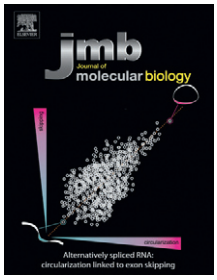
Article



Crystal Structure of Human DNA Methyltransferase 1

Zhi-Min Zhang [1], Shuo Liu [2,3], Krystal Lin [1], Youfu Luo [4], John Jefferson Perry [1], Yinsheng Wang [2,3] and Jikui Song [1]

*1 - **Department of Biochemistry,** University of California, Riverside, CA 92521, USA*
*2 - **Environmental Toxicology Graduate Program,** University of California, Riverside, CA 92521, USA*
*3 - **Department of Chemistry,** University of California, Riverside, CA 92521, USA*
*4 - **State Key Laboratory of Biotherapy/Collaborative Innovation Center for Biotherapy,** West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, PR China*

***Correspondence to Jikui Song:** jikui.song@ucr.edu*
http://dx.doi.org/10.1016/j.jmb.2015.06.001
Edited by T. Yeates

Abstract

DNMT1 (*DN*A *m*ethyl*t*ransferase *1*) is responsible for propagating the DNA methylation patterns during DNA replication. DNMT1 contains, in addition to a C-terminal methyltransferase domain, a large N-terminal regulatory region that is composed of an RFTS (*r*eplication *f*oci *t*argeting *s*equence) domain, a CXXC zinc finger domain and a pair of BAH (*b*romo *a*djacent *h*omology) domains. The regulatory domains of DNMT1 mediate a network of protein–protein and protein–DNA interactions to control the recruitment and enzymatic activity of DNMT1. Here we report the crystal structure of human DNMT1 with all the structural domains (hDNMT1, residues 351–1600) in complex with *S*-adenosyl-L-homocysteine at 2.62 Å resolution. The RFTS domain directly associates with the methyltransferase domain, thereby inhibiting the substrate binding of hDNMT1. Through structural analysis, mutational, biochemical and enzymatic studies, we further identify that a linker sequence between the CXXC and BAH1 domains, aside from its role in the CXXC domain-mediated DNMT1 autoinhibition, serves as an important regulatory element in the RFTS domain-mediated autoinhibition. In comparison with the previously determined structure of mouse DNMT1, this study also reveals a number of distinct structural features that may underlie subtle functional diversity observed for the two orthologues. In addition, this structure provides a framework for understanding the functional consequence of disease-related hDNMT1 mutations.

Introduction

DNA methylation is one of the key epigenetic mechanisms that are essential for transcriptional silencing of retrotransposons [1–3], genomic imprinting [4] and X-chromosome inactivation [5]. DNA methylation in mammals mainly occurs at the C-5 position of cytosine within the symmetric CpG dinucleotide and affects ~70–80% of the CpG dinucleotides throughout the genome [6]. The DNA methylation patterns are established by the *de novo* DNA methyltransferases DNMT3A and DNMT3B during gametogenesis and early embryogenesis and become stably inherited by the maintenance DNA methyltransferase DNMT1 (*DN*A *m*ethyl*t*ransferase *1*), in cooperation with DNMT3A and DNMT3B [7–10]. DNMT1-mediated maintenance DNA methylation is supported by both its substrate preference toward hemimethylated CpG sites [11,12] and its recruitment to DNA replication foci [13,14], through its interactions with proliferating cell nuclear antigen [15] and histone H3 ubiquitinated at lysine 23 [16].

DNMT1 is a multimodular protein that is composed of ~1620 amino acids. It contains a C-terminal methyltransferase (MTase) domain and a large N-terminal regulatory region, linked by a conserved (GK)n dipeptide repeat. The N-terminal region of DNMT1 is composed of an RFTS (*r*eplication *f*oci *t*argeting *s*equence) domain, a CXXC zinc finger domain and a pair of BAH (*b*romo *a*djacent *h*omology)

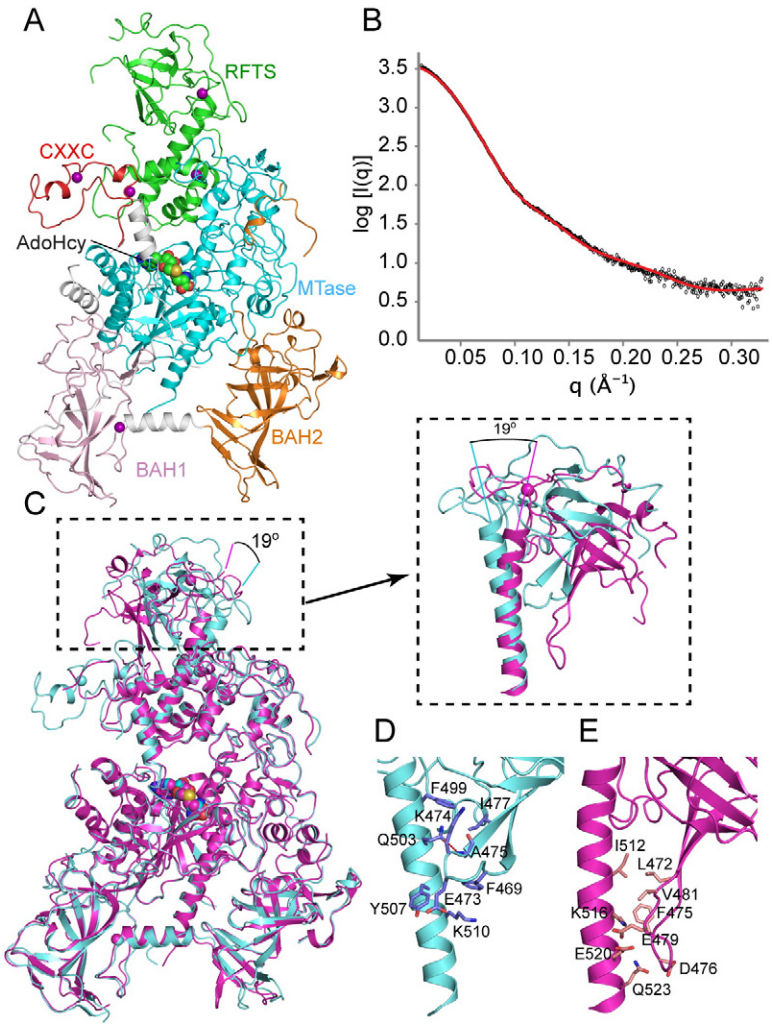


Fig. 1. Structural overview of hDNMT1$_{351\text{-}1600}$ bound to AdoHcy. (A) Ribbon representation of hDNMT1$_{351\text{-}1600}$ in complex with AdoHcy, with the RFTS, CXXC, BAH1, BAH2 and MTase domains colored green, red, pink, orange and light blue, respectively. The domain linkers and zinc ions are colored silver and purple, respectively. The bound AdoHcy is in a space-filling representation. (B) Measured scattering curve of free hDNMT1$_{351\text{-}1600}$ (black) is compared with the scattering curve calculated from the crystal structure (red). (C) Superposition of hDNMT1$_{351\text{-}1600}$ (light blue) and mDNMT1$_{291\text{-}1620}$ (magenta), with the N-terminal half of the RFTS domains highlighted in an expanded view. (D and E) Interactions between a loop from the RFTS domain and the α_{RS}-helix in hDNMT1$_{351\text{-}1600}$ (D) and mDNMT1$_{291\text{-}1620}$ (E), respectively.

domains. These N-terminal domains distinguish DNMT1 from its bacterial counterparts and impose a tight control on the recruitment and enzymatic activity of DNMT1 [7,17]. To elucidate the regulatory mechanisms of DNMT1, we have previously determined the crystal structures of a C-terminal fragment of mouse DNMT1 (residues 650–1602, mDNMT1$_{650\text{-}1602}$) in complex with an unmethylated CpG DNA, as well as the equivalent human DNMT1 (hDNMT1$_{646\text{-}1600}$)–DNA complex, at 3.0 Å and 3.6 Å resolution, respectively [18]. These structures reveal that the DNMT1 CXXC domain specifically binds to the CpG dinucleotide, which helps position the CXXC-BAH1 domain linker (also known as autoinhibitory linker) into the catalytic cleft of the MTase domain, thereby forming an autoinhibitory conformation. This observation, together with mutational studies and enzymatic activity assays, suggests that the DNMT1 CXXC domain plays an inhibitory role in DNMT1-mediated *de novo* methylation. In a separate study, Takeshita *et al.* have determined the structure of a longer mDNMT1 fragment (residues 291–1620, mDNMT1$_{291\text{-}1620}$), free and in complex with cofactor *S*-adenosyl-L-methionine (AdoMet) or cofactor product *S*-adenosyl-L-homocysteine (AdoHcy) [19]. The structure of mDNMT1$_{291\text{-}1620}$ reveals that the RFTS domain forms a DNA-competitive inhibitor through direct interaction with the MTase domain of mDNMT1. This observation suggests that the RFTS domain plays an inhibitory role in DNMT1-mediated