X-Ray Structure of the tRNA Methyltransferase Complex of Trm7 and Trm734 from *Saccharomyces cerevisiae*

Transfer RNA (tRNA) methylation, mediated by tRNA methyltransferases, is crucial for efficient and accurate protein synthesis. The tRNA methyltransferase complex between Trm7 and Trm734 (Trm7–Trm734) from *Saccharomyces cerevisiae* catalyzes 2´-*O*-methylation at position 34 in tRNA using S-adenosyl-L-methionine (SAM). Here, we determined the crystal structures of Trm7–Trm734 with and without SAM, revealing the details of the bipartite interaction between Trm7 and Trm734. Small-angle X-ray scattering analysis demonstrated that Trm7–Trm734 exists as a heterodimer in solution. These structure-guided biochemical analyses provide novel insights into the tRNA recognition mechanism of Trm7–Trm734.

Transfer RNA (tRNA) generally serves as an adapter molecule that interprets the codon of messenger RNA (mRNA) and correctly transfers an amino acid corresponding to the codon into the polypeptide chain during protein synthesis on ribosomes. Primary tRNA transcripts must undergo maturation events such as processing, splicing and post-transcriptional modification because only the mature tRNA is required for biological function. The highly conserved post-transcriptional tRNA modifications are found in all three domains of life and contribute to structural integrity, stability, decoding fidelity and efficiency during mRNA translation. More than 100 nucleoside modifications have been reported in the RNA modification database to date (see http://mods.rna.albany.edu/mods/), most of which have been found in tRNA molecules [1]. tRNA methylation is one of the most abundant modifications. The 2´-*O*-methylation in tRNA anticodon is generally observed, however, completely different types of tRNA methyltransferases function in the modifications among the three domains of life. In the case of *Saccharomyces cerevisiae*, the modification site targeted by Trm7 switches dependent on its partner subunit: a complex of Trm7 and Trm732 (Trm7–Trm732) catalyzes 2´-*O*-methylation at position 32 in tRNA using S-adenosyl-L-methionine (SAM), and a complex of Trm7–Trm734 catalyzes 2´-*O*-methylation at position 34 (Nm34) in tRNA with SAM [2]. Furthermore, it has been reported that *trm7*-gene deletion mutant strains of *S. cerevisiae* and *Schizosaccharomyces pombe* caused constitutive activation of general amino acid control response and that mutations in human FTSJ1 (ortholog of Trm7) cause non-syndromic X-linked intellectual disability [3]. Likewise, the strain containing an Alanine 26 to Proline mutation in *S. cerevisiae* Trm7 (*trm7*–A26P) shows defects in Nm34 methylation, but these are not due to the lower interaction between Trm7 and Trm734 [4]. Therefore, the structure of Trm7–Trm734 complex needs to be determined to elucidate the molecular mechanism of the bipartite interaction essential for tRNA recognition and 2´-*O*-methylation.

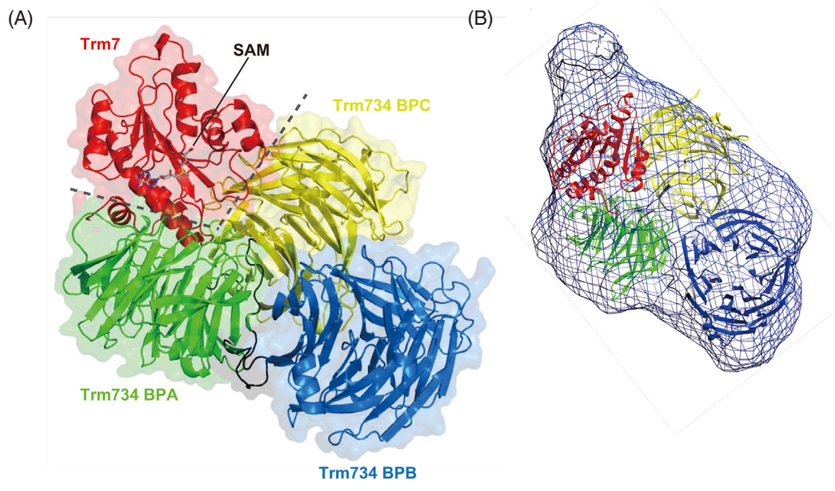

Figure 1: (A) Overall structure of Trm7–Trm734 in complex with SAM (stick model). Trm7 is colored red. The three domains of Trm734 (BPA, BPB and BPC) and linker regions are colored green, blue, yellow and black, respectively. The BPA and BPC form a V-shaped cleft for interacting with Trm7 (dotted line) **(B)** Overlay of the envelope shape (blue-mesh) of Trm7–Trm734 calculated with DAMMIF using the SAXS data and the CORAL model of Trm7–Trm734 based on its X-ray structure.

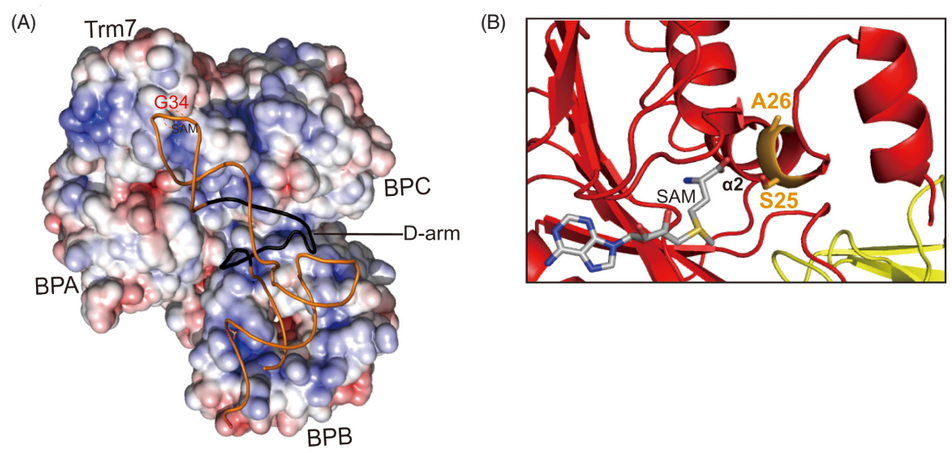

Figure 2: (A) Surface model of Trm7–Trm734 colored according to the electrostatic potential represented as a gradient from negative (red) to positive (blue). *Saccharomyces cerevisiae* $tRNA^{Phe}$ is placed along the distribution of the positively charged surface of Trm7–Trm734–SAM. The line diagram of *S. cerevisiae* $tRNA^{Phe}$ is colored orange. The D-arm of *S. cerevisiae* $tRNA^{Phe}$ is colored black. **(B)** Close-up view of the peripheral structure of α helix (α2) and SAM in Trm7. The residues S25 and A26 are shown as stick models (orange). SAM is illustrated as a stick model.

To reveal the tRNA recognition mechanism of Trm7–Trm734, we determined the crystal structure of Trm7–Trm734 in complex with SAM [**Fig. 1(A)**] [5]. Trm7 forms a heterodimer with Trm734. We also performed small-angle X-ray scattering analysis to confirm the heterodimeric structure of Trm7–Trm734 in solution at BL-10C, showing the heterodimer of the Trm7–Trm734 complex structure in solution as well as in the crystal [**Fig. 1(B)**]. The SAM is bound to the catalytic pocket of Trm7. The structural motif of Trm7 is a classical Rossmann fold, belonging to the class I type of SAM-dependent RNA methyltransferases. The structure of the C-terminal region of L260–V310 could not be built because the region was structurally disordered. Trm734 consists of three domains (BPA, BPB, and BPC), and each domain is connected by three linker regions and only the BPA and BPC domains directly interacting with Trm7. The BPA, BPB, and BPC domains are WD40 domains consisting of a seven-bladed β-propeller fold, each of the WD40 blades being composed of a four-stranded antiparallel β-sheet. Trm7 interacts with Trm734 via the V-shaped cleft of BPA and BPC [**Fig. 1(A)**], which is the most important element for the recognition of Trm7. Our mutational analysis suggests that the C-terminal region (N233–A256) of Trm7 is essential for the interaction between Trm7 and Trm734.

Next, we measured the methyl group acceptance activity of four truncated mutants in *S. cerevisiae* $tRNA^{Phe}$ transcripts, and found that the D-arm of *S. cerevisiae* $tRNA^{Phe}$ is important for tRNA recognition by Trm7–Trm734. Based on this result and those of previous reports, we manually placed the structure of *S. cerevisiae* $tRNA^{Phe}$ on Trm7–Trm734 with SAM [**Fig. 2(A)**]. The Trm734 BPB domain mainly recognizes the structural parts of the aminoacyl-stem and D-arm of *S. cerevisiae* $tRNA^{Phe}$, and Trm7 further recognizes the anticodon loop of tRNA with a methylation site of G34, which is adjacent to the bound SAM molecule. Trm734 BPC seems to slightly recognize the anticodon loop. To further clarify the details of the tRNA recognition mechanism, it is necessary to determine the structure of Trm7–Trm734 in complex with tRNA. On the other hand, our structure-guided mutagenesis suggests that the A26P of human FTSJ1 may induce the conformational change of α2 helix [**Fig. 2(B)**], as in its Trm7 counterpart. Therefore, the result may shed light on the cause of the defective Nm34 modification, because there is a striking amino acid sequence identity between *S. cerevisiae* Trm7 and human FTSJ1.

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