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How RNA modification allows non-conventional decoding in mitochondria

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Mitochondria are organelles of symbiotic origin that have retained a gene expression machinery during evolution. However, the large majority of the genes encoding mitochondrial proteins have been transferred to the nuclear genome, requiring cytoplasmic translation and mitochondrial import of about 1000 different mitochondrial proteins. In human cells, 13 proteins are encoded by the mitochondrial genome and their translation occurs on mitochondrial ribosomes in the matrix. While the mitochondrial ribosomal proteins and translation factors need to be imported from the cytoplasm, the 2 ribosomal (r)RNAs and the minimalistic set of 22 transfer (t)RNAs are encoded in the organelle and are transcribed by the mitochondrial RNA polymerase. In contrast to both the bacterial and the cytoplasmic translation systems, where separate tRNAs exist that mediate incorporation of methionine either during translation initiation or elongation, mitochondria contain only a single tRNA that facilitates incorporation of methionine during translation (Fig. 1). In addition, this single mitochondrial $tRNA^{Met}$ ($mt\text{-}tRNA^{Met}$) is employed to not only read the conventional AUG codon, but is also responsible for integration of methionine at AUA and AUU codons during translation initiation and at AUA codons during elongation, thereby playing a key role in implementing the non-conventional genetic code of mitochondria. It was previously suggested that RNA modifications in the anticodon of the $mt\text{-}tRNA^{Met}$ could expand its codon recognition, however, how the modifications are installed and which enzymes are involved had remained unknown.

In parallel with 2 independent studies, we found that cytosine 34 (C34) in the "wobble position" of $mt\text{-}tRNA^{Met}$ is methylated at position 5 of the pyrimidine ring by the RNA methyltransferase NSUN3 (Fig. 1).[1,2,3] This enzyme is a member of the Nol1/Nop2/SUN domain (NSUN) family, which also contains the RNA methyltransferases NSUN2 and NSUN6 that modify cytoplasmic tRNAs.[4,5] In contrast, NSUN3 localizes to the mitochondrial matrix where it specifically recognizes the anticodon stem loop (ASL) of $mt\text{-}tRNA^{Met}$. Interestingly, mutations that compromise basepairing in the ASL, including a pathogenic mutation, reduce C34 methylation by NSUN3, implying that lack of this modification in $mt\text{-}tRNA^{Met}$ can lead to disease.[1,2] This is further supported by Van Haute and colleagues, who describe a patient lacking functional NSUN3 and suffering from mitochondrial dysfunction. Interestingly, previous reports have suggested the presence of 5-formylcytosine (f^5C) at position 34 of $mt\text{-}tRNA^{Met}$, implying that the methyl group of m^5C installed by NSUN3 can be oxidised to generate the formyl group of f^5C. We have identified the Fe(II)/α-ketoglutarate-dependent dioxygenase, ALKBH1/ABH1 as the enzyme responsible for this oxidation (Fig. 1).[3] The related TET proteins, which oxidise m^5C in DNA, have been shown to form f^5C and 5-carboxycytosine (ca^5C) via a distributive mechanism that leads to accumulation of 5-hydroxymethylcytosine (hm^5C), as the first oxidation intermediate. In contrast *in vitro* and *in vivo* data imply that ABH1 primarily produces f^5C in $mt\text{-}tRNA^{Met}$. Cytosine 34 of $mt\text{-}tRNA^{Met}$ is almost fully modified *in vivo*,[1] however, the relative abundance of tRNAs carrying the different modifications at this position requires further clarification; while both mass spectrometry analysis of isolated $mt\text{-}tRNA^{Met}$ and bisulfite sequencing predominantly identified f^5C,[1,2,3] the presence of m^5C could also be detected,[1,3] suggesting that although the majority of $mt\text{-}tRNA^{Met}$ is oxidised by ABH1, a portion may remain in the methylated state. It has been discussed that the localization of ABH1 may differ between cell types, raising the possibility that the extent of oxidation of m^5C34 of $mt\text{-}tRNA^{Met}$ may also vary. Ribosome binding studies using differently modified forms of $mt\text{-}tRNA^{Met}$ (or the ASL) indicate that these modifications serve to expand codon recognition by $mt\text{-}tRNA^{Met}$, enabling this single methionine tRNA to fulfil its diverse functions in mitochondrial translation.[1,6] The importance of the increased decoding capacity of $mt\text{-}tRNA^{Met}$ generated by modification of C34 is highlighted by the requirement for NSUN3 and ABH1 for efficient mitochondrial translation *in vivo*.[1,2,3]

The newly identified 2-step modification pathway involving the m^5C RNA methyltransferase NSUN3 and the dioxygenase ABH1 explains how codon recognition by $mt\text{-}tRNA^{Met}$ is extended by RNA modifications at the “wobble position” of its

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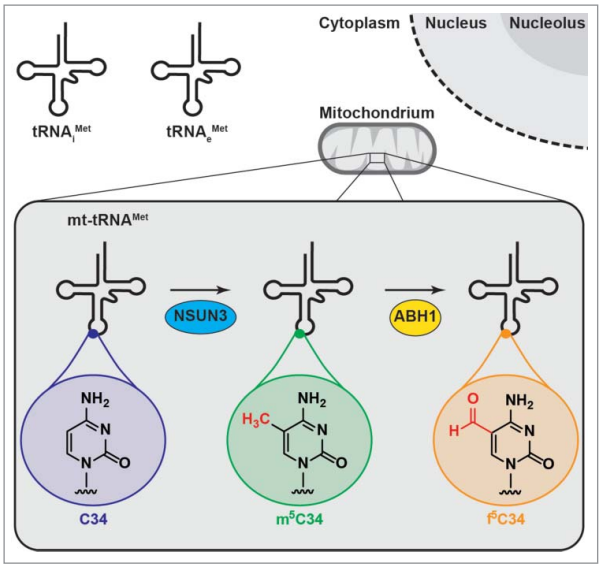

Figure 1. Wobble position modifications in the mitochondrial tRNA^{Met} expand codon recognition during translation. While cytoplasmic translation employs 2 different tRNA^{Met} for translation initiation (tRNA$_i^{Met}$) and elongation (tRNA$_e^{Met}$), mitochondria contain only one (mt-)tRNA^{Met}. Cytosine 34 (C34) of the mt-tRNA^{Met} can be methylated by the RNA methyltransferase NSUN3 to generate m^{5}C34, which can be further oxidised by the dioxygenase ABH1/ALKBH1 to 5-formylcytosine (f^{5}C34).

anticodon. This enables the single mt-tRNA^{Met} to mediate the incorporation of methionine on different codons and to act in both translation initiation and elongation in human mitochondria. Interestingly, such complex, multi-step modifications are also observed at the "wobble position" of other mitochondrial tRNAs and similarly function to alter codon recognition during mitochondrial translation (reviewed in ref.[7] and references therein). For example, the 5-taurinomethyluridine (τm^{5}U) modification at position 34 of mt-tRNA^{Trp}, mediated by GTPBP3 and MTO1, allows incorporation of tryptophan at the UGA codon, which is normally read as a stop codon by the cytoplasmic translation machinery. Analogous to NSUN3, mutations in both these enzymes have been shown to cause mitochondrial dysfunction. Therefore, RNA modifications at key positions in the anticodon emerge as important features that expand codon recognition by specific tRNAs and thereby enable use of the minimalistic mitochondrial translation system. Furthermore, these findings add to the growing body of evidence for genetic diseases that are caused by a lack of tRNA modifications or compromised mitochondrial function.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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