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Mitochondrial DNA Repair

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1. Introduction

Each mitochondrion consists of 16,569 base pairs which encodes 37 genes, all of which are essential for normal mitochondrial function (Anderson et al., 1981). Each human cell contains several hundred copies of mitochondrial DNA, encoding 13 genes that are required for oxidative phosphorylation, 22 transfer RNAs and 2 ribosomal RNAs (Anderson et al., 1981). Mitochondria are vital organelles, which generate the majority of the cells energy through oxidative phosphorylation (Wallace, 2005). During this process, reactive oxygen species (ROS) are produced, that can leak out and react with a range of cellular components, including the mitochondrial genome (Richter et al., 1988). Therefore, it has been suggested that levels of oxidative DNA damage are higher in mitochondrial DNA than in nuclear DNA, with mitochondrial DNA accumulating mutations at a 10- to 50- fold higher rate (Hudson et al., 1998; Michikawa et al., 1999; Pakendorf and Stoneking, 2005; Yakes and Van Houten, 1997). If this mitochondrial DNA damage is not repaired, it can lead to disruption of the electron transport chain and increased generation of ROS, possibly resulting in vicious cycle of ROS production and mitochondrial DNA damage, leading to energy depletion and ultimately cell death (Harman, 1972; Miquel et al., 1980). Therefore suggesting that mitochondria must employ some form of repair or defence mechanism against such forms of deleterious damage.

The integrity of mitochondrial DNA repair plays a central role in maintaining homeostasis in the cell and thus the efficient repair of mitochondrial DNA damage serves as an essential function in cellular survival. In comparison to nuclear DNA repair, our knowledge regarding mitochondrial DNA repair is limited. In fact, it was originally believed that mitochondria employed no repair mechanisms and damaged DNA was not repaired, but was merely degraded. This was primarily based on a study published in 1974, which demonstrated the inability of mitochondria to remove cyclobutyl pyrimidine dimers after exposure to ultraviolet light (Clayton et al., 1974). This theory remained for many years, but now it is abundantly clear that multiple DNA repair pathways and the controlled degradation of mitochondrial DNA, work together to maintain the integrity of the mitochondrial genome (Berneburg et al., 2006; Liu and Demple, 2010). Initially the repair of most mitochondrial DNA damage was thought to be limited to short-patch base excision repair (BER) (Stierum et al., 1999). However, the complex range of DNA lesions inflicted on mitochondrial DNA by ROS and potential replication errors indicated that such a restricted repair mechanism would be insufficient. Our knowledge of mitochondrial DNA repair has recently witnessed a rapid expansion and it is now evident that mitochondria also employ

long-patch BER (Akbari et al., 2008; Liu et al., 2008; Szczesny et al., 2008; Zheng et al., 2008), mismatch repair (de Souza-Pinto et al., 2009; Mason et al., 2003), homologous recombination and non-homologous end-joining (Bacman et al., 2009; Fukui and Moraes, 2009; Thyagarajan et al., 1996). In addition, sanitation of the mitochondrial deoxynucleotide triphosphate (dNTP) pool and selective degradation of heavily damaged mitochondrial DNA play important roles in maintaining mitochondrial DNA integrity and preventing cell death (Bacman et al., 2009; Ichikawa et al., 2008; Shokolenko et al., 2009). The majority of the proteins dedicated to DNA repair have to be transcribed and translated from nuclear DNA where they are encoded and imported into the mitochondrion (Bohr, 2002).

Many inherited diseases result from mutations in the mitochondrial genome or due to mutations in nuclear genes that encode mitochondrial components (Chan and Copeland, 2009; Horvath et al., 2009; Tuppen et al., 2010). Somatic mutations in mitochondrial DNA are increasingly linked to common diseases, including age-related degenerative disorders and cancers. Specifically, mitochondrial DNA mutations have been detected in colorectal (Habano et al., 1998; Polyak et al., 1998), breast (Parrella et al., 2001; Radpour et al., 2009) bladder (Copeland et al., 2002; Dasgupta et al., 2008; Wada et al., 2006), lung (Dai et al., 2006; Jin et al., 2007; Suzuki et al., 2003), head and neck cancers (Dasgupta et al., 2010) (Allegra et al., 2006; Mithani et al., 2007), amongst others. Furthermore, some evidence also exists suggesting that mutations in mitochondrial DNA can even accelerate disease progression (Ishikawa and Hayashi, 2010; Lee et al., 2010). Although many associations between mitochondrial DNA mutations and cancer have been shown, a functional link to mitochondrial DNA repair still requires further investigation. Increasing evidence also suggests that mitochondrial DNA damage accumulates with age. However conflicting reports argue whether aging is due to the accumulation of mitochondrial DNA damage or perhaps modifications in mitochondrial DNA repair mechanisms may cause accumulation of DNA damage associated with aging (Boesch et al., 2011; Gruber et al., 2008; Obulesu and Rao, 2010).

2. Mitochondrial DNA repair pathways

Our DNA, both nuclear and mitochondrial, is constantly exposed to endogenous and exogenous agents that induce DNA lesions and genomic instability (De Bont and van Larebeke, 2004; Sander et al., 2005). In the absence of DNA repair, the genome would be unable to survive the multitude of lesions that form throughout the cell cycle. Therefore, a range of molecular mechanisms has evolved that ensures that damaged DNA is effectively repaired. These pathways coordinate the repair of DNA lesions and the stalling of the cell cycle to allow repair to occur (Harper and Elledge, 2007). DNA repair mechanisms have been extensively studied in the nucleus and increasing data demonstrates how distinct DNA lesions are repaired by different DNA repair pathways including homologous recombination, non-homologous end joining, base excision repair, nucleotide excision repair, mismatch repair, and translesion synthesis (Hoeijmakers, 2009). The relevance of the DNA repair pathways in the maintenance of genome integrity and cellular survival is evidenced by the critical consequences in the survival of organisms when deficiencies in key enzymes of the DNA repair pathways occur (Martin et al., 2008).

In contrast to the repertoire of nuclear DNA repair pathways, for many years, the repair of mitochondrial DNA damage was thought to be limited to short-patch BER (Stierum et al., 1999). However more recently with increasing knowledge of the likely array of lesions

inflicted on mitochondrial DNA, it was suggested that such a limited repair repertoire would be insufficient. Studies have identified an expanded range of mitochondrial DNA repair processes including long-patch base excision repair, mismatch repair, homologous recombination and nonhomologous end-joining (Boesch et al., 2011; Liu and Demple, 2010; Yang et al., 2008). It is still generally considered that there is no nucleotide excision repair (NER) in the mitochondria. However, it has been shown that the NER gene, Cockayne syndrome B (CSB) is involved in the removal of oxidative DNA damage from the nucleus, such that CSB-deficient cells demonstrated reduced repair rates of 8-oxoG DNA lesions and extracts from CSB-deficient cells fail to incise oligonucleotides containing 8-oxoG (Balajee et al., 1999) (Dianov et al., 1999; Le Page et al., 2000; Selzer et al., 2002). CSB has also been shown to act in concert with OGG1 in the repair of these lesions (Tuo et al., 2002; Tuo et al., 2001). Due to the generation of ROS in the mitochondria and the increased levels of oxidative damage it was hypothesized that mitochondria-targeted CSB could have a role in repair of mitochondrial DNA. To this end, Stevnsner et al. demonstrated that CSB-deficient cells exhibited a reduced ability to repair 8-oxoG in the mitochondria, suggesting possible NER activity (Stevnsner et al., 2002a). Similarly, the presence of translesion synthesis (TLS) in mitochondria has not been fully elucidated. In the nucleus, TLS is carried out by specialized polymerases, which have the ability to copy defective DNA templates. The possibility of mitochondrial TLS has been suggested due to the fact that the mitochondrial polymerase POLG is capable of mutagenic bypass through DNA lesions introducing dA opposite an AP site or an 8-oxodG (Graziewicz et al., 2007; Pinz et al., 1995) and also opposite benzo[a]pyrene and benzo[c]phenanthrene diol epoxide adducts of deoxyguanosine and deoxyadenosine (Graziewicz et al., 2004). To date, the presence of TLS activity *in vivo* in mitochondria remains to be shown. For both NER and TLS, further research is necessary to define the precise mechanisms of these processes in the mitochondria.

2.1 Base excision repair

The mitochondrial DNA sits on the inner side of the mitochondrial inner membrane, where most reactive oxygen species (ROS) are generated, rendering it highly susceptible to oxidative damage. BER is one of the main pathways for the repair of oxidized modifications both in nuclear and mitochondrial DNA (Slupphaug et al., 2003). As mentioned above, previously the repair of mitochondrial DNA damage and in particular oxidative DNA damage was thought to be limited to short-patch BER (Stierum et al., 1999), which replaces a single nucleotide by the sequential action of DNA glycosylases, an apurinic/apyrimidinic (AP) endonuclease, a DNA polymerase, an abasic lyase activity and DNA ligase (Dianov et al., 2001)(Figure 1). In addition to oxidative DNA damage, BER is the primary pathway required for repair of small DNA modifications induced by alkylaltion and deamination. As in nuclear BER, mitochondrial BER is initiated with recognition of the modified base and its removal is followed by processing of the apurinic/apyrimidinic (AP) site, incorporation of the correct nucleotide and finally strand ligation (Chan et al., 2006; Dianov et al., 2001). A schematic representation of the BER pathway in mitochondria is illustrated in Figure 1.

The 1st step of BER is initiated by DNA glycosylases, which recognize the modified base and cleave the N-glycosidic bond, resulting in an abasic site. It has been shown that a number of glycosylases are bi-functional DNA glycosylases such that they also have AP lyase activity, which enables the cleavage of the DNA backbone (Robertson et al., 2009). Mitochondrial and

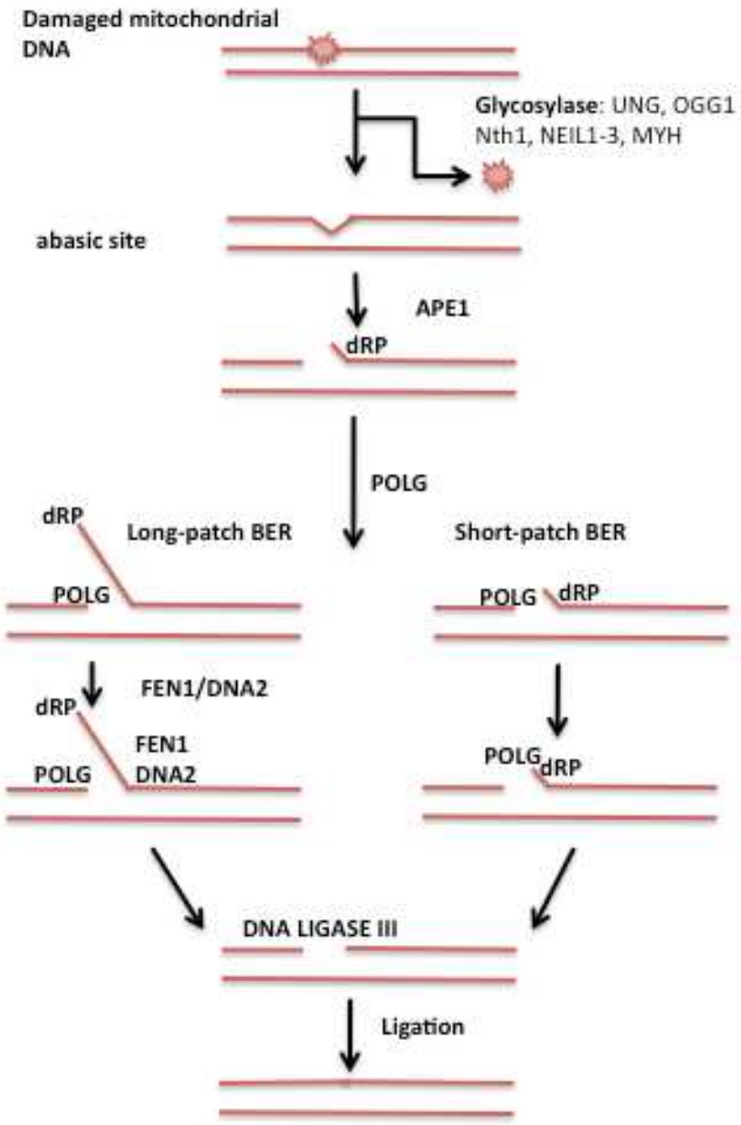

Fig. 1. Schematic representation of the BER pathway in mitochondria.

nuclear glycosylases are encoded by the same nuclear gene, however isoforms are generated by alternative transcription initiation sites and alternative splicing (Bohr, 2002; Nilsen et al., 1997). The mitochondrial DNA glycosylases include the 8-oxoguanine DNA glycosylase-1 (OGG1), the uracil DNA glycosylase (UNG), MYH, endonuclease III homolog (NTH1) and the NEIL glycosylases. OGG1 is a bi-functional glycosylase that is required for the recognition and cleavage of 8-hydroxy-guanine (8-oxoG) oxidative DNA lesions from double-stranded DNA (Kuznetsov et al., 2005). UNG was the 1st glycosylase to be identified and is involved in the removal of uracil from DNA, generated by deamination of cytosine or by misincorporation of dUMP (Lindahl, 1974). The removal of uracil is vital, because of its ability to pair with adenine resulting in GC to AT transition mutations upon replication (Darwanto et al., 2009). MYH is involved in the removal of adenine misinserted opposite 8-oxoG (Takao et al., 1999). NTH1 is also involved in the removal of oxidized DNA lesions

(Takao et al., 2002). The NEIL glycosylases are responsible for excising oxidative DNA lesions such as 2,6-diamino-5-foramidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA) (Doublie et al., 2004). There are three main isoforms, NEIL1, NEIL2 and NEIL3, which are present in both the nucleus and the mitochondria (Gredilla et al., 2010b; Hazra et al., 2002a; Hazra et al., 2002b). Whilst partial redundancy has been described for these glycosylases, NEIL1 knock-out mice accumulate mitochondrial DNA deletions to a greater extent than wild-type mice and also develop symptoms associated with metabolic syndrome (Vartanian et al., 2006).

After recognition and cleavage of the modified base by the specific DNA glycosylase, an abasic site is formed. The AP endonuclease (APE1) is involved in this step of repair. APE1 cleaves on the immediate 5′ side of the AP site, leaving a 3′ hydroxyl and 5′-deoxyribose-5-phosphate (5′-dRP) residue (Masuda et al., 1998). APE1 is the major endonuclease in mammalian cells in both the nucleus and the mitochondria (Tell et al., 2005). The functional importance of APE1 is highlighted by the findings that knockout mice for the APE1 gene are embryonic lethal at very early stages (6–8 days) suggesting that cell survival is critically compromised in the absence of APE1 (Ludwig et al., 1998; Xanthoudakis et al., 1996). Heterologous expression of APE1 restores resistance to DNA-damaging agents in AP endonuclease-deficient cells (Li et al., 2008). APE1 is the only AP endonuclease in mitochondrion, and loss of mitochondrial APE1, not of the nuclear APE1 (Chattopadhyay et al., 2006), is believed to be responsible for triggering apoptosis, therefore highlighting APE1 as a potential therapeutic target. (Li et al., 2008).

Once the AP site has been processed by APE1, the only known mitochondrial DNA polymerase, POLG is required to insert the correct nucleotide in the generated gap (Ropp and Copeland, 1996). Two different BER pathways exist depending on the number of nucleotides that is incorporated by POLG. Short-patch BER involves the incorporation of one single nucleotide into the gap, while long-patch BER involves the incorporation of several nucleotides, usually in the range of 2 to 7 (Robertson et al., 2009). During the long-patch BER process, this incorporation of multiple nucleotides results in the exposure of the original DNA strand as a single-stranded overhang or a flap structure (Xu et al., 2008). Therefore increasing the complexity of long-patch BER, as additional enzymatic activities are required to process this flap. Increasing evidence suggests that in both the nucleus and the mitochondria, this structure is recognized and cleaved by the flap endonuclease, FEN1 (Kalifa et al., 2009; Klungland and Lindahl, 1997). Although FEN1 is clearly involved in mitochondrial BER, studies have suggested the existence of additional activities involving the enzyme Dna2 can also enable the process. Dna2 was originally identified in yeast as a nuclear DNA helicase with an endonuclease activity required for removing part of an RNA or DNA flap structure (Zheng et al., 2008) and yeast Dna2 has been known for some time to function in the nucleus along with FEN1 to process 5′ flaps (Budd and Campbell, 1997). Significantly, the major isoform of Dna2 is localized to the mitochondria. (Copeland and Longley, 2008; Duxin et al., 2009). Current work implies that mammals have evolved to utilize FEN1 as the only nuclear flap endonuclease, whereas both FEN1 and DNA2 appear to function together in mitochondria (Duxin et al., 2009).

The final process in the mitochondrial BER pathway involves sealing of the nick, which requires the mitochondrial DNA ligase, Ligase III. It was shown to be an ATP independent enzyme, similar to the nuclear DNA ligase (Lakshmipathy and Campbell, 1999b). It is involved in both mitochondrial replication and repair. Recently it has been demonstrated that Ligase III is critical for mitochondrial DNA maintenance and viability, but is

dispensable for Xrcc1-mediated nuclear BER (Gao et al., 2011; Simsek et al., 2011). Depletion of DNA ligase III in the mitochondria by antisense DNA ligase III mRNA expression led to a decrease in cellular mitochondrial DNA copy number and increased levels of single-strand DNA breaks within the mitochondrial genome (Lakshmipathy and Campbell, 2001). Ongoing investigations on how the organization of mitochondrial DNA affects BER suggests that mitochondrial DNA association to the inner mitochondrial membrane may be critical for efficient BER (Boesch et al., 2010).

2.2 Mismatch repair

The presence of mismatch repair (MMR) activity in the mitochondria is a controversial area. In 2003, Mason et al. demonstrated that mitochondrial extracts from rat liver exhibited a low but significant MMR activity and that this activity was independent, of one of the main nuclear MMR proteins, MSH2 (Mason et al., 2003). Therefore suggesting that the mitochondrial MMR pathway may be distinct from nuclear MMR. To date, data suggesting the presence of the nuclear MMR proteins in the mitochondria has been conflicting. In 2009, de Souza-Pinto et al. detected the classical MMR proteins MSH3, MSH6 and MLH1 in the nuclei but not in mitochondria (de Souza-Pinto et al., 2009). However we and others, have detected the presence of MLH1, but not MSH2, in the mitochondria of human tumor cells and mouse liver, respectively (Martin et al., 2010; Mootha et al., 2003). Furthermore, our recent data suggests a role for MLH1 in mitochondrial oxidative DNA repair, such that MLH1 deficiency in combination with silencing of the mitochondrial genes, POLG and PINK1, amongst others results in an accumulation in mitochondrial 8-oxoG lesions, incompatible with cell viability (Martin et al., 2011; Martin et al., 2010). Studies have also suggested that mitochondrial DNA mismatch-binding activity is due to the Y-box-binding protein, YB-1 (de Souza-Pinto et al., 2009). Mitochondrial extracts depleted of YB-1 demonstrated a significantly reduced mismatch-binding and repair activity and also a reduced rate of cellular respiration, suggestive of mitochondrial dysfunction. Significantly, silencing of YB-1 by RNA interference (RNAi) also resulted in increased mitochondrial DNA mutagenesis, therefore suggesting that mitochondria do have a MMR pathway, which involves YB-1. The YB-1 mediated mitochondrial mismatch-binding activity was shown to have no bias in favor of the matrix strand and is therefore prone to the introduction of mutations. Recent data has suggested that it can specifically recognize and bind base mismatches and small insertion/deletion loops. In *S. cerevisiae*, Msh1 which is a homologue of the bacterial MutS component, can repair G:A mispairs in mitochondrial DNA, which are generated by replication past 8-oxodG, as well as other mismatches (Chi and Kolodner, 1994). Msh1 is also thought to be involved in mitochondrial DNA recombination, which may help prevent oxidative lesion-induced instability of the mitochondrial genome (Dzierzbicki et al., 2004; Kaniak et al., 2009; Mookerjee et al., 2005). To date the full extent of mismatch repair activity in mammalian mitochondria remains to be elucidated. BER may also be involved in repairing mitochondrial mismatches and therefore it is possible that proteins that participate in mitochondrial BER may have a role in the downstream activities of the mitochondrial MMR pathway.

2.3 Homologous recombination

Double-strand breaks (DSBs) represent one of the most lethal forms of DNA damage. In the nucleus, even one DSB can be lethal whilst in contrast because the mitochondria possess multiple copies of wild type mitochondrial DNA, this can compensate resulting in a less

critical presence of a DSB. Even so, DSB repair has been identified in the mitochondria. In general, homologous recombination (HR) is the primary mechanism for error-free repair of DSBs. HR also plays a critical role in facilitating replication fork progression when the polymerase complex encounters a blocking DNA lesion. In 1995, Ling et al identified the presence of HR in mitochondria in yeast (Ling et al., 1995). It has also been shown that mitochondria are able to repair DSBs in Chinese hamster ovary cells (LeDoux et al., 1992). Rad51, the central mediator of nuclear HR, Rad51C and XRCC3, have all been shown to localize to the mitochondria in human cells (Sage et al., 2010). Rad51 has been shown to bind mitochondrial DNA following exposure to cells upon oxidative stress. Rad51-mediated activity is necessary for regulating mitochondrial DNA copy number under conditions of oxidative stress and this activity requires the functions of Rad51C and XRCC3. In the nucleus, Rad51 and XRCC3 have been shown to cooperate in regulating replication fork progression on damaged chromosomes, therefore it has been suggested that mitochondrial Rad51, Rad51C and XRCC3 ensure faithful completion of mitochondrial DNA replication as the fork encounters blocking lesions. In addition, a study by Thyagarajan et al., have demonstrated that human mitochondrial extracts have the ability to catalyze HR of different DNA substrates (Thyagarajan et al., 1996). Further evidence of mitochondrial HR analyzed segregated mitochondrial DNA mutations in a heteroplasmic mitochondrial DNA population and identified combinations of these two mutations in different mitochondrial DNA molecules indicating HR and crossing over events between mitochondrial DNA molecules with segregated mutations (Zsurka et al., 2004). BRCA1, the breast and ovarian cancer susceptibility gene, which plays a role in the HR pathway, has also been shown to localize to the mitochondria and was found to colocalize with mitochondrial DNA clusters (Coene et al., 2005).

2.4 Non-homologous end joining

Studies have shown that mitochondrial protein extracts possess non-homologous end-joining (NHEJ) activity. NHEJ is highly precise in the case of DNA with cohesive ends while blunt-ended DNA are rejoined with less efficiency and precision (Roth et al., 1985). In mitochondrial extracts, it has been demonstrated that both cohesive and blunt-ended DNA substrates can be rejoined, although the latter with much lower efficiency (Lakshmipathy and Campbell, 1999a). Irrespective of which DNA substrate was used, the majority of recovered products were precisely repaired. Analysis of imprecisely repaired products revealed the presence of deletions that spanned direct repeat sequences. These deletions were similar to those observed in the mitochondrial DNA of certain pathological states as well as in aging cells. Ku80 is required for nuclear NHEJ due to its DNA end-joining activity. Mammalian mitochondrial DNA end-joining activity was reported to be practically indistinguishable from that of the nuclear activity. This observation led to the investigation and subsequent demonstration that Ku80 is also required for mammalian mitochondrial DNA end-joining activity (Feldmann et al., 2000).

3. Mitochondrial DNA degradation

The possibility of mitochondrial degradation was first proposed because of early studies suggesting that UV-induced pyrimidine dimmers were not repaired in mammalian mitochondria (Clayton et al., 1974). Furthermore, in response to treatment with mutagenic agents such as ethylmethane sulfonate, N-methyl-N'-nitrosoguanidine and benzo(a)pyrene, mitochondrial DNA from HeLa cells only accumulated few mutations suggesting that

mitochondrial DNA accumulating excessive amounts of damage or irreparable lesions, is not replicated (Mita et al., 1988). More recently, further investigation into this process has revealed that extensive or persistent DSBs result in mitochondrial DNA degradation (Alexeyev et al., 2008; Bacman et al., 2009; Fukui and Moraes, 2009). Such that the signal that triggers mitochondrial DNA degradation has been attributed to DSBs, generated by stalled DNA or RNA polymerases on the damaged mitochondrial DNA template. Degradation of these molecules prevents mutagenesis and maintains mitochondrial DNA integrity. In the case of UV-induced pyrimidine dimers and benzo(a)pyrene-induced adducts, the stalled RNA or DNA polymerase would trigger the degradation process. More recently, studies have suggested that oxidative stress can lead to the degradation of mitochondrial DNA and that strand breaks and abasic sites prevail over mutagenic base lesions in ROS-damaged mitochondrial DNA (Shokolenko et al., 2009). Furthermore, inhibition of abasic site processing by APE1 and inhibition of BER by methoxyamine treatment enhanced this degradation in response to oxidative damage, suggesting that the inability to repair mitochondrial DNA damage may be the signal for its degradation (Shokolenko et al., 2009). The elimination of damaged mitochondrial DNA was preceded by the accumulation of linear mitochondrial DNA molecules, which potentially represent degradation intermediates. These intermediates, unlike undamaged circular mitochondrial DNA molecules, are susceptible to exonucleolitic degradation thus ensuring the specificity of the process. Therefore supporting the observation by Suter and Richter who demonstrated that 8-oxoG content of circular mitochondrial DNA is low and does not increase in response to oxidative insult in contrast to fragmented mitochondrial DNA which had very high 8-oxoG content, that further increased after oxidative stress (Suter and Richter, 1999).

3.1 Mitochondrial DNA degradation nuclease

The Endonuclease G (EndoG) was initially proposed to be the nuclease responsible for selectively degrading non-replicable mitochondrial DNA. Such that Ikeda and Ozaki showed that mitochondrial EndoG is more active *in vitro* on oxidatively modified DNA compared to undamaged DNA suggesting that it may be involved in the degradation of oxidatively damaged mitochondrial DNA (Ikeda and Ozaki, 1997). However, more recent studies illustrated that EndoG-deficient cells or EndoG null mice showed no accumulation in mitochondria DNA mutation rate or defects in mitochondrial structure, therefore suggesting that EndoG may not be the exclusive nuclease involved (Irvine et al., 2005). Davies et al. reported that upon removal of EndoG activity from the mitochondria, another nuclease activity can be detected internal to the inner mitochondrial membrane (Davies et al., 2003). This exonuclease causes a gradual degradation of amplified DNA and linearized pBR322 plasmid DNA without the site-specific cleavage seen with EndoG. However they also showed that when supercoiled mitochondrial DNA is used as a substrate, both endo- and exonuclease activities could be detected. Whether the endo- and exonucleolytic activities arise from the same nuclease or from separate enzymes remains under investigation.

4. Sanitation of the mitochondrial deoxynucleotide triphosphate pool

So far, we have only discussed repair and damage of mitochondrial DNA, however the free deoxynucleotide triphosphate (dNTP) pool is also exposed to oxidation and other stresses.

dNTPs are the precursors used by DNA polymerases for replication and repair of nuclear and mitochondrial DNA. The cell employs specialized enzymes that remove for example, oxidized dNTPs that otherwise may be incorrectly incorporated during DNA synthesis such as 8-oxo-2'-deoxyguanosine triphosphate (8-oxo-dGTP). 8-oxo-dGTP can be potentially incorporated opposite A by POLG, resulting in 8-oxodG:dA base pairs which are resistant to the proof-reading activity of POLG, ultimately resulting in AT to CG transversions (Hanes et al., 2006; Pursell et al., 2008). As a defense to such activities, MUTYH, present in both the nucleus and mitochondria, has the ability to remove the misincorporated adenine, enabling insertion of dCMP and removal of the 8-oxoguanine by BER (Takao et al., 1999; van Loon and Hubscher, 2009). Oxidation of the mitochondrial dNTP pool represents a significant threat to mitochondrial DNA integrity with the 8-oxo-dGTP concentrations in mitochondrial extracts from rat tissues ranging from 1-10% of the total dGTP (Pursell et al., 2008).

The major defense mechanisms against 8-oxo-dGTP, is its elimination from the dNTP pool by the mitochondrial MTH1 (Kang et al., 1995; Nakabeppu, 2001). MTH1 can hydrolyze 8-oxodGTP to 8-oxodGMP, which is not a substrate for DNA polymerases and therefore would not be incorporated into the DNA. MTH1 can also hydrolyze, 8-oxo-2'-deoxyadenosine triphosphate and 2-hydroxy-2'-deoxtadenosine triphosphate to the monophosphates (Sakai et al., 2002). 8-oxoG accumulation in mitochondrial DNA was observed in MTH1-null mouse embryonic fibroblasts following hydrogen peroxide treatment and in dopaminergic neurons from MTH1-null mice following 1-methyl-4-phenyl-1,2,3,6,-tetreadropyridine treatment (Yamaguchi et al., 2006; Yoshimura et al., 2003). MTH1 was also shown to protect cells from the cytotoxicity of sodium nitoprusside by preventing 8-oxoG accumulation in mitochondrial DNA (Ichikawa et al., 2008). Taken together, this strongly suggests that MTH1 plays a critical role in protecting mitochondrial DNA from oxidized dNTPs.

The *DUT* gene, which encodes a UTPase which can remove dUTP from the nucleotide pool, also encodes an alternative splice variant that is located to mitochondria (Ladner and Caradonna, 1997). dUTP can arise from deamination of dTTP. The mitochondrial protein is 23 kDa and is constitutively expressed, in contrast to the nuclear isoform, which is cell cycle regulated. If modified dNTPs are incorporated into mitochondrial DNA they must be removed via the BER pathway, which can repair modifications of single nucleotides already incorporated in DNA.

5. Mitochondrial DNA repair and disease

Accumulating data increasingly shows the involvement of various mitochondrial DNA mutations in human diseases. Several disorders such as myopathy, optic atrophy and Leigh syndrome arise as a result of mitochondrial alterations (Edmond, 2009). In addition, a number of pathologies are also caused by mutations in nuclear genes that encode for mitochondrial proteins (Chan and Copeland, 2009; Horvath et al., 2009; Tuppen et al., 2010). The most common genetic defect seen in individuals with mitochondrial DNA-associated disease are deletions (Holt et al., 1988; Shoffner et al., 1989) or point mutations (Goto et al., 1990; Wallace et al., 1988). Mitochondrial DNA deletions have been shown to be important in pathogenesis in a number of ways. Single mitochondrial DNA deletions are a common cause of sporadic mitochondrial disease and an identical mitochondrial DNA deletion is present in all cells of the affected tissue (Schaefer et al., 2008). Some individuals with mitochondrial disease have multiple mitochondrial DNA deletions in the affected tissues,

usually the muscle and the central nervous system (Taylor and Turnbull, 2005). These involve nuclear genes encoding proteins involved in either mitochondrial nucleotide metabolism or mitochondrial DNA maintenance. There are also a number of reports of mitochondrial deletions in aged post-mitotic tissues and individuals with neurodegenerative disease (Bender et al., 2006; Kraytsberg et al., 2006; Taylor and Turnbull, 2005). These pathogenic mitochondrial DNA deletions have been suggested to be as a result of mitochondrial DNA repair. It has been postulated that mitochondrial deletions are initiated by single-stranded regions of mitochondrial DNA generated through exonuclease activity at DSBs (Krishnan et al., 2008). Ultimately, these single strands are free to anneal with microhomologous sequences such as repeat sequences on other single-stranded mitochondrial DNA or within the noncoding region (Haber, 2000). Once annealed, subsequent repair, ligation and degradation of the remaining exposed single strands would result in the formation of an intact mitochondrial genome harboring a deleted portion.

5.1 Mitochondrial DNA repair and neurodegenerative disease

Mitochondrial DNA damage is found in affected neurons in the majority of neurodegenerative disorders, and is often associated with oxidative DNA damage and mitochondrial dysfunction (de Moura et al., 2010). Accumulation of nuclear DNA and mitochondrial DNA lesions has been demonstrated to be a critical factor contributing to genomic instability and mitochondrial dysfunction in neurodegenerative diseases (Lin and Beal, 2006; Yang et al., 2008). DNA repair mechanisms are essential for the proper maintenance of the mammalian central nervous system. Therefore, deficiency in DNA repair, particularly in BER, is increasingly recognized as a major contributor to neuronal loss. Neurodegenerative diseases are increasingly associated with mutations in mitochondrial DNA strongly suggesting that neurons are particularly sensitive to mitochondrial dysfunction. Neurons in both the peripheral and central nervous systems are adversely affected by mitochondrial mutations (Wallace, 2001). Examples of neurodegenerative diseases associated with mitochondrial DNA damage and repair (Finsterer, 2006; Servidei, 2004) include but are not limited to: Alzheimers disease, Parkinsons disease and Huntingtons disease. The fact that many of these share similar neuropathological features with multiple neurodegenerative disorders, suggests a significant role for mitochondrial dysfunction in the pathogenesis of neurodegenerative disorders.

Alzheimers Disease, the most common form of age-associated dementia, is a progressive and always fatal disorder characterized clinically by memory loss and behavioral abnormalities, and histopathologically by deposition of amyloid β-peptide (Aβ), cytoskeletal pathology, degeneration of synapses and neuronal death (Mattson, 2004). Several studies have shown that oxidative modification to both nuclear DNA and mitochondrial DNA are increased in brains of Alzheimers disease patients (Gabbita et al., 1998; Mecocci et al., 1994; Wang et al., 2005). An accumulation of 8-hydroxy-2-deoxyguanosine (8-OHdG) was observed in mitochondrial DNA isolated from cortical brain regions of Alzheimers patients (Mecocci et al., 1994). Furthermore significant BER dysfunction was observed in brains of Alzheimers patients, resulting from reduced UDG, OGG1 and POLB activities (Weissman et al., 2007). Parkinson's disease is the second most prevalent neurodegenerative disease, affecting approximately 2% of individuals over the age of 65 years (de Rijk et al., 1997; Mouradian, 2002). It is clinically characterized by resting tremor, postural instability, gait disturbance, bradykinesia and rigidity. Increasing evidence suggests that oxidative damage

to DNA, both nuclear and mitochondrial, contributes to the degeneration of dopaminergic neurons in Parkinsons disease (Alam et al., 1997). Swerdlow et al. demonstrated that mitochondria from Parkinsons patients exhibit increased production of ROS, decreased activity of complex I and increased DNA damage (Swerdlow et al., 1996). Huntington's disease is a dominantly inherited neurodegenerative disorder caused by expanded CAG trinucleotide repeats in the amino-terminal coding region of the huntingtin (Htt) gene (Cepeda et al., 2007). It was suggested that expansion of the CAG trinucleotide repeats in Huntingtons disease requires DNA break repair and involves several DNA repair enzymes including FEN1 (Lee and Park, 2002; Spiro et al., 1999). It was also proposed that faulty processing of strand breaks by FEN-1, initiates CAG repeat instability in mammalian cells (Spiro and McMurray, 2003). It was recently demonstrated that the accumulation of oxidative DNA lesions in brains and livers of Huntingtons mice, including 8-oxoG, 5-hydroxyuracil (5-OHU), 5-hydroxycytosine (5-OHC), and formamidopyrimidine (FAPY), were correlated with the degree of trinucleotide expansion, suggesting that that initiation of CAG repeats may occur during removal of oxidative DNA lesions, and could be specifically associated with OGG1 activity (Kovtun et al., 2007).

5.2 Mitochondrial DNA repair and cancer

The extent to which cancer is caused by or is a consequence of mitochondrial genomic alterations is unknown, but substantial data suggest an involvement of mutations in mitochondrial DNA in the carcinogenic process. Mitochondrial defects have long been suspected to play an important role in the development and progression of cancer (Carew and Huang, 2002; Hockenbery, 2002; Warburg, 1956). However the majority of the existing data currently show an association of increased mitochondrial DNA mutations in different tumours with only little direct evidence for a functional role of these mutations. Tumour cells, in general, have increased levels of mitochondrial DNA transcripts, while both increases and decreases in the levels of tumour cell mitochondrial DNA have been reported.
ROS-triggered mutagenesis of both mitochondrial DNA and nuclear DNA has been suggested to correlate with tumourigenesis. (Klaunig et al., 2010). Decreased nuclear and mitochondrial levels of the OGG1 glycosylase were observed in human lung cancers compared with normal cells (Karahalil et al., 2010). Furthermore, decreased OGG1 expression was also observed in spontaneous hepatocellular carcinomas developed in mutant rats, in association with an accumulation of oxidative DNA damage and ROS generation (Choudhury et al., 2003). Colorectal cancers have been shown to exhibit increased somatic mitochondrial DNA mutations (Habano et al., 1998; Polyak et al., 1998). Significantly, all of these mutations were present in the majority of the tumour cells and 90% of them were detectable in all of the mitochondrial DNA present in cells, strongly suggesting that all mitochondrial DNA molecules in the mitochondrion contain the same mutation. Breast cancer also exhibit somatic mitochondrial DNA mutations (Parrella et al., 2001; Radpour et al., 2009), in addition to kidney (Meierhofer et al., 2006) (Nagy et al., 2003), stomach (Hung et al., 2010; Jeong et al., 2010), prostate (Moro et al., 2009) (Parr et al., 2006) liver (Vivekanandan et al., 2010; Zhang et al., 2010), bladder (Dasgupta et al., 2008), head and neck (Allegra et al., 2006; Dasgupta et al., 2010; Mithani et al., 2007) and lung (Dai et al., 2006; Jin et al., 2007; Suzuki et al., 2003). Furthermore increased mitochondrial DNA mutation frequencies were associated with hereditary paraganglioma (Muller et al., 2005; Taschner et al., 2001) and thyroid cancers (Abu-Amero et al., 2005; Rogounovitch et al., 2004). Clayton and Smith further expanded studies of mitochondrial DNA structural

changes in leukocytes of leukemic patients and also in patients with a variety of solid tumors (Clayton and Smith, 1975).

Data suggesting a role for mitochondrial DNA in cancer regression comes from studies with the chemotherapy drugs, bis-2-chloroethylnitrosourea (BCNU) and temozolomide. These drugs induce cell death by alkylation of DNA bases to form mutagenic O^6 methylguanine and interstrand cross-links (Ludlum, 1997; Newlands et al., 1997). The repair enzyme O^6methyloguanine DNA methyltransferase (MGMT) removes O^6methylguanine DNA damage (Bobola et al., 1995; Bobola et al., 1996). Studies have shown that transfecting haematopoietic cell lines with low repair activity for alkylated DNA damage with mitochondrial-targeted and nuclear-targeted MGMT generated resistance against the cytotoxic effects of BCNU and temozolomide (Cai et al., 2005). Significantly, this effect was more dependent on mitochondrial MGMT in comparison to the nuclear MGMT suggesting the contribution of mitochondrial DNA repair in the generation of drug-resistant tumour cells.

6. Mitochondrial DNA repair and aging

Many theories have been proposed to explain the phenomenon of aging (Kirkwood, 2005). Amongst these is the mitochondrial free radical theory of aging, which states that the accumulation of mitochondrial damage and the progressive accumulation of free radical damage in post-mitotic tissues, is the cause of aging (Harman, 1956). Because mitochondria are the main generators of ROS and consequently the main target of their DNA damaging effects, oxidative damage can result in increasing rates of mitochondrial DNA mutations. A vicious cycle can potentially occur as mitochondria encode for components of the respiratory chain and ATP synthase complexes, therefore mutations in the mitochondrial DNA may cause defects in oxidative phosphorylation resulting in an increased generation of ROS and further mitochondrial DNA damage (Miquel et al., 1980).

The mitochondrial theory of ageing has been controversial, with numerous studies performed to elucidate the precise correlation between oxidative damage, mitochondrial mutations and aging. One prominent study involves the generation of a mouse model that illustrates an increase in mitochondrial DNA mutation and oxidative phosphorylation defects. This mouse model which carries an error-prone form of POLG was generated, and correlated with decreased life expectancy and a premature ageing phenotype (Kujoth et al., 2005; Trifunovic et al., 2004). However there was little evidence of increased ROS or oxidative damage as a result of the mitochondrial DNA replication errors, suggesting the lack of the previously proposed “vicious cycle”. Studies of the various tissues of these mice, have suggested that it is the accumulation of mitochondrial DNA deletions and clonal expansion identified in the brain and heart that drive the premature aging phenotype (Vermulst et al., 2007)(Vermulst et al., 2008). More recently, an alternative study has now suggested that it is random point mutations occurring in mitochondrial DNA analyzed in the liver and heart that are the driving force behind the aging phenotype (Edgar et al., 2009). The discrepancies between the studies may be due to the analysis of either mitotic or post-mitotic tissues. Such that, it has been suggested that in post-mitotic tissues, mitochondrial DNA deletions occur initially during repair of damaged DNA whilst in mitotic tissues it is thought that mitochondrial DNA point mutations are likely to be generated during replication (Reeve et al., 2009).

A number of studies suggest that although oxidative damage of mitochondrial DNA does accumulate with age in mammalian cells, this accumulation does not regulate lifespan

(Arnheim and Cortopassi, 1992; Barja and Herrero, 2000). Similarly, in Drosophila, mitochondrial ROS production increases with age but does not influence its lifespan (Sanz et al., 2010). One reason has been postulated such that scavenging free radicals could increase life expectancy whilst increasing ROS may lead to premature cell death. To address this several transgenic models have been generated. Although over-expression of the mitochondrial Mn-superoxide dismutase (MnSOD) extends lifespan in Drosophila (Sun et al., 2002), it had no effect on lifespan in similarly over-expressing mice (Jang et al., 2009; Perez et al., 2009). An increase in ROS levels by inactivation of antioxidants does not display shortened lifespan, such that transgenic mice expressing only one allele of mitochondrial thioredoxin TRX2 do not display any decrease in life expectancy, whilst exhibiting significant defects in oxidative phosphorylation and increased hydrogen peroxide production (Jang et al., 2009). Therefore strongly suggesting that ROS generation during normal metabolism is unlikely to be the main or single cause of aging.

A causative role for mitochondrial DNA damage in the development of aging remains to be proven, however damaged mitochondrial DNA accumulates with age suggesting a potential role for mitochondrial DNA repair. Mitochondrial DNA repair defects may contribute to the accumulation of DNA damage associated with aging (Druzhyna et al., 2008; Gredilla et al., 2010a). Studies suggest that the 8-oxoG DNA lesion is one of the most abundant oxidative lesions which accumulates with age in the mitochondria. However, in apparent contrast the overall OGG1 8-oxoG glycosylase activity has been shown to increase with age in mammalian cells (Stevnsner et al., 2002b). Further studies have postulated that while the overall OGG1 content in the mitochondria increases with age, the amount of OGG1 in the mitochondrial inner compartment decreases resulting in the observed accumulation of 8-oxoG in mitochondrial DNA with a large fraction of the enzyme remaining stuck to the membrane in the precursor form, which could not be translocated to and processed in the mitochondrial matrix. (Szczesny et al., 2003). A similar observation has been reported for the mitochondrial uracil DNA glycosylase, UDG, suggesting a deficiency in import in aged cells (Szczesny et al., 2003).

Caloric restriction has been shown to reduce the accumulation of mitochondrial DNA mutations and increase lifespan (Aspnes et al., 1997; Cassano et al., 2004; Gredilla and Barja, 2005). DNA repair in the nucleus has been shown to be enhanced by caloric restriction and promote genomic stability (Heydari et al., 2007). However, studies in the mitochondria have shown that mitochondrial BER capacity did not change in liver and actually decreased in the brain and kidney of caloric restricted rats (Stuart et al., 2004). This decrease in BER correlates with the observation that mitochondria from caloric restricted rodents generate ROS and accumulate oxidative DNA damage at lower rates than non-restricted animals (Gredilla and Barja, 2005). Therefore it has been suggested that when the levels of ROS and mitochondrial DNA damage are significantly reduced, it may enable the organism to require less energy required for mitochondrial DNA repair.

7. Conclusion

Originally thought to be absent, DNA repair mechanisms in the mitochondria are now well established. Whilst all the core enzymatic components of the BER pathway have been identified in the mitochondria, the precise mechanisms of the remaining pathways have been less well investigated. For example, identification and characterization of the key players in the mitochondrial MMR pathway and a potential role for NER proteins in the

repair of oxidative damage in the mitochondria remain unclear. Inactivation of many nuclear genes encoding key proteins, can impact mitochondrial DNA maintenance and result in an accumulation of DNA damage and ultimately mutations. Controversy surrounds the pathological nature of these mitochondrial DNA mutations, however increasing evidence links mitochondrial DNA integrity with carcinogenesis, neurodegenerative disease and aging. Taken together, future work requires an in dept analysis of the functional role of these mutations in human pathologies and aging.

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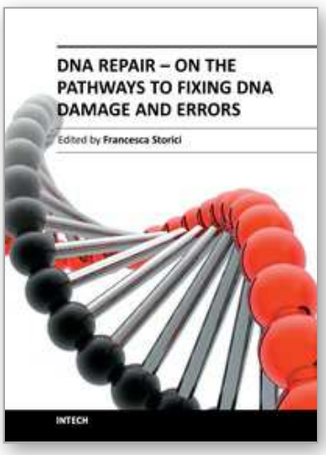

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DNA repair is fundamental to all cell types to maintain genomic stability. A collection of cutting-edge reviews, DNA Repair - On the pathways to fixing DNA damage and errors covers major aspects of the DNA repair processes in a large variety of organisms, emphasizing foremost developments, questions to be solved and new directions in this rapidly evolving area of modern biology. Written by researchers at the vanguard of the DNA repair field, the chapters highlight the importance of the DNA repair mechanisms and their linkage to DNA replication, cell-cycle progression and DNA recombination. Major topics include: base excision repair, nucleotide excision repair, mismatch repair, double-strand break repair, with focus on specific inhibitors and key players of DNA repair such as nucleases, ubiquitin-proteasome enzymes, poly ADP-ribose polymerase and factors relevant for DNA repair in mitochondria and embryonic stem cells. This book is a journey into the cosmos of DNA repair and its frontiers.

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