Mitochondrial DNA and aging

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ABSTRACT
Among the numerous theories that explain the process of aging, the mitochondrial theory of aging has received the most attention. This theory states that electrons leaking from the ETC (electron transfer chain) reduce molecular oxygen to form \( \text{O}_2^{•−} \) (superoxide anion radicals). \( \text{O}_2^{•−} \), through both enzymic and non-enzymic reactions, can cause the generation of other ROS (reactive oxygen species). The ensuing state of oxidative stress results in damage to ETC components and mtDNA (mitochondrial DNA), thus increasing further the production of ROS. Ultimately, this ‘vicious cycle’ leads to a physiological decline in function, or aging. This review focuses on recent developments in aging research related to the role played by mtDNA. Both supportive and contradictory evidence is discussed.

INVOLVEMENT OF mtDNA (MITOCHONDRIAL DNA) IN AGING
Aging can be defined as a multifactorial phenomenon characterized by a time-dependent decline in physiological function [1]. This physiological decline is believed to be associated with an accumulation of defects in the metabolic pathways. RNA, proteins and other cellular macromolecules are rapidly turned over and, consequently, are poor candidates for progressively accumulating damage over a lifetime. Therefore even early studies on mechanisms of aging focused on DNA (for example, see [2,3]). In mammalian cells, mitochondria and the nucleus are the only organelles that possess DNA. It appears obvious that the physiological integrity of the cell must critically depend upon the integrity of its genome, which is maintained by DNA repair machinery. However, although the organization, synthesis and repair of nuclear DNA have been the focus of intense studies, mtDNA has received much less attention until recently.

BASIC mtDNA BIOLOGY
Human mtDNA is a circular double-stranded molecule that is 16 569 bp long (other sequenced mammalian mitochondrial genomes have similar lengths; Figure 1). It encodes two rRNAs, 22 tRNAs and 13 polypeptides, of which seven are components of complex I (NADH dehydrogenase), three are components of complex IV (cytochrome c oxidase), two are subunits of complex V (ATP synthase) and cytochrome \( b \) (a subunit of complex III) [4]. The inheritance of mtDNA is almost exclusively maternal, although some important exceptions have been reported [5–7]. mtDNA is present in one to several thousand copies per cell [8] and is ‘encapsulated’ into mitochondria at 1–11 copies per mitochondrion with the mean being two genomes per organelle [9]. The two mtDNA strands can be separated by denaturing caesium chloride gradient centrifugation [10]. Most of the information is encoded in the heavy (purine-rich) strand (two rRNAs, 14 tRNAs and 12 polypeptides). The light (pyrimidine-rich) strand contains genetic information for only one polypeptide and eight tRNAs. Mitochondrial genes have no introns and intergenic sequences are absent or limited to a few bases. Some genes overlap and, in some instances, termination codons are not encoded, but are generated post-transcriptionally by polyadenylation [11]. mtDNA is totally dependent upon nuclear-encoded proteins for its maintenance and transcription. In fact, the mitochondrial proteome consists of an estimated 1500 polypeptides [12] of which only 13 are encoded by its own DNA. mtDNA replication is conducted by [13].

Key words: aging, DNA damage, mitochondrial DNA, oxidative stress, reactive oxygen species, repair.
Abbreviations: BER, base excision repair; CAT, catalase; ETC, electron transfer chain; \( \text{O}_2^{•−} \), superoxide anion radical; \( \text{OH}^{•−} \), hydroxyl radical; mtDNA, mitochondrial DNA; MLSP, maximum lifespan; \( \text{8-oxodG} \), 7,8-dihydro-8-oxoguanine; OGG1, 8-oxoguanine-DNA glycosylase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid.
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Figure 1  Map of human mtDNA

356 M. F. Alexeyev, S. P. LeDoux and G. L. Wilson

Figure 1  Map of human mtDNA

O

H

and O

L

, origins of heavy and light strand replication respectively; ND1–

ND6, NADH dehydrogenase (ETC complex I) subunits 1–6; Cox1–Cox3, cytochrome

oxidase subunits 1–3 (ETC complex IV); ATP6 and ATP8, subunits 6 and 8 of

mitochondrial ATPase (complex V); Cyt b, cytochrome b (complex III).

the heterodimeric DNA polymerase γ [13]. Replication

of mtDNA continues throughout the lifespan of an or-

ganism in both proliferating and post-mitotic cells. It oc-

curs bidirectionally, initiated at two spatially and tempo-

rally distinct origins of replication, O

H

and O

L

, for the

heavy and light strand origins of replication respectively

(for review, see [14]). The mitochondrial proteome is
dynamic, i.e. both the precise polypeptide composition

and the relative abundance of a given polypeptide may

vary in mitochondrial proteomes of different tissues as

well as in the same tissue over time [15]. With the dis-

ccovery of mitochondrial diseases caused by mutations of

mtDNA, it has been found that wild-type (normal) and

mutated mtDNA may coexist in the same cell, a condition
called heteroplasmy [16].

mtDNA DAMAGE AND THE MITOCHONDRIAL

THEORY OF AGING

Despite the fact that in animal cells mtDNA comprises

only 1–3 % of genetic material, several lines of evidence
suggest that its contribution to cellular physiology could
be much greater than would be expected from its size
alone. For instance, (i) it mutates at higher rates than

nuclear DNA, which may be a consequence of its close

proximity to the ETC (electron transfer chain); (ii) it

encodes either polypeptides of ETC or components

required for their synthesis and, therefore, any coding
mutations in mtDNA will affect the ETC as a whole;

this could affect both the assembly and function of the

products of numerous nuclear genes in ETC complexes;

(iii) defects in the ETC can have pleiotropic effects

because they affect cellular energetics as a whole.

Several lines of evidence indirectly implicate mtDNA

in longevity. The Framingham Longevity Study of Coro-
nary Heart Disease has indicated that longevity is more

strongly associated with age of maternal death than that

of paternal death, suggesting that mtDNA inheritance

might be involved [17]. On the other hand, longevity

was shown to be associated with certain mtDNA poly-
morphisms. Thus Italian male centenarians have increased

incidence of mtDNA haplogroup J [18], whereas French

and Japanese centenarians have increased incidences of

G → A transition at mt

9055

and C → A transversion at

mt

5178

respectively [19,20]. However, a study of an Irish

population failed to link longevity to any particular

mitochondrial haplotype, suggesting that factors other

than mtDNA polymorphism also may play a role in aging

[21].

Mitochondria have been shown to accumulate high

levels of lipophilic carcinogens such as polycyclic aro-
matic hydrocarbons [22,23]. When cells are exposed to

some of these compounds, mtDNA is damaged pre-

ferentially [24]. Other mutagenic chemicals also have

been shown to preferentially target mtDNA [23,25–29].

Therefore it is conceivable that life-long exposure to

certain environmental toxins could result in a preferential

accumulation of mtDNA damage and accelerate aging.

However, perhaps the most relevant kind of insult to

which mtDNA is exposed is oxidative damage. The lack

of protective histones and close proximity to the ETC,

whose complexes I and III are believed to be the pre-
dominant sites of ROS (reactive oxygen species) pro-
duction inside the cell, make mtDNA extremely

vulnerable to oxidative stress. Indeed, the free radical

theory of aging first put forward by Harman [30–33]

states that it is the mitochondrial production of ROS, such

as superoxide and H

2

O

2

, and the resulting accumulation

of damage to macromolecules that causes aging. Cumul-

ative damage to biological macromolecules was proposed
to overwhelm the capacity of biological systems to repair

themselves, resulting in an inevitable functional decline.
The mitochondrial theory of aging can be considered as

an extension and refinement of the free radical theory.

Its major premise is that mtDNA mutations accumulate
progressively during life and are directly responsible for
a measurable deficiency in cellular oxidative phosphe-
rilation activity, leading to an enhanced ROS production.

In turn, increased ROS production results in an increased
rate of mtDNA damage and mutagenesis, thus causing

a ‘vicious cycle’ of exponentially increasing oxidative
damage and dysfunction, which ultimately culminates in
death (Figure 2). Since Miquel et al. [34] first suggested
that mtDNA might be damaged in aging, numerous

studies over the past two decades have supplied a wealth

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Mitochondrial DNA and aging

(i) Oxidative damage in cells and, in particular, in mtDNA, is ubiquitous, substantial and, like mortality rates, increases exponentially with age [35]

It has been shown that exhalation of ethane and \( n \)-pentane, indicators of ROS-mediated lipid peroxidation increases with age [36]. mtDNA was shown to accumulate oxidative damage in an age-dependent manner in skeletal muscle [37,38], the diaphragm [39,40], cardiac muscle [41–43] and the brain [44]. Additionally, an age-related increase in oxidative damage to mtDNA appears to be greater than oxidative damage to nuclear DNA in houseflies [45,46]. In rodents, an age-related increase in 8-oxodG (7,8-dihydro-8-oxoguanine), a mutagenic DNA base lesion caused by oxidative stress, was observed in mtDNA isolated from the livers of both rats and mice [47]. Dietary restriction, which is known to retard aging and increase the lifespan in rodents, has been found to significantly reduce the age-related accumulation of 8-oxodG levels in nuclear DNA in all tissues of male B6D2F1 mice and in most tissues of male F344 rats. This study [47] also showed that dietary restriction prevented the age-related increase in 8-oxodG levels in mtDNA isolated from the livers of both rats and mice [47]. Another study [48] has found that the activities of DNA repair enzymes OGG1 (8-oxoguanine-DNA glycosylase), hypoxanthine- and uracil-DNA glycosylase increase in liver extracts of Wistar and OXYS rats with age. In both strains, OGG1 activities were approx. 10 times greater in nuclear extracts than in mitochondrial extracts [48]. However, while OGG1 activity in nuclear extracts remained relatively constant during the study, this activity increased with age in mitochondrial extracts. Importantly, in OXYS rats, which are characterized by the overproduction of ROS, high levels of lipid peroxidation, protein oxidation and decreased life span, the levels of mitochondrial OGG1 activity were greater than in normal Wistar rats, and an increase in this activity began earlier [48]. The increase in 8-oxodG levels in mtDNA with aging appears to be a general phenomenon and has been reported by de Souza-Pinto et al. [49] and Hudson et al. [50]. The steady-state concentration of 8-oxodG in mitochondrial DNA was shown to be inversely correlated with MLSP (maximum lifespan) in the heart and brain of mammals, i.e., slowly aging mammals show lower 8-oxodG levels in mtDNA than rapidly aging ones [51]. Furthermore, these authors show that this inverse relationship is restricted to mtDNA, because 8-oxodG levels in nuclear DNA were not significantly correlated with MLSP. The correlation between 8-oxodG and MLSP was better in the heart and the brain, possibly, in part, because these organs are composed predominantly of postmitotic cells [51].

(ii) Longer life expectancy in organisms belonging to the same cohort group is associated with relatively higher levels of antioxidants and lower concentrations of the products of oxygen free radical reactions

All houseflies lose the ability to fly prior to death. Therefore, in an aging population, shorter-lived flies can be identified as flightless ‘crawlers’ in contrast with their longer-lived cohorts, the ‘fliers’. The average lifespan of crawlers is about one-third shorter than the fliers. Levels of antioxidant defences [SOD (superoxide dismutase), CAT (catalase) and glutathione] and products of oxygen free radical reactions [inorganic peroxides and TBA (thiobarbituric acid) reactants] were compared between crawlers and fliers. The fliers showed greater SOD and CAT activities and glutathione concentrations than crawlers, whereas the amount of inorganic peroxides (\( \text{H}_2\text{O}_2 \)) and TBA reactants was higher in the crawlers than in fliers [52].

(iii) An experimentally induced decrease in oxidative stress retards age-associated deterioration at the organelle and organismic levels and extends chronological and metabolic life span

Simultaneous overexpression of Cu/ZnSOD and CAT in Drosophila was shown [53] to increase the maximum and average lifespan by one-third, to retard the age-related accumulation of oxidative damage to DNA and protein, to increase resistance to the oxidative effects of X-ray exposure, to attenuate the age-related increase in the rate of mitochondrial \( \text{H}_2\text{O}_2 \) generation, to increase the speed of walking and to increase the metabolic potential defined as the total amount of oxygen consumed per unit body weight during the adult life. Moreover, others have been...
able to extend *Drosophila* lifespan by overexpressing either MnSOD [54] or Cu/ZnSOD in motor neurons [55]. However, overexpression of Cu/ZnSOD or CAT alone in *Drosophila* failed to extend lifespan [56]. Additionally, overexpression of Cu/ZnSOD failed to increase longevity in mice [57]. However, MnSOD overexpression was found to extend chronological (G0) life span in yeast [58].

(iv) Variations in longevity among different species correlate inversely with the rates of mitochondrial generation of O$_2^*$ (superoxide anion radical) and H$_2$O$_2$

As has been mentioned above, a longer-lived subpopulation of *Drosophila* was found to have a lower rate of mitochondrial O$_2^*$ and H$_2$O$_2$ generation [52]. Similar results were observed in a recent cross-species study of bats, shrews and mice, where mitochondria from long-lived bats were found to produce half to one-third of the amount of H$_2$O$_2$ per unit of oxygen consumed compared with mitochondria from shrews and mice respectively [59].

(v) Restriction of caloric intake lowers steady-state levels of oxidative stress and damage, retards age-associated changes and extends the maximum life-span in mammals

Reducing dietary intake has been shown to be the most effective means for modulating the aging processes in laboratory rodents [60]. Dietary restriction has also been shown to be a modulator of membrane lipid peroxidation and cytosolic antioxidant status. Lee and Yu [61] studied the anti-ROS action of dietary restriction by quantifying the formation of the O$_2^*$, OH$^*$ (hydroxyl radical) and H$_2$O$_2$ by liver microsomes from rats of various ages. The results show that the ad *libitum*-fed group maintained a higher production of O$_2^*$ and OH$^*$ when compared with the food-restricted group of the same age. H$_2$O$_2$ formation followed the same trend but was statistically greater only at 3 and 6 months of age. The food-restricted group displayed higher SOD activity in both cytosolic and mitochondrial fractions compared with ad *libitum*-fed controls [61]. These data indicate that the ROS activity observed in liver microsomes of ad *libitum*-fed rats can be attenuated by dietary restriction, thereby providing a possible mechanism for its life-extending action.

(vi) mtDNA mutations are pathogenic and can increase ROS production by mitochondria

The discovery, in 1988, that mtDNA mutations can be pathogenic has provided a major support for the mitochondrial theory of aging. That year, several groups reported that both mtDNA point mutations [62] and deletions [63,64] could be the underlying cause of defined human pathologies. Moreover, being heteroplasmic, these diseases have revealed that not all of the mtDNA copies in a cell need to be mutated in order to achieve a disease state. The last decade has been characterized by an explosive growth in the number of mtDNA mutations implicated in human disease. The recent release of the Mitomap database lists almost 200 pathogenic point mutations, single nucleotide deletions and insertions (http://mitomap.org/). Not only have mitochondrial diseases demonstrated a causative link between mtDNA mutations and pathology, but they also have displayed, in agreement with the predictions of the mitochondrial theory of aging, an increased oxidative burden in patients suffering from these diseases [66–71]. Gross et al. [72] have reported differences in mtDNA turnover rates between tissues in the rat. Thus mtDNA turnover in postmitotic tissue (brain) was lower than that in liver. These findings led to the notion that the rate of mtDNA turnover might determine its susceptibility to oxidative damage and mutation and, thereby, control the rate of cell aging. Gene expression profiling of aging cells, performed recently, revealed an age-related decrease in the expression of genes involved in mtDNA maintenance, such as ERV1 [73]. The age-associated suppression of mtDNA replication and gene expression [74,75] may be associated with the accumulation of point mutations in the mtDNA control region.

**mtDNA REPAIR AND AGING**

In the nucleus, the steady state or induced levels of mutagenesis are determined by a balance between mutagenic insult and DNA repair. However, early studies revealed that mammalian mitochondria cannot repair UV-induced mtDNA damage [76,77]. Also, Miyaki et al. [26] suggested that repair of mtDNA after exposure to N-methyl-N'-nitro-N-nitrosoguanidine or 4-nitroquinoline-1 oxide was absent or very slow. These findings led to the belief that damaged mtDNA was destroyed and replaced with replicated undamaged DNA. However, through the use of technical advances in the analysis of DNA repair in specific sequences [78] and the treatment of the whole mitochondrial genome as simply a unique 16.5 kb DNA sequence, it was possible to show that some types of mtDNA damage can be efficiently repaired [79–88]. Currently, it is believed that mitochondria possess DNA BER (base excision repair) machinery, while lacking the nucleotide excision repair capacity [87,89].

mtDNA accumulates high levels of the mutagenic 8-oxodG, arguably the most important base damage caused by ROS, and it is widely believed to play a major
role in the aging process [47,50,90,91]. The important role played by DNA-repair enzymes in the accumulation of this lesion is underlined by the fact that liver mtDNA from knockout mice for OGG1 (the glycosylase that recognizes this lesion) accumulates 30 times as much 8-oxodG as mtDNA of wild-type control mice [91]. Interestingly, several studies have indicated that OGG1 activity in mitochondrial extracts from old rats is higher compared with extracts from young animals, which is apparently at odds with the observed accumulation of 8-oxodG in mtDNA from older animals [49,50,91,92]. However, this controversy was recently resolved by Szczesny et al. [93], who have shown that, in both hepatocytes from old mice and in senescent human fibroblasts, mitochondrial import of OGG1 is impaired and that a significant fraction of this enzyme remains localized in the outer membrane and intermembrane space in the precursor form. Because uracil-DNA glycosylase, another BER enzyme, has a similar age-dependent impairment in mitochondrial import, these authors [93] conclude that there appears to be a general deficiency in the import of BER enzymes into mitochondria from senescent animals and cells. Therefore, although age-related impairment in mitochondrial protein import does not necessarily extend beyond some DNA repair enzymes, it can explain the simultaneous accumulation of 8-oxodG and increased in vitro OGG1 activity in mitochondria from senescent animals in some settings. It should be noted, however, that other studies point out that the ability of mitochondria to import proteins is a dynamic function that can be modulated by various stimuli, such as thyroid hormone [94], which may provide an alternative explanation for the observed phenomena. Moreover, the rate of mitochondrial import of matrix chaperonins GRP75 (glucose-regulated protein 75) and HSP60 (heat-shock protein 60), which are essential for the import of precursor proteins, in fact increases with age [95]. Also, there is an apparent lack of consensus in the literature regarding the increase in mitochondrial OGG1 activity with aging in both senescent cells and animals. Some groups have reported a decline in both activity and expression of several key mitochondrial repair enzymes, including OGG1 [96–98]. Therefore, to date, there is no consensus on the question of whether DNA repair declines with age [99]. One of the possible explanations for such discrepancies is in the intrinsic variability between cell types and tissues with respect of their reliance on antioxidant defences compared with DNA repair for the maintenance of structural integrity of mtDNA. Results from our laboratory [100,101] suggest that the differential susceptibility of glial cell types to oxidative damage and apoptosis does not appear to be related to cellular antioxidant capacity, but rather to differences in their ability to repair oxidative mtDNA damage [100], and that different glial cell types differ in their capacity to repair oxidative damage [101].

CHALLENGES TO THE MITOCHONDRIAL THEORY OF AGING

Recently, the rate of ROS generation by mitochondria under physiological conditions, which is a cornerstone of the mitochondrial theory of aging, has been critically re-examined by several groups. Hansford et al. [102] have found that active H$_2$O$_2$ production (an indirect measure of O$_2^\cdot$· generation) requires both a high fractional reduction of complex I (indexed by NADH/NAD$^+$:NADH ratio) and a high membrane potential, $\Delta\Psi$. These conditions are only achieved with supra-physiological concentrations of succinate. With physiological concentrations of NAD-linked substrates, rates of H$_2$O$_2$ formation are much lower (less than 0.1 % of respiratory chain electron flux). This H$_2$O$_2$ production may be stimulated by the complex III inhibitor antimycin A, but not by myxothiazol [102]. Staniek and Nohl [103,104] reported further that mitochondria respiring on complex I and complex II substrates generate detectable H$_2$O$_2$ only in the presence of the complex III inhibitor antimycin. They also suggested that the rates of mitochondrial H$_2$O$_2$ production reported by others are artificially high due to flaws in experimental design [103,104]. Martin Brand’s group [105] capitalized on these findings and used an improved experimental design to show that mitochondria do not release measurable amounts of superoxide or H$_2$O$_2$ when respiring on complex I or complex II substrates, but release significant amounts of superoxide from complex I when respiring on palmitoyl carnitine. However, even at saturating concentrations of palmitoyl carnitine, in their estimation, only 0.15 % of the electron flow gives rise to H$_2$O$_2$ under resting conditions with a respiration rate of 200 nmol electrons·min$^{-1}$·mg$^{-1}$ of mitochondrial protein. Under physiological conditions, this rate should be even lower due to (i) lower partial oxygen pressure, (ii) lower concentration of palmitoyl carnitine and (iii) lower mitochondrial membrane potential. Therefore, under physiological conditions in cells with uncompromised antioxidant defences, ROS are produced by ETC in quantities that can be efficiently scavenged by mitochondrial antioxidant systems. As a consequence, no significant oxidative damage should be inflicted on mitochondrial components, including mtDNA due to electron leak from ETC, provided that cells have normal levels of antioxidants. This conclusion is in agreement with the observations of Orr et al. [106], who have recently re-examined their earlier findings and those of others on the effect of overexpression of antioxidant enzymes on extension of Drosophila lifespan. They have found that significant increases in the activities of both Cu/ZnSOD and CAT had no beneficial effect on survivorship in relatively long-lived y w mutant flies, and were associated with slightly decreased life spans in wild-type flies of the Oregon-R strain. The introduction of additional
The concerted effort of several groups (including that of that found in aged tissues. However, very recently [115], of mtDNA mutations to the level three times higher than significant phenotypic changes, despite the accumulation amounts of deleted mtDNA. This increase in somatic in the levels of point mutations, as well as increased mtDNA mutator phenotype with a 3–5-fold increase 2004 The Biochemical Society lived strains of type levels does not decrease the rate of aging in long-lived strains of Drosophila, although there may be some effect in relatively short-lived strains [106]. In line with this conclusion, Van Remmen et al. [107] in their study of mice heterozygous for the MnSOD gene knockout have found that, although life-long reduction of MnSOD activity leads to increased levels of oxidative damage to mitochondrial and nuclear DNA and increased cancer incidence, it does not appear to affect aging.

A report on the requirement of cytosolic CAT for phenotypic lifespan extension in Caenorhabditis elegans daf-C and clk-1 mutants [108] was recently retracted [109], dealing another blow to the notion that lifespan can be routinely enhanced by increasing antioxidant defences. Rasmussen et al. [110,111] assayed 13 different enzyme activities using optimized preparation techniques and found that the central bioenergetic systems, including pyruvate dehydrogenase, tricarboxylic acid cycle, respiratory chain and ATP synthesis, appeared unaltered with age. Maklashina and Ackrell [112] have recently critically examined the literature regarding the role of ETC dysfunction in aging. They conclude that the available evidence for age-related inactivation of the respiratory chain can be challenged because of the preparation purity and the use of inadequate assay procedures, and that recent experimental evidence does not support the mitochondrial theory of aging.

In contrast, Jacobs [113] does not so much challenge experimental evidence supporting the mitochondrial theory of aging as to point out that all the evidence available to date is indirect in its nature. He argues that studies performed so far do not address the critical issue of cause and effect, i.e. does the somatic mutation of mtDNA result in oxidative phosphorylation dysfunction and increased oxidative stress? Does increased oxidative stress promote mtDNA mutagenesis? Finally, he points out that the results from his own laboratory [114] suggest that, at least in a tissue culture model, progressive accumulation of mtDNA mutations due to expression of proof-reading-deficient DNA polymerase γ does not lead to significant phenotypic changes, despite the accumulation of mtDNA mutations to the level three times higher than that found in aged tissues. However, very recently [115], the concerted effort of several groups (including that of Howard Jacobs) led by Nils-Göran Larsson resulted in the generation of homozygous knock-in mice that express a proofreading-deficient catalytic subunit of mitochondrial DNA polymerase γ. These mice develop an mtDNA mutator phenotype with a 3–5-fold increase in the levels of point mutations, as well as increased amounts of deleted mtDNA. This increase in somatic mtDNA mutations is associated with reduced lifespan and the premature onset of age-related phenotypes such as weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spine), osteoporosis, anaemia, reduced fertility and heart enlargement [115]. Thus results of this study provide the best evidence so far for a causative link between mtDNA mutations and aging phenotypes in mammals. However, as these authors concede, the detailed kinetics of the accumulation of somatic mtDNA mutations remain to be elucidated. The mutation load in the brain of mutant mice at 2 months of age is already 2–3-fold greater than in 6-month-old wild-type littersmates. This, and the rather uniform mutation loads between tissues, suggests that much of the accumulation of mutations may occur during embryonic and/or fetal development. Also, the onset of premature aging in this model is not accompanied, temporally, by a large de novo accumulation of mtDNA mutations around 6 months. Therefore it appears plausible that the premature onset of aging in this model is the result of the cumulative physiological damage caused by the high mutation load present during adult life and/or to segregation or clonal expansion of specific mutations, as supported by the observed mosaicism for the respiratory chain deficiency found in the heart. However, since the effects of high mutational burden in mtDNA during embryonic and fetal development are poorly understood, one has to consider the possibility that premature aging in this model is predetermined at the prenatal, rather than postnatal, stage. If this holds true, then the issue of causative relationship between mtDNA mutations and normal aging remains open, since, in normal aging, accumulation of mtDNA mutations occurs postnatally. The observation that knock-in mice show some features (e.g. alopecia) that are more characteristic of human, rather than mouse aging lends some credulity to the notion that a high mutational load in mtDNA at the prenatal stage might be an important limitation of this model. Clearly, a model with inducible mtDNA mutator phenotype should help to resolve many of these outstanding issues. Finally, mtDNA mutations in this study [115] are generated by a mutator DNA polymerase γ rather than by oxidative DNA damage. Therefore this study does not address one of the central premises of the mitochondrial theory of aging, namely that oxidative mtDNA damage is the driving force behind the accumulation of mtDNA mutations. In the preceding paragraphs we have discussed support provided for the mitochondrial theory of aging by the discovery of mitochondrial disease. One might then reasonably expect, based on the mitochondrial theory of aging, that mitochondrial ROS would be causative in a significant fraction of pathogenic mtDNA mutations. However, an analysis of 188 pathogenic mtDNA point mutations [65] revealed that the mutagenic effect of 8-oxoG, widely regarded as a prime lesion resulting from an oxidative insult to DNA, can be implicated in...
the aetiology of only a few mutations. Indeed, un repaired 8-oxodG in mtDNA can pair with both C and A with almost equal efficiency resulting in G → T (and C → A on complementary strand) transversions, which account for only 5.9 % of pathogenic mtDNA mutations. Even when the potentially mutagenic pool of 8-oxodGTP (8-oxodeoxyGTP), the product of cytoplasmic/matrix dGTP pool oxidation is taken in consideration (T → G and A → C transversions), the cumulative impact of both types of mutation is still only 8.5 %. For comparison, 82 % (almost 10 times as many) of the pathogenic point mutations in mtDNA can be attributed to deamination of adenine and cytosine. 8-oxodG is not a prime oxidative mutagenic lesion, because it is efficiently repaired by BER pathways. Thus the exact factors required for the accumulation of point mutations have yet to be fully defined. Oxidative DNA damage can produce a variety of base lesions whose mutagenic potential has not been fully elucidated [116]. Therefore it is possible that other, less prominent and, at present, unidentified lesions are responsible for the bulk of ROS-mediated mutagenesis. Alternatively, it can be postulated that ROS do not play a major role in mtDNA mutagenesis.

CONCLUSIONS

Aging is a complex multifactorial process which we have only begun to understand. Although the available evidence strongly suggests that mitochondria play a role in this process, there appears to be a wide range of opinions as to the exact nature of the involvement of mitochondria in aging. In this review, we have made an attempt to present in a balanced fashion the existing views on the involvement of mtDNA in aging. Clearly, more research must be done to fully elucidate how damage to mtDNA contributes to the aging process. It is possible that the seemingly contradictory results of different studies will be reconciled or explained through the use of integrative approaches and unified model systems. The next few years of aging research should prove exciting as our knowledge expands dramatically as the results of studies from several laboratories, now experimentally testing the mitochondrial theory of aging, are published.

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Mitochondrial DNA and aging

363


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