Title

Quick Guide: Mitochondrial nucleoid

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1) What are mitochondrial nucleoids?

Mitochondrial nucleoids are large nucleo-protein complexes containing the mitochondrial DNA (mtDNA) and regulatory factors necessary for its packaging, replication, transcription, or repair. MtDNA encodes essential subunits of the electron transport chain (ETC) complexes critical to cellular energy production via oxidative phosphorylation. In most eukaryotes, many complete copies of the mitochondrial genome are individually packaged into the nucleoid complexes and distributed amongst the hundreds to thousands of individual mitochondria in each cell, through a dynamic and regulated process of mitochondrial fusion and fission (**Fig. 1**). Mitochondria descend from an endosymbiont of α-proteobacterial origin, and like their prokaryotic ancestors, they are maintained via cycles of genome duplication, membrane growth, and division.

2) Why are mitochondrial nucleoids important?

In humans, mtDNA encodes 13 essential proteins of the ETC required for adenosine triphosphate (ATP) production via oxidative phosphorylation and, thus, cellular metabolism. Mitochondrial nucleoids are the sites of mtDNA replication and transcription, which are regulated at the level of the individual complexes. While the majority of nucleoids are tightly packaged and inaccessible at any given time, a subset participates in these processes in an asynchronous manner across the mitochondrial network. Thus, mitochondrial nucleoids may be functionally non-equivalent, even when coexisting within the same mitochondrion (**Fig. 1**). How individual nucleoids undergo transitions between quiescent and active states is not well understood, though defects in the nuclear-encoded genes required for mtDNA packaging and expression cause heritable metabolic diseases, and have been linked to cancer, neurodegenerative disease, and aging.

Understanding how mitochondrial nucleoids are maintained, regulated, and positioned within mitochondrial networks is critical to further our understanding of the cellular mechanisms of mitochondrial genetics, metabolic regulation, and thus overall cell homeostasis. Mitochondrial nucleoids serve as the units of inheritance of the mitochondrial genome at mitochondrial division, when mitochondria are partitioned to daughter cells during mitosis, and during asymmetric cell divisions such as in meiosis. The subset of nucleoids engaged in mtDNA

synthesis are spatiotemporally linked to sites of mitochondrial division, and thus cellular-level mitochondrial behaviors that integrate energy production with other mitochondrial functions. The packaging and retention of mtDNA inside the mitochondrial matrix are critical. Depletion of the mitochondrial transcription factor A (TFAM), the main mtDNA packaging protein, results in reduced mtDNA integrity and pro-inflammatory release to the cytosol. In laboratory culture, cells lacking mitochondrial genomes and nucleoids (termed 'rho 0') can be viable via glycolytic or fermentative metabolism and are useful tools for studying mitochondrial biology.

3) What are mitochondrial nucleoids made of?

Mitochondrial nucleoids contain mtDNA and, depending on their replication, transcription, or repair status, a variety of proteins that interact directly or indirectly with it. Mitochondrial genome size varies depending on the organism. In human cells, *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Mus musculus*, mtDNA is between 10 and 20 kilobases; mitochondrial genome size can vary an additional order of magnitude across the broader tree of life. The multicopy nature of the mitochondrial genome means that nucleoid complexes containing either wild-type or mutant mtDNA may coexist within the same mitochondrial network, states known as homoplasmy and heteroplasmy, respectively.

A cadre of mitochondrial nucleoid proteins regulates mtDNA packaging, replication, and transcription. TFAM is a key nucleoid component that is the primary mtDNA packaging factor. TFAM also contributes to mtDNA regulation via its control of genome compaction, and thus accessibility to replication and transcription factors. Consistently, quiescent nucleoids have greater TFAM occupancy (**Fig. 1**). Beyond direct roles in mitochondrial genome maintenance, other protein constituents of nucleoids link mtDNA integrity or expression to the organelles' anabolic and catabolic functions.

4) What do we know about the sub-structure of mitochondrial nucleoids?

MtDNA and, thus, mitochondrial nucleoids are maintained in excess in many organisms; most mtDNA molecules are tightly packaged and supercoiled in a quiescent state regulated in part by the mitochondrial topoisomerase I. The transition to an "active" or "open" state is thought to be mediated by helicases such as Twinkle, and the occupancy of TFAM at non-coding regions is required for initiation of mitochondrial replication. The mitochondrial RNA polymerase POLRMT, the sole polymerase required for mtDNA transcription, also has a key role in replication. It synthesizes the RNA primers needed to start replication at the origins of replication of the heavy strand (O_H) and light strand (O_L). These primers are then extended by the DNA polymerase gamma holoenzyme (POLG) for DNA synthesis.

The nanoscale organization of mitochondrial nucleoids remains to be discovered. However, two models offer useful perspectives: (1) The "layered model" divides the nucleoid into two main layers: "core proteins" that bind directly to the mtDNA, where they participate in mtDNA transcription and replication, while "peripheral proteins" are recruited to the nucleoids to interact with core proteins to perform specific roles, such as stabilizing TFAM binding. The layered model is largely based on immunopurification-mass spectrometry studies of the total mitochondrial nucleoid population from cultured mammalian cells in which nucleoid constituents were extracted from their native milieu. Given the functional non-equivalence of individual nucleoid complexes *in situ*, nucleoid composition and structure likely vary considerably more in vivo than the layered model suggests. Recently, sequencing- and imaging-based approaches that capture individual nucleoids and their heterogeneity have been useful in refining our understanding of the differences between individual nucleoid complexes. These studies suggest model in which TFAM nucleation-and-spreading (2) determines the proportion of nucleoids that are accessible to transcription and replication machinery. According to this model, nucleoids dynamically transition between accessible and quiescent states over time. Consistently, TFAM overexpression results in an increase in the proportion of total nucleoids inactive for

transcription and replication (**Fig. 1**). This heterogeneity among nucleoids is important because it may drive compositional and functional differences between mitochondrial sub-populations, such as is seen in differentiated skeletal muscle, heart, and neuronal cells.

5) What's next?

While mitochondrial nucleoids have been described for more than half a century, they are still mysterious. First, it is unclear whether the mtDNA can be organized into higher-order structure(s) beyond TFAM compaction. Techniques such as single-molecule localization microscopy and cryo-electron tomography will likely provide welcome insights into the three-dimensional organization of the mitochondrial genome and how this is remodeled to facilitate gene expression. Another area of interest is determining how the mitochondrial DNA replisome is recruited and assembled at the specific subset of nucleoids selected for replication to maintain copy number. The importance of nucleoid position and integrity for organelle dynamics also needs to be further investigated. Indeed, mitochondrial nucleoid aggregation is a general feature of mitochondrial dysfunction. Moreover, mtDNA replication stress is linked to mitochondrial elongation in cells and animal models. These findings raise the possibility of a nucleoid integrity checkpoint on mitochondrial membrane dynamics. Finally, the majority of work characterizing mitochondrial nucleoids has been performed in immortalized mammalian cell lines and *Saccharomyces cerevisiae*. Studies in a variety of primary cells, additional studies in animal models, as well as emerging model organisms, are poised to reveal conserved principles of mitochondrial nucleoid regulation and overall mitochondrial homeostasis.

Where can I find out more?

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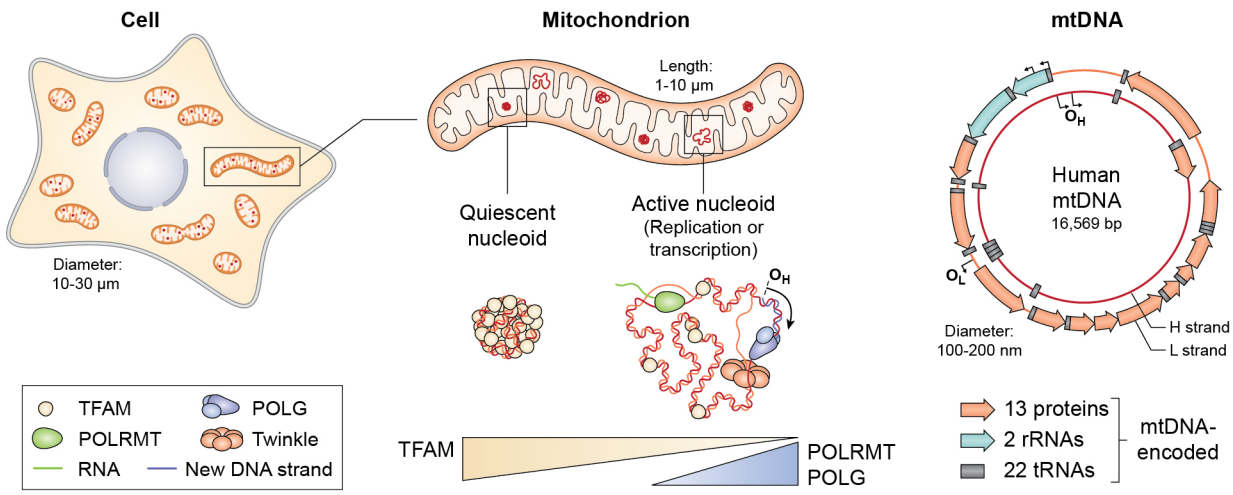
Figure Legend

Fig 1. Mitochondrial and nucleoid organization across biological scales. The multicopy mtDNA is distributed throughout mitochondrial networks in mitochondrial nucleoid complexes. Functional heterogeneity exists between individual nucleoids; these complexes can be quiescent or active in replication or transcription. Quiescent nucleoids are tightly packaged by TFAM, while active nucleoids are relatively accessible to the replication and/or transcription machinery such as RNA polymerase POLRMT, DNA polymerase POLG, and oligomeric helicase Twinkle. In human cells, the double-stranded circular mtDNA encodes 13 protein subunits of the ETC, as well as 2 ribosomal RNAs (rRNA) and 22 transfer RNAs (tRNA) for their translation on mitoribosomes. Scientific illustration by Luciana Giono.

Declaration of Interests

The authors declare no competing interests.


Cell
Mitochondrion
mtDNA
Diameter:
10-30 µm
Length:
1-10 µm
Quiescent
nucleoid
Active nucleoid
(Replication or
transcription)
O_H
Human
mtDNA
16,569 bp
O_L
Diameter:
100-200 nm
H strand
L strand
TFAM
POLG
POLRMT
Twinkle
RNA
New DNA strand
TFAM
POLRMT
POLG
13 proteins
2 rRNAs
22 tRNAs
mtDNA-
encoded