Structural and functional characterization of the TYW3/Taw3 class of SAM-dependent methyltransferases

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ABSTRACT

S-adenosylmethionine (SAM)-dependent methyltransferases regulate a wide range of biological processes through the modification of proteins, nucleic acids, polysaccharides, as well as various metabolites. TYW3/Taw3 is a SAM-dependent methyltransferase responsible for the formation of a tRNA modification known as wybutosine and its derivatives that are required for accurate decoding in protein synthesis. Here, we report the crystal structure of Taw3, a homolog of TYW3 from Sulfolobus solfataricus, which revealed a novel α/β fold. The sequence motif (S/T)xSSCxGR and invariant aspartate and histidine, conserved in TYW3/Taw3, cluster to form the catalytic center. These structural and sequence features indicate that TYW3/Taw3 proteins constitute a distinct class of SAM-dependent methyltransferases. Using site-directed mutagenesis along with in vivo complementation assays combined with mass spectrometry as well as ligand docking and cofactor binding assays, we have identified the active site of TYW3 and residues essential for cofactor binding and methyltransferase activity.

Keywords: wybutosine; tRNA; methyltransferase; TYW3; structural biology

INTRODUCTION

Methyl transfer reactions are ubiquitous in biology. They contribute to the biosynthesis of numerous essential cellular metabolites and clinically relevant small molecules and regulate other processes through the modification of biological macromolecules including DNA, RNA, proteins, polysaccharides, and lipids (Markham 2010). The majority of methyltransferases use S-adenosyl-L-methionine (SAM) as a methyl donor (Kozbial and Mushegian 2005). SAM-dependent methyltransferases can be divided into eight classes, based on their unique sequence and structural features (Schubert et al. 2003; Kozbial and Mushegian 2005; Kaminska et al. 2010; Kimura et al. 2014). The largest classes contain a Rossman fold (class I) or a SET domain (class V) (Kozbial and Mushegian 2005).

Post-transcriptional modifications are unique structural features of RNA molecules. To date, more than 100 types of modified nucleosides including methylation of bases and riboses have been found in various RNA molecules from all domains of life (Machnicka et al. 2013). About 80% of them are found in tRNAs. A wide variety of chemical modifications are found in anticodon regions of tRNAs and play critical roles in proper recognition of codons on the ribosome during protein synthesis. Aberrant tRNA modifications are associated with human diseases such as mitochondrial diseases and cancer (Kirino and Suzuki 2005; Kirino et al. 2005; Pathak et al. 2005; Guy et al. 2015; Shaheen et al. 2015), indicating that RNA modifications ensure proper functions of RNA molecules to maintain higher-ordered biological processes.

Wyosine (imG) and its derivatives such as wybutosine (yW) are hypermodified guanosines. They are found at position 37, 3′ adjacent to the anticodon of tRNA in eukarya and archaea (Thiebe and Poralla 1973; Altwegg and Kubli 1979; Keith and Dirheimer 1980; Bruce and Uhlenbeck 1982). yW stabilizes codon-anticodon base-pairing and ensures accurate translation of phenylalanine codons (Konevega et al. 2004). Early investigation of tRNA^Phe in mouse and rat revealed that many tumors are partially defective in yW synthesis, which leads to the production of immature forms of the base (Mushinski and Marini 1979). Hypomodification of yW in tRNA^Phe is known to induce −1 frameshifting (Carlson

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et al. 1999), and influence cancer pathogenesis and expression of viral proteins from human immunodeficiency viruses (Hatfield et al. 1989).

Biogenesis of yW was extensively studied in Saccharomyces cerevisiae. yW is synthesized via multistep enzymatic reactions mediated by five enzymes, TRM5, TYW1, TYW2, TYW3, and TYW4 (Fig. 1A; Noma et al. 2006; Kimura and Suzuki 2015). In the first step, TRM5 methylates G37 of tRNA•Phe in the presence of SAM to form 1-methylguanosine (m1G). TYW1, a SAM enzyme containing a radical center, which coordinates an iron–sulfur cluster, catalyzes ring formation of the tricyclic purine base on tRNA by condensation of m1G with two carbons of pyruvate to form imG-14. Then, TYW2 transfers an α-amino-α-carboxypropyl group from SAM to imG-14 forming 7-aminocarboxypropyl-demethylwyosine (yW-86). TYW3 methylates the N4 position of yW-86 in the presence of SAM to form yW-72. Finally, TYW4 catalyzes both methylation and methoxycarbonylation of yW-72 using two SAMs.

A Eukaryotes

B Sulfolobus

FIGURE 1. Wybutosine biosynthetic pathway in eukaryotes (A) and wyosine biosynthetic pathway in Sulfolobus species (B). The steps catalyzed by StTYW3 and the steps proposed to be catalyzed by SrTaw3 are shown within the gray boxes. 2-Oxoglutarate is abbreviated as 2-OG.
and one bicarbonate to synthesize yW (Suzuki et al. 2009). In mammals and several species of fungi, the β-carbon of the side chain in yW is further hydroxylated to form OHyW. TYW5, a Jumonji C domain-containing protein, catalyzes hydroxylation of yW-72 to form OHyW-72, followed by conversion to OHyW mediated by TYW4 (Noma et al. 2010).

In several archaeal species, wyosine (imG) derivatives have been found along with identification of TYW homologs by phylogenetic distribution analysis (McCloskey et al. 2001; Zhou et al. 2004; Noma et al. 2006; de Crecy-Lagard et al. 2010). TYW1-3 homologs called Taw1-3 are widely distributed in euryarchaea, whereas no ortholog of TYW4 is found in all archaea, suggesting that yW-86 and yW-72 are final products in tRNAs from several archaeal species (de Crecy-Lagard et al. 2010). In crenarchaea including *Sulfolobus* species, Taw1 and Taw3 are present, whereas no Taw2 is found, indicating that imG2 and mimG are the major wyosine derivatives (Fig. 1B).

Structural studies of tRNA-modifying enzymes in this pathway have significantly deepened our mechanistic understanding of yW formation (Bjork et al. 2001; Suzuki et al. 2007, 2009; Umitsu et al. 2009). The crystal structures have been published for TRM5, TYW1, TYW2, TYW4, and TYW5 (Goto-Ito et al. 2007; Suzuki et al. 2007, 2009; Umitsu et al. 2009; Kato et al. 2011), but not for TYW3/Taw3. Here we used X-ray crystallography to determine the structure of the Taw3 methyltransferase from *Sulfolobus solfataricus* (*Ss*Taw3) at 2.8 Å resolution. The structure revealed a novel α/β fold, which does not belong to any other class of SAM-dependent methyltransferases. Thus, Taw3 represents the founding member of the ninth class of SAM-dependent methyltransferases. It contains a mixture of α-helix and β-sheet (Fig. 2A). Two four-stranded antiparallel β-sheets line the inside of the concave front facing surface with five α-helices forming the back of the molecule (Fig. 2B). Strands 2, 3, 4, and 8 along with helices 3 and 5 comprise a RAGNYA fold domain (Balaji and Aravind 2007). The RAGNYA fold is an α/β fold composed of four strands and two helices packed against one face, which mediates critical interactions between proteins and a diverse set of ligands including nucleic acids, nucleotides, and other proteins (Balaji and Aravind 2007). Strands 5, 6, and 7 and helix 4 form an SHS2 fold domain, which is a simple modular domain that is named after its strand–helix–strand–strand (SHS2) configuration and is involved in the asymmetric unit.

**RESULTS**

**Overall structure**

We solved the X-ray crystal structure of *Ss*Taw3, a homolog of yeast TYW3 methyltransferase, to a resolution of 2.8 Å using the MAD method (Fig. 2A). The summary of the data collection and refinement statistics is shown in Table 1. The final structure contains 188 residues (4–192 of the expected 213 residues), three sulfates, and 104 water molecules. The first three N-terminal residues and C-terminal residues 192–213 are completely disordered. There are two copies of *Ss*Taw3 with domains colored as in A.

**FIGURE 2.** Structure of *Ss*Taw3. (A) Crystal structure of *Ss*Taw3, left, and zoom-in view of the crystal structure with conserved residues shown as sticks, left inset. The N-terminal extension, RAGNYA fold, and SHS2 fold domains are colored red, blue, and yellow, respectively. (B) Topology diagram of *Ss*Taw3 with domains colored as in A.
in a variety of functions ranging from protein–protein interactions to small-molecule recognition and catalysis (Anantharaman and Aravind 2004). The remaining helices 1 and 2 and sheet 1 constitute an N-terminal extension that is conserved in Taw3 proteins.

Although structural alignment did not return any published structures, the Dali server (Holm and Park 2000) did identify three structural homologs that had been deposited by structural genomics groups after our structure was deposited in the PDB database: (i) hypothetical protein APE0816 from *Aeropyrum pernix* (Z-score = 19.7; RMSD = 1.56 Å; PDB ID 2DVK), (ii) PH1069 from *Pyrococcus horikosii* (Z-score = 21.4; RMSD = 2.01 Å; PDB ID 2IT2), and (iii) AF2059 from *Archaeoglobus fulgidus* (Z-score = 22.0; RMSD = 2.66 Å; PDB ID 2QG3). All three share the same domain architecture and overall fold as SsTaw3 (Supplemental Fig. S1).

**Sequence analysis**

In addition to unique structural characteristics, methyltransferase classes can also be distinguished based on conserved sequence features (Kozbial and Mushegian 2005). The hallmark of SAM-binding Rossmann-like fold methyltransferases is a GxGxG consensus sequence, which forms part of the SAM binding site. SAM-radical methyltransferases can be recognized by the hallmark CxxxCxxC motif near their N terminus followed by a GG motif. SET domain methyltransferases can be identified by two signature motifs, ELxF/YDY and NHS/CxxPN. We performed sequence analysis using 150 sequences of TYW3 enzymes. Interestingly, we did not uncover any sequence motifs that are conserved in other classes of methyltransferases. Instead our sequence analysis revealed eight absolutely conserved residues in the TYW3 protein family: an aspartate located on the N-terminal extension, a histidine found at the end of strand 3 in the RAGNYA fold domain, and the motif (S/T)xSScxGR, which spans the junction between the SHS2 and RAGNYA fold domains (residues Asp25, Ser47, Cys48, Gly50, Arg51, and His73 in SsTaw3) (Fig. 2A, inset; Fig. 3). In addition to the novel fold, these conserved sequence elements are defining characteristics of this class of methyltransferase. The conservation and polar nature of these residues also suggests a critical role for these residues in substrate binding and catalysis. Interestingly, despite being positioned distantly in the primary sequence of the enzyme, these eight residues cluster in all of the crystal structures of the TYW3 family and form the putative active site for TYW3 enzymes (Fig. 2A, inset; Fig. 3). It has been postulated that the single absolutely conserved cysteine forms a covalent intermediate with the methyl group from SAM prior to being transferred to the target nitrogen atom on the yW precursor (Balaji and Aravind 2007), which would represent a novel methyltransferase mechanism. This intermediate would resemble the stable side chain of methionine. Another possibility is that the cysteine might form a covalent intermediate
with one of the carbons within the nucleoside rings, which facilitates nucleophilic catalysis. This type of mechanism is utilized by many nucleic acid methyltransferases (Motorin et al. 2010).

Site-directed mutagenesis and in vivo complementation studies

Since we were unable to detect enzymatic activity of StTaw3 in vitro, we decided to carry out in vivo complementation of the yeast ΔTYW3 deletion strain by a series of plasmid-encoding TYW3 variants to probe the role of the conserved putative active site residues. The ΔTYW3 deletion strain was transformed with a series of plasmids harboring wild-type TYW3 or mutants, D32A, T50A, S52A, S53A, C54A, G56A, R57A, and H88A. Total RNA was extracted from each transformant and subjected to nucleoside analysis by LC-mass spectrometry (Fig. 4). In the ΔTYW3 deletion strain, yW-86 can be detected as a proton adduct of dimer form, (yW-86)pA (m/z 752), as reported previously (Noma et al. 2006). When rescued by WT TYW3, yW-86 is methylated by TYW3 to form yW-72, and then fully modified by TYW4 to form yW, detected as a proton adduct of dimer form, yWpA (m/z 838) (Noma et al. 2006). Of the eight mutations studied, we saw varying degrees of complementation. Two mutants, S53A (S47 in StTaw3) and G56A (G50 in StTaw3) fully complemented yW formation (Fig. 4). D32, C54, and R57 (D25, C48, R51 in StTaw3), on the other hand, are essential for the activity, since TYW3 mutants with the corresponding Ala substitutions did not rescue yW formation at all (Fig. 4). In contrast, T50A, S52A, and H88A (T44, S46, and H73 in StTaw3) only partially complemented yW formation, as small levels of yW-86 were detected in these transformants (Fig. 4).

Substrate and cofactor binding sites

Cocrystallization and soaking with SAM and S-adenosyl-L-homocysteine (SAH) were attempted, but both proved unsuccessful. This may indicate the requirement for additional factors or the substrate to be bound in order to obtain stable association of the enzyme with the cofactor or the crystal packing may exclude the cofactor. Therefore, we performed molecular docking simulations with SAM and our apo-structure to help identify the cofactor binding site. Autodock placed the methyl donor in a small solvent exposed cleft adjacent to the active site (Fig. 5; Morris et al. 2009). The cleft is formed by the N-terminal extension and is conserved in all available Taw3 protein structures (Supplemental Fig. S1). The adenosyl moiety is positioned within the cleft making contact with several poorly conserved amino acids including K14, I15, D18, Y23, L24, I28, and L32. Furthermore, the sulfonium ion and methionine...
moieties extend beyond the end of the cleft, making contacts with charged residues in the active site (Fig. 5A). The conserved aspartate (D25 in SsTaw3) interacts with the sulfonium ion of SAM and was shown to be essential for TYW3 activity in our yeast complementation assay (Fig. 5B). The carboxyl group on SAM interacts with the invariant histidine (H73 in SsTaw3) in our docking model, which is more amenable to amino acid substitution showing only reduced activity in our complementation assay (Fig. 5B). Interestingly, there is a sulfate ion located in the same position as the carboxyl group of SAM in our docking model in one molecule of the asymmetric unit of our structure, which may mimic the interactions TYW3/Taw3 enzymes make with the carboxyl group of SAM. To test the role of the invariant aspartate and histidine in SAM binding we used a radioactive SAM binding assay. Mutation of either D25 or H73 reduced SsTaw3 SAM binding to the same levels as GST, which serves as a negative control (Fig. 5C), confirming their role in cofactor binding.

Figure 5A shows the surface charge distribution in the SsTaw3 structure. The solvent exposed surface of the RAGNYA fold contains a large positively charged patch that also features a well-positioned sulfate ion on each molecule in the asymmetric unit. This patch lies directly adjacent to the active site of the enzyme, which makes it likely that this serves as a binding surface for the phosphate backbone of its unidentified RNA substrate. Sulfate ions often mark the position of DNA or RNA backbone phosphates in nucleic acid binding proteins. In Supplemental Figure S1, we include the electrostatic surface potential for the three other Taw3 family members with structures available. Two of the three structures also present a large positively charged patch in the same position adjacent to the catalytic center suggesting that this may be a conserved RNA binding site in Taw3 methyltransferases. Interestingly, nucleic acid binding is among the known functions of the RAGNYA fold. Other RAGNYA fold containing proteins such as the L3-I, Tombusvirus p19, and ribosomal protein L1 interact with double-stranded regions of rRNA or siRNA–mRNA duplexes, the family Y DNA polymerase C-terminal domains and phage NinB proteins interact with DNA, and the RNA/DNA ligases interact with either RNA or DNA (Balaji and Aravind 2007). Despite similar electrostatic potential maps in the existing Taw3 structures, the only conserved residue that is a part of this charged surface is arginine, which sits at the junction of the active site and RNA binding surface. Interestingly, we show that this arginine is required for TYW3 complementation in yeast, which may be due to a role in tRNA binding (Fig. 4). However, since the substrate of SsTaw3 remains unknown we cannot directly test this hypothesis.

**DISCUSSION**

In the present study, we provide the first characterization of the most recently identified class of SAM-dependent methyltransferases, the TYW3 family, which are tRNA-\(\gamma\)-W N-4 methyltransferases involved in biosynthesis of the hypermodified \(\gamma\)W base (Noma et al. 2006). Through primary sequence analysis we show that TYW3 proteins do not contain motifs that are characteristic of the other classes of methyltransferases. Instead, we identify sequence features that are unique to TYW3 proteins including the signature motif (S/T)xSSCxGR, an N-terminal aspartate, and a C-terminal histidine. In addition, we solved the first crystal structure of a TYW3 enzyme, which reveals a novel fold with no homology to any of the known methyltransferase classes. This makes TYW3 methyltransferases the ninth class of SAM-dependent methyltransferase that appear to have independently evolved.

Interestingly, despite being separated in the primary sequence, the hallmark sequence features of TYW3 methyltransferases cluster within our structure. We propose that these residues are involved in substrate binding and/or
catalysis. Using the in vivo complementation of yeast ΔTYW3 strain combined with mass spectrometry, we revealed the importance of these conserved sequence features in TYW3 function, which also substantiates the active site identified based on the structure. The aspartate, cysteine, and arginine are required for TYW3 function and mutation of the threonine, the first serine, and histidine impair TYW3 function. Docking simulations place SAM in a surface exposed cleft in contact with both the conserved aspartate and the histidine. Mutation of either of these residues abolishes SAM binding. Therefore, we suggest that the defects in TYW3 function caused by mutating these residues are due to faulty SAM binding. Moreover, we suggest that a large basic patch on the surface of the RAGNYA fold domain may be the site of tRNA binding, which is also supported by the functional defect caused by mutating the only conserved residue on this surface.

We tested SsTaw3 activity on two different substrates yW-86 and imG-14 on tRNA$^{\text{Phe}}$ derived from yeast ΔTYW3 and ΔTYW2 strains, respectively. However, neither of these substrates was modified by SsTaw3. Which tRNAs in Sulfolobus contain mimG or imG is unknown; therefore, tRNA$^{\text{Phe}}$ might not be a substrate for Taw3 at all. Moreover, even if Taw3 methylates tRNA$^{\text{Phe}}$ in Sulfolobus species, yeast tRNA$^{\text{Phe}}$ may not be a suitable substrate for Taw3 due to the heterologous combination of enzyme and substrate. Therefore, the substrate of archaeal Taw3 activity remains an interesting and unanswered question. Each of the yeast TYW enzymes is active alone in vitro (Noma et al. 2006). However, it has been suggested that yW biosynthesis may be facilitated by the formation of a multienzymatic complex of TYW proteins (Noma et al. 2006). This is supported by the fact that the plant orthologs of TYW2, TYW3, and a part of TYW4 are present as a single large fusion protein. SsTaw3 might require other factors that we did not include in our in vitro assays to be active. Ultimately, this highlights interesting questions that still remain to be answered about the different roles of wyosine derivatives and their biosynthesis in both archaea and eukaryotes.

**FIGURE 5.** Substrate binding. (A) The electrostatic surface view of our SsTaw3 structure is shown with SAM docked. (B) Zoom in of SAM docked to SsTaw3. Side chains within bonding distance of SAM are shown as sticks. Residues labeled in red are required to complement SsTYW3 in our in vivo complementation assay. Residues labeled in orange and green only partially complemented and completely complemented SsTYW3 function in vivo, respectively. Residues labeled in black were not tested. (C) SAM binding activity was analyzed using S-[methyl-$^{\text{14}}$C]adenosyl-l-methionine and recombinant SsTaw3 and GST proteins. Values represent the average and standard deviation of two to three measurements presented as percentage of wild type.

**MATERIALS AND METHODS**

**Cloning, expression, and protein purification**

The open reading frame of the SS00622 gene from *Sulfolobus solfataricus* was PCR amplified and inserted between the NdeI and BamHI restriction sites of a modified pET-15b expression vector (Novagen) (p11) as previously described (Zhang et al. 2001). This construct generated an N-terminal hexahistidine tag joined to the SsTaw3 protein by the TEV protease recognition site (ENLYFQG). Recombinant native SsTaw3 was expressed in BL21(DE3) cells. Cells were cultured at 37°C in 1 L of Luria Bertani broth medium containing 100 μg/mL ampicillin until the OD$_{600}$ nm reached 0.6. Isopropyl-β-D-1-thiogalactopyranoside was added to the medium to a final concentration of 1 mM to induce expression and the cells were cultured for an additional 4 h. Cells were harvested by centrifugation, resuspended in Buffer A (20 mM imidazole, 0.3 M NaCl, and 20 mM Na$_2$H$_2$PO$_4$, pH 8.0), and disrupted by sonication. The crude extract was heated at 70°C for 30 min, and the denatured protein was then removed by centrifugation (3220g for 15 min). The supernatant solution was loaded on a High Performance Ni$^{2+}$-Sepharose column (HisTrapTM HP, 5 mL) equilibrated with Buffer A. Recombinant SsTaw3 was expressed in BL21(DE3) cells. Cells were cultured in minimal medium supplemented with seleno-methionine and purified under the same conditions as the native protein.

**Protein crystallization**

Protein crystals of SsTaw3 were generated through hanging drop vapor diffusion at 21°C by mixing 2 μL of protein solution (35 mg/mL) with 2 μL of well solution consisting of 23% MME 5K, 0.2 M ammonium sulfate, and 0.1 M sodium acetate, pH 4.6.
X-ray diffraction, structure determination, refinement, and modeling

SsTaw3 crystals were placed in a cryoprotectant composed of 23% sucrose added to the crystallization solution and then flash frozen in liquid nitrogen prior to data collection. Multiwavelength anomalous dispersion data were collected at three wavelengths 0.97927, 0.97948, and 0.96404 Å at the Advanced Photon Source (APS, Argonne, IL) beamline 19-ID of the Structural Biology Center-CAT with a SBC-3 CCD detector. The data were processed using HKL2000 (Otwinowski and Minor 1997). Data collection and processing statistics are shown in Table 1.

The structure of SsTaw3 was solved using the multiwavelength anomalous dispersion (MAD) method. Selenium sites were located using SOLVE (Terwilliger and Berendzen 1999; Terwilliger 2000). Selenium position refinement, phase calculation, and density modification was performed by SHARP (de La Fortelle and Bricogne 1997). The structural model was built and refined by XFIT, CNS, and Refmac (Brunger et al. 1998; McRee 1999). The final refinement statistics can be found in Table 1 and the coordinates have been deposited in the PDB (the accession code 1TLJ). SAM docking experiments were performed with AutoDock (Morris et al. 2009).

SsTaw3 activity measurements

Yeast strains were cultured and bulk tRNA was extracted as previously described (Noma et al. 2006; de Crecy-Lagard et al. 2010). SsTYW3 was prepared as before (Noma et al. 2006). In vitro reactions were carried out in a total of 25 µL containing 100 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 0.5 mM DTT, 1 mM spermidine, 5% glycerol, with or without 10 µg of protein, with or without 2 mM SAM, with 40 µg of yeast derived tRNAs. Reactions were incubated at 60°C for 10 min at 30°C. The mixture was passed 7 times with 300 µL of the binding buffer, and the bound S-[methyl-14C]adenosyl-l-methionine was quantified by liquid scintillation counting using a LS 6500 multipurpose scintillation counter (Beckman Coulter).

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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REFERENCES

Alteweg M, Kubli E. 1979. The nucleotide sequence of phenylalanine tRNA2 of Drosophila melanogaster: four isoacceptors with one basic sequence. Nucleic Acids Res 7: 93–105.

Anantharaman V, Aravind L. 2004. The SHS2 module is a common structural theme in functionally diverse protein groups, like Rpb7p, FtsA, Gyrl, and MTH1598/TM1083 superfamilies. Proteins 56: 795–807.

Balaji S, Aravind L. 2007. The RAGNYA fold: a novel fold with multiple topological variants found in functionally diverse nucleic acid, nucleotide and peptide-binding proteins. Nucleic Acids Res 35: 5658–5671.

Bjork GR, Jacobsson K, Nilsson K, Johansson MJ, Bystrom AS, Persson OP. 2001. A primordial tRNA modification found in functionally diverse nucleic acid, nucleotide and peptide-binding proteins. Nucleic Acids Res 35: 5658–5671.

Bruce AG, Uhlenbeck OC. 1982. Enzymatic replacement of the antico- don of yeast phenylalanine transfer ribonucleic acid. Biochemistry 21: 855–861.

Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, et al. 1998. Crystallography & NMR system: a new software suite for macromolecular structure determination. Acta Crystallogr D Biol Crystallogr 54: 905–921.

Carlson BA, Kwon SY, Chamorro M, Oroszlan S, Hatfield DL, Lee BJ. 1999. Transfer RNA modification status influences retroviral ribosomal frameshifting. Virology 255: 2–8.
de Creyc-Lagard V, Brochier-Armanet C, Urbanovicius J, Fernandez B, Phillips G, Lyons B, Noma A, Alvarez S, Droogmans J, Armengaud J, et al. 2010. Biosynthesis of wyosine derivatives in tRNA: an ancient and highly diverse pathway in Archaea. *Mol Biol Evol* 27: 2062–2077.

de La Fortelle E, Bricogne G. 1997. Maximum-likelihood heavy-atom parameter refinement for multiple isomorphous replacement and multiwavelength anomalous diffraction methods. *Methods Enzymol* 276: 472–494.

Goto-Ito S, Ishii R, Ito T, Shibata R, Fusatomi E, Sekine SI, Besho Y, Yokoyama S. 2007. Structure of an archaeal TYW1, the enzyme catalyzing the second step of wyb-base biosynthesis. *Acta Crystallogr D Biol Crystallogr* 63: 1039–1068.

Guy MP, Shaw M, Weiner CL, Hobson L, Stark Z, Rose K, Kalscheuer VM, Gez J, Phizicky EM. 2015. Defects in tRNA anticodon loop 2′-O-methylation are implicated in nonsyndromic X-linked intellectual disability due to mutations in FTSJ1. *Hum Mutat* 36: 1176–1187.

Hatfield D, Feng YX, Lee BJ, Rein A, Levin JG, Oroszlan S. 1989. Chromatographic analysis of the aminoacyl-tRNA which are required for translation of codons at and around the ribosomal frame-shift sites of HIV, HTLV-1, and BLV. *Virology* 173: 736–742.

Holm L, Park J. 2000. DaliLite workbench for protein structure comparison. *Bioinformatics* 16: 566–567.

Kaminska KH, Purta E, Hansen LH, Bujnicki JM, Vester B, Long KS. 2000. DaliLite workbench for protein structure comparison. *Bioinformatics* 16: 566–567.

Kato M, Araiso Y, Noma A, Suzuki T, Ishitani R, Nureki O. 2010. Crystal structure of a novel JmjC-domain-containing protein, 354 RNA, Vol. 23, No. 3

Kimura S, Miyauchi K, Ikeuchi Y, Thiaville PC, Crecy-Lagard V, Schubert HL, Blumenthal RM, Cheng X. 2003. Many paths to methyltransfer: a distinct form of microcephalic primordial dwarfism. *Genome Biol* 16: 210.

Kimura S, Noma A, Suzuki T, Senda M, Senda T, Ishitani R, Nureki O. 2009. Structural basis of AdoMet-dependent aminocarboxypropyl transfer reaction catalyzed by tRNA-wybutosine synthesizing enzyme, TYW4. *Nucleic Acids Res* 37: 9350–9365.

Kirino Y, Suzuki T. 2005. Human mitochondrial diseases associated with tRNA wobble modification deficiency. *RNA Biol* 2: 41–44.

Kirino Y, Goto Y, Campos Y, Arenas J, Suzuki T. 2005. Specific correlation between the wobble modification deficiency in mutant tRNAs and the clinical features of a human mitochondrial disease. *Proc Natl Acad Sci* 102: 7127–7132.

Konevega AL, Soboleva NG, Makhno VI, Semenkov YP, Wintermeyer W, Rodchina MV, Katunin VI. 2004. Purine bases at position 37 of tRNA stabilize codon-anticodon interaction in the ribosomal A site by stacking and Mg2+-dependent interactions. *RNA* 10: 90–101.

Kozbial PZ, Mushegian AR. 2005. Natural history of S-adenosylmethionine-binding proteins. *BMC Struct Biol* 5: 19.

Machnicka MA, Milanowska K, Osman Ogliou O, Purta E, Kurkowski M, Olchowik A, Januszewski W, Kalinin S, Dunin-Horkawicz S, Rother KM, et al. 2013. MODOMICS: a database of RNA modification pathways. *RNA Biol* 10: 1204–1214.

Mcllsekey JA, Graham DE, Zhou S, Crain PF, Ibbia M, Konisky J, Soll D, Olson GJ. 2001. Post-transcriptional modification in archael tRNAs: identities and phylogenetic relations of nucleotides from mesophilic and hyperthermophilic Methanococcales. *Nucleic Acids Res* 29: 4699–4706.

McBee DE. 1999. XtalView/Xfit—a versatile program for manipulating atomic coordinates and electron density. *J Struct Biol* 125: 156–165.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. 2009. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem* 30: 2785–2791.

Motorin Y, Lyko F, Helm M. 2010. 5-methylcytosine in RNA: detection, enzymatic formation and biological functions. *Nucleic Acids Res* 38: 1415–1430.

Mushinski JF, Marinii M. 1979. Tumor-associated phenylalanyl transfer RNA found in a wide spectrum of rat and mouse tumors but absent in normal adult, fetal, and regenerating tissues. *Cancer Res* 39: 1253–1258.

Noma A, Kirino Y, Ikeuchi Y, Suzuki T. 2006. Biosynthesis of wybutosine, a hyper-modified nucleoside in eukaryotic phenylalanine tRNA. *EMBO J* 25: 2142–2154.

Noma A, Ishitani R, Kato M, Nagao A, Nureki O, Suzuki T. 2010. Expanding role of the jumonji C domain as an RNA hydroxylase. *J Biol Chem* 285: 34503–34507.

Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 276: 307–326.

Pakh H, Iaiswal YK, Vinayak M. 2005. Hypomodification of transfer RNA in cancer with respect to queuosine. *RNA Biol* 2: 143–148.

Schubert HL, Blumenthal RM, Cheng X. 2003. Many paths to methyltransfer: a chronicle of convergence. *Trends Biochem Sci* 28: 329–335.

Shaheen R, Abdel-Salam GM, Guy MP, Alomar R, Abdel-Hamid MS, Afifi HH, Ismail SI, Emam BA, Phizicky EM, Alkuraya FS. 2015. Mutation in WDR4 impairs RNA m1G46 methylolation and causes a distinct form of microcephalic primordial dwarfism. *Genome Biol* 16: 210.

Suzuki Y, Noma A, Suzuki T, Senda M, Senda T, Ishitani R, Nureki O. 2009. Crystal structure of the radical SAM enzyme catalyzing tricyclic modified base formation in tRNA, *J Mol Biol* 372: 1204–1214.

Suzuki Y, Noma A, Suzuki T, Ishitani R, Nureki O. 2009. Structural basis of tRNA modification with CO2 fixation and methylation by wybutosine synthesizing enzyme TYW4. *Nucleic Acids Res* 37: 2910–2925.

Terwilliger TC. 2000. Maximum-likelihood density modification. *Acta Crystallogr D Biol Crystallogr* 56: 965–972.

Terwilliger TC, Berendzen J. 1999. Automated MAD and MIR structure solution. *Acta Crystallogr D Biol Crystallogr* 55: 849–861.

Thibie R, Poralla K. 1973. Origin of the nucleoside Y in yeast tRNA^Phe*. *FEBS Lett* 38: 27–28.

Umitsu M, Nishimatsu H, Noma A, Suzuki T, Ishitani R, Nureki O. 2009. Structural basis of AdoMet-dependent aminocarboxypropyl transfer reaction catalyzed by tRNA-wybutosine synthesizing enzyme, TYW2. *Proc Natl Acad Sci* 106: 15616–15621.

Zhang RG, Skarina T, Katz JE, Beasley S, Khachatryan A, Vyas S, Arrowsmith CH, Clarke S, Edwards A, Joachimiak A, et al. 2001. Structure of *Thermotoga maritima* stationary phase survival protein SurE: a novel acid phosphatase. *Structure* 9: 1095–1106.

Zhou S, Sitaramaiah D, Noon KR, Guymon R, Hashizume T, McCloskey JA. 2004. Structures of two new "minimalist" modified nucleosides from archaeal tRNA. *Bioorg Chem* 32: 82–91.