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## Pathophysiology of human mitochondrial tRNA metabolism

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### Abstract

Mitochondria play multiple critical roles in cellular activity. In particular, mitochondrial translation is pivotal in the regulation of mitochondrial and cellular homeostasis. In this forum article we discuss human mitochondrial tRNA metabolism and highlight its tight connection with various mitochondrial diseases caused by mutations in aminoacyl-tRNA synthetases, tRNAs, and tRNA-modifying enzymes.

### Glossary

**Aminoacylation:** an enzymatic reaction to ligate an amino acid to its cognate tRNA; it involves amino acid activation and the subsequent transfer of the activated amino acid moiety to the 3'-CCA terminus of the tRNA.

**Editing:** an enzymatic reaction to remove the incorrect amino acid ligated with the 3'-CCA terminus of a tRNA (post-transfer editing) or to hydrolyze the aminoacyl-AMP intermediate due to amino acid misactivation (pre-transfer editing).

**Heteroplasmy:** mitochondria that contain a mixture of wild-type and mutated mtDNA.

**Homoplasmy:** mitochondria that contain only wild-type or mutant mtDNA.

**Oxidative phosphorylation (OXPHOS):** the process by which electrons derived from NADH and FADH<sub>2</sub> combine with oxygen, and the energy released by these redox reactions is used to synthesize ATP from ADP.

**tRNA modopathies:** diseases caused by defects in tRNA modifications.

## Introduction

Mitochondria are responsible for numerous pivotal roles supporting life, including cellular ATP supply through oxidative phosphorylation (OXPHOS) (see Glossary) [1]. The OXPHOS machinery comprises five inner membrane (IM)-embedded complexes (I, II, III, IV, and V), encoded by nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), and inserted into the IM with the assistance of various assembly cofactors. The mitochondrial proteome comprises >1100 proteins. mtDNA expression involves a separate translation system in the matrix [1]. All RNA components of this system – including two rRNAs, 22 tRNAs, and 11 mRNAs (generating 13 proteins) – are encoded by mtDNA. Additional components, including aminoacyl-tRNA synthetases (aaRSs) and tRNA-modifying enzymes, are encoded by nDNA (Figure 1A) [2].

Recently, accumulating evidence has shown that dysfunction of the mitochondrial translation machinery, particularly defects in tRNA metabolism, leads to diverse mitochondrial pathologies [3]. However, our understanding of the molecular etiology of these pathologies remains incomplete. Mutations in mitochondrial aaRSs (mt-aaRSs) lead to multiple defects in structure and function. nDNA-encoded mt-aaRSs charge tRNAs with cognate amino acids to supply aminoacyl-tRNAs for translation via a two-step aminoacylation reaction. There are 19 mt-aaRSs, including 17 exclusively mitochondrial and two bifunctional aaRSs (shared between the cytosol and mitochondria) [4]. Mt-aaRSs play pivotal roles in mitochondrial structure and function. Indeed, deletion of mitochondrial aspartyl-tRNA synthetase disrupts dietary lipid processing and transport in intestinal epithelial cells [5].

Due to the existence of structurally and/or chemically similar amino acids, and the inherent difficulty in aaRSs distinguishing cognate amino acids from noncognate ones, some aaRSs have evolved proofreading (editing) activity to remove errors in amino acid selection. This proofreading activity involves hydrolyzing misactivated aminoacyl-adenylate or mischarged tRNAs (such as Ser-tRNA<sup>Thr</sup>).

Approximately half of extant aaRSs carry editing domains and exhibit editing activities. However, during the evolution of mt-aaRSs, only four have retained intact editing domains, and two of them – alanyl- and threonyl-tRNA synthetases (ThrRS) – have been demonstrated to be capable of editing [4]. Impairment of ThrRS editing leads to early embryonic lethality and cardiomyopathy, derived from oxidative-stress-induced cell cycle arrest [6]. Other mt-aaRSs, including leucyl-, prolyl- and phenylalanyl-tRNA synthetases, carry a truncated editing domain or have lost their editing domains [4]. The rationale for the loss of editing function by these mt-aaRSs is unclear, but it probably reflects a more stringent amino acid selection mechanism or tolerance for decoding errors at these codons [6]. Non-canonical functions of mt-aaRSs have been recently reported [7,8].

Mutations in all mt-aaRS genes have been identified, causing a wide variety of clinical manifestations and varying degrees of severity (Figure 1B) [9]. The main organs affected

include the central or peripheral nervous system, heart, muscle, and spleen, which consume large amounts of ATP. A few studies have elucidated the molecular effects of these mutations on the structure and function of mt-aaRSs. Several mechanisms underlie the pathogenesis of mt-aaRS mutations. Reduced aminoacylation activity of mutant mt-aaRSs is the most frequently observed functional defect. Several studies have reported a lower protein level, possibly due to accelerated protein degradation [9]. For mutations in editing-competent mt-aaRSs, impaired editing activity contributes to disease onset and development [6]. Mutations in the mitochondrial targeting sequence (MTS) could disrupt correct organellar distribution. Mutations in the intronic regions lead to aberrant mRNA splicing [9]. Notably, a decreased level of cognate tRNA is frequently observed with mt-aaRS mutations [10]. Finally, the influence of putative non-canonical functions may constitute a mechanism, but this requires further investigation.

### **Mitochondrial tRNA (mt-tRNA) mutations impair translational efficiency and fidelity**

Mutations in mt-tRNA are carried by the mtDNA, leading to dramatic variability in the phenotypic presentation of mitochondrial genetic defects. Each human cell contains variable amounts of mitochondria with different mtDNA copies. During cell division, mitochondria behave stochastically, separating randomly between daughter cells [11]. This has important consequences for the distribution of mtDNA mutations in daughter cells, which may exhibit variable mutation loads. When the mutant load reaches a certain threshold, which differs from tissue to tissue, respiratory chain function collapses and clinical consequences appear. mtDNA heteroplasmy is therefore a critical parameter that further complicates our understanding of the pathophysiological mechanisms associated with these mutations, as the different ratios of mutant to wild-type mtDNA significantly affect disease expression and severity in patients carrying the same mtDNA mutation. While most mutations occur in heteroplasmic form, a smaller number occur in homoplasmic form. Factors modulating the penetrance of a mutation include increased mitochondrial biogenesis to partially compensate for the deficit in OXPHOS activity, or the nuclear background, which may contain additional mutations. Epigenetic or environmental factors have also been found to play a role in the severity and tissue specificity of homoplasmic mtRNA mutations.

After transcription, mt-tRNAs undergo several 5' and 3' end-processing steps, as well as various post-transcriptional modifications. Mechanistically, pathogenic tRNA mutations destabilize the tRNA structure, prevent aminoacylation, impair modifications, and affect interactions with elongation factors, ribosomes, and mRNA during decoding [12]. The two best-studied mt-tRNA mutations are m.3243A>G and m.8344A>G in tRNA<sup>Leu</sup>(UUR) and tRNA<sup>Lys</sup>, respectively [2,12]. They are frequently associated with mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) and myoclonic epilepsy and ragged red fiber disease (MERRF) syndromes. Both mutations are heteroplasmic and can lead to other syndromes, depending on the degree of heteroplasmy and the cell type. At the molecular level, the m.3243A>G mutation disrupts the tertiary interaction between A14 and U8, leading to a tRNA folding defect and defects in aminoacylation, taurine modification at U34 ( $\tau\text{m}^5\text{U34}$ ), tRNA processing, and codon decoding [12]. The m.8344A>G variant also affects tertiary tRNA folding and  $\tau\text{m}^5\text{U34}$  biogenesis [12]. The broad clinical spectra of the m.3243A>G and m.8344A>G mutations partly overlap, and the identification of a patient's disease often depends on the

molecular diagnosis. Finally, these two commonest mutations are surrounded by hundreds of mutations in other tRNAs with strict pathogenicity criteria (Table 1).

### **tRNA modification defects cause tRNA modopathies tRNAs are the most highly modified RNAs**

tRNA modifications regulate the physical and biochemical properties of tRNAs by stabilizing their local or tertiary structures, serving as recognition determinants for other proteins, and modulating the interactions between codons and anticodons or between tRNA and ribosomes. In human mitochondria, 18 types of modification have been identified at 137 positions [12]. mtDNA-derived tRNA mutations – such as m.3243A>G and m.8344A>G – frequently lead to modification defects.  $\tau\text{m}^5\text{U34}$  guarantees the reading specificity of codons ending with a purine. Loss of  $\tau\text{m}^5\text{U34}$  impairs the reading of UUG codons by tRNA<sup>Leu</sup>(UUR) or AAA and AAG codons by tRNA<sup>Lys</sup> [12]. nDNA-derived mutations in mitochondrial tRNA-modifying enzymes cause a reduction in or loss of tRNA modifications via multiple mechanisms, which ultimately impairs the efficiency or fidelity of mitochondrial translation. Defects in more than nine mitochondrial tRNA-modifying enzymes have been documented [13]. For example, variations in the gene TRMU, which is responsible for 2-thiouridylation of specific tRNAs at position 34, lead to acute infantile liver failure. Mutations in GTPBP3 and MTO1 (both involved in  $\tau\text{m}^5\text{U34}$  biogenesis) cause hypertrophic cardiomyopathy [13]. The resulting pathologies are known as ‘tRNA modopathies’ and are at the origin of the mitochondrial diseases typical of energy-demanding organs (Figure 1B).

### **Concluding remarks and future directions**

AaRSs and tRNA-modifying enzymes are key players in mt-tRNA metabolism and translation. Accordingly, they are hotspots of pathogenic mutations. Previous studies have provided valuable insights into physiology and pathology of mitochondrial translation. However, the detailed molecular mechanisms of mt-tRNA modification and aminoacylation are far from completely understood. Future research should focus on the following aspects. (i) What are the basic physiological mechanisms of various components of human mt-tRNA metabolism? Recurring problems include the difficulty in expressing and purifying mitochondrial enzymes (in part due to the uncertainty of the precise cleavage site of MTS and poor solubility in the bacterial expression system) and the inherent structural instability of mt-tRNAs [2] which make activity reconstitution difficult. (ii) What are the pathogenic mechanisms of mutations in mt-tRNAs, aaRSs, and tRNA-modifying enzymes? Most genetic mutations have been reported in clinics as case reports, but the underlying pathogenic studies are urgently needed [3]. The etiology studies may be performed at the levels of enzymology, molecular biology, cell biology, and organismal models (such as yeasts, lower eukaryotes, or mice). Several mutant mice and zebrafish models of mt-aaRSs and tRNA-modifying enzymes have been constructed; however, animal models with specific mt-tRNA mutations are limited. Induced pluripotent stem cells and organoids are an alternative to bridge the gap between animal and human disease models. (iii) How can we develop effective treatment strategies for relevant diseases? So far, no effective treatment strategies for mitochondrial diseases have been established. Mitochondrial nuclease therapy approach and gene editing exhibit valuable potentials to clear or correct specific mtDNA mutations, but they need to be optimized in nucleotide conversion types, editing efficiency, and potential off-targets [14]. Additionally, mitochondrial replacement therapy is a clinical application but this suffers from technical flaws (such as carrying of perinuclear mtDNA into the reconstituted zygote or oocyte) and ethical Q5

concerns [15]. In future, based on a clearer understanding of the pathophysiology of mt-tRNA metabolism, promising targeted therapies could be developed, including but not limited to adeno-associated virus (AAV)-based gene therapy, addition of substrates of tRNA-modifying enzymes to enhance modification efficiencies, administration of small molecules to interfere with enzyme degradation, and lipid nanoparticle-mediated tRNA therapy.

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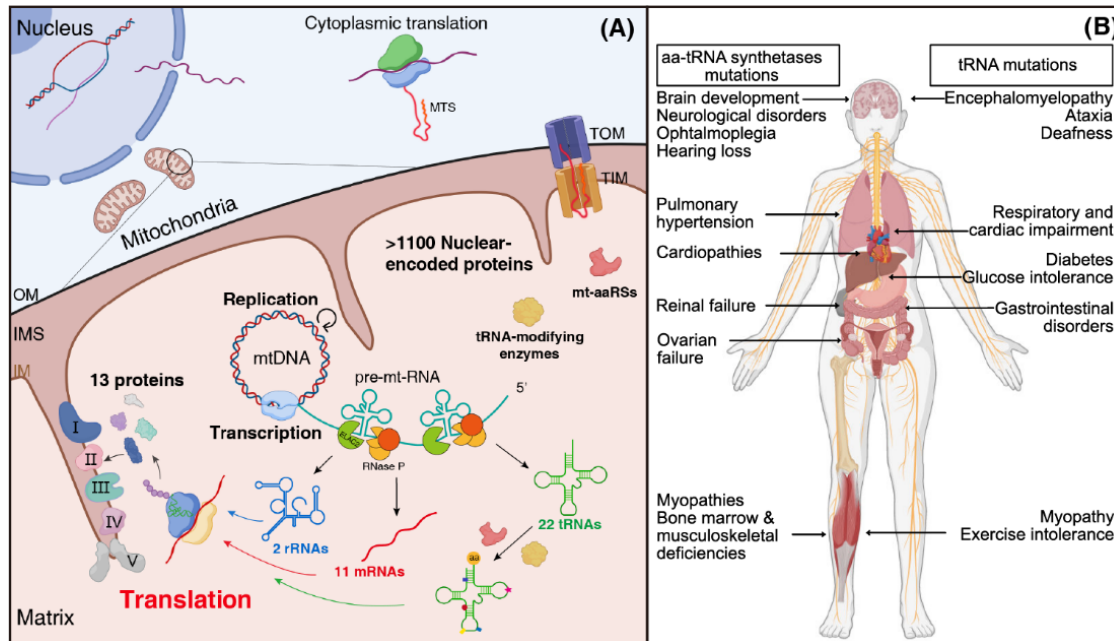
## Declaration of interests

No interests are declared.

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**Figure 1.** Human mitochondrial translation and its connection with diseases. (A) A global picture of mitochondrial translation. All protein components (including mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) and tRNA-modifying enzymes) of mitochondrial translation are synthesized in the cytosol and then transported into mitochondria. All RNA molecules (including tRNAs, mRNAs, and rRNAs) of mitochondrial translation are transcribed from mtDNA and matured in mitochondria. In total, 13 proteins are generated for oxidative phosphorylation (OXPHOS) chain complexes I, III, IV, and V. Note that mitochondrial ribosomes are tethered to the inner membrane (IM) to facilitate membrane insertion of nascent polypeptides. The mitochondrial targeting sequence (MTS) represents the targeting sequence of nuclear DNA (nDNA)-encoded mitochondrial proteins. (B) Pleiotropic effects of mitochondrial diseases resulting from mutations in mt-aaRSs and tRNAs. Multiple organs can be affected, but often one type of mutation leads to targeted deregulations and alterations. Abbreviations: OM, outer membrane; TIM, translocase of the inner membrane; TOM, translocase of the outer membrane. Figure created with BioRender.

**Table 1.** Top mt-tRNA gene mutations<sup>a</sup>

<b>SYNDROMES</b>	<b>Disease</b>	<b>tRNA</b>	<b>Mutation</b>	<b>Ho<sup>b</sup></b>	<b>He</b>
<b>CARDIOMYOPATHY</b>					
Mitochondrial Myopathy and Cardiomyopathy	MMC	Leu (UUR)	A3260G	-	+
Mitochondrial Myopathy and Cardiomyopathy	MMC	Leu (UUR)	C3303T	+	+
Maternally Inherited Cardiomyopathy	MICM	Ile	A4300G	+	+
<b>DEAFNESS/ SENSORINEURAL HEARING LOSS</b>					
Deafness/Sensorineural Hearing Loss	SNHL	Ser (UCN) precursor	A7445G	+	+
Deafness/Sensorineural Hearing Loss	SNHL	Ser (UCN)	T7511C	+	+
<b>DIABETES MELLITUS</b>					
Diabetes mellitus and deafness	DM/DMDF/MIDD/SNHL/FSGSCardiac+ multi-organ dysfunction	Leu (UUR)	A3243G	-	+
<b>ENCEPHALOMYOPATHY</b>					
Encephalomyopathy, MELAS	MELAS/MM & EXIT	Phe	G583A	-	+
Encephalomyopathy, ataxia, myoclonus, and deafness	AMDF	Val	G1606A	-	+
Encephalomyopathy, MELAS	MELAS/LS	Leu (UUR)	A3243G	-	+
Encephalomyopathy, MELAS	MELAS	Leu (UUR)	C3256T	-	+
Encephalomyopathy, MELAS	MELAS	Leu (UUR)	T3271C	-	+
Encephalomyopathy, MELAS	MELAS/Myopathy/Deafness + Cognitive Impairment	Leu (UUR)	T3291C	-	+
Encephalomyopathy, MELAS	MELAS/encephalopathy	Gln	G4332A	-	+
Encephalomyopathy, Leigh syndrome	MILS	Trp	A5537insT C7472insC	-	+
Encephalomyopathy	PEM/AMDF/Motor neuron disease-like	Ser (UCN)	C7471CC (=7472insC')	+	+
Encephalomyopathy, MERRF	MERRF	Lys	A8344G	-	+
Encephalomyopathy, MERRF	MERRF	Lys	T8356C	-	+
Encephalomyopathy, MERRF	MERRF/MICM?DEAF/Autism /LS/Ataxia + Lipomas	Lys	G8363A	-	+
Encephalomyopathy	PEM	Gly	T10010C	-	+
Encephalomyopathy, MERRF	MERRF-MELAS/cerebral edema	His	G12147A	-	+
<b>MITOCHONDRIAL MYOPATHY</b>					
Mitochondrial Myopathy	MM/CPEO	Leu (UUR)	A3243G	-	+
Mitochondrial Myopathy	MM	Leu (UUR)	A3302G	-	+
Mitochondrial Myopathy, CPEO	CPEO/MS	Ile	G4298A	-	+
Mitochondrial Myopathy, CPEO	CPEO	Ile	G4308A	-	+
Mitochondrial Myopathy	Myopathy	Ala	G5650A	-	+
Mitochondrial Myopathy, CPEO	CPEO/MM	Asn	G5703A	-	+
Mitochondrial Myopathy	MM / EXIT	Ser (UCN)	G7497A	+	+
Mitochondrial Myopathy, CPEO	CPEO / KSS	Leu (CUN)	G12315A	-	+
Mitochondrial Myopathy	Reversible COX deficiency myopathy	Glu	T14674C	+	-
Mitochondrial Myopathy	MM+DM / Encephalomyopathy	Glu	T14709C	+	+



<sup>a</sup> Data are adapted from MITOMAP (<http://www.mitomap.org>).

<sup>b</sup> Abbreviations: AMDF, ataxia, myopathy, and deafness; CPEO, chronic progressive ophthalmoplegia; DM, diabetes mellitus; DMDF, diabetes mellitus and deafness; FSGS, focal segmental glomerulosclerosis; He, heteroplasmy; Ho, homoplasmy; MERRF, myoclonic epilepsy and ragged red fiber disease; MELAS, mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes; MICM, maternally inherited cardiomyopathy; MIDD, maternally inherited diabetes and deafness; MILS, maternally inherited Leigh syndrome; MMC, mitochondrial myopathy and cardiomyopathy; PEM, progressive encephalomyopathy; SNHL, sensorineural hearing loss.