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Mitochondrial DNA Damage, Repair, Degradation and Experimental Approaches to Studying These Phenomena

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1. Introduction

In mammalian cells, genetic information is stored in two locations: in the nucleus and in mitochondria. Nuclear DNA (nDNA) is organized into chromosomes of which two sets are present per cell: one paternal, and one maternal. In contrast, mitochondrial DNA (mtDNA) inheritance is (with few exceptions) exclusively maternal, and is highly redundant, typically a few hundred to a few thousand copies per cell. In many (but not all, (Noll et al., 1990)) cell types the bulk of ATP is produced by oxidative phosphorylation (OXPHOS) in mitochondria. Since mtDNA encodes components of four out of five mitochondrial respiratory complexes, it is not surprising that alterations in mtDNA result in (mitochondrial) disease (Holt et al., 1988; Lestienne & Ponsot, 1988; Wallace et al., 1988). Apart from mitochondrial disease, mutations in mtDNA are linked to a spectrum of diseases including cancer, diabetes, cardiovascular diseases and neurodegenerative disorders, as well as the normal process of aging (Wallace, 2005). Importantly, it has been established that not only mtDNA mutations, but also reduction in the mtDNA copy number can be pathogenic (Clay Montier et al., 2009; Rotig & Poulton, 2009). Understanding cellular mechanisms for the maintenance of mtDNA integrity and copy number is, therefore, of utmost importance since it can provide targets for clinical interventions aimed at prevention and treatment of human disease.

2. Organization of the mitochondrial genome

Human mtDNA (Figue 1) is approximately 16.6 kbp long and encodes two rRNAs, 22 tRNAs and 13 polypeptides of which 7 are subunits of complex I (NADH dehydrogenase), 3 are subunits of complex IV (cytochrome c oxidase), 2 are subunits of complex V (ATP synthase), and cytochrome b (a subunit of complex III). The density of genetic information in mtDNA is relatively high, with very short intergenic regions. To increase this density some genes overlap, and some others lack complete termination codons, which are created by polyadenylation of corresponding mRNAs (Ojala et al., 1981). A short noncoding regulatory region in mtDNA harbours an origin of replication plus two promoters, one on each of the two complementary strands. These promoters generate polycistronic transcripts that are processed to produce mature rRNAs, tRNAs, and mRNAs and also are involved in the generation of the primer for replication of one of the strands.

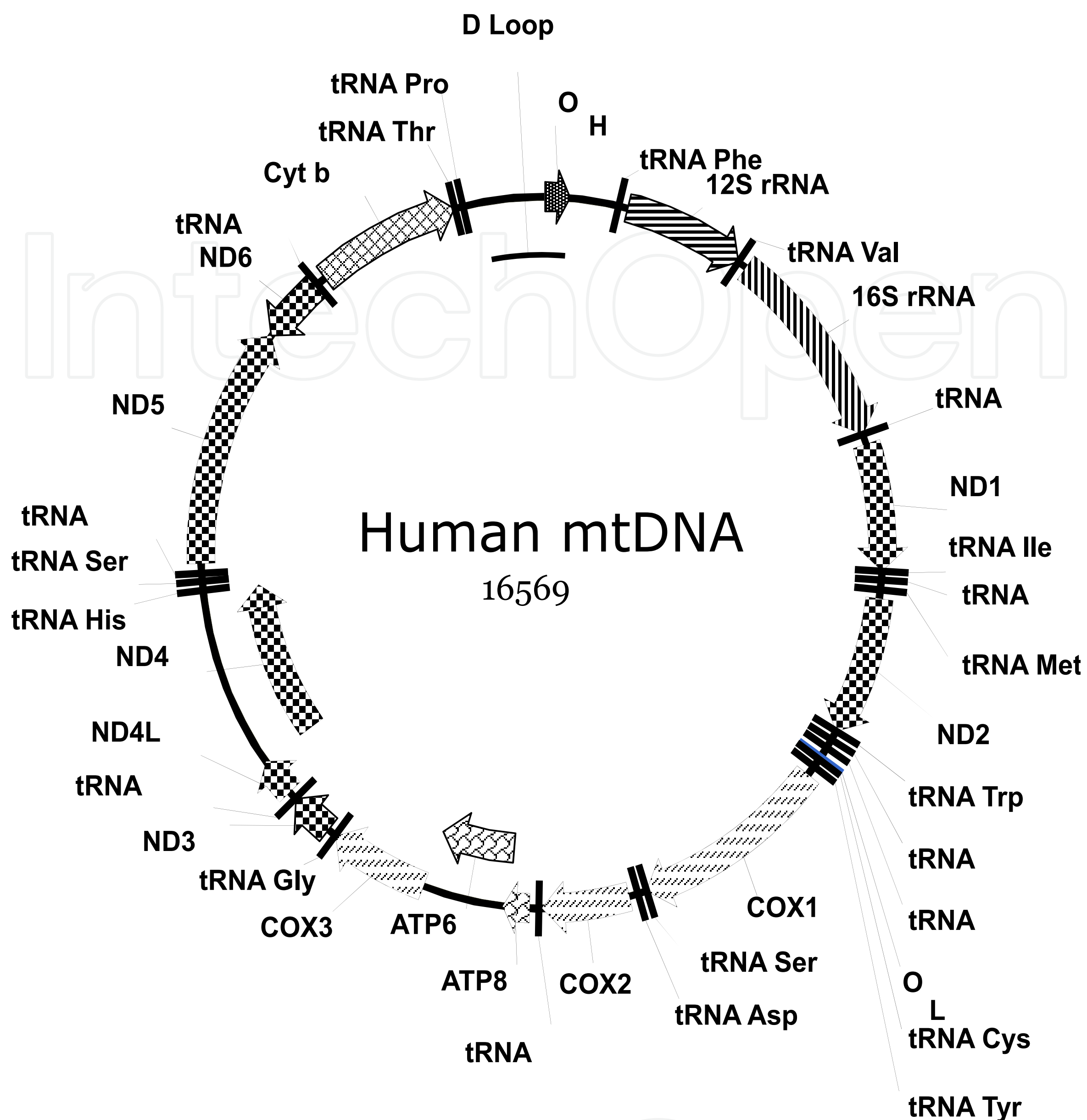

Fig. 1. The map of human Mitochondrial DNA. O_H and O_L, origins of heavy and light strand replication, respectively; ND1-ND6, subunits of NADH dehydrogenase (ETC complex I) subunits 1 through 6; COX1-COX3, subunits of cytochrome oxidase subunits 1 through 3 (ETC complex IV), ATP6 and ATP8, subunits 6 and 8 of mitochondrial ATPase (complex V), Cyt b, cytochrome b (complex III).

It has been determined that mitochondria contain, on average, two molecules of mtDNA (Cavelier et al., 2000). However, mitochondria form a dynamic network which, in different cell types and under different physiological conditions, can assume a variety of conformations, the two extremes being “reticular” (mitochondria in the cell are fused to form a network of extended filaments) and “particular” (network is disintegrated into short fragments). In both conformations, mitochondria perpetually undergo the processes of fission and fusion, thus mixing their contents. Therefore, the above definitions of “reticulate” and “particulate” mitochondrial conformations are relative terms referring to a snapshot of the mitochondrial network in a cell. Nevertheless, these terms are useful as they

describe the prevalence of either mitochondrial fission ("particulate" conformation) or fusion ("reticulate" conformation) in a given cell under given physiological conditions. In this light, the average number of mtDNA copies per mitochondrion determined in some studies (Cavelier et al., 2000) may simply reflect the extent of mitochondrial fragmentation under the assay conditions, which is defined by two factors: a) the mitochondrial conformation inside the cell, and b) the extent of mitochondrial fragmentation during isolation for the analysis of mtDNA content.

Nuclear genetic material is represented by nucleoprotein complexes consisting of DNA wrapped around a core octamer of histones forming "beads on a string". This nucleosomal chromatin is further organized to form chromosomes. In contrast, the mitochondrial genome lacks histones, which has led to the widespread belief that the observed high rate of mtDNA mutagenesis (approximately 10-fold greater than in nDNA (Brown, W.M. et al., 1979; Ballard & Whitlock, 2004; Tatarenkov & Avise, 2007) can be explained by the lack of "protective" histones. This belief lacks direct experimental support and remains controversial as it contradicts some experimental evidence, which suggests that histones may enhance, rather than reduce DNA damage (Liang, R. et al., 1999; Liang, Q. & Dedon, 2001), at least under some conditions, and that mtDNA-associated proteins are at least as protective against mutagenic insults as histones under other conditions (Guliaeva et al., 2006). Moreover, mtDNA may be physically covered with TFAM (Alam et al., 2003), an HMG-like protein involved in mtDNA transcription and replication, a notion which is consistent with the limited accessibility of mtDNA to methytransferases (Rebelo et al., 2009).

Considering the endosymbiotic theory of mitochondrial origin from an ancient prokaryote, it is perhaps not surprising that recent studies revealed similarities in packaging of mtDNA and bacterial chromosomes. Thus, it has been established that in the ECV304 cell line the 3,500 copies of mtDNA are organized into ~475 nucleoids about 70 nm in diameter, each of them carrying 6-10 copies of mtDNA (Iborra et al., 2004). This organization insures similar DNA densities in mitochondrial and E. *coli* nucleoids, about 35 mg/ml (Iborra et al., 2004). Mitochondrial nucleoids are spaced more uniformly than would be expected by random distribution. This uniformity likely results from inability of nucleoids to diffuse freely due to their anchoring in the mitochondrial inner membrane. Nucleoids are found in close association with both microtubules and with KIF5B, a kinesin motor responsible for the movement of mitochondria along microtubules. (Iborra et al., 2004). Subsequent studies refined this model, and now mitochondrial nucleoids are viewed as layered structures consisting of a core, where replication and transcription of mtDNA occur, and peripheral regions, where translation of mitochondrial transcripts and assembly of newly synthesized polypeptides into respiratory complexes occurs (Bogenhagen, D.F. et al., 2008).

3. Maintenance of mtDNA

Normal functioning of the cell and organism critically depends upon proper maintenance of mtDNA integrity and copy number. This is achieved through intricate coordination of the processes of mtDNA replication, repair, and degradation (turnover). Below, we will review each of these processes in some detail.

3.1 mtDNA replication

It is generally accepted that replication of mtDNA is not linked to the cell cycle as strictly as replication of nDNA is. In fact, mtDNA replication occurs in all stages of the cell cycle and

persists even in nondividing cells (Bogenhagen, D. & Clayton, 1977; Clayton, 1982). DNA polymerase γ (Pol γ) is the sole DNA polymerase identified in mitochondria. This enzyme is heterotrimeric and consists of a single 140 kDa catalytic subunit encoded by the POLG gene and two 55 kDa accessory subunits, encoded by POLG2. As the only DNA polymerase found in mitochondria, Pol γ is responsible for both replication and repair of mtDNA. Several other proteins play prominent roles in the mtDNA replication process. These are the DNA helicase Twinkle, a mitochondrial single-strand-binding protein (mtSSB), which mediates unwinding of mtDNA through its physical interaction with Twinkle (St John et al., 2010), and a mitochondrial RNA polymerase, which generates primers for mtDNA replication with the assistance of mitochondrial transcription factors A (TFAM), B1 (TFB1M), and B2 (TFB2M). While the major players in mtDNA replication are fairly well known, the exact mechanism remains controversial (reviewed in (Holt, 2009)).

Electron microscopic observations of purified mtDNA molecules led to the adoption of the strand-displacement model (Robberson et al., 1972). In these experiments, the observation of extensive single-strand regions in mtDNA suggested that synthesis of the leading strand is uncoupled from that of the lagging strand. The leading strand synthesis is initiated at a fixed point and advances about two-thirds of the way around the mtDNA molecule before second strand synthesis is initiated (Holt, 2009). Recently, however, analysis of mtDNA replication intermediates in both mammalian tissues and cultured cells by two-dimensional agarose gel electrophoresis revealed the presence of products consistent with a strand-coupled mechanism of replication (Holt et al., 2000). Subsequently, it was found that RNA is incorporated throughout the lagging strand (RITOLS mechanism, (Yasukawa et al., 2006)). This raised the possibility that the abundant single-strand regions observed in the earlier studies could be an artifact of RNA loss during DNA isolation and processing, and suggested that strand-coupled and RITOLS could be the only two mechanisms involved in mtDNA replication, thus excluding the earlier strand-displacement mechanism (Yasukawa et al., 2006). RITOLS appears to be initiated at several sites in the D-loop and proceeds unidirectionally (Yasukawa et al., 2006), whereas initiation of strand-coupled replication occurs over a broad region and is bidirectional (Yasukawa et al., 2005). However, the observation of stable non-replicative DNA-RNA hybrid loops formed by some mitochondrial transcripts casts a shadow on the authenticity of RITOLS in favor of the original asynchronous strand-displacement mechanism (Brown, T.A. et al., 2008).

3.2 Damage and repair of mtDNA

Mitochondrial genomes accumulate mutations approximately one order of magnitude faster than nDNA (Brown, W.M. et al., 1979; Ballard & Whitlock, 2004; Tatarenkov & Avise, 2007). This could be caused by a variety of factors, including an intrinsically lower fidelity of replication by mitochondria-specific DNA polymerase γ (Pol γ), a lower efficiency of mtDNA repair, or chronic exposure of mtDNA to noxious factors, such as Reactive Oxygen Species (ROS) or environmental genotoxins. However, attempts to experimentally link mtDNA mutagenesis to exposure to carcinogens (Mita et al., 1988) or to reactive oxygen species (Shokolenko et al., 2009) proved unsuccessful, leading to the notion that mtDNA may be resistant to mutagenesis. To confound things even further, several studies have reported that nDNA is at least as sensitive to oxidative damage as mtDNA (Anson et al., 1999; Anson et al., 2000; Lim et al., 2005), which undermines the earlier notion that the higher susceptibility of mtDNA to damage by ROS is the driving force behind its higher rate of mutagenesis (Richter et al., 1988).

The current progress in our understanding of mtDNA repair pathways has been reviewed recently (Liu & Demple, 2010). Historically, the discovery that mitochondria are unable to repair ultraviolet (UV)-induced pyrimidine dimers (Clayton et al., 1974, 1975) and some types of alkylating damage (Miyaki et al., 1977), suggested that they may contain a reduced complement of DNA repair pathways. However, Anderson and Friedberg (Anderson & Friedberg, 1980) found uracil-DNA glycosylase activity in mitochondrial extracts, suggesting the presence of the base excision repair (BER) pathway. This was followed by a report of mitochondrial repair of O^6-ethyl-2'-deoxyguanosine (Myers et al., 1988; Satoh et al., 1988). This can be processed by direct reversal using O^6-methyl guanine methyl transferase or by a nucleotide excision repair pathway. Subsequently, repair of a variety of mtDNA lesions by BER, including those arising from oxidative damage, was demonstrated (Pettepher et al., 1991; LeDoux et al., 1992; Driggers et al., 1993). Recently, long-patch BER of oxidative DNA lesions (Akbari et al., 2008; Liu et al., 2008; Szczesny et al., 2008), and mismatch repair (de Souza-Pinto et al., 2009) have been reported in mammalian mitochondria. The presence in mammalian mitochondria of a DNA end binding activity (Coffey et al., 1999), and a ligase capable of joining both cohesive and blunt ends (Lakshmipathy & Campbell, 1999) suggested the presence of a non-homologous end joining pathway in mitochondria. Similarly, detection of recombination intermediates indicated that mtDNA can be repaired through a homologous recombination pathway (Kajander et al., 2001; Kraytsberg et al., 2004). This notion was further supported by experiments on the induction of mtDNA double-strand breaks (DSBs) in vivo with the help of mitochondrially-targeted restriction endonucleases. In these experiments, DSB repair was accompanied by the formation of mtDNA deletions, some of which had breakpoints flanked by direct repeats, thus implicating homologous recombination in the repair (Srivastava & Moraes, 2005; Fukui & Moraes, 2008). To summarize, current experimental evidence suggests the presence in mitochondria of all major DNA repair pathways, with the exception of the nucleotide excision repair. Moreover, mitochondria appear to possess a unique mechanism for the maintenance of DNA integrity through degradation of damaged molecules (see below). Importantly BER, which is responsible for the repair of oxidative base lesions, is robust in mitochondria, as evidenced by observation that repair of 8-oxodG, the most prominent oxidative base lesion, is more efficient in mitochondria than in the nucleus (Thorslund et al., 2002).

3.3 Degradation and maintenance of mtDNA integrity

Unlike the nuclear genome, the mitochondrial genome is redundant, consisting of hundreds to thousands of copies per cell. Therefore, a “repair or die” constraint is not imposed on mtDNA. Conceivably, a substantial fraction of damaged mtDNA can be lost without detrimental effects, provided that this loss is compensated for by replication of new genomes. In fact, the loss and resynthesis of mtDNA was observed more than 40 years ago by Gross and Rabinowitz, who described mtDNA turnover (Gross & Rabinowitz, 1969). Many cell lines are fairly tolerant to the loss of mtDNA, and can survive both a gradual loss of mtDNA through chronic treatment with ethidium bromide (King & Attardi, 1989), and acute destruction of a fraction (Alexeyev et al., 2008) or even all of their mtDNA (Kukat et al., 2008) by mitochondrially targeted restriction endonucleases. This is in a stark contrast to nDNA, in which persistent DSB can activate apoptosis. However, the hypothesis that turnover (degradation) of damaged mtDNA can be a mechanism used by mitochondria to deal with either excessive damage, or damage that can not be repaired did not take hold in

part due to the lack of direct experimental evidence supporting it and in part due to discovery of mitochondrial BER (Pettepher et al., 1991) , which shifted attention from unrepairable lesions to those that can be repaired. However, recent evidence reignited interest in mtDNA degradation.

Ethanol has been reported to induce mtDNA loss in yeast (Ibeas & Jimenez, 1997). In mice, intragastric administration of ethanol induced oxidative stress and was accompanied by a reversible loss of mtDNA (Mansouri et al., 1999). The loss of mtDNA was approximately 50% in all organs studied. It could be partially prevented by the antioxidants melatonin, vitamin E and coenzymeQ, and was followed by adaptive mtDNA resynthesis (Mansouri et al., 2001). Lipopolysaccharide, a known inducer of in vivo oxidative stress also induced, mtDNA depletion (Suliman et al., 2003). Angiotensin II induced mitochondrial ROS production and decreased skeletal muscle mtDNA content in mice (Mitsuishi et al., 2008). Degradation of mtDNA was observed in the rat model of cerebral ischemia/reperfusion (Chen et al., 2001). Similar to mtDNA depletion induced by intragastric ethanol administration, mtDNA levels returned to normal within 24h of cerebral ischemia/reperfusion (Chen et al., 2001). Finally, H_2O_2-induced oxidative stress in hamster fibroblasts was accompanied by Ca^{2+}-dependent degradation of mtDNA (Crawford et al., 1998). Taken together, these findings strongly suggested a link between oxidative stress (which may result in oxidative mtDNA damage) and mtDNA degradation, yet they stopped short of invoking degradation as protective mechanism. In an unrelated study, it was observed that mtDNA is resistant to mutagenesis induced by alkylating agents, and the authors suggested degradation of damaged mtDNA as one of the potential mechanisms for this resistance (Mita et al., 1988). However, mtDNA degradation under the experimental conditions of that study was not demonstrated (Mita et al., 1988).

Recently, we attempted to study the relationship between experimentally induced oxidative stress and mtDNA mutagenesis. In initial experiments, superoxide radicals were generated on the matrix side of the mitochondrial inner membrane by treating cells with sublethal concentrations of the complex I inhibitor rotenone (St-Pierre et al., 2002; Muller et al., 2004). However, exposing human colon carcinoma cells or mouse embryonic fibroblasts to rotenone for 30 days did not result in a significant increase in the rate of mtDNA mutagenesis (Shokolenko et al., 2009). Similarly, repeated treatment of HCT116 colon cancer cells with H_2O_2 failed to induce significant mtDNA mutagenesis. Instead, DNA lesions that manifest themselves as strands breaks under denaturing conditions (single-strand breaks (SSBs) and DSBs, abasic sites, etc.) prevailed over premutagenic base modifications by a factor of 10. Consistent with the hypothesis that unrepairable mtDNA molecules are degraded, treatment of cells with an inhibitor of BER methoxyamine, enhanced mtDNA degradation in response to both oxidative and alkylating damage (Shokolenko et al., 2009). The elimination of damaged mtDNA was preceded by the accumulation of linear mtDNA molecules, which may represent degradation intermediates, since, unlike undamaged circular molecules, they are susceptible to exonucleolytic degradation.

The high rate of lesions (mostly, SSBs and abasic sites) in mtDNA induced by ROS suggests a mechanism by which mitochondria may maintain the integrity of their genetic information. In this model, oxidative stress induces in mtDNA lesions with a much higher (by the factor of 10, (Shokolenko et al., 2009)) frequency than mutagenic lesions. These lesions represent a block to transcription and replication of mtDNA, and when accumulated above a threshold level, they induce degradation of mtDNA molecule. Therefore,

degradation of mtDNA molecule is triggered before it accumulates mutagenic lesions. This model provides a mechanistic explanation for the observations made by Suter and Richter (Suter & Richter, 1999), who found that the 8-oxodG content of circular mtDNA is low and does not increase in response to oxidative insult. However, fragmented mtDNA had a very high 8-oxodG content, which increased further after oxidative stress. It incorporates the previously suggested notion of a possible contribution of APE1 to mtDNA degradation (Tomkinson et al., 1988; Tomkinson et al., 1990). The model is consistent with the observations of Yakes and van Houten (Yakes & Van Houten, 1997), who found that oxidative stress promoted a higher incidence of polymerase-blocking strand breaks and abasic sites in mtDNA than in nDNA. Recent studies using qPCR for the analysis of mtDNA provide further support for the notion of mtDNA degradation in response to oxidative stress (Rothfuss et al., 2010). Therefore, degradation of severely damaged mtDNA emerges as a unique, mitochondria-specific mechanism for the maintenance of DNA integrity.

Degradation of damaged organellar DNA appears not to be unique to mammalian cells. Known examples of rapid organellar DNA turnover in plants and protists in response to ROS were reviewed recently by Bendich (Bendich, 2010).

3.4 Degradation and maintenance of mtDNA copy number

In most mammalian cells, mtDNA copy number is kept relatively constant at 1,000-10,000 copies per cell, depending on the cell type and physiological conditions (Copeland, 2008). However, antiretroviral therapy (Arnaudo et al., 1991) and genetic defects in the components of the mtDNA replicating machinery (Rotig & Poulton, 2009) were demonstrated to induce a pathologic decrease in mtDNA content of the cell. Also, mtDNA copy number can be decreased in response to increased mtDNA damage, which is not met with a corresponding increase in repair (Shokolenko et al., 2009). For patients with genetic mitochondrial DNA depletion syndromes (MDS), there is no treatment other than supportive therapy (Poulton & Holt, 2009). Liver transplantation proved inefficient in two major forms of MDS associated with liver failure: Alpers-Huttenlocher syndrome and deoxyguanosine kinase (DGUOK) deficiency. In the former instance failure to achieve a therapeutic effect appears to be linked to the inevitable brain involvement, which may not be apparent until after the transplantation. Attempts to correct the hepatocerebral syndrome resulting from DGUOK deficiency through liver transplantation were reviewed recently (Rahman & Poulton, 2009). Infant death was observed in 6 out of the 9 cases reviewed.

Since mtDNA copy number is maintained through an intricate coordination between two opposing processes, mtDNA synthesis and mtDNA degradation, we suggest that MDS should not be viewed merely as diseases of reduced mtDNA synthesis but rather as diseases of imbalance between synthesis and degradation of mtDNA. This view allows for a new, so far unexplored treatment strategy, i.e. inhibition of mtDNA degradation. Indeed, suppressed mtDNA replication due to mutations in Pol γ (patients with Alpers-Huttenlocher syndrome), Twinkle helicase (patients with progressive external ophtalmoplegia), or due to ingestion of nucleotide reverse transcriptase inhibitors (AIDS patients) results in the establishment of a new, lower cellular mtDNA content, which is characterized by reduced rates of both mtDNA synthesis and degradation. Conversely, suppression of mtDNA degradation should lead to a new steady state with increased mtDNA content, and therefore could be therapeutic in patients with MDS.

4. Experimental approaches

4.1 Quantitative southern blotting

Southern Blot analysis can be used for the quantitation of various types of damage to mtDNA. This method is based on the detection of strand breaks within linearized mtDNA. Strand breaks can be generated either directly by noxious agents (e.g., by alkylating compounds or oxidative stress), or indirectly, after the treatment of damaged DNA with lesion-specific glycosylases, which remove damaged bases thus creating abasic sites. Examples of glycosylases widely used for this purpose include E. *coli* DNA-repair enzymes formamido-pyrimidine-DNA-glycosylase (FPG, recognizes oxidized purines) and endonuclease III (EndoIII, recognizes oxidized pyrimidines). Both enzymes are bifunctional glycosylases, i.e. they both remove damaged bases and incise the resulting abasic sites thus creating SSBs. Under alkaline conditions, the mtDNA strands separate and fragment at nicks resulting in a decreased hybridization signal from the treated (damaged) mtDNA (LeDoux et al., 1999). The membrane is exposed to an imaging screen, and the fraction of mtDNA remaining intact is calculated. This fraction can be used to calculate the lesion (break) frequency per length of intact fragment detected by hybridization using the formula:

$$BF = - \ln(Treated/Control) \quad (1)$$

In other words, mtDNA break frequency (BF) in treated samples equals the negative natural logarithm of the ratio of mtDNA band intensities in treated and control samples.
Several important caveats have to be noted in relation to this technique:

1. Prior to analysis, circular mtDNA is linearized by digestion with restriction endonuclease.
2. The technique relies on measuring mtDNA band intensities in treated vs. control samples. Therefore, loading equal amounts of total DNA per well of the gel, which depends on accurate DNA quantitation is very important. Since nDNA shows much lower sensitivity to oxidative damage than mtDNA, hybridization of the membrane to nDNA probe in addition to mtDNA probe can be used in addition to visual inspection of ethidium bromide stained gels as loading control when studying oxidative mtDNA damage. However, hybridization to a nDNA probe is not useful as a loading control when studying, certain types of alkylating DNA damage, when the difference in the damage of nuclear and mitochondrial genomes is not as dramatic.
3. Isolation of mtDNA is impractical and is associated with the introduction of artifacts. Therefore, in this technique total cellular DNA is subjected to Southern hybridization. The use of a mtDNA-specific hybridization probe allows one to study only changes in mtDNA integrity. In a typical cell type studied by this technique, mtDNA constitutes only about 1-2% of total DNA.
4. Quantitative Southern Blotting under denaturing (alkaline) conditions, by itself, does not discriminate between SSBs and DSBs. Therefore mtDNA containing DSBs, which repair inefficiently and therefore lead to mtDNA loss (Kukat et al., 2008), will appear the same as SSBs, which repair much better (Fig. 2, Mix 1 vs. Mix 2, left side). To discriminate between SSBs (repairable mtDNA damage) and DSBs (mtDNA degradation) we introduced an approach that involves running the same DNA samples under both alkaline and neutral conditions (Shokolenko et al., 2009). Samples containing DSBs appear the same under both conditions (Fig. 2, Mix 2, left side vs. right side). In contrast, mtDNA containing SSBs appears like mtDNA containing DSBs under

denaturing conditions, but under non-denaturing (neutral) conditions it behaves like undamaged control DNA (Fig. 2, Mix 1, left side vs. right side).

Specific types of DNA damage can be detected as follows:

1. DSBs convert circular mtDNA into a linear molecule. Therefore, qualitative detection of DSBs can be performed by Southern Blotting of total cellular DNA samples under non-denaturing conditions using linearised mtDNA as a standard. The increase in the signal corresponding to linear mtDNA is interpreted as a result of DSB. It is helpful to digest total DNA with a restriction enzyme that does not cut mtDNA (e.g., BglII for human DNA). In our experience, failure to perform this step results in an absence or in a severe reduction of the hybridization signal. However, the method is not quantitative for two reasons: a) DSB repair in mtDNA is inefficient, and most linear mtDNA is degraded fairly quickly (Shokolenko et al., 2009), and b) mtDNA can concatenate, at least in some cell lines (Bedoya et al., 2009), and electrophoretic mobility of linear concatemers is distinct from that of linear mtDNA monomers.
2. SSBs can be quantified as a difference in break frequencies detected using Southern Blotting under alkaline and neutral conditions (Fig. 2). Alternatively, it can be calculated as break frequency in sample ran under the alkaline conditions using the same sample ran under neutral conditions as a control.
3. Abasic sites. This type of lesion can be quantified as a difference in break frequency in two identical aliquots of the sample ran under alkaline conditions if one aliquot has been treated with methoxyamine prior to electrophoresis. Under alkaline conditions, abasic sites are converted into strand breaks through the process of beta-elimination. Modification of abasic sites with methoxyamine renders them alkali-resistant (Liuzzi & Talpaert-Borle, 1985; Scicchitano & Hanawalt, 1989). Alternatively, abasic sites can be quantified by comparing aliquots of methoxyamine-treated DNA run under the alkaline conditions after treatment with APE1 (control) and EndoIII (experimental). Methoxyamine-modified abasic sites are resistant to hydrolysis by APE1, but not by endoIII (Rosa et al., 1991)
4. Base modifications can be quantified using lesion-specific DNA glycosylases. One aliquot of DNA sample is treated with lesion-specific DNA glycosylase, whereas a second aliquot is left untreated. Monofunctional DNA glycosylases (e.g., uracil DNA glycosylase or methylpurine DNA glycosylase) convert a lesion into an abasic site, which can be converted into a strand break under the alkaline conditions thus allowing for the quantitation by comparing hybridization signals obrained from enzyme-treated vs. untreated controls. As indicated above, bifunctional DNA glycosylases, such as FPG or Endo III, will convert a lesion into a strand break allowing for quantitation using the same approach.

The advantages of Quantitative Southern Blotting include its robustness due to reliance on physical interactions rather than on enzymatic reactions and its ability to quantify some lesions (e.g., abasic sites), which can not be quantified by PCR-based techniques (see below). The disadvantages include the fact that the procedure involves multiple steps, is time-consuming, and requires relatively large quantities (1μg or more) of starting DNA.

4.2 Quantitative PCR

An alternative approach for the detection of DNA damage was developed by Govan (Govan et al., 1990) and modified by Yakes and van Houten for studies with mtDNA. This method, QPCR (a.k.a. QXL-PCR), is predicated upon the ability of the lesions present in mtDNA to block the progression of a thermostable DNA polymerase, resulting in a decrease of DNA

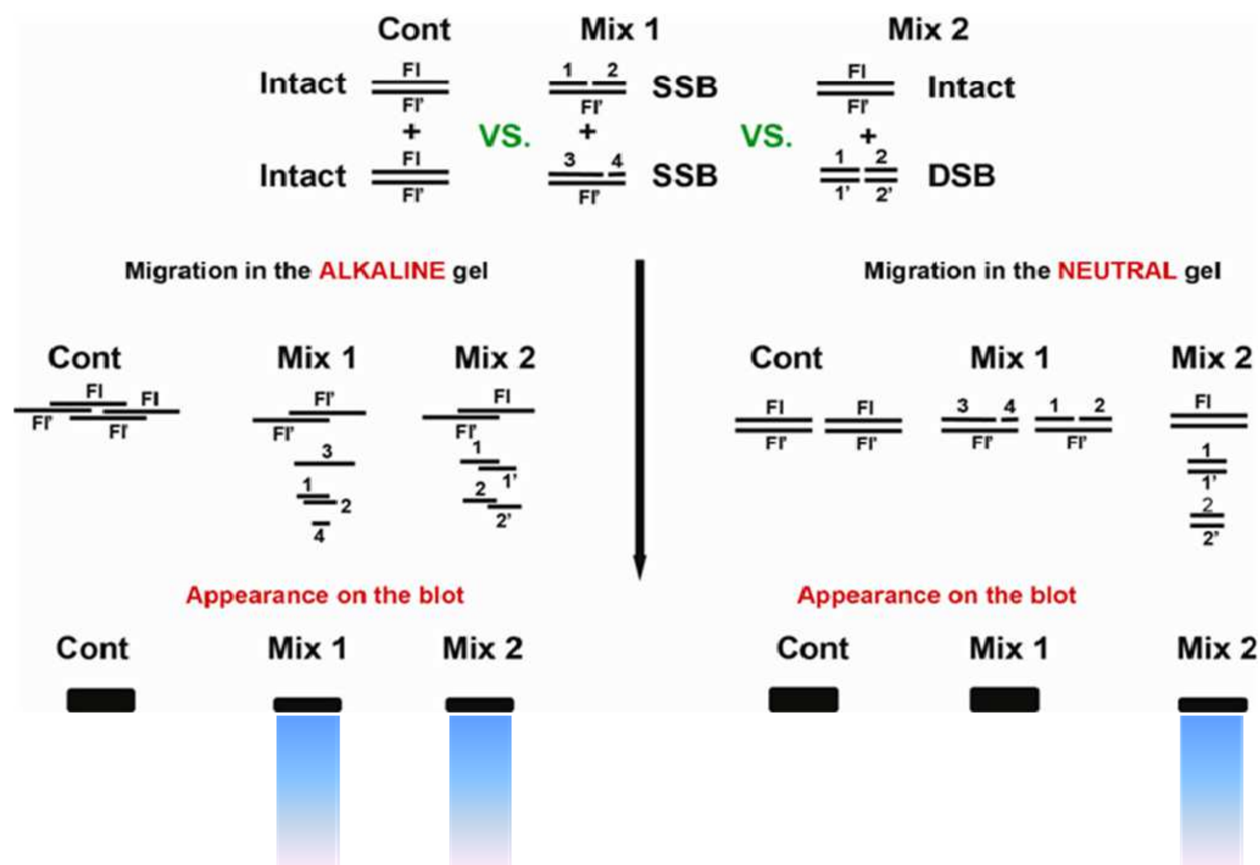

Fig. 2. Analysis of mtDNA damage by quantitative Southern Blotting under denaturing (alkaline) and non-denaturing (neutral) conditions. Behavior of the mtDNA samples that contain either no damage (Cont), SSBs (Mix 1), or a mixture of intact mtDNA and mtDNA containing DSBs (Mix 2) is presented schematically. Under the denaturing conditions (left side of the figure), mtDNA strands separate, and strands containing lesions in the form of SSBs, DSBs, or abasic sites fragment. The resulting fragments migrate faster than intact full-length (Fl) mtDNA strands in the agarose gel thus creating smears (Mix1 and Mix 2, left side). Under conditions depicted in this scheme, the intensity of the Southern Blot signal corresponding to intact mtDNA fragment from Mix 1 equals that of Mix 2, and represents half of the signal strength produced by undamaged control. When the same samples are analyzed under the non-denaturing conditions (right side of the figure), mtDNA fragmentation in Mix 1 containing SSBs does not occur. In contrast, mtDNA in Mix 2 containing DSBs fragments create a smear. As a result, the signal intensity for intact mtDNA in the Mix 1 under non-denaturing conditions is twice as high as that in the Mix 2. The arrow indicates the direction of electrophoresis; Fl′, full-length mtDNA strand complementary to Fl strand; 1, 2, 3, and 4 in Mix 1, subfragments into which Fl strand containing a lesion fragments; 1, 1′, 2, and 2′ in Mix 2, direct and complimentary strands of the subfragments resulting from a DSB in the Fl fragment.

amplification in the damaged template, when compared to undamaged control (Yakes & Van Houten, 1997). Similar to quantitative Southern Blotting, QPCR measures the fraction of undamaged amplifiable template, which decreases with increased number of lesions.

Successful outcome of experiments with either quantitative Southern Blot or QPCR is heavily dependent upon the ability to accurately measure the amount of DNA used.

Spectrophotometric methods (A_{260}) appear to be inappropriate for this purpose because of the intrinsic difficulties associated with controlling the quantity and spectrum of contaminants in DNA preparations. Fluorescense based methods (PicoGreen and Hoechst 33258 dyes), unlike spectrophotometric techniques, show little sensitivity to such contaminants as proteins, single-stranded DNA, RNA etc., which are common to genomic DNA preparations and therefore are deemed the methods of choice. Also, when using QPCR, one has to control for changes in the mtDNA copy number. Indeed, a reduction in mtDNA copy number will manifest itself as DNA damage because of the reduction in the number of amplifiable mtDNA genomes in the template. This can be controlled for by amplifying of a short (about 300bp) fragment of mtDNA-encoded gene. The rationale is that encountering DNA damage in such a short fragment is an event with a very low probability and therefore profiles of amplification of such a fragment should be essentially identical between damaged and undamaged DNA. Therefore, variations in the degree of amplification of the small fragment are assumed to be the result of fluctuations in mtDNA copy number and the results of small fragment amplification are used for the normalization of the data obtained for the large (16 kb) mtDNA fragments.
The success of the QPCR approach requires the measurements be made within the linear range of amplification. This requires optimization to find the optimal starting concentration of DNA template (Yakes & Van Houten, 1997). Alternatively, one can identify the range for linear amplification. However, both approaches require a significant amount of optimization. Recently, a real-time PCR approach has been extended to QPCR resulting in the development of the long-range PCR technique (LRPCR, (Edwards, 2008)). Two significant problems had to be addressed in the process: (1) the low processivity and polymerization rates of the DNA polymerases used in comparison to the length of the amplicons, (2) SYBR green inhibition of DNA amplification (Gudnason et al., 2007). In comparison to the earlier semi-quantitative protocols this represents a significant improvement in both the ease of data acquisition and the precision for quantification of mtDNA damage (Edwards, 2008). The most recent variation of the technique, the semi-long run real-time (SLR rt-) PCR method, further simplifies the procedure by amplifying relatively short mtDNA fragments using real-time PCR (qPCR) reagents and instruments (Rothfuss et al., 2010). In this procedure, the reduced length of amplified products enables the use of standard qPCR kits. The flip side of this improvement is the reduced sensitivity of the technique, which is directly related to the length of amplified fragments. Therefore, applicability of this technique for reliable detection of physiological (low) levels of mtDNA damage requires independent validation and is likely to strongly depend upon the instrument used. Indeed, a simple calculation shows that a fairly high level of mtDNA damage of 1 lesion/mtDNA molecule (16.5 kbp) translates into 0.061 lesion per 1 kbp fragment amplified in this method. Using "zero class" Poisson distribution used for the analysis of this type of DNA damage

$$D=-\ln(A_D/A_C) \tag{2}$$

where D= lesion frequency per length of amplified fragment (1kbp), ln is natural logarithm, A_D is amplification of the damaged DNA sample, and A_C is amplification of the control sample) we arrive at the A_D/A_C =0.94. The corresponding shift in the threshold cycle (ΔC_t, derived from the readout of the qPCR instrument) is 0.089. Therefore, a significant mtDNA damage of 1 lesion per mtDNA molecule results in less than a 0.1 threshold cycle shift between amplification curves of treated and untreated samples. This places a very high demand on the

instrument's ability to reproducibly amplify different samples. In our experience, a PCR block that allows for greater than 0.7 C_t spread between identical samples still conforms to the standards of the two major manufacturers of qPCR instruments. In this case, the instrument's well-to-well variability exceeds the measured differences by a factor of 7.

The strength of PCR-based techniques for the analysis of mtDNA damage is in the ability to work with very low starting quantities of DNA. This strength is turned into a weakness when relevant methodological precautions, such as the availability of distinct, dedicated workstations, for different steps of the procedure in physically separate laboratories (Santos et al., 2006) are considered. Another weakness of this approach is that it provides even less information about the nature of DNA damage than Quantitative Southern Blotting. E.g., abasic sites can be quantitated by Quantitative Southern Blotting under alkaline conditions by comparing lesion frequencies in DNA modified with methoxyamine vs. unmodified DNA. Methoxyamine modification protects abasic sites from being converted into strand breaks through beta-elimination under alkaline conditions. In contrast, native abasic sites, methoxyamine-modified abasic sites, and abasic sites converted into strand breaks through beta-elimination all will prevent copying by the DNA-polymerase in PCR-based techniques and therefore will be indistinguishable. Nevertheless, these techniques are the only ones available for analysis of mtDNA damage and repair when amount of the starting material is limited.

5. Conclusion

mtDNA integrity and appropriate copy number appear to be crucial for normal functioning of the cell. Therefore, understanding the processes that govern mtDNA replication, repair and degradation is of critical importance for our ability to prevent and/or clinically intervene in pathological processes associated with mutations in mtDNA and mtDNA depletion. Degradation of mtDNA is now emerging as a promising therapeutic target in the treatment of congenital mtDNA depletion syndromes and mtDNA depletion induced by antiretroviral therapy. However, the molecular identity of the nuclease involved in mtDNA degradation remains enigmatic. Future research will shed light on this and other remaining mysteries of mtDNA biology.

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7. References

Akbari, M., T. Visnes, et al. (2008). Mitochondrial base excision repair of uracil and AP sites takes place by single-nucleotide insertion and long-patch DNA synthesis. *DNA Repair (Amst),* 7, 4, pp. 605-616, 1568-7864 (Print).

Alam, T. I., T. Kanki, et al. (2003). Human mitochondrial DNA is packaged with TFAM. *Nucleic Acids Res,* 31, 6, pp. 1640-1645,

Alexeyev, M. F., N. Venediktova, et al. (2008). Selective elimination of mutant mitochondrial genomes as therapeutic strategy for the treatment of NARP and MILS syndromes. *Gene Ther,* 15, 7, pp. 516-523, 1476-5462 (Electronic).

Anderson, C. T. and E. C. Friedberg (1980). The presence of nuclear and mitochondrial uracil-DNA glycosylase in extracts of human KB cells. *Nucleic Acids Res,* 8, 4, pp. 875-888, 0305-1048 (Print).

Anson, R. M., E. Hudson, et al. (2000). Mitochondrial endogenous oxidative damage has been overestimated. *Faseb J,* 14, 2, pp. 355-360,

Anson, R. M., S. Senturker, et al. (1999). Measurement of oxidatively induced base lesions in liver from Wistar rats of different ages. *Free Radic Biol Med,* 27, 3-4, pp. 456-462, 0891-5849 (Print).

Arnaudo, E., M. Dalakas, et al. (1991). Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet,* 337, 8740, pp. 508-510, 0140-6736 (Print) 0140-6736 (Linking).

Ballard, J. W. and M. C. Whitlock (2004). The incomplete natural history of mitochondria. *Mol Ecol,* 13, 4, pp. 729-744, 0962-1083 (Print).

Bedoya, F., M. M. Medveczky, et al. (2009). Identification of mitochondrial genome concatemers in AIDS-associated lymphomas and lymphoid cell lines. *Leuk Res*, 1873-5835 (Electronic).

Bendich, A. J. (2010). Mitochondrial DNA, chloroplast DNA and the origins of development in eukaryotic organisms. *Biol Direct,* 5, pp. 42, 1745-6150 (Electronic) 1745-6150 (Linking).

Bogenhagen, D. and D. A. Clayton (1977). Mouse L cell mitochondrial DNA molecules are selected randomly for replication throughout the cell cycle. *Cell,* 11, 4, pp. 719-727, 0092-8674 (Print) 0092-8674 (Linking).

Bogenhagen, D. F., D. Rousseau, et al. (2008). The layered structure of human mitochondrial DNA nucleoids. *J Biol Chem,* 283, 6, pp. 3665-3675, 0021-9258 (Print).

Brown, T. A., A. N. Tkachuk, et al. (2008). Native R-loops persist throughout the mouse mitochondrial DNA genome. *J Biol Chem*, 0021-9258 (Print).

Brown, W. M., M. George, Jr., et al. (1979). Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci U S A,* 76, 4, pp. 1967-1971, 0027-8424 (Print).

Cavelier, L., A. Johannisson, et al. (2000). Analysis of mtDNA copy number and composition of single mitochondrial particles using flow cytometry and PCR. *Exp Cell Res,* 259, 1, pp. 79-85,

Chen, H., C. J. Hu, et al. (2001). Reduction and restoration of mitochondrial dna content after focal cerebral ischemia/reperfusion. *Stroke,* 32, 10, pp. 2382-2387, 1524-4628 (Electronic) 0039-2499 (Linking).

Clay Montier, L. L., J. J. Deng, et al. (2009). Number matters: control of mammalian mitochondrial DNA copy number. *J Genet Genomics,* 36, 3, pp. 125-131, 1673-8527 (Print) 1673-8527 (Linking).

Clayton, D. A. (1982). Replication of animal mitochondrial DNA. *Cell,* 28, 4, pp. 693-705, 0092-8674 (Print) 0092-8674 (Linking).

Clayton, D. A., J. N. Doda, et al. (1974). The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc Natl Acad Sci U S A,* 71, 7, pp. 2777-2781,

Clayton, D. A., J. N. Doda, et al. (1975). Absence of a pyrimidine dimer repair mechanism for mitochondrial DNA in mouse and human cells. *Basic Life Sci,* 5B, pp. 589-591,

Coffey, G., U. Lakshmipathy, et al. (1999). Mammalian mitochondrial extracts possess DNA end-binding activity. *Nucleic Acids Res,* 27, 16, pp. 3348-3354, 1362-4962 (Electronic).

Copeland, W. C. (2008). Inherited mitochondrial diseases of DNA replication. *Annu Rev Med,* 59, pp. 131-146, 0066-4219 (Print) 0066-4219 (Linking).

Crawford, D. R., N. E. Abramova, et al. (1998). Oxidative stress causes a general, calcium-dependent degradation of mitochondrial polynucleotides. *Free Radic Biol Med,* 25, 9, pp. 1106-1111, 0891-5849 (Print).

de Souza-Pinto, N. C., P. A. Mason, et al. (2009). Novel DNA mismatch-repair activity involving YB-1 in human mitochondria. *DNA Repair (Amst),* 8, 6, pp. 704-719, 1568-7864 (Print).

Driggers, W. J., S. P. LeDoux, et al. (1993). Repair of oxidative damage within the mitochondrial DNA of RINr 38 cells. *J Biol Chem,* 268, 29, pp. 22042-22045,

Edwards, J. G. (2008). Quantification of mitochondrial DNA (mtDNA) damage and error rates by real-time QPCR. *Mitochondrion*, 1567-7249 (Print).

Fukui, H. and C. T. Moraes (2008). Mechanisms of formation and accumulation of mitochondrial DNA deletions in aging neurons. *Hum Mol Genet*, 1460-2083 (Electronic).

Govan, H. L., 3rd, Y. Valles-Ayoub, et al. (1990). Fine-mapping of DNA damage and repair in specific genomic segments. *Nucleic Acids Res,* 18, 13, pp. 3823-3830, 0305-1048 (Print) 0305-1048 (Linking).

Gross, N. J. and M. Rabinowitz (1969). Synthesis of new strands of mitochondrial and nuclear deoxyribonucleic acid by semiconservative replication. *J Biol Chem,* 244, 6, pp. 1563-1566,

Gudnason, H., M. Dufva, et al. (2007). Comparison of multiple DNA dyes for real-time PCR: effects of dye concentration and sequence composition on DNA amplification and melting temperature. *Nucleic Acids Res,* 35, 19, pp. e127, 1362-4962 (Electronic) 0305-1048 (Linking).

Guliaeva, N. A., E. A. Kuznetsova, et al. (2006). [Proteins associated with mitochondrial DNA protect it against the action of X-rays and hydrogen peroxide]. *Biofizika,* 51, 4, pp. 692-697,

Holt, I. J. (2009). Mitochondrial DNA replication and repair: all a flap. *Trends Biochem Sci,* 34, 7, pp. 358-365, 0968-0004 (Print).

Holt, I. J., A. E. Harding, et al. (1988). Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. *Nature,* 331, 6158, pp. 717-719,

Holt, I. J., H. E. Lorimer, et al. (2000). Coupled leading- and lagging-strand synthesis of mammalian mitochondrial DNA. *Cell,* 100, 5, pp. 515-524, 0092-8674 (Print) 0092-8674 (Linking).

Ibeas, J. I. and J. Jimenez (1997). Mitochondrial DNA loss caused by ethanol in Saccharomyces flor yeasts. *Appl Environ Microbiol,* 63, 1, pp. 7-12, 0099-2240 (Print).

Iborra, F. J., H. Kimura, et al. (2004). The functional organization of mitochondrial genomes in human cells. *BMC Biol,* 2, 1, pp. 9,

Kajander, O. A., P. J. Karhunen, et al. (2001). Prominent mitochondrial DNA recombination intermediates in human heart muscle. *EMBO Rep,* 2, 11, pp. 1007-1012, 1469-221X (Print).

King, M. P. and G. Attardi (1989). Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science,* 246, 4929, pp. 500-503, 0036-8075 (Print).

Kraytsberg, Y., M. Schwartz, et al. (2004). Recombination of human mitochondrial DNA. *Science,* 304, 5673, pp. 981,

Kukat, A., C. Kukat, et al. (2008). Generation of {rho}0 cells utilizing a mitochondrially targeted restriction endonuclease and comparative analyses. *Nucleic Acids Res*, 1362-4962 (Electronic).

Lakshmipathy, U. and C. Campbell (1999). Double strand break rejoining by mammalian mitochondrial extracts. *Nucleic Acids Res,* 27, 4, pp. 1198-1204, 0305-1048 (Print).

LeDoux, S. P., W. J. Driggers, et al. (1999). Repair of alkylation and oxidative damage in mitochondrial DNA. *Mutat Res,* 434, 3, pp. 149-159,

LeDoux, S. P., G. L. Wilson, et al. (1992). Repair of mitochondrial DNA after various types of DNA damage in Chinese hamster ovary cells. *Carcinogenesis,* 13, 11, pp. 1967-1973, 0143-3334 (Print).

Lestienne, P. and G. Ponsot (1988). Kearns-Sayre syndrome with muscle mitochondrial DNA deletion. *Lancet,* 1, 8590, pp. 885,

Liang, Q. and P. C. Dedon (2001). Cu(II)/H2O2-induced DNA damage is enhanced by packaging of DNA as a nucleosome. *Chem Res Toxicol,* 14, 4, pp. 416-422,

Liang, R., S. Senturker, et al. (1999). Effects of Ni(II) and Cu(II) on DNA interaction with the N-terminal sequence of human protamine P2: enhancement of binding and mediation of oxidative DNA strand scission and base damage. *Carcinogenesis,* 20, 5, pp. 893-898,

Lim, K. S., K. Jeyaseelan, et al. (2005). Oxidative damage in mitochondrial DNA is not extensive. *Ann N Y Acad Sci,* 1042, pp. 210-220,

Liu, P. and B. Demple (2010). DNA repair in mammalian mitochondria: Much more than we thought? *Environ Mol Mutagen,* 51, 5, pp. 417-426, 1098-2280 (Electronic) 0893-6692 (Linking).

Liu, P., L. Qian, et al. (2008). Removal of Oxidative DNA Damage via FEN1-Dependent Long-Patch Base Excision Repair in Human Cell Mitochondria. *Mol Cell Biol*, 1098-5549 (Electronic).

Liuzzi, M. and M. Talpaert-Borle (1985). A new approach to the study of the base-excision repair pathway using methoxyamine. *J Biol Chem,* 260, 9, pp. 5252-5258, 0021-9258 (Print).

Mansouri, A., C. Demeilliers, et al. (2001). Acute ethanol administration oxidatively damages and depletes mitochondrial dna in mouse liver, brain, heart, and skeletal muscles: protective effects of antioxidants. *J Pharmacol Exp Ther,* 298, 2, pp. 737-743, 0022-3565 (Print).

Mansouri, A., I. Gaou, et al. (1999). An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice. *Gastroenterology,* 117, 1, pp. 181-190, 0016-5085 (Print).

Mita, S., R. J. Monnat, Jr., et al. (1988). Resistance of HeLa cell mitochondrial DNA to mutagenesis by chemical carcinogens. *Cancer Res,* 48, 16, pp. 4578-4583,

Mitsuishi, M., K. Miyashita, et al. (2008). Angiotensin II Reduces Mitochondrial Content in Skeletal Muscle and Affects Glycemic Control. *Diabetes*, 1939-327X (Electronic).

Miyaki, M., K. Yatagai, et al. (1977). Strand breaks of mammalian mitochondrial DNA induced by carcinogens. *Chem Biol Interact,* 17, 3, pp. 321-329,

Muller, F. L., Y. Liu, et al. (2004). Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J Biol Chem,* 279, 47, pp. 49064-49073,

Myers, K. A., R. Saffhill, et al. (1988). Repair of alkylated purines in the hepatic DNA of mitochondria and nuclei in the rat. *Carcinogenesis,* 9, 2, pp. 285-292,

Noll, T., P. Wissemann, et al. (1990). Hypoxia tolerance of coronary endothelial cells. *Adv Exp Med Biol,* 277, pp. 467-476, 0065-2598 (Print) 0065-2598 (Linking).

Ojala, D., J. Montoya, et al. (1981). tRNA punctuation model of RNA processing in human mitochondria. *Nature,* 290, 5806, pp. 470-474,

Pettepher, C. C., S. P. LeDoux, et al. (1991). Repair of alkali-labile sites within the mitochondrial DNA of RINr 38 cells after exposure to the nitrosourea streptozotocin. *J Biol Chem,* 266, 5, pp. 3113-3117,

Poulton, J. and I. J. Holt (2009). 163rd ENMC International Workshop: nucleoid and nucleotide biology in syndromes of mitochondrial DNA depletion myopathy 12-14 December 2008, Naarden, The Netherlands. *Neuromuscul Disord,* 19, 6, pp. 439-443, 1873-2364 (Electronic) 0960-8966 (Linking).

Rahman, S. and J. Poulton (2009). Diagnosis of mitochondrial DNA depletion syndromes. *Arch Dis Child,* 94, 1, pp. 3-5, 1468-2044 (Electronic) 0003-9888 (Linking).

Rebelo, A. P., S. L. Williams, et al. (2009). In vivo methylation of mtDNA reveals the dynamics of protein-mtDNA interactions. *Nucleic Acids Res*, 1362-4962 (Electronic).

Richter, C., J. W. Park, et al. (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A,* 85, 17, pp. 6465-6467,

Robberson, D. L., H. Kasamatsu, et al. (1972). Replication of mitochondrial DNA. Circular replicative intermediates in mouse L cells. *Proc Natl Acad Sci U S A,* 69, 3, pp. 737-741, 0027-8424 (Print) 0027-8424 (Linking).

Rosa, S., P. Fortini, et al. (1991). Processing in vitro of an abasic site reacted with methoxyamine: a new assay for the detection of abasic sites formed in vivo. *Nucleic Acids Res,* 19, 20, pp. 5569-5574, 0305-1048 (Print).

Rothfuss, O., T. Gasser, et al. (2010). Analysis of differential DNA damage in the mitochondrial genome employing a semi-long run real-time PCR approach. *Nucleic Acids Res,* 38, 4, pp. e24, 1362-4962 (Electronic) 0305-1048 (Linking).

Rotig, A. and J. Poulton (2009). Genetic causes of mitochondrial DNA depletion in humans. *Biochim Biophys Acta,* 1792, 12, pp. 1103-1108, 0006-3002 (Print) 0006-3002 (Linking).

Santos, J. H., J. N. Meyer, et al. (2006). Quantitative PCR-based measurement of nuclear and mitochondrial DNA damage and repair in mammalian cells. *Methods Mol Biol,* 314, pp. 183-199, 1064-3745 (Print) 1064-3745 (Linking).

Satoh, M. S., N. Huh, et al. (1988). Enzymatic removal of O6-ethylguanine from mitochondrial DNA in rat tissues exposed to N-ethyl-N-nitrosourea in vivo. *J Biol Chem,* 263, 14, pp. 6854-6856,

Scicchitano, D. A. and P. C. Hanawalt (1989). Repair of N-methylpurines in specific DNA sequences in Chinese hamster ovary cells: absence of strand specificity in the

dihydrofolate reductase gene. *Proc Natl Acad Sci U S A,* 86, 9, pp. 3050-3054, 0027-8424 (Print).

Shokolenko, I., N. Venediktova, et al. (2009). Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Res,* 37, 8, pp. 2539-2548, 1362-4962 (Electronic).

Srivastava, S. and C. T. Moraes (2005). Double-strand breaks of mouse muscle mtDNA promote large deletions similar to multiple mtDNA deletions in humans. *Hum Mol Genet,* 14, 7, pp. 893-902, 0964-6906 (Print).

St-Pierre, J., J. A. Buckingham, et al. (2002). Topology of superoxide production from different sites in the mitochondrial electron transport chain. *J Biol Chem,* 277, 47, pp. 44784-44790,

St John, J. C., J. Facucho-Oliveira, et al. (2010). Mitochondrial DNA transmission, replication and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. *Hum Reprod Update,* 16, 5, pp. 488-509, 1460-2369 (Electronic) 1355-4786 (Linking).

Suliman, H. B., M. S. Carraway, et al. (2003). Postlipopolysaccharide oxidative damage of mitochondrial DNA. *Am J Respir Crit Care Med,* 167, 4, pp. 570-579, 1073-449X (Print).

Suter, M. and C. Richter (1999). Fragmented mitochondrial DNA is the predominant carrier of oxidized DNA bases. *Biochemistry,* 38, 1, pp. 459-464,

Szczesny, B., A. W. Tann, et al. (2008). Long patch base excision repair in mammalian mitochondrial genomes. *J Biol Chem*, 0021-9258 (Print).

Tatarenkov, A. and J. C. Avise (2007). Rapid concerted evolution in animal mitochondrial DNA. *Proc Biol Sci,* 274, 1619, pp. 1795-1798, 0962-8452 (Print).

Thorslund, T., M. Sunesen, et al. (2002). Repair of 8-oxoG is slower in endogenous nuclear genes than in mitochondrial DNA and is without strand bias. *DNA Repair (Amst),* 1, 4, pp. 261-273,

Tomkinson, A. E., R. T. Bonk, et al. (1990). Mammalian mitochondrial endonuclease activities specific for ultraviolet-irradiated DNA. *Nucleic Acids Res,* 18, 4, pp. 929-935, 0305-1048 (Print).

Tomkinson, A. E., R. T. Bonk, et al. (1988). Mitochondrial endonuclease activities specific for apurinic/apyrimidinic sites in DNA from mouse cells. *J Biol Chem,* 263, 25, pp. 12532-12537, 0021-9258 (Print).

Wallace, D. C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet,* 39, pp. 359-407,

Wallace, D. C., G. Singh, et al. (1988). Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science,* 242, 4884, pp. 1427-1430,

Yakes, F. M. and B. Van Houten (1997). Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A,* 94, 2, pp. 514-519,

Yasukawa, T., A. Reyes, et al. (2006). Replication of vertebrate mitochondrial DNA entails transient ribonucleotide incorporation throughout the lagging strand. *Embo J,* 25, 22, pp. 5358-5371, 0261-4189 (Print) 0261-4189 (Linking).

Yasukawa, T., M. Y. Yang, et al. (2005). A bidirectional origin of replication maps to the major noncoding region of human mitochondrial DNA. *Mol Cell,* 18, 6, pp. 651-662, 1097-2765 (Print) 1097-2765 (Linking).

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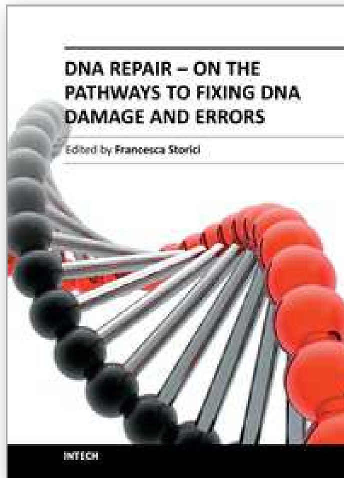

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DNA repair is fundamental to all cell types to maintain genomic stability. A collection of cutting-edge reviews, DNA Repair - On the pathways to fixing DNA damage and errors covers major aspects of the DNA repair processes in a large variety of organisms, 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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