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Bridging the Gap between tRNA Modifications and the Respiratory Chain

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Mitochondria, the powerhouses of the cell, generate the majority of the ATP used in cells by oxidative phosphorylation (OXPHOS), a process catalyzed by the respiratory chain. Because of its complexity, OXPHOS is carefully orchestrated and requires a number of respiratory proteins encoded in mitochondria, making mitochondrial translation an essential process. The mitochondrion is the only organelle in mammalian cells outside the cytoplasm that has its own protein synthesis machinery. In addition to more than 1500 proteins that are synthesized in the cytoplasm and imported into the organelle, mitochondria also encode 13 essential peptides that form critical parts of the respiratory chain.[1,2] These respiratory chain proteins, together with a subset imported from the cytoplasm, play a role in the electron transport chain, and therefore ATP generation. Significantly, mutations in the electron transport chain are linked to many human diseases especially in tissues that utilize a lot of energy, such as the brain.[1,2]

The mammalian mitochondrion translation system has only 22 tRNAs to decode the 64 codons of the genetic code.[1] These tRNAs rely on post-transcriptional modifications to accurately decode the 42 remaining codons.[3] Within the mitochondria, tRNAs can be modified with 15 distinct modifications at 118 different positions on tRNAs.[1] In the case of tRNAs encoding the amino acids Leu, Glu, Gln, Lys, and Trp, the first position of the anticodon, U_{34}, is usually modified at the C-5 position of the base with taurine forming 5-taurinomethyluridine (tm^5U). Taurine addition requires precursor methylation, and without this, the addition of taurine is defective, which has been correlated with mitochondrial disease. Until recently, the carbon source for this methylation had not been identified.[4]

In a breakthrough study, Morscher et al.[5] identified the source of this modification using innovative biochemical techniques. Initially, the authors set out to determine the role of methylene-tetrahydrofolate (methylene-THF) in mitochondria. It has been known for some time that methylene-THF is exported from the mitochondrion to the cytosol for nucleotide synthesis; however, the role of THF within the mitochondrion had yet to be elucidated. Methylene-THF is generated by the enzyme SHMT2, which catabolizes serine to generate one-carbon units and transfers the carbon to THF. To elucidate the role of methylene-THF in the mitochondria, Morscher et al. began by deleting SHMT2 in HCT116 colon cancer cells, which then favors the use of glycolysis for ATP production through enhanced glucose uptake and lactate secretion. This was paired with reduced basal respiration, low respiratory capacity, decreased NAD^+/NADH ratios, and low levels of respiratory complex I, IV, and V proteins, which is indicative of a respiratory chain deficiency (Figure 1).

Curiously, this effect on the respiratory chain is specific to SHMT2, and gene deletions downstream of SHMT2 in mitochondrial 1C metabolism did not have the same deleterious effect. This led the authors to focus on the catalytic activity of SHMT2 and how it impacts the electron transport chain. As expected, introduction of a catalytically inactive protein did not rescue phenotypes associated with the SHMT2 deletion, indicating that the function of SHMT2 is critical for maintaining the respiratory chain. Methylene-THF, the product of SHMT2 catabolism, is responsible for producing deoxythymidine triphosphate (dTTP); therefore, SHMT2 could potentially be playing a broad role in DNA replication or having a more direct impact on nucleotide production. In mitochondrial 1C metabolism, methylene-THF can be converted by MTHFD2 into formyl-THF to generate f-Met, the amino acid used to initiate protein synthesis. Therefore, production of either dTTP or f-Met synthesis could both be critical in maintaining the respiratory chain. Deletion of MTHFD2 had no effect on the respiratory chain, indicating that dTTP production, and not f-Met generation, is essential for electron transport chain function. Mysteriously, upon examination of how DNA copy number and transcript levels are effected by SHMT2 deletion, no significant decrease was observed.

The authors then moved their search for the role of SHMT2 to mitochondrial protein synthesis. Overall, deletion of SHMT2 reduced protein levels in complex I and IV of the respiratory chain. To determine how SHMT2 deletion leads to a decreased rate of protein synthesis, Morscher et al. applied mitochondrial ribosome profiling enriching for 55S mitochondrial ribosomes. Protected mRNA footprints were sequenced to identify stalling sites on a particular message. This revealed that ribosomes were stalled at lysine and leucine codons, AAG and UUG, respectively. Interestingly, the decrease in the level of translation was not due to a decrease in amino acid availability or tRNA abundance but rather due to difficulty decoding the message. The 3′ guanosine specifically is problematic because it requires a methylene modification to allow wobble base pairing. Therefore, Morscher et al. set out to determine if there was a change in the modification state of tRNA. They established a liquid chromatography−mass spectrometry (LC−MS) method to detect modified tRNA bases and found that the tm^5U

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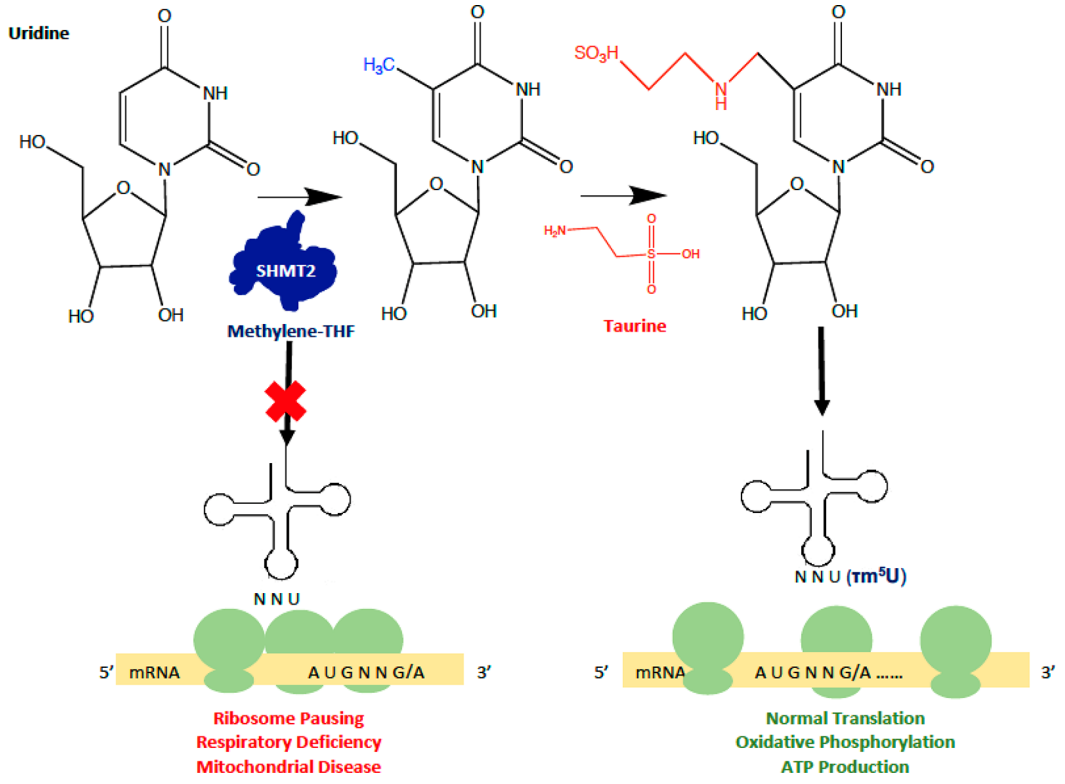

Figure 1. Stepwise tRNA modification of U^{34} and the repercussions of knocking out SHMT2 and not preforming the required precursor of tm^5U, methylated U^{34}.

modification was depleted in these SHMT2 knockout cells while other modifications were unaffected. They then went on to show that the methyl group at C5 of U_{34}, a forcible precursor to taurine addition, was provided by serine and therefore pausing is a direct consequence of SHMT2 deletion.

In addition to discovering the link among SHMT2, tRNA modifications, and the respiratory chain, these findings can be directly linked to human disease. MERRF and MELSA are human diseases linked to mutations in $tRNA^{Leu/Lys}$ that cause ribosome stalling. However, the extent of ribosome stalling in these diseases is reduced compared to that seen for the direct deletion of SHMT2 or the modification enzyme due to changes in the modification state of tRNA. Understanding how the catabolism of serine affects the mitochondrial translation system could provide insight into understanding disease progression and reveal potential targets for the development of new treatments. This work provides an understanding of how biological systems are closely linked and raises questions about how this may serve as a method for regulation of mitochondrial translation.

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