4956–4963.
[93] T.W. O'Rourke, N.A. Doudican, M.D. Mackereth, P.W. Doetsch, G.S. Shadel, Mitochondrial dysfunction due to oxidative mitochondrial DNA damage is reduced through cooperative actions of diverse proteins, Mol. Cell. Biol. 22 (2002) 4086–4093.
[94] S. Meeusen, Q. Tieu, E. Wong, E. Weiss, D. Schieltz, J.R. Yates, J. Nunnari, Mgm101p is a novel component of the mitochondrial nucleoid that binds DNA and is required for the repair of oxidatively damaged mitochondrial DNA, J. Cell Biol. 145 (1999) 291–304.
[95] K. Nishioka, T. Ohtsubo, H. Oda, T. Fujiwara, D. Kang, K. Sugimachi, Y. Nakabeppu, Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs, Mol. Biol. Cell 10 (1999) 1637–1652.
[96] A. Denaut, S. Boiteux, J.P. Radicella, Characterization of the hOOG1 promoter and its expression during the cell cycle, Mutat. Res. 461 (2000) 109–118.
[97] M.-R. Lee, S.-H. Kim, H.-J. Cho, K.-Y. Lee, A.R. Moon, H.G. Jeong,

J.-S. Lee, J.-W. Hyun, M.-H. Chung, H.J. You, Transcription factors NF-YA regulate the induction of human OGG1 following DNA-alkylating agent methylmethane sulfonate (MMS) treatment, J. Biol. Chem. 279 (2004) 9857–9866.
[98] R. Venugopal, A.K. Jaiswal, Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes, Oncogene 17 (1998) 3145–3156.
[99] M. Inoue, G.P. Shen, M.A. Chaudhry, H. Galick, J.O. Blaisdell, S.S. Wallace, Expression of the oxidative base excision repair enzymes is not induced in TK6 human lymphoblastoid cells after low doses of ionizing radiation, Radiat. Res. 161 (2004) 409–417.
[100] P. Mistry, K.E. Herbert, Modulation of hOGG1 DNA repair enzyme in human cultured cells in response to pro-oxidant and antioxidant challenge, Free Radic. Biol. Med. 35 (2003) 397–405.
[101] C. Lundby, H. Pilegaard, G. van Hall, M. Sander, J. Calbet, S. Loft, P. Moller, Oxidative DNA damage and repair in skeletal muscle of humans exposed to high-altitude hypoxia, Toxicology 192 (2003) 229–236.
[102] L.-H. Lin, S. Cao, L. Yu, J. Cui, W.J. Hamilton, P.K. Liu, Up-regulation of base excision repair activity for 8-hydroxy-2′-deoxyguanosine in the mouse brain after forebrain ischemia–reperfusion, J. Neurochem. 74 (2000) 1098–1105.
[103] B. Tyrberg, K.A. Anachkov, S.A. Dib, J. Wang-Rodriguez, K.H. Yoon, F. Levine, Islet expression of the DNA repair enzyme 8-oxoguanosine DNA glycosylase (Ogg1) in human type 2 diabetes, BMC Endocr. Disord. 2 (2002) 2.
[104] N.C. deSouza-Pinto, B.A. Hogue, V.A. Bohr, DNA repair and aging in mouse liver: 8-oxodG glyosylase activity increase in mitochondrial but not in nuclear extracts, Free Radic. Biol. Med. 30 (2001) 916–923.
[105] D. Chen, G. Cao, T. Hastings, Y. Feng, W. Pei, C. O'Horo, J. Chen, Age-dependent decline of DNA repair activity for oxidative lesions in rat brain mitochondria, J. Neurochem. 81 (2002) 1273–1284.
[106] B. Szczesny, T.K. Hazra, J. Papaconstantinou, S. Mitra, I. Boldogh, Age-dependent deficiency in import of mitochondrial DNA glycosylases required for repair of oxidatively damaged bases, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10670–10675.
[107] J. Hu, S.Z. Imam, K. Hashiguchi, N.C. de Souza-Pinto, V.A. Bohr, Phosphorylation of human oxoguanine DNA glycosylase (alpha-OGG1) modulates its function, Nucleic Acids Res. 33 (2005) 3271–3282.
[108] M. Jaiswal, N.F. LaRusso, N. Nishioka, Y. Nakabeppu, G.J. Gores, Human Ogg1, a protein involved in the repair of 8-oxoguanine, is inhibited by nitric oxide, Cancer Res. 61 (2001) 6388–6393.
[109] R. Radi, A. Cassina, R. Hodara, C. Quijano, L. Castro, Peroxynitrite reactions and formation in mitochondria, Free Radic. Biol. Med. 33 (2002) 1451–1464.
[110] Z. Lacza, E. Pankotai, A. Csordas, D. Gero, L. Kiss, E.M. Horyath, M. Kollai, D.W. Busija, C. Szabo, Mitochondrial NO and reactive nitrogen species production: Does mtNOS exist? Nitric Oxide 25 (2005 July) (Electronic publication).
[111] A.R. Evans, M. Limp-Foster, M.R. Kelley, Going APE over ref-1, Mutat. Res. 461 (2000) 83–108.
[112] K.G. Pinz, D.F. Bogenhagen, Characterization of a catalytically slow AP lyase activity in DNA polymerase γ and other family A DNA polymerases, J. Biol. Chem. 275 (2000) 12509–12514.
[113] M.J. Longley, R. Prasad, D.K. Srivastava, S.H. Wilson, W.C. Copeland, Identification of 5′-deoxyribose phosphate activity in human DNA polymerase gamma and its role in mitochondrial base excision repair in vitro, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 12244–12248.
[114] D.K. Srivastava, B.J. Berg, R. Prasad, J.T. Molina, W.A. Beard, A.E. Tomkinson, S.H. Wilson, Mammalian abasic site base excision repair. Identification of the reaction sequence and rate-determining steps, J. Biol. Chem. 273 (1998) 21203–21209.
[115] M. Ogihara, M. Tanno, N. Izumiyama, H. Nakamura, T. Taguchi, Increase in DNA polymerase gamma in the hearts of adriamycin-administered rats, Exp. Mol. Pathol. 73 (2002) 234–241.
[116] T. Kaneko, S. Tahara, M. Tanno, T. Taguchi, Age-related changes in the induction of DNA polymerases in rat liver by gamma-ray irradiation, Mech. Ageing Dev. 123 (2002) 1521–1528.
[117] H.B. Suliman, M.S. Carraway, K.E. Welty-Wolf, R. Whorton, C.A. Piantadosi, Lipopolysaccharide stimulates mitochondrial biogenesis via activation of nuclear respiratory factor-1, J. Biol. Chem. 278 (2003) 41510–41518.
[118] H.B. Suliman, K.E. Welty-Wolf, M.S. Carraway, L. Tatro, C.A. Piantadosi, Lipopolysaccharide induces oxidative cardiac mitochondrial damage and biogenesis, Cardiovasc. Res. 64 (2004) 279–288.
[119] J.A. Stuart, S. Mayard, K. Hashiguchi, N.C. Souza-Pinto, V.A. Bohr, Localization of mitochondrial DNA base excision repair to an inner membrane-associated particulate fraction, Nucleic Acids Res. 33 (2005) 3722–3732.
[120] R.K. Naviaux, Assay of mtDNA polymerase gamma from human tissues, Methods Mol. Biol. 197 (2002) 259–271.
[121] N. Garrido, L. Griparic, E. Jokitalo, J. Wartiovaara, A.M. van der Blick, J.N. Spelbrink, Composition and dynamics of human mitochondrial nucleoids, Mol. Biol. Cell 14 (2003) 1583–1596.
[122] F. Legros, A. Lombes, P. Frachon, A. Lombès, M. Rojo, Organization and dynamics of human mitochondrial DNA, J. Cell Sci. 117 (2004) 2653–2662.
[123] H.T. Jacobs, S.K. Lehtinen, J.N. Spelbrink, No sex please, were mitochondria: a hypothesis on the somatic unit of inheritance of mammalian mtDNA, BioEssays 22 (2000) 564–572.
[124] I.R. Boldogh, D.W. Nowakowski, H.-C. Yang, H. Chung, S. Karmon, P. Royes, L.A. Pon, A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery, Mol. Biol. Cell 14 (2003) 4618–4627.
[125] N. Phadnis, E.A. Sia, Role of the putative structural protein Sed1p in mitochondrial genome maintenance, J. Mol. Biol. 342 (2004) 1115–1129.
[126] S. Meeusen, J. Nunnari, Evidence for a two membrane-spanning autonomous mitochondrial DNA replisome, J. Cell Biol. 163 (2003) 503–510.
[127] A.W. Dobson, Y. Xu, M.R. Kelley, S.P. LeDoux, G.L. Wilson, Enhanced mitochondrial DNA repair and cellular survival after oxidative stress by targeting the human 8-oxoguanine glycosylase repair enzyme to mitochondria, J. Biol. Chem. 275 (2000) 37518–37523.
[128] A.W. Dobson, V. Grishko, S.P. LeDoux, M.R. Kelley, G.L. Wilson, M.N. Gillespie, Enhanced mtDNA repair capacity protects pulmonary artery endothelial cells from oxidant-mediated death, Am. J. Physiol.: Lung Cell. Mol. Physiol. 283 (2002) L205–L210.
[129] L.I. Rachek, V.I. Grishko, S.I. Musiyenko, M.R. Kelley, S.P. LeDoux, G.L. Wilson, Conditional targeting of the DNA repair enzyme hOGG1 into mitochondria, J. Biol. Chem. 277 (2002) 44932–44937.
[130] L.I. Rachek, V.I. Grishko, M.F. Alexeyev, V.V. Pastukh, S.P. LeDoux, G.L. Wilson, Endonuclease III and endonuclease VIII conditionally targeted into mitochondria enhance mitochondrial DNA repair and cell survival following oxidative stress, Nucleic Acids Res. 32 (2004) 3240–3247.
[131] N.C. de Souza-Pinto, L. Eide, B.A. Hogue, T. Thybo, T. Stevnsner, E. Seeberg, A. Klungland, V.A. Bohr, Repair of 8-oxodeoxyguanine lesions in mitochondrial DNA depends on the oxoguanine DNA glycosylase (OGG1) gene and 8-oxoguanine accumulates in the mitochondrial DNA of OGG1-defective mice, Cancer Res. 61 (2001) 5378–5381.
[132] A. Klungland, L. Rosewell, S. Hollenbach, E. Larsen, G. Daly, B. Epe, E. Seeberg, T. Lindahl, D.E. Barns, Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13300–13305.
[133] K. Sakumi, Y. Tominaga, M. Furuichi, P. Xu, T. Tsuzuki, M. Sekiguchi, Y. Nakabeppu, Ogg1 knockout-associated lung tumorigenesis and its suppression by Mth1 gene disruption, Cancer Res. 63 (2003) 902–905.
[134] M. Endres, D. Biniszkiewicz, R.W. Sobol, C. Harms, M. Ahmadi, A. Lipski, J. Katchanov, P. Mergenthaler, U. Dirnagl, S.H. Wilson, A. Meisel, R. Jaenisch, Increased postischemic brain injury in mice deficient in uracil-DNA glycosylase, J. Clin. Invest. 133 (2004) 1711–1721.
[135] S. Katchhap, K.K. Singh, Mitochondrial inhibition of uracil-DNA glycosylase is not mutagenic, Mol. Cancer 3 (2004) 32.

[136] M. Gaspari, N.G. Larsson, C.M. Gustafsson, The transcription machinery in mammalian mitochondria, Biochim. Biophys. Acta 1659 (2004) 148–152.
[137] Y.-H. Chen, D.F. Bogenhagen, Effects of DNA lesions on transcription elongations by T7 RNA polymerase, J. Biol. Chem. 268 (1993) 5849–5855.
[138] R.M. Elliott, S. Souton, D.B. Archer, Oxidative insult specifically decreases levels of a mitochondrial transcript, Free Radic. Biol. Med. 26 (1999) 646–655.
[139] Z. Cui, T.L. Mason, A single nucleotide substitution at the rib2 locus of the yeast mitochondrial gene for 21S rRNA confers resistance to erythromycin and cold-sensitive ribosome assembly, Curr. Genet. 16 (1989) 273–279.
[140] S.E. Kearsey, I.W. Craig, Altered ribosomal RNA genes in mitochondria from mammalian cells with chloramphenicol resistance, Nature 290 (1981) 607–608.
[141] P. Nagley, R.M. Hall, B.G. Ooi, Amino acid substitutions in mitochondrial ATPase subunit 9 of *Saccharomyces cerevisiae* leading to oligomycin or venturicidin resistance, FEBS Lett. 195 (1986) 159–163.
[142] U.P. John, P. Nagley, Amino acid substitutions in mitochondrial ATPase subunit 6 of *Saccharomyces cerevisiae* leading to oligomycin resistance, FEBS Lett. 207 (1986) 79–83.
[143] A. Chatterjee, K.K. Singh, Uracil-DNA glycosylase-deficient yeast exhibit a mitochondria mutator phenotype, Nucleic Acids Res. 29 (2001) 4935–4940.
[144] R. Vongsamphanh, P.-K. Fortier, D. Ramotar, Pir1p mediates translocation of the yeast Apn1p endonuclease into the mitochondria to maintain genomic stability, Mol. Cell. Biol. 21 (2001) 1647–1655.
[145] K.K. Singh, B. Sigala, H.A. Sikder, C. Schwimmer, Inactivation of *Saccharomyces cerevisiae* OGG1 DNA repair gene leads to an increased frequency of mitochondrial mutants, Nucleic Acids Res. 29 (2001) 1381–1388.
[146] N.W. Chi, R.D. Kolodner, Purification and characterization of MSH1, a yeast mitochondrial protein that binds to DNA mismatches, J. Biol. Chem. 269 (1994) 29984–29992.