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Mitochondrial RNA metabolism, a potential therapeutic target for mitochondria-related diseases

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Abstract

In recent years, the roles of mitochondrial RNA and its associated human diseases have been reported to increase significantly. Treatments based on mtRNA metabolic processes and nuclear gene mutations are thus discussed. The mitochondrial oxidative phosphorylation process is affected by mtRNA metabolism, including mtRNA production, maturation, stabilization, and degradation, which leads to a variety of inherited human mitochondrial diseases. Moreover, mitochondrial diseases are caused by mitochondrial messenger RNA, mitochondrial transfer RNA, and mitochondrial ribosomal RNA gene mutations. This review presents the molecular mechanisms of human mtRNA metabolism and pathological mutations in mtRNA metabolism-related nuclear-encoded/nonencoded genes and mitochondrial DNA mutations to highlight the importance of mitochondrial RNA-related diseases and treatments.

Keywords: Mitochondrial RNA; Mitochondrial diseases; Mutation; Metabolism; Modification; Mitochondria dysfunction

Introduction

Mutations in mitochondrial DNA (mtDNA) or genes related to mitochondrial RNA (mtRNA) metabolism lead to a series of diverse human genetic diseases. In 1971, human mtRNA was reported to be produced symmetrically by bacterial-like polycistronic transcription using mtDNA as a template.[1] The oxidative phosphorylation (OXPHOS) system consists of 13 mitochondrial messenger RNAs (mt-mRNAs) for protein subunit translation, including NADH-ubiquinone oxidoreductase core subunits (ND1–ND6 and ND4L), cytochrome B (CytB), cytochrome c oxidase subunits (CO1–CO3), and ATP synthase subunits (ATP6 and ATP8). mtRNAs also include 12S and 16S mitochondrial ribosomal RNAs (mt-rRNAs), 22 mitochondrial transfer RNAs (mt-tRNAs), and some mitochondrial noncoding RNAs (mt-ncRNAs).[2] The precise production of mtRNAs is essential for cellular homeostasis since the subsequent translation of mtRNAs results in the synthesis of proteins that constitute an integral part of the mitochondrial respiratory complex. Human mtRNA metabolism involves formation, maturation, and degradation. mtRNA formation is the process of mtDNA transcription, after which mtRNAs are cleaved to release different types of mtRNAs, which are then modified by a variety of specialized enzymes for maturation, and mtRNAs that are damaged during the process are degraded. Dysfunction in any part of the mtRNA metabolic process can lead to disease. Recent data have shown that mtRNAs play important roles in the innate immune response, particularly in the antiviral response.[3]

In this article, human mtRNA metabolism, including mtRNA formation, maturation, and degradation, was reviewed. The link between mtRNA metabolism and human diseases was explored. The mutations in mtRNA metabolism-related nuclear-encoded proteins and pathological mutations in mtDNA as well as diseases associated with mt-ncRNA were described.

mtRNA Metabolism

mtRNA production

The transcription of mtDNA includes promoter recognition, promoter-specific transcription initiation, elongation, and termination. The proteins involved in mtDNA transcription include DNA-directed RNA polymerase mitochondrial (POLRMT),[4] transcription factor A mitochondrial (TFAM),[5] transcription factor B2 mitochondrial (TFB2M),[6] transcription elongation factor mitochondrial (TEFM),[7] and mitochondrial transcription termination factor 1 (MTERF1)[8] [Figure 1].

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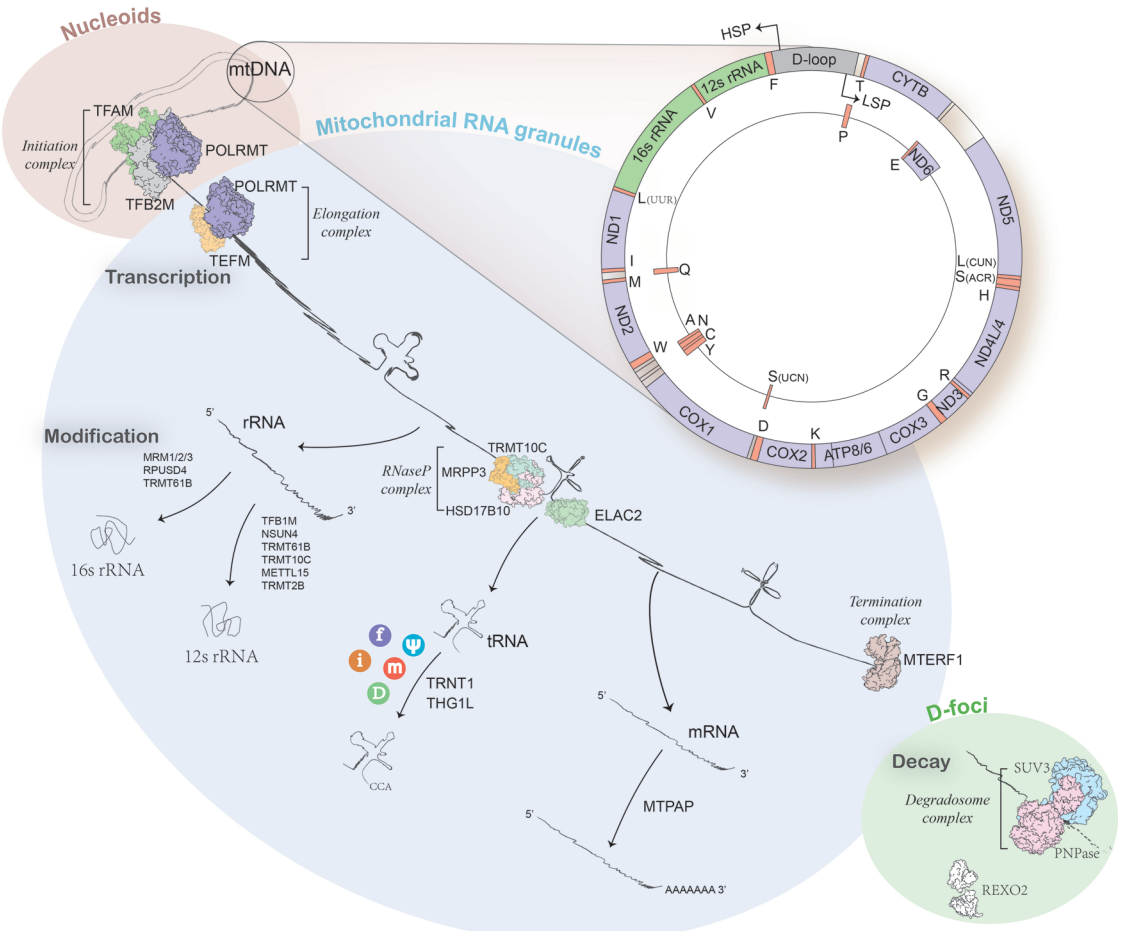

Figure 1: Schematic representation of the human mitochondrial genome and key steps in mtRNA expression and maturation. The genes encoding the two rRNAs are indicated in green, the 13 mRNAs are indicated in purple, and the 22 tRNAs are indicated in orange. ATP: Adenosine triphosphate; CYTB: Cytochrome B; ELAC2: RNase Z; HSP: Heavy-stranded promoters; LSP: Light-stranded promoter; METTL15: Methyltransferase like 15; MRM1/2/3: 2′-O-ribose methyltransferases; mRNA: Messenger RNA; mtDNA: Mitochondrial DNA; MTERF1: Mitochondrial transcription termination factor 1; MTPAP: Mitochondrial poly(A) polymerase; ND1-ND6 and ND4L: NADH- ubiquinone oxidoreductase core subunits; NSUN: NOP2/Sun RNA methyltransferase; PNPase: Polyribonucleotide nucleotidyltransferase 1; POLRMT: RNA polymerase mitochondrial; PRORP (MRPP3): Mitochondrial ribonuclease P catalytic subunit; REXO2: RNA exonuclease 2; RPUSD4: RNA pseudouridine synthase D4; rRNA: Ribosomal RNA; SUV3: Suv3 like RNA helicase; TEFM: Transcription elongation factor mitochondrial; TFAM: Transcription factor A mitochondrial; TFB1M: Transcription factor B1 mitochondrial; THG1L: tRNA-histidine guanylyltransferase 1 like; TRMT2B: tRNA methyltransferase 2 homolog B; TRMT10C (MRPP1): tRNA methyltransferase 10C; TRMT61B: tRNA methyltransferase 61B; tRNA: Transfer RNA; TRNT1: tRNA-nucleotidyl transferase 1.

The transcription start site [light-stranded transcription start site (ITL); heavy-stranded transcription start site (ITH)] of the mtDNA strand is located in the noncoding regulatory region (NCR), namely, the light-stranded promoter (LSP) and the heavy-stranded promoters (HSP1 and HSP2).[9] The binding of the promoter-binding protein TFAM to the mtDNA upstream of the transcription start site induces bending of the mtDNA at the binding site and recruits it to the promoter via the N-terminal extension of POLRMT, forming a closed preinitiation complex.[5,10] Immediately afterward, the initiation factor TFB2M (containing an rRNA methyltransferase structural domain) induces structural changes in POLRMT and melting of the double-stranded DNA, thereby opening the initiation complex. The active site of POLRMT then interacts with the RNA–DNA heterodimer, and mtRNA synthesis is initiated accordingly.[10]

Upon the initiation of mtRNA synthesis, TFAM and TFB2M are released, and the elongation factor TEFM is recruited. Structural studies have shown that TEFM contains a pseudonuclease core that forms a "sliding clamp" around the mtDNA downstream of POLRMT and interacts with POLRMT through its C-terminal structural domain, thus forming an elongation complex (EC, also known as the antitermination complex).[7] During the elongation phase, TEFM is required for the sustained synthesis of mitochondrial transcripts,[7] especially in promoting the formation of longer transcripts,[11] in the absence of TEFM, POLRMT terminates prematurely in the conserved G-quadruplex-forming sequence downstream of the LSP, producing short transcripts that can act as primers for mtDNA H-strand replication.[12] This role of TEFM effectively prevents EC breakage and ensures the uninterrupted production of nascent transcripts.[13] In addition, TEFM also enhances the transcriptional capacity of POLRMT by reducing the frequency and shortening the duration of long-lived pauses.[14]

Upon the completion of each transcription cycle, POLRMT stops RNA synthesis and dissociates from mtDNA. The exact mechanism of transcription termination remains

unclear, although researchers have proposed that the Zurdo structural domain of MTERF1 binds to double-stranded DNA (dsDNA) containing the $tRNA^{Leu\ (UUR)}$ gene sequence, thereby terminating transcription through base flipping (i.e., a nucleotide base everts from the DNA double-helix) and DNA deconvolution.[15] In addition, MTERF1 can halt the transcription of LSP prematurely at the 3′-end of the mt-rRNA coding sequence (CDS).[16]

mtRNA modification

The mt-rRNA sequence and the majority of the protein CDSs are separated by mt-tRNA in the long polycistronic transcript, and the excision of the mt-tRNA nucleic acid releases both mt-mRNA and mt-rRNA, a concept known as the "tRNA punctuation model".[17] Mammalian mitochondrial RNase P, a heterotrimeric nucleic acid endonuclease composed of tRNA methyltransferase 10C (TRMT10C/MRPP1), hydroxysteroid 17-beta dehydrogenase 10 (HSD17B10/MRPP2), and mitochondrial ribonuclease P catalytic subunit (PRORP/MRPP3), cleaves primary transcripts at the 5′-end of tRNA.[18] RNase Z (ELAC2) is an endonuclease that performs 3′-end maturation of mitochondrial and nuclear tRNA precursors.[19] RNase P and RNase Z process mt-tRNA from primary transcripts at the 5′- and 3′-ends, respectively, followed by the posttranscriptional modifications required for mtRNA maturation. This maturation process occurs within nonmembrane structures known as mitochondrial RNA granules (MRGs)[20] [Figure 1].

Polyadenylation at the 3′-end is a common posttranscriptional modification of mt-mRNAs, except for *MT-ND6*.[21] This process, which is performed by mitochondrial poly(A) polymerase (MTPAP),[22] plays a crucial role in the completion of the missing stop codons that are absent in certain mt-mRNAs following the processing of polycistronic RNA.[17] MTPAP localizes to MRGs after import into mitochondria and processes mt-mRNAs carrying a U or UA at the 3'-end.[23]

The posttranscriptional modifications of mt-mRNAs also include methylation and pseudouridylation. Two tRNA methyltransferases, TRMT61B and TRMT10C, are responsible for the N1-methyladenosine (m^1A) modifications of mt-mRNAs. Li *et al*[24] identified 22 m^1A sites from 10 mitochondrial genes, 21 of which are located in the CDS and one in the 3′ untranslated region (UTR). TRMT61B is likely responsible for catalyzing m^1A methylation at specific sites, such as base 1472 in the *MT-CO1* mRNA, base 707 in the *MT-CO3* mRNA, base 252 in the *MT-ND4L* mRNA, and base 617 in the *MT-CYTB* mRNA[24], whereas TRMT10C methylates *MT-ND5* mRNA at position 1374.[25]

Pseudouridine synthases (PUSs) are enzymes that are responsible for pseudouridylation by converting a specific uridine to pseudouridine (Ψ). The RNA pseudouridine synthases D3 (RPUSD3) and TRUB2 introduce Ψ at position 391 of *MT-CO1* and positions 698–700 of the *MT-CO3* mRNA. RPUSD3 is thought to be the primary enzyme responsible for mt-mRNA pseudouridylation, whereas TRUB2 appears to play a secondary role.[26]

The mitochondrial 55S ribosome consists of two subunits: the large 39S subunit (mtLSU), which is involved in catalyzing the peptidyl transferase reaction, and the small 28S subunit (mtSSU), which provides the platform for mRNA binding and decoding. The 39S subunit is composed of 16S mt-rRNA, whereas the 28S subunit is composed of 12S mt-rRNA.[27] The maturation of mitochondrial ribosomes requires posttranscriptional modifications of mt-rRNA, including methylation, 2′-O-ribose methylation, and pseudouridylation.

In 12S mt-rRNA, TFB1M is responsible for the dimethylation of adenine ($m^{6,2}A$) at bases 936 and 937, which is essential for the assembly and maturation of human mitochondrial ribosomes.[28] NSUN4 methylates cytosine (m^5C) at position 841, and its knockdown in mice affects mitochondrial genome translation.[29] Additionally, Li *et al*[24] and Safra *et al*[25] identified m^1A modification sites at positions 282, 433, and 490 of the 12S mt-rRNA, where m^1A433 is likely catalyzed by TRMT61B, and m^1A288 and m^1A490 are likely catalyzed by TRMT10C. Methyltransferase-like 15 (METTL15), an N4-methylcytidine (m^4C) methyl methyltransferase, is responsible for methylation at position 839 in the mitochondrial 12S rRNA, and METTL15 deletion results in the impaired translation of mitochondrial protein-coding mRNAs and reduces the mitochondrial respiratory capacity.[30]

In 16S mt-rRNA, a group of 2′-O-ribose methyltransferases (MRM1, MRM2/FtsJ2, and MRM3/RNMTL1) has been shown to be associated with modifications at three nucleotide positions (Gm1145, Um1369, and Gm1370) situated in the peptidyl transferase center. The absence of these modifications detrimentally impacts the assembly of the large subunit of the mitochondrial ribosome, consequently disrupting mitochondrial translation.[31] The RNA pseudouridine synthase D4 (RPUSD4) was identified as the enzyme that may provide the Ψ 1397 modification.[26] Another enzyme, TRMT61B, is the enzyme responsible for 16S m^1A947.[32] In addition, the newly detected m^1A sites (such as at positions 363, 1162, and 1360) have a relatively low level of modification, and TRMT61B may also be responsible for the modification of m^1A363 and m^1A1360 among them.[24,25]

mt-tRNA undergoes extensive posttranscriptional modifications, including the addition of deadenylated CCA trinucleotide at the 3′-end and chemical nucleotide modifications.

The CCA sequence is present at the 3′-end of all mature mt-tRNAs and is not encoded by mtDNA. Instead, it is synthesized posttranscriptionally by tRNA-nucleotidyl transferase 1 (TRNT1). This enzyme does not require a template sequence but preferentially selects cytidine triphosphate (CTP) and adenosine triphosphate (ATP) for polymerization.[33] Additionally, the noncoding 5′ guanine present in $mt\text{-}tRNA^{His}$ is added posttranscriptionally by tRNA-histidine guanylyltransferase 1 like (THG1L) via 3′–5′ polymerase activity.[34]

Position 34, also known as the "wobble position", within the anticodon sequence is a crucial site for the modification

of mt-tRNA. Various nucleoside modifications, including 5-formylcytidine (f^5C), queuosine (Q), and 5-taurine methylation (τm^5U), which increase codon recognition selectivity, occur at this site. For example, human mtDNA contains only one gene encoding mitochondrial $tRNA^{Met}$, which is responsible for the initiation and elongation phases of mitochondrial protein synthesis, and recognizes the unconventional start codons AUA, AAU, and AUC, in addition to decoding the universal AUG codon. *In vitro* studies have shown that the f^5C modification of human mt-$tRNA^{Met}$ at the anticodon "wobble position" is required for decoding AUG and unconventional AUN codons.[35] Enzymes responsible for the modification of this position include NOP2/Sun RNA methyltransferase 3 (NSUN3) and alpha-ketoglutarate-dependent dioxygenase (ABH1/ALKBH1), where the cytosine residue is initially modified to 5-methylcytosine (m^5C) by NSUN3 and subsequently oxidized to 5-formylcytosine (f^5C) by ABH1.[36] Queuosine is also present at position 34 of the four mt-tRNA species ($tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asp}$, and $tRNA^{Asn}$). tRNA-guanine transglycosylase (TGT), composed of queuine tRNA-ribosyltransferase catalytic subunit 1 (QTRT1) and queuine tRNA-ribosyltransferase accessory subunit 2 (QTRT2), is responsible for replacing guanine with queuosine in the mt-tRNA.[37] Mitochondrial tRNA translation optimization 1 (MTO1) and tRNA-modifying GTP-binding protein 3 (GTPBP3) catalyze the uridine 5-taurine methylation (τm^5U) modification at the "wobble position" with 5,10-methylenetetrahydrofolate and taurine as substrates.[38] Mitochondrial tRNA-specific 2-thiouridylase (MTU1/TRMU) and cysteine desulfurase (NFS1) are responsible for further 2-thiolation (τm^5s^2U) modifications of $tRNA^{Lys}$, $tRNA^{Glu}$, and $tRNA^{Gln}$ at position 34.[39]

In addition to the modification of the "wobble position", position 37 downstream of the anticodon is often chemically modified to promote stable codon–anticodon interactions. This position is subject to various modifications, including N1-methylation of guanosine (m^1G) by tRNA methyltransferase 5 (TRMT5)[40] and N6-isopentenylation of adenosine (i^6A) by tRNA isopentenyltransferase 1 (TRIT1).[41] Subsequently, the mt-tRNA with i^6A37 is methylated by cell cycle protein-dependent kinase 5 regulatory subunit-associated protein 1 (CDK5RAP1) to generate 2-methylthio-N6-isopentenyladenosine (ms^2i^6A37).[42] Another modification at position 37 is N6-threonylcarbamoyladenosine (t^6A), which is essential for translation accuracy and fidelity. Two enzymes are responsible for this modification in human mitochondria. First, yrdC N6-threonylcarbamoyltransferase domain containing (YRDC) synthesizes the L-threonylcarbamoyl adenosine (TC-AMP) intermediate and then transfers the TC portion to the mt-tRNA via a possible tRNA N6-adenyltransferase (OSGEPL1).[37]

Methylation is prevalent in mt-tRNA. For example, the TRMT10C-HSD17B10 (MRPP1-MRPP2) complex methylates adenine or guanine at position 9 of mt-tRNA to form N1-methyladenine (m^1A) and N1-methylguanine (m^1G), respectively.[43] Furthermore, NOP2/Sun RNA methyltransferase 2 (NSUN2) is required for the 5-methylcytidine (m^5C) modification of mt-tRNA at positions 48, 49, and 50.[44] Pseudouridine modifications, which are catalyzed by PUSs to produce rotational isomers of uridine, are common in noncoding RNAs.[45,46] PUS1 modifies U27 and U28 in mt-tRNA, heterodimerizing uridine to pseudouridine,[45] while RPUSD4 introduces pseudouridine at positions 39 and 50.[46] A comprehensive analysis by Suzuki *et al*[37] involving mass spectrometry, biochemical mapping of Ψ in mt-tRNA, and previously published data revealed a total of 52 Ψ sites in human mt-tRNAs across all species, 44 of which were further confirmed by tRNA-Ψ sequencing.

Stabilization of mtRNA

Considering the inherent instability of RNA molecules, mitochondria have evolved a variety of mechanisms for mtRNA stabilization.

The stability of mitochondrial transcripts is regulated by leucine-rich pentapeptide-rich structural domain protein (LRPPRC) and steroid receptor RNA activator (SRA) stem-loop-interacting RNA-binding protein (SLIRP), both of which contain a pentapeptide repeat (PPR) sequence and an RNA recognition motif (RRM), respectively. Upon import into the mitochondria, LRPPRC interacts with SLIRP to form a complex that enhances the stability of mt-mRNAs.[47] As a molecular chaperone of mt-mRNAs, the LRPPRC–SLIRP complex stabilizes the RNA structure mainly by regulating the secondary structure of mitochondrial transcripts and exposing sites required for translation, stabilization, and polyadenylation.[48] Studies have shown that the deletion of LRPPRC reduces the steady-state levels of mt-mRNAs, leading to translation anomalies and the absence of the respiratory chain complex, while having no impact on rRNA and tRNA.[49] In addition, the LRPPRC–SLIRP complex inhibits the degradation of mt-mRNAs by degradosomes. As two closely related molecules, the levels of LRPPRC and SLIRP are interdependent, with LRPPRC silencing leading to a decrease in SLIRP expression and vice versa.[47] SLIRP stabilizes LRPPRC by protecting it from degradation.[50]

Fas-activated serine/threonine kinase (FASTK) and its homologs, FASTKD1–5, are RNA-binding proteins located in the mitochondria that participate in mtRNA stabilization, precursor processing, translation, and assembly of proteins.[23,51,52] FASTKD is characterized by a mitochondrial targeting signal at the N-terminus and the presence of three conserved structural domains, FAST_1, FAST_2, and RAP (an RNA-binding domain of approximately 60 amino acids).[53] FASTK interacts with the 3′-end of the *MT-ND6* mRNA via the RAP-dependent structural domain and protects the *MT-ND6* mRNA from degradation.[51] Furthermore, an analysis employing cross-linked immunoprecipitation (CLIP) techniques has identified 16S rRNA and *MT-ND6* mRNA as targets for *FASTKD2* binding, and *FASTKD2* deletion resulted in aberrant processing and expression of 16S rRNA and *MT-ND6* mRNA, underscoring its critical role in their regulation.[52] In addition, the disruption of the *FASTKD3* gene has been found to prolong the half-life and steady-state levels of the *MT-ND2*, *MT-ND3*, *MT-CYTB*,

MT-CO2, and *MT-ATP8/6* mRNAs.[54] Boehm *et al*[53] found that FASTKD4 binds to most of the heavy-strand encoded transcripts, contributing significantly to the stable expression of the *MT-ATP8/6*, *MT-CO1*, *MT-CO2*, *MT-CO3*, *MT-ND3*, *MT-CYTB*, and *MT-ND5* mRNAs. Conversely, FASTKD1 negatively impacts the stability of the *MT-ND3* mRNA, and FASTKD1 deletion increases *MT-ND3* mRNA levels. Similar to FASTKD4, FASTKD5 regulates the maturation process of precursor RNAs that cannot be processed by RNase P and ELAC2.[51]

As the name suggests, G-rich RNA sequence binding factor 1 (GRSF1) is an RNA-binding protein that interacts with RNAs rich in guanine sequences and is localized to the MRG.[55] Recent studies have reported the participation of GRSF1 in the RNA surveillance pathway. The mitochondrial genome has a specific GC skew, i.e., a relatively high guanine content on the heavy strand. Consequently, transcripts derived from templates lacking guanine (i.e., the light strand) manifest as guanine-rich RNAs, which possess an inherent propensity to form stable G-quadruplex structures, thereby impeding RNA degradation. In this context, GRSF1 collaborates with mitochondrial degradosomes to facilitate the degradation of mtRNAs harboring G4 structures.[56]

Mitochondrial transcription rescue factor 1 (MTRES1) interacts with POLRMT and TFAM to prevent stress-induced mtRNA loss at mitochondrial transcription initiation and enhances mitochondrial transcription without affecting the stability of mitochondrial transcripts.[57] Mitochondrial ribosomal protein L12 (MRPL12) is a part of the large subunit of the mitochondrial ribosome. However, it also exists in the mitochondrial matrix in a ribosome-independent "free" form, where it may interact directly with POLRMT to regulate POLRMT transcriptional activity, ribosome biogenesis, and protein synthesis. Furthermore, MRPL12 knockdown decreases POLRMT stability and reduces mitochondrial transcript levels,[58] suggesting that MRPL12 is required for POLRMT stability.

Surveillance and degradation of mtRNA

The precise mechanisms underlying RNA degradation in human mitochondria have not yet been fully elucidated. Recent studies have revealed that mtRNA degradation relies primarily on the mitochondrial degradosome (also referred to as the mitochondrial exoribonuclease complex (mtEXO)), which consists of the Suv3-like RNA helicase (SUPV3L1/SUV3)[59] and the polyribonucleotide nucleotidyltransferase 1 (PNPT1/PNPase)[60] [Figure 1]. SUV3 is an ATP-dependent multisubstrate helicase that catalyzes the unwinding of double-stranded RNA (dsRNA), dsDNA, and RNA/DNA heteroduplexes in the 5′–3′ direction,[61] while PNPase is a phosphate-dependent 3′–5′ exoribonuclease localized in the intermembrane space and mitochondrial matrix that catalyzes the degradation of phosphodiester bonds in RNA.[62] *In vitro* analyses have shown that the interaction between SUV3 and PNPase is a prerequisite for mtRNA degradation, and PNPase must form a complex with SUV3 prior to unwinding the substrate for degradation.[63] Subsequently, products of several nucleotides in length are produced and further removed by RNA exonuclease 2 (REXO2), an oligoribonuclease located in the mitochondrial matrix.[64] The entire degradation process occurs in a specific location in the mitochondrial matrix called D-foci.[60]

In mitochondria, abnormal or damaged mRNAs, tRNAs, and rRNAs are subject to degradation. Exposure to ethidium bromide (EtBr) disrupts the tRNA secondary structure, resulting in polyadenylation, and the subsequent withdrawal of EtBr leads to rapid degradation of polyadenylated tRNA by PNPase-SUV3 degradosomes.[65] In addition, human mtDNA has bidirectional transcriptional properties, and the mtRNA formed by transcription and its complement are highly susceptible to producing mitochondria-encoded double-stranded RNA (mt-dsRNA),[66] which can be released from the mitochondria into the cytoplasm and trigger interferon (IFN) responses via pattern recognition receptors (PRRs)[67] [Supplementary Figure 1, http://links.lww.com/CM9/C347]. The depletion of either PNPase or SUV3 leads to the accumulation of mt-dsRNA and IFN induction.[67] In addition, the knockdown of SUV3 or PNPase results in the accumulation of antisense transcripts, mtRNAs with extended poly(A) tails, and the appearance of degradation intermediates.[59,60] Similarly, REXO2 silencing leads to reduced levels of mtRNA and mtDNA, impaired translation processes, negative effects on cell growth and the accumulation of short RNAs and dsRNAs.[68] Hence, the mtRNA surveillance function of the degradosome is essential for maintaining mitochondrial gene expression and normal metabolism.

mtRNA-Related Diseases

Diseases associated with mutations in mtRNA metabolism-related genes

The mitochondrial genome exclusively encodes proteins related to OXPHOS, whereas proteins related to mtRNA metabolism are encoded by nuclear genes and imported from the cytoplasm to the mitochondria. Defects in nuclear genes encoding mtRNA metabolism-associated proteins result in impaired mtRNA metabolism and the inability to produce mitochondrial proteins with normal function, further contributing to incurable mitochondrial diseases. These heterogeneous disorders are observed in patients during childhood or adulthood and have clinical significance because they affect specific organs or systems [Supplementary Table 1, http://links.lww.com/CM9/C347].

TFAM gene mutations that are key for mtRNA transcription lead to a reduction in mtDNA copy number and progressive liver failure in newborns.[69] The alterations in TFAM levels and the associated changes in mtDNA copy number are associated with neurodegeneration.[70] The subunits of the RNase P complex, TRMT10C, HSD17B10, and PRORP, are critical for processing polycistronic mtRNA, and mutations in the genes encoding these proteins result in a variety of phenotypes. As an mtRNA modifier, mutations in TRMT10C lead to phenotypes such as dysplasia, lactic acidosis, hypotonia, and liver dysfunction.[71] Mutations in HSD17B10 lead

to HSD10 mitochondrial disease with complete development delays, hypertrophic cardiomyopathy (HCM), and progressive neurodegeneration.[72] PRORP mutations lead to sensorineural hearing loss, appendicular hypertonia, seizures, and ovarian failure.[73]

Mutations in the LRPPRC gene underlies the pathogenesis of Leigh syndrome of the French–Canadian type,[74] with clinical symptoms of dysplasia, microcephaly, HCM, dysphagia, poor feeding, hypotonia, psychomotor retardation, and lactic acidosis. Recent studies suggest that LRPPRC may be linked to tumors or neurodegenerative pathologies.[75] FASTKD2 mutations lead to mitochondrial encephalomyopathy, lactic acidosis, stroke-like seizures, seizures, optic nerve atrophy, developmental delay, and asymmetric brain atrophy.[76]

Mutations in multiple proteins involved in modifying mtRNAs can also lead to different mitochondrial disease phenotypes. In particular, given the extensive modification of mt-tRNAs, defects in tRNA modification can have various types of severe pathological consequences, hence the term "RNA modopathies" for this class of diseases. For example, TRNT1 is involved in the addition of CCA at the 3′-end of mt-tRNA, and its mutation at different positions can lead to retinitis pigmentosa and erythrocyte microcytosis or borderline anemia with B-cell immunodeficiency, periodic fever, and developmental delay.[77] Mutations in the methylesterase TRMT1 cause autosomal recessive mental retardation,[78] whereas TRMT5 mutations cause peripheral neuropathy with spasticity, motor intolerance, and developmental delay.[79] Mutations in MTO1, an enzyme involved in "wobble position" modification, can lead to lactic acidemia, HCM, psychomotor retardation, and epilepsy.[80]

Diseases associated with mtRNA alterations due to mtDNA mutations and deletions

Numerous pathogenic variants in human mtDNA affect mt-tRNA, mt-rRNA, and mt-mRNA genes, resulting in oxidative phosphorylation defects, further reducing ATP production, and increasing reactive oxygen species production. The tissues that are heavily dependent on aerobic metabolism for ATP production, such as the central nervous system and skeletal muscle, are particularly susceptible to oxidative phosphorylation defects (e.g., encephalomyopathy).

mt-mRNA mutations result in various syndromes, such as neuropathy ataxia retinitis pigmentosa (NARP) syndrome (multiple mutations in the *MT-ATP6* mRNA-encoding gene),[81] sensorineural deafness (pathogenic variants in the *MT-CO1* mRNA),[82] exercise intolerance (mutations in the *MT-CYTB* mRNA),[83] and Leber hereditary optic neuropathy (mRNA mutations in *MT-ND3*)[84] [Supplementary Table 2, http://links.lww.com/CM9/C347]. Pathogenic variants in genes encoding mt-rRNAs are seldom reported. Mutations in MT-RNR1, such as m.827A ψ G and m.1095T>C, are mostly associated with hearing loss.[85,86] Heteroplasmic point mutations in the mt-tRNA gene are a common cause of mitochondrial disease, and

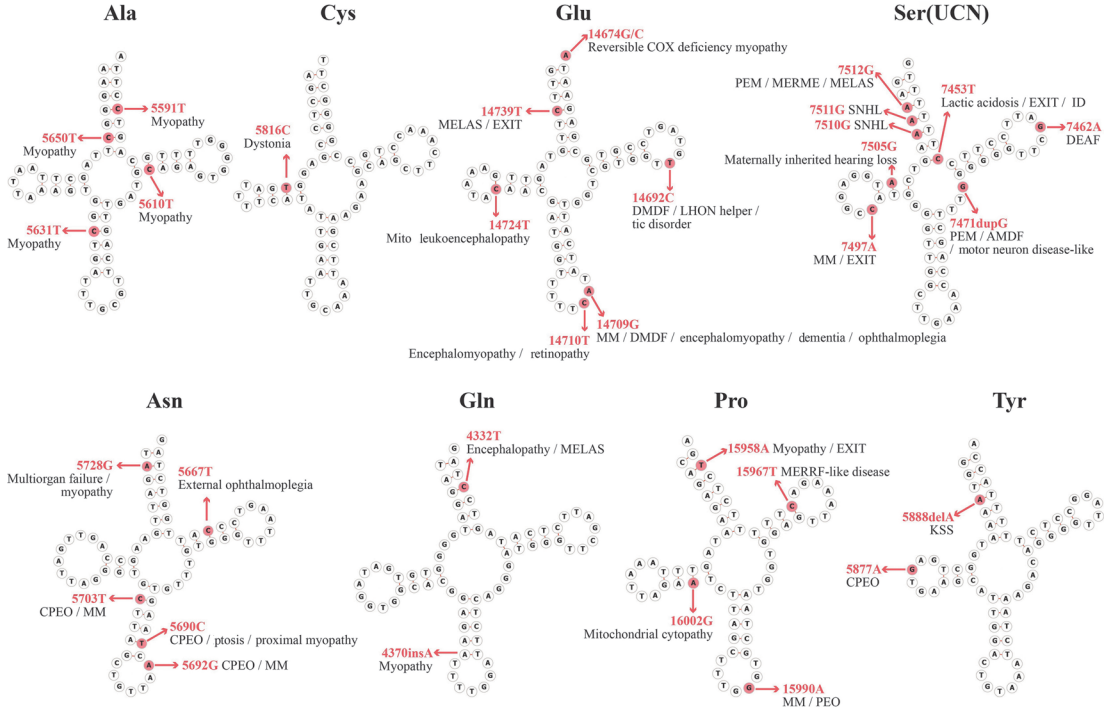

Figure 2: Diseases associated with mt-tRNA alterations due to mtDNA heavy strand point mutations. The red circles indicate the locations of the variants in the mt-tRNA. Red arrows point to the disease caused by the mutation. Ala: Alanine; AMDF: Ataxia, myoclonus and deafness; Asn: Asparagine; CPEO: Chronic progressive external ophthalmoplegia; Cys: Cysteine; DEAF: Maternally inherited deafness; DMDF: Diabetes mellitus and deafness; EXIT: Exercise intolerance; Gln: Glutamine; Glu: Glutamate; ID: Intellectual disability; KSS: Kearns-Sayre syndrome; LHON: Leber hereditary optic neuropathy; MELAS: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERME: MERRF/MELAS overlap disease; MERRF: Myoclonic epilepsy and ragged red muscle fibers; MM: Mitochondrial myopathy; PEM: Progressive encephalopathy; PEO: Progressive external ophthalmoplegia; Pro: Proline; Ser: Serine; SNHL: Sensorineural hearing loss; Tyr: Tyrosine.

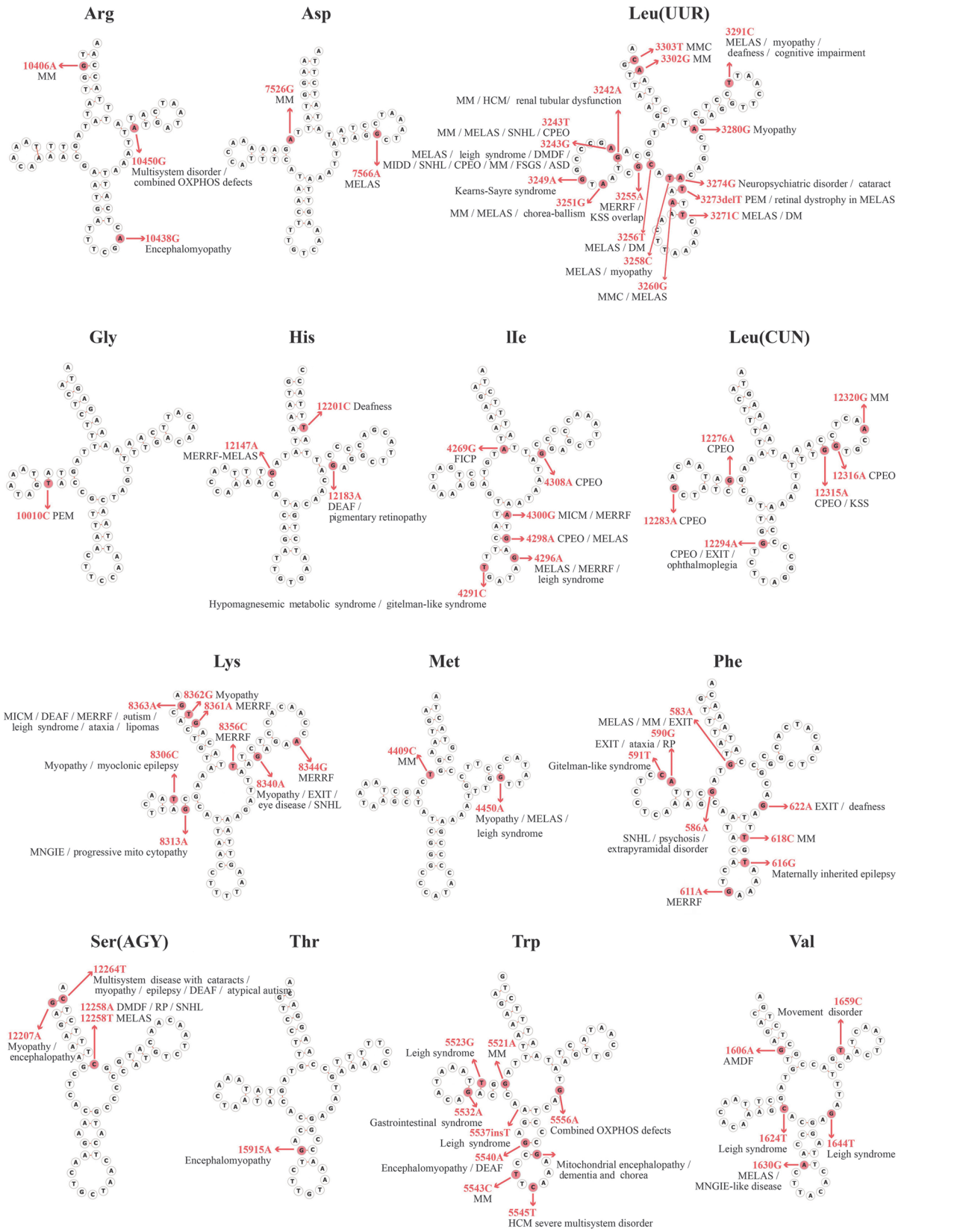

Figure 3: Diseases associated with mt-tRNA alterations due to mtDNA light strand point mutations. The red circles indicate the locations of the variants in the mt-tRNA. Red arrows point to the disease caused by the mutation. AMDF: Ataxia, myoclonus and deafness; Arg: Arginine; Asp: Aspartate; CPEO: Chronic progressive external ophthalmoplegia; DEAF: Maternally inherited deafness; DM: Diabetes mellitus; DMDF: Diabetes mellitus and deafness; EXIT: Exercise intolerance; Gly: Glycine; HCM: Hypertrophic cardiomyopathy; His: Histidine; Ile: Isoleucine; KSS: Kearns-Sayre syndrome; Leu: Leucine; Lys: Lysine; MELAS: Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MERME: MERRF/MELAS overlap disease; MERRF: Myoclonic epilepsy and ragged red muscle fibers; Met: Methionine; MICM: Maternally inherited cardiomyopathy; MM: Mitochondrial myopathy; MMC: Maternal myopathy and cardiomyopathy; MNGIE: Mitochondrial neurogastrointestinal encephalopathy disease; OXPHOS: Oxidative phosphorylation; PEM: Progressive encephalopathy; Phe: Phenylalanine; RP: Retinitis pigmentosa; Ser: Serine; SNHL: Sensorineural hearing loss; Thr: Threonine; Trp: Tryptophan; Val: Valine.

the phenotypes caused by different tRNA point mutations vary significantly [Figures 2 and 3]: $tRNA^{Leu\ (UUR)}$ point mutations encoded by the light strand of mtDNA are found in patients with mitochondrial myopathies, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome, Kearns-Sayre syndrome (KSS), etc.

On the other hand, mtDNA deletions appear to lead to more severe clinical manifestations. The three classic phenotypes caused by mtDNA deletions are KSS, Pearson syndrome, and progressive external ophthalmoplegia (PEO). For example, the more common 4977 bp deletion can lead to classic KSS, manifesting as chronic progressive external ophthalmoplegia, myopathy, cerebellar ataxia, and short stature.[87] The deletion of 5400 bp of mtDNA results in Pearson syndrome, which manifests as pancytopenia, hyperlactatemia, steatorrhea, insulin-dependent diabetes mellitus, liver function abnormalities, and Fanconi syndrome.[88]

Diseases linked to defective mtRNA surveillance

As previously mentioned, the proper function of mtRNA surveillance is crucial for maintaining mitochondrial gene expression and normal metabolism. Alterations in this function may be involved in the development of several diseases, particularly those arising from the abnormal activation of the IFN response pathway. The IFN response pathway is part of the innate immune response, wherein the PRR recognizes pathogen-associated molecular patterns (PAMPs), including viral or bacterial nucleic acids or other microbe-specific molecules, thereby inducing the upregulation of IFN and IFN-stimulated genes. While the detection of PAMPs and activation of defense responses are essential for organismal homeostasis, they also increase the risk of the harmful recognition of host-produced biomolecules such as mt-dsRNAs [Supplementary Figure 1, http://links.lww.com/CM9/C347].

Recently, the expression of mt-dsRNAs in the synovial fluid of patients with osteoarthritis was shown to be significantly increased compared with that of patients with acute gouty arthritis or rheumatoid arthritis; furthermore, the severity of cartilage damage in patients with osteoarthritis was positively correlated with the expression of mt-dsRNAs.[89] One study revealed that increased mt-dsRNA expression and decreased PNPase expression were observed in the renal tissues of patients with various renal diseases, such as acute tubular necrosis, diabetic nephropathy, lupus nephritis, immunoglobulin A nephropathy, membranous nephropathy, and focal segmental glomerulosclerosis, suggesting that mt-dsRNAs may be involved in the process of tubular injury.[90] Taken together, mt-dsRNAs may serve as potential targets for intervention in a variety of inflammation-associated diseases.

Finally, the treatment of patients with mitochondrial diseases remains a challenge and the treatment recommendations focus on improving quality of life and prolonging life expectancy, such as anticonvulsants for mitochondrial epilepsy and insulin or oral hypoglycemic agents for patients with diabetes.

Conclusion

Oxidative phosphorylation is affected by mtRNA production and regulation. This process involves steps such as transcription, modification, stabilization, and RNA enzyme-mediated degradation of abnormal mtRNAs. mtRNA metabolism is regulated by a complex mechanism that involves not only mtDNA-encoding genes but also numerous nuclear-encoded genes, which are involved in all aspects of mtDNA transcription, mtRNA modification, maturation, and degradation. Defects in these proteins are related to mtRNA metabolism, causing predominantly autosomal recessive human diseases that typically exhibit a neonatal or infant onset and are often accompanied by a severe disease phenotype. In addition, mutations or deletions of large segments of mtDNA impair mtRNA, leading to diseases associated with respiratory chain defects. Due to the difficulties in treating diseases caused by mutations, current therapies center around the early treatment of complications. In conclusion, improving our understanding of the regulation of mtRNA metabolism may contribute to the development of specific therapeutic strategies to alleviate mitochondrial dysfunction in patients with mitochondria-related diseases. The "atypical" mtRNAs in human diseases have become an emerging research target.

Conflicts of interest

None.

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