m1A RNA Modification in Gene Expression Regulation

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Contributor: Shanshan Xie , , Tianhua Zhou

N^1-methyladenosine (m^{1}A) is a prevalent and reversible post-transcriptional RNA modification that decorates tRNA, rRNA and mRNA. Studies based on technical advances in analytical chemistry and high-throughput sequencing methods have revealed the crucial roles of m^{1}A RNA modification in gene regulation and biological processes.

N1-methyladenosine(m1A) RNA modification gene expression

1. Introduction

Cellular RNAs contain more than 170 different types of chemical modifications across species [1]. *N*^{1}-methyladenosine(m^{1}A) is a reversible methylation involving the addition of a methyl group at the *N*^{1} position of adenosine in cellular transcripts [2]. The methyl group can block the normal Watson–Crick base pairing of A:T or A:U, resulting in an unstable mismatch with other nucleosides by forming Hoogsteen base pairs [3]. The secondary structure and RNA–protein interaction of m^{1}A-modified RNAs are also altered under physiological conditions [4]. As a dynamic and reversible post-transcriptional RNA modification, m^{1}A can be installed by methyltransferases, removed by demethylases and recognized by m^{1}A-dependent RNA-binding proteins [2][5]. m^{1}A RNA modification affects RNA metabolism, including RNA structure, stability and mRNA translation, thereby regulating gene expression and several fundamental cellular processes [6].

m^{1}A RNA modification has been found with high abundance in transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) but at low levels in messenger RNAs (mRNAs) [7][8][9][10][11][12]. It occurs in the tRNA of bacteria, archaea and eukaryotes at positions 9, 14, 16, 22, 57 and 58 (m^{1}A9, m^{1}A14, m^{1}A16, m^{1}A22, m^{1}A57, and m^{1}A58, respectively) [13]. In cytosolic (cyt) tRNAs, m^{1}A RNA modification occurs at five different positions (9, 14, 22, 57, and 58) [14][15]. Among them, m^{1}A14 has only been identified in cyt(tRNA)^{Phe} from mammals, m^{1}A22 has only been identified in bacteria tRNAs, and m^{1}A57 has been identified in archaea existing only transiently as an intermediate of 1-methylinosine (m^{1}I) [14][15]. In mitochondria, m^{1}A9 is quite abundant and found in 14 species of mt-tRNA, while m^{1}A58 is a minor modification with a 17% frequency found in four species of mt-tRNAs [16]. Additionally, m^{1}A16 is unique to human mt-tRNA^{Arg}, and its frequency is approximately 20% [16]. For rRNAs, the nuclear-encoded large subunit rRNA m^{1}A645 in 25S rRNA and m^{1}A1322 in 28S rRNA located in the peptidyl transfer center of the ribosome are conserved in budding yeast and humans, respectively [17][18][19], and m^{1}A is conserved at position 947 of 16S rRNA in the mitochondrial ribosome of vertebrates [20]. Regarding mRNAs, m^{1}A in mRNA accounts for approximately 0.015–0.054% of all adenosines in mammalian cell lines and 0.05–0.16% in mammalian tissues [9]

[10][21]. m^1A sites are usually located near the translation start site and the first splice site of mRNA, and they are associated with the translation of coding transcripts [9][10].

2. m^1A RNA-Modifying Proteins

Reversible m^1A methylomes in nuclear- and mitochondrial-encoded transcripts are achieved via the dynamic regulation of m^1A RNA-modifying proteins (m^1A methyltransferases, m^1A demethylases and m^1A-dependent RNA-binding proteins). The characterization of m^1A-modifying proteins is crucial for understanding the mechanisms underlying m^1A-mediated gene regulation and the biological roles of m^1A RNA modification. To date, several m^1A RNA-modifying proteins responsible for nuclear- and mitochondrial-encoded transcripts have been identified in humans (**Figure 1**).

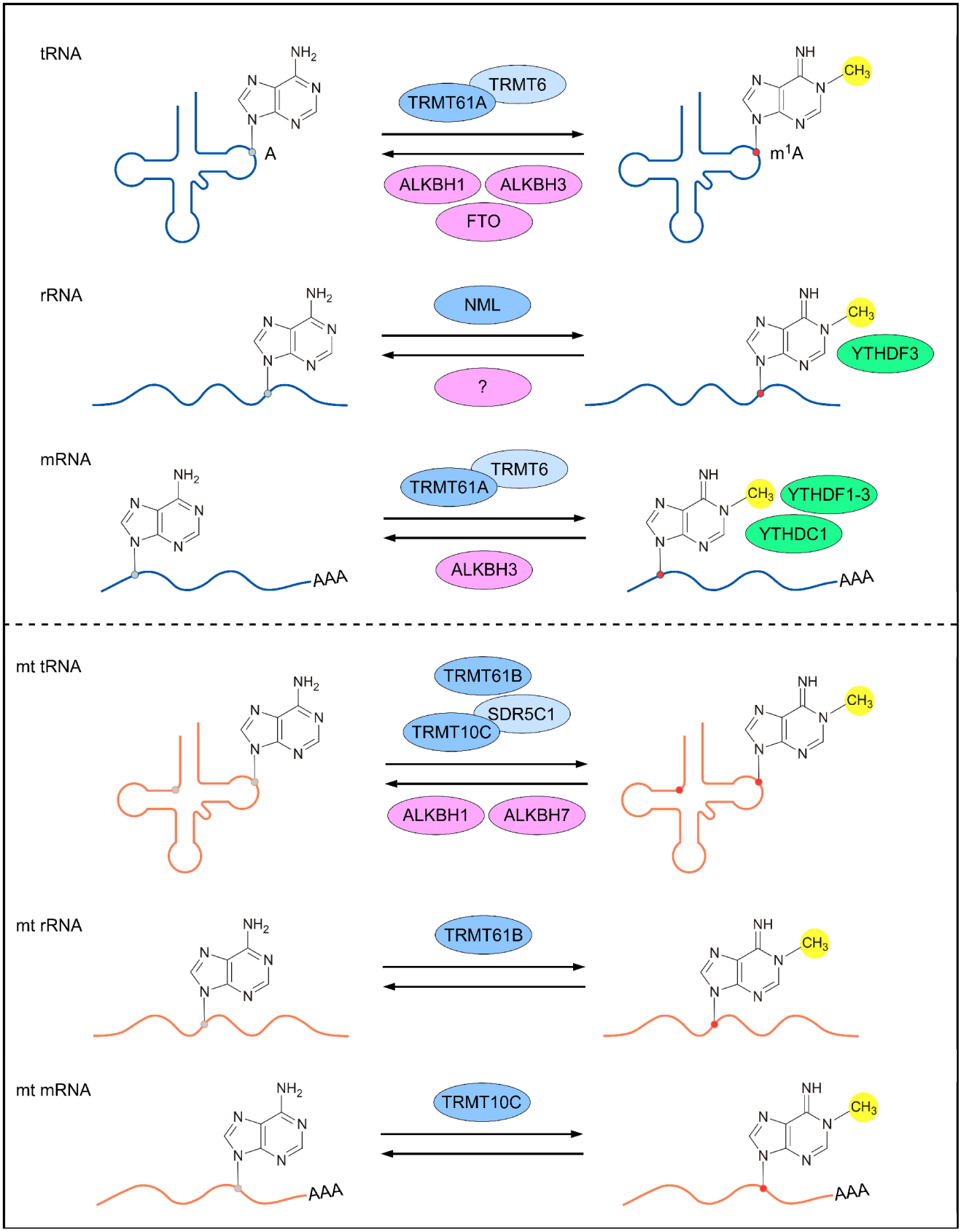

Figure 1. m^{1}A-modifying proteins for different types of RNAs. The nuclear-encoded (top panel) and mitochondrial (bottom panel) RNAs are reversibly methylated by m^{1}A methyltransferases (blue; dark blue represents catalytic core of the methylase complex), demethylased by m^{1}A demethylases (pink), and bound by m^{1}A-dependent RNA-

binding proteins (green). A, adenosine; m^1A, N^1-methyladenosine; TRMT, tRNA (adenine (58)-N (1))-methyltransferase subunit; ALKBH, α-ketoglutarate-dependent dioxygenase alkB homolog; FTO, α-ketoglutarate-dependent dioxygenase alkB homolog FTO; NML, nucleomethylin; YTHDF, YTH domain-containing family protein; YTHDC1, YTH domain-containing protein 1; SDR5C1, 3-hydroxyacyl-CoA dehydrogenase type-2.

3. Biological Functions of m^1A RNA Modification

Since the discovery of m^1A RNA modification as a chemical modification of RNAs, efforts have been taken to understand the functional characterization of this dynamic methylation in RNA metabolism and gene expression regulation.

3.1. m^1A RNA Modification in RNA Metabolism

m^1A RNA modification is a pivotal regulator of RNA metabolism, including RNA structure alteration, decay and translation (**Figure 2**).

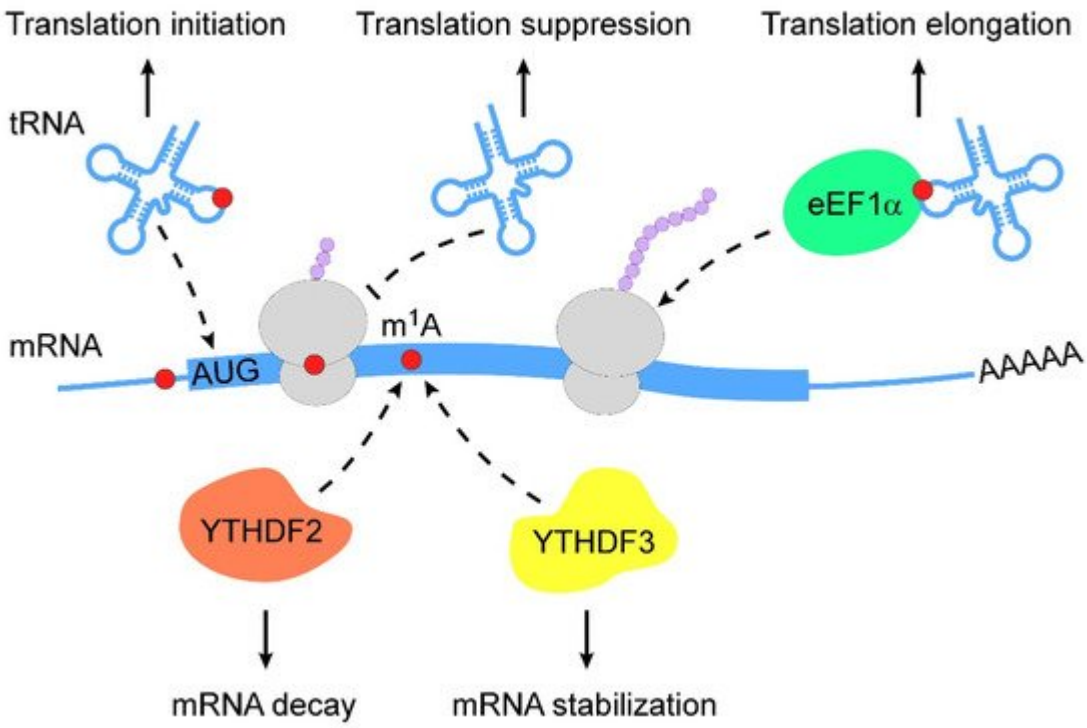

Figure 2. Action mechanisms of m^1A in RNA metabolism. m^1A RNA modification regulates RNA metabolism in multiple layers (from top to bottom: (1) m^1A RNA modification stabilizes tRNAs to promote translation initiation; (2) m^1A-modified mRNAs interfere with Watson–Crick base-pairing with tRNA to suppress translation; (3) m^1A-modified tRNAs are coupled with eEF1α to polysomes to promote translation elongation; (4) m^1A-modified mRNAs are subjected to degradation by interacting with YTHDF2; (5) m^1A-modified mRNAs become stable when they bind to YTHDF3). m^1A, N^1-methyladenosine; eEF1α, eukaryotic elongation factor 1-α; YTHDF, YTH domain-containing family protein.

The chemical properties of m^1A RNA modification enable changes in RNA secondary structure. For instance, m^1A9 and m^1A58 in tRNAs are required for the conformational shift of mitochondrial $tRNA^{Lys}$ and $tRNA^{iMet}$, respectively, which contribute to the stabilization of alternative native structures [22][23][24][25]. The loss of m^1A645 has been shown to affect the topological structure of 28S rRNA and alter the RNA interactome [26]. m^1A was also found to favor the hairpin structure of palindromic RNA sequences, wherein m^1A can stably localize within apical loops [27]. A recent study revealed that m^1A RNA modification controlled RNA conformational equilibrium by blocking base-pairing to modulate the RNA duplex [3].

The regulation of m^1A-modified mRNA decay is mediated by m^1A-dependent RNA-binding proteins. Limited evidence suggests that the knockdown of YTHDF2 increases the abundance of 7 out of 8 m^1A-modified transcripts and 2 out of 3 transcripts that bear only the m^1A but not m^6A (N^6-methyladenosine) modification [28]. In addition to YTHDF2, YTHDF3 overexpression has been reported to decrease the abundance and decay rate of *insulin like growth factor 1 receptor* (*IGF1R*) mRNA [29].

Translational regulation by m^1A modification varies among different RNA types. The m^1A demethylases ALKBH1 and FTO have been reported to control specific tRNA m^1A demethylation and decrease translation initiation [30][31]. Eukaryotic elongation factor 1-α (eEF1α) immunoprecipitation was used to reveal that m^1A-methylated tRNAs are enriched in polysomes, indicating the role of m^1A RNA modification in translation activation [30]. During retroviral reverse transcription in early human immunodeficiency virus 1 (HIV-1) replication, TRMT6-mediated m^1A58 of $tRNA_3^{Lys}$ acted as a stop site that contributed to genome integration [32]. Further, mRNAs carrying m^1A undergo translation repression because of interfered Watson–Crick base pairing [8][12][33].

3.2. m^1A RNA Modification in Biological Processes

Post-transcriptional modifications are involved in various biological processes, and recent evidence showed the importance of m^1A RNA modification in this field. In a high-temperature-sensitive *Thermococcus kodakarensis* strain, decreased m^1A58 and melting temperature of tRNA were observed, suggesting the relevance of m^1A58 and the growth ability of this strain at high temperatures [34]. m^1A RNA modification was found to exhibit its protective ability of RNAs under stress conditions. During heat shock, m^1A-harbouring transcripts were found to preferentially accumulate in stress granules, subsequently resulting in a shorter time to restore the translation state during recovery [35]. Alkylating agents induced m^1A modification in RNAs and orchestrated translational suppression by recruiting the ASCC damage repair complex (activating signal cointegrator 1 complex) [36]. The tRNA modification profiles of the *Aplysia* central nervous system showed increased m^1A RNA modification levels in animals after behavioral training [37]; this was the first study to characterize the variable pattern of m^1A RNA modification during defensive reflex-associated behavioral sensitization. *Petunia* TRMT61A catalyzed m^1A RNA modification in mRNAs, and the knockdown of TRMT61A decreased the chlorophyll content and changed chlorotic and wrinkled leaf phenotype [38]. A recent study showed that the m^1A demethylase ALKBH3 functioned as a negative regulator of ciliogenesis by removing the m^1A sites on *Aurora A* mRNA (a key regulator of cilia disassembly) in mammalian cells, which was further involved in cilia-associated developmental processes in zebrafish [39].

4. m^1A RNA Modification in Diseases

The limited exploration of m^1A RNA modification as a pathological feature has mainly focused on tumor progression (**Table 1**). It was reported that the knockdown of m^1A demethylase ALKBH3 increased the abundance of m^1A RNA modification in small RNAs (< 200 nucleotides) along with suppressed nascent protein in pancreatic cancer cells [40]. The ALKBH3-dependent m^1A demethylation of macrophage colony-stimulating factor 1 (*CSF1*) mRNA enhanced its mRNA stability and thus promoted the invasion of breast and ovarian cancer cells [41]. In addition, ALKBH3 removed the m^1A RNA modification of $tRNA^{GlyGCC}$ to promote tRNA cleavage by angiogenin. The generation of excessive tRNA-derived small RNAs may affect ribosome assembly and apoptosis in HeLa cells [42]. Furthermore, ALKBH3 promoter CpG island hypermethylation and transcriptional silencing were found in Hodgkin lymphoma cells, which were identified as a potential prognostic biomarker associated with poor clinical outcomes in patients with Hodgkin lymphoma [43]. A recent study found that levels of tRNA m^1A modification were upregulated in hepatocellular carcinoma (HCC) tissues. The TRMT6/TRMT61A complex mediated increased m^1A58 levels in tRNA, which then triggered *peroxisome proliferator-activated receptor delta* (*PPARδ*) mRNA translation in HCC stem cells. PPARδ promoted cholesterol biogenesis to activate the Hedgehog pathway, thereby initiating the self-renewal of HCC stem cells [44].

Table 1. Dysregulation of m^1A RNA modification in human cancers.

Cancers	m^1A-Modifying Proteins	Roles	Targets	Mechanisms	Refs
Pancreatic cancer	ALKBH3	Oncogene	small RNAs	Unknown	[40]
Breast and ovarian cancer	ALKBH3	Oncogene	CSF1	mRNA decay	[41]
Cervical cancer	ALKBH3	Oncogene	tRNAs	tRNA cleavage	[42]
Hodgkin lymphoma	ALKBH3	Tumor suppressor	COL1A1, COL1A2	Unknown	[43]
Hepatocellular carcinoma	TRMT6/TRMT61A	Oncogene	tRNAs	Unknown	[44]

ALKBH, α-ketoglutarate-dependent dioxygenase alkB homolog; TRMT, tRNA (adenine(58)-N(1))-methyltransferase subunit; CSF-1, macrophage colony-stimulating factor 1; COL1A1, collagen α-1(I) chain; COL1A2, collagen α-2(I) chain.

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