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Crystal structures of *N*2,*N*2-dimethyltransferase (Trm1) from *Pyrococcus horikoshii*

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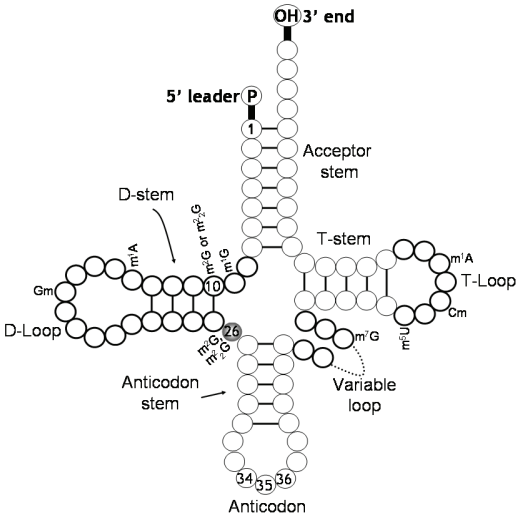
Transfer RNA (tRNA) is a crucial adapter molecule in protein biosynthesis. For being the adapter molecule, tRNAs should be extensively processed such as removal of the 5' and 3' additional sequences by RNaseP, endo and exonucleases and nucleotide modifications by corresponding modification enzymes.[1; 2] Modified nucleosides in tRNAs are commonly found in the core (including the D- and T-stems interactions) and anticodon regions. In the core region, modified nucleosides such as m^1A58, Gm at 18 and 19, m^2G and m^2_2G at 10 and 26 are linked to the correct folding and stability of the three-dimensional (3D) structure of tRNAs. While modifications in the anticodon stem and anticodon region (position 34-36) have functions in repairing the decoding process and avoiding codon-anticodon mismatch.[3] Thus in general, modified nucleosides in tRNAs are important for the fidelity and efficiency of the protein biosynthesis although as yet mostly unknown. Trm1 catalyses mono (m^2G) and dimetylation (m^2_2G) of tRNA guanine at position 26, a junction between the D- and anticodon-stems, employing S-adenosyl-L-Methionine (SAM) as a methyl donor.[4] Both m^2G26 and m^2_2G26 modifications are found in most archaeal, eukaryotic and *Aquifex aeolicus* tRNAs.[4; 5]

To explore the Trm1 methyl transfer mechanism, we determined crystal structures of Trm1 from *Pyrococcus horikoshii* (*PhoTrm1*) in an apo, SAM and two S-adenosyl-L-Homocysteine (SAH) forms. The *PhoTrm1* structure consists of two domains: the N-terminal domain (NTD) with the classical methyltransferase (MTase) fold and the C-terminal domain (CTD) with a new fold. The CTD contains a putative DNA-binding subdomain at the C-termini and together with NTD may mediate protein-tRNA interactions. The SAH or SAM molecule was found at NTD and the methyl moiety of SAM faced to two strictly-conserved phenylalanines. In the front of the SAM binding pocket, these two phenylalanines form a narrow pocket which is likely a putative G26 pocket. Further, our models of Trm1-substrate complex offer possible explanation why Trm1 requires tRNAs with five or more nucleotides at the variable loop.



References

1. Hopper, A. K. & Phizicky, E. M. (2003). tRNA transfers to the limelight. *Genes Dev* **17**, 162-80.
2. Nakanishi, K. & Nureki, O. (2005). Recent progress of structural biology of tRNA processing and modification. *Mol Cells* **19**, 157-66.
3. Grosjean, H. (2005). *Fine-Tuning of RNA Functions by Modification and Editing*, NY Springer Verlag, Berlin-Heidelberg.
4. Constantinesco, F., Motorin, Y. & Grosjean, H. (1999). Characterisation and enzymatic properties of tRNA(guanine 26, N (2), N (2))-dimethyltransferase (Trm1p) from *Pyrococcus furiosus*. *J Mol Biol* **291**, 375-92.
5. Awai T, Takehara T, Takeda H & Hori H. (2005). *Aquifex aeolicus* Trm1[tRNA (m22G26) methyltransferase] has a novel recognition mechanism of the substrate RNA. *Nucleic Acids Symp Ser (Oxf)* **49**, 303-304.