Mitochondrial DNA, base excision repair and neurodegeneration

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Introduction

Neurodegenerative diseases, in particular those associated with aging, represent a significant public health concern in the developed world. The prevalence of dementia doubles every five years after the age of 60, with an estimated 30 to 45 percent of those age 85 and older suffering from some form of dementia, most commonly Alzheimer's disease [1]. As mean and maximum life expectancy have increased significantly in the last century, the prevalence of such age-associated dementias has followed course. The development of effective treatments is, however, hindered by the complex, multigenic nature of these diseases and by their relatively poorly understood molecular pathophysiology. One aspect of the biology of neurodegenerative diseases that may have important implications for better understanding the underlying molecular mechanisms causing neurodegeneration is that, in spite of the diverse clinical and cellular manifestations, mitochondrial dysfunction is a ubiquitous feature of neurodegenerative diseases, detected both in neurons and in non-neuronal cells.

There is still some discussion in the field as to whether mitochondrial dysfunction is the major cause of neurodegeneration; but the pervasive observation of dysfunctional mitochondria in affected brain regions and cells clearly indicate that mitochondria are essential for neuronal survival, and suggest that pathways involved in maintaining mitochondrial integrity function to prevent or delay the onset of neurodegeneration. This review will examine the evidence that links mitochondrial genome instability to neuronal loss and neurodegeneration. We will explore DNA damage accumulation in mitochondrial DNA (mtDNA) in several pathological conditions, and whether changes in DNA repair activities may play a role in these events (see also Yang et al, this volume, for additional

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discussion of mitochondrial damage and neurological pathology) . And while it is not the scope of this review, it is noteworthy that specific defects in the repair of DNA single-strand breaks in the brain have been directly associated with the pathophysiology of some types of ataxias, namely spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) and ataxia oculomotor apraxia-1 (AOA1) [2] (see Lavin et al in this volume).

Mitochondrial dysfunction in neurodegeneration

The central nervous system has a very high energetic demand, used mostly to maintain ionic distribution across the membranes and for synaptic function. Glucose is virtually the sole fuel for the human brain, except during prolonged starvation. Moreover, the brain lacks fuel stores and hence requires a continuous supply of glucose. These characteristics render brain cells particularly dependent on mitochondrial function for the generation of ATP. Thus, it is not surprising that loss of mitochondrial integrity has been widely associated with neurodegeneration.

Mitochondrial dysfunction in the brain can stem from inherited or spontaneous mutations in mtDNA or in nuclear genes encoding mitochondrial proteins, or from direct damage to mtDNA caused by endogenous or exogenous toxic agents. The "*Mitochondrial Theory of Aging*" proposes that "normal aging" is caused by the steady accumulation of mitochondrial damage caused by endogenous agents, most likely reactive oxygen species (ROS), over an organism's life span [3, 4]. Interestingly, diseases stemming from mitochondrial defects are characterized by a wide range of neuro-muscular phenotypes, and a large number of them are presented with at least one of several neurological symptoms, ranging from hearing loss and mild ophtalmoplegia to severe stroke, epilepsy, ataxia and neuropathy [5–7]. Moreover, the disruption of the mitochondrial respiratory chain by toxins leads to neuronal cell death and phenotypes that often resemble neurodegenerative diseases, such as Parkinson's (PD) and Huntington's (HD) diseases.

PD is a progressive movement disorder that is believed to be caused by the selective loss of dopaminergic neurons, particularly in the substantia nigra region of the brain. Several lines of evidence support the hypothesis that a deficiency in the mitochondrial respiratory Complex I (NADH Ubiquinone Oxireductase) is an underlying feature of PD (for review see [8–10]). This is strongly corroborated by the observation that inhibitors of Complex I induce a motor disorder that clinically and pathologically mimic PD. The effect of the compound 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is a classical example. MPTP-induced Parkinsonism was first identified in the late 1970's, in drug addicts who consumed a synthetic heroine derivative [11], where MPTP was a small contaminant generated unexpectedly during the synthetic process. The toxicity of MPTP is conferred by its metabolite 1-methyl-4-phenylpyridinium ion (MPP$^+$) [12], which accumulates selectively in mitochondria from dopaminergic neurons. MPP$^+$ is a strong inhibitor of Complex I, resulting in decreased oxidation of NAD-linked substrates, disruption of the electron transport chain, reduced oxidative phosporylation and ATP synthesis [13–15]. Increased oxidative stress as a result of MPP$^+$ toxicity has also been postulated to play a causative role in the loss of dopaminergic neurons, as supported by the observation that MPTP toxicity is significantly exacerbated in mice deficient for anti-oxidant enzymes including glutathione

peroxidase, Cu- and Mg-Superoxide Dismutase. Interestingly, the chemical inhibition of poly-ADP-ribose polymerase (PARP) significantly attenuates MPTP toxicity [16, 17]; since PARP is activated by DNA strand breaks and oxidative damage, these findings suggest that mitochondrial-induced oxidative DNA damage also play a role in the etiology of PD (see Woodhouse et al in this volume for detailed discussion of PARP and neurological disease) . Another well known inhibitor of Complex I, rotenone, has been shown to cause PD-like pathology as well. This antibiotic is a common component of pesticides, and several epidemiological studies have implicated accidental exposure to rotenone as a risk factor in the development of PD [8].

Inhibitors of the mitochondrial respiratory Complex II (Succinate-Ubiquinol Oxireductase, or Succinate Dehydrogenase-SDH) also induce neurodegeneration in animal models (for review, see [10, 18]). Most notably, inhibition of Complex II by 3-nitropropionic acid (3-NPA) and malonate greatly resemble those seen in HD, another movement disorder characterized by cell death in the striatum and disruption of the cortical-striatal circuitry. While 3-NPA and malonate inhibit SDH by distinct molecular mechanisms, the similarities between their pathological outcomes strongly suggests that SDH inhibition is a central molecular event in HD etiology. This is consistent with Complex II and III deficiencies observed in post-mortem striatum samples [19]. Moreover, oxidative stress indicators, particularly in mitochondria, are also elevated in animal models of 3-NPA-induced toxicity as well as in HD samples [20].

The findings discussed above indicate a strong correlation between disruption of the mitochondrial electron transport chain and neurodegeneration. They suggest a scenario in which mitochondrial dysfunction, induced by toxins as discussed above, or by yet unknown mechanisms in the case of the idiopathic diseases, is an initial event in a cascade that leads to neuronal loss. This initial mitochondrial inhibition would lead to energetic crisis and elevated oxidative stress, accumulation of oxidative DNA damage and mutations, and ultimately to neuronal cell death. In this scenario, it is critical to understand how these events are linked to cellular dysfunction, since they could provide targets for clinical interventions that could block or attenuate the progression of these diseases.

Mitochondrial oxidative DNA damage accumulation

The mtDNA is situated at the inner side of the mitochondrial inner membrane. It is believed to be covalently associated with the membrane, if not always, then at least during some metabolic transactions, such as DNA replication [21]. In eukaryotic cells, the inner mitochondrial membrane is the major cellular site for generation of ROS, such as superoxide anion, hydrogen peroxide and the hydroxyl radical [22]. ROS are very reactive towards biomolecules, and can randomly attack lipids, proteins and nucleic acids. The hydroxyl radical will react with any molecule in a radius of 15 Å of its site of formation, with a rate constant only limited by diffusion [23]. The oxidative attack of biomolecules results in a variety of potentially harmful consequences, such as 1) lipid peroxidation, which leads to the generation of highly reactive aldehyde by-products; 2) oxidation of SH groups, which causes protein aggregation; 3) oxidation of amino acid side chains forming carbonyl groups, which target proteins for degradation; 4) oxidation of the sugar in nucleic acids, leading to

breakage of the phosphodiester backbone; and 5) oxidation of the bases in nucleic acids, with the concomitant formation of a plethora of base modifications that can be either mutagenic or cytotoxic (reviewed in [24]).

The physical proximity of the mtDNA to the sites of ROS production in the inner mitochondrial membrane renders the mtDNA more vulnerable to being damaged by such species in comparison to nuclear DNA. Several groups, including ours, have demonstrated that the levels of oxidized bases in mtDNA are 2–3 times greater than in nuclear DNA [25, 26], likely reflecting the issue of accessibility.

From the large number of different oxidized DNA modifications detected in DNA exposed to oxidizing agents [27] , only a few have been, so far, extensively studied in regards to their biological significance. Eight-hydroxy-guanine (8-oxoG) was one of the first oxidized bases to be recognized as biologically relevant [28, 29] and has since been considered a biomarker of oxidative stress. 8-oxoG is a mutagenic lesion when present in double-stranded DNA, because in *syn* configuration it can mispair with adenine, leading to a G:C→T:A transversion mutation [30]. For instance, DNA polymerase gamma (pol γ), the only DNA polymerase identified in mammalian mitochondria so far (see more below), will insert a wrong base opposite 8-oxoG in the template about 27% of the time [31], resulting in a mutagenic event. Recent data, however, suggest that 8-oxoG may not be as deleterious *in vivo* as previously believed. Maga and colleagues showed that 8-oxoG is faithfully bypassed by the nuclear DNA polymerases lambda and eta when the auxiliary factors PCNA and RPA are present [32]. It has also been shown that the transcription machinery can bypass 8-oxoG *in vitro* [33]. We observed normal mitochondrial bioenergetic function in mitochondria isolated from livers and hearts of mice lacking the DNA repair enzyme Oxoguanine DNA Glycosylase (OGG1) [34]. These mice show no removal of 8-oxoG from their mtDNA and accumulate approximately 20 fold higher levels of 8-oxoG than wild type controls [35], indicating that such high levels of 8-oxoG did not compromise the integrity of the respiratory chain.

Other oxidized bases have also been detected in DNA, even in the absence of any exogenous insult. For example, thymine glycols (TG) can be detected both in genomic as well as in mtDNA [36]. TG is a polymerase-blocking lesion, causing aborted DNA and RNA synthesis [37], which could ultimately lead to cell death. In addition, we have recently shown that ring-opened formamidopyrimidine lesions (4,6-diamino-5-formamidopyrimidine, FapyA; and 2,6-diamino-4-hydroxy-5-formamidopyrimidine, FapyG) are detected in mouse liver genomic DNA at levels that are similar, or even slightly higher, than those of 8-oxoG [38]. Both FapyA and FapyG are strong mutagenic bases *in vitro*, directing the incorporation of adenine opposite the modified purine [39, 40].

Nonetheless, 8-oxoG quantification has been extensively used as a marker of oxidative DNA damage accumulation and several groups have demonstrated that the levels of this lesion are elevated in mtDNA both during normal aging as well as in neurodegenerative disorders. Ames and colleagues [25, 41] were the first to show that mtDNA accumulated 8-oxoG at higher levels than the nuclear DNA, and although there has since been much discussion as to

the absolute levels of oxidative lesions in mtDNA from various sources [42, 43], it is well accepted that oxidative damage increases with age in the mtDNA [26, 44].

Base excision repair in mitochondria

Because mammalian cells harbor genetic material in both their mitochondrial and nuclear compartments and each genome is vulnerable to various types of spontaneous and exposure-dependent DNA lesions, organisms have evolved protective systems for both partitions. However, the spectrum of the DNA damage responses varies between the mitochondria and nucleus (Table 1), and in many cases, the precise molecular pathway and specific repair proteins involved are distinct. For the purpose of this review, we focus exclusively on the Base Excision Repair (BER) pathway in mitochondria, but refer to the following reviews for discussion on nuclear DNA repair [45] and mtDNA repair in general [46]. BER is the predominant pathway for coping with most spontaneous decay, alkylation and oxidative DNA lesions.

Nuclear or mitochondrial BER, like all DNA repair responses, involves a cascade of recognition and enzymatic processing steps that aim to remove the target damage and restore genome integrity. In BER is initiated by the excision of a modified or inappropriate base from the sugar-phosphate DNA backbone by a DNA glycosylase. The resulting apurinic/apyrimidinic (AP) site is subsequently cleaved by an AP endonuclease, creating a strand break with a 3'-hydroxyl end and a 5'-deoxyribose phosphate (dRP) residue. The single nucleotide gap is then filled by a specific DNA repair polymerase, and the 5'-dRP fragment is excised to create a normal 5'-phosphate terminus. To complete short patch (or single nucleotide) BER, the final nick is sealed by a DNA ligase (Figure 1). It is worth emphasizing that the mitochondrial genome does not encode DNA repair proteins, and thus, as will be discussed next, most of the mitochondrial BER proteins are splice-variants, alternative translation-initiation products, or post-translationally modified versions of the nuclear-encoded protein forms.

DNA Glycosylases

Several DNA glycosylases have been identified in mammalian mitochondria (Figure 2). The gene encoding hOGG1, which excises, among others, 8-oxoG from DNA, creates at least four mRNA isoforms by alternative splicing of distinct 3' exons [47, 48]. Most of these isoforms, such as type 2a/OGG1β (Figure 2), which is one of the major expression products, encode proteins that localize to the mitochondria, presumably via a shared N-terminal mitochondrial targeting sequence (MTS). However, the type 1a protein (also known as OGG1α; Figure 2), which contains a unique C-terminal domain that harbors a strong nuclear localization signal (NLS), sorts mainly to the nucleus, yet to a lesser extent to the mitochondria. Recent evidence suggests that the β form is a non-active splice variant with unknown function and that OGG1α accounts for both the nuclear and mitochondrial 8-oxoG repair activities [49]. In addition to 8-oxoG [50], mammalian OGG1 has been shown to exhibit glycosylase activity for the oxidized base lesions 8-oxoA and FapyG, as well as a weak AP lyase function [51–53].

Human MYH, a homolog of the *Escherichia coli* DNA glycosylase mutY, excises adenine opposite 8-oxoG as part of the defense system against the mutagenic consequences of this oxidative base damage (i.e. the so-called GO system [54]). The human *MYH* gene produces three major transcripts with unique 5' mRNA sequences, i.e. α, β and γ, each of which is alternatively spliced to create as many as 10 different splice variants [55, 56] (see example in Figure 2). Despite initial studies indicating that hMYH resides mainly in the mitochondria [47], western blot analysis uncovered the presence of hMYH protein forms in both nuclei (p52/53) and mitochondria (p57) [56]. The p52 nuclear species has been suggested to be the product of the hMYHβ1, β3 or γ2 transcript(s). The origin of the mitochondrial MYH is likewise unclear, but studies suggest that it may be the larger protein created by the hMYHα3 mRNA (p54) or the 60 kDa species predicted to be generated by the hMYHoα1 transcript. Determination of the primary amino acid sequences of the purified MYH glycosylases from both nuclear and mitochondrial extracts will shed light on the nature of the different compartmental forms.

Mammalian NTH1, an ortholog of *Escherichia coli* endonuclease III (Nth), is a DNA glycosylase/AP lyase that excises a wide range of pyrimidine lesions, including TG, 5-hydroxy cytosine and fapy intermediates. Current evidence suggests that hNTH1 protein is localized almost exclusively to the nuclues, likely due to the presence of a weak MTS [47, 57, 58]. Notably, no alternative splicing of the hNTH1 gene has been reported so far, and the disparate protein isoforms of hNTH1 generated by multiple translation initiation sites do not exhibit differences in localization (Figure 2). In contrast, mouse NTH1 appears to localize mostly in mitochondria [58], which is in agreement with the abrogation of TG and 5-OHU incision activity in liver mitochondria from NTH1 knockout mice [59].

A new set of DNA glycosylases with specificity for oxidative DNA lesions has been recently identified, NEIL1, 2 and 3 [60–63]. These are mammalian homologues of the *Escherichia coli* DNA glycosylase Fpg/Nei, which excise primarily ring-opened oxidized purines such as FapyG and FapyA [64] . We have recently shown that NEIL1 localizes to mouse liver mitochondria, where it is likely to provide the residual incision activity observed with a FapyG containing oligomer in extracts from mice lacking both OGG1 and NTH1. The importance of NEIL1 for the maintenance of mtDNA has also been implied by the observation that mtDNA deletions accumulate in the livers of NEIL1 knockout mice, and that these mice have symptoms often associated with mitochondrial disease [63].

The uracil-DNA glycosylases (UDG) UNG1 (mitochondria) and UNG2 (nuclear) are encoded by the *UNG*-gene (Figure 2). These enzymes remove uracils generated by spontaneous deamination of cytosine in DNA. The mitochondrial and nuclear isoforms of UDG share a common catalytic domain, but have different N-terminal sequences for subcellular sorting as a result of the use of alternative promoters and mRNA splicing [65]. In addition to excising uracil from U:G > U:A base pairs, the UNG proteins have the ability to remove oxidized cytosines from DNA as well, including isodialuric acid, alloxan and 5-hydroxyuracil [66].

In the nucleus alkylation base damage is repaired mainly by methylpurine DNA glycosylase (*MPG*). While the *MPG* gene encodes alternative transcripts that produce proteins with

putative nuclear and mitochondrial targeting sequences, MPG has not been shown explicitly to localize to the mitochondria [67]. However, efficient repair of methylated and ethylated bases in mtDNA has been documented [68–71].

AP Endonuclease

Following base excision by a DNA glycosylase, the resulting abasic site is typically incised by an AP endonuclease (Figure 1). In humans, the major, if not only significant, AP site incision activity is intrinsic to APE1. Initial studies revealed that translocation of the only known human APE1 protein species (a 318 amino acid product), which was engineered to harbor a FLAG-tag, scarcely occurred into the mitochondria of COS-7 cells [47]. APE1 harbors a NLS in its N-terminal sequence and no major alternatively-expressed forms have been reported [72]. However, endonucleolytic AP site cleavage activity had been observed in mitochondrial extracts [73], and immunohistochemistry with APE1-specific antibodies suggested the presence of an APE1-like protein in this cellular compartment [74, 75]. Mitra and colleagues recently reported that the mitochondrial AP endonuclease is derived from the main APE1 polypeptide by protease removal of the 33 N-terminal amino acid residues that contain the NLS [76] (Figure 2). It is important to emphasize that the truncated APE1 protein described above was only seen at a significant level in bovine and mouse liver and the NIH 3T3 mouse cell line, whereas only full-length APE1 was detected in a variety of human cell lines. These results may indicate that the full-length form is the major protein species in cultured cells and/or has a more pronounced role in mtDNA repair in humans. A second protein with homology to APE1, as well as its bacterial ancestor exonuclease III, APE2, has been shown to localize partially to the mitochondria, but its contribution as an AP endonuclease is in question [77, 78].

DNA Polymerase

Mitochondria possess a single DNA polymerase, POLγ (Figure 2), which assumes sole responsibility for DNA synthesis in all replication, recombination, and repair transactions involving mtDNA [79]. POLγ is homologous to the family A of DNA polymerases, which include the Klenow fragments of *Escherichia coli* and *Bacillus stearothermophilus* polymerase I and the polymerases of *Thermus aquaticus* and bacteriophage T7. In humans, POLγ holoenzyme is a 195 kDa heterotrimer, consisting of a catalytic subunit (p140, coded by *POLG* on chromosome 15q25) and two identical accessory subunits (p55, coded by *POLG2* on chromosome 17q). The accessory subunit (p55) is a DNA binding factor that activates both the polymerase and exonuclease activities of the catalytic subunit, and confers high processivity by increasing the affinity of the heterotrimer for template DNA [80, 81]. The catalytic subunit (p140) possesses DNA polymerase, 3'→,5' exonuclease activity and a 5'dRP lyase function [82], all of which are important to the accurate and productive execution of gap-filling and termini processing in mitochondrial BER (Figure 1). The accessory subunit has been shown to improve the efficiency of dRP removal (the rate-limiting step in mitochondrial BER) by increasing the lyase reaction and the ability of the enzyme to locate the damage site on DNA, presumably by enhancement of DNA binding [83].

DNA Ligase

The final step of BER is sealing of the remaining single-strand nick (Figure 1). In mitochondria, this molecular process is performed by a variant of the nuclear DNA ligase III (LIG3). In particular, the human DNA *LIG3* gene encodes both nuclear and mitochondrial enzymes (Figure 2), which arise from the use of alternative-translation initiation start sites in a single mRNA transcript [84]. Interestingly, the mitochondrial DNA LIG3 protein apparently functions without XRCC1 [85], a key single-strand break repair factor required for the stability of nuclear DNA LIG3oα via its direct physical association [86].

Mitochondrial DNA instability in neurodegenerative disorders

Increased oxidative DNA damage, mutations and deletions have been observed in the aging brain and neurodegenerative diseases. Alzheimer's disease (AD) is a progressive age-dependent neurodegenerative disease that leads to cognitive and behavioral impairment. Pathologically, AD is characterized by protein aggregates (amyloid plaques and neurofibrillary tangles) and neuronal cell death that is thought to initiate in the hippocampus and spread throughout the cortex. On the other hand, the cerebellum often does not display any of those hallmarks, at least until much later in the progression of the disease. Lovell and colleagues found that the levels of several oxidized bases in AD brains were significantly higher in frontal, parietal, and temporal lobes compared to control subjects and that mtDNA had approximately 10-fold higher levels of oxidized bases than nuclear DNA. Moreover, DNA from temporal lobe showed the most oxidative damage, whereas cerebellum was only slightly affected in the AD brains, correlating with these regions being respectively the most and least affected in human AD brain [87]. This same group also measured oxidative damage in samples from patients suffering from Mild Cognitive Impairment (MCI), the earliest clinical manifestation of AD. Eight-hydroxy-guanine was significantly higher in MCI nuclear DNA from the frontal and temporal lobe and in mtDNA from the temporal lobe compared with age-matched control subjects, and statistically significant elevations of FapyA were also observed in mtDNA of MCI temporal, frontal and parietal lobes [88]. Because MCI is considered to be a transient stage between normal aging and dementia, these results indicate that accumulation of oxidative damage in the mtDNA is an early event in AD manifestation and may have a causative role.

The results discussed above raise the question of whether this accumulation of oxidative DNA damage in AD patients is the result of enhanced damage formation or decreased repair. We addressed this question by looking at BER activities in short post-mortem brain samples from AD, MCI and age-matched control subjects. Using *in vitro* assays we analyzed each step of BER independently, as well as the process as a whole, and found significant changes in both AD and MCI samples [89]. We observed lower activity and protein levels of UDG, OGG1 and DNA polymerase β, as well as total BER, not only in an affected brain region (cortex) but, also in the least affected region (cerebellum). Interestingly, in MCI subjects there was an inverse correlation between total BER activity and the Braak stage, a quantification of the number of neurofibrillary tangles. Since neurofibrillary tangle pathology in AD is associated with cognitive decline [90], these findings suggest a link

between BER capacity and the degree of neurological impairment, as measured by the Braak stage.

A role for oxidative DNA damage in PD pathology is much less well documented. Nonetheless, Zhang and colleagues observed increased cytoplasmic 8-oxoG immunoreactivity, indicating increased levels of this oxidized base in the mtDNA and/or in cytosolic RNA [91].

Accumulation of mutations and deletions of the mtDNA have also been associated with degenerative diseases, particularly AD and PD. However, the significance of these associations is still unclear [92]. Lin and colleagues [93] were the first to report on the association of one particular mtDNA mutation, in this case at codon 331 of NADH dehydrogenase 2, with AD, even though this finding was shortly thereafter contested [94, 95]. Since then, several reports have associated mtDNA mutations [96–100] and deletions [101–103] with AD. On the other hand, other studies found none or inconclusive associations between mtDNA instability and neurodegenerative diseases [104–108]. This conflicting range of results has led to the speculation that this variability may be due to the misleading amplification of ancient mtDNA sequences present in the nuclear DNA [109] or to great mosaicism of mtDNA among single cells in the brain [110].

While several mutations in genes with yet unclear function (alpha-synuclein, parkin, DJ-1, PINK-1 and LRRK2) have been associated with PD, the vast majority of cases are sporadic, without any relevant family history [111]. Mutations in the mtDNA have also been found in brains from PD patients [112–116], however recent results suggest that mtDNA deletions play a more significant role in the pathology of the disease [117]. Two studies published in parallel [118, 119] report very high levels of deleted mtDNA genomes in the substantia nigra region of brains from PD patients. These deletions are clonally expanded and are particularly enriched in cytochrome oxidase-negative cells, indicating that they may be directly responsible for impaired cellular respiration.

While the findings discussed above are correlative, and do not address the issue of causation directly, some results obtained with transgenic mouse models lend further support for a role of mtDNA damage accumulation in neuronal loss. For instance, alpha-synuclein (the primary component of Lewy bodies, which are seen in the brains of patients suffering from several neurodegenerations [120]) mutations have been linked to familial PD and transgenic mice expressing human mutated genes develop a severe motor disorder (for review, see [121]). Interestingly, in one of these mouse models (the A53T mutant), the mitochondria in a subset of neurons from the neocortex, brainstem and spinal cord ventral horn were positive for deoxynucleotidyl transferase-mediated UTP nick end labeling, indicating accumulation of mtDNA damage [122]. Using another approach, mice with a conditional knockout of the mitochondrial transcription factor A (TFAM) in dopaminergic (DA) neurons show reduced mtDNA expression and respiratory chain deficiency in midbrain DA neurons, which, in turn, lead to parkinsonism with adult onset of slow, progressive impairment of motor function accompanied by formation of intraneuronal inclusions and dopamine nerve cell death [123].

Changes in BER with age and in neurodegeneration

The "*Mitochondrial Theory of Aging*" has been extensively discussed lately, with conflicting observations emerging. Two independent groups [124, 125] have generated transgenic mice expressing a mutated, proof-reading deficient POLγ. These animals exhibit severely elevated levels of mtDNA mutations and deletions and a premature aging phenotype that is strikingly similar to normal aging. These observations were initially hailed as "definitive proof" that mtDNA mutations cause aging. However, further investigation found that animals heterozygous for the mutated POLγ accumulate nearly as many mutations as homozygous littermates, but show no clear aging phenotypes, thus dissociating the mutational load from the premature aging outcome [126]. One implication of this theory is that the age-associated accumulation of mtDNA damage and mutations leads to increased oxidative stress because of faulty expression of mitochondrial genes that encode for components of the mitochondrial respiratory complexes. There is a great deal of experimental evidence that age is associated with increased mitochondrial generation of ROS [127], but, again, the recent observation that the mitochondrial POLγ mutant mice do not show enhanced oxidative stress [128], despite the elevated mutations, casts doubt on the direct relationship between these events. Nonetheless, oxidative damage in the mtDNA has been shown to increase significantly with age and yeast require BER activities to attain a full chronological lifespan, implying that accumulation of damage hinders life span [129].

Since the first demonstration by Hart & Setlow that DNA repair activities are inversely correlated to life span in mammals [130], the general consensus had been that DNA repair capacity decreases with age. However, we demonstrated that OGG1 activity rather increases with age in liver and heart mitochondria and does not change significantly in the nuclear extracts of rats [131] and mice [132]. Interestingly, the rodent mtUDG activity did not change with age in either compartment, indicating that the increased capacity to repair 8-oxodG does not reflect a general up-regulation of DNA repair in mitochondria with age in these tissues. Thus, a steady-state accumulation of 8-oxoG in mtDNA in mouse liver and heart is therefore likely to be caused by an increased rate of damage formation, which exceeds the mtDNA repair capacity. The induction of the mitochondrial OGG1 activity may represent a cellular response in an attempt to counteract the increased DNA damage formation. Interestingly, Szczesny and Mitra demonstrated that mouse hepatocyte AP endonuclease activity is increased in nuclei as well as in mitochondria with age, whereas the total APE1 activity/level was unchanged [133].

A high level of oxidative damage can be particularly deleterious in post-mitotic tissue, such as the heart and the brain, because they cannot self-renew through cell proliferation. When nuclear and mitochondrial BER activities were evaluated in five distinct mouse brain regions, a significant age-dependent decrease in mitochondrial OGG1, UDG and NTH1 activity was observed for all regions [134]. Another study of rat cerebral cortices showed that mitochondrial BER activities gradually declined with age, and this decline in activity parallels decreased expression of repair enzymes, such as OGG1 and POLγ [135].

Together these results point to a very important aspect in aging research, which is that there is a great degree of variability among different tissues in an organism. While the implication

that different cell types will show specific responses to DNA damage must be appreciated, these results also suggest that changes in mitochondrial BER with age may contribute to the accumulation of DNA damage, and the increase in oxidative damage to mtDNA might contribute to age-related decline, particularly in the brain.

Only a few studies have been performed to address age-associated changes in BER in humans. However, using a biotin-containing aldehyde-reactive probe for measuring AP sites in living cells, Ames and co-workers have shown that leukocytes from old human donors possess reduced AP site repair and a reduced activity of DNA glycosylases that remove methylated bases [136]. Moreover, cultured human fibroblasts showed decreased BER activity both in the nucleus and mitochondria with higher passage number [137], suggesting that senescence is also associated with decreased BER. However, *in vitro* cellular senescence may not fully emulate the molecular changes that take place *in vivo*, and this issue still needs to be further addressed using human samples.

Genetically inherited premature aging syndromes are very valuable tools when aiming to understand the normal aging process in humans. Cell lines from patients suffering from such syndromes have been established and isogenic cell lines with the functional gene transfected back into the deficient line are employed as controls. In the human disorder Cockayne syndrome, complementation group B (CSB), which is characterized by segmental premature aging and neurological dysfunction, there appears to be a deficiency in 8-oxoG repair. In particular, whole cell extracts from CSB-deficient cells display reduced 8-oxoG incision [138] and CSB mutant cells exhibit impaired *in vivo* repair of oxidative damage in nuclear genes [139]. More recently, we reported that the mitochondrial repair of 8-oxoG is also deficient in CSB-deficient cells [140]. While this deficiency in repairing oxidative damage, especially from mtDNA, may be a major underlying cause of the disease, the precise molecular role of CSB in the BER pathway is, however, still unclear. Based on identified interaction partners of the CSB protein, it has been proposed that CSB is an auxiliary factor in BER [141].

Work from several groups has proposed that changes in BER play a causative role in neurodegeneration. Iida and colleagues found changes in expression of BER enzymes, including the mitochondrial β-OGG1 in neuronal cytoplasm and reduced Polβ in midtemporal cortex samples of affected AD tissue [142]. Lower OGG1 activity was also reported for nuclear lysates from affected human AD brain regions using a sodium borohydride trapping assay, which, however, only measures the robustness of the AP lyase activity of OGG1 [143]. Moreover, the same group recently reported that mutations in the OGG1 gene that affect its activity are found preferentially in AD patients [144].

The results discussed here provide strong support to the hypothesis that mtDNA instability contributes to neurodegeneration. Our results showing lower BER activity in AD brains further suggest that lower BER may be a predisposing factor in the development of AD. Together, these shed some light in the sequence of events and factors that may be involved in the complex cascade that leads to neurodegeneration, and hopefully contribute to the development of risk assessment tools as well as preventive drug therapy.

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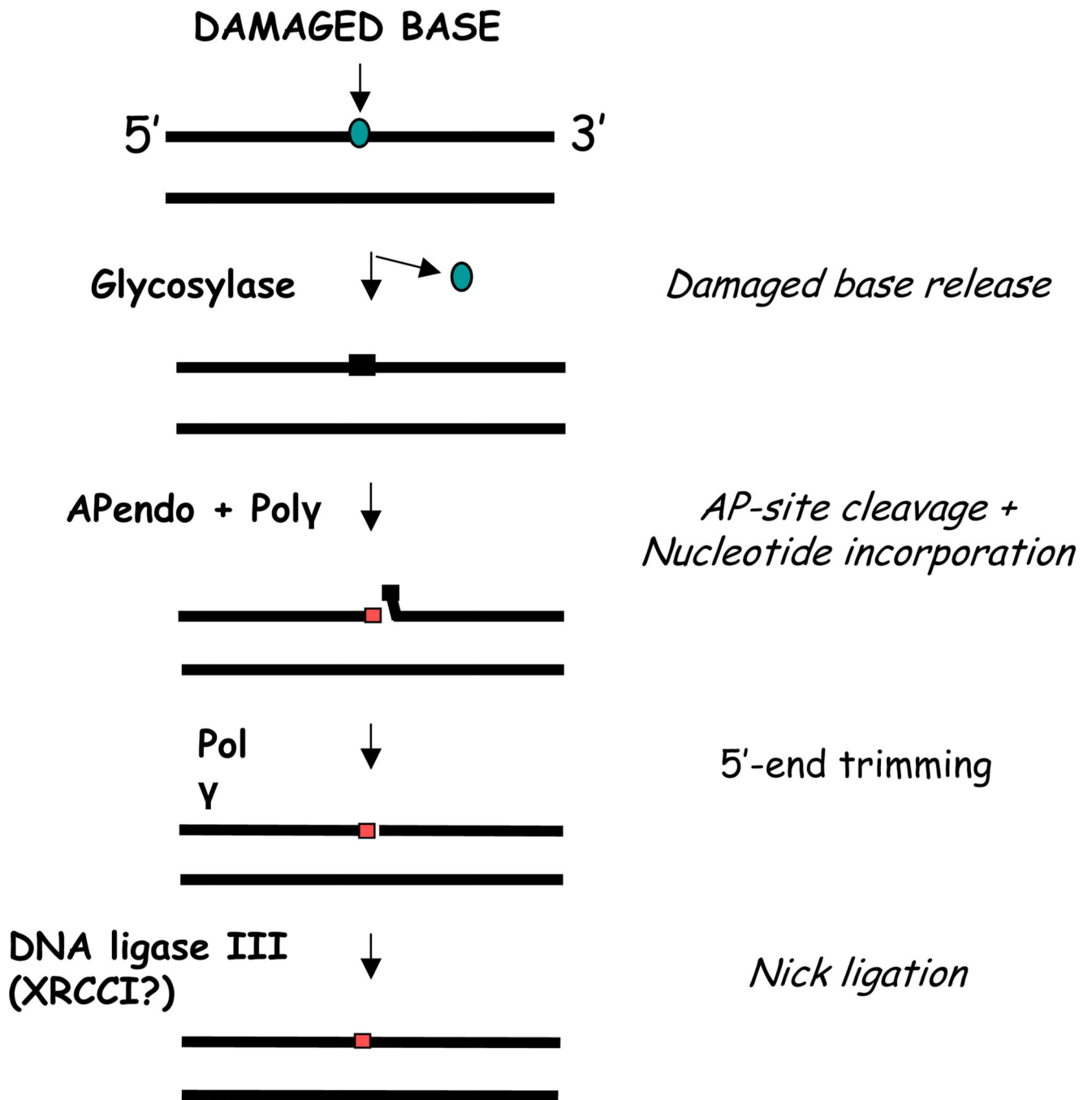
Figure 1.
Schematic representation of the Base Excision Repair Pathway in Mammalian Mitochondria. Each enzymatic step and the proteins involved are further described in the text.
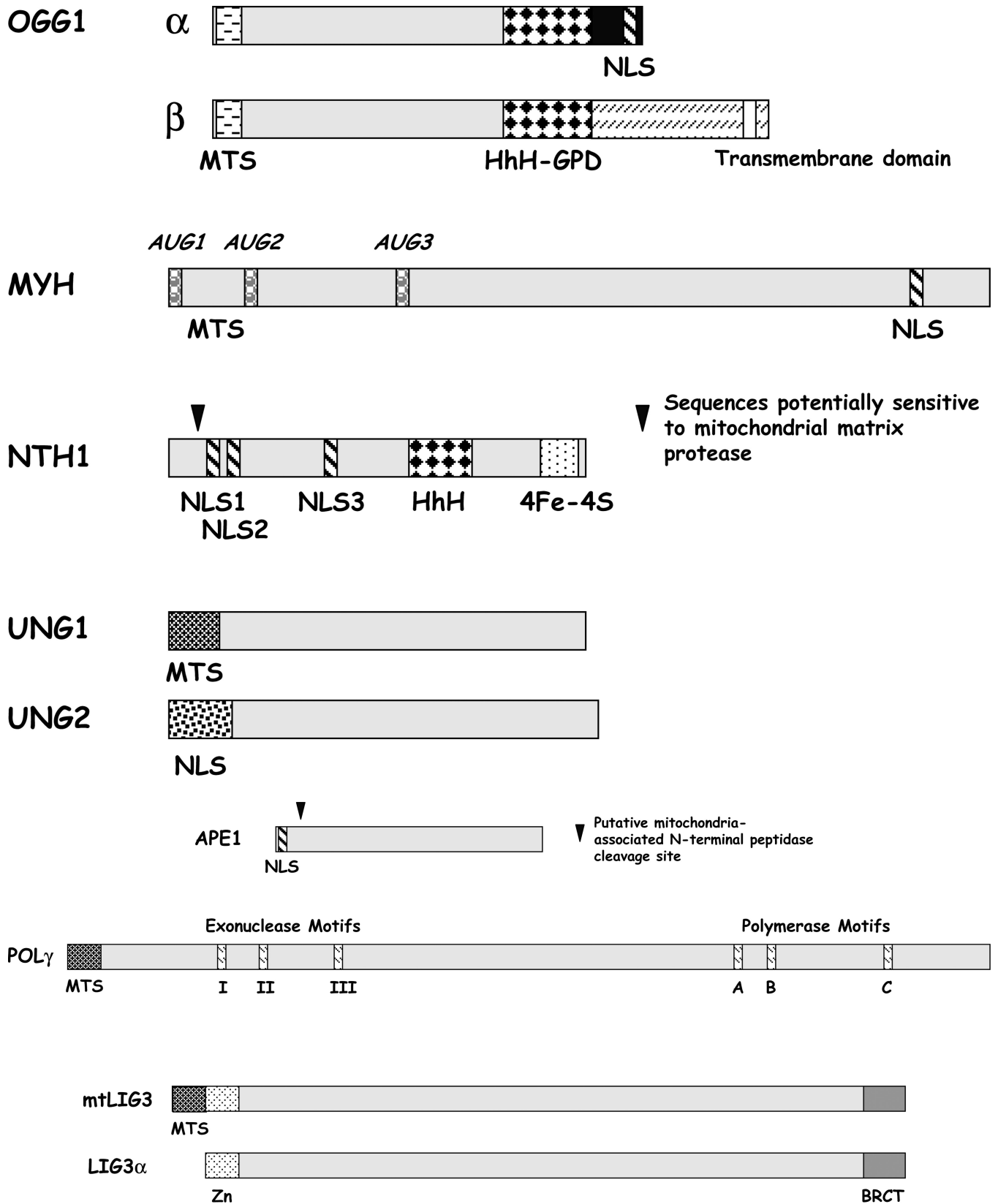
Figure 2.
Splice variants of DNA repair enzymes found in mammalian mitochondria.

Table 1

Comparison of the DNA repair pathways identified so far in the nucleus and mitochondria of mammalian cells.

Mammalian DNA Repair Pathways			
PATHWAY	**SUBPATHWAYS**	**Nuclear**	**Mito**
BER/SSBR	Long patch	Y	N
	Short patch	Y	Y
NER	General genome	Y	N
	Gene-specific	Y	N
	Transcription associated	Y	N
MMR		Y	Y?
Recombination	HR	Y	?
	NHEJ	Y	Y
Direct damage reversal		Y	Y