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Review Article

DNA damage related crosstalk between the nucleus and mitochondria☆

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ABSTRACT

The electron transport chain is the primary pathway by which a cell generates energy in the form of ATP. Byproducts of this process produce reactive oxygen species that can cause damage to mitochondrial DNA. If not properly repaired, the accumulation of DNA damage can lead to mitochondrial dysfunction linked to several human disorders including neurodegenerative diseases and cancer. Mitochondria are able to combat oxidative DNA damage via repair mechanisms that are analogous to those found in the nucleus. Of the repair pathways currently reported in the mitochondria, the base excision repair pathway is the most comprehensively described. Proteins that are involved with the maintenance of mtDNA are encoded by nuclear genes and translocate to the mitochondria making signaling between the nucleus and mitochondria imperative. In this review, we discuss the current understanding of mitochondrial DNA repair mechanisms and also highlight the sensors and signaling pathways that mediate crosstalk between the nucleus and mitochondria in the event of mitochondrial stress.

1. Introduction

A few decades after the nucleus was discovered in the 1830s [1], another granular-looking organelle termed “bioblasts” by Richard Altmann was discovered [2]. In 1898, the term mitochondria was coined by Carl Benda who named this organelle after the Greek words “*mitos*” meaning thread and “*chondros*” meaning granule [2]. The discovery that mitochondria contained nucleic acids was made in the 1960s and the development of cloning and sequencing techniques aided in a much better understanding of these organelles [3,4]. Despite their primary role in ATP generation, mitochondria also play critical roles in aspects of cellular signaling, fatty acid oxidation, calcium signaling, heme biosynthesis, and the assembly of iron-sulfur clusters in proteins [5–7]. These essential functions make mitochondria indispensible for cellular function; however, the first example of a eukaryotic micro-organism that lacks mitochondria was recently reported [8].

The circular ~16.5 kb human mitochondrial DNA (mtDNA) is maternally inherited and encodes for 13 polypeptides, 22 tRNAs, and 2 rRNAs that participate in oxidative phosphorylation (OXPHOS) via the electron transport chain (ETC) [9]. Unlike nuclear DNA, which is packaged into nucleosomes, mtDNA molecules are tightly associated with the mitochondrial matrix and form compact structures called nucleoids [10]. Nucleoids are composed of mtDNA-protein complexes that include proteins involved in replication and transcription such as mitochondrial single-strand binding protein (mtSSB), DNA polymerase gamma (POLG), and mitochondrial transcription factor A (TFAM) [11].

Just like its nuclear counterpart, mtDNA is subjected to genotoxic assaults from exogenous sources such as exposure to chemotherapeutic drugs as well as from endogenous sources including reactive oxygen species (ROS) that form as byproducts of mitochondrial respiration [12]. While evidence suggests that mtDNA molecules are likely to be more susceptible to oxidized DNA damage than nuclear DNA owing to their proximity to sites of oxidative phosphorylation, our current knowledge on the extent of mtDNA damage is limited owing to the lack of experimental approaches to accurately detect oxidatively generated mtDNA damage [7,12]. Several DNA repair mechanisms exist within a cell to restore DNA integrity and while these pathways have been extensively studied in the nucleus (reviewed in [13,14]), the base excision repair (BER) pathway has been established as the primary repair pathway in the mitochondrion [15]. Evidence for DNA repair pathways occurring in the mitochondria has been presented where mtDNA repair enzymes are encoded by nuclear genes and translocate to the mitochondria [15,16]. With the exception of the 13 mtDNA encoded polypeptides, the mitochondrial proteome comprises an estimated > 1500 proteins encoded by nuclear genes that are exported to the mitochondria for mtDNA maintenance [17,18]. Multiple mitochondrial protein import pathways participate in protein translocation to the mitochondria [19]. For instance, many proteins possess a

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet and Davies [252].

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mitochondrial targeting signal (MTS) that enables the protein to be transported via translocases of the outer and inner membranes (TOM and TIM), while others rely on the redox-mediated MIA pathway for import into the mitochondria [18–20]. Web servers like MitoProt, MitoFates, and TPpred2 that predict mitochondrial localization by analysis of the primary amino acid sequence, are becoming useful tools to theoretically evaluate the probability of mitochondrial targeting of a protein [21–23].

Mitochondria are unique in their genome organization in that they can contain multiple copies of mtDNA molecules within a single mitochondrion. Therefore, mtDNA damage if left unrepaired can lead to mutations that could result in heteroplasmy, a condition where both undamaged and damaged mtDNA molecules co-exist within the same mitochondrion [24]. Mitochondrial mutations can cause coding errors in the 13 polypeptides involved in ATP generation via the ETC and in the 22 tRNA molecules encoded by the mitochondrial genome [25,26]. Depending on the extent of DNA damage, heteroplasmy can lead to mitochondrial dysfunction and the fine threshold between normal mitochondria and those that develop into a disease state remains intriguing [27]. Several chronic human diseases including diabetes, aging, neurodegenerative disorders (such as Alzheimer's and Parkinson's disease), and cancer are believed to be associated with mitochondrial dysfunction [17,26,28–30]. Mitochondria signal and communicate with the rest of the cell through numerous signaling pathways in response to dysfunction caused by physiological stimuli, stresses, and biological events [31–33]. In this review, we discuss our current knowledge of mtDNA repair and highlight some of the signaling pathways that coordinate stress responses between the nucleus and mitochondria in the event of DNA damage.

2. Mitochondrial DNA genome maintenance

Mitochondrial antioxidant pathways present the first line of defense that protects mitochondrial genome integrity (reviewed in [34,35]). These include superoxide dysmutases (SODs), and the glutathione (GSH) peroxidase, thioredoxin (Trx), and peroxiredoxin (Prx) pathways [36–39]. Manganese SOD, an example of a member of this family, is present in the mitochondrial matrix and converts superoxide ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) [40]. The H_2O_2 generated can be neutralized to water by either GSH or the thiol-specific peroxidases, Prx [39]. If the H_2O_2 generated in the mitochondria is not neutralized to water, it can be readily converted to the hydroxyl radical ($^{\bullet}OH$), a strong oxidant, in the presence of Fe^{2+} via the Haber-Weiss Fenton reaction [41]. Since the mitochondria play a critical role in FeS cluster biogenesis and in iron homeostasis, these organelles are likely to be more susceptible to damage by H_2O_2[12].

Oxidative stress results when the equilibrium between the antioxidant systems and ROS production is perturbed. Guanine has the lowest redox potential of the four DNA bases and is prone to oxidatively generated DNA damage produced by one-electron oxidants or singlet oxygen species [42–44]. The most common base modification that results from oxidation of guanine is 8-oxo-7,8-dihydroxyguanine (or 8-oxoG, Fig. 1) [45,46]. Thymine on the other hand is susceptible to $^{\bullet}OH$-mediated damage resulting in lesions such as 5,6-dihydroxy-5,6-dihydrothymine [43]. Other modifications including alkylation, bulky adducts, and deamination also occur within mtDNA (Fig. 1). To combat DNA damage, like the nucleus, mammalian mitochondria also possess DNA repair pathways, which involve repair proteins encoded by nuclear genes that are transported to the mitochondria (see Table 1). Whether these repair enzymes pre-exist in mitochondria at the time of damage or translocate into the mitochondria in response to damage signals is still not clear. The DNA repair pathways in mammalian mitochondria include base excision repair (BER), direct reversal (DR), mismatch repair (MMR), translesion synthesis (TLS), and possibly double-strand break repair (DSBR) [16,47–49]. Thus far, there is no evidence for the repair of helix-distorting bulky adducts and ultraviolet-induced photodimers by the nucleotide excision repair (NER) pathway in mitochondria [50]. However, in one study, XPD, an NER protein was seen to protect mtDNA from oxidative DNA damage [51]. These data need to be further clarified and substantiated [52]. Furthermore, bulky adducts such as benzo[a] pyrene diol epoxide that are also formed within mtDNA (Fig. 1) have no known mechanism of repair within the mitochondria, and are susceptible to destruction by autophagy [17,53]. Overall, of the DNA repair pathways available in the mitochondria, BER appears to be the major pathway for eliminating ROS-induced oxidative damage.

2.1. Base excision repair (BER)

The BER pathway is a well-characterized, tightly-coordinated process that is carried out in a step-wise manner and includes: recognition and excision of the damaged DNA base, removal of the resulting abasic (apurinic/apyrimidinc or AP) site, end processing, gap filling, and ligation (summarized in Fig. 2) [16,48,54]. BER can proceed via three sub-pathways, short-patch repair (SP-BER, 1-nt), long patch repair (LP-BER, 2 or more nt), and single-stranded break repair (SSBR) (extensively reviewed in [55–57]).

The initiation step of BER is carried out by DNA glycosylases, specialized enzymes, which recognize the damaged base and catalyze the cleavage of the N-glycosyl bond between the damaged base and its 2-deoxyribose resulting in an AP site [58,59]. Glycosylases are either monofunctional or bifunctional, depending on whether they possess an intrinsic lyase activity. Seven of the eleven known mammalian DNA glycosylases contain an MTS that allows them to translocate to the mitochondria (summarized in Table 1 and Fig. 2) [54]. The translocation of some DNA glycosylases to their respective nuclear and mitochondrial compartments is enabled by mechanisms of alternative splicing and different transcription start sites as observed with the uracil-DNA glycosylase (UDG), 8-oxoG DNA glycosylase 1 (OGG1), and MUTYH DNA glycosylase [60–62]. Monofunctional glycosylases excise non-oxidized damaged bases and rely on AP endonuclease (APE1) to complete the lyase elimination reaction whereas bifunctional DNA glycosylases are involved in the removal of oxidized DNA bases and nick the DNA backbone 3' to the lesion [63,64]. End processing following base excision and backbone cleavage by bifunctional glycosylases either involves APE1 as is seen in the case of OGG1 and NTHL1 (Nth-like 1) or polynucleotide kinase phosphate (PNKP) that processes the ends generated by the NEIL (Nei-like) enzymes [64,65]. Both APE1 and PNKP have been identified in the mitochondria and play critical roles in mitochondrial BER [66–69]. DNA POLG, the primary polymerase in the mitochondrion, is responsible for the gap-filling synthesis step in the mitochondria [70,71]. During SP-BER, POLGA, the catalytic subunit of heterotrimeric POLG, possesses lyase activity and is able to excise the 5′-phosphodeoxyribose (dRP) moiety generated by APE1 and fills the 1-nt gap [72]. During LP-BER, the strand displacement synthesis activity of POLGA is utilized, which generates flaps of 2–6 nt that are good substrates for the flap endonuclease 1 (FEN1) [73–75]. Although early reports suggested that SP-BER was the primary mitochondrial BER pathway, the choice between LP-BER and SP-BER is determined by several factors that are present in both the nucleus and mitochondria [64,74]. For instance, certain types of oxidized abasic sites (such as 2-deoxyribonolactone) that are not suitable substrates for POLG undergo FEN1-dependent LP-BER [75]. Another enzyme DNA2, also found in mitochondrial extracts, assists in the FEN1-mediated cleavage of the DNA flaps by maintaining the length of single-stranded flap so that it is not immediately coated by mtSSB [76]. In contrast, another study demonstrates that 5'-exo/endonuclease (EXOG) removes 5' flaps during mitochondrial LP-BER independently of FEN1 and DNA2 [77]. The final ligation step in the mitochondria, which is common in all BER sub-pathways, is carried out by DNA ligase III (LigIII) [78,79].

The SSBR pathway is considered to be a form of SP-BER that involves detection of SSBs, end processing, gap filling, and ligation