

N⁶-methyladenosine Modification in Bacterial mRNA

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Abstract

Among more than 140 naturally occurring RNA modifications have been identified, N⁶-methyladenosine (m⁶A) is the most abundant messenger RNA (mRNA) modification in eukaryotic organisms. A group of demethylases, methyltransferase and m⁶A-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 m⁶A modification in bacteria RNA, particularly mRNA. This review summarizes the recent research advances of eukaryotic m⁶A modification, and outlines the optimized analysis techniques together with recent progress on m⁶A modification in bacterial mRNA.

Keywords: N⁶-methyladenosine; mRNA; Bacteria

Introduction

Among more than 140 naturally occurring RNA modifications have been identified [1], N⁶-methyladenosine (m⁶A) is the most abundant messenger RNA (mRNA) modification in eukaryotic organisms. A group of demethylases, methyltransferase and m⁶A-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 [2].

However, we have little understanding of m⁶A modification in bacteria RNA, particularly mRNA. This review summarizes the recent research advances of eukaryotic m⁶A modification, and outlines the optimized analysis techniques together with recent progress on m⁶A 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, m⁶A became the most important modification in eukaryotes, thanks to the identification of a group of m⁶A-associated proteins, including METTL3, METTL14, FTO and ALKBH5 [10-14]. METTL3 and METTL14 form a heterodimer that catalyzes m⁶A RNA methylation, while WTAP (Wilms tumor 1 associated protein) interacts with the complex and affects the mRNA methylation [15].

FTO and ALKBH5 are m⁶A RNA demethylases, which are involved in development, RNA metabolism and fertility [11,16]. Moreover, two

m⁶A-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 N⁶-adenosinemethylation by the METTL3-METTL14 complex for the first time, which demonstrates the molecular basis of the m⁶A methylase reaction [19].

Although fast and exciting breakthroughs have been made on m⁶A in eukaryotic mRNA, very little is known on m⁶A in bacterial RNA. This review focuses on the bacterial m⁶A 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 ³²P-orthophosphate and either ³H or ¹⁴C-(methyl)-methionine) to detects m⁶A modification in RNA [20]. Later, the subsequent approach of primer extension assay was used for the detection of chemical modification of m²G, m¹A, m⁷G, and pseudouridine [21]. However, m⁶A modification of RNA does not interfere the reverse transcription, thus primer extension assay cannot detect the methylation of m⁶A [21].

The continuous optimization of m⁶A-seq or MeRIP-seq technology (A combination of anti-m⁶A antibody immunoprecipitation with next-generation sequencing) revealed the first m⁶A 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 m⁶A/A (mRNA) level in a wide range of bacterial species, and found high level of m⁶A (~0.2%) in tested Gram-negative bacteria. In both *E. coli* and *P. aeruginosa*, hundreds of m⁶A sites are enriched inside ORF with a consensus motif of GCCAG,

suggesting the importance of bacterial m⁶A modification in mRNA [24].

The Functional Roles of RNA m⁶A 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 m⁶A 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 m⁶A modification in bacterial mRNA, and found that m⁶A widely exists in a variety of bacteria [24]. A m⁶A-seq has identified 265 and 109 m⁶A sites in *E. coli* and *P. aeruginosa*, respectively. The functional enrichment analysis of these sites revealed that most of m⁶A-modified genes are involved in pathways such as respiration metabolism, amino acids metabolism, stress response and small RNAs, which suggests important functional roles of m⁶A in these genes [24]. A recent work demonstrates that several mRNA modifications (including m⁶A) 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 m⁶A in bacterial mRNA is key to understand the selectivity and mechanism of this modification. Unfortunately, although three m⁶A 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 m⁶A machinery is largely different from that in bacteria. Thus far the information on m⁶A 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 m⁶A-associated proteins has led to exciting discoveries of regulatory role of m⁶A modifications in eukaryotes. In prokaryotes, a crucial question is whether m⁶A plays functional roles in bacterial mRNA. We envision that genetic and phenotypic characterizations of aforementioned m⁶A-associated proteins would help to draw a comprehensive picture of this emerging modification in bacterial mRNA.

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