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ADF Perspectives

'To repair or not to repair – no longer a question': repair of mitochondrial DNA shielding against age and cancer

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Abstract: The role of mitochondria in energy production and apoptosis is well known. The role of mitochondria and particularly the role of the mitochondria's own genome, mitochondrial (mt) DNA, in the process of ageing were postulated decades ago. However, this was discussed, debated and more or less disposed of. Recent data from elegant mouse models now confirm that mutations of mtDNA do indeed play a central and pivotal role in the ageing process. Newer reports also indicate a possible role of mtDNA mutations in the carcinogenesis of several organs. But is damaged mtDNA repaired, or is it simply degraded and discarded? This question appears to be answered now. According to recent data, mitochondria possess functional repair mechanisms such as base excision repair, double-strand break repair and mismatch repair, yet nucleotide excision repair has so far not been detected.

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Introduction

The life of a mitochondrion is a cyclic one – in science as well as in biology. Biologically, when more energy production is needed, mitochondria can be increased in number or size or they can be degraded when less necessary. Furthermore, the mitochondria's own genome is a double-stranded, circular molecule that is continuously replicated and degraded. In addition, recent evidence suggests that in contrast to former paradigms, mitochondria do contain the machinery to repair damage to their own genome. In order to achieve this, the repair proteins have to be transcribed and translated from nuclear DNA where they are encoded and imported into the mitochondrion through import complexes which guide them through the outer and inner membrane of this organelle.

The good and bad sides of mitochondria

Mitochondria are organelles which, according to the endosymbiosis theory, evolved from purpurbacteria approximately 1.5 billion years ago. Their main function, among others, is the generation of energy for the cell in the form of adenosine triphosphate (ATP) (Fig. 1). This energy is generated through the respiratory chain (RC) located at the inner mitochondrial membrane, consisting of five protein complexes (complexes I–V). Electrons derived from different reduction equivalents such as NADH move along these protein complexes reducing molecular oxygen at complex IV. If single electrons leave the RC earlier (complexes I and III), reactive oxygen species (ROS) are generated (1–6). Incompletely reduced oxygen (superoxide radicals: O_2^-) can be transformed subsequently to

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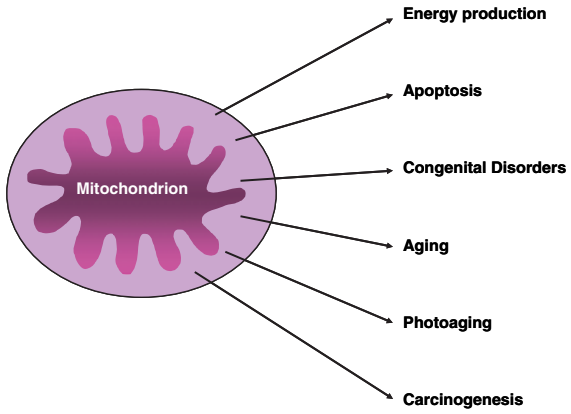

21 *Figure 1.* Influence of mitochondria or mitochondrial DNA on cellular processes. Listed processes are shown in the order of controversy (energy production: lowest, carcinogenesis: highest) regarding a causal role of mitochondria.

H_2O_2 by superoxide dismutase then leading to free hydroxyl radicals ($^{\bullet}OH$) via Fenton chemistry (3). While hydroxyl radicals only have a very short lifetime of 1 ns or shorter, they are still one of the most aggressive forms of ROS, interacting with the deoxyribosyl backbone or the DNA bases themselves. 8-Oxo-guanine (8oxoG) and 8-oxo-adenine are the best characterized oxidative products of DNA bases and particularly 8oxoG is highly mutagenic, mispairing with adenine to lead to G $\rightarrow$ T and C $\rightarrow$ A substitutions. If 8oxoG is incorporated opposite to adenosine, it can lead to A $\rightarrow$ C substitutions (7,8). In addition to endogenous ROS, environmental stressors such as ultraviolet (UV) and ionizing radiation (IR) represent the most important exogenous sources of ROS (9–12).

Measurement of ROS production under physiological conditions is difficult as they are rapidly transformed to O_2 or H_2O by ROS detoxifying enzymes. There is still controversy about the actual amount of cellular ROS production. Newer reports indicate that the amount of ROS under physiological conditions is low (13–15). But as oxidative damage to tissues (lipid peroxidation, protein oxidation and oxidative DNA damage) increases with age (16), it is likely that even low amounts of ROS have detrimental long-term effects on cells.

Mitochondrial DNA and its mutations in health and disease

The mitochondrial genome is directly situated at the site of continuous ROS production. The mitochondrial (mt) DNA is a 16.559-bp, double-stranded, circular molecule existing in about 4–10 copies per mitochondrion. It exclusively encodes proteins for the RC. All other proteins needed for mitochondrial maintenance are encoded by the nucleus. The genes on the mitochondrial genome are highly sensitive to mutation and it has been estimated that the mutation frequency of mtDNA is considerably higher than that of the nuclear genome (17).

The most frequent and best characterized mutation of mtDNA is a 4977-bp deletion, also called 'common deletion'. This deletion includes genes for the NADH dehydrogenase complex, cytochrome-*c* oxidase and ATP-synthetase, all of them functionally important enzymes in the RC. The common deletion is associated with congenital disorders such as Kearns–Sayre syndrome, Alzheimer's disease and diabetes (18), the general ageing process and in particular photoageing (19–23), but its functional role is not clear.

Repair mechanisms of mitochondrial DNA do indeed exist

Base excision repair (BER) is the best characterized mechanism in mitochondria

It is well established that nuclear DNA requires repair mechanisms to maintain normal function. In sharp contrast, it was previously thought that damaged mtDNA was not repaired. The prevailing concept was that mtDNA molecules carrying an excess of damage would simply be discarded, if not the entire mitochondrion, to be replaced by newly generated successors. This notion was based on a study published in 1974 showing that cyclobutyl pyrimidine dimers (CPDs), induced by UV radiation, are not removed from mtDNA (24). This led to the hypothesis that mtDNA is never repaired. It is still valid that CPDs are not removed from mtDNA and degradation is certainly an option for damaged mtDNA. But a recent publication demonstrated that mitochondria do indeed contain repair factors that promote the removal of damage and restoration of intact mtDNA molecules. First in this line, the existence of a BER system specific for the removal of 8oxoG was reported by Nishioka et al. (Fig. 2). They found that mitochondria contain 8oxoG glycosylase (OGG) (25,26). For this type of repair, the nuclear protein human 8oxoG glycosylase (hOGG-1) is targeted to mitochondria via an N-terminal mitochondrial localization signal (MLS). Following this

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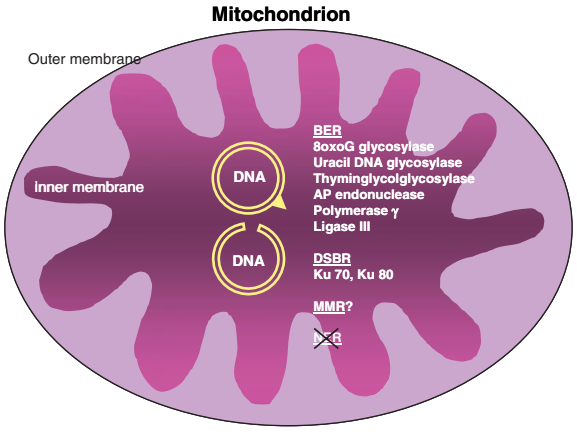


Figure 2. Mechanisms for the repair of mitochondrial (mt) DNA damage. Due to its location next to the respiratory chain in the inner membrane, mtDNA is particularly exposed to DNA damaging reactive oxygen species. Some damage can be compensated through other non-damaged mtDNA, but the mitochondrion has two further options to deal with damage. It can (i) simply degrade damaged mtDNA molecules or (ii) repair them. It has been shown that base excision repair and double-strand break repair exist and several molecules involved in these processes have been demonstrated to be present in mitochondria. Conversely, it has been shown that mismatch repair may be carried out, although proteins of this mechanism have not been detected in mitochondria and very early work has shown that mitochondria do not repair cyclobutylpyrimidine dimers, indicating that complete nucleotide excision repair does not exist in mitochondria.

publication, other glycosylases, uracil DNA glycosylase and thyminglycolglycosylase, were detected in mitochondria (27–29). Knockout mice have been created for DNA uracilglycosylase, 8-oxo-guanineglycosylase and thyminglycolglycosylase which demonstrate the importance of these glycosylases in vivo. In the laboratory of Bohr and co-workers (30), it was shown that mitochondrial thyminglycolglycosylase mNTH1 is active in the removal of mitochondrial thyminglycol damage in vivo. A variety of different phenotypes have been detected in these murine knockout models: modulation of inflammatory regulation (31), increased post-ischaemic brain injury (32) and increased incidence of lung and small intestine cancer (33–35). Other enzymes for BER have also been detected in mitochondria. Pinz and Bogenhagen (36) detected efficient repair of abasic sites in DNA by mitochondrial extracts. They also purified an AP endonuclease from mitochondrial extracts and demonstrated that the mitochondrial polymerase γ (Pol γ) is capable of filling small gaps in a DNA substrate. The proofreading function of Pol γ also serves a repair function. Recently, the importance of this repair function was shown in mice lacking the proofreading function of Pol γ. These mice showed signs of premature ageing and accumulated mutations in their mtDNA (37). Further studies by Lakshmipathy and Campbell revealed that the nuclear gene DNA ligase III also encodes a functionally active mitochondrial ligase (38) and that this ligase could be reduced in mitochondria by antisense DNA ligase III (39). Mitochondria of cells treated with antisense ligase III have a reduced respiratory rate, numerous mtDNA strand nicks and a diminished capacity to restore irradiated mtDNA. In addition to the repair of oxidative damage, several publications show that methylated purines are repaired in mtDNA, indicating that there is also a mitochondrial repair pathway for alkylation-mediated DNA damage (40–44).

Is there mismatch repair (MMR) activity in mitochondria?

Recently, it was shown that purified human mitochondria also possess MMR activities in vitro. Even though its presence has been shown, this system appears to be different from nuclear MMR as it seems not to be nick directed. No homologues of nuclear MMR proteins have been reliably detected in mitochondria so far (45,46). There is controversy whether MSH 2, an essential protein for nuclear MMR, is located in mitochondria. Therefore, it is unclear if an MMR system responsible for the repair of misincorporated nucleotides is present in mammalian mitochondria.

Double-strand break repair (DSBR) is backing up BER

Double-strand breaks (DSBs), generated by ROS or IR (47), represent one of the most dangerous forms of DNA damage. In the nucleus, one DSB can be lethal. In mitochondria, however, DSBs are less dangerous because multiple copies of wild-type mtDNA can compensate for DSBs. Nevertheless, there is evidence for the repair of DNA DSBs in mitochondria (Fig. 2). Ling et al. (48) reported a yeast mutant defective in a subpathway of DSBR, homologous recombination (HR). These mutant cells showed UV-light-induced respiratory deficiency and a decrease in oxidative stress-associated mtDNA repair. Generally, DSBs are repaired via HR (49,50) and there is evidence for HR in mitochondria of mammalian cells. It was reported that mitochondria are capable of repairing DSBs in Chinese hamster ovary cells (41) and it

could be shown that human mitochondrial extracts are able to catalyse HR of different DNA substrates (51). Other evidence for mitochondrial HR came from Zsurska et al. They investigated segregated mtDNA mutations (one on each mtDNA molecule) in a heteroplasmic mtDNA population (52,53). They also found a combination of these two mutations on a different mtDNA molecule indicating HR and crossing over events between mtDNA molecules with segregated mutations. Another subpathway of DSBR mechanism is non-homologous end joining. In nuclear DNA, this process involves the DNA end binding proteins Ku70 and Ku80 which detect and bind free DNA ends. Subsequently, the DNA protein kinase
6 DNA PK_{CS} and the XRCC4/ligaseIV complex reseal the DNA ends (47). Campbell and co-workers (54) showed that mitochondrial extracts are capable of reannealing ends of artificially cleaved plasmids. Thus, repair of DSBs in mtDNA is another type of repair reported to be present in mitochondria. This does not come as a surprise because mtDNA is constantly exposed to ROS-mediated oxidative stress, and DSBR as a backup repair mechanism for BER in the repair of oxidatively induced mtDNA lesions is obligatory. Thus, more evidence of DSBR will probably appear in the future.

Is the nucleotide excision repair (NER)-associated Cockayne syndrome protein also involved in mtDNA repair?

As described above, there is no efficient repair
7 of UV-induced CPDs in mtDNA (24). Yet, evidence increases that NER proteins are also involved in the repair of oxidative DNA damage. Information on the repair of oxidative damage comes from the DNA repair disorder Cockayne syndrome, a rare autosomal recessive disease with growth and mental retardation and increased UV photosensitivity (55). It is a progeroid syndrome as it shows signs of premature ageing (56,57). Defects in two genes, CSA and
8 CSB, have been found to be responsible for this disorder. Little is known about the function of the CSA protein while for the CSB protein,
9 which belongs to the SWI/SNF subfamily with DNA-dependent ATPase activity, important nuclear functions have been reported (58). CSB is involved in the transcription process, is associated with RNA polymerase II and functions as transcription elongation factor.

10 Cells from patients with CS are defective in a subpathway of NER, the so-called transcription-coupled repair (TCR) (55). CSB is involved in the removal of oxidative DNA damage from the nucleus, as CSB-deficient cells have reduced repair rates of 8oxoG lesions (59–62). In other experiments, extracts of CSB-deficient cells fail to incise oligonucleotides containing 8oxoG, while the incision of oligonucleotides containing uracil lesions is normal (60). Further studies assessed a functional crosstalk between CSB and hOGG-1, as electromobility shift assays showed that CSB null or helicase motif mutant cell extracts fail to bind to 8oxoG-containing oligonucleotides (63,64). Tuo et al. also demonstrated that CSB-deficient cells fail to incise 8OH-adenine-containing oligonucleotides. 8OH-adenine is also a DNA lesion induced by ROS which is removed in CSB wild-type cells (65). Taken together, CSB protein, a 'classical' nuclear NER protein, is also involved in the repair of oxidative DNA damage and acts in concert with hOGG. As mtDNA is close to sites of ROS generation, it could also be possible that mitochondria-targeted CSB is associated with mtDNA repair in NER. If this is the case, it would also shed new light on possible NER mechanisms in mitochondria. CSB contribution to mtDNA repair is underlined by the findings of Stevnsner et al., who found reduced repair capacity of 8oxoG in mitochondrial extracts of CSB-deficient cells. However, the exact repair mechanism is still unknown and data showing the presence of CS proteins in mitochondria remain elusive.

Mitochondrial ageing is finally accepted

According to the free radical theory of ageing by Harman (66), free radical damage is generated and accumulates during normal metabolism and in stress situations. In agreement with this concept, the antioxidative capacity of *Dro-*
11 *sophila* and *Caenorhabditis elegans* is associated with longevity (66–68). Persistently high ROS is linked to several age-related diseases such as atherosclerosis, diabetes and Parkinsonism (66). Caloric restriction of *Drosophila*, *C. elegans*, rats and mice leads to a longer lifespan (69,70). Hamilton et al. (70) have shown that the level of 8oxoG increases with age in liver mitochondria of rats and mice, but caloric restriction of these animals reduces this age-related increase of 8oxoG. Lifespan extension due to caloric restriction can be linked to changes in the insulin receptor signalling pathways (71,72) and to decreased mitochondrial ROS production in rats (71).

Xeroderma pigmentosum, CS and trichothiodystrophy are three clinically heterogeneous syndromes (55) which are defective in NER. As all three syndromes show different signs of premature ageing such as loss of subcutaneous fat, skeletal abnormalities and neurological changes, the link between NER and ageing has been investigated by several groups. Cortopassi and Wang (73) were the first to show a positive correlation between DNA repair capacity in UV-irradiated fibroblasts from different species (mice, hamsters, rats, bovines, elephants and humans) and lifespan, while Hart and Setlow (74) showed that the rate of NER capacity was proportional to the logarithm of lifespan. Further data indicating that mice with defects of NER show signs of premature ageing (75) strengthened the link between DNA repair and ageing. Although this study only investigated nuclear DNA repair, further studies investigated the repair of mtDNA and its connection with ageing. Several groups observed increased repair capacity of 8oxoG lesions in mitochondrial extracts from old rodents (76,77). This finding is in contrast to data showing that the rate of oxidative DNA lesions in older individuals increases (70,76). This controversy was resolved by the work of Szczesny et al. (78) demonstrating that most of mitochondria-targeted OGG and uracilglycosylase are stuck at the mitochondrial membrane so that while the repair proteins are increased, functional repair of mtDNA damage within the mitochondria is decreased. In consequence, ROS-induced mutations increase in mtDNA with age (Table 1).

The potential role of mtDNA mutations in ageing was furthermore addressed by investigating a mutated, repair-associated, protein, polymerase γ. Polymerase γ is the only known polymerase involved in all repair processes of mitochondria. Trifunovic et al. investigated homozygous knock in mice expressing a proofreading-deficient polymerase γ. They observed increased levels of point mutations and deletions within mtDNA. Clinically, these mutant mice developed progeroid symptoms such as weight loss, kyphosis, osteoporosis, alopecia and subcutaneous fat reduction (79). Kujoth et al. (37) confirmed these observations but could not detect elevated levels of ROS in these knockout mice but found increased apoptosis in tissues with rapid cellular turnover. They presumed that the ageing phenotype in these mice could be due to apoptosis, which in this case could come from decreased maintenance of mtDNA through restricted repair of replicating mtDNA.

Human skin is probably the organ that is most intensively exposed to mtDNA damage due to chronic exposure to UV light (19). It has been shown that UV-treated fibroblasts show signs of premature ageing such as decreased proliferative capacity (80) and accelerated telomere shortening (81). Furthermore, UV-A-induced ROS production known to be involved in photoageing also induces the mitochondrial common deletion, suggesting that these mtDNA deletions are involved in the process of photoageing (22,82). In agreement with this, mtDNA mutations are increased in chronically UV-exposed human skin (20–23) and these mutations persist as long-term markers of UV exposure in the skin for more than 1.5 years (83). These mutations are paralleled by decreased oxygen consumption, mitochondrial membrane potential, ATP production and an increase of photoageing-relevant metalloproteinase MMP-1 (84–86). Together with the key findings on polymerase γ, these data demonstrate the critical role of mtDNA in cellular ageing.

Table 1. Reports with arguments for a causative role of mitochondria in ageing and arguments against them

For	Caveats	Based on references
Caloric restriction increases lifespan	Data only from *Drosophila*, *Caenorhabditis elegans* and mice	(69)
Association of antioxidative capacity and longevity	None, well accepted	(66–68,122,123)
Age-dependent import deficiency of glycosylases constricts repair impact 8 on mtDNA	Shown only for two repair enzymes	(78)
Age-dependent accumulation of mtDNA mutations in humans	No functional link to mitochondrial activity	(124)
Respiratory chain becomes defective with age	No functional link to mtDNA mutations	(16)
Increase of mtDNA mutations associated with decreased function	No direct link between processes, in vitro data	(84)
mtDNA mutations induced by ROS	In vitro data	(22)
Defective Pol γ in mice leads to premature ageing and infertility	None, well accepted	(37,79)

Reports are roughly given in the order of the scientific discussion.
mtDNA mitochondrial DNA; ROS, reactive oxygen species.

A potential role for mitochondrial damage in carcinogenesis

Already 50 years ago, it was suggested that mitochondria are involved in carcinogenesis (87). But up until now, there was no direct evidence for a causal role of mtDNA mutations in carcinogenesis. As discussed above, mtDNA mutations in tumors could also be due to ageing effects (37,79). Recently, Salas et al. (88) reported flaws in the study designs of work investigating mtDNA sequence data in different cancer and control tissues. Most of the existing data currently show an association of increased mtDNA mutations in different tumors with only little direct evidence for a functional role of these mutations. Mitochondria are key players in tumor control by apoptosis (89–91). Recently, it was shown that UV-B induces mitochondrial apoptotic pathways in human papillomavirus-immortalized keratinocytes, which could represent a possible regression mechanism for premalignant transformation of cells (92). Joshi et al. (93) have shown that respiratory-deficient (ρ^0) prostate tumor cells devoid of the mt genome are more resistant to the apoptosis-inducing anticancer drug BMD 188 than their parental ρ^+ tumor cells containing mtDNA. In addition, the close proximity of mtDNA to the RC, an important generator of ROS, is a likely reason for its high mutation frequency. Experimental evidence for the role of mtDNA in cancer regression comes from studies with bis-2-chloroethylnitrosourea (BCNU) and temozolomide (TMZ), chemotherapeutic drugs which induce cell death by alkylation of DNA bases to form mutagenic O^6 methylguanine and interstrand crosslinks. The repair enzyme O^6 methylguanine DNA methyltransferase (MGMT) removes O^6 methylguanine DNA damage. Cai et al. (94) carried out experiments with haematopoietic cell lines characterized by low repair activity of alkylated DNA damage. Transfection with nuclear-targeted or mitochondria-targeted MGMT generated resistance against cytotoxic effects of DNA alkylating agents such as BCNU and TMZ. Interestingly, this effect was more dependent on mitochondrial than on nuclear MGMT emphasizing the contribution of mtDNA repair mechanisms in the development of drug-resistant cancer cells. Further publications demonstrated a positive correlation between mitochondrial ROS production and tumor invasion (95). Mitochondria may also promote oestradiol-induced breast cancer. Oestradiol induces ROS formation and enhances cell motility via phosphorylation of c-Jun and cAMP response element binding protein as well as increasing DNA binding of transcription factors that mediate the activation of early cell cycle genes (96). Direct evidence for a mitogenic potential of ROS was provided by Liu et al. (97) who showed that ROS stimulated hepatoma cell proliferation via crosstalk between phosphatidylinositol-3 kinase and protein kinase B and jun N-terminal kinase signalling leading to enhanced cFos and cJun expression.

These data discussed above indicate a link between ROS formation, mitochondria and carcinogenesis. In addition, a functional link between mtDNA and the repair of oxidative damage in the nuclear genome was demonstrated by other groups. Delsite et al. (98) have shown that, in ρ^0 HeLa cancer cells, oxidative damage to nuclear DNA increased while the nuclear DNA repair capacity was decreasing. However, results obtained with ρ^0 cells should be interpreted with care as the total absence of mtDNA is an unphysiological state which could differ from that of normal and tumor cells. In other experiments, Hoffmann et al. (99) could not find elevated levels of ROS-induced oxidative mtDNA lesions. Mambo et al. (100) observed decreased nuclear and mitochondrial hOGG-1 expression in human lung cancers compared with normal cells and Choudhury et al. (101) demonstrated in a mutant rat strain that develops spontaneous hepatocellular cancers that increased ROS formation and accumulation of oxidative DNA damage in the liver of these animals were due to decreased expression of the repair enzyme OGG. Further studies have recently shown the increase of mtDNA mutations in tumors and somatic mtDNA mutations are increased in colorectal tumors (102,103). Interestingly, all of these mutations were present in the majority of the tumor cells and 90% of them were detectable in all of the mtDNA molecules present in the mitochondria of the cells (homoplasmic), suggesting that all mtDNA molecules in the mitochondrion contain the same mutation. In addition to colorectal cancer, breast cancer cells also showed somatic mtDNA mutations (104) and currently the number of mitochondrial mutations detected in different tumors is rapidly increasing. Kidney, stomach, prostate, liver, bladder, head and neck and lung as well as hereditary paraganglioma and thyroid cancers show increased mutation frequencies of mtDNA (90,105–114). Shidara and colleagues showed that specific

Table 2. Reports with arguments for a causative role of mitochondria in carcinogenesis and arguments against them

For	Caveats	Based on references
Mitochondria are involved in apoptosis	No link between apoptosis and mtDNA mutations	(89–91)
No apoptosis in prostate tumor cells lacking mtDNA	In vitro data from artificial cell line	(93)
Association of mitochondrial ROS production and tumor growth	In vitro data, no link between mtDNA mutations and carcinogenesis	(95–97)
Association of reduced mtDNA repair and lung/hepatocellular cancer	No direct functional link between mtDNA repair and tumor formation	(100,101)
Somatic mtDNA mutations in numerous tumors	No direct functional link between mutations and tumor formation	(102–114,125)

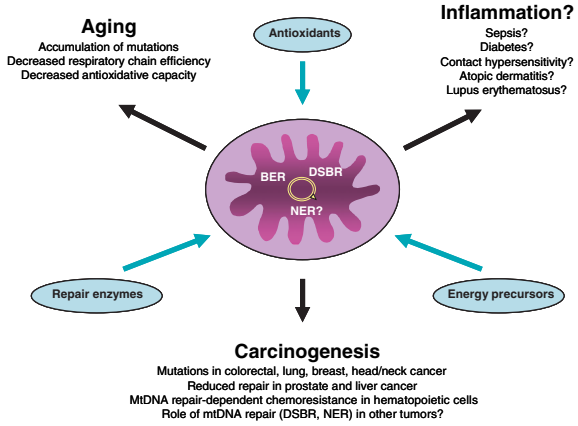
Reports are roughly given in the order of the scientific discussion.
mtDNA mitochondrial DNA; ROS, reactive oxygen species.

point mutations in mtDNA accelerate growth and reduce apoptosis in a variety of tumors (91–94). These data support the notion that point mutations occurring in tumors within mtDNA can have functional advantages that promote tumor growth. Key publications illustrating the role of mtDNA mutations in carcinogenesis are summarized in Table 2.

While the studies above investigated point mutations of mtDNA, only few studies have investigated deletions in the mitochondrial genome of tumor cells (78,119). Work by Durham et al. (106) analysed the levels of the common deletion in basal cell carcinoma and squamous cell carcinoma and found no consistent pattern. Shieh et al. (120) assessed the level of deletions in oral cancer or precancerous lesions. Their results indicate a higher level of deletions in stromal non-tumor tissue compared with tumor tissue. As high levels of large deletions result in a dysfunctional RC, tumor cells needing high energy support for proliferation might not be able to tolerate low energy levels generated by large deletions of mtDNA. Therefore, deletions could be a transient phenomenon during the early stages of transformation providing mutagenic ROS for precancerous cells, while at later tumor stages they are intolerable and deletions disappear by selection mechanisms. Nevertheless, although many associations between mtDNA mutations and cancer have been shown, a functional link to mtDNA repair still needs further investigation.

Conclusions and perspectives

Being the cell's energy supply, mitochondria have generally been seen as quiet, diligent and hardworking guests in the cell who do not deserve much attention or care. Depending on the metabolic requirements of cells in different tissues (i.e. metabolically active liver and brain cells versus dormant fibroblasts), cells can tolerate different levels of mutations while still fulfilling tissue-specific functions. Therefore, it was thought for a long time that mutated mtDNA molecules could either be tolerated or be disposed of. Although controversial data exist regarding a causative role of mtDNA repair in the processes of ageing and carcinogenesis, more and more data arise in support of (i) the presence of repair mechanisms in mitochondria and (ii) a role of these repair mechanisms in the protection against ageing, photoageing and possibly carcinogenesis. In addition, recent publications indicate that hOGG-1 is involved in inflammatory processes such as sepsis, diabetes and contact hypersensitivity (121). As this enzyme is also present in the mitochondrion, it is tempting to speculate that the mitochondrial fraction of this enzyme may also play a part in these processes (Fig. 3).



COLOUR FIG.

21 *Figure 3.* Mitochondria and repair of mitochondrial (mt) DNA in cellular processes. Mitochondria are an important generator of reactive oxygen species and mtDNA is directly exposed to these damaging agents. Recent publications indicate that in addition to widely accepted functions such as energy production or apoptosis, mutations of mtDNA or the repair of mtDNA are directly or indirectly linked to other cellular processes such as ageing, carcinogenesis and possibly inflammation. Further reports demonstrate that intervention in some of these processes is possible by antioxidants or energy precursors and it is tempting to speculate that direct mediation of these compounds as well as repair enzymes to the mitochondrion may exert protective or even therapeutic effects in some of these (patho-)physiological processes.

Future work will be able to employ existing tools to correlate mitochondrial phenotype localization with human diseases and murine models of disease for functional relevance. Future publications will determine the role of proteins involved in the repair of damaged mtDNA together with their functional role in tumors. They will also analyse the controlled targeting of antioxidants, energy precursors and repair enzymes to the mitochondrion, which may provide possible prophylactic and therapeutic tools against defective mitochondrial processes in the future.

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