Discovery of a tRNA modification enzyme that also acts on nucleosides

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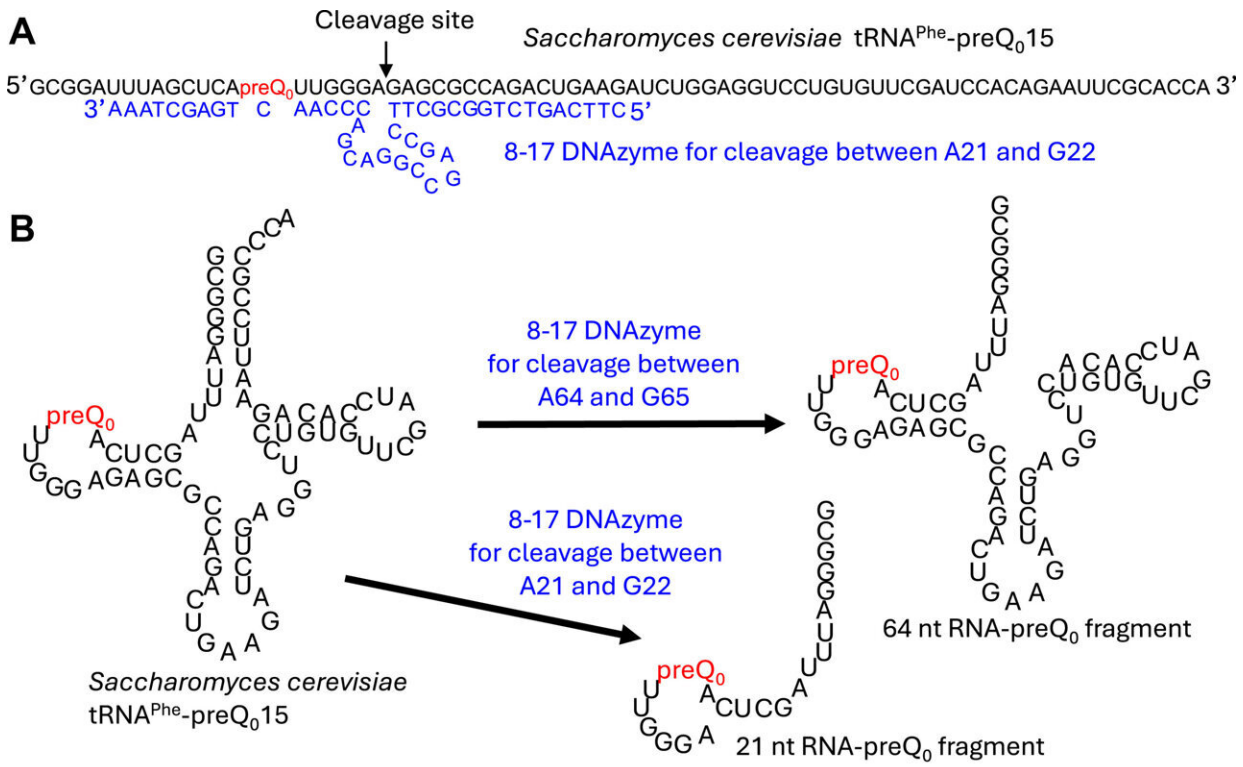

Fig. 1. ArcS does not require the L-shaped tRNA structure for the lysine-transfer reaction. Credit: *Journal of Biological Chemistry* (2024). DOI: 10.1016/j.jbc.2024.107505

The genetic information on DNA is transcribed into messenger RNA (mRNA) and translated to the amino acid sequence by transfer RNA

(tRNA) on the ribosome. Modified nucleosides within RNA are involved in maintaining and regulating the protein synthesis system.

Archaeosine is a modified nucleoside found only in the tRNAs from archaea, the so-called third domain of life, and contributes to the maintenance of the L-shaped tRNA three-dimensional structure.

The synthesis of archaeosine involves multiple steps, with the first step introducing a preQ$_0$ base into tRNA via ArcTGT. In the second step, ArcS transfers an amino acid, lysine, to the preQ$_0$ base in tRNA and synthesizes preQ$_0$-Lys as an intermediate. The resultant preQ$_0$-Lys in tRNA is then converted into archaeosine by RaSEA, the third-step enzyme.

This synthesis pathway of archaeosine was elucidated in 2019 through a collaborative study by Ehime University and Gifu University published in *Nature Chemical Biology*. However, the substrate specificity of the second-step enzyme ArcS was previously unknown.

To address this issue, a research group led by Professor Hiroyuki Hori, Lecturer Dr. Ryota Yamagami, and graduate students Shu Fujita, Yuzuru Sugio, and Dr. Takuya Kawamura (currently at Thomas Jefferson University, U.S.) at the Graduate School of Science and Engineering, Ehime University, in collaboration with Professors Takashi Yokogawa and Natsuhisa Oka from Gifu University and Associate Professor Akira Hirata from Tokushima University, conducted biochemical analyses.

The results of the study are published in the *Journal of Biological Chemistry*.

Most RNA modification enzymes recognize the three-dimensional structure around the target site of RNA and only rarely the RNA sequence itself. To investigate the substrate RNA specificity of ArcS,

preQ$_0$-modified tRNA was fragmented using DNAzymes, and lysine transfer was assessed for each fragment (see Fig. 1 above).

Surprisingly, ArcS transferred lysine to all RNA fragments containing preQ$_0$. In the 21-nucleotide (21 nt) RNA fragment, not only the whole tRNA structure, but also the D-arm structure was disrupted. This result demonstrates that ArcS does not recognize the three-dimensional structure of substrate RNA.

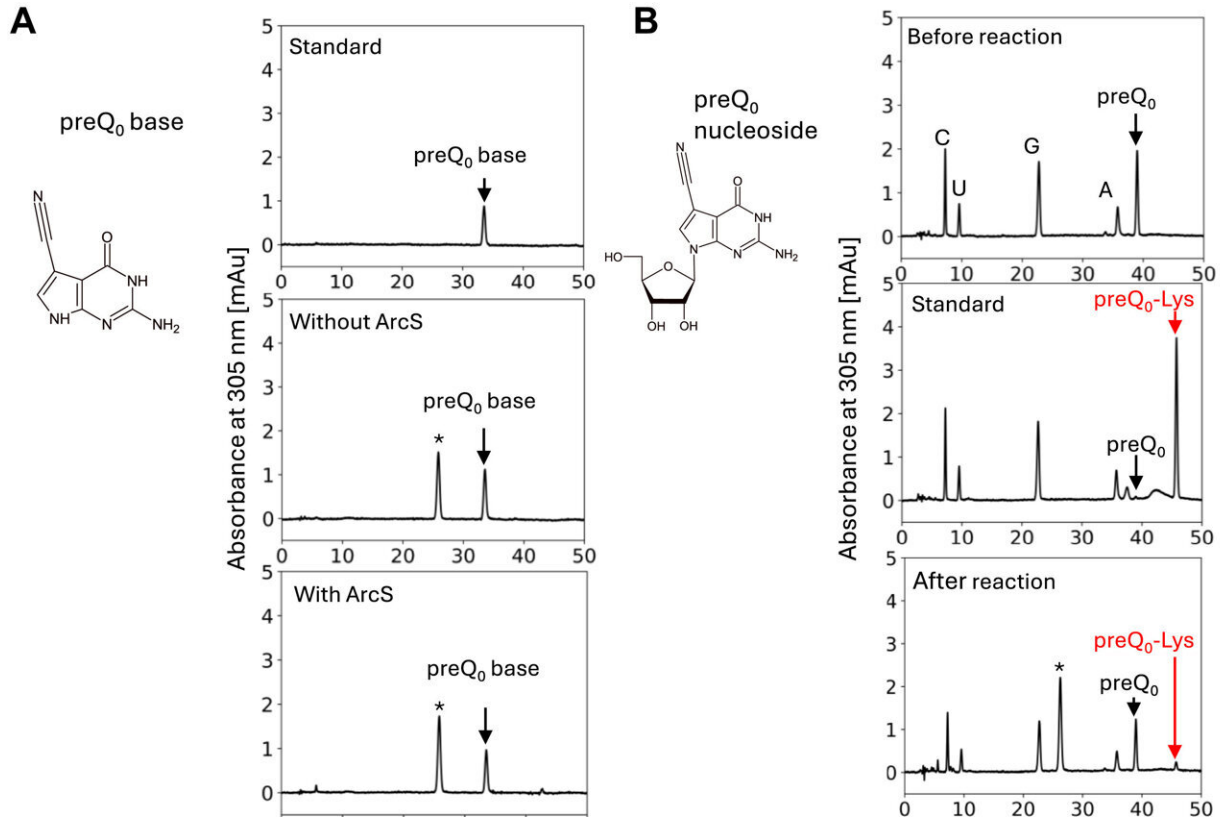

Fig. 2. preQ0 nucleoside and its derivatives are the substrates of ArcS. Credit: *Journal of Biological Chemistry* (2024). DOI: 10.1016/j.jbc.2024.107505

To identify the minimum substrate, lysine-transfer was assessed using

the $preQ_0$ base, $preQ_0$ nucleoside, 5'-phosphorylated $preQ_0$ nucleotide, and 3'-phosphorylated $preQ_0$ nucleotide (Fig. 2).

It was found that the minimum substrate was the $preQ_0$ nucleoside, with reaction efficiency increasing when a phosphate group was attached to the 5' position. Thus, ArcS is an unprecedented tRNA modification enzyme that can act on a nucleoside as the substrate.

With the development of mRNA vaccines for COVID-19, modified nucleosides like pseudouridine and 1-methylpseudouridine are effectively used, and research on introducing various modifications into target RNAs is being conducted globally. The discovery of ArcS, which can utilize a nucleoside as a minimum substrate, provides new insights into the synthesis of precursor molecules for these modified nucleosides.

More information: Shu Fujita et al, ArcS from Thermococcus kodakarensis transfers L-lysine to preQ0 nucleoside derivatives as minimum substrate RNAs, *Journal of Biological Chemistry* (2024). DOI: 10.1016/j.jbc.2024.107505

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