Clinical Microbiology: Open Access

Gao et al., Clin Microbiol 2016, 5:4
http://dx.doi.org/10.4172/2327-5073.1000256

Review Article Open Access

N^6-methyladenosine Modification in Bacterial mRNA

Ruxia Gao[1#], Heng Shi[2#], Yingchao Zhang[2], Xiaolong Shao[2] and Xin Deng[2*]

[1]*College of Life Science, Shanxi Normal University, Linfen, Shanxi, China*

[2]*Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, TEDA Institute of Biological Sciences and Biotechnology, Nankai University, 23 Hongda Street, Tianjin, China*

[*]***Corresponding author:*** *Dr. Xin Deng, TEDA Institute of Biological Sciences and Biotechnology, Nankai University, 23 Hongda Street, Tianjin 300457, China, E-mail: dengxin@nankai.edu.cn*

[#]These authors contribute equally to this work

Received date: June 16, 2016, **Accepted date:** July 13, 2016, **Published date:** July 20, 2016

Abstract

Among more than 140 naturally occurring RNA modifications have been identified, N^6-methyladenosine (m^6A) is the most abundant messenger RNA (mRNA) modification in eukaryotic organisms. A group of demethylases, methyltransferase and m^6A-specific binding proteins in mammals, plants as well as yeast are in support of the regulatory functions of this RNA modification. Recent years, with the fast development of purification methods and detecting techniques, researchers have extended the range of RNA epigenetics from the rRNA and tRNA modification to the field of mRNA and ncRNA in eukaryotes. However, we have little understanding of m^6A modification in bacteria RNA, particularly mRNA. This review summarizes the recent research advances of eukaryotic m^6A modification, and outlines the optimized analysis techniques together with recent progress on m^6A modification in bacterial mRNA.

Keywords: N^6-methyladenosine; mRNA; Bacteria

Introduction

Among more than 140 naturally occurring RNA modifications have been identified [1], N^6-methyladenosine (m^6A) is the most abundant messenger RNA (mRNA) modification in eukaryotic organisms. A group of demethylases, methyltransferase and m^6A-specific binding proteins in mammals, plants as well as yeast are in support of the regulatory functions of this RNA modification. Recent years, with the fast development of purification methods and detecting techniques, researchers have extended the range of RNA epigenetics from the rRNA and tRNA modification to the field of mRNA and ncRNA in eukaryotess [2].

However, we have little understanding of m^6A modification in bacteria RNA, particularly mRNA. This review summarizes the recent research advances of eukaryotic m^6A modification, and outlines the optimized analysis techniques together with recent progress on m^6A modification in bacterial mRNA.

The study of RNA modifications dates back to early 1950s, when Srinivasan et al. firstly found rRNA and tRNA methylations in bacteria [3-6]. In 1970s, Greenberg et al. pioneered a new research field on the methylation of rRNA and tRNA in animal cells [6-9]. Over the last two decades, m^6A became the most important modification in eukaryotes, thanks to the identification of a group of m^6A-associated proteins, including METTL3, METTL14, FTO and ALKBH5 [10-14]. METTL3 and METTL14 form a heterodimer that catalyzes m^6A RNA methylation, while WTAP (Wilms tumor 1 associated protein) interacts with the complex and affects the mRNA methylation [15].

FTO and ALKBH5 are m^6A RNA demethylases, which are involved in development, RNA metabolism and fertility [11,16]. Moreover, two m^6A-specific binding proteins (YTHDF2 and YTHDF1) have been discovered and characterized, suggesting its important roles in mRNA stability and translation efficiency [17,18]. A recent report has revealed the structural basis of N^6-adenosinemethylation by the METTL3-METTL14 complex for the first time, which demonstrates the molecular basis of the m^6A methylase reaction [19].

Although fast and exciting breakthroughs have been made on m^6A in eukaryotic mRNA, very little is known on m^6A in bacterial RNA. This review focuses on the bacterial m^6A modification (especially in mRNA), research methods, and key discoveries of this modification to promote intensive study in this field.

Methods of Detection

Initially, researchers employed a radioactive isotope labeling method （labelled by incorporation of ^{32}P-orthophosphate and either ^{3}H or ^{14}C-(methyl)-methionine） to detects m^6A modification in RNA [20]. Later, the subsequent approach of primer extension assay was used for the detection of chemical modification of m^2G, m^1A, m^7G, and pseudouridine [21]. However, m^6A modification of RNA does not interfere the reverse transcription, thus primer extension assay cannot detect the methylation of m^6A [21].

The continuous optimization of m^6A-seq or MeRIP-seq technology (A combination of anti-m^6A antibody immunoprecipitation with next-generation sequencing) revealed the first m^6A maps in bacteria [22,23]. We used a UHPLC-QQQ-MS/MS (ultra-high pressure liquid chromatography coupled with triple-quadrupole tandem mass spectrometry) to quantified the m^6A/A (mRNA) level in a wide range of bacterial species, and found high level of m^6A (~0.2%) in tested Gram-negative bacteria. In both *E. coli* and *P. aeruginosa*, hundreds of m^6A sites are enriched inside ORF with a consensus motif of GCCAG,

suggesting the importance of bacterial m^{6}A modification in mRNA [24].

The Functional Roles of RNA m^{6}A Modification in Bacteria

In recent years, a variety of methyltransferases have been found in bacteria, which play important roles in many functional pathways. RlmF and RlmJ methylate A1618 and A2030 of 23S rRNA in *Escherichia coli* [25,26]. Both *ybiN* (*rlmF*) mutant and its overexpression lead to growth defect compared to the wild-type strain [25]. In addition, KsgA (the dimethyltransferase of 16S rRNA) and the resulting modified adenosine bases appear to be conserved in all species of eubacteria, eukaryotes, and archae [27]. KsgA is also involved in antibiotic resistance [27].

Although progress has been made on m^{6}A in bacterial rRNA, its occurrence in mRNA was not clear. In light of this, our recent work employed a UHPLC-QQQ-MS/MS technology to survey the m^{6}A modification in bacterial mRNA, and found that m^{6}A widely exists in a variety of bacteria [24]. A m^{6}A-seq has identified 265 and 109 m^{6}A sites in *E. coli* and *P. aeruginosa*, respectively. The functional enrichment analysis of these sites revealed that most of m^{6}A-modified genes are involved in pathways such as respiration metabolism, amino acids metabolism, stress response and small RNAs, which suggests important functional roles of m^{6}A in these genes [24]. A recent work demonstrates that several mRNA modifications (including m^{6}A) compromise translation and amino acid incorporation *in vitro*, strongly suggesting its potential similar function *in vivo* [28].

Challenges and Prospect Forecast

Identification of the methylases, demethylases, or binding proteins of m^{6}A in bacterial mRNA is key to understand the selectivity and mechanism of this modification. Unfortunately, although three m^{6}A methylases (RlmF, RlmJ, and KsgA) have been characterized in bacterial rRNA, they do not work on mRNA [24]. There is no close homolog of either METTL3, METTL14, FTO, or ALKBH5 in bacterial genome, suggesting that the eukaryotic m^{6}A machinery is largely different from the that in bacteria. Thus far the information on m^{6}A enzymatic machinery is absent in bacterial mRNA. Protein pulldown and genetic screen can be used to identify these proteins, given the small size of proteome and efficient genetic approaches in model bacterial species such as *E. coli*.

Coupling of next generation sequencing to biochemical identification of m^{6}A-associated proteins has led to exciting discoveries of regulatory role of m^{6}A modifications in eukaryotes. In prokaryotes, a crucial question is whether m^{6}A plays functional roles in bacterial mRNA. We envision that genetic and phenotypic characterizations of aforementioned m^{6}A-associated proteins would help to draw a comprehensive picture of this emerging modification in bacterial mRNA.

References

1. Machnicka MA, Milanowska K, Osman OO, Purta E, Kurkowska M, et al. (2013) MODOMICS: a database of RNA modification pathways--2013 update Nucleic Acids Res 41: D262-D267.
2. Marbaniang CN, Vogel J (2016) Emerging roles of RNA modifications in bacteria. Curr Opin Microbiol 30: 50-57.
3. Srinivasan PR, Borek E (1964) Enzymatic Alteration Of Nucleic Acid Structure. Science 145: 548-553.
4. Starr JL, Sells BH (1969) Methylated ribonucleic acids. Physiol Rev 49: 623-669.
5. Klagsbrun M (1973) An evolutionary study of the methylation of transfer and ribosomal ribonucleic acid in prokaryote and eukaryote organisms. J Biol Chem 248: 2612-2620.
6. Ke S, Alemu EA, Mertens C, Gantman EC, Fak JJ, et al. (2015) A majority of m6A residues are in the last exons, allowing the potential for 3' UTR regulation. Genes Dev 29: 2037-2053.
7. Chandola U, Das R, Panda B (2015) Role of the N6-methyladenosine RNA mark in gene regulation and its implications on development and disease. Briefings in Functional Genomics 14: 169-179.
8. Bernhardt D, Darnell JJ (1969) tRNA synthesis in HeLa cells: a precursor to tRNA and the effects of methionine starvation on tRNA synthesis. J Mol Biol 42: 43-56.
9. Munns TW, Sims HF (1975) Methylation and processing of transfer ribonucleic acid in mammalian and bacterial cells. J Biol Chem.250: 2143-2149.
10. Liu J, Yue Y, Han D, Wang X, Fu Y, et al. (2014) A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol 10: 93-95.
11. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, et al. (2013) ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 49: 18-29.
12. Landfors M, Nakken S, Fusser M, Dahl JA, Klungland A, et al. (2016) Sequencing of FTO and ALKBH5 in men undergoing infertility work-up identifies an infertility-associated variant and two missense mutations. Fertil Steril 105: 1170-1179.
13. Aik W, Scotti JS, Choi H, Gong L, Demetriades M, et al. (2014) Structure of human RNA N(6)-methyladenine demethylase ALKBH5 provides insights into its mechanisms of nucleic acid recognition and demethylation. Nucleic Acids Research 42: 4741-4754.
14. Bokar JA, Rath-Shambaugh ME, Ludwiczak R, Narayan P, Rottman F (1994) Characterization and partial purification of mRNA N6-adenosine methyltransferase from HeLa cell nuclei. Internal mRNA methylation requires a multisubunit complex. J Biol Chem 269: 17697-17704.
15. Li R, Yang YG, Gao Y, Wang ZQ, Tong WM (2012) A distinct response to endogenous DNA damage in the development of Nbs1-deficient cortical neurons. Cell Res 22: 859-872.
16. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, et al. (2011) N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 7: 885-887.
17. Wang X, Lu Z, Gomez A, Hon GC, Yue Y, et al. (2014) N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 505: 117-120.
18. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, et al. (2015) N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell 161: 1388-1399.
19. Wang X, Feng J, Xue Y, Guan Z, Zhang D, et al. (2016) Structural basis of N6-adenosine methylation by the METTL3–METTL14 complex. Nature 534: 575-578.
20. Fellner P, Sanger F (1968) Sequence analysis of specific areas of the 16S and 23S ribosomal RNAs. Nature 219: 236-238.
21. Golovina AY, Dzama MM, Petriukov KS, Zatsepin TS, Sergiev PV, et al. (2014) Method for site-specific detection of m6A nucleoside presence in RNA based on high-resolution melting (HRM) analysis. Nucleic Acids Res 42: e27.
22. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, et al. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 149: 1635-1646.
23. Sergiev PV, Golovina AY, Osterman IA, Nesterchuk MV, Sergeeva OV, et al. (2016) N6-Methylated Adenosine in RNA: From Bacteria to Humans. J Mol Biol 428: 2134-2145.
24. Deng X, Chen K, Luo GZ, Weng X, Ji Q, et al. (2015) Widespread occurrence of N6-methyladenosine in bacterial mRNA. Nucleic Acids Res 43: 6557-6567.

25. Sergiev PV, Serebryakova MV, Bogdanov AA, Dontsova OA (2008) The ybiN gene of Escherichia coli encodes adenine-N6 methyltransferase specific for modification of A1618 of 23 S ribosomal RNA, a methylated residue located close to the ribosomal exit tunnel. J Mol Biol 375: 291-300.

26. Golovina AY, Dzama MM, Osterman IA, Sergiev PV, Serebryakova MV, et al. (2012) The last rRNA methyltransferase of E. coli revealed: the yhiR gene encodes adenine-N6 methyltransferase specific for modification of A2030 of 23S ribosomal RNA. RNA 18: 1725-1734.

27. O'Farrell HC, Scarsdale JN, Rife JP (2004) Crystal structure of KsgA, a universally conserved rRNA adenine dimethyltransferase in Escherichia coli. J Mol Biol 339: 337-353.

28. Hoernes TP, Clementi N, Faserl K, Glasner H, Breuker K, et al. (2016) Nucleotide modifications within bacterial messenger RNAs regulate their translation and are able to rewire the genetic code. Nucleic Acids Research 44: 852-862.