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Mitochondrial DNA Damage and its Consequences for Mitochondrial Gene Expression

Susan D. Cline
Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA 31207

Abstract

How mitochondria process DNA damage and whether a change in the steady-state level of mitochondrial DNA damage (mtDNA) contributes to mitochondrial dysfunction are questions that fuel burgeoning areas of research into aging and disease pathogenesis. Over the past decade, researchers have identified and measured various forms of endogenous and environmental mtDNA damage and have elucidated mtDNA repair pathways. Interestingly, mitochondria do not appear to contain the full range of DNA repair mechanisms that operate in the nucleus, although mtDNA contains types of damage that are targets of each nuclear DNA repair pathway. The reduced repair capacity may, in part, explain the high mutation frequency of the mitochondrial chromosome. Since mtDNA replication is dependent on transcription, mtDNA damage may alter mitochondrial gene expression at three levels: by causing DNA polymerase γ nucleotide incorporation errors leading to mutations, by interfering with the priming of mtDNA replication by the mitochondrial RNA polymerase, or by inducing transcriptional mutagenesis or premature transcript termination. This review summarizes our current knowledge of mtDNA damage, its repair, and its effects on mtDNA integrity and gene expression.

1. Introduction

Mitochondria harbor the small, circular genome in human cells that is essential for life, but the environment of the mitochondrial matrix is rather inhospitable to the macromolecules that reside there. The oxidative production of ATP required for cellular function also generates reactive oxygen species (ROS) that damage mitochondrial DNA (mtDNA), membrane lipids, and protein [1–6]. Additionally, environmental chemicals that enter the body can induce mtDNA damage, both by amplifying the production of endogenous DNA lesions and by generating unique DNA adducts [7]. Unlike nuclei, which have multiple mechanisms for DNA repair, mitochondria appear limited in their ability to rectify all of the possible forms of mtDNA damage [8]. Persistent chromosomal damage can impair mitochondrial DNA polymerase γ (pol γ) and RNA polymerase (POLRMT) activity and threaten mtDNA stability and gene expression. The past decade has brought a surge in research efforts to characterize the variety of biomolecular damage in mitochondria, to elucidate the mechanisms of mitochondrial DNA repair, and to determine the effects of DNA damage on the function of mitochondrial polymerases.

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Contact information: Susan D. Cline, Ph.D., Division of Basic Medical Sciences, Mercer University School of Medicine, Macon, GA 31207, 478-301-2231, Cline_sd@mercer.edu.

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2. Oxidative damage in mitochondria

A host of reactive molecules are generated within mitochondria due to the escape of electrons from the transport chain during oxidative phosphorylation [1]. Approximately 0.15% of the molecular oxygen consumed by mitochondria is reduced to superoxide anion ($O_2^{\cdot-}$) [9]. Seven potential sites for matrix $O_2^{\cdot-}$ generation exist, with complexes I and III exhibiting the highest rate of $O_2^{\cdot-}$ production [3]. Superoxide cannot diffuse through the inner mitochondrial membrane (IMM), so it is confined to the matrix where it can react with Fe-S proteins to liberate iron or be converted to hydrogen peroxide by manganese-superoxide dismutase, Mn-SOD. Hydrogen peroxide may diffuse through the IMM, but if it remains in the matrix, it can undergo Fenton chemistry with Fe(II) to yield hydroxyl radicals, which are highly reactive toward DNA and polyunsaturated fatty acids (PUFAs) [2]. Free radical attack on the DNA bases and phosphodiester backbone produce a number of oxidative lesions to the genetic material and release reactive base aldehydes, called base propenals. Polyunsaturated lipid peroxidation (LPO) in the membrane is a major source of electrophilic aldehydes that, along with base propenals, react with cysteine, lysine, arginine, and histidine side chains in proteins and with amino groups of DNA bases [2,4,6,10–13].

Polyunsaturated fatty acyl chains comprise over 50% of the total fatty acids in the IMM and are subject to free radical chain reactions that are triggered by ROS [14]. The auto-oxidation of the unsaturated bonds in the PUFAs leads to the formation of distinctive aldehyde products that are routinely neutralized by glutathione conjugation or aldehyde dehydrogenase activity in mitochondria [6,11]. However, cellular conditions that cause an increase in mitochondrial ROS may overwhelm these mechanisms. A recent study has shown that the oxidation of linoleate cardiolipin, which is the predominant cardiolipin species in the IMM, liberates 4-hydroxynonenal (4-HNE) [15,16]. Due to differences in the lipid compositions of the IMM, the mitochondrial outer membrane and the cell plasma membrane, nuclear and mitochondrial DNA may be exposed to a different mixture of the α,β-unsaturated aldehydes, the most prevalent of which are 4-HNE, malondialdehyde (MDA), acrolein and crotonaldehyde [11,13]. Each of these non-radical oxidants can induce protein and DNA modifications in the matrix and is capable of diffusing outward through the IMM [4]. Therefore, reactive aldehydes may compromise mitochondrial gene expression at multiple levels: by damaging mtDNA to induce genetic instability or interfere with polymerase function, by inactivating pol γ, POLRMT or their associated factors involved in gene expression, and by modifying proteins required for the mitochondrial import of RNA and cytosolic proteins. Mitochondrial protein modification by free radical and non-radical endogenous agents and its ramifications on cellular function are discussed in more detail in recent reviews from the Jones laboratory [4,17]. This article will continue with a focus on mitochondrial chromosome damage and its consequences for gene expression.

3. Mitochondrial DNA damage

Mitochondrial DNA polymorphisms and somatic mutations have been studied in the pursuit to understand human disease inheritance, longevity, and population migration [18–22]. Certain sequences in the mtDNA chromosome, such as the hypervariable segments of the D-loop regulatory region, appear to be unstable in the natural course of aging [21]. Many single nucleotide variations in mtDNA are benign, but some promote disease [20]. Cells may contain thousands of copies of the mitochondrial chromosome, and a threshold level of the mtDNA population must be compromised before biochemical phenotypes will arise in a cell [22,23]. There appears to be an orchestrated control of the mitochondrial matrix conditions, mitochondrial dynamics, and mtDNA maintenance mechanisms that allow natural genetic change, yet prevent pathogenic mtDNA mutations and degradation [24].

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Mitochondrial DNA is susceptible to reactive species generated from cellular metabolism and to environmental agents, including industrial byproducts, therapeutic drugs, and radiation, as will be discussed in detail below. The need for physiological measurements of mtDNA damage and for studies of mtDNA turnover in response to damage is increasing as medical science seeks to correlate the presence of modified nucleic acid metabolites in patient specimens with disease or health risks [25,26]. This is a challenge considering that mtDNA makes up a small fraction of the total cellular DNA and the relative amount of mtDNA to nuclear DNA varies between tissues. Advances in liquid chromatography and mass spectrometry techniques, specifically HPLC-MS/MS, quantitative PCR methods, and repair enzyme-based assays have allowed sensitive detection of a plethora of DNA modifications by eliminating the artifacts that commonly plagued earlier measurements [7,27–39]. In recent years, studies have focused on identifying and measuring mtDNA damage to determine whether particular lesions may play a significant role in the mtDNA mutagenesis or depletion observed in clinical disease and chemical toxicology (Table 1). The following sections discuss some of the types of DNA damage detected in human cells that are likely to impact mitochondrial gene expression.

3.1 Endogenous mtDNA damage

Free-radical and non-radical oxidants generated inside of cells produce a spectrum of DNA damage including small modifications to bases, such as 8-oxo-2′-deoxyguanosine (8-oxo-dG), formamidopyrimidines, and 8,5′-cyclo-2′-deoxynucleosides, insults to the sugar backbone, such as abasic sites, oxidized deoxyribose rings, and DNA strand breaks, and chemical adducts of bases, such as aldehyde modifications, just to name a few [29]. To date, only a limited set of these have been specifically detected in mtDNA, but most are suspected to occur in mitochondria due to the oxidative conditions of the matrix [5,13,29,30,40–52] (Table 1). It has been known for over a decade that the steady-state level of oxidative damage in mtDNA is higher than that in nDNA; however, detection methods for oxidative lesions have required improvements to prevent artifacts and to allow the necessary specificity and sensitivity for measurements [53]. Several different types of oxidative lesions may form under the same cellular conditions, complicating the effort to obtain a complete profile of metabolic DNA damage with a single technique. Another difficulty in mtDNA damage detection is that lesions may undergo additional chemistry *in vivo* that alters their structure. For example, the 8-oxo-dG in DNA is more susceptible to oxidation than the native dG, and 8-oxo-dG oxidation produces a variety of more stable and mutagenic forms guanine damage, such as hydantoin derivatives [54, 55]. Unlike 8-oxo-dG, hydantoins of DNA bases may not be readily removed from mtDNA, giving rise to their greater mutagenic potential [8, 56]. Therefore, methods that allow for the characterization of multiple lesions and their derivatives would provide the most accurate account of mtDNA damage. In addition to directly damaging the mitochondrial chromosome, biological oxidants can react with free nucleotides producing aberrant substrates for mitochondrial DNA and RNA synthesis [57, 58]. While mitochondria possess an enzyme that can clear 8-oxo-dGTP from the substrate pool, other nucleotide damage also may occur and persist. To compensate for the nucleotide pool imbalance, mtDNA degradation may occur in order to restore the ratios of nucleotides needed for proper synthesis [58–60].

Abasic sites, due primarily to purine loss from DNA, occur at a steady-state level of >50,000 sites per cell in mammalian tissues [61–63]. Brain, which is rich in mitochondria, has the greatest amount of abasic sites [62]. The 2′-deoxyribose of the abasic site may be oxidized to form modified sugars and single strand breaks (SSBs). In fact, a recent study found that abasic sites and SSBs appear to be the predominant forms of mtDNA damage produced by H_2O_2 and rotenone-induced oxidative stress in cultured cells [64]. The C4′-oxidation of an abasic sugar will result in the release of the adjacent base in the strand as a base propenal

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[25, 65]. The base propenals act as electrophiles to generate DNA adducts also produced by the LPO-derived aldehydes [12].

The metabolic capacity and membrane composition of mitochondria make the matrix of these organelles a likely site for the generation of a host of reactive aldehydes [11]. The formation of DNA adducts by biological aldehydes has been reviewed recently in detail; therefore, a brief summary will be offered here [13,66]. In general, aldehydes react primarily with the N^2-amine of deoxyguanosine (dG), but they also attack the amino groups of other bases. Aldehyde adducts of DNA bases may undergo additional chemistry depending on their structure. Formaldehyde, which is produced through amino acid metabolism, initiates covalent crosslinks between protein and DNA, but also produces N^2-hydroxymethyl-dG adducts [67]. Although it is produced physiologically, formaldehyde is rapidly oxidized to formate and may not be a substantial threat to DNA, unless it is encountered at higher concentrations through environmental exposure [68,69]. The α, β-unsaturated aldehydes, resulting from PUFA and DNA oxidation, each produce a characteristic set of adducts by a Michael addition to the amino group of DNA bases followed by a cyclization to form either a five-membered (etheno) or a six-membered (propano) ring on the DNA base [13]. The LPO-induced, etheno adducts, 1, N^6-ethenodeoxyadenosine and 3, N^4-ethenodeoxycytidine, are produced in the liver mtDNA of Long-Evans Cinnamon rats, animals with a genetic background resulting in the accumulation of copper and ultimately in the formation of hepatocellular carcinomas [51]. Malondialdehyde (MDA), generated from both prostaglandin biosynthesis and LPO, creates the six-membered, exocyclic adducts, M_1dG, M_1dA and M_1dC, of which M_1dG is most prevalent and occurs at a higher frequency in mtDNA than nDNA [50]. The cardiolipin LPO product, 4-HNE, forms 1, N^2- and N^2,3-etheno-dG adducts, which are also produced by acrolein and crotonaldehyde, l, N^2-propano-dG, and four unique stereoisomeric l, N^2-propano-dG adducts carrying a fatty acyl group [70]. The DNA adducts of MDA and 4-HNE, and metabolites of these adducts, are of particular interest clinically, due to their elevated levels in patients with various pathologies [25]. Acrolein creates the hydroxy-l, N^2-propano-dG adducts, α-and γ-OH-PdG, with γ-OH-PdG being the major product [13]. The γ-OH-PdG adduct may further react with nearby functional groups to form DNA interstrand crosslinks and protein-DNA crosslinks [70–72]. Considering the PUFA enrichment of the IMM, α,β-unsaturated aldehydes are likely a major threat to mtDNA stability and gene expression during tissue oxidative stress.

Interestingly, protein-DNA covalent crosslinks are also produced through the action of DNA topoisomerases, enzymes required for replication, transcription, and separation of chromosomes. The characterization of mitochondrial type I topoisomerases, Top1mt and Top 3αmt, and the subsequent detection of Top1mt cleavage sites in the D-loop of mtDNA raises the possibility that aberrant topoisomerase activity may be a cause of mtDNA deletions and chromosome degradation [73–75]. As part of their mechanism in mediating DNA topology during transcription and replication, the type I topoisomerases cut a DNA strand and create a covalent phosphotyrosyl bond with either the 5′ or 3′ end of the nick [76]. These enzyme-DNA complexes, which are normally transient intermediates in the catalytic cycle, can be stabilized by cancer chemotherapy drugs and by many forms of oxidative and environmental DNA damage [76, 77]. Extending the lifetime of the intermediates can be deleterious, as they can be broken by replication and transcription complexes leaving unique tyrosyl-DNA linkages that must be resolved before DNA strand continuity can be restored. Tdp1, a phospholipase D family member that resides in both nuclei and mitochondria, recognizes the 3′-phosphotyrosyl-DNA ends created by the disruption of type I topoisomerase catalytic intermediates [78, 79]. A loss of Tdp1 function results in spinocerebellar ataxia with axonal neuropathy (SCAN1), which resembles Friedreich ataxia, a disease that is known to be associated with mtDNA instability [26,80]. This suggests that Tdp1 plays a critical role in the processing of Top1mt-mediated DNA

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strand breaks in order to maintain mitochondrial function in neurons [79,81]. Since Top1mt alleviates torsional stress in mtDNA during replication and transcription, studies of the response of this enzyme to DNA damage and further studies of Tdp1 function in mtDNA repair may provide insight into the mechanisms for mtDNA deletions and the side effects of cancer chemotherapies targeted against topoisomerases [82].

3.2 Environmentally-induced mtDNA damage

Chemicals in the environment, metabolites of dietary components, drugs in clinical therapies, and radiation from sunlight or medical procedures are external sources of DNA damage. The benzo[*a*]pyrene and acrolein components of cigarette smoke, the fungal toxin aflatoxin B1, platinum-based chemotherapy agents, antiviral nucleoside analogs, and ultraviolet (UV) radiation all induce DNA adducts that inhibit mitochondrial transcription or interfere with DNA pol γ function [83–86](R. Kasiviswanathan and W. C. Copeland, personal communication). Therefore, disruption of mitochondrial gene expression or mutagenesis of mtDNA should be considered in assessing environmental exposure or drug toxicities.

The aldehydes, produced physiologically in our tissues, are increased during the metabolism of alcohol and can also enter our bodies from the environment, where they are present with other reactive agents [7, 87]. Acetaldehyde, also called ethanal, is generated at a high concentration in the liver following the ingestion of alcohol and is converted to acetate in mitochondria by aldehyde dehydrogenase 2, ALDH2. If ALDH2 cannot efficiently oxidize acetaldehyde, it forms a number of adducts, including N^2-ethylidene-dG, 1, N^2-propano-2′-dG and their derivatives [88–91]. The 1, N^2 -propano-2′-dG adduct can combine with a second aldehyde molecule to make an interstrand DNA crosslink that will inhibit strand separation by helicases [92]. In industrial pollutants, formaldehyde coexists with alkylating agents, a combination that forms N^2-hydroxymethyl-dG and its derivatives from subsequent methylation events [69]. Cigarette smoke contains acrolein, crotonaldehyde and formaldehyde along with polycyclic aromatic hydrocarbons (PAH), such as benzo[*a*]pyrene, and several tobacco-specific *N*-nitrosamines (TSNA). Benzo[*a*]pyrene (BaP) is metabolized by the liver cytochrome P450 CYP1A1 to benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide (BaPDE), and both BaP and BaPDE have been shown to accumulate in mitochondria and inhibit mtDNA synthesis [93–96]. The TSNA compounds, also activated by CYP enzymes, form DNA adducts that have been detected in the lung and liver mtDNA of carcinogen exposed rats, however the effects of TSNA adducts on mtDNA synthesis and gene expression have not been determined [97]. The BaP effects on mtDNA replication likely stem from BaPDE modifications of dG and dA, which block DNA pol γ and inhibit topoisomerase activity. However, in smoke exposed tissues, the coexisting aldehyde and TSNA-induced damage may act synergistically with BaPDE adducts to hinder mitochondrial polymerases [85, 98–103].

Hepatic toxicity and carcinogenesis from environmental exposures may be due in part to mtDNA damage by xenobiotic derivatives, as in the case of BaP or aflatoxin B1, or by ROS generated during CYP activity. Polychlorinated biphenyls (PCBs), which are widespread in our environment, increase ROS production during their metabolism by liver CYP enzymes [104]. Rats chronically exposed to PCBs were found to have elevated levels of M_1dG in their livers, possibly due to the release of MDA and base propenals by lipid and DNA sugar oxidation, respectively [105]. In fact, this finding suggests that toxic environmental agents that increase ROS by the disruption of mitochondrial membrane potential, such as dioxins in herbicides, may also induce oxidative mtDNA damage that inhibits mitochondrial gene expression [106].

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The nucleoside analogs designed as antiviral drugs and some DNA damaging agents employed in cancer therapies have off-target effects on mitochondria that may be attributed to direct and indirect damage to mtDNA and its RNA transcripts. Nucleotide reverse transcriptase inhibitors (NRTIs) against HIV have common side effects that resemble symptoms of mitochondrial diseases, including skeletal muscle and heart myopathies, peripheral neuropathy, lactic acidosis, lipodystrophy and hepatic steatosis [107]. The mitochondrial pol γ is the only human DNA polymerase that effectively uses NRTIs as substrates, and many of the clinically utilized NRTIs are incorporated into mtDNA and cause the termination of strand synthesis [108, 109]. Other ribonucleoside analogs intended for the treatment of RNA viral infections, but found to be highly toxic to patients in clinical trials, may be substrates for POLRMT and inhibit mitochondrial transcription [110]. Indeed, the future development of therapeutic nucleoside analogs may benefit from initial testing of substrate utilization by mitochondrial polymerases now that purified recombinant systems, as described in Section 5, make these assessments possible.

Many anticancer agents directly or indirectly damage DNA to inhibit tumor growth and induce apoptosis. A few of these have side effects that suggest that they may have effects on mitochondrial function. Bleomycin and neocarzinostatin cause oxidative damage to DNA through ROS and reactive aldehyde generation [12, 111]. These drugs are known to cause pulmonary fibrosis, and bleomycin is used to generate fibrosis in animal models for the study of this disease process [112,113]. While no direct evidence exists, it is possible that mtDNA damage may contribute to the cellular transformation and apoptosis observed in the lung tissue of bleomycin-treated patients [25,65]. The platinum (Pt)-containing chemotherapy drugs, such as cisplatin, carboplatin, and oxaliplatin, bind directly to DNA to form single base adducts and intra- and interstrand crosslinks (ICL) between guanine bases [114]. For cisplatin, the intrastrand l,2-d(GpG) crosslink appears to be the most prevalent modification following drug exposure; however, even in minor amounts, ICL adducts are catastrophic to replication and transcription as they prevent the strand separation required for polymerase function [115–118]. Cisplatin adducts appear in the mtDNA of fetal tissues following the treatment of pregnant rats and monkeys, indicating that the drug may alter development through inhibition of mitochondrial gene expression [119,120]. Patients treated with Pt agents often experience peripheral neuropathy, which may relate to the observation of cisplatin-induced mtDNA damage in dorsal root ganglion neurons [121]. Cisplatin exposure reduced mtDNA replication and transcription in the cultured neurons, which correlates with the *in vitro* finding that pol γ nucleotide insertion is inhibited by GG intrastrand crosslinks [84,122]. In the nucleus, repair of the full spectrum of Pt-DNA adducts requires a concerted effort by the Fanconi pathway for ICL repair, homologous recombination, mismatch repair and nucleotide excision repair mechanisms [118,123–125]. Cells of the body that are reliant on oxidative metabolism, such as neurons and muscle cells, may be highly susceptible to Pt-drug chemotherapy, because mitochondria do not have the full complement of these repair pathways [126].

4. Mitochondrial DNA repair mechanisms

Historically, the high DNA mutation rate in mitochondria has been attributed to oxidative damage in the context of limited mtDNA repair. However, in the last two decades, studies have revealed that mitochondria possess several mechanisms for genetic maintenance, including a robust base excision repair (BER) mechanism that utilizes both mitochondrial proteins and nuclear proteins that translocate into mitochondria. Additionally, mitochondrial fusion and fission events and mtDNA degradation may allow the organelles to lessen the toxic effects of damage left unresolved by mitochondrial repair pathways [64,127,128]. Several reviews published in the last two years have described in detail the known repair

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mechanisms in mitochondria, so only a brief synopsis of mtDNA repair and the latest findings are reported here [8,129–131].

The DNA repair repertoire of mitochondria currently includes single-nucleotide base excision repair (SN-BER) [43,126,132,133], long-patch BER (LP-BER) [134–138], single-strand break repair (SSBR) [131], YB-1-mediated mismatch repair [139,140], removal of adenine opposite 8-oxo-dG [141,142], and MTH1 removal of 8-oxo-dGTP and 8-oxo-2′-dATP from the mitochondrial nucleotide pool [143,144]. Nonhomologous end joining and homologous recombination activities, which can repair double-stranded DNA breaks, have been detected in mammalian mitochondria, and recent studies suggest a role for the RecQ helicase, RECQL4, in mitochondrial double-strand break repair [145–150]. Strikingly, mitochondria lack nucleotide excision repair activity, which resolves bulky DNA damage, such as UV photoproducts, BaPDE adducts, cisplatin intrastrand crosslinks, and the oxidative lesions, 8,5′-cyclo-2′-deoxypurine and M_1dG [98–100,124,126,151–156]. The mitochondrial limitations for excision repair are discussed below, along with some recent findings that add insight into the relationship between nuclear and mitochondrial DNA damage in preserving cellular function.

4.1 Base Excision Repair

Base excision repair (BER) is primarily responsible for the removal of DNA bases altered by oxidation, alkylation, or deamination, and the repair of abasic sites and single-strand breaks resulting from spontaneous hydrolysis and oxidation, respectively (reviewed in [130]). The mitochondrial BER mechanisms, SN-BER and LP-BER, closely resemble the nuclear pathways and utilize some of the same proteins, but DNA pol γ conducts the 5′-deoxyribose phosphate lyase and polymerase activities performed by DNA polymerase β, in the nucleus. Several nuclear DNA glycosylases, which catalyze damage-specific N-glycosylic bond cleavage, have been found in mitochondria, including uracil DNA glycosylase, UNG 1, nth endonuclease III-like 1 glycolysase, NTHL1, MutY homolog glycosylase, MUTYH, and the bifunctional glycosylases OGG1, NEIL1 and NEIL2 ([130] and references therein). The latter three enzymes have overlapping specificity for 8-oxo-dG, but differ in their recognition of other oxidative lesions. Together, they rectify most of the prevalent forms of free radical induced oxidative DNA damage. In fact, the level of 8-oxo-dG in the intact mtDNA isolated from the liver of *OGG1*$^{-/-}$ mice is only slightly elevated above that in mtDNA from the liver of wild type animals, suggesting the efficient removal of 8-oxo-dG by the NEIL enzymes or the degradation of highly damaged mitochondrial chromosomes in the absence of OGG1 [30]. While mitochondria have redundant glycosylase activity for 8-oxo-dG removal, they appear to lack the monofunctional N-methylpurine DNA glycosylase, MPG, that is responsible for the BER processing of alkylpurines, including methylated bases and larger alkylations, such as the l, N^6-etheno-dA and l, N^2-etheno-dG adducts generated by reactive aldehydes [157–159]. Therefore, it appears that these forms of base damage pose a persistent threat to mtDNA gene expression and stability.

OGG1, NEIL1 and NEIL2 all conduct N-glycosylic bond cleavage at oxidized bases, but NEIL1 and 2 catalyze both β- and δ-elimination reactions to excise the abasic sugar without recruiting AP endonuclease 1 (APE1). OGG1 performs only β-elimination at the sugar and relies on APE1 to complete sugar excision from the DNA strand. Nuclear BER initiated by either the OGG1 or NEIL glycosylases converge at the recruitment of XRCC1/ligase III and DNA pol β for restoring continuity to the gapped DNA strand produced by damage removal [130]. The recognition of the single-base, gapped intermediate is believed to involve a poly(ADP-ribose) polymerase-1 (PARP1) complex [160], and in the case of the NEIL-initiated BER pathway, also requires the activity of polynucleotide kinase 3′-phosphatase, PNKP, an enzyme that prepares the DNA ends at a strand gap for extension by pol β [161,

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162]. Interestingly, mitochondria do not contain the XRCC1 protein, which acts as a scaffold for assembly of nuclear BER factors and brings in ligase III to complete repair. Mitochondrial ligase III is essential for mouse embryo viability; therefore, it must be recruited into the BER process by a surrogate for XRCC1 [163,164].

Recently, aprataxin, PARP1, and PNKP have been located in mitochondria [160,165–167]. Mutations in the gene for aprataxin, *APTX,* is associated with the disorder ataxia oculomotor apraxia 1, or AOA1, which is an autosomal recessive cerebellar ataxia with peripheral neuropathy and eye movement limitations [168–171]. Aprataxin shares homology with PNKP, histidine-triad (HIT) proteins, and zinc-finger DNA binding proteins and interacts with PARP1 at single-strand breaks where it removes 5′-adenylation damage to prepare DNA ends for strand ligation by DNA ligase III [160,171,172]. Based on the mitochondrial localization and the molecular interactions of aprataxin, this protein is a candidate for the recognition and processing of single-stranded breaks in a mitochondrial BER mechanism [173]. While the ADP ribosylation of mitochondrial proteins has been reported by several groups, the mitochondrial presence of PARP1 and its role in mtDNA expression is still a matter of debate [165,174–176]. PARP1 appears to enter the organelle through an interaction with the mitochondrial import protein, mitofillin; however, a recent report suggests that PARP1 affects mitochondrial repair and transcription through its regulation of nuclear genes for mitochondrial proteins, instead of by mitochondrial interactions [165,176]. PNKP, on the other hand, appears to participate in mitochondrial BER through a direct association with NEIL2 [167]. The mitochondrial PNKP is identical to the nuclear protein, which contains a C-terminal mitochondrial localization sequence. The depletion of PNKP from human cells causes an increase in both mitochondrial and nuclear DNA damage following H_2O_2 treatment [166]. Since aprataxin, PARP-1, and PNKP appear to act in restoring continuity to DNA strands, these proteins may play a broad role in recruiting pol γ and ligase III for the repair of strand breaks formed by many insults to mtDNA.

The discrepancies found in the mitochondrial localization of repair proteins may be due to a tissue-specificity for their function in mitochondrial DNA repair. Equally as plausible is the possibility that DNA damaging agents may trigger the mitochondrial localization of specific repair proteins, such as PARP1. While this idea is merely speculation, a recent paper showing that PARP-1 binds to DNA containing cisplatin l, 2-d(GpG) intrastrand crosslinks, taken along with the reports of mitochondrial PARP1, suggests that this hypothesis may be a valid explanation for inconsistencies in PARP1 localization studies [177,178]. PARP-1 may enter the mitochondria in response to mtDNA damage in order to facilitate mtDNA repair or to initiate a signal for mtDNA destruction. Further studies in this area may bring to light the link between the DNA damage binding and ADP ribosylation properties of PARP1 in mitochondria.

4.2 Nucleotide Excision Repair

It was established in 1974, and reaffirmed in two later studies, that mitochondria lack the NER mechanism responsible for the removal of UV-induced pyrimidine dimers [126,151,152]. Consistent with these findings, mitochondria display very poor repair of cisplatin intrastrand crosslinks but efficiently remove interstrand crosslinks, which are repaired by recombination instead of NER [126, 153]. Since both types of cisplatin adducts are efficiently removed by nuclear repair mechanisms, mitochondrial damage is likely to be the major mechanism for cisplatin-induced neurotoxicity [121,123,124,179]. In light of the apparent mitochondrial deficiency in NER, 8,5′-cyclo-2′-deoxypurines (cPu), M_1dG and BaPDE adducts, would also pose problems for mtDNA gene expression in many tissues of the body [98–100,124,154–156]. While cPu have yet to be detected in mtDNA, these oxidative lesions accumulate in the liver, kidney, and brain of aging mice and are known to

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inhibit DNA transcription [155,180]. M_1dG occurs at a two-fold higher frequency in mtDNA than nuclear chromosomes and likely co-exists with other DNA damage requiring NER removal, including endogenous aldehyde adducts and adducts generated by polyaromatic hydrocarbons (PAH) [13, 50, 105]. Benzo[*a*]pyrene and BaPDE accumulate in mitochondria, and a 40- to 90-fold higher level of BaP covalent adducts has been observed in the mtDNA relative to the nDNA of mammalian cells upon exposure to BaPDE [93, 94, 96]. This difference may be due to a higher binding affinity of PAH compounds for mtDNA [181]. A more recent study of Atlantic killifish confirmed that BaP-induced adducts accumulate in the mtDNA of liver, brain and muscle tissue following intraperitoneal BaP injection, but no significant difference between mtDNA and nDNA levels were observed [182]. Although there are inconsistencies between cellular and whole animal studies of PAH adducts, both systems revealed that mtDNA is highly susceptible to PAH damage due to the absence of mitochondrial NER.

The only evidence to date for an NER-like mechanism in mitochondria is the presence of the Cockayne syndrome proteins, CSA and CSB, which are required for nuclear transcription-coupled NER (TC-NER) [183–185]. TC-NER is detected in nuclear chromosomes as the more rapid repair of the transcribed strands of genes relative to non-transcribed DNA and is initiated by the arrest of an elongating RNA polymerase II (RNAP II) complex at a site of DNA damage [186]. The CSB protein recruits CSA and chromatin remodeling proteins to the stalled RNAP II complex, while CSA, a ubiquitin E3 ligase, brings in other essential factors that assist in reversing RNAP II and processing the RNA transcript to clear the site of damage for template strand repair [185]. The CSB protein has been implicated in the repair of 8-oxo-dG, and the loss of CSB in an *Ogg1*$^{-/-}$ background increases mutation rates [187, 188]. However, *Ogg1*$^{-/-}$ *Csb*$^{-/-}$ mice have no significant increase in 8-oxo-dG in their liver mtDNA relative to wild type animals [30]. The NEIL glycosylases in mitochondra likely compensate for Ogg1 loss in removing 8-oxo-dG, and the elevated mutation frequencies with CSB loss may be due to the persistence of other oxidative lesions. Recently, the NEIL1 glycosylase, which is found both in the nucleus and in mitochondria, has been shown to mediate nuclear NER of 8′, 5′-cyclo-2′ deoxyadenosine (cyclo-dA), an endogenous oxidative DNA lesion formed by intramolecular cyclization between C5′ of the deoxyribose and C8 of the purine ring [189]. Interestingly, mice lacking CSB accumulate cyclo-dA, indicating a role for CSB in its repair. While cyclo-dA has yet to be detected in mtDNA, the finding that CSB participates in the removal of this oxidative lesion suggests that mitochondria have an NER-like mechanism [184,189]. Another intriguing aspect of the *Ogg1*$^{-/-}$ *Csb*$^{-/-}$ mouse study is that a portion of the mtDNA oxidative damage in the mutant animals appears to be due to 4′-oxidation resulting in base loss, a process that would generate base propenals and aldehyde-induced DNA adducts [65]. Since aldehydes primarily damage dG, the formation of aldehyde adducts in mtDNA may, in part, explain the prevalent G→T transversions and mtDNA deletions observed in *Ogg1*$^{-/-}$ *Csb*$^{-/-}$ mice [187]. Further studies of mtDNA from *Ogg1*$^{-/-}$ *Csb*$^{-/-}$ mice are required to determine whether the persistence of mtDNA damage due to the lack of a CSB-dependent mechanism may contribute to the enhanced mutation frequency in these animals.

Mitochondria may have NER-like mechanism that resolves a different spectrum of DNA damage than that handled by the nuclear NER pathway, while some nuclear targets for NER may trigger mtDNA deletion or degradation. Indeed, the recent finding of PARP-1 recognition of 1,2-d(GpG) platinum crosslinks hints at the possibility for alternative processing of NER-targeted adducts in mtDNA [178]. As discussed below, the arrest of POLRMT by the aldehyde adduct, M_1dG, also suggests that a mitochondrial transcription-coupled mechanism for mtDNA damage signaling may exist. Our current knowledge of mtDNA repair, however, leaves us to conclude that metabolically- and environmentally-induced adducts requiring NER for their removal simply will persist in mitochondrial

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chromosomes where they can disrupt mtDNA replication and gene expression, cause mtDNA mutation, and may ultimately lead to mitochondrial chromosome degradation.

5. DNA damage effects on gene expression

Many types of DNA damage have been studied for their effects on mammalian nuclear DNA polymerases and RNAP II [185,190–194]. These studies are the basis for understanding the process of translesion DNA synthesis, which serves as a tolerance mechanism for DNA damage, and for the mechanism of TC-NER, which was discussed above. To date, we have only a minimal knowledge of how DNA damage affects pol γ or POLRMT function. Additionally, proteins that support mtDNA replication and transcription, namely mtTop1 and the nucleoid/transcription factor, TFAM, interact with DNA damage, and those interactions may alter mtDNA processing [73, 102, 179, 195]. The structural and biochemical properties of pol γ and POLRMT are discussed by R. Kasiviswanathan and J. J. Arnold, respectively, elsewhere in this issue [82, 110]. The following sections of this review summarize the behavior of these enzymes when encountering DNA damage and the possible ramifications of mtDNA on mitochondrial gene expression.

5.1 Mitochondrial DNA replication

DNA pol γ performs all of the replicative and repair-associated DNA synthesis in mitochondria. In all of the proposed models for mtDNA replication, DNA strand synthesis begins from an RNA primer generated by POLRMT (reviewed by [82] in this issue). This requirement intimately links the functions of these enzymes and presents two avenues by which DNA damage may affect mtDNA replication, by interfering with POLRMT RNA primer synthesis or by disrupting pol γ processivity and fidelity in DNA synthesis. In the nucleus, a plethora of DNA polymerases are part of the ‘tool kit’ for lesion bypass mechanisms, which are activated through ubiquitinylation of the PCNA sliding clamp [192, 196]. However, it appears that pol γ acts alone when negotiating DNA damage in mitochondrial chromosomes, and any replication-mediated signals that may trigger the repair or bypass of mutagenic damage have yet to be elucidated.

For mtDNA replication, DNA pol γ forms a highly processive holoenzyme complex with two molecules of its accessory subunit, p55. The polymerase possesses $3' \rightarrow 5'$ proofreading ability that confers fidelity for nucleotide insertion, but the activity is reduced when pol γ is bound with p55 [197–200]. This property indicates that pol γ may bypass some forms of DNA damage through either correct or errant nucleotide incorporation. The proofreading activity of the enzyme competes with its nucleotide insertion activity, complicating the kinetic analysis of either process in isolation. Therefore, an exonuclease-deficient (exo$^-$ p140) pol γ has been purified for the study of nucleotide insertion opposite DNA adducts and of nucleotide analog utilization during strand synthesis [201]. The exo$^-$ p140 enzyme retains its ability to associate with p55 in a holoenzyme complex and has been characterized relative to wild-type pol γ for fidelity and nucleotide insertion kinetics. For studies of pol γ interaction with DNA damage, primer:template duplex substrates are used that contain a single lesion in the template strand either at the position immediately past the end of the primer or at a position that is a few residues downstream of the primer terminus. The placement of the damaged nucleotide in these locations allows for the assessment either single nucleotide insertion kinetics or kinetics after a ‘running start’ to DNA synthesis, respectively. Additionally, undamaged primer:template substates are employed to analyze pol γ binding and incorporation of damaged nucleotide triphosphates that may be present in the mitochondrial dNTP pool [202]. These reconstituted systems have provided the basis for our understanding of pol γ interaction with DNA damage and of therapeutic drug effects on mtDNA synthesis.

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Among the forms of DNA damage investigated for their effects on pol γ are abasic sites, the oxidation adducts, 8-oxo-dG, M_1dG, and γ-OH-PdG, the UV-induced CPDs, the carcinogenic adducts of benzo[*a*]pyrene- and benzo[*c*]phenanthrene diol epoxide, and the intrastrand crosslinks induced by platinum-based chemotherapy agents ([84–86]; R. Kasiviswanathan and W. C. Copeland, personal communication; S. D. Cline, unpublished) (see Table 2). Abasic sites are likely to be the single greatest threat to mtDNA stability, based on their prevalence in human cells [62]. Early studies with *Xenopus laevis* pol γ showed that abasic sites inhibited 80% of strand synthesis and caused nearly exclusive insertion of dATP when translesion synthesis did occur [203]. Abasic sites frequently arise from depurination, so most of these events would be mutagenic. The same study showed a similar degree of pol γ inhibition by 8-oxo-dG; however, lesion bypass occurred primarily through dCTP insertion, indicating that coding errors would be avoided during replication of a damaged chromosome. Following these studies, a correlation was made between a mtDNA 4977 bp deletion and elevated levels of 8-oxo-dG in aging human brain tissue suggesting that oxidative lesions in DNA may trigger substantial loss of genetic material through their effects on DNA pol γ [46]. MTH1 expression is increased in Parkinson disease patients, indicating that oxidation of the nucleotide pool is part of the neurodegenerative mechanism [204]. While mitochondrial MTH1 can effectively clear 8-oxo-dGTP from the nucleotide pool and 8-oxo-dG is efficiently removed from mtDNA by BER, pol γ likely encounters these damaged substrates in tissues under oxidative stress [143]. Compared to wild-type mice, *MTH1*$^{-/-}$ animals show an accumulation of 8-oxo-dG in the mtDNA of their dopaminergic neurons following treatment with 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), an agent used to mimic the effects of Parkinson's disease. MPTP-induced 8-oxo-dG accumulation presumably occurs through pol γ incorporation of 8-oxo-dGTP from the dNTP pool, since BER is not compromised in the MTH1 deficient mice [58, 202, 205, 206]. This finding indicates that the levels of ROS generated by MPTP may overwhelm BER capacity to remove 8-oxo-dG from mtDNA. Studies such as these suggest that the neurodegeneration observed in aging and disease may arise due to multiple insults on mtDNA, some of which may inhibit pol γ synthesis to induce mutagenesis and mtDNA deletions [22, 46, 207, 208].

Several oxidative lesions have been shown to disrupt DNA synthesis by human DNA pol γ using the reconstituted system described above (see Table 2). An 8-oxo-dG in the template strand prevents 95% of pol γ molecules from proceeding past insertion at the lesion [202, 206]. As seen with the *Xenopus* enzyme, human pol γ inserts dCTP opposite 8-oxo-dG, so that the rare bypass events are error-free. This suggests that 8-oxo-dG primarily inhibits replication, and other types of damage, produced concurrently with 8-oxo-dG, may be responsible for mtDNA mutagenesis. The adducts γ-OH-PdG and M_1dG, produced by the aldehyde byproducts of lipid peroxidation, likely form along with 8-oxo-dG in human tissues. Although these adducts are much less prevalent in mtDNA than 8-oxo-dG, they have substantial effects on pol γ activity. Human pol γ shows a strong preference for the incorporation of purines over the correct insertion of dC opposite both γ-OH-PdG and M_1dG. Interestingly, the enzyme can continue strand synthesis from γ-OH-PdG:purine pairs making this adduct highly mutagenic, while M_1dG:purine formation seems to terminate replication (R. Kasiviswanathan and W.C. Copeland, personal communication; S. D. Cline, unpublished). These findings indicate that endogenous adducts formed in mtDNA during oxidative stress have very specific effects on DNA integrity, and that less prevalent, but persistent aldehyde adducts, may contribute to mtDNA instability.

Environmental agents and therapeutic drugs also have substantial effects on pol γ function. While UV-induced CPDs form only in sun exposed tissues, their impact on mtDNA replication may contribute to dermatological aging, neoplasia and cancer [209–213]. Recently, it was shown that pol γ incorporates adenines opposite thymine-thymine CPDs;

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however, the observed 800-fold decrease in efficiency for adenine insertion suggests that the majority of pol γ replication will stall at CPDs, preventing replication of UV-damaged chromosomes [86]. These data point to CPD encounter by pol γ as a source of the mtDNA instability associated with UV exposure. For other human tissues, ingestion, inhalation or injection are the most likely delivery mechanisms for environmental mtDNA damaging agents. Two prevalent PAH compounds, BaP and benzo[*c*] phenanthrene (BcPh), are converted by liver cytochromes P450 and epoxide hydrolyases to their respective diol epoxides (BaPDE and BcPhDE), which react with purines to generate a host of stereisomeric DNA adducts [214–216]. The BaPDE and BcPhDE adducts of guanine and adenine are substantial blocks to pol γ strand extension, findings consistent with the inhibition of mtDNA synthesis observed in cultured hepatocytes treated with BaP [95,217]. When translesion synthesis does occur opposite these PAH adducts, pol γ primarily inserts purine nucleotides resulting in strand mutagenesis or in a base pair that cannot be extended [217]. Therefore, the instability of mtDNA is likely to be a part of the carcinogenic mechanism of PAH exposure.

In the therapeutic realm, two drug classes appear to have effects on mtDNA, the Pt-based anticancer agents and the NRTIs used against HIV. As mentioned above, cisplatin can cause peripheral neuropathy in patients and reduced mtDNA replication and transcription in cultured dorsal root ganglion neurons [121]. Previous *in vitro* studies had shown that cisplatin or oxaliplatin intrastrand d(GpG) crosslinks arrest pol γ synthesis either just prior to or immediately after nucleotide incorporation opposite the 3′-G in the d(GpG) [84]. Taken together, these findings indicate that termination of mtDNA replication by pol γ is the underlying mechanism for the side effects of cisplatin.

Many NRTIs utilized clinically for HIV therapy have the off-target effect of mitochondrial toxicity resulting in lactic acidosis, liver failure and granolocytopenia in patients. These nucleoside analogs are incorporated into mtDNA by pol γ and cause the termination of DNA synthesis [108, 109]. NRTIs display the following order of pol γ inhibition: zalcitabine (ddC) = didanosine (ddI) = stavudine (D4T) ≫ lamivudine (3TC) > tenofovir (PMPA or TDF) > zidovudine (AZT) > carbovir (CBV, the active form of abacavir, ABC) [108, 109]. Consistent with these findings, a high level of mtDNA depletion was observed in peripheral blood mononuclear cells from children treated with ddI, and the agents ddC, ddI, and D4T induced mtDNA depletion in cultured human skeletal muscle cells [218, 219]. In the muscle cells, ddI decreased the RNA for mitochondrial-encoded *MTCYB* and *MTC03* genes without altering mRNA levels for *POLG* and *TFAM* [219]. A loss of mtDNA was also seen in lymphocytes treated with ddC, ddI, and D4T, while lymphocytes exposed to AZT, which has lesser effects on pol γ, retained their mtDNA [107]. From this body of work, it may be concluded that NRTI mitochondrial toxicity results from mtDNA depletion triggered by the direct effects of the agents on DNA synthesis by pol γ.

5.2 Mitochondrial DNA transcription

Less is known about the response of POLRMT to DNA or nucleotide pool damage than about that of pol γ or other RNA polymerases. The RNA polymerases of the T7 bacteriophage, *Escherichia coli,* yeast, and human cells have been characterized for their ability to facilitate TC-NER and for their response to a wide variety of DNA damage [185, 186, 193, 220, 221]. Although NER of UV damage is absent from mitochondria, making the existence of TC-NER unlikely, the presence of CSA and CSB suggests that other transcription-coupled processes may exist. Even in the nucleus, the exact mechanism that triggers repair following CSA and CSB interaction with a damage-arrested RNAP II elongation complex has not been elucidated [185]. Mitochondrial CSA and CSB have yet to be analyzed for their association with POLRMT, so whether they play a similar role in a mitochondrial transcriptional response to mtDNA damage remains uncertain. Indeed, the

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polycistronic nature of mitochondrial transcription on both mtDNA strands, along with the dependence of replication priming on transcription, make the study of POLRMT interaction with DNA damage essential to our understanding of mitochondrial chromosome maintenance and gene expression.

If POLRMT arrest at DNA damage does not trigger repair, the POLRMT elongation complex may abort transcription, preventing efficient gene expression, or may remain stalled at the site of damage, potentially emanating a signal for mitochondrial chromosome degradation or for mitophagy [222]. Such a “self-destruct” signal is issued by the sustained arrest of nuclear RNAP II complexes, which triggers apoptosis, and recently, p53 has been found in mitochondria and shown to control mtDNA copy number [223–226]. So, in a similar way, POLRMT arrest might protect the cell by starting a signaling cascade that would eliminate populations of defective mtDNA, in lieu of their repair, and prevent the expression of aberrant mtDNA transcripts [226, 227]. DNA damage that does not halt transcript elongation may cause POLRMT to insert incorrect nucleotides, creating an errant template for protein translation. This outcome has been termed ‘transcriptional mutagenesis’ and has been documented for *E. coli* RNA polymerase and eukaryotic RNAP II enzymes in their interactions with several forms of DNA damage [228–230]. As discussed above for DNA pol γ, oxidative damage to the mitochondrial nucleotide pool or the presence of nucleotides derived from therapeutic nucleosides might also foul RNA synthesis, as the POLRMT binds and inserts the modified NTPs. Since transcriptional disruption could have both detrimental and protective consequences, experimental systems that allow for the isolation and sequencing of POLRMT transcripts and for the isolation of factors that bind to damage-arrested POLRMT are needed in order to elucidate transcription-mediated events resulting from mtDNA damage. Reconstituted *in vitro* systems with purified human mitochondrial proteins have been employed for the analysis of POLRMT initiation, elongation and nucleotide utilization [231–235]. The few studies completed thus far have provided insight not only by showing the effects of DNA and nucleotide pool damage on POLRMT transcript elongation but also by revealing functional differences between POLRMT and the T7RNAP and RNAP II enzymes.

The *in vitro* arrest of POLRMT by DNA damage is the most tangible evidence that transcription-coupled mechanisms for mtDNA maintenance might exist and that the inhibition of POLRMT by mtDNA damage may contribute to mitochondrial dysfunction in human aging and disease. Since the mechanisms of POLRMT initiation and elongation are still being refined and the mitochondrial promoters appear to impart different characteristics to POLRMT initiation, the study of POLRMT reaction to DNA damage must be conducted in a way that allows the findings to be placed into context of promoter-driven POLRMT function on the mtDNA chromosome [232, 233]. With the apparent absence of NER-like mechanisms in mitochondria, mtDNA damage by endogenous aldehydes may pose a significant threat to mtDNA gene expression, as most other oxidative lesions are repaired by BER.

The M_1dG adduct formed by MDA nearly halts transcription by RNAP II and is a substantial impediment to T7RNAP [236]. To characterize the encounter of POLRMT with the M_1dG, our group employed purified human POLRMT, its transcription factors, TFAM and TFB2M, and DNA templates containing a site-specific adduct placed in either the transcribed or non-transcribed strand downstream of either the LSP or HSP1 mitochondrial chromosome promoter sequences [234]. The exocyclic ring of M_1dG opens to form an acyclic adduct, N^2-OPdG, when it is paired with C; therefore, the adduct was placed in the template opposite either C or T in order to elucidate the structural effects of N^2-OPdG and M_1dG, respectively, on POLRMT transcript elongation. The acyclic N^2-OPdG arrested >60% of POLRMT complexes, which was at least two-fold greater than T7RNAP arrest in a

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similar promoter-driven context [236]. Likewise, M_1dG blocked >80% of POLRMT complexes, again twice exceeding the extent of T7RNAP arrest. These findings indicate that structural differences in the active sites of elongating T7RNAP and POLRMT result in different responses of the enzymes to DNA damage [237]. In fact, POLRMT sensitivity to the N^2-OPdG and M_1dG adduct conformations more closely resembled that of RNAP II, a multi-subunit enzyme with transcription factor requirements for initiation and elongation [236]. Interestingly, the sensitivity of POLRMT to the aldehyde adducts appeared to be dependent upon the initiating promoter [232–234]. POLRMT displayed a 10% greater degree of transcriptional arrest at both N^2-OPdG and M_1dG following initiation at LSP than after HSP1 initiation. This finding, in conjunction with the recent studies showing that LSP and HSP1 differ in their response to TFAM during POLRMT initiation, suggests that POLRMT may adopt promoter-specific conformations for elongation. This hypothesis may be addressed through experiments using templates containing PdG, a saturated analog of M_1dG, that arrests >95% of POLRMT in stable elongation complexes with DNA [234]. Biochemical mapping of arrested POLRMT complexes at PdG following initiation at LSP or HSP1 should provide structural and mechanistic insight into POLRMT elongation. The fact that POLRMT complexes can bypass N^2-OPdG, the form of the aldehyde adduct that would be present in double-stranded mtDNA, suggests that transcriptional mutagenesis is likely during mitochondrial RNA synthesis. The characteristics of the errant transcripts generated from POLRMT insertion at N^2-OPdG may also be readily addressed using the reconstituted system.

As discussed above, oxidized nucleotides, such as 8-oxoGTP, are likely the most prevalent type of aberrant nucleotides in mitochondria. However, NRTIs and other therapeutic nucleoside analogs infiltrate the substrate pools for mitochondrial replication and transcription. Indeed, analogs that were developed for the treatment of RNA viral infections, but were found to have unacceptable toxicity for clinical use, are substrates for POLRMT. The antivirals inhibit transcript elongation or completely terminate RNA synthesis in a manner that correlates with their structure (J. J. Arnold, personal communication). These findings may guide the design of new nucleoside analogs, which may be readily tested in the POLRMT reconstituted system, in order to develop therapies with fewer undesired effects on patients.

In addition to its effects on POLRMT, DNA damage also alters the nucleic acid binding properties of TFAM, a dual HMG-box transcription factor that forms nucleoids and interacts with a host of other proteins involved in DNA transactions [23]. It preferentially binds to DNA containing either an 8-oxo-dG lesion or a cisplatin intrastrand crosslink and appears to inhibit the excision repair of these forms of DNA damage [84, 179, 195, 238]. A purpose for TFAM inhibition of excision repair is unclear at this time; however, TFAM may serve to balance mtDNA repair and gene expression on mitochondrial chromosomes for maintenance of the total mtDNA population. TFAM dissociates from DNA in the presence of p53, which has been shown to translocate to mitochondria during cell stress [225]. Since p53 also participates in mitochondrial BER, an interplay between p53 and TFAM may determine whether damaged mtDNA chromosomes are repaired or degraded [239]. TFAM may associate more strongly with oxidatively damaged chromosomes to sequester them into nucleoids and prevent their transcription until p53 releases TFAM from the DNA allowing BER to occur, thus explaining why TFAM inhibits 8-oxodG incision. This idea is plausible based on the nucleoid heterogeneity of mammalian cells and the observation that nucleoids with lesser amounts of TFAM have greater replication activity. A threshold level of TFAM per chromosome may exist for an optimal mtDNA topology that allows polymerase binding and translocation. Therefore, in addition to directly inhibiting RNA synthesis by POLRMT, DNA damage could alter mitochondrial gene expression through its effects on the distribution of TFAM.

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6. Conclusion

Over three decades ago researchers began to consider the adverse effects of DNA damage on mitochondria and the ramifications of drug therapies on the mitochondrial nucleotide pool. Today, advances in biophysical techniques allow us to detect and even measure the levels of damaged DNA bases in mitochondria. We now know that both endogenous and environmental agents, including drug therapies, pose a threat to mitochondrial health by their chemical modification of mtDNA or through their incorporation by mitochondrial polymerases. Recent discoveries in mitochondrial biology include the elucidation of the repair pathways in and the analysis of DNA damage effects on DNA pol γ and POLRMT. This research has shown numerous insults to mtDNA may interfere with mitochondrial biogenesis and gene expression in a tissue-specific way. For tissues that are highly dependent on oxidative phosphorylation, such as brain, muscle and kidney, the metabolic processes in mitochondria generate DNA lesions that cannot be repaired and interfere with mtDNA replication and transcription. Other polymerase-disrupting adducts are produced by environmental agents that are likely to have greatest effects in the epithelium of the respiratory tract, liver and gut. Additionally, therapies targeted against viruses and human cancers also interfere with mitochondrial polymerase function and inadvertently produce harmful side effects. Research focused on understanding the prevalence and repair of the many forms of DNA damage in human tissues will not only advance our understanding of DNA repair, but also of environmental and drug toxicology and of the process of human aging and disease progression. Findings in this field, taken together with advances in the study of nuclear-mitochondrial signaling in response to cellular redox state and energetics, will provide the most complete picture of how mitochondrial DNA repair, dynamics, biogenesis and destruction act together to maintain mtDNA integrity and gene expression to support cellular function.

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Highlights

Mitochondrial DNA is susceptible to endogenous and environmental damage.

Mitochondrial lack the full cohort of nuclear DNA repair mechanisms.

Persistent mtDNA damage poses a threat to mitochondrial gene expression.

Mitochondrial polymerase disruption by mtDNA damage may underlie human disease and environmental toxicity.

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Table 1

Mitochondrial DNA Damage

Type of Damage	mtDNA Source	Reference
8-oxo-2′-deoxyguanosine (8-oxo-dG)	Alzheimer's and non-diseased human brain	[40,41, 46,49]
	HeLa cells	[42]
	Rat kidney	[48]
	Rat liver	[45, 52]
	Rat heart	[44]
	Mouse liver	[47]
8-hydroxy-2′-deoxyadenine	Alzheimer's and non-diseased human brain	[49]
5-hydroxy-2′-deoxycytosine	Alzheimer's and non-diseased human brain	[49]
5-hydroxy-2′-deoxyuracil	Alzheimer's and non-diseased human brain	[49]
2,6-diamino-4-hydroxy-5- formamidopyrimidine (FapyG)	Alzheimer's and non-diseased human brain	[49]
4,6-diamino-5-formamido-pyrimidine (FapyA)	Alzheimer's and non-diseased human brain	[49]
Fpg Sensitive Sites (includes 8-oxo-dG, 5- hydroxy-2′-purines, and formamidopyrimidines)	Mouse liver	[30]
	CHO cells	[43]
1, N^6-ethenodeoxyadenosine[b]	Rat liver	[51]
3, N^4-ethenodeoxycytidine[b]	Rat liver	[51]
M_1dG	Rat liver	[50]
BaPDE[b]	C3H10T ½ mouse embryonic cells	[94]
	Rat hepatocytes	[95]
	Rat liver, lung, & kidney	[96]
	Atlantic killifish liver, brain & muscle	[182]
N-nitrosamine adducts[b]	Rat liver & lung	[97]
Aflatoxin B1 adducts[b]	Rat liver	[83]
Cisplatin adducts[b]	CHO cells	[153]
	Mouse dorsal root ganglion neurons	[121]
	Maternal and fetal rat tissues	[119]
	Fetal monkey tissues	[120]

[a] DNA damage detected by damage-specific methods.

[b] Exposure conditions given in the corresponding reference.

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Table 2

DNA Pol γ Responses to DNA Damage

Damage	Inhibition[a]	Insertion Preference[b]	Reference
Abasic sites[c]	n.d.[d]	dATP	[203]
8-oxo-dG	+	dCTP≫dGTP>dATP>dTTP	[202, 203, 206]
γ-OH-PdG	+	dATP>dGTP≫dTTP>dCTP	R. Kasiviswanathan and W.C. Copeland, personal communication
M_1dG	n.d.	dATP>dGTP≫dCTP>dTTP	Cline, unpublished
CPD (T^T)[e]	+		[86]
T-T 3′-T	+	dATP≫dGTP≫dTTP>dCTP	
T-T 5′-T	++	dATP	
PAH			[85]
BaP *trans R* dG	+++	dATP>dGTP>dTTP>dCTP	
BaP *trans S* dG	+++	dATP>dGTP>dTTP>dCTP	
BaP *trans R* dA	++	dATP>dTTP	
BaP *trans S* dA	++	dATP>dTTP	
BaP *cis R* dA	++	dATP > dTTP>dGTP>dCTP	
BaP *cis S* dA	++	dATP > dGTP>dTTP>dCTP	
BcPh *cis R* dA	++	dATP≫dTTP>dGTP>dCTP	
BcPh *cis S* dA	++	dATP≫dCTP>dGTP>dTTP	
Pt-GG intrastrand[f]			[84]
oxaliplatin	85%	n.d.	
cisplatin	91%	n.d.	
JM216 (satraplatin)	95%	n.d.	

[a] Based on the translesion insertion efficiency which is the ratio: (kcat/Km) preferred dNTP at normal base/(kcat/Km) preferred dNTP at the damaged base. Translesion insertion efficiency indicates the extent of synthesis inhibition and is expressed as: + = up to 1000-fold; ++ = 1000 to 10,000-fold; and +++ = 10,000 to 100,000-fold inhibition.

[b] Preference for nucleotide insertion opposite the adduct; ≫ indicates at least an order of magnitude difference in either V_{max}/K_m or k_{cat}/K_m for insertion. Insertion was not detectable for dNTPs not listed.

[c] Results only for *Xenopus laevis* pol γ

[d] *n.d.* indicates 'not determined'.

[e] Information provided for insertion opposite the 3′-T or 5′-T of the TT dimer. Only dATP was studied for insertion at the 5′-T.

[f] Insertion kinetics for individual dNTPs were not determined; therefore, the percentage inhibition of primer extension by the Pt adduct relative to the undamaged GG in the presence of all four dNTPs and a 1:1 enzyme-to-template ratio is shown.