Our Ancestral Heritage: Mitochondrion, Its Genome, and a Story of Intercompartmental Voyage

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Abstract

Mitochondria is an energy-generating organelle that contains its own genetic material mitochondrial DNA (mtDNA). Although strictly confined within the mitochondrial network, mtDNA sometimes escapes from this confinement to the cytoplasm. Since such mtDNA escape has serious influences on different cellular pathways, it is important to understand the underlying mechanisms and consequences of this process. In order to understand the mtDNA leakage phenomenon better, in this article, we review the research that explores unique features of mitochondria, including its origin, nuclear gene transfer, and different escape mechanisms. Notably, we highlight the Bax/Bak-dependent and VDAC-dependent escape of the mtDNA into the cytoplasm and subsequent activation of the cGAS/STING pathway. Several studies suggest that Bax/Bak pores and VDAC channels are active under extreme and moderate stress levels, respectively, and this activation leads to mtDNA leakage to the cytoplasm. Further investigations have to be conducted to have a more comprehensive view of the flow of mtDNA from mitochondria to cytoplasm and possibly even to the nucleus. Given that mitochondrial malfunctioning has been broadly implicated in a myriad of physiological and pathological conditions, mtDNA leakage and its prevention can be a very attractive target for clinical interventions.

> "It seems that all eukaryotic cells either have, or once had (and then lost) mitochondria. In other words, possession of mitochondria is a *sine qua non* of the eukaryotic condition"
>
> Nick Lane, *Power, Sex, Suicide: Mitochondria and the Meaning of Life*

Introduction

Mitochondria is an essential organelle of the eukaryotic cell that produces more than 60% of cellular ATP through oxidative phosphorylation (**Figure 1**). For this reason, it is commonly referred to as "powerhouse of the cell" [Kim, 2014]. However, latest studies clearly indicate that this organelle does more than just energy production. For example, mitochondria are actively involved in TCA cycle, calcium storage and apoptosis. Even

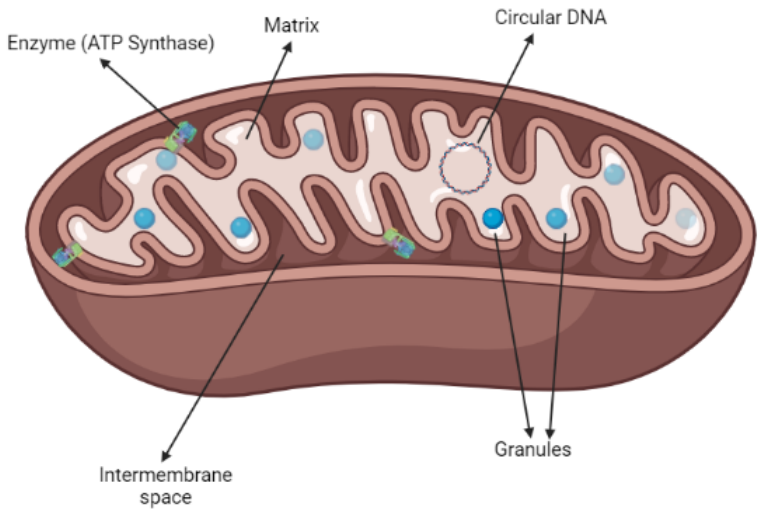

Figure 1. Schematic representation of a mitochondrion

though general visualization of this organelle reminds a single shoe with a folded interior, in a living cell, mitochondria appear as highly dynamic tubular networks that undergo cycles of fusion and fission events. Inspired by this dynamic morphology of the organelle, in 1898, microbiologist Carl Benda coined the name mitochondria (from *mitos* thread and *chondros* granules in Greek). Moreover, due to its endosymbiotic origin, mitochondria possess some unique characteristics, such as double membrane, independent DNA genome and uniparental inheritance, aspects which will be discussed in further chapters. Since mitochondria increases the efficiency of energy production and contributes to the cell's genetic diversity with its own genome (mtDNA), it can be considered as the main driving force of multicellular life and sex formation in eukaryotic cells.

It is important to mention that the mtDNA replication occurs independently of the nuclear DNA replication. Such uncommon properties of mtDNA are important for our understanding of mtDNA mutations as several mitochondrial diseases arise as a result of these mutations. The mammalian mitochondrial genome is 15 000 - 17 000 bp long and consists of thirty-seven genes [Ballard and Whitlock, 2004]. Thus, mtDNA encodes for less than 1% of all proteins in the cell. Additionally, most of the >1,000 mitochondrial proteins are synthesized as precursors in the cytosol and then transported into mitochondria [Wiedemann and Pfanner, 2017].

Another unique characteristic of mitochondria is its uniparental inheritance, meaning that mtDNA only passes down from a single parent – apart from some bivalve mollusks – multicellular organisms obtain their mtDNA from the egg cell, a phenomenon called maternally uniparental inheritance (MUI) [Wen et al., 2016]. This tendency has also been observed in some species without sexual gamete dimorphism. A good example of this is the unicellular green alga *Chlamydomonas reinhardtii*, which has a distinct mode of gamete differentiation [Gillham, 1994]. In some cases, however, failure of the mechanism that excludes the paternal mtDNA entrance to the zygote can

lead to presence of both maternal and paternal mtDNA haplotype - a condition that is referred as heteroplasmy [Herst et al., 2017]. There are several mitochondrial diseases associated with mtDNA heteroplasmy.

Several types of cancer are also related to mitochondrial dysfunction. Even though mtDNA mutations in cancer cells have been studied for more than three decades, discovery of mitochondrial tricarboxylic acid (TCA) cycle gene mutations brought attention to the role of mitochondrial alterations in cancer anew [Wallace, 2012]. Because the mitochondrial genome has 10–17 times higher mutation rate than the nuclear genome [Tuppen et al., 2010], it is more likely to accumulate mutations than its nuclear counterpart. This can be the result of oxidative damage caused by reactive oxygen species (ROS), which are products of the oxidative phosphorylation and are highly reactive molecules that can damage biomolecules such as DNA. Since mitochondria are one of the main sites of ROS production, the mitochondrial genome is very susceptible to oxidative damage [Craven et al., 2017].

In this minireview, we summarized cytoplasmic mtDNA leakage and pathways involved in this process. But the endosymbiosis phenomenon and main features of the mitochondrial genome, including nuclear gene transfer are also mentioned in the article. In the end, we will also present some open questions in the field and some experimental approaches to address them.

mtDNA - a unique feature of mitochondria

We cannot properly describe mitochondria without mentioning views about its origin. Just like chloroplast, mitochondria are also thought to be the product of endosymbiosis. At one period of life, a free-living prokaryote had been taken up by a simple eukaryotic cell and in time turned into its essential compartment [Allen et al., 2007]. Originally, there were two main hypotheses regarding the endosymbiotic origin of mitochondria: the Archezoa hypothesis and the fusion hypothesis. In the Archezoa hypothesis, Cavalier-Smith suggested that the eukaryotes had appeared prior to the endosymbiotic events that gave rise to mitochondrial eukaryotes. According to his hypothesis, an archaebacterium gave rise to a eukaryotic organism through the elaboration of a cytoskeleton and development of an internal membrane system that included the formation of a nucleus [Cavalier-Smith, 1987]. The fusion hypothesis, on the other hand, is primarily based upon molecular phylogenetic data [Zillig et al., 1989]. According to this hypothesis, Archezoa is the starting point of eukaryotic evolution, but eukaryotes came from a fusion event between an archaebacteria and eubacteria. The resulting chimaera is thought to have then evolved eukaryotic structures and acquired the mitochondrion. However, none of these hypotheses examined the energy metabolism of the host or the endosymbiont. Consequently, W. Martin and M. Müller approached the subject from different perspectives and suggested the hydrogen hypothesis in 1998. According to their view, the first eukaryote possessed a nucleus but lacked mitochondria. Eventually, the eukaryotic cell engulfed a primitive bacterium, which gave rise to the mitochondrion. The future mitochondrion was a facultatively anaerobic eubacterium which produced hydrogen and carbon dioxide as byproducts of anaerobic respiration. A symbiotic relationship between two organisms started based on the host's hydrogen dependence [Martin and Müller, 1998] (**Figure 2**).

One of the main factors that led to the endosymbiosis theory is the independent DNA of the mitochondria. The mtDNA is mostly single, short, circular and contains about 13 protein-coding genes in most animals [Ladoukakis and Zouros, 2017]. In some cases, mtDNA can be separated into minichromosomes [Shao et al., 2009]. Almost all the genes encoded by mtDNA are involved in oxidative phosphorylation (OXPHOS) — the process also known as mitochondrial respiration. However, this is not the only

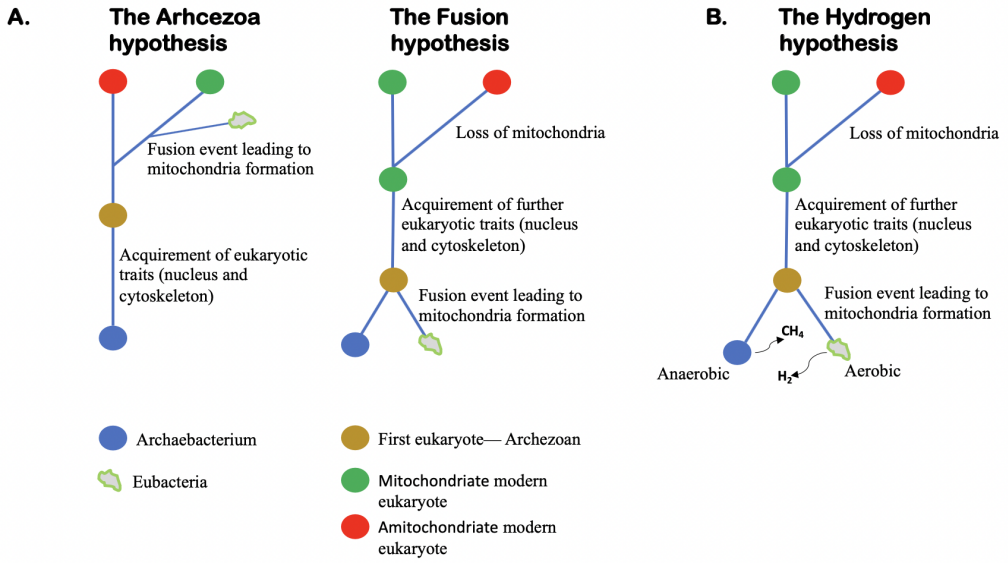

Figure 2. Different hypothesis on the endosymbiotic origin of mitochondria: A) Schematic representations of the Archezoa and Fusion hypotheses **B)** Schematic representation of the Hydrogen hypothesis - a version of the Fusion hypothesis that takes into account the metabolic needs of the archaebacterial and eubacterial components of the endosymbiotic event

process that the mitochondria are responsible for and it is involved in other important cellular activities as well. As an example, we can show apoptosis [Sinha et al., 2013], ageing [Bratic and Larsson, 2013] and various other signaling pathways [Chandel, 2015]. As mtDNA is in charge of coding certain proteins, mutations in these genes can cause defects in the cell. These mutations are generally observed as point mutations [Lightowlers et al., 2015]. Mechanisms of mitochondrial DNA replication also differ in different groups of organisms [Ciesielski et al., 2016].

Studies show that the original α-proteobacteria genome shrank over the time and reached its current size found in mitochondria as a result of gene loss and gene transfer over millions of years. Main transfer target of these genes is the nucleus. Examination of some genes in the nucleus reveals that they have a mitochondrial origin, because they are present in the mitochondria of other species [Lane, 2006]. The nuclear genomes of primates, rodents, cats, birds and even plants certainly carry mtDNA sequences [Perna and Kocher, 1996]. The research by Peter E. Thorsness and Thomas D. Fox on Saccharomyces cerevisiae in 1990 showed that the transfer is unidirectional, from mitochondria to nucleus, but not the other way around [Thorsness and Fox, 1990]. This transfer is also associated with certain genes in the nuclear DNA, such as YME1 (yeast mitochondrial escape) and YME2. The inactivation of the yeast nuclear genes YME1 and YME2 causes an increased rate of DNA escape from mitochondria to the nucleus [Hanekamp and Thorsness, 1996]. Both genes are coding for mitochondrial inner membrane proteins. YME1 is a catalytic subunit of the i-AAA protease complex, which is responsible for the degradation of unfolded or misfolded mitochondrial gene products. YME2, on the other hand, plays a crucial role in maintaining mitochondrial nucleoid structure and number.

During the human genome project, at least 354 different mitochondria-to-nucleus DNA transfers have been identified [Lane, 2006]. These transferred pieces of DNA sequences are called NUMTs (Nuclear-mitochondrial sequences) (**Figure 3**). In 2002, Einat Hazkani-Covo et. al. revealed 82 large NUMTs within the human

genome [Hazkani-Covo et al., 2003]. NUMTs are also called pseudogenes because they are nonfunctional in the nuclear DNA. This can be the result of the differences between the nuclear and mitochondrial genetic codes. However, in their research paper about the presence of mitochondrial DNA sequences inside nuclear DNA in rat tissues, Caro, et.al., show that in the Wistar rats analyzed, all mtDNA are present at the centromeric region and hence they are not inserted at random [Caro et al., 2010]. Therefore, they hypothesize that the repetitive mtDNA might help the centromere function because this part always contains a large number of repetitive sequences [Caro et al., 2010]. Nuclear gene transfer is still an ongoing process and sometimes can even cause health issues. In 2003, [Turner et al., 2003] characterized a patient with a gene transferred from the mtDNA to the nuclear genome. The transfer caused a condition called Pallister-Hall syndrome, which is usually inherited in an autosomal-dominant mode [Turner et al., 2003]. As a result of the study on rats, a new hypothesis is proposed, which states that mtDNA fragments may be inserted into nuclear DNA and in return may contribute to the aging and age-related diseases by altering the nuclear genome [Caro et al., 2010]. In order to affect the nuclear genome, mtDNA fragments first have to be physically carried to the nucleus. This process requires a few key steps such as:

1. degradation of abnormal mitochondria [Campbell and Thorsness, 1998];
2. lysis of mitochondrial compartment [Hazkani-Covo et al., 2010];
3. encapsulation of mitochondrial DNA inside the nucleus [Hazkani-Covo et al., 2010].

While the cDNA-mediated transfer is the current suggestion on the mechanisms of gene transfer from organelles to the nucleus in plants, an alternative mechanism, such as direct DNA transfer is also possible [Henze and Martin, 2001]. Only in the 21st century did we begin to approach these problems. Progress in molecular techniques, coupled with advances in computer science and information technologies set the stage for a new generation of analysis tools to be developed.

A novel road in the old town—cytoplasmic mtDNA leakage

As mentioned earlier, under physiological conditions, mtDNA is contained within the mitochondrial network; however, segments of the mtDNA can leak to the cytoplasm in case of the mitochondrial damage. This can happen in multiple cell types. Later, the cytosolic mtDNA initiates several different cellular responses depending on the cell type. One study shows that Streptococcus pneumoniae (S. pn) secretes H_2O_2 (an example of ROS), which causes mitochondrial damage and consequently affects mouse lung tissue. This H_2O_2 oxidizes mtDNA in alveolar epithelial cells and causes release of mtDNA into the cytosol [Gao et al., 2019]. It is also clear that mitochondrial MRE11A protein, which is a DNA repair nuclease, protects mtDNA from cytoplasmic leakage. Therefore, if there is a loss of function in the MRE11A gene, mtDNA leaks to the cytoplasm and initiates inflammation [Li et al., 2019]. Another source of mitochondrial damage and consequent mtDNA leakage can be the Palmitic acid. Investigations show that it induces the damage to the mitochondria and causes leakage of mtDNA to the cytosol. As a result of this process cGAS–STING–IRF3 pathway is activated and triggers endothelial activation and inflammation [Yun et al., 2017]. Another study by Maekawa et. al., also supports that the cytosolic mitochondrial DNA activates cGAS-STING pathway and causes inflammation in the kidney [Maekawa et al., 2019].

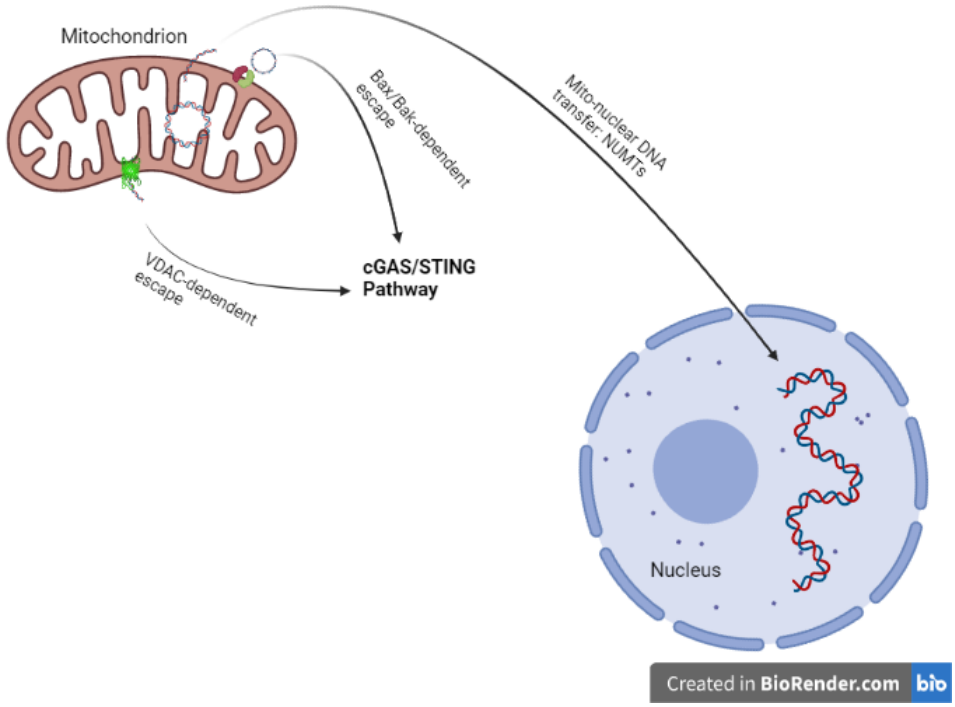

Figure 3. A schematic model representing some of the most prominent routes of mtDNA escape

It is thought that mtDNA leaks through the BAX pores in the mitochondrial membrane to the cytosol [Maekawa et al., 2019]. Stephen W. G. Tait and Douglas R. Green, in their review article about the mitochondrial membrane permeabilization, summarized two possible mechanisms for the pore formation process of BAX and BAK proteins [Tait and Green, 2010]. First mechanism suggests that, because BAX and BCL-XL proteins share similar structure with bacterial pore-forming toxins, they too might directly form pores in the mitochondrial outer membrane [Suzuki et al., 2000]. But another model displays a different approach. According to this model, interaction of activated BAX and BAK with outer membrane lipids cause membrane bending, in turn this leads to the formation of transient lipid pores, as the result of this intermembrane space proteins (IMS proteins) are released into the cytosol [Hardwick and Polster, 2002] (**Figure 3**). It is thought that not only proteins, but also mtDNA can use these pores to escape from the organelle. BAK/BAX pores, however, are not the only possible way out from the mitochondria. Recent study demonstrates that the pores formed by VDAC, Voltage-Dependent Anion-Selective channel, oligomerization can be another gateway for mtDNA [Kim et al., 2019] (**Figure 3**). Authors of the study suggest that these pores are active under moderate stress, whereas the BAK/BAX pores are active under extreme stress levels. Based on its frequent involvement in the cytoplasmic mtDNA detection/sensing, downstream of mtDNA leakage, cGAS–STING pathway can be a promising target for new cancer therapy approaches. For a detailed review of this topic see [Hu et al., 2021].

Conclusion and Future Perspective

Although the brief description of mitochondria, mtDNA, and its cytoplasmic leakage we provide in this mini review is far from being all-encompassing, it does allow us to see

that the exchange between this "alien, endosymbiotic entity" and the nuclear genome of the modern eukaryotic cell is still ongoing. The continuous inflow of NUMTs into the nDNA is a strong testimony to this. Moreover, cytoplasmic leakage of mtDNA under different stress and pathological conditions and the concomitant cellular responses to the presence of such cytoplasmic mtDNA is another attestation to the dynamic interplay between mitochondria and the cell. Overall, considering the permanent effects of NUMTs on the cellular genome and the emerging link between the mtDNA leakage and DNA damage responses (DDR), it wouldn't be too far-fetched to conclude that mtDNA is still a major driver of evolution. Despite their paramount importance, both fields are relatively novel; naturally, there is a lot to be investigated further. Especially the comprehensive mechanistic understandings of questions such as how does mtDNA leak to cytoplasm and how does the cytoplasmic mtDNA find its way to the nucleus or is incorporated to the nDNA are still elusive. A logical next step in the field would hence be to investigate and elucidate the underlying mechanism(s) of the mtDNA flow in mitochondria → cytoplasm → nucleus direction, which doubtlessly is(are) a promising target(s) for the clinical research as well.

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