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# The Role of Genome Instability in Frailty: Mitochondria versus Nucleus

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**Abstract**

Late-life aging in humans is often associated with severe frailty. This suggests catastrophic events reaching an undeniable biological threshold in cellular stability and a rapidly diminished homeostasis. The driving force of the syndrome is likely 'genetic instability' or 'genomic instability', a high frequency of mutations and deletions within the genome (both nuclear and mitochondrial DNA) of bodily somatic cells caused by DNA damage and inefficient repair. Reactive oxygen species, calcium deregulation, and iron dyshomeostasis are potential chemical triggers of nucleic acid sequence alterations and chromosomal rearrangements. These include mutations, deletions, translocations, chromosomal inversions, and single- and double-strand DNA breaks. Nuclear damage, such as telomere shortening, also appears to cause an abnormal expression of several proteins, including p53, which leads to impaired mitochondrial biogenesis, mitochondrial permeability transition pore opening, apoptosis, and other biological events. Moreover, mitochondrial DNA damage could produce inaccurate translation and synthesis of proteins important for energy production in the inner mitochondrial membrane. Another cause of genomic instability may be a reduced expression and function of DNA repair genes, especially when stressful events trigger slow responses. With late-life frailty, overall endogenous damage occurs much more frequently and repair is much less efficient, which further accelerates genomic instability.



## Introduction

Frailty increases an individual's susceptibility to age-associated cardiovascular, metabolic, and inflammatory disorders. Moreover, a strong association between frailty and cognitive impairment was observed in elderly human populations,

suggesting frailty is not just limited to pathophysiological impairment, but also attributes to psychological weakness caused by hormonal/neural changes. Phenotypic characteristics of frailty, therefore, accompany multidimensional impairments in immune/inflammatory control and hormonal/neuromuscular regulation, and are associated with alterations in cellular metabolic/redox responses that occur during aging [1].

## Cellular and Mitochondrial Mechanisms of Nuclear Genome Instability

Several lines of correlative evidence indicate that genomic instability gradually increases with increasing age in humans. Thought to be a causative driver of frailty, genomic instability reprograms nuclear gene expression, thereby impairing cellular homeostatic maintenance/repair function (reviewed by Bellizzi et al. [2]). Various in vitro studies suggest that alterations in genomic integrity by abnormal DNA methylation deregulate genes related to cell cycle/death regulation, DNA damage repair, and cell metabolic homeostasis in aging humans [3]. Besides this epigenetic deregulation, intrinsic chromosomal DNA instability generates an extrachromosomal DNA structure, potentially decreasing nuclear genome stability, but these mechanisms are not yet understood. A recent study reported that microDNA (extrachromosomal DNA structure) occurs in mouse/human cell lines and adult brain tissues [4], suggesting a potential risk factor that increases nuclear genome instability during the human aging process. A similar deteriorative role of extrachromosomal DNA structures in nuclear genome integrity is well described in a budding yeast-replicative aging model [5]. Ribosomal DNA (rDNA) circles, which inherently form due to yeast genomic instability of the rDNA locus, propagate while yeast cells undergo asymmetric division cycles. This asymmetric cell division unequally accumulates rDNA circles mostly in mother cells, and rDNA circles diminish their nuclear genome stability and cell viability. Interestingly, preventing abnormal accumulation of this extrachromosomal circular DNA by genetic, pharmacological, or metabolic means (e.g. redistribution of sirtuin proteins to the nucleolus, rapamycin, and calorie restriction) significantly increases the mitotic capacity and viability of cells. This suggests that intrinsic risks in nuclear DNA possibly increase chromosomal abnormality with advancing age in humans. Whether extrachromosomal DNA directly or indirectly impacts nuclear genome instability and drives frailty in human aging remains to be tested.

Another genetic factor that directly compromises eukaryotic genome integrity is the progressive loss of telomeres (repetitive nucleotide sequences at each end of the chromatids). Telomeres protect chromosomes from being progressively shortened or fused into adjacent chromosomes while cells replicate their nuclear DNA.

Loss of telomeric tails perturbs genomic DNA replication and promotes cell death signals, especially in the non-germ line stem cell population; this is due to the lack of telomerase (the enzyme that extends telomere length) activity. Shortening telomeres can also increase chromosome abnormality by inappropriate DNA recombination and thus increase the cancer risk [6]. Loss of telomere integrity during aging or aging-related disease processes, therefore, possibly forms a cellular basis for the mechanisms of frailty. Indeed, few studies have attempted to reveal a biological association between telomere length and frailty phenotype in humans [7].

A recent remarkable study by DePinho and colleagues further connects telomere abnormality to subcellular organellar dysfunction by identifying a mechanistic link between telomere instability and mitochondrial dysfunction [8]. Using telomerase reverse transcriptase knockout (*Tert*$^{-/-}$) mice, whose telomere becomes extremely short due to the lack of telomere biogenesis after several generations, the authors found that telomere dysfunction deactivates peroxisome proliferator-activated receptor-γ co-activator (PGC) 1α and PGC1β, and accelerates aging phenotypes across different tissues in premature *Tert*$^{-/-}$ mice. Since PGC1α and PGC1β regulate mitochondrial protein and genome (mitochondrial DNA, mtDNA) biogenesis, *Tert*$^{-/-}$ mice exhibit defective mitochondrial biogenesis and metabolic activity. Surprisingly, activation of p53 caused by telomere dysfunction transcriptionally represses both PGC1α and PGC1β activities. These data indicate that loss of telomere integrity can lead to mitochondrial dysfunction through the negative effect of p53 on mitochondrial biogenesis. It is therefore possible that loss of nuclear genome integrity activates p53 and represses mitochondrial biogenesis while accumulating damaged mitochondria in aging cells and tissue (fig. 1). This would diminish mitochondrial metabolic function and lead to a gradual decline in overall cell/tissue functions in aged organisms. To the best of our knowledge, this report is the first to link and unify the important theories of aging, such as the mitochondrial and nuclear damage event theories.

A master regulator of programmed cell death pathways, p53, functions either by increasing the levels of apoptosis inducers such as AIP1, Bax, and Fas, or by modulating the activities of Bcl-2 family signals [9]. In addition to having these canonical roles in the regulation of apoptosis, p53 can directly impact mitochondrial signals and their metabolic function. Recently, Vaseva et al. [10] reported that p53 activation upon DNA damage redistributes cytosolic p53 into the mitochondrial matrix, and mitochondrial p53 destabilizes the mitochondrial permeability transition pore (mPTP) complex by interacting with one of the mPTP components (i.e. cyclophilin D). A nonselective pore that resides on mitochondrial inner and outer membranes, mPTP, regulates mitochondrial membrane potential by channeling nonorganic ions, such as calcium. This p53-cyclophilin D interaction triggers mPTP opening, increases cytosolic calcium levels, and

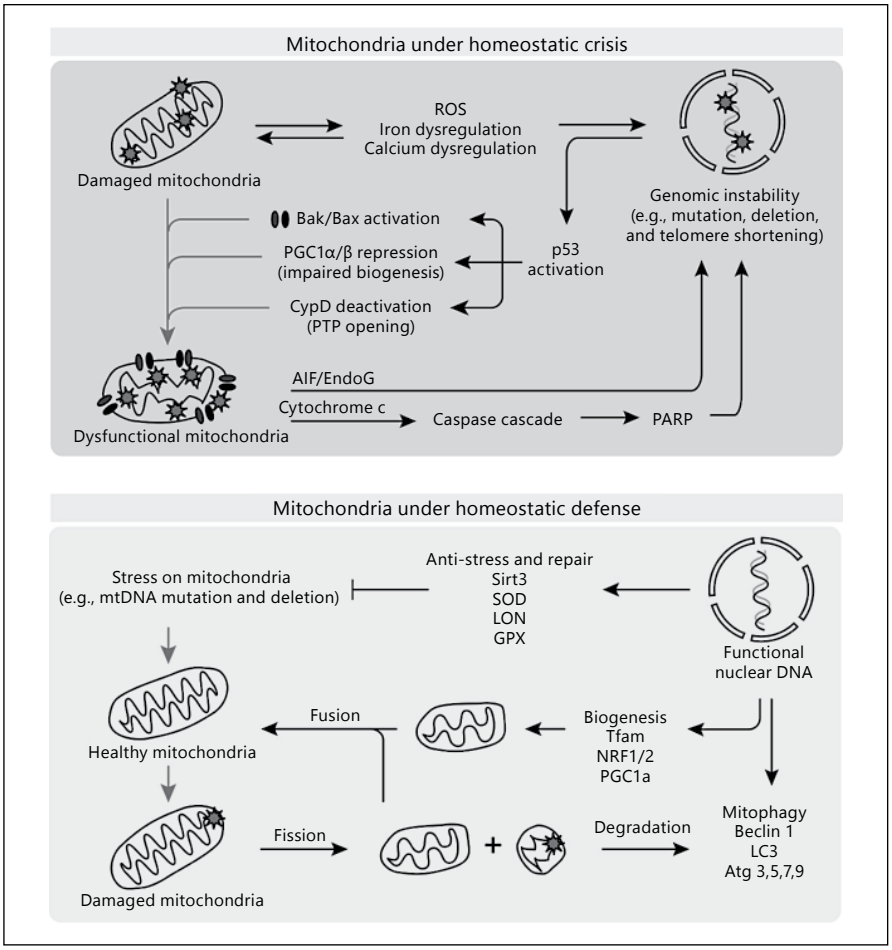


**Fig. 1.** Homeostatic interplay between mitochondria and nucleus for the maintenance of genomic integrity in aging cells. Age-associated mitochondrial damage (e.g. mtDNA mutations and deletions) gradually increase mtDNA instability, which eventually augment nuclear DNA damage and genomic instability along with telomere shortening in aging cells. Without successful DNA damage repair, accumulating abnormalities in the genome (both mitochondria and nucleus) activate p53. Then, activated p53 increases cellular susceptibility towards cell death by inducing apoptosis-regulatory genes (e.g. Bax/Bak) and triggering mitochondrial permeability transition through opening of a channel, the mPTP. As a transcriptional repressor, p53 decreases mitochondrial biogenesis by inhibiting PGC1α and PGC1β activity, leading to a global loss of mitochondrial structural/functional fidelity. Dysfunctional mitochondria also directly affect nuclear genome integrity by perturbing ISC biogenesis or activating regulators in subcellular endonuclease pathways [e.g. apoptosis-inducing factor (AIF), endonuclease G (EndoG), and poly(ADP-ribose) polymerase (PARP)]. In contrast, keeping healthy/functioning mitochondria allows aging cells to increase genome stability by homeostatic defense pathways. A coordinated interplay between mitochondrial dynamics, mitochondrial biogenesis, and degradation (i.e. mitophagy) would play a pivotal role in maintaining mitochondrial quality and genomic stability. CypD = Cyclophilin D.

induces necrotic cell death, although cyclosporine A (an mPTP inhibitor) abolishes this cascade of cell death reactions. A direct interplay, therefore, exists between nuclear DNA damage, p53 activation, and mitochondrial dysfunction. Augmented nuclear genomic instability caused by telomere shortening or other damage occurring in DNA can initiate p53 activation and subsequently turn on cell death signaling (fig. 1a). Concurrently, p53 activation diminishes mitochondrial function by destabilizing mPTP, eventually resulting in overall physical, physiological, or psychological weakness in the old and frail.

Given the fact that even healthy mitochondria inevitably leak electrons during metabolic activity, mitochondrial dysfunction can significantly proliferate cellular reactive oxygen species and damage various subcellular compartments, including endogenous nuclear DNA. This can gradually increase nuclear genome instability. In addition, recent studies suggest that defective mitochondrial activity can increase nuclear genome instability independent of the redox status, which is regulated by low-weight molecular compounds, such as NADPH, GSH, and key antioxidant enzymes. Veatch et al. [11] reported that defective iron-sulfur cluster (ISC) biogenesis due to mtDNA loss decreases cytosolic ISC and impairs the assembly of ISC-containing protein(s) specifically required to maintain nuclear genome stability in yeasts. A similar mechanism by which ISC availability affects nuclear genome stability is conserved in higher organisms [12], suggesting that a decrease in mitochondrial function can directly impair nuclear genome integrity through defective ISC synthesis by mitochondria. These findings provide another cell mechanism by which mitochondrial metabolic function, although not necessarily limited to energy metabolism, plays a pivotal role in the maintenance of nuclear genome stability.

Taken together, intrinsic defects in genomic DNA structures (e.g. telomere shortening) and extrinsic damage to the nuclear genome due to defective mitochondrial function (e.g. impaired redox status, metabolic function, and cell death homeostasis) seem to be largely responsible for the age-dependent loss of nuclear genomic stability. Thus, keeping healthy mitochondria during the cell senescence process is critical not only for enhancing the mitochondrial canonical function in cell metabolism and survival, but also for maintaining an intact nuclear genome (fig. 1).

## Mitochondrial Quality Control and Nuclear Genome Stability

While there are multidimensional processes required for maintaining healthy mitochondrial pools in cells, at least three different pathways must interplay correctly: mitochondrial fusion and fission dynamics, biogenesis, and degradation

(reviewed by Seo et al. [13]). Recent studies reveal that mitochondria are highly motile and remarkably plastic in their structure. This dynamic property, which is regulated by the fusion and fission processes, is critical for active changes in mitochondrial function under various environmental stimuli (i.e. oxidative stress and apoptotic signals). Without this structural dynamic, mitochondria immediately lose their genomic integrity, increase redox and cell death stress, and become metabolically dysfunctional. Thus, it is not surprising to observe that genetic defects in the fusion and fission processes are often related to severe developmental abnormalities, neuromuscular degeneration, and metabolic disorders in various animal models and in humans. Furthermore, gradual deregulation of mitochondrial dynamics might be one of the intrinsic causes of mitochondrial malfunction in the aging process. How the fusion and fission dynamics modulate mitochondrial function, however, is still unclear, and whether this structural dynamism can directly or indirectly induce any changes in nuclear genome integrity in aging cells remains unknown.

An aberrant mitochondrial structure caused by defective fusion and fission events potentially impairs mitochondrial quality control. By accumulating unrepaired (malfunctioning) mitochondria in aging cells, this indirectly diminishes nuclear genomic stability, as suggested previously. To remove dysfunctional mitochondria, cells operate a lysosome-mediated degradation pathway called autophagy. Autophagy degrades entire mitochondria when autophagosomes (specialized double-membrane structures) encapsulate and deliver them into the lysosome. During this process, mitochondria must undergo constant division and fusion cycles to isolate the damaged mitochondria from the healthy ones. Unopposed mitochondrial fusion leads to mitochondrial enlargement and inhibits autophagic mitochondrial turnover, since autophagosomes cannot engulf enlarged mitochondria that are larger than their physical limit [14]. Indeed, enlarged (or swollen) mitochondria often accumulate in aging cells and exhibit diminished metabolic activity, possibly due to inefficient mitochondrial turnover [15]. On the other hand, mitochondrial fusion must follow immediately after mitochondrial division to protect isolated healthy mitochondrial pools from being randomly digested by nonselective autophagic activity [14]. Without fusion, unwanted mitochondrial degradation would occur, which can accelerate mitochondrial loss in aging organisms. Therefore, both mitochondrial fission and fusion play an important role in degrading and protecting mitochondria during the organellar quality control and in maintaining their function (fig. 1b), thus securing nuclear genome integrity.

While mitochondrial fission increases the organellar number, the fusion process might be involved in mitochondrial biogenesis [13]. Since fused mitochondria can synchronize their electrochemical gradient potential, which is

required for active import and/or exchange of genetic/enzymatic materials between organelles, mitochondrial biogenesis should be more efficiently facilitated in the fused mitochondrial network than the unfused ones. Furthermore, the fact that some mitochondrial fusion components (e.g. MFN2) colocalize onto both endoplasmic reticulum and mitochondria suggests that mitochondrial fusion might help their biogenesis by forming a direct bridge between mitochondria and endoplasmic reticulum by enhancing the protein import efficiency of nuclear-encoded mitochondrial gene products to newly growing mitochondria. Reinforcing this, activation of AMP-activated protein kinase, which is the master regulator of mitochondrial biogenesis, concurrently promotes mitochondrial fusion activity by posttranslational deactivation of fission machineries [unpubl. data]. Moreover, PGC1α directly regulates mitochondrial fusion gene (i.e. MFN2) expression together with triggering mitochondrial biogenesis genes, which suggests that mitochondrial fusion and biogenesis potentially depend on each other [16]. Given the fact, then, that proper mitochondrial biogenesis is necessary for maintaining healthy mitochondria, mitochondrial quality control relies on a well-balanced mitochondrial fission and fusion activity. In support of this, impaired mitochondrial fusion and fission activities coincide with diminished mitochondrial quality, content, and function in age-related metabolic diseases (e.g. obesity and type 2 diabetes) [17].

Taken together, a coordinated interplay between mitochondrial fission and fusion dynamics, degradation, and biogenesis allows aging cells to maintain mitochondrial repair and quality control (fig. 1b). This delicate balance is lost in old age, and especially in frail elderly under acute stress, leading to comorbidities and increased mortalities. Several combination therapies (nutritional, hormonal, and pharmaceutical) implemented earlier in life could alter the number of comorbidities and improve the quality of life of the elderly.

## Concluding Remarks

Late-life aging in humans is often associated with severe frailty, which increases an individual's susceptibility to age-associated cardiovascular, metabolic, and inflammatory disorders as well as cognitive and physical weakness. Although cellular mechanisms underlying the frailty syndrome are still largely unknown, an altered balance between damage repair of mitochondrial and nuclear material as well as associated altered cellular signaling play a driving force to aging and frailty. Upon augmented nuclear genome instability, nuclear DNA signals (e.g. p53, Bax, and Bak) become overactivated, resulting in impaired

mitochondrial biogenesis, mPTP opening, apoptosis, and an overall rapid decline in cellular viability. The healthy interaction between the mitochondria and nuclear events (mitochondrial dynamics/mitophagy) are lost during cellular aging. These are critical processes for maintaining an intact nuclear genome, preventing loss of mtDNA and nuclear DNA content, and quality. In this review, we briefly discussed potential molecular mechanisms related to changes in genomic instability (mitochondria/nucleus) and their interactive roles in the causes of frailty.


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## Disclosure Statement

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