Biosynthesis of tRNA thiouridine in *Thermus thermophilus* and its evolutionary relationship with eukaryotic ubiquitin system

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Post-transcriptional RNA modifications, a characteristic structural feature of RNA molecules, play critical roles in biogenesis, metabolism, structural stability, and function of RNA molecules. To date, more than 100 species of RNA modifications have been reported (http://biochem.ncsu.edu/RNAmods/). Most of these modifications have been identified and characterized in transfer RNA (tRNA) molecules. These are required for several functions in translation: codon recognition, maintenance of reading-frame, stabilization of tertiary structure, and serving as identity elements recognized by the translational apparatus. In particular, 2-thiouridine (s^2U) functions in codon recognition in almost all organisms and in stabilization of the tertiary structure of tRNAs in some thermophiles [Fig. 1]. The biosynthesis pathways of these thiouridine have been partly unraveled by recent studies. For example, in the biosynthesis of 2-thiouridine at anticodon (s^2U34) of $tRNA^{Glu}$, $tRNA^{Gln}$, and $tRNA^{Lys}$ in *E. coli*, the sulfur atom of cysteine is activated by cysteine desulfurases as persulfide (R-SSH) and the persulfide sulfur is transferred to specific sulfur-carrier proteins and a modification enzyme which incorporates the sulfur into tRNA [1].

Here, we identified another type of biosynthesis pathway for s^2U derivative, 2-thioribothymidine (s^2T, also called as 5-methyl-2-thiouridine, m^5s^2U) at position 54 of tRNAs of some thermophilic organisms such as *Thermus thermophilus* and *Pyrococcus furiosus* [2] [Fig. 1]. The rigid conformation of s^2T stabilizes the D loop-T loop interaction in tRNA, conferring a thermostable tertiary structure to the tRNAs. The 2-thiolation of s^2T54 is a partial modification, and the ratio of 2-thio modification increases along with elevation of the cultivation temperature [3]. Therefore, the translational apparatus adapts higher temperatures.

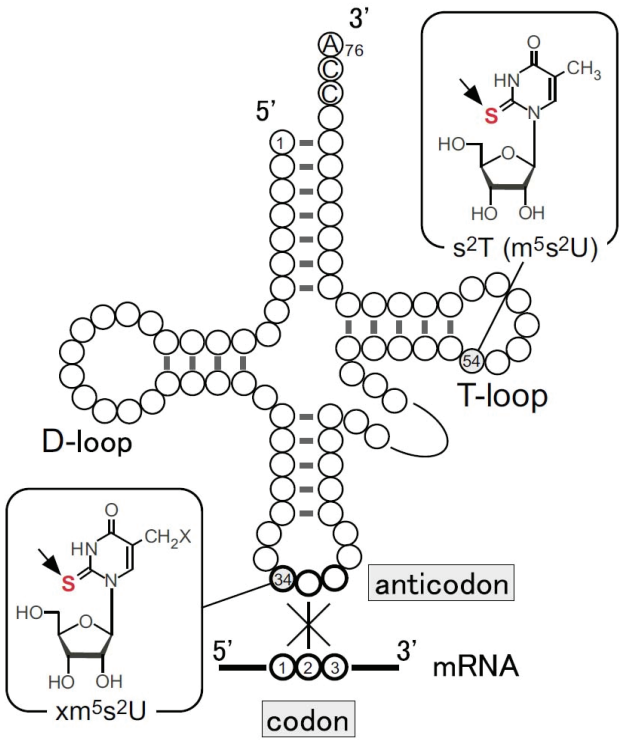

Fig.1 Chemical structures of 2-thiouridine derivatives and their positions in tRNA.

First, we identified five proteins required for the 2-thiolation of s^2T in *T. thermophilus* (cysteine desulfurase (IscS or SufS), tRNA-two-thiouridine A (TtuA), TtuB, and TtuC), and examined *in vitro* s^2T formation [4-6] [Fig. 2A, C]. TtuB is activated as an acyl-adenylate by TtuC and thiocarboxylated

by cysteine desulfurases. The sulfur atom of TtuB-COSH is then transferred to tRNA by TtuA. The sulfur-carrier protein TtuB may have evolved to deliver reactive sulfur atoms to specific target and avoid nonspecific transfer of activated sulfur atoms, which could inactivate other biomolecules. We also found that s^2T deficient strain could not grow over 80 ^oC, indicating that 2-thio modification of tRNAs is indispensable for growth at high temperatures. In a *ttuC* mutant of *T. thermophilus*, not only s^2T, but also sulfur-containing cofactors (molybdenum cofactor and thiamin) were not synthesized, suggesting that TtuC is shared among these biosynthetic pathways. This finding implies the tight and evolutionary relationship between these pathways.

TtuB is estimated to have ubiquitin fold structure [Fig. 2A, B] and TtuC possesses homologous sequence with ubiquitin activating enzyme (E1). Ubiquitin is acyl-adenylated by E1 and covalently linked to E1 via thioester bond [Fig. 2D]. Finally ubiquitin is attached to a lysine residue in the target protein by E2 and E3 enzymes. We confirmed that TtuB is also acyl-adenylated by TtuC and TtuB-TtuC thioester is formed *in vitro*. These results imply the evolutionary relationship between the biosynthesis pathways of sulfur-compounds in eubacteria and ubiquitin system of eukaryote. Now we are investigating the possibility that TtuB is attached to the target proteins in *T. thermophilus* like ubiquitin in eukaryote.

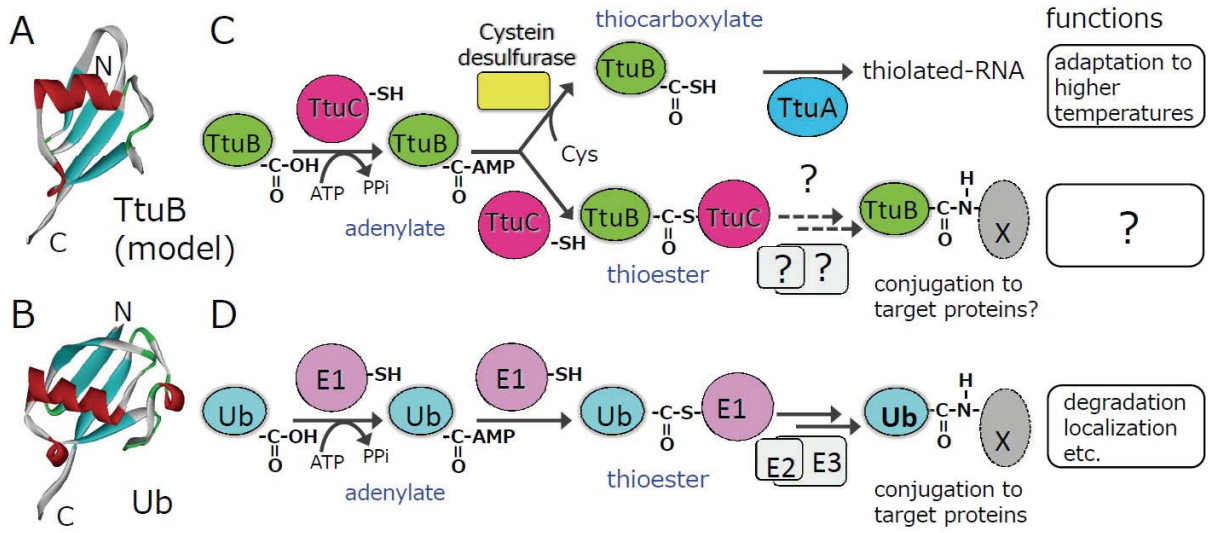

Fig. 2 Comparison of the s^2U biosynthesis and eukaryotic ubiquitin system.
A. structural model of *T. thermophilus* TtuB. **B**. structure of yeast ubiquitin.
C. the biosynthesis pathway of s^2U in eubacteria (*T. thermophilus*). **D**. ubiquitin system in eukaryote.

References

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