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N^6-methyladenosine Modification in Bacterial mRNA

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Abstract

Among more than 140 naturally occurring RNA modifications have been identified, N^6-methyladenosine (m^{6}A) is the most abundant messenger RNA (mRNA) modification in eukaryotic organisms. A group of demethylases, methyltransferase and m^{6}A-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^{6}A modification in bacteria RNA, particularly mRNA. This review summarizes the recent research advances of eukaryotic m^{6}A modification, and outlines the optimized analysis techniques together with recent progress on m^{6}A 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^{6}A) is the most abundant messenger RNA (mRNA) modification in eukaryotic organisms. A group of demethylases, methyltransferase and m^{6}A-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^{6}A modification in bacteria RNA, particularly mRNA. This review summarizes the recent research advances of eukaryotic m^{6}A modification, and outlines the optimized analysis techniques together with recent progress on m^{6}A 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^{6}A became the most important modification in eukaryotes, thanks to the identification of a group of m^{6}A-associated proteins, including METTL3, METTL14, FTO and ALKBH5 [10-14]. METTL3 and METTL14 form a heterodimer that catalyzes m^{6}A RNA methylation, while WTAP (Wilms tumor 1 associated protein) interacts with the complex and affects the mRNA methylation [15].

FTO and ALKBH5 are m^{6}A RNA demethylases, which are involved in development, RNA metabolism and fertility [11,16]. Moreover, two m^{6}A-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^{6}A methylase reaction [19].

Although fast and exciting breakthroughs have been made on m^{6}A in eukaryotic mRNA, very little is known on m^{6}A in bacterial RNA. This review focuses on the bacterial m^{6}A 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^{6}A modification in RNA [20]. Later, the subsequent approach of primer extension assay was used for the detection of chemical modification of m^{2}G, m^{1}A, m^{7}G, and pseudouridine [21]. However, m^{6}A modification of RNA does not interfere the reverse transcription, thus primer extension assay cannot detect the methylation of m^{6}A [21].

The continuous optimization of m^{6}A-seq or MeRIP-seq technology (A combination of anti-m^{6}A antibody immunoprecipitation with next-generation sequencing) revealed the first m^{6}A 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^{6}A/A (mRNA) level in a wide range of bacterial species, and found high level of m^{6}A (~0.2%) in tested Gram-negative bacteria. In both *E. coli* and *P. aeruginosa*, hundreds of m^{6}A 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.

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