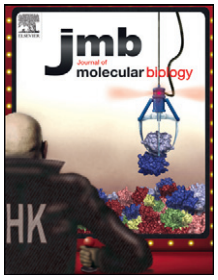

Crystal Structure of the Human tRNA m^{1}A58 Methyltransferase–tRNA$_3^{Lys}$ Complex: Refolding of Substrate tRNA Allows Access to the Methylation Target

Janet Finer-Moore†, Nadine Czudnochowski†, Joseph D. O'Connell III, Amy Liya Wang and Robert M. Stroud

Department of Biochemistry and Biophysics, *University of California San Francisco, San Francisco, CA 94143, USA*

Correspondence to Janet Finer-Moore: *finer@msg.ucsf.edu*
http://dx.doi.org/10.1016/j.jmb.2015.10.005
Edited by G. Schulz

Abstract

Human tRNA$_3^{Lys}$ is the primer for reverse transcription of HIV; the 3′ end is complementary to the primer-binding site on HIV RNA. The complementarity ends at the 18th base, A58, which in tRNA$_3^{Lys}$ is modified to remove Watson–Crick pairing. Motivated to test the role of the modification in terminating the primer-binding sequence and thus limiting run-on transcription, we asked how the modification of RNA could be accomplished. tRNA m^{1}A58 methyltransferase (m^{1}A58 MTase) methylates N1 of A58, which is buried in the TΨC-loop of tRNA, from cofactor *S*-adenosyl-L-methionine. This conserved tRNA modification is essential for stability of initiator tRNA in *Saccharomyces cerevisiae*. Reported here, three structures of human tRNA m^{1}A58 MTase in complex with human tRNA$_3^{Lys}$ and the product *S*-adenosyl-L-homocysteine show a dimer of heterodimers in which each heterodimer comprises a catalytic chain, Trm61, and a homologous but noncatalytic chain, Trm6, repurposed as a tRNA-binding subunit that acts *in trans*; tRNAs bind across the dimer interface such that Trm6 from the opposing heterodimer brings A58 into the active site of Trm61. T-loop and D-loop are splayed apart showing how A58, normally buried in tRNA, becomes accessible for modification. This result has broad impact on our understanding of the mechanisms of modifying internal sites in folded tRNA. The structures serve as templates for design of inhibitors that could be used to test tRNA m^{1}A58 MTase's impact on retroviral priming and transcription.

Introduction

In all kingdoms of life, tRNA is extensively modified posttranscriptionally by an array of enzymes. Each enzyme is specific for one or a few tRNA sites. The modifications contribute to translational fidelity, recognition of aminoacyl-tRNA synthetases, tRNA stability and tRNA structure. Much research has focused on tRNA m^{1}A58 methyltransferase (m^{1}A58 MTase), which catalyzes the transfer of a methyl group from the cofactor *S*-adenosyl-L-methionine (SAM) to N1 of adenine 58 to give 1-methyladenosine (m^{1}A). This modification is essential in *Saccharomyces cerevisiae* because it is required for the stability of initiator methionine tRNA [1].

m^{1}A58 modification of tRNA$_3^{Lys}$ also has a role in replication of HIV in humans. Immunodeficiency viruses, such as HIV-1, coopt m^{1}A58-modified tRNA$_3^{Lys}$ as a primer for the initiation of reverse transcription and rely on the modified base to terminate the base-paired region after 18 bases at the 3′ end [2]. Thus, m^{1}A58 MTase may be a new human host enzyme that can be targeted for therapeutic intervention in HIV infection. However, m^{1}A58 MTase has not yet been validated as a drug target. Contradictory reports as to whether A58 methylation is the sole determinant for termination of viral (+) strand strong-stop DNA synthesis and thus for reverse transcription exist. The mutation A58U inhibits replication of HIV *in vivo* [3]; on the other hand, a complementation system in which a HIV-1 proviral genome with altered primer-binding site uses *Escherichia coli* tRNA$_3^{Lys}$ added *in trans* to prime reverse transcription is insensitive to the A58U mutation in the *E. coli* tRNA$_3^{Lys}$ [4].

In humans, m^{1}A58 MTase is composed of two subunits, a catalytic component, Trm61, and an RNA-binding component, Trm6 [5]. Bacterial and

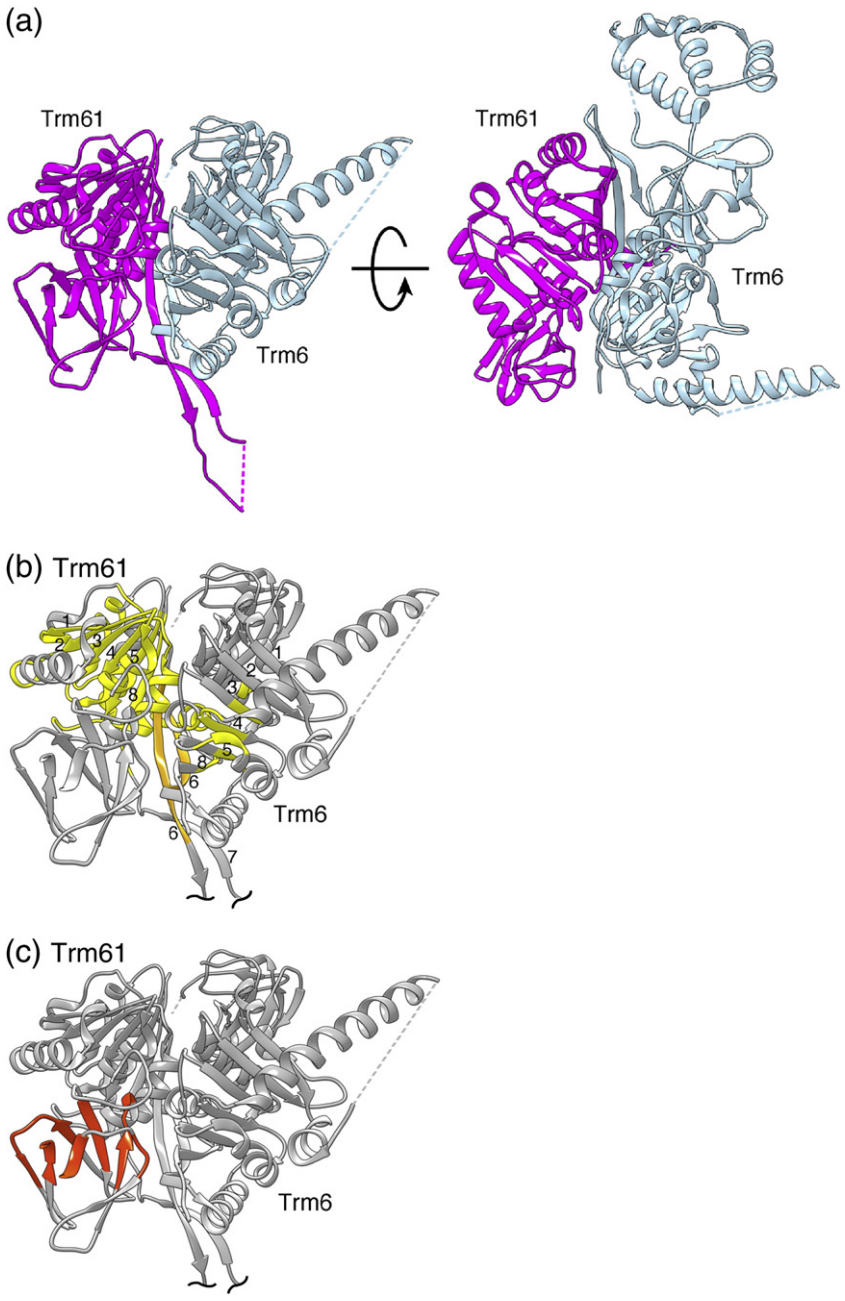

Fig. 1. Structure of the human m^1A58 MTase dimer. (a) Ribbon drawing of the tight Trm61/Trm6 heterodimer of human m^1A58 MTase shown in two views. The views are related by an approximately 90° counterclockwise rotation around the horizontal axis. In the view on the left, the insert in the N-terminal domain of Trm6 is behind the clipping plane, thus not visible. The catalytic subunit, Trm61, is magenta and the noncatalytic subunit, Trm6, is gray-blue. (b) Structurally highly conserved core, with respect to *T. thermophilus* m^1A58 MTase, spanning the C-terminal domains of the human m^1A58 MTase heterodimer. The core is colored gold except for the β6 strands that form the dimer interface, which are highlighted in light orange. β-Strands in the cross-subunit β-sheet of the C-terminal domains are numbered as in TrmI [6]. (c) Conserved structural core of the N-terminal RNA-binding domain of Trm61, colored orange-red.

archaeal m^1A58 MTases comprise only one subunit, called TrmI, and crystal structures of four such m^1A58 MTases reveal that the enzymes assemble as homotetramers [6–9]. None of these structures include tRNA so that the mechanism and selectivity even for the bacterial enzymes is as yet