



Mechanism for METTL6-Mediated m³C RNA Methylation in Terms of its Structure

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ABSTRACT

RNA modifications are critical in mediating how RNAs perform biological functions. Despite being less common, 3-methylcytidine (m³C) is discovered to be widely present in tRNAs, rRNAs, and mRNAs. A m³C methyltransferases for tRNAs, including transfer, is human METTL6 (UGA). In the presence of S-adenosyl-L-methionine, we were able to solve the structure of human METTL6, and an enzyme assay revealed that recombinant human METTL6 is active towards transfer (UGA). A putative tRNA binding area on the surface of METTL6 was identified by structural analysis, which also revealed the specific interactions between S-adenosyl-L-methionine and METTL6. In the near future, the design of effective METTL6 inhibitors will undoubtedly become clearer as a result of structural study that is accompanied by biochemical enzyme testing.

Keywords: Plant Biology, biochemistry, METTL6

INTRODUCTION

Numerous RNA modifications, such as localization, splicing, translation, and turnover, have been found to often occur inside practically all types of RNAs to mediate there in vivo functions [1,2]. The most popular kind is methylation of RNA. The two most common internal RNA modifications, N⁶-adenosine (m⁶A) and 5-methylcytosine (m⁵C) are specifically linked to the post-transcriptional control of gene expression in higher eukaryotes [1,3,4].

Some RNA modifications are known to be installed and deleted by RNA methyl Transferase (“writers”) and Demethylases (“erasers”), similarly to covalent changes on proteins and DNAs. Some RNA alterations may be able to function biologically by being recognized by particular elements, or “readers,” that are referred to as such. For instance, YTH domain proteins act as m⁶A reader proteins whereas METTL14-METTL3 [5-7], METTL5-TRMT112 [8, 9], and METTL16 [10]. are recognized as m⁶A methyl transferase and ALKBH5 and FTO function as demethylases [9,10], Other RNA methylation marks, such as 3-methylcytidine (m³C), are far less common than m⁶A and, until very recently, their biochemistry was not well known [5, 6]. This is in contrast to m⁶A, which may be found in a range of RNA types.

It has been discovered that m³C, which was first discovered to be a particular alteration inside rRNAs and tRNAs [7, 8], also occurs in mammalian mRNAs to control RNA fates [8]. Trm140 and Trm140/Trm141, which catalyse the m³C methylation on the tRNAs of *Saccharomyces pombe* and *S. cerevisiae*, respectively, were discovered to function as the mammalian tRNA m³C methyl transferase. To be more precise, METTL2 adds m³C to tRNA Thr(UGU) and tRNA Arg(CCU), while METTL6 catalyses the m³C32 of tRNA Ser(AGA/UGA) found in is acceptors [6, 9]. Mettl6 deficiency has been shown to affect pluripotency and slow the proliferation of tumors cells [9], making it a possible target for pharmaceutical companies for developing inhibitors. However, due to a paucity of structural knowledge, it is still unclear how METTL6 detects and catalyses’ its tRNA substrates. Here, using the co-factor S-Adenosyl methionine (SAM), we were able to solve the crystal structure of human METTL6 and discovered that it displays a non-traditional Rossmann fold with seven beta strands. It was determined by the biochemistry enzyme test that METTL6 is a functional methyltransferases for transfer (UGA). As a result, our biochemistry and structural studies shed light on the inhibitor design for this m³C methyltransferases in addition to the catalytic mechanism of METTL6.

MATERIALS AND METHODS

Expression and purification of proteins In order to transform *E. coli* BL21, a cDNA fragment encoding the complete human

METTL6 (residues 1 to 284) was sub cloned into the pET28-MHL vector (DE3). After being induced by 1 mM (final concentration) isopropyl—D-1-thiogalactopyranoside, the recombinant protein was overexpressed at 16°C for 18 hours (IPTG). The cells were then suspended in a solution containing 20 mM Tris-HCl (pH 7.5), 400 mM NaCl, and lysed by centrifugation at 3600 g, 4°C, for 15 minutes.

RESULTS AND DISCUSSION

METTL6's identification as the transfer (UGA) m³C methyltransferases in earlier research served as inspiration. To examine the catalytic activity of human *METTL6* towards transfer (UGA) in vitro, we generated full-length recombinant human *METTL6* protein through cloning, expression, and purification. We acquired two sets of *k*_{cat} and *K*_M values by employing the MTase-GloTM Methyltransferases assay, one for tRNASER (UGA) and the other for SAM. The transfer *k*_{cat}/*K*_M values (UGA).

Data Availability

The RCSB protein data bank has accession number 7F1E for the coordinates and structural elements of the structure of *METTL6* bound with SAM.

REFERENCES

1. Otwinowski Zbyszek, and Wladek Minor. "Processing of X-ray diffraction data collected in oscillation mode." *Methods in enzymology*. Vol. 276. Academic press, (1997). 307-326.
2. Iwanami Yasuo, and Gene M. Brown. "Methylated bases of ribosomal ribonucleic acid from HeLa cells." *Archives of biochemistry and biophysics* 126.1 (1968): 8-15.
3. Xu Luang, et al. "Three distinct 3-methylcytidine (m³C) methyltransferases modify tRNA and mRNA in mice and humans." *Journal of Biological Chemistry* 292.35 (2017): 14695-14703.
4. Liu Fange, and Chuan He. "A new modification for mammalian messenger RNA." *Journal of Biological Chemistry* 292.35 (2017): 14704-14705.
5. Xu Chao, et al. "Structural basis for the discriminative recognition of N⁶-methyladenosine RNA by the human YT521-B homology domain family of proteins." *Journal of Biological Chemistry* 290.41 (2015): 24902-24913.
6. Zheng Guanqun, et al. "ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility." *Molecular cell* 49.1 (2013): 18-29.
7. Kim Young-Kook, Inha Heo, and V. Narry Kim. "Modifications of small RNAs and their associated proteins." *Cell* 143.5 (2010): 703-709.
8. Roundtree Ian A, et al. "Dynamic RNA modifications in gene expression regulation." *Cell* 169.7 (2017): 1187-1200.
9. Zhao, et al. "Post-transcriptional gene regulation by mRNA modifications." *Nature reviews Molecular cell biology* 18.1 (2017): 31-42.
10. Yang Xin, et al. "5-methylcytosine promotes mRNA export—NSUN2 as the methyltransferase and ALYREF as an m⁵C reader." *Cell research* 27.5 (2017): 606-625.