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Title: Small mitochondrial RNAs as mediators of nuclear gene regulation, and potential implications for human health.

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Abstract

Much research has focused on the effects of pathogenic mitochondrial mutations on health. Notwithstanding, the mechanisms regulating the link between these mutations and their effects remain elusive in several cases. Here, we propose that certain mitochondrial mutations may disrupt function of a set of mitochondrial-transcribed small RNAs, perturbing communication between mitochondria and nucleus, leading to disease. Our hypothesis synthesizes two lines of supporting evidence. Firstly, several mitochondrial mutations cannot be directly linked to effects on energy production or protein synthesis. Secondly, emerging studies have described the existence of small RNAs encoded by the mitochondria and proposed their involvement in RNA interference. We present a roadmap to testing this hypothesis.

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Introduction

The mitochondria are intracellular organelles that have retained their own genome of mitochondrial DNA (mtDNA), encoding 37 known functional products; 13 proteins involved in oxidative phosphorylation (OXPHOS), and 24 functional (mt-)RNAs involved in the synthesis of these proteins. OXPHOS, the metabolic pathway used to oxidize nutrients for the ultimate production of ATP, takes place across five enzyme complexes, four of which rely on synergistic interactions between protein subunits transcribed by the mtDNA within the mitochondria and subunits transcribed from the nuclear genome, the latter of which are translated in the cytoplasm and then transported into the mitochondria to exert their function [1;2]. Due to the close synergistic coordination required between the mitochondrial and nuclear proteins that comprise these enzyme complexes, defects in one mitochondrial protein are sufficient to cause a deficiency in OXPHOS [2]. One of the best-known examples of a single defect causing deficiency in OXPHOS and debilitating symptoms is the mutation T8993G [2]. This mutation changes one of the amino-acids in the protein MT-ATP6 from a hydrophobic leucine to a hydrophilic arginine, disrupting the function of this protein; when this mutation is present in over 70% of the mtDNA copies of a cell, it causes a range of symptoms such as Neurogenic muscle weakness, ataxia and retinitis pigmentosa syndrome (NARP)[2].

Similarly, the current paradigm indicates that mutations impairing the function of mt-rRNAs and mt-tRNAs will affect the synthesis of the 13 mtDNA-encoded proteins, leading to the impairment of OXPHOS function. The mechanisms leading to impaired OXPHOS function include mutations disrupting RNA editing sites on the mt-tRNA, and mutations that interfere with the interaction between mt-tRNAs and mitochondrial elongation factor (eg. mtEF-Tu)

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[3, 4]. There are over 200 mutations in the mt-tRNAs that have been associated with diseases, and in numerous cases mutations have been described that unambiguously affect OXPHOS function, with clear negative consequences for human health. However, the causal links between these mutations and subsequent diseases are sometimes weak, and less than half of these 200 mutations have a known mechanism of action [4]. Studying these mechanisms of action is sometimes challenging due to the presence of mitochondrial heteroplasmy within cells. Mitochondrial heteroplasmy is a phenomenon in which multiple copies of mtDNA within the same cell carry different sets of mutations, rendering it difficult to disentangle the effects of mutant mtDNA from the original wild-type mtDNA copies with which they coexist. This phenomenon is likely to have significantly contributed to the presence of discrepancies in several cases between the theorised mechanisms by which these mutations are predicted to affect human health and the observed results of experimental studies. In this paper, we document and further explore discrepancies in links between mtDNA mutations and mitochondrial diseases, and present evidence for an alternative mechanism through which mtDNA mutations might generally affect health; a mechanism that is independent of direct alterations to OXPHOS function.

The first discrepancy: linking mitochondrial mutations to energy production

The first discrepancy to have emerged between theory and experimental observation is that, contrary to expectation, disease-associated mtDNA mutations linked to impaired energy production are often located within mitochondrial genes that are not directly associated with the mitochondrial complex exhibiting OXPHOS deficiency. An example is the missense mutation T3094C located within the gene MT-ND5, which encodes one of the subunits at the core of the mitochondrial Complex I. Indeed, there are multiple studies linking this mutation to mitochondrial diseases such as MELAS and Leigh Syndrome (LS), where the authors

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found OXPHOS deficiency in presence of this mutation [5–8]. Theoretically, it is expected that the OXPHOS deficiency in the case of this mutation will be directly linked to dysfunction of Complex I. Yet, associations between this mutation, the activity of individual OXPHOS complexes, and overall OXPHOS deficiency have been shown to be highly variable across patients with MELAS and LS. For example, Ng et al. 2018 found that in the skeletal tissues of 10 patients affected by either MELAS or LS, only half exhibited an associated OXPHOS deficiency. Surprisingly, across the patients with normal OXPHOS, there were patients with high frequency of this mutation (~80% in heteroplasmy). Furthermore, across the five of the ten patients with a clear OXPHOS deficiency, only one had exhibited deficiency to Complex I only, while the other four exhibited deficiencies to Complex I in tandem with other complexes (variable across patients). Indeed, in one of the patients, both complex I and II were affected, even though Complex II is formed only by nuclear proteins and it is therefore expected to function independently of mutations in the mtDNA. If the mutation T3094C affected the assembly of Complex I, then we would expect this complex to be consistently affected and to observe frequent OXPHOS deficiency, however, the effects of this mutation on OXPHOS function generally, and Complex I function specifically, are inconsistent regardless of heteroplasmy levels or pedigree of the patients [5]. Furthermore, if Complex I deficiency were to create consistent downstream effects on the function of the other four complexes, we would expect most of the other complexes to be consistently affected, rather than the high variation in affected complexes across the patients studied. Thus, while mutation T3094C is known to cause OXPHOS deficiency by directly affecting Complex I, there is some inconsistency with this interpretation.

Other studies present similar discrepancies when it comes to linking mitochondrial genes harbouring candidate pathogenic mutations to their effects on phenotype. We can see clear

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examples of these discrepancies in studies of the A3243G mutation. A3243G is located on the mt-tRNA Leu and is one of the most common mutations in mitochondrial disease, and the major known genetic cause of MELAS [9, 10]. However, studies investigating this mutation have revealed several unexpected findings. For example, Li and Guan (2010) reported that overexpression of Leucyl-tRNA synthetase 2 (LARS2) partially recovered mt-tRNA Leu production in cybrid cells carrying the A3243G mutation. Along with an increase in mt-tRNA Leu expression, the authors found a slight increase in ATP production in the cybrid cells, thus supporting a predicted link between the expression of mt-tRNA Leu and ATP production. However, closer scrutiny of their study challenges this interpretation. When overexpressing LARS2, the authors observed a range of effects that cannot be linked to mt-tRNA Leu, but that might have caused the observed changes in ATP production. Among these effects, there was an increase in acetylation of mt-tRNAs Met and Ala, and increased synthesis of proteins MT-COI and MT-COII [11]. Due to the similar amount of Leucine across all mtDNA-encoded proteins, there is no obvious connection between the increased synthesis of these two proteins and the recovery in expression of mt-tRNA Leu. However, the increased synthesis of these proteins can be easily linked to the slight increase in ATP production, thus challenging the idea that the recovery of mt-tRNA production was the direct cause of either increased protein synthesis or ATP production. Similarly, in another study investigating the role of A3243G in pigmentary retinopathy, Chichagova *et al.* [12] discovered cells with this mutation produced as much energy as healthy cells, albeit while still presenting pathogenic features [9]; thus providing a practical case in which the A3243G mutation does not cause detectable decreases in energy production *per se,* but is nonetheless still presumed as the cause of the disease.

The second discrepancy: variable positions of pathogenic mutations across mt-tRNAs

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The mitochondrial genome harbours 22 mt-tRNAs, which play a fundamental role in the translation of proteins. Mutations in mitochondrial mt-tRNA genes have been linked to impairment in mitochondrial protein synthesis through a range of mechanisms [12]. Indeed, mutations on mt-tRNAs can involve sites that require RNA editing, or sites that are important for the correct three-dimensional structure of the RNA, thus potentially altering the interactions of mt-tRNAs with the ribosomal proteins and other RNA-binding proteins. Among the RNA-binding proteins able to bind mt-tRNAs, probably the most important are the aminoacyl-tRNA synthetases responsible for protein translation in the mitochondria, and interfering with the binding between mt-tRNAs and these proteins is a possible cause of diseases [13]. A discrepancy between theory and experimental observation, however, arises when considering the highly conserved function and structure of different mt-tRNAs. Such strong conservation implies that the pathogenic potential of a mutation at a particular point in the mt-tRNA sequence should be similar among mt-tRNAs; thus, a mutation associated with impaired protein synthesis at one position of the nucleotide sequence in one mt-tRNA should in theory be associated with similar impairment when found at the identical position of other mt-tRNAs. Contrary to expectations, however, the position of pathogenic mutations is specific to each mt-tRNA.

Indeed, many mutations found within the mtDNA sequence are not pathogenic and are widespread across populations. Mutations that are shared by many people, and that can be traced back to a common ancestor, are classified in groups named mtDNA haplogroups [14]. Haplogroup mutations are generally used for phylogenetic purposes to infer evolutionary relationships between related taxonomic units; [15], and traditionally different haplogroups were not considered to incur differences in their effects on components of health and fitness (neutral *sensu lato* ; [16]). Noticeably, positions of neutral and pathogenic mutations are similar, highlighting the tRNA-specific nature of pathogenic mutations (**Fig.1A**). An

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interesting example is represented by mutations found near nucleotide positions 27,36 on mt-tRNA Histidine and 31,42 respectively on mt-tRNAs Leucine. These mutations are in similar positions in the mt-tRNA structure, however, the mutations in the mt-tRNA for the amino acid Leucine cause MELAS, while those on the mt-tRNA for histidine do not have any known negative effects, and are indeed widespread among populations with haplogroup L1 (position 27) and L3 (position 36; [14]). Furthermore, some mt-tRNAs (e.g. mt-tRNA Leu1) are associated with many more pathogenic mutations compared to other mt-tRNAs, suggesting that mt-tRNA conservation is not as strict for all mt-tRNAs despite them having similar function and structure.

Indeed, when it comes to the role of mt-tRNA mutations in mitochondrial disease, the discrepancy between theory and experimental observation extends to the function of these RNAs. Indeed, if pathogenic mutations in the mt-tRNAs exerted their negative effects through impairment of their ability to translate mRNAs into proteins, we would then predict that all mtDNA-encoded proteins possessing the particular amino acid translated by the mutant mt-tRNA variant would be affected. This prediction is not upheld. For example, biochemical studies comparing mitochondrial enzyme functionality in tissue samples of patients suffering from MELAS relative to control samples, brought about by the A3243G mutation in mt-tRNA Leucine 1, reported that only Complex I and IV are affected by this mutation, while Complex III and V were unaffected [17]; despite each of the complexes comprising similar leucine content (ranging from 13 to 20%). Taken together, the data highlight the presence of a discrepancy between theoretical expectation and experimental observation, where researchers have by tradition assumed that mitochondrial mutations in mt-tRNAs will lead to impaired protein synthesis. However, if this assumption were to be the case, then the associated impairment would be expected to be general across multiple protein complexes exhibiting high representation of the relevant amino acids. Instead, we propose

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that mutations in mt-tRNAs, which have previously been associated with mitochondrial diseases, do not consistently lead to impaired OXPHOS, and thus the reasons for the pathogenicity of these mutations are yet to be adequately explained, and warrant consideration of an alternative hypothesis.

An alternative hypothesis

We propose the existence of a mechanism we call 'mitochondrial interference' to explain the discrepancies discussed above (**Fig.1B**). Under this hypothesis, we contend that some occurrences of mitochondrial disease may originate from mutations within small RNAs encoded in the mitochondrial genome. In particular, we hypothesize the existence of functional small RNAs encoded in the mitochondria that are able to interfere with nuclear regulation. We propose that these (mt-)small RNAs can modify nuclear protein expression through RNA interference. RNA interference is a process in which a small RNA leads a protein complex to a target mRNA, blocking its translation [18]. The mechanism used to block mRNA translation varies depending on both the proteins and small RNAs involved. For example, in humans, miRNAs can guide both Argonaute 1 and Argonaute 2 to a target mRNA, but they block translation using different mechanisms. Argonaute 1 blocks mRNA translation by binding to the target mRNA and physically preventing the ribosome from translating it. In contrast, Argonaute 2 has a catalytic domain in its structure that enables the degradation of the target mRNAs, thus both eliminating the mRNA itself and blocking its translation. Nonetheless, in all types of RNA interference, the small RNA recognizes the target mRNA thanks to the partial complementarity of their sequences, and therefore mutations of either sequence, small RNA or target, could disrupt RNA interference function. It is well known that disruption of the interaction between miRNAs (nuclear small RNAs) and their target mRNAs can be pathogenic [19]. We propose that mutations in the mtDNA

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sequence that do not affect either protein structure or synthesis, may affect other currently cryptic products in the mitochondrial genome, including the expression and function of small non-coding RNAs. According to our hypothesis, and supported by the emerging evidence presented below, we propose that these mitochondrial small RNAs are encoded within other known mtDNA genes, such as mt-tRNAs and coding genes. The occurrence of mutations within the small RNAs nested within known mt-tRNA and mt-coding genes, might explain the discrepancy of how these mutations can consistently confer mitochondrial disease without necessarily causing an associated effect on protein structure or synthesis.

The mitochondrial interference hypothesis provides an alternative explanation for the observed pathogenicity of several mitochondrial mutations. Indeed, the wide range of targets that mt-small RNAs could affect, unrelated to ATP production, could explain many of the various symptoms observed in humans. Indeed, most nuclear mRNAs have tissue-specific expression, and specific small RNAs likely affect different mRNA targets depending on the tissue considered [20, 21]. Therefore, the disruption of small RNA-mediated regulation could be expected to cause tissue-specific effects, and be pathogenic only in specific tissues, consistent with what is commonly observed in the case of many mitochondrial diseases [2]. Importantly, the effects of mutations in these small RNAs will not only be expected to reduce the affinity with their target, but may also enhance their affinity with other targets, thus creating a wide range of possible, and complex, effects, which may explain the wide range of disease symptoms linked to mitochondrial mutations. Moreover, our hypothesis provides an alternative explanation for the observation of pathogenic mitonuclear interactions. In fact, while mutations that disrupt fundamental cellular function, such as energy production, cannot be expected to be widespread in a population due to strong purifying selection against them [22, 23], the force of purifying selection may conceivably be less intense on mutations affecting small RNAs if disruption of the target mRNAs does not cause systemic cellular

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dysfunction. While this parallel hypothesis provides a theoretical explanation for the discrepancies that we have outlined above, it ultimately hinges on solid evidence of the existence of functional small mitochondrial RNAs. Such evidence is starting to emerge, and we discuss it in the next section.

Recent discoveries supporting the existence of mitochondrial interference.

Recently, a new class of mitochondrial small non-coding RNAs of unknown function was identified in humans [24]. These small RNAs are between 20 and 30 nucleotides long and usually encoded within mt-tRNAs. These mitochondrial RNAs are similar in structure to other well-known nuclear small RNAs such as microRNAs and piwi-interacting RNAs, having similar size, being transcribed in small clusters, and having longer RNA precursors [24–27]. Thus, we contend it is possible that they interact with the same proteins as do nuclear small RNAs. Several lines of evidence already support this contention [28]. We note that most of the proteins that are associated with regulation by piwi-interacting RNAs are localized on the mitochondrial membrane [29–31]. Furthermore, mt-tRNAs interacting with Argonaute (1 to 4), a key protein in the RNA interference mechanism, were recently identified in humans [28]. Additionally, a recent study found evidence that changes in cellular mitochondrial content have profound effects on global variability in protein expression [32], indicating a pervasive effect of the mitochondria on patterns of gene expression across the nuclear genome. Although there are multiple possible pathways that the mitochondria might use to influence gene regulation, these effects align with the mitochondrial interference hypothesis. In fact, the amount of small mitochondrial RNAs within any particular tissue is correlated with mtDNA copy number [25], and thus the abundance of small mitochondrial RNAs present within a cell might directly underlie changes

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in protein expression. Moreover, several studies have now reported the widespread presence of small mitochondrial RNAs across vertebrates, including humans, and that the sequences encoding these small RNAs lie within other mitochondrial genes [25–27, 33–35]. Taken together, these emerging studies indicate that mitochondrial small RNAs exist and are widespread. Although their function remains to be verified, these small RNAs have some parallels to nuclear small RNAs involved in modulation of gene expression, and thus may play a role in the manipulation of nuclear expression on behalf of the mitochondria. These studies provide a body of preliminary evidence to suggest that mitochondrial interference may be a previously overlooked genetic mediator of mitochondrial effects on human health.

Solving the discrepancies

We propose that the mitochondrial interference hypothesis has capacity to resolve at least some of the discrepancies between theory and experimental observation outlined above. The A3243G mutation, which is located within the mt-tRNA Leu1, has been consistently associated with many different symptoms, spanning from retinal neurodegeneration to stroke-like episodes [9, 36]. The diversity of symptoms associated with this mutation is difficult to explain based on the current paradigm, which assumes that variation in levels of tissue-specific heteroplasmy will confer differences in the degree of OXPHOS impairment across tissues. However, this diversity could be explained if the mutation A3243G was located within a small mitochondrial RNA. Indeed, genes of both the mitochondrial and nuclear genomes exhibit tissue-specific expression [25], thus the interactions between the RNAs of both genomes will be predicted to lead to tissue-specific effects. We predict that functional effects resulting from mutations in mitochondrial small RNAs and their mRNA targets will be similar to effects resulting from mutations in nuclear miRNAs, or in their targets.

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We propose that mutation A3243G may lie within a small mitochondrial RNA and predict that the abundance of both this small RNA and its target within any given cell or tissue lineage is tied to pathology of that lineage. Thus, we predict that mutations within this small mitochondrial RNA may affect different nuclear mRNA transcripts in each tissue, depending on which transcript is expressed, and cause the various symptoms associated with the mutation A3243G. These predictions are supported by one, previously ignored, but intriguing published finding. The first study reporting the presence of small mitochondrial RNAs found that mt-tRNA Leu1 encodes a small mitochondrial RNA precisely in the region where the mutation A3243G is located [24]. This small mitochondrial RNA is differentially expressed across multiple cell lines, supporting the presence of tissue-specific interactions [24]. Furthermore, the authors of that study found no correlation between the expression of mt-tRNAs and the expression of small mitochondrial RNAs, suggesting that these small mitochondrial RNAs were not simply bioproducts of RNA turnover from the mt-tRNAs. Therefore, some evidence already exists that patients harbouring the mutation A3243G are expressing a mutated small mitochondrial RNA with unknown function, which might explain the pathogenicity of the mutation A3243G.

Population-specific effects

The mitochondrial interference hypothesis could help advance understanding of observed variation in penetrance in effects of mtDNA mutations across populations, where the same mutations are known to confer different effects depending on the individual ancestry. For example, a recent study found that a specific mt-haplogroup (N9a) is associated with increased resistance to Type 2 diabetes in people of Asian descent [37]. There is increasing evidence of cases in which the effects of pathogenic mitochondrial mutations changes depending on the genetic context and environment in which these mutations are expressed. The mutation T3394C is often found in patients suffering from Leber hereditary optic

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neuropathy (LHON). Ji et al. (2012) studied the pathogenicity of this mutation in different Asian population contexts – Han and Tibetan, creating cell lines that carried the mutation on different mtDNA haplogroup backgrounds, M9 (enriched in Tibetan populations), B4c and F1 (enriched in Han populations). They found complex I-specific activity and basal NADH-linked respiration was substantially higher (and representative of levels in control lines) when the mutation was placed on the M9 background relative to the other two backgrounds, revealing a haplotype-specific effects of this mutation. Intriguingly, the M9 haplotype with the T3394C is greatly enriched in high-altitude Tibetan populations, suggesting that it has been shaped under positive selection in these populations (i.e. adaptive in high altitude, but pathogenic in low altitude populations). According to Ji et al. (2012) this observation suggests that the effect of specific mitochondrial mutation on human health depends on the genetic background (population and haplotype) and environment (altitude) [38]. Indeed Kang et al. (2015) identified a candidate list of numerous mtDNA mutations that are enriched in high altitude Tibetan populations, which they contend may be involved in adaptation to hypoxia, and thus serve beneficial effects in these populations, despite being pathogenic in other human populations. This suggests that these mutations may have coevolved with nuclear targets to moderate these effects at high altitude. The contention that nuclear loci will moderate the effects of mtDNA mutations is not new; indeed, there are many known nuclear modifiers of mtDNA mutations associated with human disease [39]. The observation that the pathogenicity of mtDNA mutations depends on the genetic background might be explained by the mitochondrial interference hypothesis. Indeed, under the mitochondrial interference hypothesis, we would propose that at least some of the mutations defining the mt-haplogroup, such as T3394C, lie within small mitochondrial RNAs, and that Tibetans and other Asian populations have different nuclear alleles interacting with the mutated small mitochondrial RNA. Therefore, the pathogenic phenotype should not have the same penetrance across all populations carrying the mutated small mitochondrial RNA but would have high penetrance only when a particular target nuclear allele is paired with the mutated small mitochondrial RNA, creating a population-specific effect. This hypothesized population-specific effect

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depends on the interactions of the RNAs made by both the mitochondria and nucleus. Indeed, according to our hypothesis, the small mitochondrial RNA should bind a non-coding region of the target nuclear mRNA, and if this region has a population-specific polymorphism that increases the affinity for the mutated small mitochondrial RNA, then the regulation of the mRNA having such polymorphism would be disrupted when in presence of the mutated small mitochondrial RNA, thus manifesting the pathogenic phenotype.

Testing the Mitochondrial Interference hypothesis

Demonstrating both the existence and pervasiveness of mitochondrial interference is complex given that any such interference would involve interactions between two genomes – mitochondrial and nuclear. However, our hypothesis provides three key predictions that can be tested (**Fig.2**). Our first prediction is that small mitochondrial RNAs can interfere with the translation of nuclear mRNAs. This prediction can be tested by leveraging bioinformatic analyses to identify the targets of these small mitochondrial RNAs, then injecting small mitochondrial RNAs whose sequences specifically target candidate mRNAs of interest into cell cultures, and, finally, by performing a western blot to verify the downregulation of the translated proteins. A similar experiment and proof-of-concept was performed recently, where the authors synthesized candidate small mitochondrial RNAs as single-strand RNAs and injected these into the gonads of a species of clam, finding that the injected RNAs disrupted a specific pathway of methylation in the clam gonads in a time-sensitive manner [40]. Performing a similar experiment in human cell culture would require modifications, such as using transient transfection to deliver the small RNAs [41]. However, these experiments carry several risks. While using transient transfection to deliver synthetic small RNAs might deliver some results, researchers found that these methods induce the presence of high-molecular RNA complexes, which are not induced with other methods such as

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plasmid transfection [41]. Unfortunately, since the mechanisms underlying the biogenesis of small mitochondrial RNAs remain unknown, researchers are currently unable to leverage methods that utilize small RNA biogenesis pathways (e.g. plasmid transfection) to consistently transcribe a target small mitochondrial RNA, but must instead introduce the target small RNAs transiently into the focal cells and tissues (e.g. transient transfection). Other risks in these experiments are linked to the ability to accurately identify the mRNAs targeted by the small mitochondrial RNAs; as previously seen with miRNAs, modern methods have strong confirmation bias and do not perform well with novel RNAs [42]. Nonetheless, these risks might be offset by using high-throughput approaches, such as analysing the entire proteome of multiple injected cell cultures, thus avoiding the necessity of a target identification step [43]. By testing this prediction experimentally, it would be possible to verify whether or not small mitochondrial RNAs may act as inhibitors of mRNA translation.

Our second prediction is that the small mitochondrial RNAs can bind to key proteins of RNA interference. This prediction can be tested through RNA immunoprecipitation sequencing (RIP-seq) experiments, with nuclear proteins key to RNA interference– such as Argonaute (1 to 4), or Piwi. The RIP-seq technique involves an RNA and RNA-binding protein co-immunoprecipitation, followed by RNA sequencing, thereby revealing all the RNAs able to bind the RNA-binding protein [44–46]. Testing the ability of small mitochondrial RNAs to bind to Argonaute (1 to 4), or Piwi, would have power to resolve the question of whether small mitochondrial RNAs may regulate patterns of nuclear gene and protein expression through RNA interference. Furthermore, by investigating if the small mitochondrial RNAs bind to multiple Argonaute and Piwi proteins, it would be possible to distinguish between specific interactions, wherein a small mitochondrial RNAs binds to a specific Argonaute or

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Piwi protein consistently, from potentially false interactions, where a small mitochondrial RNAs is found to bind all proteins with similar abundance.

The third prediction is that the function of cells that harbour pathogenic mtDNA mutations (located within candidate small mitochondrial RNAs) can be restored through injection of the small mitochondrial RNA counterparts that lack the mutation. However, experiments investigating the effect of pathogenic mtDNA mutations usually measure the recovery of the cells by measuring their OXPHOS activity, which might not work when investigating the mitochondrial interference hypothesis. Indeed, OXPHOS activity might not be affected, either directly or indirectly, from mutations within small mitochondrial RNAs, as we do not know the extent of their effect on gene regulation. Therefore, other methods should be used to verify the effect of small mitochondrial RNAs injections, such as measuring if the cell transcriptome and proteome are restored upon injection of the small mitochondrial RNAs to levels similar to that observed in wild-type cells. Nonetheless, designing a reliable experiment would be challenging, given we currently have no understanding of mechanisms of small mitochondrial RNA biogenesis and transport [47], thus providing synthetic small mitochondrial RNAs directly in the cytoplasm cannot accurately mimic the cell 'healthy state'. The endogenous small mitochondrial RNAs, or their precursors, have to move through the mitochondrial membrane to interact with nuclear transcripts, and a mechanism for such a process is unknown. If this process exists, it might not only transport the small mitochondrial RNAs, but it might be involved in their regulation, for example through RNA modifications, thus making restoration of cellular function through small mitochondrial RNAs challenging for now. Notwithstanding, while the implementation of these approaches is not without its challenges, they offer promising avenues to experimentally test the key predictions of the mitochondrial interference hypothesis, and therefore we believe deserve research attention.

Conclusion

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In sum, the mitochondrial interference hypothesis proposes a new framework for the investigation of mitochondrial diseases. Current studies of the genetics underpinning the expression of mitochondrial diseases focus mostly on effects of sequence mutations on OXPHOS dysregulation. In addition, we propose a role for the mitochondria in the regulation of the nuclear gene expression through mitochondrial small RNAs. We contend that recently discovered small mitochondrial RNAs may interfere with nuclear regulation and may therefore confer mitochondrial disease in a hitherto undescribed biological context; a context independent of OXPHOS functionality. Here, we have discussed small mitochondrial RNAs and their potential biomedical implications, highlighting their similarity with nuclear RNAs involved in the RNA interference mechanism. However, while focusing our attention on the potential role of these small RNAs in mitochondrial diseases, the potential implications extend beyond mitochondrial diseases *per se*, influencing common diseases such as Type 2 Diabetes as well as a range of late-onset diseases recently identified to have mitochondrial involvement [48]. In conclusion, we hope that the mitochondrial interference hypothesis will inspire clinicians, biomedical scientists, and the general scientific community to pursue new pathways in the study of mitochondrial biology in general, and mitochondrial diseases in particular.

References

1. **Anraku Y**. 1988. Bacterial electron transport chains. *Annu. Rev. Biochem.* **57**: 101–32.
2. **Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, McFarland R, … Turnbull DM.** 2016. Mitochondrial diseases. *Nat Rev Dis Primers* **2**: 16080.
3. **Richter U, Evans ME, Clark WC, Marttinen P, Shoubridge EA, Suomalainen A, … Battrsby BJ.** 2018. RNA modification landscape of the human mitochondrial tRNALys regulates protein synthesis. *Nat. Commun.* **9**: 3966.
4. **Blakely EL, Yarham JW, Alston CL, Craig K, Poulton J, Brierley C, … Taylor RW.** 2013. Pathogenic mitochondrial tRNA point mutations: nine novel mutations affirm their importance as a cause of mitochondrial disease. *Hum. Mutat.* **34**: 1260–8.
5. **Ng YS, Lax NZ, Maddison P, Alston CL, Blakely EL, Hepplewhite PD, … Gorman GS.** 2018. MT-ND5 Mutation Exhibits Highly Variable Neurological Manifestations at Low Mutant Load. *EBioMedicine* **30**: 86–93.
6. **Blok MJ, Spruijt L, de Coo IFM, Schoonderwoerd K, Hendrickx A, Smeets HJ.** 2007. Mutations in the ND5 subunit of complex I of the mitochondrial DNA are a frequent cause of oxidative phosphorylation disease. *J. Med. Genet.* **44**: e74.

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7. **Ching CK, Mak CM, Au KM, Chan KY, Yuen YP, Yau EK, … Chan AY.** 2013. A patient with congenital hyperlactataemia and Leigh syndrome: an uncommon mitochondrial variant. *Hong Kong Med. J.* **19**: 357–61.

8. **Valente L, Piga D, Lamantea E, Carrara F, Uziel G, Cudia P, … Tiranti V.** 2009. Identification of novel mutations in five patients with mitochondrial encephalomyopathy. *Biochim. Biophys. Acta* **1787**: 491–501.

9. **Chichagova V, Hallam D, Collin J, Buskin A, Saretzki G, Armstrong L, … Steel DH.** 2017. Human iPSC disease modelling reveals functional and structural defects in retinal pigment epithelial cells harbouring the m.3243A > G mitochondrial DNA mutation. *Sci. Rep.* **7**: 12320.

10. **Majamaa K, Moilanen JS, Uimonen S, Remes AM, Salmela PI, Karppa M, Hassinen IE.** 1998. Epidemiology of A3243G, the mutation for mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes: prevalence of the mutation in an adult population. *Am. J. Hum. Genet.* **63**: 447–54.

11. **Li R**, **Guan M-X.** 2010. Human mitochondrial leucyl-tRNA synthetase corrects mitochondrial dysfunctions due to the tRNALeu(UUR) A3243G mutation, associated with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like symptoms and diabetes. *Mol. Cell. Biol.* **30**: 2147–54.

12. **Yarham JW, Al-Dosary M, Blakely EL, Alston CL, Taylor RW, Elson JL, McFarland R.** 2011. A comparative analysis approach to determining the pathogenicity of mitochondrial tRNA mutations. *Hum. Mutat.* **32**: 1319–25.

13. **Sissler M**, **González-Serrano LE**, **Westhof E**. 2017. Recent Advances in Mitochondrial Aminoacyl-tRNA Synthetases and Disease. *Trends Mol. Med.* **23**: 693–708.

14. **van Oven M**, **Kayser M**. 2009. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum. Mutat.* **30**: E386–94.

15. **Rubinoff D**, **Holland BS**. 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. *Syst. Biol.* **54**: 952–61.

16. **Ballard JW**, **Kreitman M**. 1995. Is mitochondrial DNA a strictly neutral marker? *Trends Ecol. Evol.* **10**: 485–8.

17. **Lin D-S, Kao S-H, Ho C-S, Wei Y-H, Hung PL, Hsu MH, … Chiang MF.** 2017. Inflexibility of AMPK-mediated metabolic reprogramming in mitochondrial disease. *Oncotarget* **8**: 73627–39.

18. **Ha M**, **Kim VN**. 2014. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **15**: 509–24.

19. **Ardekani AM**, **Naeini MM**. 2010. The Role of MicroRNAs in Human Diseases. *Avicenna J. Med. Biotechnol.* **2**: 161–79.

20. **Ovando-Vázquez C**, **Lepe-Soltero D**, **Abreu-Goodger C**. 2016. Improving microRNA target prediction with gene expression profiles. *BMC Genomics* **17**: 364.

21. **Alberti C, Manzenreither RA, Sowemimo I, Burkard TR, Wang J, Mahofsky K, … Cochella L.** 2018. Cell-type specific sequencing of microRNAs from complex animal tissues. *Nat. Methods* **15**: 283–9.

22. **Stewart JB**, **Freyer C**, **Elson JL**, **Larsson N-G**. 2008. Purifying selection of mtDNA and its implications for understanding evolution and mitochondrial disease. *Nat. Rev. Genet.* **9**: 657–62.

23. **Cooper BS, Burrus CR, Ji C, Hahn MW, Montooth KL.** 2015. Similar Efficacies of Selection Shape Mitochondrial and Nuclear Genes in Both Drosophila melanogaster and Homo sapiens. *G3* **5**: 2165–76.

24. **Mercer TR, Neph S, Dinger ME, Crawford J, Smith MA, Shearwood AM, … Mattick JS.** 2011. The human mitochondrial transcriptome. *Cell* **146**: 645–58.

25. **Pozzi A**, **Dowling DK**. 2019. The Genomic Origins of Small Mitochondrial RNAs: Are They Transcribed by the Mitochondrial DNA or by Mitochondrial Pseudogenes within the Nucleus (NUMTs)? *Genome Biol. Evol.* **11**: 1883–96.

26. **Pozzi A, Plazzi F, Milani L, Ghiselli F, Passamonti M.** 2017. SmithRNAs: Could Mitochondria "Bend" Nuclear Regulation? *Mol. Biol. Evol.* **34**: 1960–73.

27. **Ro S, Ma H-Y, Park C, Ortogero N, Song R, Hennig GW, … Yan W.** 2013. The mitochondrial genome encodes abundant small noncoding RNAs. *Cell Res.* **23**: 759–74.

28. **Maniataki E**, **Mourelatos Z**. 2005. Human mitochondrial tRNAMet is exported to the cytoplasm and associates with the Argonaute 2 protein. *RNA* **11**: 849–52.

29. **Shiromoto Y, Kuramochi-Miyagawa S, Daiba A, Chuma S, Katanaya A, Katsumata A, … Nakano T.** 2013. GPAT2, a mitochondrial outer membrane protein, in piRNA biogenesis in germline stem cells. *RNA* **19**: 803–10.

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30. **Ding D, Liu J, Dong K, Melnick AF, Latham KE, Chen C.** 2019. Mitochondrial membrane-based initial separation of MIWI and MILI functions during pachytene piRNA biogenesis. *Nucleic Acids Res.* **47**: 2594–608.

31. **Nishimasu H, Ishizu H, Saito K, Fukuhara S, Kamatani MK, Bonnefond L, … Nureki O.** 2012. Structure and function of Zucchini endoribonuclease in piRNA biogenesis. *Nature* **491**: 284–7.

32. **Guantes R, Rastrojo A, Neves R, Lima A, Aguado B, Iborra FJ.** 2015. Global variability in gene expression and alternative splicing is modulated by mitochondrial content. *Genome Res.* **25**: 633–44.

33. **Larriba E, Rial E, Del Mazo J**. 2018. The landscape of mitochondrial small non-coding RNAs in the PGCs of male mice, spermatogonia, gametes and in zygotes. *BMC Genomics* **19**: 634.

34. **Riggs CL, Summers A, Warren DE, Nilsson GE, Lefvre S, Dowd WW, … Podrabsky JE.** 2018. Small Non-coding RNA Expression and Vertebrate Anoxia Tolerance. *Front. Genet.* **9**: 230.

35. **Bottje WG, Khatri B, Shouse SA, Seo D, Mallmann B Olowski SK, … Kong BC.** 2017. Identification and Differential Abundance of Mitochondrial Genome Encoding Small RNAs (mitosRNA) in Breast Muscles of Modern Broilers and Unselected Chicken Breed. *Front. Physiol.* **8**: 816.

36. **Lax NZ, Hepplewhite PD, Reeve AK, Nesbitt V, McFarland R, Jaros E, … Turnbull DM.** 2012. Cerebellar ataxia in patients with mitochondrial DNA disease: a molecular clinicopathological study. *J. Neuropathol. Exp. Neurol.* **71**: 148–61.

37. **Fuku N, Park KS, Yamada Y, Nishigaki Y, Cho YM, Matsuo H, Tanaka M.** 2007. Mitochondrial haplogroup N9a confers resistance against type 2 diabetes in Asians. *Am. J. Hum. Genet.* **80**: 407–15.

38. **Ji F, Sharpley MS, Derbeneva O, Alves LS, Qian P, Chalkia D, … Wallace DC.** 2012. Mitochondrial DNA variant associated with Leber hereditary optic neuropathy and high-altitude Tibetans. *Proc. Natl. Acad. Sci. U. S. A.* **109**: 7391–6.

39. **Morrow EH, Camus MF**. 2017. Mitonuclear epistasis and mitochondrial disease. *Mitochondrion* **35**: 119–22.

40. **Passamonti M, Calderone M**, **Delpero M**, **Plazzi F**. 2020. Clues of in vivo nuclear gene regulation by mitochondrial short non-coding RNAs. *Sci. Rep.* **10**: 1–12.

41. **Jin HY, Gonzalez-Martin A, Miletic AV, Lai M, Knight S, Sabouri-Ghomi M, … Xiao C.** 2015. Transfection of microRNA Mimics Should Be Used with Caution. *Front. Genet.* **6**: 340.

42. **Cloonan N.** 2015. Re-thinking miRNA-mRNA interactions: intertwining issues confound target discovery. *Bioessays* **37**: 379–88.

43. **Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH.** 2017. Proteomics: Technologies and Their Applications. *J. Chromatogr. Sci.* **55**: 182–96.

44. **Lu Z**, **Guan X**, **Schmidt CA**, **Matera AG**. 2014. RIP-seq analysis of eukaryotic Sm proteins identifies three major categories of Sm-containing ribonucleoproteins. *Genome Biol.* **15**: R7.

45. **Clark PM, Loher P, Quann K, Brody J, Londin ER, Rigoutsos I.** 2014. Argonaute CLIP-Seq reveals miRNA targetome diversity across tissue types. *Sci. Rep.* **4**: 5947.

46. **Zhang X, Zuo X, Yang B, Li Z, Xue Y, Zhou Y, ... Fu XD.** 2014. MicroRNA directly enhances mitochondrial translation during muscle differentiation. *Cell* **158**: 607–19.

47. **Gammage PA**, **Moraes CT**, **Minczuk M**. 2018. Mitochondrial Genome Engineering: The Revolution May Not Be CRISPR-lzed. *Trends Genet.* **34**: 101–10.

48. **Hudson G**, **Gomez-Duran A**, **Wilson IJ**, **Chinnery PF**. 2014. Recent Mitochondrial DNA Mutations Increase the Risk of Developing Common Late-Onset Human Diseases. *PLoS Genet.* **10**: e1004369.

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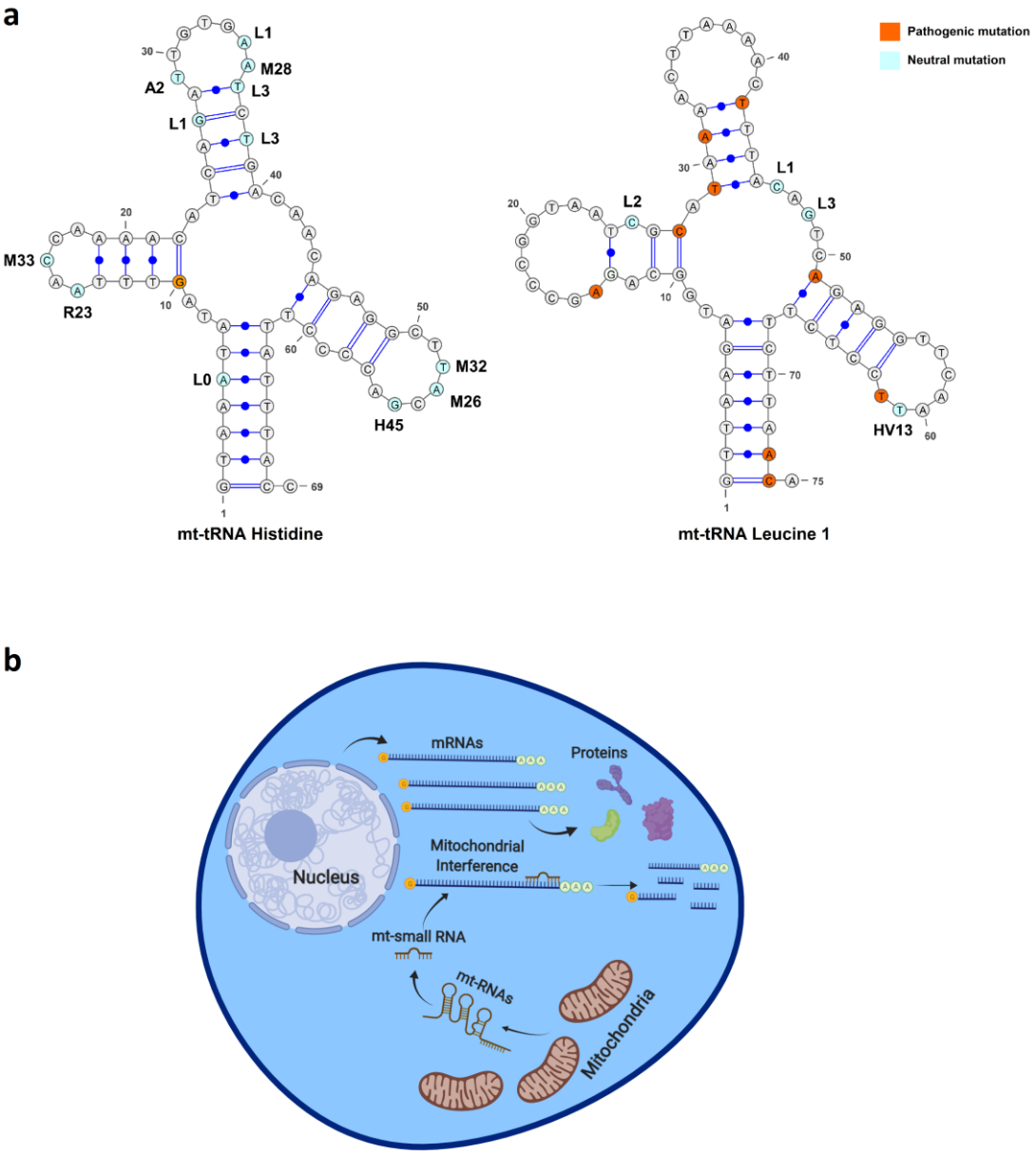

FIGURE 1 In the first figure a) we represented the variability in the position of mtDNA mutations across mt-tRNAs. Two human mt-tRNAs are shown (mt-tRNA Histidine on left, and Leucine 1 on right), in which nucleotides with different colors represent neutral (light blue) and pathogenic (orange) mutations. Neutral mutations are mutations without any known negative effects on fitness. The labels next to the neutral mutations indicate the haplogroup in which that mutation is common. These haplogroups have different frequencies depending on the population considered. For example, haplogroup L is the most common in African populations. In the second figure b) we show a schematic representation of the mitochondrial interference hypothesis. The mitochondrial genome transcribes small mitochondrial RNAs (mt-small RNA) that can bind a complementary mRNA made by the nucleus. Then the mtsmall RNA binds the target mRNA in a region with similar sequence. This binding stops the translation of the nuclear

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mRNA, thus changing the expression of a nuclear gene. This process could in theory target virtually any nuclear gene, and thus could result in the dysfunction of innumerable phenotypes

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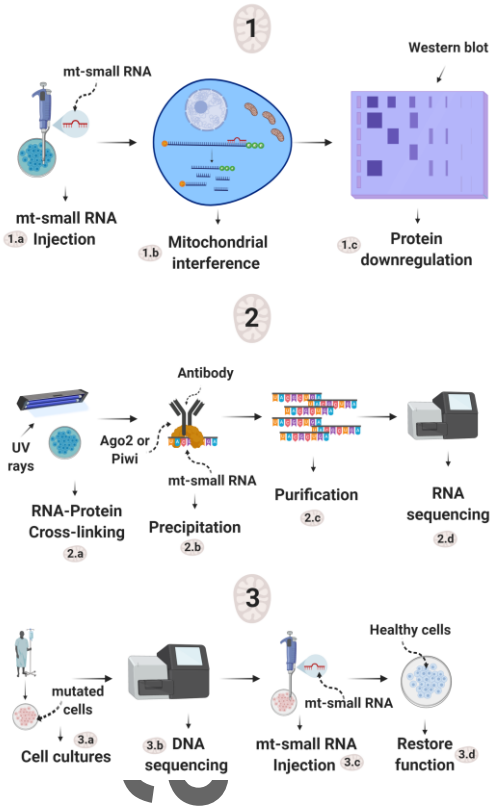

FIGURE 2 A schematic representation of the experiments necessary to verify the three key predictions of the mitochondrial interference hypothesis. Prediction 1 is that the small mitochondrial RNAs can interfere with mRNA translation. It can be tested by measuring the expression of target proteins after injection of candidate mt-small RNAs, proving that their action is to inhibit protein expression. This involves injecting small mitochondrial RNAs (1.a) targeting specific mRNAs, which will cause mitochondrial interference (1.b), with protein downregulation then verified through western blot analysis (1.c). Prediction 2 is that the small mitochondrial RNAs can bind to key proteins of RNAi. It can be tested by determining whether small mitochondrial RNAs are bound to an Argonaute protein (eg Ago2), or Piwi, in vivo. It involves cross-linking Argonaute protein (eg Ago2) with the small mitochondrial RNAs (2.a), precipitating them using antibodies (2.b), purifying the RNAs by removing the proteins (2.c), and, ultimately, sequencing the small mitochondrial RNAs (2.d). Prediction 3 is that the function of cells that harbour pathogenesis-inducing mtDNA mutations can be restored through injection of the small mitochondrial RNA counterparts that lack the mutation. This experiment involves two stages. In the first stage, the pathogenic mutations in the patient are identified though cell cultures (3.a) and sequencing (3.b). In the second stage, unmutated small mitochondrial RNAs corresponding to the mutated mitochondrial regions are injected into the cell cultures (3.c), then the function of the healthy cells verified through histological and physiological analyses depending on the pathology investigated (3.d)

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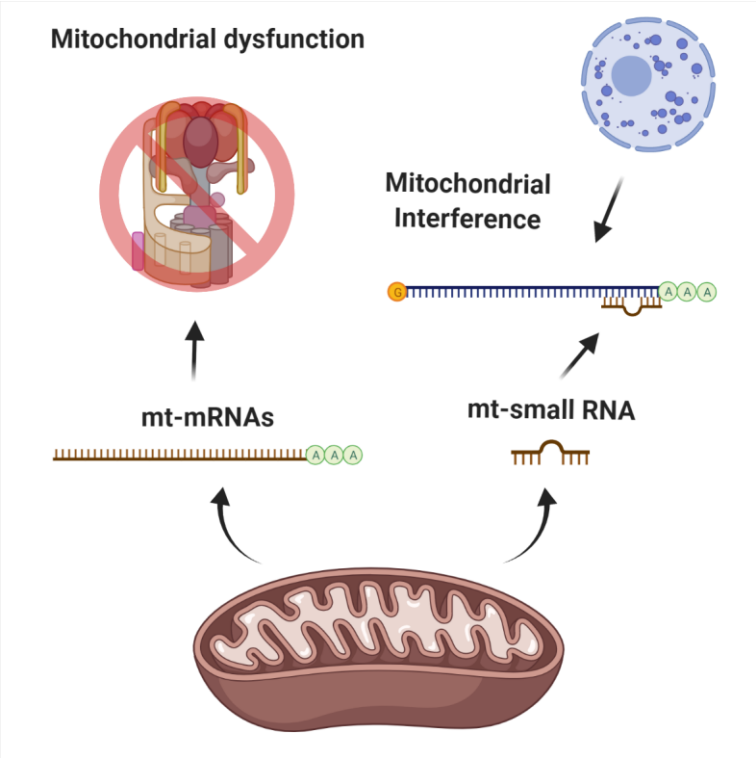

Recently a new type of small RNA encoded within the mitochondrial genome has been discovered. In this work we hypothesize that these small mitochondrial RNAs can interfere with nuclear gene expression through RNA interference. We discuss the implications of this hypothesis and how to test it.

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