Published in final edited form as:

Free Radic Biol Med. 2017 June ; 107: 146–150. doi:10.1016/j.freeradbiomed.2016.11.025.

Risky Repair: DNA-protein Crosslinks Formed by Mitochondrial Base Excision DNA Repair Enzymes Acting on Free Radical Lesions

Rachel Audrey Caston and Bruce Demple*

Department of Pharmacological Sciences, Stony Brook University School of Medicine, Stony Brook, NY, 11794, USA

Abstract

Oxygen is both necessary and dangerous for the aerobic cell function. ATP is most efficiently made by the electron transport chain, which requires oxygen as an electron acceptor. However, the presence of oxygen, and to some extent the respiratory chain itself, poses a danger to cellular components. Mitochondria, the sites of oxidative phosphorylation, have defense and repair pathways to cope with oxidative damage. For mitochondrial DNA, an essential pathway is base excision repair, which acts on a variety of small lesions. There are instances, however, in which attempted DNA repair results in more damage, such as the formation of a DNA-protein crosslink trapping the repair enzyme on the DNA. That is the case for mitochondrial DNA polymerase γ acting on abasic sites oxidized at the 1-carbon of 2-deoxyribose. Such DNA-protein crosslinks presumably must be removed in order to restore function. In nuclear DNA, ubiquitylation of the crosslinked protein and digestion by the proteasome are essential first processing steps. How and whether such mechanisms operate on DNA-protein crosslinks in mitochondria remains to be seen.

Keywords

oxidized abasic sites; 2-deoxyribonolactone; AP lyase; DNA polymerase beta; DNA polymerase gamma

Oxidative Damage in the Mitochondria

Cells depend strongly on the electron transport chain to generate ATP. Oxygen is used in this process as an electron acceptor, and the overall process generates the proton gradient that powers ATP synthase. While efficient for producing ATP, oxidative phosphorylation potentiates side reactions that “leak” electrons via non-enzymatic reactions with oxygen to generate reactive oxygen species (ROS) (Figure 1). This is not the only mechanism that produces ROS in the cell, but it is an significant pathway in mitochondria[1].

*Corresponding author: bruce.demple@stonybrook.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

It is important to note that mitochondria also use ROS generation as a signal. When mitochondria become damaged, the increased release of ROS is a signal to the cell that these mitochondria need to be eliminated [2]. The complex role of ROS in mitochondria is indicated, for example, in *C. elegans*, where both the elimination *and* the upregulation of the mitochondrial superoxide dismutase increases life span [3,4].

All components of the mitochondrion are at risk of damage from ROS, but the mitochondrial DNA (mtDNA) is of particular interest. The mitochondrial genome encodes some components of the electron transport chain, the tRNAs that mediate a separate genetic code, and ribosomal RNAs for translation in the organelle [5]. The mtDNA is associated with the inner membrane of mitochondria, placing it in close proximity to the electron transport chain, which presumably increases exposure of the DNA to ROS [6]. The mtDNA further lacks the extensive packing that constitutes nuclear chromatin [7]. While it would seem that mtDNA should have a greater frequency of oxidatively generated lesions compared to nuclear DNA, definitive evidence of this has been elusive. The level of mtDNA lesions reported varies widely, depending on the technique used for measurement [6,8].

When mitochondrial proteins are altered by mutation, faults in the electron transport machinery can result, which increases the formation of ROS. It has been speculated that this generates a vicious cycle causing further mutations, which may contribute to aging phenotypes [9,10]. Oxidative damage in mtDNA is frequently cited as being correlated with neurodegeneration. When mutations accumulate faster in certain cell types, for example the substantia nigra in the brain, the rapid amassing of failing mitochondria may underlie neurodegeneration [11]. An increased load of oxidatively generated lesions in mtDNA has also been shown in Parkinson's, Huntington's, and Alzheimer's diseases [12–14]. However, it is not clear whether the mutations are the cause of the degeneration, or only a symptom of a process already in motion.

Mitochondrial Base Excision DNA Repair

As lesions arise in mitochondrial DNA, repair pathways are engaged to maintain the integrity of the DNA. Mitochondria do not have the full complement of repair options available in the nucleus, but they do have a subset of those pathways [15]. Base excision DNA repair (BER) is responsible for correcting small, non-distorting lesions, such as those that would be formed by ROS. BER (Figure 2) begins with the recognition of a DNA lesion by a DNA glycosylase. Each glycosylase recognizes a limited range of base damages, with some overlap among the enzymes. The glycosylase removes the base by breaking the N-glycosylic bond between the base and the sugar-phosphate backbone, leaving an apurinic/aprimidinic (AP) site in the DNA [16]. After the damaged base is removed, Ape1 (in mammalian cells) nicks the DNA on the immediate 5' side of the AP site, which generates a normal 3' OH that can be used as a primer by DNA polymerase, and a 5' end bearing the abasic 2-deoxyribose-5-phosphate (5'-dRP).

The glycosylase can be either monofunctional or bifunctional. Monofunctional glycosylases remove the damaged base by hydrolysis, which produces an unmodified 2-deoxyribose in the AP site, which is then incised by Ape1. In contrast, bifunctional DNA glycosylases act

via a covalent intermediate to effect base removal, sometimes cleaving the DNA backbone in a β -elimination reaction to produce a 3' end bearing a 2,3-unsaturated derivative of 2-deoxyribose. This lyase-generated 3'-blocking group must be removed, and a number of candidate enzymes are proposed for this role, notably Ape1, which has a significant 3'-processing activity [17]. It is also possible for certain lyases, such as Neil1 and Neil2, to remove the base using β , δ -elimination; the reactions eliminate the 2-deoxyribose, although a 3'-phosphate remains that has to be removed by an enzyme such as polynucleotide kinase-phosphatase before ligation can occur [18][19]. Mitochondria do not contain all 11 of the glycosylases found in the nucleus, but 7 of them are localized to the organelle in mammalian cells, including several that have lyase activity (Table 1)[20].

In the mitochondria, DNA repair polymerase activity has been attributed to DNA polymerase γ (Pol γ) [31]. However, with the recent discovery of PrimPol, this issue should be revisited to clarify whether the newly discovered polymerase is involved in the gap-filling step of BER [32,33]. PrimPol has the ability to copy past abasic sites and photolesions in the DNA [32,33], so it could assist repair at sites where there are lesions in both DNA strands, for example.

At this point BER branches into "short-patch" and "long-patch" sub-pathways. During short-patch (single-nucleotide) BER, the DNA polymerase adds one nucleotide to replace the damaged one. Enzymes such as Pol γ can then remove the 5'-dRP via a lyase activity, which allows the nick to be ligated, evidently by DNA ligase III, to complete the repair. In long-patch BER, the DNA polymerase adds two or more nucleotides, which displaces an oligonucleotide retaining the 5'-dRP. This displaced flap needs to be excised by a structure-specific nuclease. Several enzymes have been proposed to fill this roll in mitochondria: Fen1, DNA2, and ExoG [34,35]. Another recently identified mitochondrial nuclease, MGME1, has enzymatic properties also appropriate to this function [36]. A case can be made for each of these enzymes to function in mitochondrial BER, which is discussed below. Flap excision by Fen1 acts on relatively short displaced strands to generate a ligatable product [34]. The process can also occur in multiple steps, with (for example) DNA2 removing a flap down to 2 nucleotides, followed by Fen1 to excise the remaining dinucleotide [37], allowing DNA ligase III to seal the nick. The fate of the excised oligonucleotide containing a 5'-terminal abasic site has not been determined.

As the only proteins encoded by mtDNA are components of the respiratory apparatus, nuclear-encoded BER enzymes must all be imported into mitochondria. In general, the mitochondrial and nuclear repair proteins are quite similar, aside from their targeting sequences (Table 1). A clear example is UNG1 and UNG2, which are expressed from alternatively spliced mRNAs to yield proteins with different N-termini [38,39]. UNG1 contains a mitochondrial targeting sequence that is not found in UNG2. The UNG1 mitochondrial targeting sequence is cleaved as the protein enters the mitochondria [40]. There is also a set of proteins for which the mitochondrial localization is not associated with a recognizable mitochondrial targeting sequence, such as Fen1. Alternative splicing of Fen1 mRNA does result in a distinct mitochondrial form with a preference for binding RNA over DNA, but it seems to lack the nuclease activity of the full-length protein [27].

With regard to the mitochondrial structure-specific nucleases, Fen1 and DNA2 are primarily nuclear, while ExoG and MGME1 are mostly localized to mitochondria. Fen1 and DNA2 are good candidates for the long patch BER nucleases, and their activity on flap substrates in the nucleus is well documented [41]. However, cell cycle and differentiation processes may affect the expression of these proteins, as has been seen with other BER proteins [42,43]. ExoG and MGME1, being mitochondria-specific, may not fluctuate with the cell cycle. [26,36,44,45]. ExoG has not proven to be efficient at cleaving a flap substrate longer than 2 nucleotides [26,35], and there is only limited evidence to support the enzyme's involvement in BER [35]

Formation of DNA-Protein Crosslinks (DPC)

In the nucleus, the majority of lesions handled via BER can in principle be repaired using the short-patch pathway [46][47]. However, some lesions can be repaired only by the long-patch pathway. One reason is that BER polymerases, and certain DNA glycosylases, form covalent links to the DNA via transient Schiff base intermediates during their lyase reactions. However, enzyme attack on some lesions forms an amide linkage that traps the protein in a stable covalent link to the DNA (Figure 3A). A clear example is the trapping of DNA polymerase β (Pol β) [48] or *Escherichia coli* endonuclease III [49] by 2-deoxyribonolactone (dL). The dL lesion was the first characterized oxidative product in DNA, and it results from hydroxyl radical attack on the C1' carbon of a nucleotide [50]. The 5'-dRP of Pol β attacks the oxidized carbon using a lysine nucleophile, generating a DPC anchored by an amide bond. Recently, the in vivo formation and removal of Pol β -DPC formed by oxidative agents was characterized in human and mouse cells [51,52]. Formation of Pol β DPC was a result of Pol β 's mechanistic role in BER. Cells treated with oxidizing agents generated Pol β DPC, with a low background level of Pol β DPC even in untreated cells. The effectiveness of an agent in producing the Pol β -DPC in cells correlated directly with the agent's ability to generate dL lesions, with copper-orthophenanthroline being especially effective compared to H₂O₂, and non-oxidative agents such as methylmethane sulfonate ineffective [51]. A substitution of the Pol β lyase active-site lysine by alanine prevented the formation of oxidatively generated DPC. Even the background DPC in untreated cells was eliminated for the lyase-defective protein [51], which indicates that this spontaneous accumulation is due to lesion processing by the enzyme. In the nucleus, DPC formation with dL can be avoided by repair using the long-patch BER pathway, which is also observed in mitochondrial extracts [34].

Some DNA glycosylases can also be trapped by dL or other oxidatively generated lesions. Oxanine, generated in DNA by nitric oxide attack on guanine, traps bacterial Fpg, Nei/ endonuclease VIII, and AlkA proteins, as well as eukaryotic Ogg1 [53]. 5-Hydroxy-5-methylhydantoin traps Fpg, Nei/Endo VIII, and mammalian NEIL1 [54]. The DPC formed with oxanine and 5-hydroxy-5-methylhydantoin differ from those formed by Pol β with dL in two ways: the mechanism of trapping in these two cases is via direct reaction with the modified base; and they occur in an unbroken DNA strand. Relatively stable DPC can even be formed by some lyases acting on normal AP sites, as reported for the lyase activity of poly(ADP-ribose) polymerase-1 [55].

There is evidence for the generation of mitochondrial DPC. Mitochondrial Pol γ is trapped in vitro by dL, either with the purified protein or in mitochondrial extracts from HeLa cells [34]. Preliminary evidence from our lab has also revealed the formation of DPC containing Pol γ in oxidant-treated whole mitochondria. The other mitochondrial DNA polymerase, PrimPol, has not been shown to have lyase activity. Although no studies have been reported on them, other mitochondrial lyases would likely also be trapped by dL in mitochondrial DNA.

Repair of Oxidatively Generated DPC

It seems likely, even obvious, that DPC would need to be removed from DNA to prevent the disruption of transcription and replication. Nuclear Pol β -DPC accumulate in oxidant-treated cells incubated with the proteasome inhibitor MG132, indicating that proteolysis is an early step in their excision from the DNA [51]. The accumulated DPC, which are cytotoxic, also contain ubiquitin, of which 65–75% depends on the Pol β lyase: the lyase-inactivating K72A substitution (preventing the formation of oxidative Pol β -DPC) eliminates the bulk of ubiquitin from oxidatively generated DPC in MG132-treated human cells [51]. Similarly, most of the ubiquitin trapped in DPC in oxidant-treated murine fibroblasts depends on Pol β [51]. These data imply that ubiquitylation of Pol β -DPC targets their processing by the proteasome.

Mitochondria contain their own set of proteases, located in the intermembrane space, the inner membrane, and the matrix. [56]. So far, E3-ubiquitin ligases are reported only for the outer membrane of mitochondria, so their involvement in processing DPC in mtDNA seems unlikely. In the matrix, where the mtDNA is located, AAA proteases perform quality control degradation and the removal of mitochondrial targeting sequences. LONP1 and ClpP proteases degrade oxidized proteins in the mitochondrial matrix [56]. It is possible that one or more of these proteases also degrades the protein component of DPC.

In the absence of repair, cells may have damage tolerance pathways. For example, translesion DNA polymerases can synthesize past a DPC or its residual peptide in an unbroken DNA strand, thus using proteasome-coupled repair as a form of damage tolerance [57]. This process would not remove the peptide-DNA adduct, but it could prevent cytotoxicity. However, it is uncertain whether appropriate translesion DNA polymerases to support such a process occur in mitochondria. There is some evidence that PrimPol is capable of translesion synthesis across UV products, 8-oxo-7,8-dihydroguanine, or an abasic site [32,58,59]. In this context, though, it should be realized that the Pol β -DPC differ in an important way from the DPC processed by translesion synthesis: the former are trapped at sites of a strand break, which is expected to prevent DNA synthesis across them.

Concluding Remarks and Future Directions

The generation of oxidatively generated DPC during mitochondrial BER is coming into focus. Pol γ -DPC are formed in mitochondrial extracts incubated with the oxidatively generated lesion dL [34,48], and preliminary data indicate the formation of Pol γ -DPC in isolated mitochondria treated with dL-forming oxidants. Whether Pol γ -DPC can be detected

in intact cells remains to be seen. In the nucleus, the lyase active site of Pol β is essential for the observed protein trapping. The active site of the lyase in Pol γ has yet to be characterized, however, making a similar mechanistic test of Pol γ trapping difficult. Moreover, processes for removing mitochondrial DPC have not yet been described. The nuclear DPC degradation mechanism is dependent upon E3-ubiquitin ligase activity and the proteasome, which do not seem to have mitochondrial counterparts. Thus, new mechanisms for coping with DPC may emerge as the processing of these lesions in mitochondria is defined.

Acknowledgments

We are grateful to our laboratory colleagues for helpful discussions, and to Prof. Orlando Schärer for his comments on a figure. Our work is supported by grants to B.D. from the U.S. National Institutes of Health (R21CA198752 and R21CA191856), and by a grant from the U.S. National Aeronautics and Space Administration (NNA14AB04A; P.I. Prof. Tim Glotch, Stony Brook University).

References

1. Murphy MP. How mitochondria produce reactive oxygen species. *Biochemical Journal*. 2009; 417:1–13. [PubMed: 19061483]
2. Sena L, Chandel N. Physiological roles of mitochondrial reactive oxygen species. *Molecular Cell*. 2012; 48:158–167. [PubMed: 23102266]
3. Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, Papp D, Braeckman BP, Gems D. Increased life span from overexpression of superoxide dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. *Free Radical Biology and Medicine*. 2011; 51:1575–1582. [PubMed: 21839827]
4. Hekimi S, Lapointe J, Wen Y. Taking a “good” look at free radicals in the aging process. *Trends in Cell Biology*. 2011; 21:569–576. [PubMed: 21824781]
5. Copeland WC, Longley MJ. Mitochondrial genome maintenance in health and disease. *DNA Repair (Amst)*. 2014; 19:190–198. [PubMed: 24780559]
6. Alexeyev M, Shokolenko I, Wilson G, LeDoux S. The Maintenance of Mitochondrial DNA Integrity-Critical Analysis and Update. *Cold Spring Harb Perspect Biol*. 2013; 5:a012641. [PubMed: 23637283]
7. Bogenhagen DF. Mitochondrial DNA nucleoid structure. *Biochim Biophys Acta*. 2011; 1819:914–920. [PubMed: 22142616]
8. Anson RM, Hudson E, Bohr VA. Mitochondrial endogenous oxidative damage has been overestimated. *FASEB J*. 2000; 14:355–360. [PubMed: 10657991]
9. Liochev SI. Which Is the Most Significant Cause of Aging? *Antioxidants (Basel)*. 2015; 4:793–810. [PubMed: 26783959]
10. Harman D. Aging: A Theory Based on Free Radical and Radiation Chemistry. *Journal of Gerontology*. 1956; 11:298–300. [PubMed: 13332224]
11. Sanders LH, McCoy J, Hu X, Mastroberardino PG, Dickinson BC, Chang CJ, Chu CT, van Houten B, Greenamyre JT. Mitochondrial DNA damage: molecular marker of vulnerable nigral neurons in Parkinson's disease. *Neurobiology of Disease*. 2014; 70:214–223. [PubMed: 24981012]
12. Ayala-Peña S. Role of oxidative DNA damage in mitochondrial dysfunction and Huntington's disease pathogenesis. *Free Radical Biology and Medicine*. 2013; 62:102–110. [PubMed: 23602907]
13. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*. 2006; 443:787–795. [PubMed: 17051205]
14. Riemer J, Kins S. Axonal Transport and Mitochondrial Dysfunction in Alzheimer's Disease. *Neurodegenerative Diseases*. 2013; 12:111–124. [PubMed: 23037012]

15. Kazak L, Reyes A, Holt IJ. Minimizing the damage: repair pathways keep mitochondrial DNA intact. *Nature Reviews Molecular Cell Biology*. 2012; 13:659–671. [PubMed: 22992591]
16. Dianov GL, Hübscher U. Mammalian base excision repair: the forgotten archangel. *Nucleic Acids Research*. 2013; 41:3483–3490. [PubMed: 23408852]
17. Brooks SC, Adhikary S, Robinson EH, Eichman BF. Recent advances in the structural mechanisms of DNA glycosylases. *Biochim Biophys Acta*. 2012; 1834:247–271. [PubMed: 23076011]
18. Das A, Wiederhold L, Leppard JB, Kedar P, Prasad R, Wang H, Boldogh I, Karimi-Busheri F, Weinfeld M, Tomkinson AE, Wilson SH, Mitra S, Hazra TK. NEIL2-initiated, APE-independent repair of oxidized bases in DNA: Evidence for a repair complex in human cells. *DNA Repair (Amst)*. 2006; 5:1439–1448. [PubMed: 16982218]
19. Izumi T, Boldogh I, Imhoff B, Kow YW, Jaruga P, Dizdaroglu M, Mitra S. Identification and characterization of a human DNA glycosylase for repair of modified bases in oxidatively damaged DNA. *Proceedings of the National Academy of Sciences*. 2002; 99:3523–3528.
20. Prakash A, Doublié S. Base Excision Repair in the Mitochondria. *J Cell Biochem*. 2015; 116:1490–1499. [PubMed: 25754732]
21. Otterlei M, Terje T, Toril TS, Slupphaug G, Tore L, Krokan HE. Nuclear and mitochondrial uracil-DNA glycosylases are generated by alternative splicing and transcription from different positions in the UNG gene. *Nucleic Acids Research*. 1998; 25:4611–4617.
22. Nishioka K, Ohtsubo T, Oda H, Fujiwara T, Kang D, Sugimachi K, Nakabeppu Y. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Mol Biol Cell*. 1999; 10:1637–1652. [PubMed: 10233168]
23. Takao M, Zhang QM, Yonei S, Yasui A. Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine: 8-oxoguanine DNA glycosylase. *Nucleic Acids Research*. 1999; 27:3638–3644. [PubMed: 10471731]
24. Mandal SM, Hegde ML, Chatterjee A, Hegde PM, Szczesny B, Banerjee D, Boldogh I, Gao R, Falkenberg M, Gustafsson CM, Sarkar PS. Role of human DNA glycosylase Nei-like 2 (NEIL2) and single strand break repair protein polynucleotide kinase 3'-phosphatase in maintenance of mitochondrial genome. *Journal of Biological Chemistry*. 2011; 287:2819–2829. [PubMed: 22130663]
25. Li M, Zhong Z, Zhu J, Xiang D, Dai N, Cao X, Qing Y, Yang Z, Xie J, Li Z, Baugh L, Wang G, Wang D. Identification and characterization of mitochondrial targeting sequence of human apurinic/apyrimidinic endonuclease 1. *The Journal of Biological Chemistry*. 2010; 285:14871–14881. [PubMed: 20231292]
26. Cymerman IA, Chung I, Beckmann BM, Bujnicki JM, Meiss G. EXOG, a novel paralog of Endonuclease G in higher eukaryotes. *Nucleic Acids Research*. 2008; 36:1369–1379. [PubMed: 18187503]
27. Kazak L, Reyes A, He J, Wood SR, Brea-Calvo G, Holen TT, Holt IJ. A cryptic targeting signal creates a mitochondrial FEN1 isoform with tailed R-Loop binding properties. *PLoS ONE*. 2013; 8:e62340. [PubMed: 23675412]
28. Kornblum C, Nicholls TJ, Haack TB, Schöler S, Peeva V, Danhauser K, Hallmann K, Zsurka G, Rorbach J, Iuso A, Wieland T, Sciacco M, Ronchi D, Comi GP, Moggio M, Quinzii CM, DiMauro S, Calvo SE, Mootha VK, Klopstock T, Strom TM, Meitinger T, Minczuk M, Kunz WS, Prokisch H. Loss-of-function mutations in MGME1 impair mtDNA replication and cause multisystemic mitochondrial disease. *Nature Genetics*. 2013; 45:214–219. [PubMed: 23313956]
29. Ropp PA, Copeland WC. Cloning and characterization of the human mitochondrial DNA polymerase, DNA polymerase gamma. *Genomics*. 1996; 36:449–458. [PubMed: 8884268]
30. Lakshminpathy U, Campbell C. The Human DNA Ligase III Gene Encodes Nuclear and Mitochondrial Proteins. *Molecular and Cellular Biology*. 1999; 19:3869–3876. [PubMed: 10207110]
31. Stumpf JD, Copeland WC. Mitochondrial DNA replication and disease: insights from DNA polymerase γ mutations. *Cell Mol Life Sci*. 2010; 68:219–233. [PubMed: 20927567]

32. Bianchi J, Rudd S, Jozwiakowski S, Bailey L, Soura V, Taylor E, Stevanovic I, Green A, Stracker T, Lindsay H, Doherty A. PrimPol Bypasses UV Photoproducts during Eukaryotic Chromosomal DNA Replication. *Molecular Cell*. 2013; 52:566–573. [PubMed: 24267451]
33. García-Gómez S, Reyes A, Martínez-Jiménez MI, Chocrón ES, Mourón S, Terrados G, Powell C, Salido E, Méndez J, Holt IJ, Blanco L. PrimPol, an archaic primase/polymerase operating in human cells. *Molecular Cell*. 2013; 52:541–553. [PubMed: 24207056]
34. Liu P, Qian L, Sung J-S, de Souza-Pinto NC, Zheng L, Bogenhagen DF, Bohr VA, Wilson DM, Shen B, Demple B. Removal of oxidative DNA damage via FEN1-dependent long-patch base excision repair in human cell mitochondria. *Molecular and Cellular Biology*. 2008; 28:4975–4987. [PubMed: 18541666]
35. Tann AW, Boldogh I, Meiss G, Qian W, van Houten B, Mitra S, Szczesny B. Apoptosis induced by persistent single-strand breaks in mitochondrial genome: critical role of EXOG (5'-EXO/endonuclease) in their repair. *The Journal of Biological Chemistry*. 2011; 286:31975–31983. [PubMed: 21768646]
36. Uhler JP, Thörn C, Nicholls TJ, Matic S, Milenkovic D, Gustafsson CM, Falkenberg M. MGME1 processes flaps into ligatable nicks in concert with DNA polymerase γ during mtDNA replication. *Nucleic Acids Research*. 2016; 44:5861–5871. [PubMed: 27220468]
37. Zheng L, Zhou M, Guo Z, Lu H, Qian L, Dai H, Qiu J, Yakubovskaya E, Bogenhagen DF, Demple B, Shen B. Human DNA2 is a mitochondrial nuclease/helicase for efficient processing of DNA replication and repair intermediates. *Molecular Cell*. 2008; 32:325–336. [PubMed: 18995831]
38. Bharati S, Krokan HE, Kristiansen L, Otterlei M, Slupphaug G. Human mitochondrial uracil-DNA glycosylase preform (UNG1) is processed to two forms one of which is resistant to inhibition by AP sites. *Nucleic Acids Research*. 1998; 26:4953–4959. [PubMed: 9776759]
39. Otterlei M, Haug T, Nagelhus TA, Slupphaug G, Lindmo T, Krokan HE. Nuclear and mitochondrial splice forms of human uracil-DNA glycosylase contain a complex nuclear localisation signal and a strong classical mitochondrial localisation signal, respectively. *Nucleic Acids Research*. 1998; 26:4611–4617. [PubMed: 9753728]
40. Dudek J, Rehling P, van der Laan M. Mitochondrial protein import: common principles and physiological networks. *Biochim Biophys Acta*. 2012; 1833:274–285. [PubMed: 22683763]
41. Finger LD, Attack JM, Tsutakawa S, Classen S, Tainer J, Grasby J, Shen B. The wonders of flap endonucleases: structure, function, mechanism and regulation. *Subcell Biochem*. 2012; 62:301–326. [PubMed: 22918592]
42. Narciso L, Fortini P, Pajalunga D, Franchitto A, Liu P, Degan P, Frechet M, Demple B, Crescenzi M, Dogliotti E. Terminally differentiated muscle cells are defective in base excision DNA repair and hypersensitive to oxygen injury. *Proc Natl Acad Sci USA*. 2007; 104:17010–17015. [PubMed: 17940040]
43. Fortini P, Ferretti C, Pascucci B, Narciso L, Pajalunga D, Puggioni EMR, Castino R, Isidoro C, Crescenzi M, Dogliotti E. DNA damage response by single-strand breaks in terminally differentiated muscle cells and the control of muscle integrity. *Cell Death and Differentiation*. 2012; 19:1741–1749. [PubMed: 22705848]
44. Szczesny B, Olah G, Walker DK, Volpi E, Rasmussen BB, Szabo C, Mitra S. Deficiency in repair of the mitochondrial genome sensitizes proliferating myoblasts to oxidative damage. *PLoS ONE*. 2013; 8:e75201. [PubMed: 24066171]
45. Iyama T, Wilson DM III. DNA repair mechanisms in dividing and non-dividing cells. *DNA Repair (Amst)*. 2013; 12:620–636. [PubMed: 23684800]
46. Dianov G, Lindahl T. Reconstitution of the DNA base excision-repair pathway. *Curr Biol*. 1994; 4:1069–1076. [PubMed: 7535646]
47. Fortini P, Dogliotti E. Base damage and single-strand break repair: mechanisms and functional significance of short- and long-patch repair subpathways. *DNA Repair (Amst)*. 2006; 6:398–409. [PubMed: 17129767]
48. DeMott MS, Beyret E, Wong D, Bales BC, Hwang J-T, Greenberg MM, Demple B. Covalent trapping of human DNA polymerase beta by the oxidative DNA lesion 2-deoxyribonolactone. *The Journal of Biological Chemistry*. 2002; 277:7637–7640. [PubMed: 11805079]

49. Hashimoto M, Greenberg MM, Kow YW, Hwang J-T, Cunningham RP. The 2-Deoxyribonolactone Lesion Produced in DNA by Neocarzinostatin and Other Damaging Agents Forms Cross-links with the Base-Excision Repair Enzyme Endonuclease III. *J Am Chem Soc.* 2001; 123:3161–3162. [PubMed: 11457038]
50. Sonntag, von C. *The Chemical Basis of Radiation Biology.* Taylor & Francis. 1989
51. Quinones JL, Thapar U, Yu K, Fang Q, Sobol RW, Demple B. Enzyme mechanism-based, oxidative DNA-protein cross-links formed with DNA polymerase β in vivo. *Proc Natl Acad Sci USA.* 2015; 112:8602–8607. [PubMed: 26124145]
52. Quinones JL, Demple B. When DNA repair goes wrong: BER-generated DNA-protein crosslinks to oxidative lesions. *DNA Repair (Amst).* 2016; 44:103–109. [PubMed: 27264558]
53. Nakano T, Terato H, Asagoshi K, Masaoka A, Mukuta M, Ohyama Y, Suzuki T, Makino K, Ide H. DNA-Protein Cross-link Formation Mediated by Oxanine: A NOVEL GENOTOXIC MECHANISM OF NITRIC OXIDE-INDUCED DNA DAMAGE. *Journal of Biological Chemistry.* 2003; 278:25264–25272. [PubMed: 12719419]
54. Le Bihan Y-V, Izquierdo MA, Coste F, Aller P, Culard F, Gehrke TH, Essalhi K, Carell T, Castaing B. 5-Hydroxy-5-methylhydantoin DNA lesion, a molecular trap for DNA glycosylases. *Nucleic Acids Research.* 2011; 39:6277–6290. [PubMed: 21486746]
55. Prasad R, Horton JK, Chastain PD, Gassman NR, Freudenthal BD, Hou EW, Wilson SH. Suicidal cross-linking of PARP-1 to AP site intermediates in cells undergoing base excision repair. *Nucleic Acids Research.* 2014; 42:6337–6351. [PubMed: 24771347]
56. Hamon M-P, Bulteau A-L, Friguet B. Mitochondrial proteases and protein quality control in ageing and longevity. *Ageing Research Reviews.* 2015; 23:56–66. [PubMed: 25578288]
57. Duxin JP, Dewar JM, Yardimci H, Walter JC. Repair of a DNA-protein crosslink by replication-coupled proteolysis. *Cell.* 2014; 159:346–357. [PubMed: 25303529]
58. Martínez-Jiménez MI, García-Gómez S, Bebenek K, Sastre-Moreno G, Calvo PA, Díaz-Talavera A, Kunkel TA, Blanco L. Alternative solutions and new scenarios for translesion DNA synthesis by human PrimPol. *DNA Repair (Amst).* 2015; 29:127–138. [PubMed: 25746449]
59. García-Gómez S, Reyes A, Martínez-Jiménez MI, Chocrón ES, Mourón S, Terrados G, Powell C, Salido E, Méndez J, Holt IJ, Blanco L. PrimPol, an archaic primase/polymerase operating in human cells. *Molecular Cell.* 2013; 52:541–553. [PubMed: 24207056]

Highlights

- > Some DNA lesions generated by oxidants can trap certain base excision DNA repair enzymes via their active sites during attempted repair
- > Mitochondria contain DNA repair enzymes that can form DNA-protein crosslinks in such trapping reactions
- > Systems in mitochondria that can repair these mechanism-based crosslinks remain to be identified

Figure 1. Formation of ROS from "Leaks" in The Electron Transport Chain

Transfer of electrons during oxidative phosphorylation creates the risk of ROS formation by reaction with oxygen, which initially generates superoxide as shown. This occurs mostly for Complex I and Complex III. The arrows indicate pathways producing first H_2O_2 , then the far more reactive hydroxyl radical. The close proximity of the mtDNA may enhance damage due to ROS generated locally.

Figure 2. Base Excision DNA Repair in Mitochondria

A damaged base (red) is removed by a DNA glycosylase. The Ape1 endonuclease incises the resulting AP site (or one generated by non-enzymatic, hydrolytic base loss) at the 5' phosphodiester, leaving behind a 5'-dRP (green). In short-patch BER, one new nucleotide is added (orange) by Pol γ , and the DNA is ligated by Lig III. For long-patch BER, multiple nucleotides are added, and the resulting displaced flap is excised by Fen1, DNA2, MGME1 or ExoG, followed by ligation by Lig III.

Figure 3. Schematic of DNA-Protein Crosslink Formation and Removal

A. A lyase-active DNA polymerase excises the 5'-dRP from an AP site via a Schiff base intermediate, finally releasing the unsaturated product, a "clean" 5' terminus on the DNA, and the free enzyme. B. With the dL lesion, the DNA polymerase forms a stable amide linkage to the lesion via its lyase active site residue. C. In the nucleus, DPCs are initially digested by the proteasome, expected to leave a small peptide attached to the DNA 5' terminus. Mitochondria do not have the proteasome, but other proteases in the mitochondrial matrix could perform this function.

Table 1
Localization of Base Excision Repair Proteins

Mitochondrial and nuclear isoforms are known for many BER proteins. Some enzymes are localized to the mitochondria, but not the nucleus. For those localized to the mitochondria, not all have a confirmed mitochondrial targeting sequence (MTS).

Mitochondrial Isoform	Nuclear Isoform	Function	Known MTS
<i>DNA N-glycosylases</i>			
UNG1	UNG2	Removal of Uracil	Yes [21]
AAG-A, AAG-B	AAG (MPG)	Removal of 3-meA, hypoxanthine	Yes
NTHL1	NTHL1	Removal of 5-hC, 5-hU, Fapy	Unknown
OGG1 1b,1c,2a-e	OGG1 1a	Removal of 8-oxoG, FapyG	Yes [22]
MUTYH α	MUTYH B, γ	Removal of A:8-oxoG	Yes [23]
NEIL1	NEIL1	Removal of 5-hU, hoC, urea, FapyG, FapyA	Predicted from MitoProt [20]
NEIL2	NEIL2	Removal of 5-hU, 5-hC, urea, FapyG, FapyA	Unknown [24]
<i>Endonucleases</i>			
APE1	APE1	Incision of AP site	Yes [25]
ExoG	None	Excision of displaced oligonucleotide flaps	Predicted[26]
Fen1, FENMIT	Fen1	Excision of displaced oligonucleotide flaps	Unknown [27]
DNA2	DNA2	Excision of displaced oligonucleotide flaps	Unknown
	MGME1	Excision of displaced oligonucleotide flaps	Predicted [28]
<i>DNA Polymerases</i>			
DNA Polymerase γ	None	Gap filling	Predicted[29]
PrimPol	PrimPol	Translesion synthesis?	Unknown
DNA Ligase	DNA Ligase III	Nick sealing	Yes [30]

Abbreviations: 3-methyladenine (3-meA), 5-hydroxycytosine (5-hC), 5-hydroxyuracil (5hU), formamidopyrimidine (Fapy), 8-oxoguanine (8-oxoG).