Mechanism of methyltransferase METTL8-mediated mitochondrial RNA m3C modification and its relaxed substrate specificity

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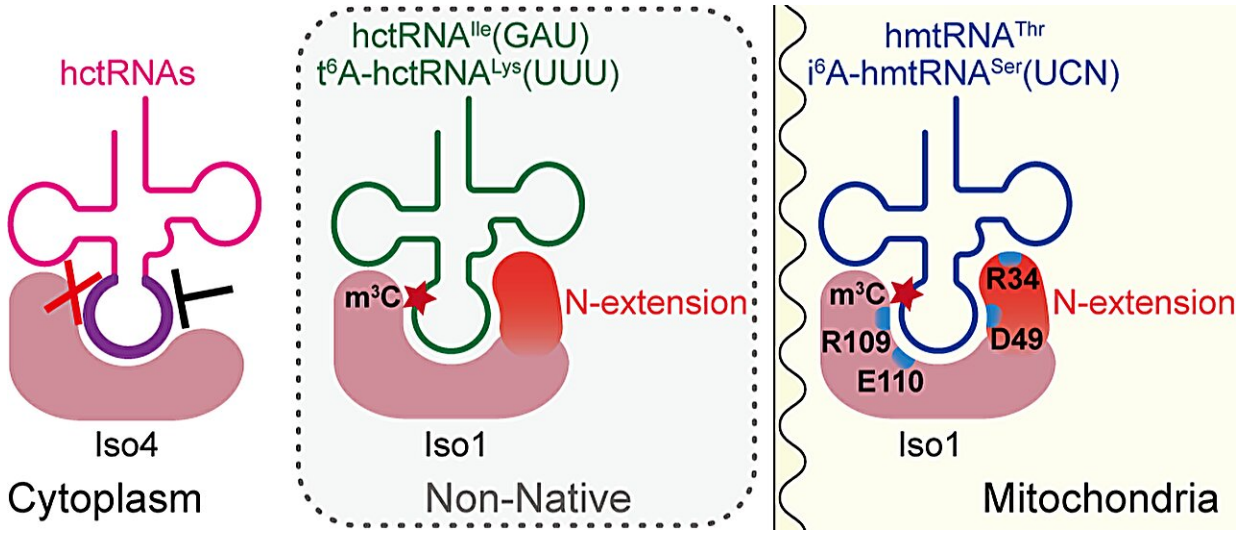

N-extension of METTL8-Iso1 is critical in m3C32 biogenesis while METTL8-Iso4 is inactive in m3C32 modification activity due to absence of the N-extension. METTL8-Iso1 exhibited a relaxed tRNA substrate specificity to modify several cytoplasmic or even bacterial tRNAs. Credit: Science China Press

A study published in the journal *Science Bulletin* was led by Profs. Xiao-Long Zhou and En-Duo Wang (CAS Center for Excellence in Molecular

Cell Science, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences).

tRNA is a key adaptor molecule in mRNA translation. There are a large number of post-transcriptional modifications on tRNA, which regulate the speed and fidelity of protein synthesis. 3-Methylcytosine (m^3C) modification is widely found at position 32 (m^3C32) of the anticodon loops of several cytoplasmic and mitochondrial tRNAs in eukaryotes.

A previous study by the same lab has found that the m^3C32 modification of human cytoplasmic tRNAs was mediated by METTL2A/2B and METTL6, while that of human mitochondrial $tRNA^{Thr}$ ($hmtRNA^{Thr}$) and $tRNA^{Ser}(UCN)$ ($hmtRNA^{Ser}(UCN)$) is catalyzed by METTL8; Human *METTL8* generates two protein isoforms of different lengths by alternative splicing of mRNA.

The long-length form, METTL8-Iso1, was targeted into mitochondria to catalyze the m^3C32 modification of $hmtRNA^{Thr}$ and $hmtRNA^{Ser}(UCN)$; while the short-length form, METTL8-Iso4, was located in the nucleolus with unknown function.

The only difference between the two isoforms is a 28-amino acid N-terminal extension peptide in METTL8-Iso1. Whether METTL8-Iso4 has m^3C32 methyltransferase activity and the role of the N-terminal extension of METTL8-Iso1 in mitochondrial tRNA m^3C32 modification is unknown.

It is also unclear whether cytoplasmic or mitochondrial m^3C32 modification enzymes can cross-recognize tRNAs from different cellular compartments. In addition, since most tRNA m^3C32 modifications require N^6-threonylcarbamoyl adenosine modification at position 37 (t^6A37) in the anticodon loop as a prerequisite, the preparation of tRNA molecules containing only m^3C32 modification has not been fully

achieved.

To address these questions, the researchers confirmed the conservation of the N-terminal extension (N-extension) of METTL8-Iso1 through multiple sequence alignment. In vitro enzyme activity determination revealed that METTL8-Iso4 had no m^3C32 modification activity. They further proved that the N-extension of METTL8-Iso1 acted as a key tRNA-binding element in the catalytic process.

Two completely conserved amino acid residues in all METTL2A/2B/8 proteins were identified. METTL8-Iso1 was able to mediate m^3C32 modification for both cytoplasmic and *E. coli* tRNAs, which was not reliant on t^6A37.

However, cytoplasmic m^3C32 modification enzymes METTL2A and METTL6 were unable to catalyze m^3C32 modification of mitochondrial tRNA, indicating that METTL8-Iso1 has a more relaxed substrate specificity. The m^3C32 modification did not affect the t^6A37 modification and aminoacylation levels of $hmtRNA^{Thr}$.

Finally, they also revealed that METTL8-Iso1 interacted with mitochondrial seryl-tRNA synthetase (SARS2) and mitochondrial threonyl-tRNA synthetase (TARS2), respectively, and significantly promoted aminoacylation activity of SARS2 and TARS2.

In summary, this work reveals the molecular mechanism of mitochondrial tRNA m^3C32 biogenesis mediated by METTL8, which relies on a specific N-extension as a key RNA-binding element. METTL8 had a broad spectrum of heterogenous tRNA substrates, which provided a basis for preparation of tRNAs containing only a m^3C moiety. This work provides a comprehensive understanding of the conservation and difference between cytoplasmic and mitochondrial tRNA m^3C modification.

More information: Meng-Han Huang et al, Mitochondrial RNA m3C methyltransferase METTL8 relies on an isoform-specific N-terminal extension and modifies multiple heterogenous tRNAs, *Science Bulletin* (2023). DOI: 10.1016/j.scib.2023.08.002

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