Chapter

Mitochondrial Genome Maintenance: Damage and Repair Pathways

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Abstract

The mitochondrial genomic material (mtDNA), similarly to nuclear genome, is exposed to a plethora of exogenous and endogenous agents, as well as natural processes like replication that compromise the integrity and fidelity of the mtDNA, despite the abovementioned, the mtDNA does not contain genes involved in DNA repair, therefore mitochondria completely depend on the importation of nuclear-encoded elements to achieve genome maintenance, which implies a coordinated crosstalk between these two organelles. It has been determined that to counteract damage, mitochondria possess well-defined repair pathways quite similar to those of the nucleus, among which are: base excision repair (BER), mismatch repair (MMR), single-strand break repair (SSBR), microhomology-mediated end joining (MMEJ), and probably homology recombination dependent repair (HRR). If these repair pathways are nonfunctional and the lesions remain unrepaired, the emergence of mutations, deletions, and other insults may result in compromised cellular viability and disease.

Keywords: mitochondria, mtDNA, damage, repair, BER, MMR, SSBR, HRR, MMEJ

1. Introduction

The mitochondrion is an essential organelle involved principally in the production of ATP and other metabolites which are important to several cellular functions, besides this organelle participates in other processes as iron-sulfur cluster biogenesis, heme production, and calcium regulation [1]. The mitochondrion possesses its own circular genomic material (mtDNA), which is exposed to the same DNA lesions as nuclear genome is, however, unlike the latter, mtDNA does not encode for genes involved in DNA maintenance or repair which implies that these processes completely depend on nuclear-encoded elements translocated to mitochondria. It was first thought that mitochondria lacked the ability to repair its DNA material, and this assumption was originated due to the observation of the absence of pyrimidine dimer resolution after ultra violet light exposition in mammalian cells [2]; however, nowadays, the study of mtDNA repair pathways has evolved into a complete research area that is constantly growing, since it has been observed that mitochondria not only possess some of the nuclear-conserved mechanisms like: base excision repair (BER), mismatch repair (MMR), single-strand break repair (SSBR), microhomology-mediated end joining (MMEJ), and homologous recombination

dependent repair (HRR), additionally mitochondria have evolved specific unique methods to deal with mtDNA insults based on the redundancy nature of mtDNA and mitochondrion itself, if the damage surpasses its repair capabilities, the mtDNA molecules can be destroyed and replicated again or even the whole organelle can be degraded [3]. Of importance, lesions that remain unrepaired in mtDNA such as deletions, mutations, inversions, and other rearrangements have been linked to several heritable disease syndromes [4]; further, mtDNA rearrangements and deletions have been associated with aging and cancer (www.mitomap.org/org/MITOMAP) [5]. In this chapter, we will summarize the different mechanisms by which the mammalian mtDNA can be damaged and the described pathways that are involved in maintenance of fidelity and integrity of mitochondrial genome.

2. The mitochondrial genome

One of the features of mammalian cells is that they have two DNA-containing compartments: nuclei and mitochondria. Nuclear genome is large, diploid, and linear; in contrast, mitochondrial genome is polyploid and quite small, since is formed by a 16,569 pb circular molecule that accounts for 0.0005% of the human genome and 0.1% of the total number of genes in the human; mtDNA is redundant, since a few hundred to few thousand copies can be found per cell [3], when all the copies are identical, the genotype is termed homoplasmy, instead when multiple forms exist within the same tissue or cell; the genotype is called heteroplasmy [6]. The mitochondrial genome presents 37 genes, 13 of which encode for proteins oxidative phosphorylation chain specific and the remaining are implicated in translation: 2 ribosomal RNAs (small of 12S and large of 16S) and 22 tRNAs. The grade of compaction of mtDNA is interesting since it has no introns, and the intergenic regions are almost absent, additionally there are two noncoding regions: one of approximately 1 kb known as noncoding region (NCR) and another small of 30 bp, both implicated in regulation of replication and transcription [7]. The NCR presents a triple stranded region, named D-loop, which occupies most of its extension and is related to the start of transcription [8], besides it has been observed that some genes overlap and others lack termination codons; therefore, it has been established that the promoters produce polycistronic transcripts which are further processed to generate mature RNA molecules [9]. As mentioned above, some of the proteins involved in respiratory system and ATP synthesis, which are extremely important to cellular functions, are encoded by mtDNA and not the nuclear genome, thus it is important to maintain mitochondrial genome integrity to preserve homeostasis [10].

Despite the advances made in the study of mtDNA replication mechanism, the exact machinery and steps involved in this procedure are not fully known; however, it has been determined a general head core to this process which consists of the polymerase gamma (Polγ), a DNA helicase named Twinkle, and the mitochondrial single-stranded binding protein (mtSSB) [11]. Nowadays, there are three proposed models to explain mtDNA replication: (1) the first is quite similar to nucleus DNA replication, with standard leading and lagging strand replication, (2) a strand displacement model, where the lagging strand is synthetized once the leading has advanced and synthetized a long fragment, and (3) in this model, the lagging strand is hybridized with complementary RNA, a mechanism termed RNA incorporation throughout the lagging strand (RITOL) [8]. Another interesting feature about mtDNA replication is that contrary to what occurs in nuclear genome, mitochondrial genome replication is not limited to S phase of the cell cycle [12].

Unlike the nucleus, where the DNA forms part of nucleoprotein complexes, consisting of DNA molecules wrapped around histone structures, the mitochondrial

genome does not present histones. It has been thought that this lack is responsible of the high rate of mtDNA mutagenesis, which is 10-fold greater than that in nucleus; however, this hypothesis is controversial since experimental evidence has suggested that histones might provoke DNA damage instead of preventing it [3]. Despite the above, mitochondrial genome is not naked; it is packaged into protein-DNA complexes, which are termed mitochondrial nucleoids due to its similarity to bacterial chromosomes [13]. The most abundant nucleoid-associated proteins are mtSSB, transcription factor A of mitochondria (TFAM), Polγ, mitochondrial RNA polymerase (POLRMT), and Twinkle DNA helicase [14].

3. Sources of mtDNA damage

Mitochondrial genome is exposed to almost the same insults that nuclear genome is, which can be originated by internal and external sources. Six types of DNA damage have been proposed to be the more relevant in mitochondria [3].

3.1 Alkylation damage

This kind of lesion may be due to exposition to exogenous agents as chemotherapeutic drugs, diet, and tobacco smoke; however, DNA alkylation damage can also be generated from the interaction of DNA with endogenous molecules [15], such as betaine, choline, and S-adenosylmethionine (SAM); the latter is the most relevant alkylating agent in the cell; SAM is a co-substrate involved in the transfer of methyl groups, when incubated with DNA in aqueous solutions leads to base modification, forming small amounts of 7-methylguanine and 3-methyladenine nonenzymatically, therefore SAM acts as a weak DNA-alkylating agent [16]. Of interest, these DNA modifications, in specific 7-methylguanine can trigger the formation of mutagenic apurinic sites (AP) and imidazole ring opening which results in the stoppage of replication machinery [17]; moreover, 3-methylguanine itself is a cytotoxic DNA lesion that also blocks replication [15]. Interestingly, mitochondria store about 30% of total hepatic SAM [18], thus mtDNA is constantly exposed to this alkylating agent, which threats its stability and integrity.

3.2 Hydrolytic damage

There are two types of hydrolytic damage, the first is the formation of AP sites as a product of hydrolysis of the glycosidic bonds between bases and deoxyribose, and these lesions could appear due to heating, alkylation damage (previously mentioned) or by the action of N-glycosylases [19]. It has been estimated that AP is one of the most frequent lesions in the DNA, with approximately 10,000 lesions per cell, per day [20]. Interestingly, typical AP sites generate base pair modifications, since there is a preference to incorporate adenine opposite to AP by polymerases during DNA replication [21]. The other form of hydrolytic damage is the hydrolytic deamination of bases, where cytosine and its homolog 5-methylcitosine are mainly affected. It is noteworthy that the conversion of cytosine to uracil may introduce punctual mutations to the genome during replication if left unrepaired [20].

3.3 Formation of adducts

This type of lesions can be generated for exposition to ultraviolet type B and C light which produce bulky DNA adducts termed photodimers, in addition, activated metabolites of several organic contaminants, for example, polycyclic aromatic

hydrocarbons and mycotoxins may bring about adducts [1]. On the other hand, adduct formation can also be stimulated by endogenous factors, for example, it has been demonstrated that reactive intermediate products of diethylstilbestrol metabolization form DNA adducts preferentially with mitochondrial genome, where these insults are suggested to avoid replication and/or transcription, thus producing mtDNA instability in vivo [22].

3.4 Mismatches

During replication, polymerases can introduce base to base mismatches as well as generate nucleotide insertions or deletions in mitochondria, which are normally known as insertion-deletion loops (IDLs). One important source of mismatches are damaged deoxyribonucleotide triphosphates (dNTPs), predominantly oxidized, which can be incorporated to DNA during synthesis [3, 10].

3.5 DNA strand breaks

These injuries are divided based on the breaking of one or both strands. Single strand breaks (SSBs) can be generated by normal cellular procedures that went wrong, such as erroneous or abortive activity of DNA topoisomerase I (Top1), which presents mitochondrial localization, and when it fails may produce protein-linked DNA breaks [23], also SSBs are produced by ineffective base excision repair (BER), or by oxidative stress [24]. One lesion related to SSBs is the formation of a covalently linked AMP to a 5′ phosphate, product of an unsuccessful DNA ligase activity [25]. On the other hand, double strand breaks (DSBs) are the most harmful, since they can provoke global cellular responses that involve many aspects of cell metabolism [26]. These lesions may occur by endogenous agents like reactive oxygen species (ROS), errors in DNA metabolism by topoisomerases, and nucleases or detention of replisome. On the other hand, lesion can be caused by exogenous insults such as ionizing radiation and chemotherapeutic drugs [1].

3.6 Oxidative damage

In living organisms, ROS are normally produced as a consequence of endogenous metabolic reactions and also by external factors. ROS include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and single oxygen (O_2), all of them can oxidize DNA molecules and generate several types of damage including oxidized bases and single- and double-strand breaks [15]. Oxidative damage is by far the most prevalent and studied in mtDNA, since mitochondria are an important contributor in the creation of ROS [27], generated by the leakage of electrons from the electron transportation chain (ETC) [28], and there are at least nine sites responsible for generating the superoxide anion [29]. The importance of ROS affecting mtDNA lies in the observation that oxidative damage accumulates in several human diseases [30]. Of interest, it has also been reported that reactive nitrogen species (RNS) are able to oxidize or deaminate DNA and produce strand breaks, lesions that could be possible in mtDNA since these RNS can be found in mitochondria [31, 32].

4. Mitochondrial DNA repair machinery

Most of the repair pathways used by mitochondria to deal with its damaged DNA are quite similar to those operating in the nucleus; this observation makes

sense when we realize that mitochondrion relies completely in import of DNA repair elements encoded by nuclear genome, despite that mitochondrial genome does not contain any gene implicated in repair; it appears that the mitochondrial version of the repair machinery operates with fewer proteins than the nuclear counterparts [33] (**Figure 1**).

4.1 Base excision repair (BER)

Base excision repair (BER) is the commissioned pathway to removed nonbulky lesions like alkylated, deaminated, and oxidized bases from the DNA. This pathway has been well studied in the nucleus and was the first repair mechanism reported in mitochondria and to date the best characterized in this last organelle [33, 34]. BER mechanism is highly conserved from bacteria to humans and basically comprises five stages: (1) recognition and excision of the damaged base, (2) removal of the abasic site formed, (3) DNA end processing, (4) repair synthesis, and (5) ligation [35], and at the same time, BER can be divided into two branches (both founded in mitochondria): short patch and long patch, where the difference lies in the procedure used to repair, while short patch forms a single nucleotide gap, long patch forms a bigger one of 2–10 nucleotides [36]. One of the main elements involved in BER processes are the DNA glycosylases, enzymes that catalyze the excision of the N-glycosidic bond between the altered base and its corresponding carbohydrate, thus creating an abasic site, and activity that was first observed by Lindahl et al. [37]. These glycosylases can be subdivided into monofunctional or bifunctional, depending on whether they have lyase activity or not, ability that determines the type of damage they can repair, while monofunctional glycosylases focus on nonoxidative damage, bifunctional exert their action against oxidized DNA bases [35]. In mammalian mitochondria, seven glycosylases have been reported, three monofunctional: uracil-DNA glycosylase (UNG1), *E. coli* MutY homolog (MYH), and N-methylpurine DNA glycosylase (AAG/MPG) and four bifunctional: 8-oxoguanina-DNA glycosylase (OGG1), *E. coli* endonuclease III homolog (NTH1) endonuclease VIII-like glycosylase 1 and 2 (NEIL1 and 2) [38–41]. After the action of glycosylases, the next step, DNA end processing, is catalyzed by nuclear BER enzymes that are also found in mitochondria, as apurinic-apyrimidinic endonuclease 1 (APE1) and polynucleotide kinase-3′-phosphatase (PNKP) [35], further the

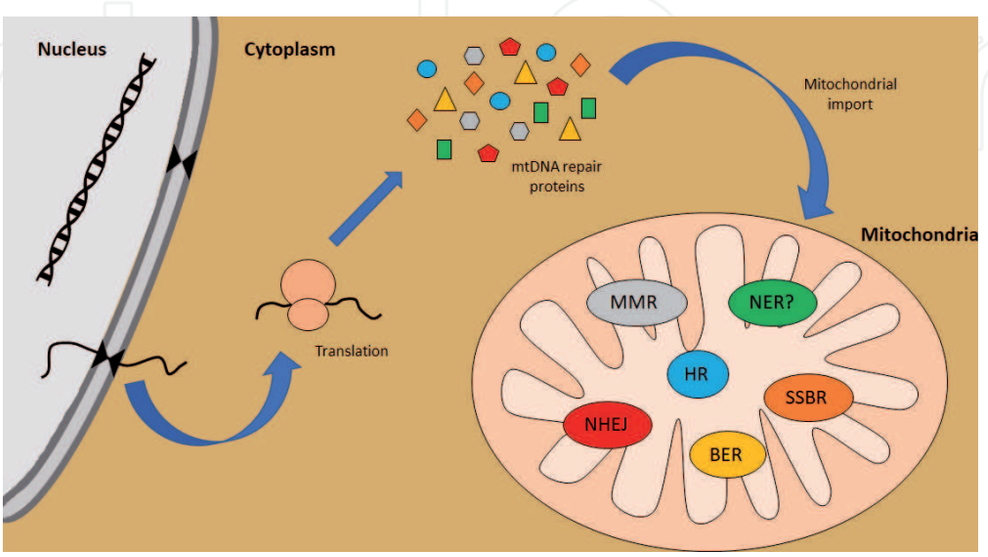

Figure 1.
Mitochondrial import and mtDNA repair pathways. The elements that participate in mitochondrial genome maintenance are nuclear encoded; therefore, mitochondria need to import these effector proteins, which are involved in different repair pathways.

synthesis is carried out by the main mitochondrial polymerase (Polγ). It was recently reported that polymerase beta (Polβ) also localizes within mitochondria, whereas similar to the nucleus, it has a relevant role in mtDNA maintenance and mitochondrial homeostasis through its participation in BER [42]. It has also been observed that FEN1, DNA2, and EXOG are involved in the removal of DNA flap generated by polymerase strand displacement synthesis [43]. Finally, the ligation activity is made by mitochondrial ligase III [44]. In conclusion, despite that BER is the most characterized pathway in mitochondria, it is still unknown whether all the nuclear elements are conserved in mitochondrial BER, or on the contrary, if this pathway have specific proteins that do not participate in nuclear version of BER, it is not well understood either which are the regulatory signals that controls the import of these elements to mitochondria.

4.2 Nucleotide excision repair (NER)

Nucleotide excision repair (NER) pathway is a complex machinery involved in recognition of lesions, adducts or structures that modifies the DNA double helix structure, having the possibility of blocking transcription, replication, and thus affecting DNA stability. One of the most important features of NER is its versatility on a wide kind of lesions since they are detected and repaired. Roughly, the process starts with damage recognition, next, the damaged strand is cleaved at both sides of the lesion to be removed later, then the missing sequence is synthesized using the complementary strand as a template, and finally the ends are ligated, thus restoring DNA sequence and integrity [45].

There is a large body of evidence reporting the absence of nucleotide excision repair activity in mitochondria. The first observation about this lack was made by Clayton et al. in 1974 [2], who reported that mitochondria were unable to remove UV-induced pyrimidine dimers. As a result, lesions that are normally repaired in nucleus by NER could persist in mitochondrial genome; this damage is not only restricted to photodimers, since NER also participates in repair of other bulky lesions and a subset of oxidative DNA damage [1]. In the nucleus, bulky lesions can also be overcome through the use of specialized translesion synthesis (TLS) polymerases; however, in mitochondria, the major polymerase (Polγ) presents a weakly thymine dimer bypass activity in vitro and at present, it is unknown whether this activity is conserved in vivo [46]. On the other hand, it was recently reported that polymerase theta (Polθ), an enzyme that acts as a translesion bypass polymerase, thus promoting the pass of replicative stalling lesions in the nucleus [47], is localized to mitochondria. Additionally, it was observed that there is an increase of Polθ localization to mitochondria after treating the cells with an oxidative agent, suggesting that the enzyme is recruited to the organelle when this kind of damage is inflicted, where it could facilitate translesion bypass synthesis. Interestingly, in POLQ KO cells, the rate of point mutation in mtDNA was significantly reduced after oxidative treatment; this observation indicates that Polθ is involved in a pathway of error-prone DNA synthesis that may facilitate replication in mitochondria [48].

Despite that the nucleotide excision repair pathway has not been clearly recognized in mitochondria, there are several proteins involved in the nuclear version of this pathway that not only localize to the organelle but also they accumulate upon oxidative damage, and when they are depleted from cells, the number of point mutation is increased, observations that strongly suggest their participation in mtDNA maintenance. Some of these proteins are: Cockayne syndrome A (CSA), Cockayne syndrome B (CSB), and Rad23A [48–50]. Therefore, further investigation is needed to completely rule out NER pathway from mitochondria, or to elucidate whether these mentioned proteins are involved in other pathways different to nucleus.

4.3 Mismatch repair (MMR)

MMR is a highly conserved pathway involved in the correction of misincorporation and slippage mistakes committed by polymerases during DNA replication, and base mismatches generated by base deamination, alkylation, and oxidation [3]. In general terms, MMR process presents the next steps: localization of the mismatch and identification of the newly synthesized strand, excision at both extremes of the mismatch lesion, DNA resynthesis, and finally ligation to complete the process [10].

The first demonstration of mammalian mitochondria capable to repair mismatch lesions was done by Mason et al. [51]. They observed that rat liver mitochondrial lysates repaired G-T and G-G mismatches; however, by using immunodetection no MSH2, a key nuclear element in MMR, was detected in these lysates, suggesting that mitochondrial MMR activity uses different elements to those of the nucleus. Posteriorly, in the pursuit of proteins responsible for the activity observed, one study reported that mismatches and small IDLs in mitochondrial genome are recognized by the Y-box binding protein 1 (YB1), which also localizes to nucleus where it exerts other functions, and its depletion in cultured cells triggers an increased mutagenesis in mtDNA. In addition, it was demonstrated that MMR activity is independent of MSH2 and that MSH3, MSH6 or MLH1 are not present in human mitochondria, at least under the experimental conditions employed [52]. In contrast to the previous observation, it was later demonstrated that MLH1 do localizes to mitochondria [53]. Through overexpression of *Mlh1* or *Msh2* in retinal endothelial cells, it was determined that MLH1 has a protective role in mtDNA after glucose-induced DNA damage, and on the other hand, this protective effect was not detected when *Msh2* was overexpressed, observation that suggests no participation in mtDNA maintenance [54], in accordance with previous studies. Additionally, it was reported that the incidence of base-mismatches in mtDNA in diabetic retina is a consequence of expression silencing of MLH1 by methylation of its promoter, activity performed by Dnmt1, enzyme overexpressed in diabetes. Thus, these observations propose that MLH1 has an important role in mtDNA maintenance, since its silencing by methylation triggers mtDNA damage [55]. In summary, MMR pathway is involved in mitochondrial genome maintenance; however, not all the elements implicated have been found. It could be possible that the proteins of mitochondrial MMR may have a different splicing or post translational versions than nuclear ones, which impairs their identification through antibody-based techniques, or in the other hand, mitochondrial MMR could not depend of all the elements involved in the canonical nuclear form, as it was seen with the participation of YB1 [52]. In any case, more research is needed to find more MMR nuclear factors within mitochondria or to discover new ones and be able to catalog the mitochondrial MMR as an original pathway.

4.4 Single strand break repair (SSBR)

The repair of SSBs in mitochondria is achieved through a BER subpathway known as base excision/SSB repair (which is also present in nucleus), since both mechanisms share common component, especially in the last steps: gap filling and DNA ligation [10, 24]. Indeed, most of the SSBs can be repaired by elements of BER pathway: APE1, PKPK, and Polγ [24]. Other members of mitochondrial SSB repair include: PARP1, a protein implicated in the detection of SSBs in the nucleus and also more recently observed in mitochondria, where not only it binds to mtDNA, but also when is depleted, this provokes accumulations of DNA damage, thus confirming its participation in mtDNA maintenance [56]. Besides, the participation of ExoG in SSB repair has been elucidated, since its depletion induces mitochondrial persisting SSBs that eventually lead to apoptosis [57].

As was previously mentioned, there are some lesions associated to SSB, like trapped topoisomerase 1 (Top1), damage that can be repaired through the action of tyrosil-DNA phosphodiesterase 1 (TDP1), an important enzyme involved in the release of covalently trapped Top1 with DNA that was first described in yeast [58]. In addition to its well characterized function, it has been observed that TDP1 also removes other types of 3′-blocking lesions, resulting oxidative damage [59–61]. A fraction of TDP1 (nuclear encoded) localizes to the mitochondria, where it has been implicated in mtDNA repair, since the treatment with chain terminator nucleotide analogs (CTNAs), which are also substrates of this enzyme, in *tdp1*$^{-/-}$ cells generate a reduction in mtDNA copy number, whereas wild type cells remain unaffected [61, 62]. These findings confirm the involvement of TDP1 in mtDNA damage repair, in this case induced by CTNAs. Another SSB-related lesion is the generation of a covalent binding of adenine monophosphate (AMP) to the 5′end of mtDNA, and this error is promoted by abortive ligase activity. The resolution of such damage relies in aprataxin (APTX) protein that is able to remove 5′-adenylate groups. APTX localizes to mitochondria, whereas its depletion generates a decline of mtDNA copy number as well as higher levels of DNA damage, observations that suggest a direct role of this enzyme in mtDNA maintenance [63]. If any of the lesions mentioned remains unrepaired, further complications may appear, since SSBs may progress to DSBs, which are more deleterious to cells.

4.5 Double strand break repair (DSBR)

In general, cells of higher eukaryotes use two main approaches to repair DSBs. The first approach is through the union of the ends in a nonhomologous dependent way, this pathway is termed nonhomologous end joining (NHEJ); it has been determined that NHEJ possesses some alternatives versions that use noncanonical elements, these sub pathways are known as alternative NHEJ (A-NHEJ); the repair with these mechanisms guarantee the restoration of DNA integrity but not sequence.

4.5.1 Nonhomologous end joining (NHEJ)

Nonhomologous end joining (NHEJ) is one of the two main pathways used by the cells to repair DNA double strand breaks. Similar to most DNA repair processes, NHEJ is based on three general steps: the action of a nuclease to resect the damaged DNA, next, the fill-in to make new DNA by a polymerase, and finally the participation of a ligase to restore the integrity of the strands. One of the most interesting features of NHEJ is the diversity of substrates that can use and convert to joined products [64]. By virtue of its template-independent operation, NHEJ is associated with insertions and deletions and hence with a lack of reliable restoration [26].

It has been observed that mammalian mitochondria do possess the capacity to bind DNA ends, activity that is retained even in Ku-deficient cells [65], additionally, the efficiency and precision of this activity apparently depend on the structure of the ends generated, since blunt-ended DNA fragment repaired are less conserved than sticky ends in comparison to the original [66]. Tadi et al. [67] demonstrated that mitochondria have a noncanonical version of NHEJ, also named alternative NHEJ (A-NHEJ). The repair by this pathway is based on microhomology and is sometimes associated with long deletions, and hence it is described as microhomology-mediated end joining (MMEJ). In this same study, using rodents and human mitochondrial extracts, a lack of end-to-end joining of nonligatable broken DNA was observed, the fact that suggests the absence of a functionally operative canonical NHEJ in mitochondria, or at least is undetected with the techniques used. In contrast, mitochondria have the ability to join oligomeric dsDNAs

harboring direct repeats (microhomology) that vary in length, from 5 to 22 nt, with an efficiency that is enhanced with the increase in the length of homology. These results are supported by a previous observation, where DSBs are induced in mice through mitochondrially targeted restriction endonuclease (*Pst* I), and the repaired mtDNA presented small directed repeats (a few nucleotides) at the breakpoint [68], resolution that fits with the repair manner of MMEJ, and in addition, these repair products have also been observed in most of the mtDNA deletions associated with human diseases, which are mostly (~85%) flanked by small direct repeats [69]. Besides, it has been determined that this mitochondrial MMEJ activity involved the proteins CtIP, FEN1, Mre11, PARP1, and ligase III [67].

Therefore, MMEJ has been proposed as a central pathway in the repair of double strand breaks and maintenance of mammalian mitochondrial genome, and the use of this pathway and possibly not C-NHEJ may explain the Ku independence proteins to exert the joining activity as was previously mentioned. The possible absence of C-NHEJ activity in mitochondria is contrasting with the observation that antibodies to KU70 and KU80 cross-react with proteins from mitochondrial extracts with DNA end-binding activity [10, 70]. Furthermore, it has been determined that XRCC4, a mediator protein of nuclear DSB repair pathway, is present in mitochondrial, where it is indeed involved in mtDNA repair and possibly associated with DNA ligase III [48]. In summary, at present no NHEJ activity has been described in mitochondria; however, this organelle presents the ability to join broken DNA ends, and it appears that this action depends on the structure of the ends generated and in the presence of homology at both ends of the DSB, further, the repair by MMEJ may explain the deletions observed in the majority of mitochondrial diseases.

4.5.2 Homologous recombination (HR)

Homologous recombination (HR) is a ubiquitous process conserved from bacteriophages to humans and is one of the most important pathways used by the cells to deal with DNA double strand breaks. To achieve the restoration of molecular integrity and sequence in a free-error manner, HR needs a homologous sequence to use it as a template [26, 71].

It has been determined that HR is essential for preservation of mtDNA in plants, yeast, and fungi, and on the other hand, although there is evidence about HR in mammalian, its significance in vivo is not clear [1]. One of the first reports about mitochondrial homologous repair capabilities was made by Thyagarajan et al. [72] where they observed that mitochondrial protein extracts from mammalian cells catalyzed homologous recombination repair of plasmid DNA substrates, therefore concluding that mitochondria do possess the machinery to perform this process. Additionally, after preincubating protein extracts with anti-RecA antibodies, an inhibition of the reaction was observed, fact that suggests the participation of a mammalian mitochondrial RecA homolog. Supporting this evidence, in 2010, Sage et al. [73] demonstrated that Rad51 and the related proteins, Rad51C and XRCC3, localize to human mitochondria, and they also reported that the protein levels were enriched after stress induction and that depletion of any of these elements generates a dramatic decrease in mtDNA copy number, these results strongly suggest some type of HR participation in mitochondrial genome maintenance. Other proteins involved in HR have been observed in mitochondria, and their participation in mtDNA repair has been validated, such as Dna2 [74] and Mre11 [67, 75, 76]; moreover, it has been suggested that ExoG could supply Exo1, and therefore, many of the factors needed to perform HR process are present in mitochondria.

Recently, using biochemical assays, it was determined that HR is the major DSBR mechanism, where it has a role in maintenance of mitochondrial genome

integrity, since the induction of DSBs significantly enhanced this process. Besides the participation of Rad51, Mre11 and Nibrin relevance in HR was confirmed by suppression of HR-mediated repair after immunodepletion of these proteins in the mitochondrial extracts [76]. The process of mitochondrial HR may proceed in two ways, one through intramolecular recombinant events, where a sole mtDNA molecule recombines with itself, and a second form, where a molecule can recombine with another one homologous or heterologous [77, 78]. Despite the knowledge of the elements involved in mitochondrial HR have increased over the last years, the exact mechanism about how HR is achieved in mitochondria is lacking in comparison to the nuclear models [33].

The second approach is through the use of nondamaged homologous sequences; this kind of repair restores molecular integrity as well as sequence; another pathway that uses homology sequences is single strand annealing (SSA), which needs directed repeats in both ends of the DSB, in such a way that when repairing, it restores integrity and sequence but at the expense of a variable length deletion [26].

4.6 Other pathways

As it has been previously described in this chapter, mitochondria have a repertoire of elements to deal with DNA damage, even it has been observed that mitochondria possess a mechanism to "prevent" further lesions (described below). However, if the mtDNA lesions surpass the mitochondrial repair capabilities, the cell maintains other options to avoid a higher damage, and in these circumstances, it is possible to degrade the unrepairable mtDNA, the organelle or even the whole cell [10].

4.6.1 Sanitation of the dNTP pool

The DNA is not the only molecule susceptible to chemical damage, the deoxyribonucleotide triphosphates (dNTPs) pool is also affected, being oxidative damage one of the most recurrent alterations [79]. If unrepaired, these lesions could become a source of mismatch errors during DNA synthesis [3]. To cope with this threat, mitochondria have MTH1, an specialized enzyme also found in the nucleus, which can hydrolyze oxidized dNTPs such as 8-oxo-20-deoxyguanosine triphosphate (8-oxo-dGTP), 8-oxo-20-deoxyadenosine triphosphate (8-oxo-dATP), and 2-hydroxy-20-deoxtadenosine triphosphate (2-hydroxy-ATP) to corresponding monophosphates, which cannot be assembled in the DNA by polymerases [41, 80]. In 2008, Pursell et al. [81] reported that 8-oxo-dGTP exists in some rat tissues at levels that are potentially mutagenic; therefore, these data suggest that oxidized dNTP precursors could generate mutagenesis in vivo and consequently promote mitochondrial dysfunction. In addition, it was reported that a pathogenic variant of Polγ, which is present in patients with progressive external ophthalmoplegia (PEO), increases 8-oxo-dGTP misincorporation, observation that establishes a relationship between the oxidative lesions and increased mtDNA damage observed in other models with this pathogenic version, and misincorporation of oxidized nucleotides [82]. In summary, although sanitation of premutagenic free nucleotides is not properly a DNA repair mechanism, its participation prevents the formation of mismatches in mitochondrial genome and therefore reduces the probability of mutagenesis.

4.6.2 mtDNA degradation

Compared to nuclear genome, mitochondrial genomic material has a remarkable advantage about DNA damage and repair, its redundancy, consisting of hundreds to thousands of copies per cell. Due to this characteristic, mitochondria

can dispose of a considerable fraction of mtDNA, where its repair capabilities were exceeded; however, it does not compromise organelle functions, and this is not an option for nucleus, where the diploid genome cannot be submitted to degradation without affecting the cellular homeostasis [3]. It is thought that after mtDNA degradation, the lost molecules are restored by mitochondrial genome turnover, a process that was first described several decades ago [83] (**Figure 2**). There is a wide body of evidence that supports this hypothesis of mtDNA degradation after unrepairable insults; for instance, it was observed that when one of the initial steps of the BER repair pathway is inhibited by methoxyamine drug, the increase of incidence of oxidative and alkylating damage enhanced the mtDNA degradation [84]; additionally, through qPCR analysis, it has been shown a mtDNA amount decrease after persisting exposure with the oxidizing agent hydrogen peroxide [85, 86]. Furthermore, the absence of mutation fixation after persisting cell treatment with alkylating agents which have a high mutagenic potential suggests that due to the lack of mechanisms for repairing bulky lesion, the mtDNA could be selectively degraded and to prevent further modifications [87]. Nowadays, it is not completely clear how the mitochondrion degrades its damaged DNA; however, it has been recently determined that endonuclease G (EndoG) has an important role in mtDNA depletion, since it promotes cleavage of mtDNA as a response to oxidative and nitrosative stress, action that subsequently generates an upregulation of mtDNA replication as an indirect outcome [88]. This evidence is supported by the fact that endo G is the most abundant and active nuclease within mitochondria, and it has a preference on oxidized DNA harboring single-strand breaks or distorted DNA product of crosslinking agents to exert its endonuclease activity in vitro [89], also it has been reported that this nuclease preferentially cleaves 5-hydroxymethylcytosine an oxidized product of 5-methylcytosine [90]. In conclusion, despite that additional research is needed to elucidate the whole mechanisms and elements that participate in mtDNA degradation, this pathway emerges as a unique and mitochondrial specific method to maintain DNA integrity.

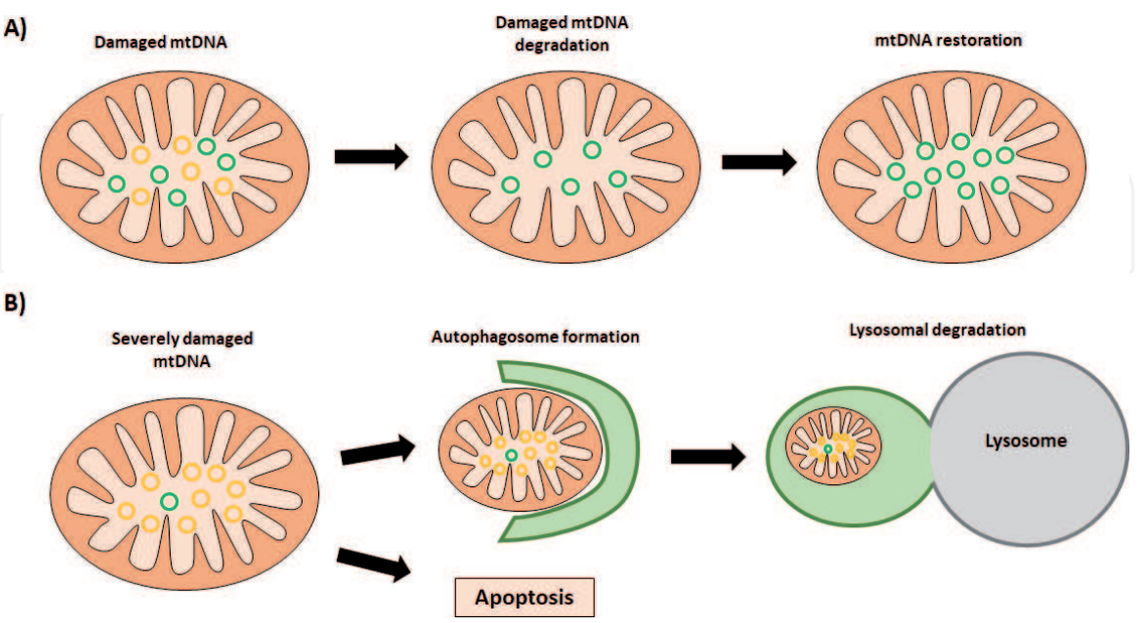

Figure 2.
mtDNA degradation and mitophagy. (A) Damaged mtDNA (yellow circles) can be selectively degraded inside mitochondria, thus keeping "healthy" mtDNA (green circles), then this can replicate to re-establish mitochondrial genome homeostasis. (B) If the mtDNA is severely damaged and the repair mechanism is surpassed, injured mitochondria can be selectively degraded by the formation of an autophagosome and subsequent fusion with lysosomes [91]. On the other hand, the mtDNA lesions can also trigger cell apoptosis.

4.6.3 Mitochondrial clearance, dynamics, and apoptosis

In general terms, autophagy is a highly conserved degradative mechanism used by cells to maintain homeostasis [92]. This is a finely regulated process that takes part in cell growth, development, and in the maintenance of an equilibrium between synthesis, degradation and recycling of cellular elements including whole organelles [11]. There is a specialized sub pathway of autophagy, which is specifically involved in degradation of damaged and dysfunctional mitochondria, and this procedure is known as mitophagy or mitochondrial clearance. Although mitophagy can emerge as a programmed cellular event, like the one that is observed during erythroblast maturation in order to generate mature red blood cells lacking mitochondria [11], it has been proposed that mitophagy could participate in the elimination of organelles harboring low levels of DNA damage stress. On the other hand, when the DNA lesions are too many to handle with mtDNA repair mechanism or by mitochondrial clearance, the cellular response could trigger apoptosis [93], therefore the choice of which pathway must be used depends on the degree of DNA damage (**Figure 2**). In accordance with the previous mechanism, Suen et al. [94] observed selectively degradation by mitophagy of organelles harboring deleterious COXI mutations after overexpressing the protein Parkin, which translocated to affected mitochondria and induced autophagic elimination, thus this selection enriched cells for nonmutated mtDNA and restoring cytochrome c oxidase activity [95].

It appears that mitophagy is closely associated with mitochondrial dynamics processes: fission and fusion [96]. Fusion is the joining of two organelles to form one, this mechanism allows mitochondria to distribute mtDNA and to replenish it when is damaged, therefore safeguarding mtDNA integrity and protecting it from mutations [97]. On the other hand, fission is the division of a single organelle to create two, this process is very important to cellular viability, it contributes to symmetrical distribution of mitochondria during mitosis, and promotes the removal of lesioned organelles by partitioning the damaged elements (like mtDNA) to a derived mitochondria that can fuse to a healthy one with the intention of recovering functionality or to be degraded by mitophagy. Therefore, mitochondrial removal by mitophagy is preceded by mitochondrial fission, which is capable of dividing the organelle into smaller pieces to be degraded easily [98]. When mitochondrial clearance, fusion, or fission are dysfunctional, the cells could be severely affected, since it has been observed that in these situations, an increase in mtDNA instability and generation of neurodegenerative, cardiovascular, and age-related diseases were obtained [99].

5. mtDNA repair, diseases, and aging

Mitochondrial diseases are a heterogeneous group of illnesses affecting multiple organs and leading to eventual degeneration and in some cases premature death. These affectations have origin in mutations on mtDNA, which are generated by errors during DNA replication, exogenous sources, and ROS; however, mitochondrial dysfunction may also arise from mutations in nuclear genes which encode proteins with mitochondrial function, involved in several processes like biogenesis, transcription, replication, mitochondrial dynamics, and mtDNA repair, among others. Of interest, neurodegeneration is a prevalent trait in mitochondrial diseases, maybe because the brain needs a higher demand of energy in comparison with other tissues [11]. On the other hand, there is a large body of evidence that underscores the relationship between mitochondrial disorders and aging; however, there is still

controversy about whether these mutations in the mtDNA are the product of age-related disorders or they are themselves the cause [100].

About the genes involved in mtDNA maintenance, it has been well established that failure of the mtDNA repair pathways may promote diseases and age-related disorders in humans [11, 28]; in addition to mutations, the reduction of mtDNA copy number has also been associated with neurodegeneration, aging, diabetes, and cancer [101]. For example, it has been observed that the lack of proofreading activity of Polγ in mice generates multi-systemic disease and phenotypes resembling to premature aging [102], furthermore, over 200 mutations in POLG have been associated with mitochondrial diseases, these POLG-related disorders can be classified into five main phenotypes of neurodegeneration: Alpers-Huttenlocher syndrome (AHS), childhood myocerebrohepatopathy spectrum (MCHS), myoclonic epilepsy myopathy sensory ataxia (MEMSA), ataxia neuropathy spectrum (ANS), and PEO [28], besides, mutations in Twinkle helicase often causes infantile onset spinocerebellar ataxia (IOSCA), which usually appears in early childhood [103]. Other mtDNA repair elements, such as APTX and TDP1, implicated in SSBR, are related with the generation of ataxia with ocular motor apraxia (AOA1) when are mutated [10, 104]. Also, defects in the proteins CSA and CSB, implicated in the possibly mitochondrial DNA repair transcription coupled-NER pathway, are related with the development of progressive cerebellar pathology [105]. Furthermore, alterations in fusion, fission, or mitophagy processes due to mutations in the proteins involved generate mtDNA instability, which in turn may induce neurodegenerative, cardiovascular, and age-related diseases [99], such is the case of MFN2, which is implicated in mitochondria fusion, and its alteration lead to organelle fragmentation and causes axonal Charcot-Marie-Tooth disease (CMT2A) [106], also mutations in OPA1, lead to optic atrophy, affectation that can be accompanied with hearing loss and ophthalmoplegia [107]. Additionally, mutations in DNA2 and mitochondrial genome maintenance exonuclease 1 (MGME1) nucleases are implicated in ophthalmoplegia, myopathy, and mtDNA depletion [108, 109]. In conclusion, the importance of mtDNA maintenance lies in the observation that when the repair elements are affected, or the mechanisms exceeded, the risk of disease development increases, thus the understanding of these alterations may shed light for clinical targets to prevent diseases or treat them.

6. Concluding remarks

After decades of study, it has been concluded that like nucleus, mitochondria do possess specific mechanisms to maintain integrity of its small and polyploid genome, and although nowadays, the complete repertoire of elements participating in mtDNA repair has not been identified, it appears that these pathways resemble those of nucleus but operating with fewer elements. In addition, mitochondria have evolved organelle specific mechanisms which work as a backup when the repair pathways are surpassed by the amount of damage and that would be impossible to carry out in nuclear genome. In conclusion, the repair of mitochondrial genome is a field in continuous growth that promises new discoveries in the years to come.

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