Review

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Review

Mitochondrial Epitranscriptomics: The Role of RNA Modifications in Cellular Energy Regulation and Aging

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Abstract: Mitochondria, often referred to as the powerhouses of the cell, are essential for cellular metabolism and energy production. While their function is primarily regulated by both nuclear and mitochondrial DNA, recent research suggests that modifications to mitochondrial RNA (mtRNA) play a crucial role in shaping mitochondrial activity, maintaining cellular energy balance, and influencing the aging process. This review delves into the emerging field of mitochondrial epitranscriptomics, focusing on key RNA modifications such as N6-methyladenosine (m6A), pseudouridylation, and 5-methylcytosine (m5C), and their role in regulating mitochondrial gene expression and function. We explore how these modifications affect oxidative phosphorylation (OXPHOS), the balance of reactive oxygen species (ROS), and cellular senescence. Additionally, we highlight advanced techniques used to study mitochondrial RNA modifications and examine how their dysregulation is linked to aging and age-related diseases. Finally, we discuss the potential of targeting these modifications for therapeutic interventions. A deeper understanding of mitochondrial epitranscriptomic changes could pave the way for novel approaches to treating neurodegenerative diseases, metabolic disorders, and promoting healthy aging.

Keywords: mitochondrial epitranscriptomics; RNA modifications; aging; oxidative phosphorylation; m6a; pseudouridylation; m5c; neurodegeneration; metabolic disorders; longevity

1. Introduction

Mitochondria are highly dynamic organelles that play a crucial role in cellular energy production, metabolic regulation, and apoptosis. They generate ATP through oxidative phosphorylation (OXPHOS), a process that relies on the coordinated expression of genes encoded both in the nucleus and the mitochondria [1]. While mitochondrial function has traditionally been studied from a genetic perspective, recent findings highlight the importance of post-transcriptional RNA modifications in fine-tuning mitochondrial gene expression and function [2]. This emerging field, known as mitochondrial epitranscriptomics, examines chemical modifications in mitochondrial RNA (mtRNA) that impact RNA stability, translation, and overall mitochondrial efficiency [3].

RNA modifications have long been recognized as key regulators of gene expression in the nuclear transcriptome. However, advances in sequencing technologies have revealed that mitochondrial RNAs also undergo extensive chemical modifications [4]. These include N6-methyladenosine (m6A), 5-methylcytosine (m5C), and pseudouridylation (Ψ), all of which play essential roles in regulating mitochondrial translation and maintaining metabolic homeostasis [5]. Disruptions in these modifications have been linked to aging, metabolic disorders, and neurodegenerative diseases [6].

Aging is a complex biological process marked by a gradual decline in cellular function, increased oxidative stress, and mitochondrial dysfunction. Mitochondria play a central role in aging by regulating energy metabolism, reactive oxygen species (ROS) production, and apoptosis [7]. Age-related mitochondrial dysfunction has been associated with changes in mtRNA modifications, potentially contributing to reduced ATP production, the accumulation of mitochondrial DNA

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(mtDNA) mutations, and elevated ROS levels [8]. However, the precise molecular mechanisms linking mtRNA modifications to mitochondrial aging remain unclear, necessitating further research [9]. Recent studies suggest that modulating mitochondrial RNA modifications could offer a promising therapeutic approach for combating age-related diseases. For example, targeting m6A-modifying enzymes has shown potential in restoring mitochondrial function in models of metabolic dysfunction and neurodegeneration [10]. Furthermore, mitochondrial epitranscriptomics is being explored in the context of mitochondrial stress responses, autophagy, and cellular senescence, shedding new light on its role in longevity [11].

This review provides a comprehensive overview of mitochondrial epitranscriptomics, focusing on key mitochondrial RNA modifications, their role in cellular energy homeostasis, and their implications in aging and age-related diseases. We also discuss recent advancements in techniques for studying mtRNA modifications and explore emerging therapeutic strategies targeting mitochondrial epitranscriptomic pathways. A deeper understanding of the regulatory role of mitochondrial RNA modifications could pave the way for novel interventions aimed at mitigating mitochondrial dysfunction in aging and disease.

2. Major Mitochondrial RNA Modifications and Their Functions

Mitochondrial RNA undergoes various post-transcriptional modifications that influence RNA stability, translation efficiency, and mitochondrial function. These modifications regulate the expression of mitochondrially encoded proteins required for oxidative phosphorylation (OXPHOS) and cellular metabolism. Recent advances in RNA sequencing and mass spectrometry have identified several key epitranscriptomic modifications in mitochondrial RNA, including N6-methyladenosine (m6A), pseudouridylation (Ψ), and 5-methylcytosine (m5C), which play significant roles in mitochondrial gene regulation and aging [12], see Table 1.

Table 1. Mitochondrial RNA Modifications and Their Impact on Aging.

Modification	Function	Impact on Aging
N6-Methyladenosine (m6A)	One of the most prevalent RNA modifications in both nuclear and mitochondrial transcripts. Regulates mtRNA stability, processing, and translation efficiency by influencing RNA-protein interactions and ribosomal recruitment [13].	Dysregulation of mitochondrial m6A is associated with metabolic decline, impaired mitochondrial function, and age-related diseases. A decrease in m6A methylation leads to defective mitochondrial translation and reduced ATP production, contributing to cellular aging [14]. m6A-modifying enzymes such as FTO and METTL3 are implicated in mitochondrial metabolic regulation and longevity [15].
Pseudouridylation (Ψ)	Conversion of uridine to pseudouridine (Ψ), enhancing RNA structural stability and translation accuracy. Catalyzed by pseudouridine synthases, which play a crucial role in mitochondrial ribosome function [16].	Reduced pseudouridylation is linked to mitochondrial dysfunction, increased oxidative stress, and accelerated aging. A decline in Ψ modifications leads to defects in mitochondrial tRNA and rRNA processing, affecting mitochondrial translation and OXPHOS efficiency [17].

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		Studies suggest that restoring pseudouridylation levels may improve mitochondrial function and promote cellular longevity [18].
5-Methylcytosine (m5C)	Methylation of cytosine residues in mitochondrial tRNAs and mRNAs. Plays a role in RNA stability, tRNA processing, and mitochondrial protein synthesis, ensuring proper translation of key respiratory chain components [19].	Changes in m5C levels are associated with reduced ATP production, mitochondrial fragmentation, and cellular senescence. Dysregulated m5C modifications contribute to mitochondrial stress and are linked to neurodegenerative diseases such as Alzheimer's and Parkinson's [20]. Modulating m5C methylation levels is considered a potential therapeutic strategy for mitochondrial-related disorders and aging [21].

3. Mitochondrial Epitranscriptomics and Cellular Energy Regulation

Mitochondrial RNA (mtRNA) modifications have emerged as key regulators of mitochondrial gene expression, energy metabolism, and cellular stress responses. These modifications play a crucial role in shaping the function of the electron transport chain (ETC), oxidative phosphorylation (OXPHOS), and mitochondrial dynamics, ultimately influencing cellular energy production and homeostasis. Recent studies suggest that mtRNA modifications are highly responsive to changes in cellular metabolism and environmental stress, allowing mitochondria to adapt to fluctuating energy demands and oxidative challenges [22].

3.1. Regulation of Mitochondrial Gene Expression and ETC Activity

- The ETC is composed of protein complexes encoded by both nuclear and mitochondrial genomes, and the proper expression of mtDNA-encoded subunits is essential for efficient ATP synthesis and metabolic stability [23].
- Modifications such as N6-methyladenosine (m6A) and 5-methylcytosine (m5C) regulate mitochondrial mRNA translation and stability, directly impacting ETC activity and ATP production [24].
- A reduction in mtRNA modifications has been linked to decreased expression of OXPHOS components, leading to mitochondrial dysfunction and energy deficits commonly associated with aging and metabolic disorders [25].

3.2. Impact of Dysregulated mtRNA Modifications on Mitochondrial Function

When mtRNA modifications become dysregulated, mitochondrial function is significantly compromised, contributing to cellular dysfunction and disease progression.

- Abnormal mtRNA modifications have been associated with mitochondrial fragmentation, ATP depletion, and elevated reactive oxygen species (ROS) production—key features of aging and neurodegenerative diseases [26].
- Impairments in pseudouridylation (Ψ) and m5C methylation have been linked to defective mitochondrial ribosomal assembly, disrupting the translation of ETC proteins and compromising energy metabolism [27].

 doi:10.20944/preprints202502.0449.v1

- Studies in cellular and animal models indicate that restoring mtRNA modifications through genetic or pharmacological interventions can enhance mitochondrial efficiency and mitigate oxidative stress, highlighting their potential as therapeutic targets [28].

3.3. Adaptive Role of mtRNA Modifications in Metabolic Stress

Mitochondria constantly adjust to changes in cellular energy demands and metabolic stress, and mtRNA modifications play a crucial role in this adaptive response.

- Under metabolic stress conditions, cells regulate mtRNA modification levels to maintain mitochondrial function and support energy balance [29].
- Dynamic fluctuations in m6A modifications enable mitochondria to optimize the translation of key OXPHOS components, ensuring efficient energy production during nutrient deprivation [30].
- Stress-responsive mtRNA modifications also activate mitochondrial quality control pathways, such as mitophagy and the mitochondrial unfolded protein response (UPRmt), which help protect mitochondria from damage and maintain cellular health [31].
- Given their role in metabolic adaptation, targeting mtRNA modifications is being explored as a potential therapeutic strategy for restoring mitochondrial function in metabolic and age-related diseases [32].

4. Mitochondrial Epitranscriptomics and Aging

Aging is a complex, multifactorial process marked by progressive mitochondrial dysfunction, increased oxidative stress, and the accumulation of cellular damage. Mitochondria play a critical role in aging by regulating energy metabolism, reactive oxygen species (ROS) homeostasis, and apoptosis. In recent years, research has shown that mitochondrial RNA (mtRNA) modifications are closely involved in these aging-related processes, influencing mitochondrial gene expression, protein synthesis, and overall mitochondrial function [33].

As organisms age, a decline in mtRNA modifications has been linked to impaired mitochondrial translation, reduced ATP production, and elevated ROS levels—factors that contribute to cellular senescence and age-related diseases [34]. Additionally, mtRNA modifications are implicated in pathways related to autophagy, apoptosis, and cellular stress responses, reinforcing their central role in the aging process [35].

4.1. Mitochondrial RNA Modifications and Cellular Aging

- Studies have shown that aging is associated with alterations in key mtRNA modifications, including N6-methyladenosine (m6A), 5-methylcytosine (m5C), and pseudouridylation (Ψ). These modifications regulate mitochondrial transcript stability and translation efficiency, ensuring proper mitochondrial function [36].
- Reduced m6A methylation in aged cells has been linked to impaired oxidative phosphorylation (OXPHOS), ATP depletion, and mitochondrial fragmentation, all of which contribute to cellular senescence [37].
- Dysregulation of pseudouridylation (Ψ) in mitochondrial tRNAs negatively affects ribosomal function, leading to disruptions in mitochondrial proteostasis and further exacerbating age-related mitochondrial decline [38].

4.2. mtRNA Modifications in Autophagy, Apoptosis, and Senescence

- Autophagy plays a crucial role in clearing damaged mitochondria through mitophagy, a process essential for maintaining mitochondrial quality. Age-related declines in mtRNA modifications have been associated with reduced mitophagy efficiency, leading to the accumulation of dysfunctional mitochondria and cellular decline [39].

 doi:10.20944/preprints202502.0449.v1

- Apoptosis, or programmed cell death, is tightly controlled by mitochondrial signaling pathways. Aberrant mtRNA modifications have been linked to dysregulated apoptosis, increasing susceptibility to age-related neurodegenerative and metabolic disorders [40].
- Cellular senescence, a state of irreversible cell cycle arrest, is influenced by changes in mitochondrial metabolism driven by mtRNA modifications. Research suggests that alterations in m6A and m5C levels contribute to mitochondrial dysfunction and the activation of the senescence-associated secretory phenotype (SASP) [41].

4.3. Mitochondrial Epitranscriptomics in Neurodegenerative and Metabolic Disorders, as Shown in Table 2

Table 2. Mitochondrial Epitranscriptomics in Neurodegenerative and Metabolic Disorders.

Disorder	Mitochondrial Epitranscriptomic Implications
Alzheimer's Disease (AD)	Reduced mitochondrial function and alterations in mtRNA modifications have been implicated in synaptic dysfunction and neuroinflammation, key hallmarks of AD [42].
Parkinson's Disease (PD)	Dysregulated mtRNA methylation affects mitochondrial complex I activity, contributing to dopaminergic neuron loss and the progression of PD [43].
Metabolic Disorders	Impaired m5C and pseudouridylation modifications in mitochondrial tRNAs have been linked to insulin resistance, diabetes, and obesity; conditions that share common mitochondrial dysfunction features with aging [44].

4.4. Therapeutic Potential of Targeting mtRNA Modifications in Aging

- Restoring mitochondrial RNA modifications is being actively explored as a potential therapeutic approach for treating age-related diseases and promoting longevity [45].
- Pharmacological interventions targeting m6A- and m5C-modifying enzymes have shown promise in improving mitochondrial function in aging models, highlighting their potential as anti-aging strategies [46].
- Future research should focus on developing precision epitranscriptomic therapies that leverage RNA-modifying enzymes as therapeutic targets to enhance mitochondrial function and slow age-related decline [47].

5. Techniques for Studying Mitochondrial RNA Modifications

Advancements in RNA modification detection technologies have significantly improved our understanding of mitochondrial epitranscriptomics. These techniques enable researchers to identify, quantify, and map specific RNA modifications within mitochondrial transcripts, providing critical insights into their regulatory roles in mitochondrial function and aging. Below are the key methodologies used to study mtRNA modifications [48], see Table 3.

Table 3. Techniques for Analyzing Mitochondrial RNA Modifications.

Technique	Purpose	Methodology	Application
LC-MS/MS (Liquid Chromatography-Mass Spectrometry)	Quantifies specific RNA modifications at the nucleotide level.	RNA is enzymatically digested into nucleosides, which are analyzed using liquid chromatography coupled with mass spectrometry (LC-MS/MS) to detect and quantify modifications such as m6A, m5C, and pseudouridylation (Ψ) [49].	LC-MS/MS has been instrumental in identifying age-related changes in mitochondrial RNA modifications, providing insights into their role in metabolic regulation [50].
MeRIP-Seq (Methylated RNA Immunoprecipitation Sequencing)	Maps m6A modifications in mtRNA at a transcriptome-wide scale.	Uses m6A-specific antibodies to immunoprecipitate methylated mtRNAs, followed by high-throughput sequencing (RNA-Seq)	MeRIP-Seq has revealed that m6A methylation plays a critical role in mitochondrial gene expression, particularly

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		to determine the distribution and abundance of m6A sites [51].	under conditions of oxidative stress and aging [52].
Ribo-Seq (Ribosome Profiling)	Analyzes mitochondrial translation dynamics and ribosome occupancy on mtRNAs.	Deep sequencing of ribosome-protected mRNA fragments (RPFs) provides a high-resolution view of active translation sites and reveals how mtRNA modifications impact mitochondrial protein synthesis [53].	Ribo-Seq has demonstrated that dysregulated mtRNA modifications alter mitochondrial ribosome activity, contributing to age-related mitochondrial dysfunction [54].

5.4. Additional Techniques for Studying mtRNA Modifications

Advancements in sequencing technologies and biochemical methods have significantly improved our ability to detect and analyze mitochondrial RNA (mtRNA) modifications. Several specialized techniques have been developed to map these modifications with high precision, shedding light on their roles in mitochondrial function and disease.

5.4.1. Nanopore Direct RNA Sequencing

- Provides real-time detection of RNA modifications without the need for chemical conversion.
- Enables the identification of modifications in full-length mitochondrial transcripts at single-molecule resolution, offering a comprehensive view of mtRNA modifications [55].

5.4.2. Bisulfite Sequencing (BS-Seq) for m5C Detection

- A widely used method for detecting and quantifying 5-methylcytosine (m5C) in mitochondrial RNA.
- Provides base-resolution mapping of m5C modifications, particularly in mitochondrial tRNAs and mRNAs, helping to understand their functional significance [56].

5.4.3. Pseudo-Seq for Pseudouridylation Mapping

- Utilizes chemical treatments (CMCT modification followed by reverse transcription stops) to precisely map pseudouridine (Ψ) residues in mtRNA.
- Enables high-resolution profiling of Ψ modifications, linking them to mitochondrial translation efficiency and overall gene regulation [57].

5.5. Future Perspectives in mtRNA Modification Detection

- The development of **single-cell RNA modification profiling** techniques will provide insights into mtRNA heterogeneity, revealing cell-type-specific differences in mitochondrial epitranscriptomics.
- **Multi-omics integration** (combining transcriptomics, proteomics, and metabolomics) will help uncover how mtRNA modifications regulate mitochondrial function in aging and disease, providing a more comprehensive understanding of their biological impact [58].
- **Machine learning-based models** are being explored to predict novel mitochondrial epitranscriptomic markers, offering potential applications in disease diagnostics and targeted therapies [59].

6. Therapeutic Implications and Future Directions

The recognition of mitochondrial RNA (mtRNA) modifications as critical regulators of energy metabolism, oxidative stress, and aging has opened exciting new possibilities for therapeutic interventions targeting mitochondrial dysfunction. Emerging research suggests that modulating mtRNA modifications could offer novel strategies for treating age-related diseases, neurodegenerative disorders, and metabolic syndromes [60]. Future directions in mitochondrial epitranscriptomics focus on developing small-molecule modulators, exploring the interplay between

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nuclear and mitochondrial RNA modifications, and identifying potential biomarkers for disease diagnosis and targeted therapy [61].

6.1. Targeting Mitochondrial RNA Modifications for Therapeutic Strategies

- Small-molecule inhibitors and activators of RNA-modifying enzymes, such as m6A demethylases FTO and ALKBH5, are being explored as potential therapeutic agents for mitochondrial disorders and aging-related diseases [62].
- CRISPR-based RNA editing technologies are being investigated for their ability to precisely correct mtRNA modifications, paving the way for gene therapy approaches to treat mitochondrial diseases [63].
- Nutritional and pharmacological interventions that influence mitochondrial epitranscriptomics—such as NAD+ supplementation, calorie restriction, and mitochondrial-targeted antioxidants—are being studied for their potential to enhance mitochondrial function and promote healthy aging [64].

6.2. Development of Small-Molecule Epitranscriptomic Modulators

The identification of mtRNA-modifying enzymes has created opportunities for targeted drug development. Recent studies have shown that:

- **m6A-modulating compounds** can restore mitochondrial function in models of metabolic syndrome and neurodegeneration, suggesting their potential as therapeutic agents [65].
- **m5C and pseudouridine analogs** are being investigated for their ability to stabilize mitochondrial transcripts and enhance cellular energy production [66].
- **Mitochondria-targeted RNA-binding proteins** could be engineered to selectively regulate mitochondrial transcript stability and translation, offering new approaches for mitochondrial disease treatment [67].

6.3. Investigating the Interplay Between Nuclear and Mitochondrial Epitranscriptomes

Since nuclear and mitochondrial genomes are highly interdependent, coordinated regulation of gene expression is essential for maintaining mitochondrial function. Future research should:

- Examine how nuclear RNA modifications, such as m6A in nuclear-encoded mitochondrial genes, impact mitochondrial activity and energy metabolism [68].
- Investigate the role of nuclear-encoded RNA-modifying enzymes (e.g., METTL3, TRMT10C) in modifying mitochondrial transcripts and influencing longevity pathways [69].
- Develop **systems biology approaches** integrating transcriptomics, proteomics, and metabolomics to better understand how mitochondrial epitranscriptomics contribute to age-related diseases [70].

6.4. Future Directions in Mitochondrial Epitranscriptomics

- **Expanding RNA modification databases** to include mitochondrial-specific modifications, improving our understanding of their physiological roles.
- **Leveraging single-cell and spatial transcriptomics** to study heterogeneity in mitochondrial RNA modifications across different tissues and aging models.
- **Utilizing machine learning and AI-driven predictive models** to identify novel therapeutic targets within the mitochondrial epitranscriptome.
- **Advancing personalized medicine approaches** to target mtRNA modifications for precision treatment of age-related metabolic and neurodegenerative disorders.

 doi:10.20944/preprints202502.0449.v1

7. Conclusions

Mitochondrial epitranscriptomics is an emerging field that has transformed our understanding of mitochondrial gene regulation, energy metabolism, and aging-related diseases. The discovery of key RNA modifications in mitochondrial transcripts—such as N6-methyladenosine (m6A), pseudouridylation (Ψ), and 5-methylcytosine (m5C)—has provided new insights into how mitochondria respond to metabolic stress, regulate oxidative phosphorylation (OXPHOS), and influence cellular lifespan. These modifications are not merely passive chemical markers but actively contribute to maintaining mitochondrial homeostasis and cellular function.

Aging is closely linked to progressive mitochondrial dysfunction, with alterations in mtRNA modifications playing a critical role in this decline. Changes in these modifications contribute to ATP depletion, elevated reactive oxygen species (ROS) levels, impaired mitophagy, and metabolic imbalances—hallmarks of age-related neurodegenerative and metabolic disorders. A deeper understanding of the molecular mechanisms underlying mitochondrial RNA modifications presents new opportunities for therapeutic interventions in aging-associated diseases such as Alzheimer's disease, Parkinson's disease, type 2 diabetes, and cardiovascular disorders.

Recent advances in RNA modification detection techniques, including LC-MS/MS, MeRIP-Seq, and Ribo-Seq, have enabled high-resolution mapping of mtRNA modifications, shedding light on their physiological roles. However, despite these breakthroughs, several critical gaps remain. Future research should prioritize:

- **Deciphering the functional interplay between nuclear and mitochondrial epitranscriptomes**, as their coordinated regulation is essential for maintaining cellular energy balance.
- **Developing precise RNA modification-targeting therapies**, such as small-molecule modulators and CRISPR-based RNA-editing technologies, to correct mitochondrial dysfunction.
- **Exploring tissue-specific and single-cell epitranscriptomic landscapes** to understand how mtRNA modifications vary across different organs and aging models.
- **Leveraging AI-driven predictive models** to identify novel biomarkers and therapeutic targets within the mitochondrial epitranscriptome.

With growing evidence supporting the role of mtRNA modifications in mitochondrial homeostasis, longevity, and age-related diseases, targeting mitochondrial epitranscriptomics represents a promising avenue in precision medicine. Continued research in this field could lead to innovative mitochondria-based anti-aging therapies, ultimately improving healthspan and quality of life in aging populations.

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