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Structural Basis for Mitochondrial Disease in Large Subunit (LSU) Assembly of Mitochondrial Ribosomes

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Abstract

The mitochondria are a unique organelle, due to the fact that it possesses its own DNA and thus is involved in producing its own protein building blocks. The mitochondrial ribosomes play a key role in the protein synthesis mechanism of the organelle, meaning a dysfunction in the large or small subunits eventually impacts this process. Throughout the past decades, scientists and researchers have been monitoring the mitochondrial disease, its types and effects on the human organism. The mitochondrial DNA-related disorders are divided into two key groups: those resulting from mutations in genes encoding individual proteins of the respiratory chain, and those resulting from mutations in genes involved in mitochondrial protein synthesis. Among these, mutations in the mitochondrial translation apparatus, the mitochondrial ribosome structural components and assembly factors, cause mitochondrial diseases such as cardiomyopathy and protein synthesis deficiencies. The primary focus of this research work is the relationship

between the mitochondrial disease and the large subunit assembly in mitochondrial ribosomes. Understanding the composition of the mitochondria, its DNA, and the mitochondrial ribosomes, and studying the protein synthesis and large subunit assembly from a structural perspective, the selected example of a mutation in the MRPL3 (uL3m) is extensively explored.

Introduction

The double-membraned mitochondria is a semi-autonomous organelle present in nearly all eukaryotic organism cells (with the exception of the erythrocytes of most vertebrates). Originating about 2 billion years ago, mitochondria are hypothesized to have derived from a bacteria belonging to a sister group of α-proteobacteria that was engulfed by an archaeal host cell to develop into the first aerobic eukaryotic cell, meaning it had the ability to adapt to and exploit the rising oxygen levels in the environment (Bykov et al., 2021). Widely known as the "powerhouse" of the cell, mitochondria's key role is to manufacture energy in the form of ATP (adenosine triphosphate), required by the human organism to carry out nerve impulse transmissions, active transport, muscle movement, and other metabolic processes in the body. In addition to this, it is responsible for functions beyond ATP production: it is a biosynthetic hub for nucleotides, lipids, and amino acids.

The organelle resembles a bean by shape in a size range from 0.5 to 10 μm., and consists of an inner and outer membranes, cristae, matrix, intermembrane space, and the mitochondrial DNA (mtDNA). Mitochondria are the only cell organelles apart from chloroplasts (both considered semi-autonomous) which harbor their own DNA and are able to manufacture proteins

independently. Since genes manage the mitochondrial protein synthesis, their mutation impacts this vital manufacturing mechanism in the mitochondria (Wiese & Bannister, 2020).

Mitochondrial Ribosome

Introduction to Mitochondrial Ribosomes

The ribosome is a crucial molecular machine responsible for translating messenger RNA (mRNA) into proteins. It consists of a small subunit (SSU) that binds to and reads the genetic information from mRNA, and a large subunit (LSU) that catalyzes the formation of peptidyl bonds between amino acids for protein synthesis (Scaltsoyiannes et al., 2022). The mammalian mitochondrial ribosomes (mitoribosomes) are made up of the 22S SSU and the 39S LSU, forming a 55S protein complex, which is present in mitochondria and is responsible for the translation of mitochondrial mRNAs encoded in mtDNA.

Ribosomes are complex particles composed of ribosomal RNAs (rRNAs) and ribosomal proteins (r-proteins). The r-proteins are typically located on the periphery of the ribosomes, with globular domains facing the solvent and long domains extending into the ribosome (between the RNA helices). Due to the diversity of rRNA composition across mitochondrial ribosomes, many of these proteins have been recruited to fulfill specific needs, such as stabilizing an rRNA extension or compensating for rRNA loss. While the precise function of r-proteins is not fully explored, it is hypothesized that their positively charged nature helps stabilize the negatively charged rRNAs, promoting efficient protein synthesis.

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Mitoribosomes involve protein synthesis within the mitochondrial genome which construct oxidative phosphorylation complexes. Thus, mitoribosome biogenesis is key for ATP (adenosine triphosphate) production and overall cellular metabolism. The central role of mitoribosomes to cellular metabolism is highlighted by several human diseases caused by mutations in either motor-proteins or assembly factors.

Mitochondrial ribosomes play a crucial role in synthesizing proteins encoded within the mitochondrial genome, which are essential for oxidative phosphorylation and ATP production. Defects in mitoribosome biogenesis, such as mutations in their assembly factors, can lead to human diseases. This stresses the importance of understanding the principles of mitoribosome assembly and their associated molecular processes from a structural perspective.

Structural Basis for the LSU Assembly

This paper overviews the LSU assembly process, as it connects to the mitochondrial disease. According to the SILAC proteomics study, mt-LSU assembly is complex and involves protein modules and individual proteins incorporated during three defined stages, early, intermediate, and late (Moran et al., 2023).

In the initial stages of assembly, a total of three major modules consisting of 24 proteins were identified to join together with similar speed, indicating a potential synchronized binding. These proteins are primarily located in a region encompassing the 5’ rRNA domain. The first module, comprised of rRNA binding (uL3m, bL19m) and other proteins (uL14m, bL17m, uL22m, and bL32m), acts as an anchor for mL39 and eventually mL45 **(**Rebelo-Guiomar et al.,

2022). It is suggested that the protein mL45 may secure the mt-LSU at the inner membrane during subsequent assembly steps. MALSU1 (Mitochondrial Assembly of ribosomal Large Subunit 1) plays a role in the insertion of uL14m during the assembly of this module (Hillen et al., 2021). Additionally, the DEAD-box RNA helicase DDX28 and the Fas Activated serine/threonine (FAST) kinase family protein FASTKD2 interact with and stabilize the 16S rRNA, indicating an early involvement, although their exact functions are not yet known (Moran et al., 2023). DDX28 remains attached to the developing mt-LSU particle until the later stages of maturation. A second early MRP visualization demonstrates the mRNA-binding bL20m protein also with bL21m, mL42, mL43, and mL44. The third module is composed of the mRNA binding heterodimer uL4m-uL15m, which recruits mL49 and mL50. The fourth assembly represents proteins associated with the tRNAVal (mL40, mL46, and mL48) (Moran et al., 2023).

The intermediate stage has been studied in relativity to yeast and bacteria, so some explanations in this paragraph include comparisons between the organisms. The fourth early module at the intermediate stage incorporates a second group of tRNAVal surrounding proteins, including mL38, uL18m, and bL27m. It is currently unknown how tRNAVal is recruited. Interestingly, human mitoribosomes can use mt-tRNAPhe instead of mt-tRNAVal when the latter is limited, showcasing unique plasticity (Rebelo-Guiomar et al., 2022). During this stage, the dimer uL13m-mL66 and uL11m bind the uL10m stalk through interacting with RNA and early-binding proteins. Even though the protein uL12m was initially categorized as an early protein based on kinetic proteomics data in HeLa cells, it can actually incorporate before uL11m and uL10m, which are situated at the base of the L12 stalk. The uL12m assembles later in

ribosomes similarly from bacteria and yeast mitochondria. Following this, a large module of intermediate-late proteins (mL41, uL23m, uL24m, uL29m, and bL34m) is proposed to be recruited to form the polypeptide exit tunnel. Additionally, there are differences in the timing of assembly of the polypeptide exit tunnel between mitoribosomes, bacteria, and yeast mitochondria. Notably, mitochondria-specific proteins, such as mL44 and mL50 in yeast, and mL45 in human cells, are involved in forming the polypeptide exit tunnel. These proteins assemble early and are related to the membrane-facing protuberance in yeast and the membrane-anchoring site in human cells (Moran et al., 2023; Hillen et al., 2021).

In the later stages of assembly, proteins near the interface with the mt-SSU are added, forming a large module consisting of uL2m, uL28m, uL29m, mL37, and mL65. These proteins contribute to intersubunit bridges, indicating that the intersubunit interface becomes well organized only at this late stage. Certain assembly factors work at this point to complete the maturation of the mt-LSU particle and establish quality-control checkpoints during the formation of the mt-LSU catalytic site, the peptidyl transferase center (PTC). Some of the late assembly factors are GTPases, including GTPBP7/MTG1, GTPBP5/MTG2, GTPBP10, and GTPBP6. Proteomics, biochemical, and structural studies have suggested a sequential recruitment of these assembly factors. GTPBP10 is the first to be recruited and binds to the 16S rRNA; its absence prevents the incorporation of bL33m and bL34m (Hillen et al., 2021; Rebelo-Guiomar et al., 2022).

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Protein Synthesis Background

Although the key focus is on the LSU assembly itself, it is helpful to have a foundation in the protein synthesis mechanism in mitochondria to understand the role mitoribosomes and their LSUs play in this process.

The translation process in human mitochondria involves several phases, including initiation, elongation, termination, and recycling. Each phase is carried out by specific components and factors. The complex in mitochondria is made up of specialized mitoribosomes, which consist of large and small subunits containing rRNA and proteins. Mutations in genes encoding mitoribosomal proteins and mitochondrial DNA can lead to various genetic diseases.

When the translation complex reaches the stop codon, release factors are required to separate the finished protein from the final tRNA, ribosome, and mRNA. The termination stage in human mitochondria has some unique aspects, such as certain codons not functioning as expected, and the involvement of specific release factors. After the termination of protein synthesis, the ribosome forms a post-termination complex, which needs to be disassembled and recycled for the next round of protein synthesis. This disassembly process involves the cooperation of specific factors to release the mRNA, tRNA, and ribosome subunits (Wang et al., 2021).

The Mitochondrial Disease

Introduction to Mitochondrial DNA

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The mitochondrial disease has been a serious issue for the past decades, and while there still remains a room for discovery and research, there is a sufficient background to this pathological condition. The vast majority of the mitochondrial diseases originate from genetic mutations within the mitochondria, which highlights the crucial role of the mitochondrial DNA (mtDNA) governing the organelle.

Due to the fact that the genetic materials of eukaryotic cells are stored in either the nucleus or the mitochondria, it may be concluded that the two are similar. However, the mammalian mitochondrial DNA is about 16,500 DNA building blocks (base pairs) in length, representing a small fraction of the total DNA in cells. In contrast, nuclear DNA comprises about 3 billion base pairs and contains thousands of genes, including those responsible for producing structural mitochondrial proteins and enzymes essential for maintaining mtDNA.

The mitochondrial DNA contains 37 genes, which are essential for maintaining homeostasis in mitochondria. Thirteen of these genes encode enzymes involved in oxidative phosphorylation, a process that produces adenosine triphosphate (ATP) — the primary energy source for cells — using oxygen and simple sugars. The other genes provide instructions for creating molecules called transfer RNA (tRNA) and ribosomal RNA (rRNA), crucial for assembling protein building blocks (amino acids) into functional proteins (Lax et al., 2011).

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Inheritance of mtDNA mutations differs from that of nuclear DNA due to the multi-copy nature of mtDNA and its exclusive maternal inheritance. Since sperm mitochondria are degraded after fertilization, all mitochondria and mtDNA copies in the fertilized egg come from the mother. As a result, a mother with an mtDNA point mutation will pass it to all her offspring, but only female offspring can transmit these mutations to future generations. Most individuals have identical mtDNA molecules in their cells, a condition known as homoplasmy. The uniparental inheritance pattern of mtDNA suggests that mutations can slowly accumulate over generations, potentially leading to the development of a disease as mtDNA mutates at a high rate.

Specific Mutation Affecting the LSU Assembly

The mtDNA-related disorders fall into two major groups: those due to mutations in genes encoding individual proteins of the respiratory chain, and those due to mutations in genes involved in mitochondrial protein synthesis (Chinnery, 2021). Among them, mutations in the mitochondrial translation apparatus, the mitoribosome structural components and assembly factors, cause mitochondrial diseases such as cardiomyopathy and protein synthesis deficiencies, as observed in the presented example of the mutation in MRPL3 (uL3m).

The current understanding is that numerous mitochondrial assembly factors are RNA-binding proteins that are associated with 12S and 16S rRNA. These proteins exhibit molecular chaperone activity and aid in the proper folding of the ribosomal RNA. Research indicates that these mitoribosome assembly factors are crucial for mitochondrial translation and are implicated in diseases related to mitochondrial translation disorders.

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The protein uL3m interacts extensively with the 16S rRNA and neighboring proteins bL17m, bL19m, bL32m, and mL39. Genetic mutations in uL3m are usually associated with myo-, cardio-, and encephalopathy — muscle, heart and brain dysfunction, respectively. A study examined four siblings with a large-scale deletion at the MRPL3 (uL3m) locus, leading to hypertrophic cardiomyopathy, failure to thrive, and liver dysfunction. Analysis of patient fibroblasts revealed reduced levels of 16S rRNA and proteins uL3m and bL12m, affecting protein synthesis and OXPHOS complex function. In a structural representation of uL3m in a human mitoribosome, a mutation occurs in a coiled region with disordered loops, which results in protein destabilization and alterations in the mt-LSU assembly. The uL3m mutation's variant position is 317, at which the residues change from Proline (P) to Arginine (R) (Ferrari et al., 2020), from the codon CCT to the codon CGT, respectively. The frequency of the alternation from Cytosine to Guanine in uL3m can be observed in the following table (dbSNP, 2022). It can be observed that the Alt. allele (found at the locus) Cytosine is present in the European population, with a 0.00007 result, while the rest of the sample size has the reference allele Guanine. The other populations examined, however, demonstrate Guanine as the reference allele, without any Cytosine found at the locus.

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Table 1

Allele Frequency

Population	Sample Size	Ref Allele	Alt Allele
Global	14050	G=0.9993	C=0.00007
European	9690	G=0.9999	C=0.0001
African	2898	G=1.0000	C=0.0000
African American	2784	G=1.0000	C=0.0000
African Others	114	G=1.000	C=0.000
Asian	112	G=1.000	C=0.000
Latin American 1	146	G=1.000	C=0.000
Latin American 2	610	G=1.000	C=0.000
Other	496	G=1.000	C=0.000
East Asian	86	G=1.00	C=0.00
Other Asian	26	G=1.00	C=0.00
South Asian	98	G=1.00	C=0.00

Furthermore, an alteration occurring in the 39S ribosomal protein L3 mitochondrial (Gene MRPL3: uL3m) will interfere with the rRNA stabilizing mechanism and the rRNA binding in the initial stage of LSU ribosomal assembly. This disruption in the mitoribosomal structure will subsequently impact the protein synthesis process in mitochondria, as mitoribosomes are key to driving the process successfully.

Conclusion

The importance of the mitoribosome and mitochondrial protein synthesis in the biomedical field has been recognized for many years. With current advancements in full-genome sequencing, identifying pathogenic alleles in mitoribosome components and assembly factors for patients with mitochondrial disorders has become a more achievable task. Through fundamental research, an insight into the pathogenic mechanisms of known factors can be gained and the new candidates linked to mitochondrial translation defects can be discovered through investigating the genetic causes of mitochondrial disorders. Furthermore, mitochondrial medicine offers opportunities to study the relationship between structure and function in subjects carrying mutations, which contributes to the understanding of mitoribosome biogenesis.

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