The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations

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Mitochondria are cellular energy factories that generate ATP via the reaction of hydrocarbons with oxygen. Every human cell contains hundreds of mitochondria, and each mitochondrion contains multiple copies of mitochondrial DNA (mtDNA). The ancestry of the mitochondrial genome can be traced to early eubacteria, and it is therefore unexpected that this organelle may have a major role in governing the pace of human aging. Three recent papers (1–3) plus a work published in a recent issue of PNAS (4) have demonstrated that accelerating the mtDNA mutation rate can result in some features suggestive of premature aging, consistent with the view that loss of mitochondrial function is a major causal factor in aging.

mtDNA polymerase (Pol-γ) is the only DNA polymerase that is known to be targeted to and that resides in mitochondria (5). In the absence of other known mtDNA polymerases, it is assumed that Pol-γ is responsible for all aspects of mtDNA synthesis, including replication of the mitochondrial genome and repair of DNA damage. As is the case for many other DNA polymerases, the high fidelity of Pol-γ derives from both selection for the correct incoming nucleotide and excision of misincorporated nucleotides by a 3′ → 5′ exonucleolytic proofreading activity (6). Elimination of proofreading by replacement of critical aspartic acid residues in the exonuclease domain with alanine increases misincorporation; *in vitro*, this misincorporation is manifested predominantly as single base substitutions (7). Both the Larsson (1) and Prolla (2) groups replaced in embryonic stem cells POLG, the gene encoding wild-type POLG, with a mutant that encodes an error-prone active Pol-γ and generated mice that accumulate mitochondrial mutations with increasing age. The mutations were predominantly single-base substitutions and deletions, the latter presumably retained by *in vivo* selection. Although apparently normal at birth, the mice exhibited at an early age many of the phenotypes characteristic of human aging (but perhaps not characteristic of murine aging). The results clearly indicate that Pol-γ is responsible for the attenuated lifespan and progeroid features in these mice. Conversely, Schriner *et al.* (3) targeted catalase to mitochondria and observed an extension of both the median and maximum lifespan in mice. The extension of lifespan was in association with reduced damage to mtDNA and increased mitochondrial resistance to exogenous reactive oxygen species (ROS) damage.

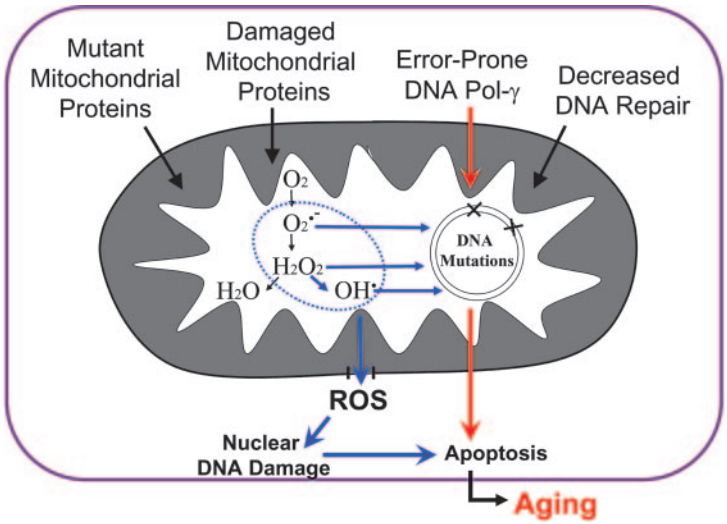

Fig. 1. Mitochondrial DNA damage and aging. Multiple factors may impinge on the integrity of mitochondria that lead to loss of cell function, apoptosis, and aging. The classical pathway is indicated with blue arrows; the generation of ROS (superoxide anion, hydrogen peroxide, and hydroxyl radicals), as a by-product of mitochondrial oxidative phosphorylation, results in damage to mitochondrial macromolecules including the mtDNA, the latter leading to deleterious mutations (3). When these factors damage the mitochondrial energy-generating apparatus beyond a functional threshold, proteins are released from the mitochondria that activate the caspase pathway leading to apoptosis, cell death, and aging. The findings of Trifunovic *et al.* (4) as well as those of Kujoth *et al.* (2) now demonstrate that the introduction of excessive mutations in the mtDNA (red arrows) via an error-prone Pol-γ can sufficiently impair mitochondrial function as to result in many of the manifestations of aging without causing a further increase in ROS.

Trifunovic *et al.* (4) now address the mechanism for accelerated aging in POLG mutator mice. Do the mitochondrial mutations introduced by Pol-γ result in mutant mitochondrial proteins that are defective in coupling of oxygen metabolism with ATP causing increased ROS production, DNA damage, and mutations? This scenario would be consistent with the venerable "free radical theory of aging" (8). However, they find little or no evidence for the key intermediates in this cycle, ROS, although the production of ROS in very old animals was not examined. Despite an accumulation of mitochondrial mutations in POLG mice and the presence of "respiratory-chain dysfunction," as measured by a 95% reduction in O_2 consumption, they observed no increase in damaged proteins in the heart and liver in the mutator mice. Moreover, the mice did not exhibit a reduction in mitochondrial aconitase activity, a classic marker of oxidative damage to protein. In addition, mRNA levels of enzymes that scavenge ROS were unaffected, suggesting the lack of an ROS-induced stress response. Embryonic fibroblasts derived from the POLG mice and pre-

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sumably cultured in ambient oxygen [to which primary mouse fibroblasts are exceptionally sensitive (9)] exhibited no heightened sensitivity to H_2O_2 and no enhanced generation of superoxide. Thus, it was concluded that the mice bearing the mutant Pol-γ exhibited substantial respiratory dysfunction in the absence of evidence of enhanced ROS production. Presumably, the respiratory dysfunction in these mutant animals is the result of mitochondrial mutations generated by the mutant polymerase during mtDNA replication. This same inference applies to comparable experiments carried out by Kujoth *et al.* (2), although those authors emphasized an important role for apoptosis in the generation of progeroid features and reduced lifespan.

There are several explanations that could account for the lack of increased production of ROS and oxidatively damaged macromolecules in POLG mutator mice. First, there may be many pathways that operate concertedly to induce aging in mice. The introduction of an error-prone Pol-γ may be one of these and is able to produce manifestations of premature aging in the absence of enhanced ROS production by artificially increasing the mutation rate (Fig. 1). It would now be important to determine whether mutations in Pol-γ that render the enzyme more accurate might reduce the accumulation of mtDNA mutations and extend lifespan. Second, alterations in Pol-γ may be downstream from mechanisms that generate ROS. Thus, damage to Pol-γ that renders the enzyme error-prone might be the re sult of protein damage by ROS. Experimental verification of the latter mechanism would involve the demonstration that Pol-γ is damaged by ROS and that this damage results in an error-prone polymerase. Third, the introduction of an error-prone DNA polymerase may cause extensive mutations throughout the mitochondrial genome that prevent the generation of ROS. This premise is consistent with the 95% reduction in oxygen consumption in POLG mouse embryo fibroblasts (4).

Many factors have the potential to shorten lifespan, and mutations in POLG may be one factor.

Although we feel that the results of these important experiments confirm that the loss in mtDNA integrity and mitochondrial function are central factors in aging, they do not necessarily negate a role for mitochondrial-generated ROS in the normal aging process. In the course of normative aging, mtDNA mutations, respiratory dysfunction, and apoptosis mediated by the mitochondrial permeability transition pore (10) could result from oxidative damage to mitochondrial macromolecules rather than from infidelity of Pol-γ. In normal aging, a role for the emergence of mutator POLG molecules also needs to be considered, and it will be important to determine whether Pol-γ is rendered inaccurate either by mutation or by protein oxidation during aging. The generation of an error-prone Pol-γ could result in further mutations and increased cell loss during aging. Finally, there are many factors that have the potential to shorten lifespan, and indeed mutations in POLG may be one of these factors. As a result, experiments that shorten lifespan are less informative than those that extend healthy lifespan. It therefore will be of great interest to evaluate the life histories of mice bearing Pol-γs with unusually robust fidelities.

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