Chapter 7

The role of DNA repair in maintaining mitochondrial DNA stability

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Abstract

Mitochondria are a vital organelle as a “Power House” inside the cell and have a double membrane organelle with essential roles to maintain cellular functions, e.g. ATP production. iron-sulfur synthesis metabolism, and steroid synthesis. An important difference with other organelles is to contain their own mitochondrial DNA (mtDNA). Such powerful organelle is also sensitive to both endogenous and exogenous factors that can cause lesions to structural components of mitochondrial and their mtDNA, resulting in gene mutations and eventually leading to diseases. In this review, we will mainly focus on mammalian mitochondrial DNA repair pathways that safeguard mitochondrial DNA integrity and several important factors involved in the repair process, especially on an essential pathway, base excision repair. We eagerly anticipate to explore more methods to treat related diseases by constantly groping for these complexes and precise repair mechanisms.

Key words: mitochondrial DNA, lesions, DNA repair, BER, major pathway

1 Introduction

The nucleus contains most of the genetic material while only a small fraction is

present in mitochondria. Nuclear DNA (nDNA) is inherited from both parents unlike mitochondrial DNA (mtDNA) that is essentially maternally inherited [1]. Human mtDNA is a circular genome about 16.5 kb long, containing 37 genes: 13 coding for proteins which are structural components of the oxidative phosphorylation system, 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs (rRNAs) essential for mitochondrial translation (Figure 1) [2]. There are > 100 mitochondria, of which each is comprised of 10 DNAs in eukaryotic cells [3]. Of genetic materials, mtDNA molecules with high mitochondrial densities are small in contrast to the size of the nuclear genome, accounts for only about 1%. nDNA contains 6 billion base pairs, coding with 25000 protein coding genes.

Despite representing a small amount as compared to nDNA, mtDNA plays an important role in maintaining the normal functioning of the organism. Once mtDNA is damaged exceeding the upper limit of repair or cannot be repaired in time, it can accumulate genetic mutations to eventually lead to various diseases involving diabete, cancer, neurodegenerative disorders and aging [4-9]. The mutation rate of mtDNA is estimated to be 10-100 times higher than nDNA [10]. mtDNA is more susceptible to damage (e.g. oxidative damage) as it is linked to the electron transport system while nDNA is less vulnerable to such damage. mtDNA lacks certain protective proteins like histone [11]. Because of the biological characteristics of mitochondria, there are many channels to produce reactive oxygen species (ROS). For instance, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes produce ROS in

phagocytic cells, to fight against microbial infections [12]. Moreover, epithelial and endothelial cells can also produce ROS, e.g. Nox1, Nox4, respectively, by NADPH oxidase enzymes [13, 14].

Mitochondria are the major site of ROS production. Superfluous ROS may lead to DNA damage in the form of oxidized bases and DNA strand breaks [12]. However, a variety of DNA repair systems are in pale in mitochondria in order to maintain the integrity of mtDNA and the normal function of mitochondria. Base excision repair (BER) contains two ways of repairing DNA, the short-path BER (SP-BER) and long-path BER (LP-BER), existed in the nucleus and in the mitochondrion. Other repair systems like non-homologous end joining, which is crucially important for the repair of DNA double strand break [15], have been less studied, and related mechanism remain unclear. Our purpose of this review is to sketch several types of mtDNA damage and the differentiation between nDNA and mtDNA as well as summarize several repair pathways and major enzymes.

2 Characteristics of mitochondria

Mitochondria are involved in the synthesis of heme, cholesterol, and phospholipids, in immune responses [16], and in aging and apoptosis [17]. Despite mitochondria contain less genetic materials, their genetic information is at a higher density than the nDNA, since there are no introns, reduced or absent UTRs, no or very small

intergenic regions, small regulatory regions, and some genes overlap [1]. As a consequence, damage to mtDNA is likely to happen in a coding region. mtDNA damage often occurs in individual cells, due either to intracellular processes (e.g. DNA replication mismatch, bases deamination, oxidative damage, depurination or depyrimidination) [18] or to external factors (e.g. ionizing radiation, ultraviolet radiation, cisplatin) [19]. mtDNA damage can result in base substitutions, deletions and missense mutations, which lead to mtDNA malfunction [20]. This does not mean that the damage of a single base definitely causes a disease. Only when mutations load in mtDNA reaches a high level, about 75% for most cases, to have the impact in mitochondrial function [21]. Although DNA repair was present in the nucleus, there are a number of repair systems in mitochondria after the discovery of the presence of uracil–DNA glycosylase (Ung) within mitochondria [22].

3 Types of damage

Mitochondrial DNA damage can be caused by endogenous or exogenous factors, and either biological or chemical agents. Additionally, the proximity of mtDNA to damaging agents presents within mitochondria and the high replication rate of mtDNA may also contribute to higher levels of DNA damage compared to the nucleus.

The alkylation is a type of mtDNA methylation by internal agents [18] like S-

adenosylmethionine [23], and byexternal factors like chemotherapy for tumors [1]. Alkylation damage can lead to modified DNA bases, genetic instability, the normal function of cells, and development of cancer [24]. The alkylation-associated mtDNA damage is repaired by BER [25]. Hydrolytic damage includes the formation of abasic sites produced by hydrolysis of glycosidic bonds [26] and hydrolytic deamination of bases, mostly cytosine [27]. The formation of adducts can be generated by endogenous ROS (14) or estrogens [28] or by exogenous chemical agents [1] and are removed by the nucleotide excision repair system (NER) [29, 30]. However, there is no evidence of nucleotide excision repair in mitochondria. Mismatched bases of mtDNA damage originate from DNA replication errors, by either incorporation of wrong nucleotides or nucleotides mainly in oxidized bases [31]. Single base mismatches are identified by Y-box binding protein 1 (YB1) [32] and repaired by the mismatch repair system (MMR) [33]. DNA strand breaks includes single-strand (SSBs) [34] and double-strand breaks (DSBs) [35]. DNA strand breaks may originate from ROS-induced DNA lesions [17] or during the BER repair if the repair intermediates cannot accomplish the proper steps [12]. Oxidative damage in mtDNA may occur as base modifications, abasic sites, and a variety of lesions [36]. Of those, repair of 8-oxoguanine is the most comprehensively studied one [4], and oxidative damage is the most prevalent damage in mitochondria [37]. The increased oxidative damage can lead to mitochondrial dysfunction and repaired BER or NER [38]. nDNA possess unique types of injury with altered 3D chromatin architecture, rather than mtDNA [39]. Such a high-order chromatin structure is damaged by radical and

prevented by antioxidant treatment [40].

4 Major enzymes involved in mtDNA

BER is one of the most studied DNA repair pathways both in the nucleus and mitochondria involved with a large number of proteinases, e.g. DNA glycosylases, AP-endonuclease, DNA polymerase, DNA ligase, and other synergistic effect enzymes. Roles of those proteinases in the development of mtDNA damage and repair pathways remain unclear.

4.1 **DNA glycosylases**

DNA glycosylase can damage and remove bases from the DNA and can be divided into four superfamilies [41], e.g. uracil DNA glycosylase (UDG) family, alkyladenine DNA glycosylase (AAG) family, helix-hairpin-helix (HhH) family, and formamidopyrimidine DNA glycosylase (Fpg)/ Nei (endonuclease VIII) or helix two-helix-hairpin-helix (H2TH) family.

The UDG family: UDG family can be further subdivided into six subfamilies of monofunctional glycosylases, e.g. uracil N-glycosylase (UNG), thymine DNA glycosylase (TDG) or MUG (mismatch uracil DNA glycosylase), single-strand specific monofunctional uracil DNA glycosylase (SMUG), and DNA glycosylases. Of those, only UNG was found in human mitochondria [41], of which UNG1 has a 30-amino acid leader peptide that targets the protein to mitochondria while UNG2 lacks

this domain and is targeted to the nucleus. The main role of UNG is to dislodge uracil from mtDNA [2], and remove other substances, e.g. isodialuric acid, 5-hydroxiuracil and alloxan, albeit with low efficiency [42]. UNG has a single domain made of a β-sheet with four parallel β-strands flanked by two sets of α-helices and contribute to narrow and shallow DNA binding groove [30].

The AAG family: AAG, also known as MPG/MDG, is a monofunctional glycosylase able to recognize alkylated and deaminated bases. There are 3 isoforms, AAG-A, AAG-B, AAG-C originated by post-transcriptional processing of a single transcript [43]. Targeting to mitochondria happens thanks to a N-terminal mitochondrial targeting signal in isoforms A and B [43]. Similarity to UDG, AAG also has a single domain, but in this cases it has a mixed α/β topology comprising a positive DNA-binding groove [30].

The HhH family: HhH family comprises a diverse group of glycosylase that is further split into six subfamilies, e.g. endonuclease III (EndoIII or Nth) in several species, 8-oxo-7,8-dihydroguanine DNA glycosylase 1 (Ogg1) in eukaryotes, A/G mismatch-specific adenine glycosylase (MutY/Mig), alkyladenine DNA glycosylase (AlkA) in bacteria and eukaryotes but not in mammals, 8-oxoG DNA glycosylase 2 (Ogg2) in archaeal, and Nmethylpurine-DNA glycosylase II (MpgII) in bacteria and archaea. In the human genome, there are four representatives of these HhH family: NTHL1, OGG1, MUTYH, and MBD4 (methyl-binding domain protein 4). Human

NTHL1 or NTH1 (E. coli endonuclease III-like 1) is present in the nucleus and mitochondria, while mouse Nth1 primarily in mitochondria [44]. Human NTH1 is a bifunctional glycosylase to excise oxidized DNA bases such as 5-hydroxycytosine, 5-hydroxyuracil, as well as the ring-opened 2,6-diamino-4-hydroxy-5-formamidopyrimidine [45]. NTH1 contains an iron-sulfur (4Fe-4S) cluster formed by the N- and C- terminal ends for the protein involved in DNA binding [46]. Unlike NTH1, OGG1 can remove damaged DNA bases induced by ROS, 7,8-dihydro-8-oxoguanine (8-oxoG) [47]. DNA glycosylase removes basesl and has AP lyase activities [46]. Up to eight different isoforms containing an MTS are generated by alternative splicing but their precise function is still to be established. MUTYH is a monofunctional glycosylase and plays a role in removing adenine opposite 8-oxoG, guanine, or cytosine. Unlike other members of this family, MUTYH has a unique mechanism to cleave the undamaged bases from the DNA opposite the damaged ones, such as a mismatch A: 8-oxo-G [48], and can prevent mutations in DNA. Alternative splicing generates three primary transcripts that can further be spliced into an over 15 different transcripts in total. However, there seems to be a main mitochondrial isoform that contains a N-terminal MTS. The glycosylase also contains an iron-sulfur cluster within the catalytic region [47]. MBD4 is a monofunctional glycosylase with the peculiarity of having two functional domains: a methyl-binding domain at the N-terminus (MBD) and a glycosylase domain at the C-terminus [49]. Another feature is that this glycosylase was not found in mitochondria [30]. All these subfamilies have a common HhH motif with two α-helices that are important for DNA contact. The iron-

sulfur clusters are not present in every subfamily and may play a role in lesion detection since the redox potential shift was detected upon DNA binding [47].

The Fpg/Nei family: Fpg and Nei are three human Neil (Nei-like) DNA glycosylases in the nucleus: NEIL1, NEIL2, and NEIL3, of which NEIL1 and NEIL2 present in human mitochondria, and NEIL1 has with a long N-terminal MTS [50]. Double strand DNA (dsDNA) lesions, are mainly cleaved by NEIL1 while lesions in single strand DNA (ssDNA) are mostly excised by NEIL2. Both are able to recognize the same type of structures, e.g. bubbles, forks, and bulges [30]. Fpg has no homologous enzyme in humans. All family members present an N- and a C-terminal domain linked by a flexible region with the long axis of the protein placed orthogonal to DNA binding groove.

DNA glycosylases play a role in the identification of damaged bases and BER processes via creating intermediates or transferring the lesion to other repair methods [46]. X-ray repair cross-complementing protein 1 (XRCC1) [51], for example, acts as a BER scaffolding protein, but it can also team up with AAG, NTHL1, or NEIL1 to enhance the repair activity of BER.

4.2 **AP endonuclease**

AP endonuclease exists both in the nucleus and in the mitochondria and is encoded by a nuclear gene. In addition to excising abasic sites created during the repair process, it can regulate different transcription factor, and inhibit ROS production as a way of

controlling redox status in the cell [52]. The main hydrolytic AP endonuclease in mammalian cells is APE/Ref-1 (APE1) [26] and functions throughout the BER pathway [53]. The AP sites are left after the excision of the damaged bases by monofunctional glycosylases (e.g. UDG1) and excised by APE1 through a hydrolytic mechanism. By contrast, bifunctional glycosylases (e.g. OGG1, NTHL1) have both the capabilities of incising the damaged bases and cleaving the AP site from 3' side in DNA backbone by the phosphodiesterase activity of APE1. AP site disrepair may result in blocking DNA transcription and replication [54]. APE1 can be subject of different post-translational modifications and phosphorylation in repair activity, S-nitrosation targets the protein to the cytoplasm and binds to certain regulatory elements promoted by acetylation [55].

4.3 **DNA polymerase γ**

The DNA polymerase γ (polγ) is encoded in nucleus and consists of the catalytic and the accessory subunit [56] and plays a role in BER in mitochondria to fill the gaps created during repair [57]. The dRP lyase activity is essential to end the process of a combination of monofunctional gycosylases and APE1 [3]. Polγ is able to perform translesion synthesis (TLS), i.e., nucleotide incorporation opposite a damaged template and past the damage via extension of a DNA primer [56]. Polymerase β is present in the BER process in nucleus and enhances mtDNA BER activity to maintain mitochondrial function [57].

4.4 **DNA ligase**

Nuclear mammalian DNA ligases include ligases I, IV, and III, of which ligase III (Lig 3) only confined to vertebrates [58]. Alternative splicing of the human DNA ligase III gene originates both the nuclear and mitochondrial form of the protein [59]. It plays a role in cell replication, recombination, and DNA repair (single-strand DNA repair and BER) [12]. Mitochondrial Lig 3 also plays a role in maintaining cell survival under long-term oxidative stress and removing dysfunctional mitochondria via autophagy, rather than nuclear Lig3. The overexpression of Lig3 increases the rate of base excision repair pathway in mitochondria and is associated with XRCC1 in various DNA repair activities [58]. XRCC1 can protect mammalian cells from ionizing radiation [60] and can participate in the DNA repair process of alkylation or ultraviolet light [61] to maintain the integrity of DNA [62]. DNA Lig3 is closely bound to XRCC1, dependent on the expression of XRCC1 in the nucleus [63], but not in the mitochondria [64].

5 mtDNA repair pathways

Lose of mtDNA integrity leads to mitochondrial dysfunction and ultimately to mitochondrial diseases. Reduced activity for DNA glycoxylases, OGG1, NTH1, or UNG1, is accompanied by increased APE1 activity in aged brain mitochondria [65]. In the following section, we will discuss several significant repair pathways in mitochondria (Figure 2).

5.1 **SP-BER**

BER repairs spontaneous base damages at a rate of more than 20000 events per cell daily [66], associated with the repair of oxidation, alkylation, deamination, and SSBs. Uracil-DNA glycosylase could catalyze and cleave the mutagenic uracil DNA base [67]. SP-BER and LP-BER are present in mitochondria. Although the BER pathways present in the nucleus and mitochondria contain similar steps [30], there are still significant differences. Mitochondrial BER has lower efficiency to repair oxidative DNA lesions than nuclear BER. The major type of DNA damage caused by ROS, 8-oxoguanine (8-oxoG), or thymine glycol (TG) [68], is more efficient in mitochondrial BER than nuclear BER [12]. In the nucleus, there are multiple polymerases involved, including pol β, δ, ε, ϰ and λ that connect with various sub-pathways, while human mitochondrial BER contains mainly Polγ [1], pol β, and PrimPol. BER comprises the recognition and incision of the damaged base by DNA glycosylases generating an abasic or AP-site, removal of the AP-site by AP-endonuclease 1(APE1), gap filling by DNA polymerase, and finally ligation by DNA ligase [12].

Damaged bases are recognized by either mono- or bifunctional DNA glycosylases. Monofunctional DNA glycosylases are uracil DNA glycosylase 1(UDG1/ UNG1) [22] and human MutY homolog glycosylase MUTYH (MYH) [69] in mitochondrial BER. After then, monofunctional DNA glycosylases remove the damaged base by hydrolysis of the N-glycosidic bond, leaving in the DNA a baseless or AP-site (Figure 3a). AP-site is created and subsequently cleaved by APE1 from the 5' end after the action of a monofunctional DNA glycosylase. A 5'-dRP is generated at one side and a

3’-OH at the other side [70]. Types of damages are instead recognized by bifunctional DNA glycosylases in mitochondria. There are four types of mitochondrial bifunctional glycosylases, e.g. 8-oxoguanine DNA glycosylase (OGG1) [71], NTH1/ NTHL1 [72], NEIL1, or NEIL2 [50, 73] and have an inherent AP-lyase activity. Bifunctional DNA glycosylases can recognize, remove the damaged base, and cleave the AP-site in patterns of β- or β, δ-eliminations [74]. The specific process is shown in Figure 1. Bifunctional glycosylases can be further subdivided into OGG1 and NTHL1 to possess β-lyase activity, while NEIL1 and NEIL2 are β, δ-lyases. Those different activities produce different cleaved products at the 3’ end. β-lyases produce 5’P and 3’-phospho-α, β-unsaturated aldehyde (3′ PUA) ends while β, δ-lyases generate 5’P and 3’P at each end, respectively. The specific process is shown in Figure 3b.

A 3’-OH and a 5’dRP are generated after the action of UNG1 or MUTYH1, and APE1 and accomplished by the mitochondrial polγ, thanks to its dRP lyase activity. Either a 3′ PUA or a 3’P is generated after the action of bifunctional DNA glycosylases, and completed by the 3′ phosphodiesterase activity of APE1[75] and the phosphatase activity of the polynucleotide kinase 3’-phosphatase (PNKP) [3], respectively. The single-nucleotide gaps with 5’P and 3’-OH groups are generated and can be filled by the mitochondrial polγ [70]. After gap filling by Polγ, a nick left in mtDNA is carried out by Lig3 to complete the DNA repair process, where Lig3 plays an essential role in SP-BER of mtDNA repair.

5.2 **LP-BER**

LP-BER is initiated when mtDNA is damaged beyond the capacity of SP-BER or the type of damage is not suitable for this type of repair system. 2-deoxyribonolactone-induced damage is repaired via LP-BER pathway in mitochondria [76]. This repair process requires APE1, Polγ, and Lig3 [21], as well as other factors (Figure 3c). In the LP-BER pathway, the 5′ blocking group becomes a single-stranded DNA flap with 6–9 nucleotides after incision by APE1 and displacement by Polγ as a consequence of the extension from the 3'-OH [17]. Then, the flap endonuclease 1(FEN1) [77] acts as 5'-endonuclease cooperating with DNA2 [78] or 5' EXO/endonuclease (EXOG) [79] to inciss the flap. The Lig3-dominant repair pathway and the machinery can be also caused by other oxidative lesions and could be involved in mtDNA replication [76]. The involvement in mtDNA replication is similar to the nucleus [78]. LP-BER exists both in nucleus and in mitochondria, although there are differences regarding the enzymes or specific mechanisms. LP-BER occurs in the nucleus mainly in cell proliferation with the involvement of PCNA, FEN-1, polδ (ε), polβ and Lig1 [75]. Polδ/ε is the preferred polymerase in proliferating cells, while Polβ is the main polymerase for repair in nonproliferating cells. Due to the weak dRP lyase of Polγ, it would be possible that LP-BER can actually be the predominant mode of repair damaged bases in mitochondria.

5.3 **mtDNA Degradation**

mtDNA has many copies within mitochondria and is degraded without compromising organelle and cell function, different from in nDNA. When mtDNA suffered from serious excessive injuries and repair is no longer possible or damage cannot be repaired, the occurrence of cell degradation can prevent from further damage. Degradationto repair mtDNA plays a role in the maintenance of DNA stability and can improve the ability to repair damages caused by oxidative and alkylation, when BER is inhibited by compounds like methoxyamine or the repair system is overwhelmed. mtDNA degradation can process some damages of pyrimidine dimers induced by UV-light and numerous chemical carcinogens, which otherwise are not able to be dealt with in mitochondria. UV-related DNA lesions can also be repaired in mitochondria by polymerase zeta (Pol ζ) subunit REV3, a translesion DNA polymerase [80]. Indirect mtDNA degradation may also result in mitophagy. Damaged mtDNA leads to mitochondrial dysfunction and decreased membrane potential, directing the organelle and mtDNA to degradation.

5.4 **Direct DNA repair**

There are three main repair mechanisms for DNA direct repair: UV light-induced photolesions are repaired by photolyases, a series of O-alkylated damaged DNA are reversed via O_6-alkylguanine-DNA alkyltransferases, and N-alkylated base adducts are reversed by AlkB family dioxygenases [81]. The photoreactivation of cyclobutene pyrimidine dimers by photolyase were described in yeast [82] or plant mitochondria [82]. Other direct reversal enzymes such as DNA methyltransferase and ABH2

dioxygenase are recently identified [83], although their mitochondrial localization is not described. However, the repair pathways in human mitochondria remain unclear.

5.5 **Nucleotide Excision Repair**

NER exists in the nucleus to repair damages induced by UV and various chemicals, while seems not in human mitochondria [84]. Despite of this,, two related proteins, Cockayne syndrome A and B, form complexes with DNA glycosylase Ogg1 and ssDNA binding protein SSBP1 during oxidative stress and accumulate in mitochondria [70]. In addition, another NER related protein, RAD23A is present in human cadiomyocyte mitochondria co-localizing with mtDNA [85].

5.6 **Mismatch repair**

MMR exists in the nucleus and in the mitochondria to repair mismatches caused by alkylation, oxidation, deamination of bases, erroneous insertions or slippage errors caused by dysfunction of the DNA polymerase in the DNA replication. Mitochondrial MMR is strand unbiased and not nick-directed unlike bacterial or nuclear counterparts, while nuclear MMR proteins are not found in mitochondria, suggesting a completely independent machinery. mtDNA instability is rarely associated with alterations in nuclear MMR genes, due to the variation between nuclear and mitochondria repair systems [70]. MMR begins with the recognition of mismatches and insertion/deletion loops in the nucleus by heterodimeric complexes MutSα and MutSβ, while those mismatches are recognized in mitochondria by the

multifunctional Y-box binding protein 1 [86, 87], although the mechanism by which other mitochondrial MMR proteins are recruited has been poorly understood. Y-box binding protein 1 is also present in the nucleus and functions as a transcription factor.

5.7 **DNA Strand Break Repair**

Single-strand break repair: The single stranded DNA is more susceptible to damage as compared to double stranded DNA. SSB repair can be induced by ROS-induced disintegration of oxidized deoxyribose, genotoxic elements, aberrant hydrolytic processes, or ineffective enzymatic activities. The latter group includes abortive DNA topoisomerase 1 (TOP1) activity during replication and transcription [89], abortive DNA ligase activity at existing SSBs and inefficient BER repair [70]. SSB repair pathway consists of break detection, DNA end-processing, gap filling, and finally DNA ligation, similar to base excision repair pathway, of which the latter two are the same as BER [89]. The enzymes involved in SSB repair are different to distinct enzymes such as aprataxin [90] and tyrosyl-DNA phosphodiesterase [69] in DNA ligase and TOP1 related SSBs, respectively, rather than DNA glycosylases.

Double-strand break repair: In the nucleus, double-strand breaks can be repaired in non-homologous end-joining (NHEJ), homologous recombination [91], or microhomology-mediated end-joining (MMEJ). In mitochondria, NHEJ and homologous recombination are initially described [4], and MMEJ is recently repaired as a very efficient repair system in mitochondria [92].

Homologous recombination

Compared to the NHEJ and MMEJ of DSB repair, homologous recombination maintains genetic information and requires an intact copy of the DNA as template, of which the activity is restricted to S and G2 phases. The DSB is recognized by a complex of MRE11, RAD50, and NBS1. Then exonuclease 1 or STR-DNA2 produce an ssDNA which invades a homologue molecule of DNA with RAD51 to create a crossing the break site from DNA polymerase to synthetize DNA. Homologous recombination exists in yeast, Chlamydomonas reinhardtii, and plant mitochondria [93, 94]. Although homologous recombination occurs not widely in mammalian mitochondria, the large amounts of recombination junctions and catenation are often seen in human heart and associated to the initiation of replication in adult human heart, while not in infants and rodents [95]. Proteins involved in homologous recombination, e.g. RAD51, DNA2, and EXOG, are equivalent to the mitochondrial exonuclease 1. We believe that homologous recombination plays a role in the maintenance of human mtDNA integrity.

Non-homologous End Joining

NHEJ is the main form of DNA strand break repair in the nucleus of mammalian cells and is performed by direct rapid ligation of chromosome broken ends with no template requirement, and at stages of the cell cycle except for M phase [96]. The repair process of NHEJ includes Ku heterodimer to prevent DSBs from degradation

and promote the recruitment of other mediator elements. Of those, the DNA dependent protein kinase catalytic subunit forms a bridge between two blunt DNA ends, XRCC4, and XLF (XRCC4-like factor) then binds to the complex and facilitates sealing by DNA Ligase IV (Lig 4). The meticulous process of NHEJ seems to be involved in other repair processes, such as BER, NER [15]. Mitochondrial extracts also have blunt-ended DNA binding activities, and Ku proteins and MRE11 were identified in mitochondria [97, 98]. It is evidenced that NHEJ is an important repair pathway in mitochondria, albeit not the main one for resolving DSB.

Microhomology-mediated end-joining

In addition to the classical NHEJ repair pathway, MMEJ functions in the cell with or without the deficiency of factors required for NHEJ. The junctions are often associated with larger deletions and the use of 5-25 nucleotide microhomology. The microhomology in regions is associated with mtDNA deletions in patients [99]. Proteins involved in mitochondrial MMEJ (MRE11, FEN1, PARP1, LIG3) may also contribute to this repair, instead of NHEJ [92].

6 Conclusion

The understanding of mtDNA repair experiences from the initial thought of no DNA repair pathway in mitochondria to the discovery of the BER repair pathway and to multiple mtDNA repair pathways. NER proteins are present in mitochondria, while their activities and functions are not clear [45]. The integrity of mtDNA is a

prerequisite for maintaining mitochondrial function, dependent upon a variety of DNA repair pathways. Mitochondrial gene mutations cause mitochondrial diseases and other diseases, including cancer [100], diabetes [101], cardiovascular diseases, and neurodegenerative disorders, as well as aging [68]. There are the clear differences of mtDNA function, regulation, and repair from nDNA, even though the repair machinery and some proteins can be shared between the nucleus and mitochondria. Such variations can reflect different environments and factors to DNA damage.

7 Future outlooks

There are urgent and great needs to explore more precise mechanisms by which DNA is involved in the repair of various pathways, and mtDNA components interact between them owns and between mitochondria and nuclear. It is questioned whether actually NER exists in mitochondria and how mitochondrial DNA repair pathways function in pathological states. We should pay more attentions to factors to control and regulate the balance between mtDNA injury and repair. In addition, new technical developments will benefit the understanding and potential application of gene editing and gene therapy as well as molecular mechanisms of mtDNA damage and repair. More efforts should be offer to investigate mtDNA damage dependent crosstalk, signaling, and interaction between mitochondria and nucleus [102, 103].

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9 Figure legends

Figure1 The human mtDNA

The human mtDNA. It is a circular genome about 16.5 kb long which contain the heavy and the light strand, respectively. This mtDNA containing 37 genes, containing 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs), 13proteins which all connect with oxidative phosphorylation system. ND1-6, NADH dehydrogenase, forming complex I; CYT b, cytochrome b of coenzyme Q, forming complex III; COX1-3, cytochrome c oxidase, forming complex IV. ATP8, ATP6, ATP synthetase 8 and 6, forming complex V.

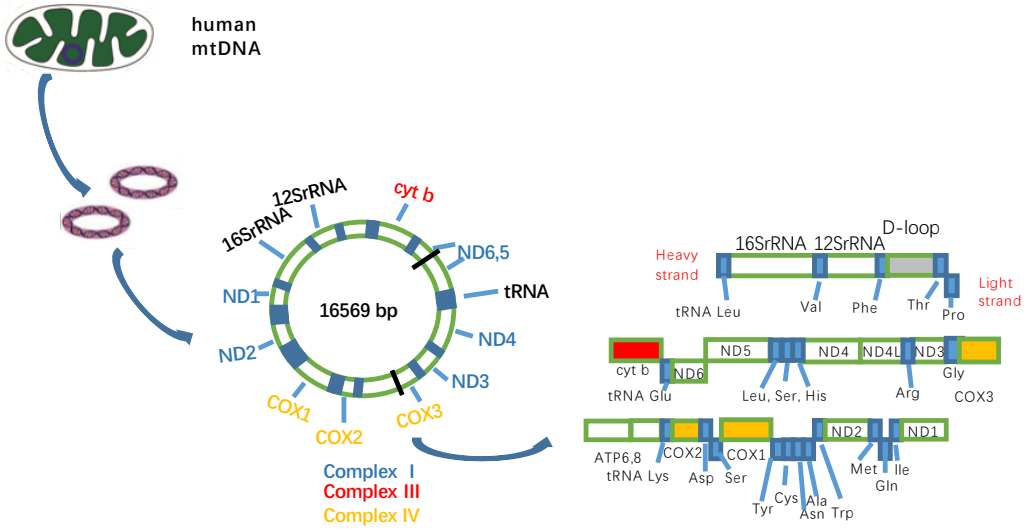
Figure 2 MtDNA repair pathways

MtDNA repair pathways. In mitochondrial DNA, there are many pathways of repair, including BER (SP-, LP-), mtDNA degradation, direct DNA repair, mismatch repair as well as DNA strand break repair (single-, double-).

Figure 3 BER

The BER pathways. In SP-BER, the first step is the damaged base recognition and removed by monofunctional DNA glycosylase, remaining an AP-site. This site is cleaved by APE1 from 5' to the site. This step generating 5'-dRP at one side and a 3'-OH at another side. Due to 5'-dRP cannot be ligated by DNA ligase, it must be removed. This step accomplishes by mitochondrial polγ, which possess dRP lyase

activity. But for bifunctional DNA glycosylases, other than that, it also has another character of inherent AP-lyase activity. The bifunctional DNA glycosylases cleave the AP-site through two methods: a β-elimination or β, δ-elimination reaction. Incision via the β-elimination reaction results in a strand break at the AP site with 3'PUA and 5'P, whereas β, δ-elimination leads to a single nucleotide gap with 3'P and 5'P termini. In both cases, the ends cannot be ligated and have to be further processed. This procedure is completed by the 3′ phosphodiesterase activity of APE1(71), phosphatase activity of the PNKP, respectively. As a result, this step generates a single-nucleotide gap with 5'P and 3'-OH groups. After gap filling by Polγ, leaving nick completed by the last step. This procedure via DNA Lig3, close the remaining nick. If the 5′ ends produced by base excision and strand cleavage cannot be used to generate a sequent DNA strand, repair this damage via LP-BER. For instance, formation of the 2-deoxyribonolactone damage is not associated with glycosylase activity because the base is lost as a result of hydrogen abstraction and oxidation of the C1' of deoxyribose and cannot be removed by dRP-lyase activity of polγ. During LP-BER, the 5'-blocking group is displaced by DNA polymerase extending from the 3'-OH ending and forming a so called "flap". And the 5'-blocking group becomes part of a single strand DNA flap. This flap in mitochondria has a length of up to 6–9 nucleotides. FEN1 acts as 5'-endonuclease cooperate with DNA2 or 5' EXO/endonuclease (EXOG) incising the flap. Finally, ligase III is used to fill the rest of the nick.


human
mtDNA
12SrRNA
16SrRNA
cyt b
ND6,5
ND1
tRNA
16569 bp
ND4
ND2
ND3
COX1
COX2
COX3
Complex I
Complex III
Complex IV
D-loop
16SrRNA 12SrRNA
Heavy
strand
Light
strand
tRNA Leu
Val
Phe
Thr
Pro
cyt b
ND6
ND5
ND4
ND4L
ND3
Gly
tRNA Glu
Leu, Ser, His
Arg
COX3
ATP6,8
tRNA Lys
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Gln
Tyr
Cys
Ala
Asn
Trp

Figure 1

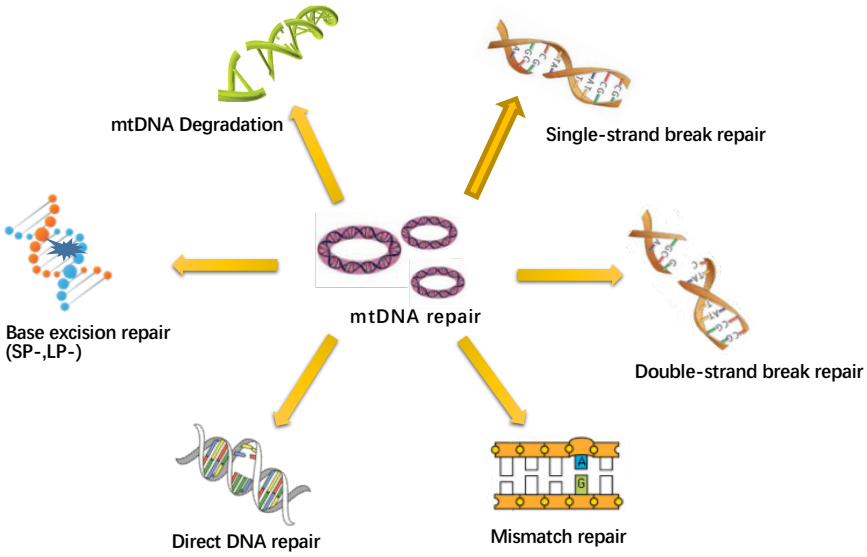
mtDNA Degradation
Single-strand break repair
Base excision repair
(SP-,LP-)
mtDNA repair
Double-strand break repair
Direct DNA repair
Mismatch repair
A
G

Figure 2

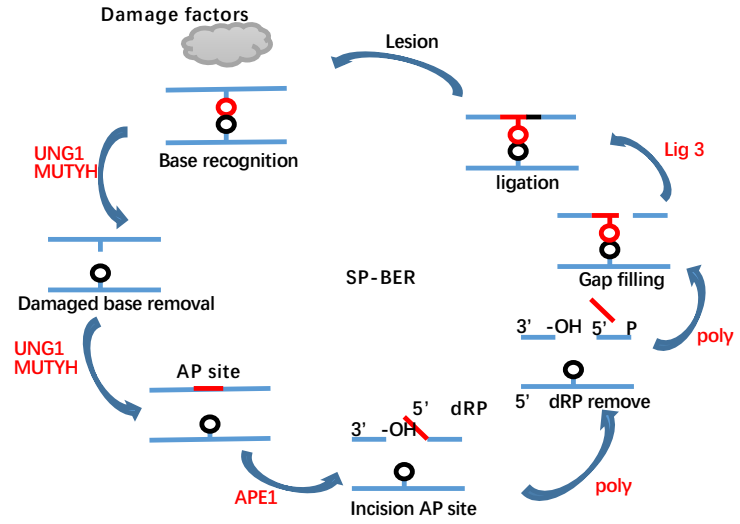

Figure 3a

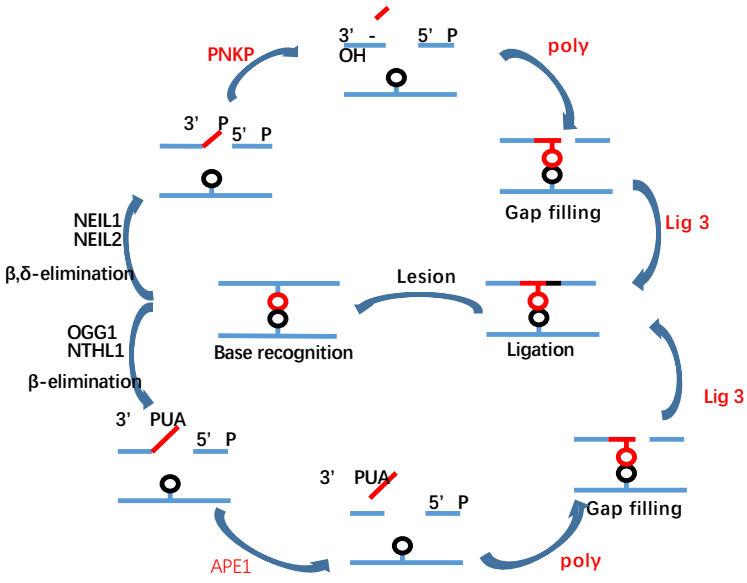

Figure 3b

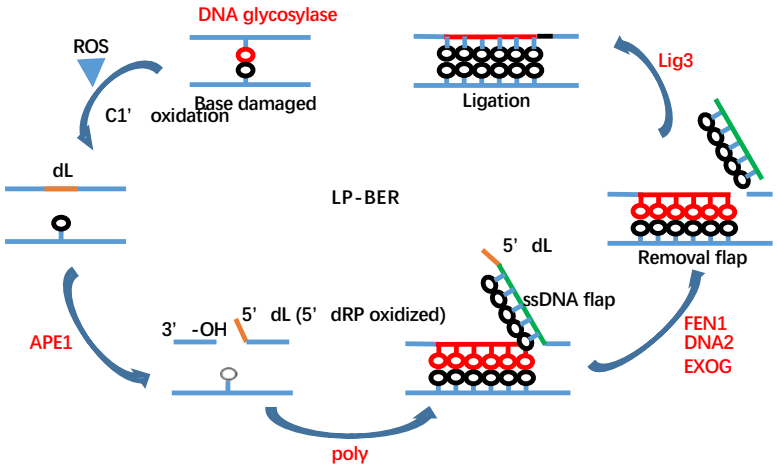
DNA glycosylase
ROS
C1' oxidation
Base damaged
Ligation
Lig3
dL
LP-BER
5' dL
Removal flap
ssDNA flap
3' -OH
5' dL (5' dRP oxidized)
APE1
FEN1
DNA2
EXOG
poly

Figure 3c