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REVIEW

Mechanisms of Intracellular Selection of Mitochondrial DNA

Georgii Muravyov[1,2] and Dmitry A. Knorre[2,a]*

[1]*Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, 119234 Moscow, Russia*

[2]*A. N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119234 Moscow, Russia*

[a]*e-mail: knorre@belozersky.msu.ru*

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Abstract—Eukaryotic cells contain multiple mitochondrial DNA (mtDNA) molecules. Heteroplasmy is coexistence in the same cell of different mtDNA variants competing for cellular resources required for their replication. Here, we review documented cases of emergence and spread of selfish mtDNA (i.e., mtDNA that has a selective advantage in a cell but decreases cell fitness) in eukaryotic species, from humans to baker's yeast. The review discusses hypothetical mechanisms enabling preferential proliferation of certain mtDNA variants in heteroplasmy. We propose that selfish mtDNAs have significantly influenced the evolution of eukaryotes and may be responsible for the emergence of uniparental inheritance and constraints on the mtDNA copy number in germline cells.

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INTRODUCTION

Mitochondria are semi-autonomous organelles with their own genome. Mitochondrial DNA (mtDNA) has been found in a vast majority of eukaryotes, except few species [1]. It encodes proteins necessary for oxidative phosphorylation, as well as components of the mitochondrial translation system [2]. Typically, a single eukaryotic cell contains several mtDNA copies, although their exact number may depend on the conditions, cell type, and prior cell history [3-5]. New mtDNA variants appear through mutations. The state when a cell has several mtDNA variants is called **heteroplasmy** [6]. During cell division, mtDNA molecules are distributed randomly between the new cells. After several (sometimes, many) generations, due to random genetic drift, the descendants of a heteroplasmic cell retain only one of the mtDNA variants, the state referred to as **homoplasmy** [7]. As a result, the emergence of heteroplasmy (due to mutations or cell fusion) is balanced by random genetic drift in the frequencies of mtDNA mitotypes, leading to the preservation of one or another mtDNA mitotype in the cell descendants (Fig. 1).

mtDNA is a subject of natural selection at multiple organizational levels: molecular, organelle, cellular, or organismal [7]. Therefore, the selection process can be multidirectional, i.e., the same mtDNA variant might have a high fitness at one organizational level and low fitness at another [7]. To describe selection at the cellular level, it is important to introduce the concept of mutant mtDNA **pathogenicity threshold**. Cell phenotype is a complex function of mtDNA genotypes, as it reflects manifestations of these genotypes at the levels of transcription, translation, enzymatic activity, respiratory chain function, and cell as a whole. However, when the proportion of mutant mtDNA variants is less than a certain threshold for a given heteroplasmic cell, harmful mutations may not manifest themselves at the cellular or organismal

* To whom correspondence should be addressed.

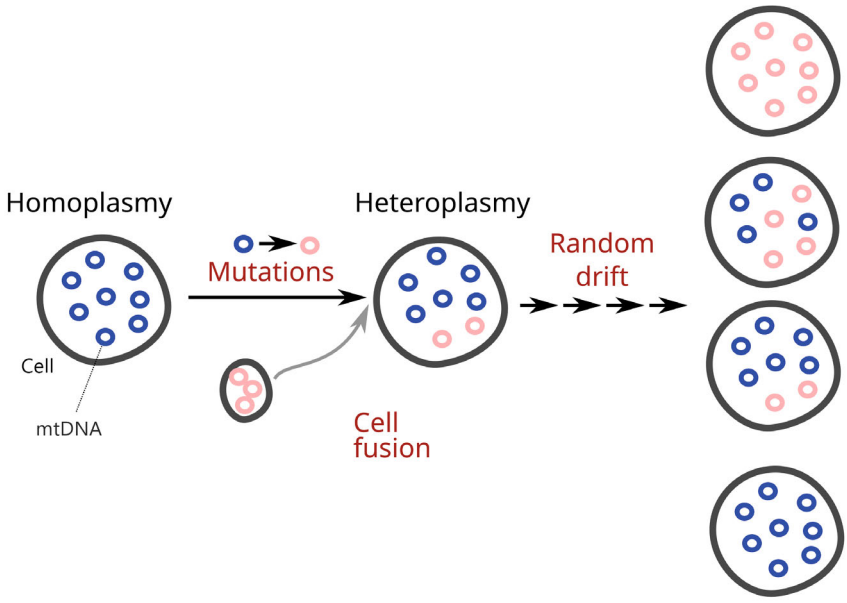

Fig. 1. Heteroplasmy arises as a result of mtDNA mutations in a cell or via cell fusion with other cells. Over generations, random drift may cause descendants of the heteroplasmic cells to revert to homoplasmy. Mitochondria are not shown in this figure, and it is assumed that mtDNAs constitute a single population within each cell.

levels [8, 9], usually indicating an excess number of mtDNA copies per cell. As a result, mtDNA variants carrying mutations harmful to the cell and, at the same time, providing an advantage in fitness at the intracellular level, can avoid selection in individual cells until their allele frequency reaches a specific threshold. Such mutations can persist for a long time or even gain an advantage in organisms and populations. Furthermore, in some cases, deleterious mtDNA mutations harmful to the multicellular organism, may gain a fitness advantage at the cellular level. This is exemplified by the spread of colonic crypts containing cells with mutations in the mitochondrial cytochrome oxidase *CO1* gene [10].

mtDNA variants characterized by a high fitness at the intracellular level but not beneficial (or even deleterious) to the cell or entire organism fall under the definition of "selfish genetic elements" [11]. In this work, we will refer to such mitotypes (mtDNA variants) as **selfish mtDNA**. There have been numerous descriptions of the emergence and spreading of selfish mtDNA in both nature and artificial experimental systems. In the next section, we discussed examples of intracellular selection of mtDNA enabling proliferation of selfish mtDNA.

INTRACELLULAR SELECTION AND SELFISH mtDNA IN NATURE AND EXPERIMENTAL SYSTEMS

One evidence of mtDNA selection at the intracellular level is clonal expansion of deletion-containing mtDNA variants in the cells of postmitotic tissues of multicellular animals. Clonal expansion is the process where a mutated mtDNA molecule multiplies within some cells, leading to a higher concentration of that specific mtDNA, while in other cells, this mtDNA variant is absent [12, 13]. Analysis of sequences of mtDNA control regions in individual human somatic cells has shown that proportion of mutant mtDNA variants (i.e., those differing from the main mitotype in a given organism) in some cells can significantly increase with age [14]. In most cases, it is difficult to distinguish whether the clonal expansion is caused random stochastic drift or results from positive intracellular selection. Nonetheless, the mutation patterns found in individual cells from different tissues differ markedly, providing suggestive evidence for the presence of positive intracellular selection. For example, it has been shown that in cardiomyocytes, most *de novo* mutations occurred in the mtDNA genome region with the coordinates 16,025-16,055 bp, whereas in buccal epithelial cells, this region contained no mutations [14]. This result suggests that either (1) the spectrum of mutations varies greatly among different tissues or (2) due to the intracellular selection, some mtDNA variants undergo clonal expansion. Both these processes – selection and genetic drift – have been demonstrated in mice with artificially created heteroplasmy. The animals showed an increase in the frequency of certain mtDNA variants in some tissues, but not in others [15]. In another study, sequencing mtDNA from individual cells of aged (2-year-old) and young mice revealed mutant mtDNA variants, the proportion of which increased with age faster than

can be explained by random drift [16]. These mutations, which were under positive selection in somatic cells, were predominantly located in the mtDNA control region containing the origin of replication. Interestingly, in some cases, the relative content of associated passenger mutations also increased [16].

Finally, comparison of frequencies of different mtDNA variants in mothers and offspring suggested the presence of both purifying [17, 18] and positive [19, 20] selection of mtDNAs in mammalian germlines. The direction of selection largely depends on the frequencies of mtDNA alleles in germline cells and specific mtDNA mitotypes (see discussion in [21, 22]). If a mutant mtDNA variant (mitotype) constitutes a significant fraction of all molecules, the purifying selection may occur at the whole-cell level. The selection is enabled because, during the development of follicular cells, the mtDNA diversity within individual cells decreases due to the genetic bottleneck effect (see review [23]), linking the mtDNA genotype to the phenotype of the entire cell. At the same time, both purifying selection against deleterious mtDNAs with low allele frequencies and any kind of positive selection are most likely restricted to the intracellular level.

Selfish mtDNA is one of the challenges in the mitochondrial replacement therapy. Mitochondrial heteroplasmy is associated with a large number of human hereditary diseases. Such diseases can be transmitted from a mother to child, and their heritability depends on the ratio of mutant (pathogenic) and normal mtDNA variants in the oocytes [24]. To prevent the spread of such diseases, scientists have been actively developing the methods for mitochondrial replacement therapy, leading to the first live birth of a “three-parent” baby whose nuclear DNA originated from the parents, while mtDNA was from a donor (“third parent”) [25]. The transfer of the spindle from the cytoplasm of the mother’s cell, who carried the pathogenic variant of mtDNA, into the cytoplasm of the donor cell resulted in the donor’s mtDNA constituting over 99% mtDNA in the developing embryo. However, in some cases, embryonic stem cell lines derived by this method demonstrated a gradual increase in the pathogenic variant of the mother’s mtDNA and loss of “healthy” donor’s mtDNA [26], suggesting that the mutant mtDNA variant associated with the disease had a higher intracellular fitness compared to the donor mtDNA.

Examples of selfish mtDNA can be found among invertebrates. For instance, in *Caenorhabditis briggsae*, an mtDNA variant with a large deletion might have an advantage in the cell, but was harmful at the organismal level [27]. In *Caenorhabditis elegans*, mtDNA variants with a large deletion have been maintained in the state of heteroplasmy with the wild-type mtDNA due to the effects of multidirectional intra- and interorganismal selection. [28]. Remarkably, mtDNA from one species can displace native mtDNA in another species, as has been demonstrated in heteroplasmic fruit flies harboring two different mtDNAs from two closely related species, *Drosophila mauritiana* and *Drosophila melanogaster* [29].

Finally, an example of an organism in which selfish mtDNA variants can arise as a result of mutations is baker’s yeast *Saccharomyces cerevisiae*. Yeast cells inherit mtDNA from both parents (mating haploid cells) [30]. If these parental haploid cells have different mitotypes, the progeny will have mitochondrial heteroplasmy. At the same time, some variants of yeast mtDNA with extensive deletions (*rho*$^-$) demonstrated a bias in the mtDNA inheritance: crossing *rho*$^-$ cells with wild-type *rho*$^+$ cells led to the formation of almost exclusively *rho*$^-$ diploid cells [31-33]. We have recently shown that this bias can be explained by both an increased number of mtDNA copies in *rho*$^-$cells and elevated intracellular fitness of *rho*$^-$ mtDNA variants compared to wild-type *rho*$^+$ mtDNA variants [34]. Mutant mtDNA variants also emerged and rapidly become fixed (remained the only variants) in yeast cells grown in the absence of selection pressure at the whole-cell level, indicating the existence of intracellular selection favoring proliferation of mtDNA variants with extensive deletions [35].

HYPOTHETICAL MECHANISMS PROVIDING AN ADVANTAGE TO SELFISH mtDNA IN THE CELL

While the presence of selfish mtDNAs in multiple cell types and species has been experimentally confirmed, the mechanisms that confer their competitive advantage over wild-type DNA remain poorly understood. Nonetheless, there are several hypotheses, with a varying degree of indirect experimental support, that aim to explain the differences in the intracellular fitness between the wild-type and mutant mtDNA mitotypes.

Increased replication initiation frequency. The mechanism allowing one mtDNA variant to displace another mtDNA variant may involve the differences in the replication initiation frequency. This mechanism implies that mutations in the mtDNA replication origin may result in the increased replication frequency for one mtDNA variant compared to another (Fig. 2a). Even a slight increase in the replication frequency, if it occurs over many replication rounds, could lead to the displacement of one mtDNA variant by another during either individual development or across the generations. In support of this assumption, it has been found that the drivers for the age-associated

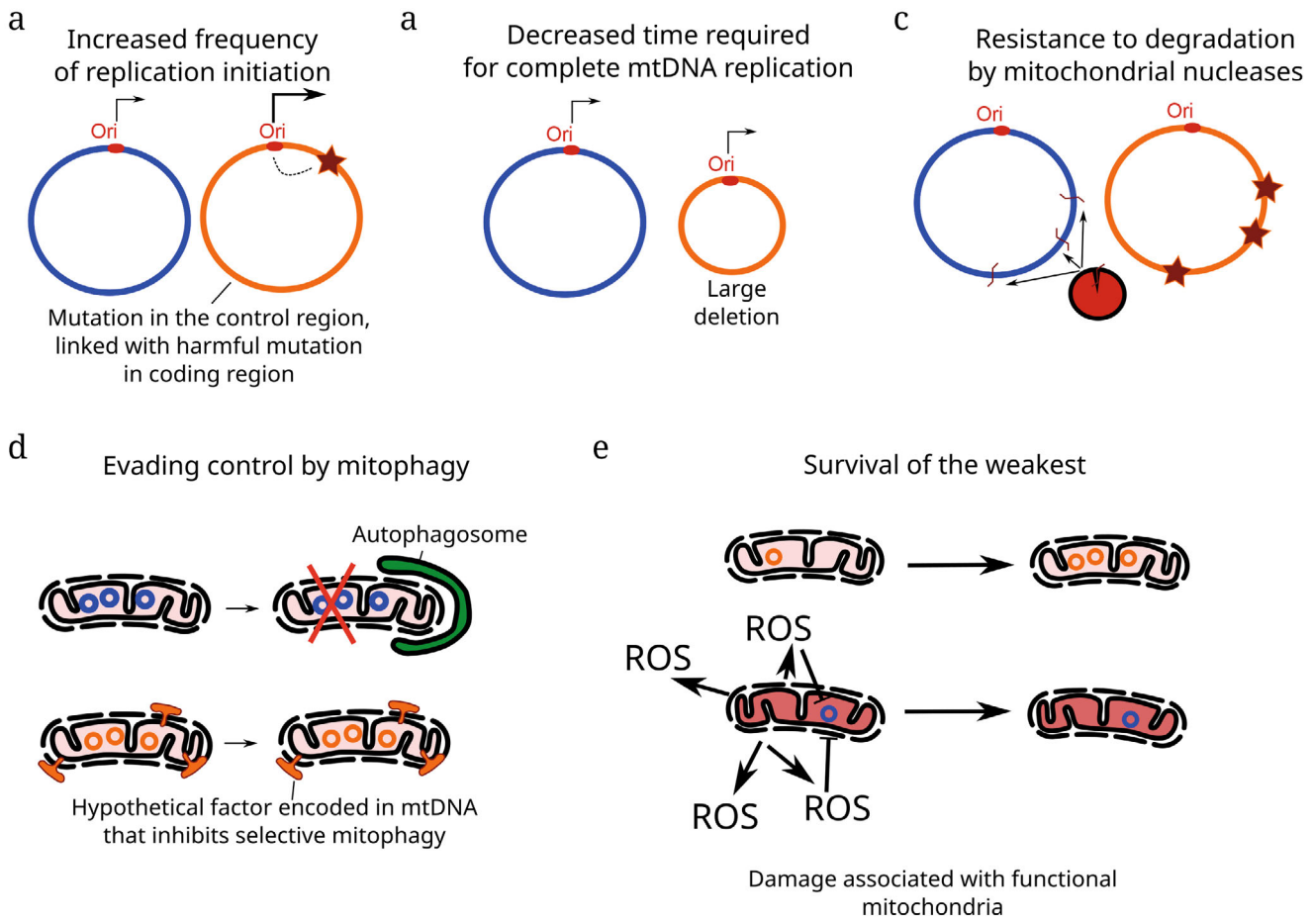

Fig. 2. Hypothetical mechanisms allowing a particular variant of mtDNA to have a higher intracellular fitness despite the presence of harmful mutations (see the text for discussion).

accumulation of mutant mtDNA are mtDNA variants with mutations in the non-coding region containing the *OriH* sequence responsible for the initiation of mtDNA replication [16]. In *D. melanogaster* fruit flies with artificially created heteroplasmy, the "winning" mtDNA variant was determined, at least in some cases, by the sequence of the non-coding region [29].

However, the possibility of emergence of mtDNA variants with "more active" replication origins leaves open the question of why such variants had not appeared and been fixed during the evolution. We suggested that mutations increasing mtDNA fitness at the intracellular level inevitably reduce the fitness of these variants at the cellular or organismal levels. For instance, an increased replication frequency could reduce the transcription rate, since initiation of one process delays the other [36]. Excessive replication initiation may increase the amount of mtDNA per cell beyond the optimal levels, potentially causing detrimental effects.

Advantages in the replication rate of mtDNA molecules with large deletions. Another hypothetically possible mechanism allowing some mtDNA variants to have a higher intracellular fitness is the difference in the duplication rate, as large deletions reduce the time required for complete mtDNA replication (Fig. 2b). However, the key factor in this case is the relation between the mtDNA duplication time and replication initiation frequency, which determines the proportion of replicating mtDNA molecules at a given moment of time. If the fraction of simultaneously replicating molecules is small, then the time required for the replication of the entire molecule should not have a significant effect on their intracellular fitness. To illustrate this, let us consider an extreme case, when there is only one functional replisome in the mitochondria and the replication of the next molecule does start until the replication of the previous molecule is complete. Obviously, in this case, the replication time will have no effect on the relative intracellular fitness of the mtDNA molecules.

The elongation rate of human mitochondrial DNA polymerase is 180-270 bases per minute [37, 38], suggesting that the replication of entire human mtDNA molecule takes no more than 2.5 h. This is expected to be true even considering that individual

mtDNA strands replicate asynchronously (see reviews [39, 40]). At the same time, the half-life of mtDNA in post-mitotic mammalian tissues is 1 to 3 weeks [41]. According to the results of computer modelling (taking into account several additional assumptions on the mtDNA copy number in a cell), the time required for the complete displacement of normal mtDNA by deletion-carrying mtDNA molecules would be decades [41]. Hence, it is unlikely that this mechanism has a significant effect on the spread of mutant mtDNA variants with deletions in postmitotic human tissues.

On the other hand, if mitochondrial biogenesis and associated proliferation of mtDNA proceed rapidly, mutant deletion-carrying mtDNA variants might gain an advantage. For example, when the content of mtDNA was reduced by treatment with the DNA intercalating agent ethidium bromide and then ethidium bromide was removed, mtDNA variants with large deletions repopulated the cells faster than the full-length mtDNA molecules [42]. Following this line of reasoning, mtDNA variants with deletions would also gain an advantage in the case of elevated mtDNA turnover, i.e., when the rates of mtDNA replication and degradation increase simultaneously. Indeed, mtDNA is actively degraded as a result of mitophagy activation or mtDNA damage [3, 43]. Moreover, mtDNA variants with deletions may gain a greater relative advantage in species with a large mitochondrial genome and short cell cycle, which implies a high proportion of replicating mtDNA molecules at any given time. Such organisms include yeast species, some of which have mitochondrial genomes exceeding 100 kb [44].

Avoidance of mtDNA degradation. mtDNA is constantly synthesized and degraded, even in postmitotic tissues [41]. mtDNA is also actively degraded in germline cells, ensuring the mtDNA inheritance strictly along the maternal line [45]. mtDNA degradation occurs predominantly in the mitochondrial matrix due to the activity of mitochondrial nucleases [46] or during autophagy, along with the degradation of mitochondria [47]. Theoretically, both these processes can be selective towards some mtDNA variants compared to others (Fig. 2, c, d). Let us consider these processes separately.

Selective autophagy of mitochondria (mitophagy) can ensuring the quality control of mtDNA, but this requires specific mtDNA molecules to be “linked” to protein variants encoded in these molecules [48, 49]. Such linkage is possible due to restrictions on the diffusion of mtDNA-encoded complexes through the mitochondrial network [50] or when the mitochondrial dynamics processes (continuous mitochondrial fusion and fission) are limited. For example, in *Drosophila*, mutant mtDNA variants with harmful mutations are eliminated via mitophagy during oogenesis [51]. This process is preceded by a decrease in the amount of mitofusin protein responsible for the mitochondrial fusion, leading to the fragmentation of mitochondrial reticulum. It is important to note that this mechanism takes place only in germline cells, but not in somatic ones [51]. Therefore, selective mitophagy in the absence of mitochondrial dynamics can lead to the elimination of mutant mtDNA variants, thus preventing their proliferation.

At present, no mechanism has been identified by which selfish mtDNA could evade the control by mitophagy and gain advantage over the wild-type mtDNA. However, it could be assumed that if an mtDNA variant encodes a factor that inhibits mitophagy or promotes mitochondrial fusion, it can increase its own intracellular fitness by evading the quality control at the level of individual organelles (Fig. 2d). Despite that mtDNA typically encodes a strictly defined set of genes, mtDNA of some invertebrate species contains ORFans – open reading frames that encode proteins with no homology to any known protein, but subjected to the purifying selection pressure [52, 53]. Although the functions of the encoded proteins remain unknown, we propose that they might modulate the mitophagy or mitochondrial dynamics, thereby contributing to the increased intracellular fitness of mtDNA variants harboring them.

Another mechanism of mtDNA degradation is associated with the activity of nucleases located in the mitochondrial matrix. The presence of double-strand breaks in mtDNA triggers rapid degradation of linear mtDNA fragments, driven by the exonuclease activities of mtDNA polymerase gamma and MGME1 nuclease [54]. A deficit in the exonuclease activity can drive accumulation of deletion-carrying mtDNA variants, as was found in mice with a homozygous mutation in the mitochondrial mtDNA polymerase gene ($PolG^{D257A/D257A}$), leading to the disruption of its exonuclease activity [55]. The degradation of male mtDNA in *Drosophila* spermatids requires the presence of another mitochondrial endonuclease, EndoG [56], and exonuclease Poldip2 [46]. Finally, the mitochondrial genomes of some fungal species contain homing endonucleases capable of cleaving DNA and then integrating their coding sequence at the site of the double-strand break. This allows endonuclease genes to spread in the population as a selfish element of mitochondrial genome [57, 58]. Taken together, these data suggest that mtDNA variants whose sequences lack the sites for hydrolysis with mitochondrial nucleases may gain an advantage under conditions when mtDNA is actively degraded (Fig. 2c), e.g., as a result of damage or during certain life cycle stages.

Survival of the weakest. In 1996, Aubrey de Grey proposed that the wild-type mtDNA associated with a fully functional respiratory chain might be more

susceptible to degradation than mutant mtDNA unable to provide an assembly of the functional respiratory chain [59]. This hypothesis was named "survival of the weakest" (Fig. 2e). Indeed, in some cases, mitochondrial respiratory chain can be a source of reactive oxygen species (ROS) [60, 61] that cause mutations in mtDNA. This is consistent with the fact that the C to T mutations typical of oxidative damage in a single-stranded DNA, dominate in vertebrates [62, 63]. At the same time, the damage to mtDNA caused by hydrogen peroxide (one of ROS forms) can induce mtDNA degradation [64].

However, it should be noted that this hypothesis has not been confirmed experimentally, likely because there are other factors that can exert a strong effect in the opposite direction. Indeed, the import of mitochondrial proteins essential for mtDNA replication depends on the transmembrane potential at the inner mitochondrial membrane and ATP content in the mitochondrial matrix [65]. Therefore, mutant variants unable to provide formation of the functional respiratory chain and ATP synthase will be at a disadvantage in this respect. Moreover, mitochondria in cells actively fuse and divide, which equalizes their content throughout the cell's mitochondrial network [66, 67] and makes it difficult to link specific mtDNA molecules to the areas of mitochondria enriched with proteins encoded in them.

CONCLUSION

mtDNA selection occurs simultaneously at several organizational levels. Consequently, the emergence of selfish mtDNA variants that have a replicative advantage but are detrimental to the cell or multicellular organism is possible. Despite the mechanisms that eliminate mutant mtDNA, selfish mtDNAs can emerge in nature and experimental systems, that might have an advantage due to a faster replication or ability to evade degradation. Therefore, eukaryotic organisms are under continuous pressure created by the potential emergence of such mtDNA.

We propose that during the early stages of eukaryotic evolution, before the mitochondrial quality control systems evolved to suppress proliferation of mutant mtDNA, selfish mtDNAs had posed a significant challenge to eukaryotic cells. This pressure has likely drove the evolution of cellular mechanisms protecting against selfish mtDNA. Such mechanisms may include control of mtDNA copy number in the germlines of multicellular animals [68], selective mitophagy [69], and uniparental inheritance of mtDNA in most eukaryotic species [70], which prevents the horizontal transfer of selfish mtDNA within populations.

Abbreviations

mtDNA mitochondrial DNA

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Contributions

G.M. and D.K. analyzed the published articles and wrote the manuscript; D.K. prepared the figures.

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Ethics approval and consent to participate

This work does not contain any studies involving human or animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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