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Review

Mitochondrial DNA: A disposable genome?

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ABSTRACT

In mammalian cells, mitochondria are the only organelles besides the nucleus that house genomic DNA. The mammalian mitochondrial genome is represented by prokaryotic-type, circular, highly compacted DNA molecules. Today, more than a half-century after their discovery, the biology of these small and redundant molecules remains much less understood than that of their nuclear counterparts. One peculiarity of the mitochondrial genome that emerged in recent years is its disposable nature, as evidenced by cells abandoning a fraction of their mitochondrial DNA (mtDNA) in response to various stimuli with little or no physiological consequence. Here, we review some recent developments in the field of mtDNA biology and discuss emerging questions on the disposability and indispensability of mtDNA.

1. Introduction

In mammalian cells, mitochondria are cellular organelles that generate the bulk of ATP required to sustain a plethora of diverse cellular processes. Besides generating ATP, mitochondria also play important roles in intracellular calcium signaling, apoptosis, reactive oxygen species (ROS) production and detoxification, and biosynthesis of heme and iron–sulfur clusters.

Mitochondria are encapsulated by two membranes which create four compartments: 1) the outer membrane, 2) the intermembrane space, 3) the inner membrane and 4) the matrix. The inner mitochondrial membrane houses five complexes of the oxidative phosphorylation (OXPHOS) system, of which complexes I–IV comprise an electron transport (respiratory) chain. The mitochondrial matrix houses numerous anabolic and catabolic enzymes, most prominently enzymes involved in the Krebs cycle and β-oxidation of fatty acids. The matrix is also the compartment that houses mitochondrial DNA (mtDNA).

In humans, mtDNA is a circular 16,569 bp molecule (Fig. 1) which encodes 37 "formal" genes: 13 polypeptides and 24 RNA components of the mitochondrial translational apparatus (2 rRNAs and 22 tRNAs). mtDNA is characterized by a tight packaging of genetic information: mitochondrial genes in mammals lack introns, and intergenic sequences are either absent or limited to a few bases. In some instances, genes overlap (*MTATP8/6* and *MTND4L/4*), and translational termination codons of six mitochondrial genes are not encoded in the mitochondrial genome, but are completed by the addition of a polyadenine (polyA) tail to a terminal uracil encoded in mtDNA (thus generating sequence $(UAA)_{TER}A_n$). At the same time, mtDNA harbors a large ~1100 bp region that does not code for any genes, a so-called control region, which overlaps with a D-loop, a ~1000 nucleotide triple-helical region, which is formed by abortive initiation of replication.

The base distribution between two strands of human mtDNA (44% vs. 56% purine content, GenBank NC_012920.1) is asymmetric enough to allow for their separation in denaturing buoyant density gradients of cesium chloride. It is frequently stated that the 'Heavy' (H) strand encodes the majority of mitochondrial genes (12 polypeptides, 2 rRNAs and 14 tRNAs), and the 'Light' (L) strand encodes the rest. This notation is historical [1], and according to present-day convention (and the convention used in the original report on the sequence of human mtDNA [2]), the L-strand is the coding strand for the majority of mitochondrial genes. All of the mitochondrially encoded polypeptides are components of the OXPHOS complexes I (MTND1-6 plus MTND4L), III (MTCYB), IV (MTCO1-3) and V (MTATP6 and 8). Notably, all subunits of the mitochondrial complex II, all proteins responsible for mtDNA replication and repair, as well as all proteins responsible for transcription and translation of the mitochondrial genome are encoded in the nucleus.

1.1. Non-canonical mitochondrial genes

In recent years, it became increasingly apparent that in addition to its 37 "formal" genes, mtDNA may encode short open reading frames (ORFs) which can be translated into peptides with important biological

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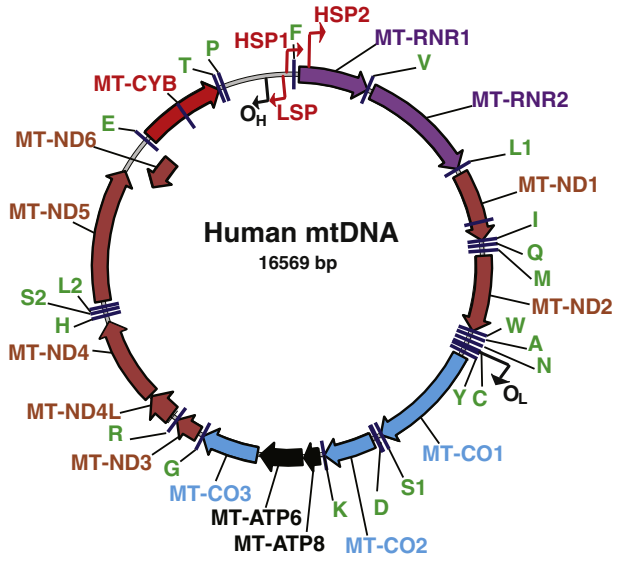

Fig. 1. The map of human mtDNA. The orientation is according to the revised Cambridge Reference Sequence (rCRS, GenBank NC_012920.1). Black bent arrows designate origins and direction of new H-strand (O_H) and L-strand (O_L) synthesis during replication (or, conversely, origins of L-strand and H-strand replication), respectively. Red bent arrows designate mitochondrial promoters that transcribe either H-strand (HSP1 and HSP2) or L-strand (LSP), respectively. Brown arrows: MT-ND1-MT-ND6, NADH dehydrogenase subunits 1 through 6. Blue arrows: MTCO1-MTCO3, cytochrome oxidase subunits 1 through 3. Black arrows: MT-ATP6 and MT-ATP8, subunits 6 and 8 of the mitochondrial ATPase, red arrow: MT-CYB, cytochrome b. tRNAs are designated by green letters using single-letter codes for corresponding amino acids.

functions. The first such peptide, humanin, was identified 14 years ago in an unbiased functional screen for clones that protect neuronal cells from death induced by amyloid precursor protein (APP) mutants, which are associated with early-onset familial Alzheimer's disease [3]. Humanin is encoded by a 75 bp ORF within the gene for 16S rRNA, and was independently isolated in a yeast two-hybrid screen as a partner of the insulin-like growth factor-binding protein-3 (IGFBP-3). Humanin has since been shown to exert cytoprotective effects against not only mutant APPs, but also against neuronal cell death induced by other stimuli such as mutant presenilins 1 and 2, cytotoxic Aβ peptides, Aβ1-42, Aβ1-43, and Aβ25-35. It has also been shown to protect against IGFBP-3 induced apoptosis. Another short ORF encoding the 16 amino-acid-long peptide mitochondrial open reading frame of the 12S rRNA-c (MOTS-c) has been recently discovered within the gene for mitochondrial 12S rRNA. This peptide targets skeletal muscle, and its cellular actions inhibit the folate cycle and de novo purine biosynthesis, leading to activation of the AMP-activated protein kinase. MOTS-c treatment in mice prevented age-dependent and high-fat-diet-induced insulin resistance, as well as diet-induced obesity [4].

1.2. mtDNA organization

mtDNA in mitochondria is organized into compact structures called nucleoids. Nucleoids can be visualized by labeling with various DNA stains, including anti-DNA antibodies, BrdU, or fluorescent intercalators, such as DAPI and Pico Green followed by microscopy. Therefore, the number of nucleoids detected per cell (and thus, estimates of the number of mtDNA molecules per nucleoid) depends on the properties of the optical system used, such as optical resolution and signal-to-noise ratio. This may explain why values for mtDNA content of nucleoids vary greatly in the literature. The lowest reported estimate of 1.45 mtDNA molecules per nucleoid, obtained with the help of the most advanced microscopy techniques [5], is likely be the most accurate, although we cannot exclude the possibility that this number is variable and/or depends on the cell type.

Nucleoids are ovoid structures diverse in size with an average diameter of about 100 nm [5,6]. They are associated with the mitochondrial inner membrane and are often wrapped around cristae or cristae-like membrane invaginations [6]. Experimental evidence suggests that there is little, if any, exchange of mtDNA between nucleoids. The packaging density of DNA in mitochondrial nucleoids is greater than that in the *Escherichia coli* nucleoid or human nucleus, and is comparable to the packaging density in the papillomavirus capsid [7]. This high degree of compaction is achieved with the help of the mitochondrial transcription factor A (TFAM), a high mobility group (HMG)-box DNA binding protein with functions in mtDNA packaging, replication and transcription. This protein binds mtDNA with a footprint of 23 bp or 30 bp [8], and is present in mitochondria in 1000-fold molar excess with respect to mtDNA molecules, which is sufficient for the complete coating of mtDNA [5]. TFAM can bind mtDNA specifically, at the H-strand promoter 1 (HSP1) and at the L-strand promoter (LSP) promoters to facilitate transcription and replication, and non-specifically, all over the mitochondrial genome [9], to induce mtDNA compaction. mtDNA compaction by TFAM depends on TFAM dimerization, which is mediated by HMGA domains [10]. Upon both specific and non-specific binding, TFAM imposes a sharp U-turn on its substrate mtDNA, which is essential for both transcription and packaging [10]. Interestingly, mitochondrial TFAM is predominantly DNA-bound. The release of TFAM from complexes with mtDNA is mediated by phosphorylation on Ser55 and Ser56 by PKA followed by Lon-mediated degradation [11].

1.3. mtDNA replication

Only one replicative DNA polymerase, DNA polymerase γ (PolG), has been described in mammalian mitochondria. This holoenzyme mediates both replication and repair of mtDNA, and consists of a large catalytic subunit and two accessory subunits. The catalytic subunit possess the polymerase, 3′ → 5′ exonuclease (proofreading), and 5′-deoxyribose phosphate (dRP) lyase activities, while the accessory subunits enhance DNA binding and processivity. Also, a novel DNA polymerase/primase, PrimPol, has been recently identified in mitochondria. This enzyme is believed to facilitate replication fork progression by acting as a translesion DNA polymerase or as a specific DNA primase reinitiating downstream of lesions that block synthesis during both mitochondrial and nuclear DNA replication. Unlike PolG$^{-/-}$, PrimPol$^{-/-}$ mice are viable indicating accessory role for the enzyme [12].

The mitochondrial replisome consists of PolG, the mitochondrial single-stranded DNA binding protein (mtSSB), mitochondrial DNA helicase, topoisomerases and RNaseH [13]. The lack of dedicated primase, an enzyme that generates an RNA primer for replication, is a unique feature of mtDNA replication. Instead of being synthesized by a dedicated enzyme, the primer for H-strand replication is generated by transcription initiated by mitochondrial RNA polymerase on an L-strand promoter (see below). Thus, the RNA polymerase serves to generate not only transcripts, but also the primers needed for mtDNA replication.

The mode of mtDNA replication remains controversial, and at least three models of replication have been described (recently reviewed in [14]). The original asynchronous strand-displacement model suggests that mtDNA replication is primed by an L-strand transcript. Interaction of the transcription elongation factor TEFM with POLRMT and the nascent transcript prevents the generation of replication primers and increases transcription processivity to enable synthesis of a near-genomic length L-strand transcript. Therefore, TEFM serves as a molecular switch between replication and transcription [15]. The primer generated at LSP is extended by the mitochondrial replisome and displaces H-strand over ~70% of mtDNA length. Then H-strand replication exposes the origin of the L-strand replication (O_L), and the synthesis of a new L-strand is initiated in the opposite direction. The asymmetry of strand synthesis leaves one segregated daughter molecule with an incompletely synthesized L-strand, called a gapped circle (GpC).

In the alternative strand-coupled (synchronous) model, there is thought to be a zone of replication initiation within a broad area beyond the D-loop. Within this zone, both strands are synthesized bidirectionally as the conventional double-stranded replication forks advance through continuous synthesis of leading and discontinuous synthesis (through Okazaki fragments) of lagging strands. However, this model relies on continuous ligation of Okazaki fragments during lagging strand synthesis and appears to be inconsistent with recent findings that 100-fold reduction in mitochondrial DNA ligase III does not appreciably affect the rate of mtDNA replication or copy number [16]. A third model is based on the observation of RNA incorporation throughout the lagging strand (RITOLS) [17]. According to this model, replication proceeds as in the strand-displacement model, except that the displaced H-strand is present not as single-stranded DNA, but rather as a DNA/RNA hybrid sensitive to RNase H, until it is made duplex by PolG. It is not clear whether RITOLS serve as primers for Okazaki fragments, but it appears unlikely due to the low response of mtDNA replication to DNA ligase III (see above). Recently, it was demonstrated that the in vivo occupancy profile of mtSSB displays a distinct pattern, with the highest levels of mtSSB close to the mitochondrial control region and with a gradual decline towards OriL. This pattern correlates with the replication products expected for the strand displacement mode of mtDNA synthesis thus lending strong in vivo support [18].

1.4. mtDNA copy number control

mtDNA is maintained at different copy numbers in different tissues, the two extremes of this spectrum being mammalian erythrocytes and sperm which have no mtDNA and ~5 copies of mtDNA per cell [19], respectively, and oocytes, which may contain >500,000 copies [20]. In *Drosophila*, mtDNA is eliminated from the sperm in the male genital tract prior to fertilization, so that fertilizing sperm may contain no mtDNA at all [21]. Curiously, human oocyte quality directly correlates with mtDNA copy number, whereas this correlation is inverse for the human sperm [20].

It is often overlooked that normal mtDNA copy number in a given tissue is not a set figure, but is rather a range. Therefore, in most population studies mtDNA copy number in apparently healthy individuals varies over a 2–10 fold range [22], and mtDNA content between 40 and 150% of the average is considered clinically normal [23]. Apart from normal variation in mtDNA copy number, mtDNA content can be altered under various pathologic scenarios. For example, mtDNA depletion syndromes [24] are associated with a profound loss of mtDNA, down to as low as 2% of normal [25]. Even though mtDNA depletion in these patients is frequently associated with perinatal lethality, long survival has been reported in a 29 year old patient with 76% depletion, who was observed for this condition since early childhood [26]. Also, a profound (91%) loss of mtDNA in a 47 year old patient was associated with relatively mild symptoms such as daytime sleepiness, exercise intolerance, and myalgias in the lower-limb muscles [27]. Paradoxically, both increased [28] and decreased skeletal muscle mtDNA content have been reported in patients with mutations in mitofusin 2 (MFN2). Changes in mtDNA content in tissues of aged individuals have been widely reported, although the direction of these changes remains controversial. Some studies report increased mtDNA copy number in the elderly [29], while others report a decrease and associate frailty with either lower [30] or higher [31] mtDNA copy number.

Regulation of mtDNA copy number is complex and remains incompletely understood. Nitric oxide, PGC1α, NCOR1 and their coactivators have been implicated as regulators through their effects on mitochondrial biogenesis. A systematic analysis in yeast revealed 102 ORFs whose deletion led to the loss of mtDNA. Remarkably, 55% of those ORFs were associated with biosynthesis of mitochondrial proteins [32]. In humans, sex-specific quantitative trait loci for mtDNA content have been identified on chromosomes 1, 2 and 3 [33]. Recently, epigenetic modification of exon 2 of the gene for the catalytic subunit of PolG has been implicated in mtDNA copy number regulation [34].

1.5. mtDNA repair and abandonment

Mitochondria contain a reduced complement of DNA repair pathways as compared to those present in the nucleus (reviewed in [35]). As mtDNA encodes only 13 out of ~1500 polypeptides that comprise mitochondria, all components of mtDNA repair machinery are encoded in the nucleus, translated on cytoplasmic ribosomes, and post-translationally imported into mitochondria. Mitochondria are proficient in both short-patch and long-patch subpathways of the Base Excision Repair (BER) pathway, which is responsible for the repair of oxidative and alkylating lesions as well as single strand breaks [35]. Importantly, some oxidized base lesions are repaired in mitochondria more efficiently than they are in the nucleus [35]. The presence of any other complete DNA repair pathway in mitochondria has not been demonstrated yet, although some components of the mismatch repair pathway have been identified in these organelles. How, then, do mitochondria cope with the mutagenic effects of DNA lesions that they are unable to repair? It turns out that in mammalian cells, the high redundancy of mtDNA enables a unique, mitochondria-specific pathway for the preservation of DNA integrity through degradation of damaged molecules. This pathway is nonspecific to the type of lesion and is mobilized not only in response to lesions that mitochondria are unable to repair, but also in response to the presence of an overwhelming amount of lesions that mitochondria are normally able to repair in moderate quantities, such as oxidative lesions [36], abasic sites, or gapped duplexes [37]. The kinetics of this process is different in different cell lines, and in some cell lines mtDNA loss can be detected as soon as 5–10 min after challenge with H_2O_2 [38]. Strikingly, almost no information exists on the mechanism of induced mtDNA degradation or nucleases involved in the process, although it appears unlikely that mitophagy can account for the rapid mtDNA turnover observed in some experiments [38].

1.6. Extramitochondrial mtDNA

Circulating cell-free mtDNA (mtDNAcf) was described in blood plasma 15 years ago by Zhong et al. [39], and has been suggested to have prognostic value in cancer, cardiac arrest and severe sepsis [40,41]. Recently, mtDNAcf emerged as a danger-associated molecular pattern (DAMP), a major mediator of innate immunity and systemic inflammatory response syndrome (SIRS). Upon tissue damage (e.g., blunt-force trauma), blood levels of mtDNAcf dramatically increase leading to toll-like receptor 9 (TLR-9)-mediated neutrophil activation and systemic inflammation [42]. Curiously, there appears to be discordance between the reported activation of TLR-9 exclusively by DNA containing unmethylated CpG islands and the well-documented cytosine methylation in mtDNA, which is mediated by an organellar isoform of the nuclear methyltransferase DNMT1 [43]. Since both nuclear and mitochondrial CpG islands can be methylated, one possible explanation for the selective activation of the immune system by mtDNA is in the greater content of unmethylated CpGs in mtDNA.

Extramitochondrial mtDNA has also been reported in the cytosol. Cytosolic release of mtDNA can be mediated by strong insults like oxidative stress, or by subtle changes like altered compaction of mitochondrial nucleoids secondary to TFAM haploinsufficiency [44]. This intracellular release of mtDNA has been implicated in cell-intrinsic innate immune responses [45,46].

1.7. mtDNA is disposable but indispensable

The prevailing evidence indicates that mtDNA is disposable and has a limited life span. In unstressed cells and tissues, it turns over with relatively short half-lives [47]. The substantial variability of mtDNA

content in the same tissue of different healthy individuals revealed by epidemiological studies suggests that, within limits, alterations in mtDNA copy number do not affect normal mitochondrial function [22,30,31,48]. In support of this notion, it has been observed that reduced mtDNA copy number has no major effect on mitochondrial transcript levels or enzyme activities in various tissues [49,50]. It has also been shown that some cells can survive after losing their mtDNA. For example, in response to stimulation, eosinophils abandon their mtDNA by catapulting it into extracellular milieu, while remaining viable [51,52]. In response to a heat shock and upon antibody-induced clustering of GPI-anchored antigens, ciliated protozoa jettison mitochondria out of the cells without losing viability [53]. In transgenic mice, doxycycline-induced expression of the mutant UNG1 resulted in hippocampal mtDNA depletion [54], which was accompanied by relatively mild behavioral changes such as increased locomotor activity, impaired cognitive abilities, and lack of anxietylike responses [55]. Lastly, mitochondria are normally shed together with enclosed mtDNA by retinal ganglion cell axons for degradation by surrounding astrocytes [56]. Reversible loss of mtDNA in experimental animals in response to treatments also supports the notion that mtDNA molecules are disposable. In mice, intragastric administration of ethanol was accompanied by a ~50% depletion of mtDNA in all organs studied, which reversed 24 h after discontinuation of treatment [57]. Degradation of mtDNA was also observed in a rat model of cerebral ischemia/reperfusion [58]. Similarly to mtDNA levels following depletion induced by intragastric ethanol administration, mtDNA levels following cerebral ischemia returned to normal within 24 h of reperfusion [58]. Unaccustomed resistance exercise can induce up to 32% mtDNA depletion in mouse muscle [59], whereas prolonged endurance training results in a 50% increase in mtDNA copy number in human muscle [60]. These examples demonstrate that a partial mtDNA loss is relatively common in some cells and tissues and that this loss, at least in some instances, 1) can be accompanied by either mild or no consequences and 2) can be transient and accompanied by compensatory adaptive mtDNA resynthesis.

While the evidence reviewed above suggests that individual mtDNA molecules are disposable, it does not answer the question of whether or not mtDNA in general is superfluous for cell viability: in other words, is mtDNA dispensable? For mammalian erythrocytes, which contain no mtDNA, it is. Also, cultured animal cells can be induced to undergo a complete loss of mtDNA. The resulting ρ^0 cells become auxotrophic, but retain viability. Interestingly, however, these cells fail to form tumors in experimental animals unless they capture mtDNA from surrounding tissues [61]. Yet, successful attempts to induce global mtDNA loss in multicellular organisms have not been reported. As noted above, infants with mtDNA depletion syndromes, which are accompanied by severe loss of mtDNA, usually die in the first year of life. Also, whole-body ablation of TFAM, a protein required for mtDNA replication, results in severe mtDNA depletion and is embryonically lethal in mice [62]. These observations suggest that mtDNA is indispensable for the viability of multicellular organisms.

2. Conclusion

More than 50 years of research have led to dramatic progress in our understanding of mtDNA biology. Our views have evolved from dismissing the mitochondrial genome as vestigial to appreciating the key role of mtDNA and its alterations in cellular bioenergetics and mitochondrial disease. Yet it appears that mammalian cells have a relaxed reliance on mtDNA, which is perpetually destroyed and resynthesized. Under physiological and pathophysiological conditions, the balance between these two processes can be shifted and restored, often with mild or no consequences. The absence of a direct correlation between mtDNA copy number and levels of mitochondrially encoded transcripts or proteins, which may remain unaltered during mtDNA depletion [49,50], provides a mechanistic explanation for this relaxed reliance. Yet, some key questions remain unanswered. What are the triggers and mechanism(s) of mtDNA degradation? What enzymes are involved in the process? Is mtDNA degraded down to nucleotides, or to oligonucleotides? Is degrading mtDNA contained inside mitochondria or released intra/extracellularly? Does degrading mtDNA contribute to innate immune responses? These and other questions will be addressed in the coming years.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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