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m^{6}A RNA Methylation Controls Neural Development and Is Involved in Human Diseases

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Abstract

RNA modifications are involved in many aspects of biological functions. N6-methyladenosine (m^{6}A) is one of the most important forms of RNA methylation and plays a vital role in regulating gene expression, protein translation, cell behaviors, and physiological conditions in many species, including humans. The dynamic and reversible modification of m^{6}A is conducted by three elements: methyltransferases ("writers"), such as methyltransferase-like protein 3 (METTL3) and METTL14; m^{6}A-binding proteins ("readers"), such as the YTH domain family proteins (YTHDFs) and YTH domain-containing protein 1 (YTHDC1); and demethylases ("erasers"), such as fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5). In this review, we summarize the current knowledge on mapping mRNA positions of m^{6}A modification and revealing molecular processes of m^{6}A. We further highlight the biological significance of m^{6}A modification in neural cells during development of the nervous system and its association with human diseases. m^{6}A RNA methylation is becoming a new frontier in neuroscience and should help us better understand neural development and neurological diseases from a novel point of view.

Keywords N6-methyladenosine (m^{6}A) · Methyltransferase · Demethylase · METTL3 · FTO · Neural development

Introduction

Posttranscriptional modification of RNAs has been known for over 70 years. Several modifications have been identified in native cellular RNAs including mRNAs, tRNAs, rRNAs, small nuclear RNAs (snRNAs), and small nucleolar RNAs (snoRNAs) [1, 2]. RNA modifications have enriched the diversity of RNA functions and processing of RNA genetic information. m^{6}A modification of mRNAs is the major form of RNA modification compared to other types of RNA modifications, such as N1-methyladenosine (m^{1}A), N7-methyladenosine (m^{7}A), N7-methylguanosine (m^{7}G), 5-methylcytosine (m^{5}C), pseudouridine (Ψ), N6,2-Odimethyladenosinez (m^{6}A$_m$), and 2′-O-methylation (2′OMe) (Fig. 1).

m^{6}A is the most abundant modification in eukaryotic mRNAs, which accounts for more than 80% of RNA base methylations, and exists in various species [3–9]. m^{6}A modification draws significant attention after nuclear RNA m^{6}A was reported to be one of the major substrates for FTO, which was identified as a demethylase that regulates m^{6}A methylation in a dynamic and reversible manner [10]. With advances in technology, a complex of methyltransferase including METTL3, METTL14, Wilms tumor1-associating protein (WTAP), and KIAA1429 has been uncovered [11–14]. In addition, YTHDF1, YTHDF2, YTHDF3, YTHDC1, and heterogeneous nuclear ribonucleoprotein A2B1 (HNRNPA2B1) and HNRNPC have been demonstrated as m^{6}A-binding proteins, which play a regulatory role by influencing mRNA stability, export, translation, splicing, and decay [2, 15–19]. On the other hand, ALKBH5 is found to be a FTO-like demethylase [20].

Furthermore, recent studies have shown that dysregulation of RNA methylation is associated with many biological processes including neurodevelopment and neurodegenerative diseases [21, 22], even though m^{6}A does not alter the ability of base pairing [23, 24].

In this review, we summarize the molecular processes of m^{6}A modifications, biological roles of m^{6}A in regulating gene

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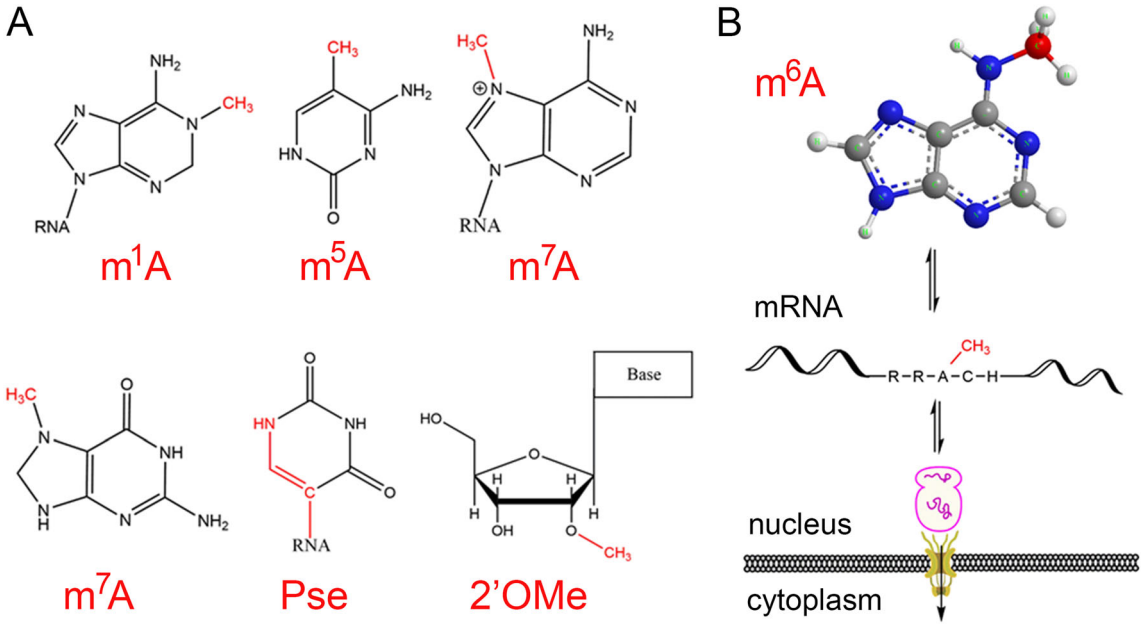

Fig. 1 Different types of mRNA modifications. **a** N1-methyladenosine (m^1A), 5-methylcytosine (m^5C), N7-methyladenosine (m^7A), N7-methylguanosine (m^7G), pseudoridine (Ψ), and 2′-O-methylation (2'OMe) are methylated at different positions (red) on mRNAs. **b** The m^6A modification is the most abundant methylation on mRNAs and is mainly present on the conserved sequence RRACH (R = G/A; H = A/C/U). The process of m^6A modification mostly occurs in the nucleus of a cell and is dynamically and reversibly modified

expression, and behaviors in cells and organisms. In particular, we highlight m^6A modification in neural development and its dysregulation in human disorders.

Mapping of m^6A Modification in RNAs

Determining positions of m^6A in the gene transcript is a key step to understand the fundamental roles of m^6A. Transcriptome-wide m^6A profiling has shown that m^6A modification is present in thousands of RNA transcripts with unique distribution patterns [25–27].

Two independent transcriptome studies have revealed that m^6A modification on mRNAs occurs at a frequency of 1 m^6A per 2000 ribonucleotides (NTs) on average [25, 26]. To identify and localize m^6A sites at a transcriptome-wide level, m^6A sequencing (m^6A-seq) approach has been applied. Methodically, total RNA is fragmented into about 100 NTs and immunoprecipitated using an anti-m^6A affinity-purified antibody. Libraries are prepared from immunoprecipitated NTs for sequencing, and subsequently, m^6A sites are identified using a peak-detection algorithm [25]. Motif search from m^6A peak sites has revealed the tendency of m^6A appearance in the RRACH (R = G/A; H = A/C/U) consensus sequence [10, 13, 25].

Early research with m^6A-seq indicated that m^6A modification mainly occurs in intragenic regions, including coding sequences (CDSs), stop codon flanking regions, and 3′ untranslated regions (3′UTRs) with a bias in the 3′ end of CDSs and the first quarter of the 3′UTR [25, 26] (Fig. 2a). Interestingly, follow-up studies have shown that m^6A is highly enriched in 3′UTRs (> 40%) of mRNAs, especially in the beginning (within 150–400 NTs) of the last exon, where it rises sharply (6-fold), while there is no preference for location of m^6A sites around stop codons [28]. Moreover, most exonic m^6As are located in the last exon in genes expressed in mouse brains (Fig. 2b). When the stop codon is not in the last exon, m^6A is enriched in the last exon but not around the stop codon [28].

Dynamic and Reversible Modification of m^6A

m^6A modification is a dynamic and reversible process in cells because of the presence of methyltransferase and demethylase (Fig. 3). For easy understanding of RNA modifications, researchers have called m^6A methyltransferases as "writers," their binding proteins as "readers," and demethylases as "erasers."

The "Writers" of m^6A Modification

m^6A generally refers to the methylated site of the sixth nitrogen of adenosine distributed in an mRNA. Methylation of m^6A is catalyzed by several proteins to form complexes, including METTL3 [29], METTL14 [12], WTAP [30, 31], KIAA1429 [14, 32], RNA-binding motif protein 15 (RBM15) [33, 34], and its paralogue RBM15B [34]

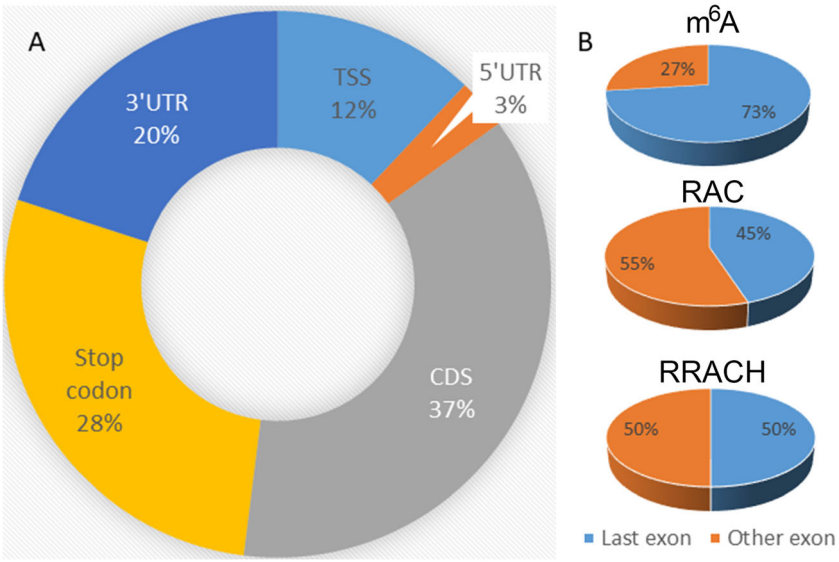

Fig. 2 Mapping m^{6}A modification on RNAs. **a** Fractions of m^{6}A peaks in each of the five nonoverlapping transcript segments, including coding sequences (CDSs), 3′ untranslated regions (3′UTRs), 5′UTR, transcription start site (TSS), and near stop codons. **b** Relative proportion of m^{6}A peaks and the RAC and RRACH motifs in the last exon and other exons

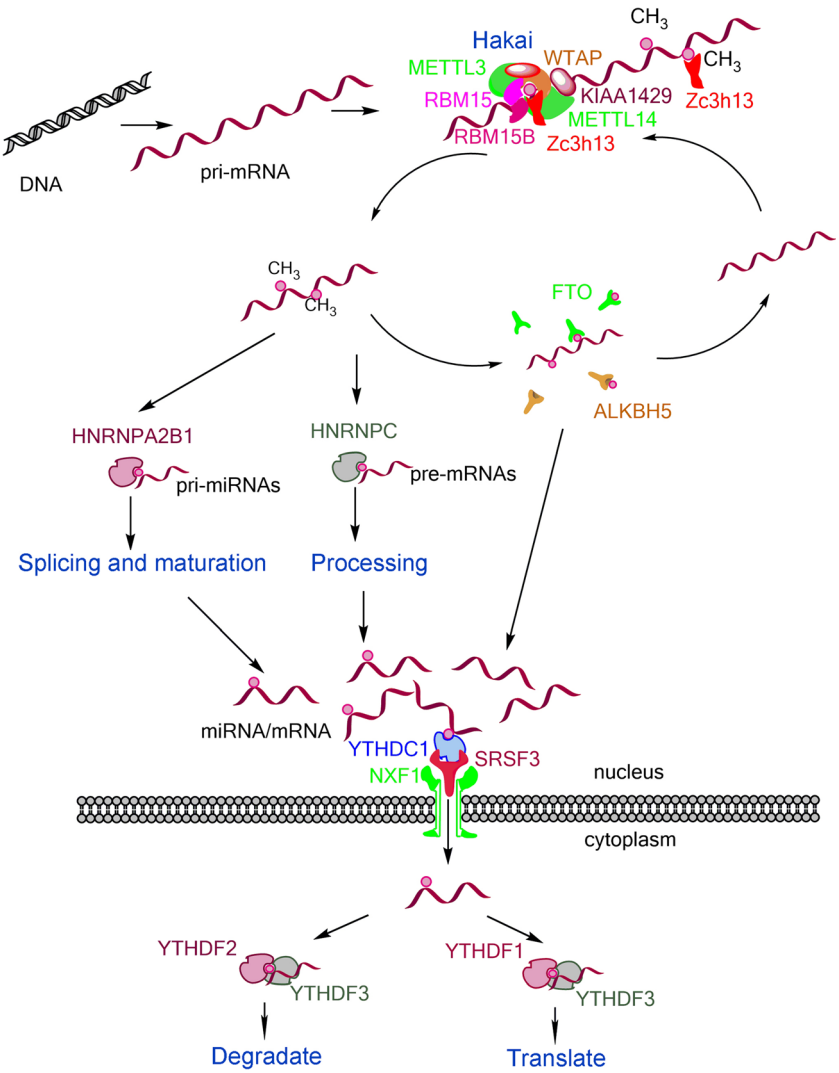

Fig. 3 Biological process of m^{6}A modification. In the nucleus, DNA is transcribed into primary mRNA (pri-mRNA). For easy understanding of RNA modifications, m^{6}A methyltransferases are named as "writers," their binding proteins named as "readers," and demethylases named as "erasers." The "writers" complex includes METTL3, METTL14, WTAP, KIAA1429, Hakai, RBM15, and RBM15B. The complex is translocated by Zc3h13 to the precursor mRNAs (pre-mRNAs) to conduct m^{6}A modification. On the other hand, m^{6}A can be eliminated by "erasers" FTO and ALKBH5. Furthermore, m^{6}A is recognized by the "readers." The HNRNPC protein affects pre-mRNA stability, splicing, export, and translation by binding to nascent RNA transcripts. HNRNPA2B1 recognizes m^{6}A sequences and facilitates the processing of a set of primary miRNAs (pri-miRNAs). YTHDC1 incorporates bound mRNAs into a dephosphorylated SRSF3-containing complex and facilitates their export via the NXF1. In addition, YTHDF proteins recognize m^{6}A on mRNAs in the cytoplasm and control degradation and translation through a cooperative regulation

(Fig. 3).Although METTL3 and METTL14 both have methyltransferase domains, crystal structure studies demonstrate that only METTL3 has catalytic activity, while METTL14 plays a role in stabilizing METTL3 and its interaction with the RNA molecule [35, 36]. METTL3 and METTL14 form heterodimers and then bind to WTAP to form a methyltransferase complex, while KIAA1429 and Hakai (also known as CBLL1, an ubiquitin ligase) function as components associated with WTAP in mammalian cells [37]. Additionally, Zc3h13 plays a role in nuclear localization of other components in the methyltransferase complex [38] (Fig. 3).

A proteomics analysis of WTAP has identified KIAA1429, RBM15, and RBM15B as interactors [34, 37]. Depletion of KIAA1429 resulted in substantial loss of m^{6}A, suggesting an essential role of KIAA1429 in the methylation process [14, 19]. Moreover, knockdown of RBM15 and RBM15B caused a significant reduction of m^{6}A in mRNAs [34]. The individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) study has shown that RBM15 and RBM15B bind to U-enriched regions adjacent to the m^{6}A site on mRNAs, rather than directly to the RRACH motif [34]. The WTAP/METTL3/METTL14 complex is subsequently recruited to methylation sites nearby the RRACH sequence. Interestingly, the RRACH site is not fully methylated near the U-rich region, so it seems that RBM15/RBM15B-mediated methylation may only be present in a subset of cellular RNAs [34].

The "Readers" of m^{6}A Modification

The presence of m^{6}A produces preferential binding sites for certain proteins that recognize and combine with the modified RNA base [39]. At the same time, this combination can affect many aspects of RNA function. For example, YTHDC1 has been identified as a reader of m^{6}A marks on mRNAs (Fig. 3). It displays a 10–50-fold higher affinity for m^{6}A methylated mRNAs than for unmethylated mRNAs [40–42]. YTHDC1 regulates splicing and is required for proper function of the long noncoding RNA X-inactive-specific transcript (*XIST*) [25, 43]. YTHDC1 incorporates bound mRNAs into a dephosphorylated serine and arginine-rich splicing factor 3 (SRSF3)-containing complex and facilitates their export via the nuclear export factor 1 (NXF1) [44].

Moreover, YTHDF proteins directly bind and recognize m^{6}A methylation on mRNAs [2, 15]. Studies have shown that YTHDF1, YTHDF2, and YTHDF3 provide spatiotemporal control over RNA metabolism in a collaborative way. YTHDF1-mediated translation increases translation efficiency, ensuring effective protein production from dynamic transcripts that are marked by m^{6}A [45]. In the cytosol, YTHDF1 enhances translation of its targets by interacting with initiation factors and facilitating ribosome loading [45]. The carboxy-terminal domain of YTHDF2 selectively binds to m^{6}A-containing mRNA, whereas the amino-terminal domain is responsible for the localization of the YTHDF2-mRNA complex to cellular RNA decay sites [2]. Therefore, YTHDF2 promotes mRNA degradation by localizing m^{6}A-modified mRNA to appropriate processing bodies [2]. YTHDF2 also changes its own cellular localization in response to heat-shock stress [46]. YTHDF1 and YTHDF2 share a set of common target mRNAs, and YTHDF1 binds earlier during the mRNA life cycle than YTHDF2 [45]. Moreover, YTHDF3 promotes protein synthesis in synergy with YTHDF1 and affects methylated mRNA decay mediated through YTHDF2 [47] (Fig. 3).

In addition, m^{6}A can recruit additional accessory factors, which directly recognize m^{6}A to destabilize the RNA structure and facilitate binding of HNRNPC, an abundant nuclear ribonucleoprotein responsible for pre-mRNA processing [2, 43, 48–51] (Fig. 3). Normally, HNRNPC preferably binds to single-stranded U-tracts (five or more contiguous uridines) [19, 48, 49] and affects pri-mRNA stability, splicing, export, and translation by binding to nascent RNA transcripts [43, 48, 49, 51] (Fig. 3). Posttranscriptional m^{6}A modifications can regulate RNA-HNRNPC interactions through modulating the structure of coding and noncoding RNAs, which in turn influences gene expression and maturation in the nucleus. Moreover, m^{6}A controls the RNA-structure-dependent accessibility of RBMs to affect RNA-protein interactions for biological regulation. This mechanism is termed the "m^{6}A-switch" [19].

In the nucleus, HNRNPA2B1 binds to m^{6}A-modified transcripts and regulates splicing and maturation of microRNA (miRNA) [18]. HNRNPA2B1 is a bridge between the m^{6}A mark and effectors, such as DiGeorge syndrome critical region 8 (DGCR8) and the ribonuclease type III DROSHA, which are involved in primary miRNA (pri-miRNA) processing [18, 52–56]. Reciprocal immunoprecipitation revealed that endogenous DGCR8 co-precipitates HNRNPA2B1. Consistent with this, HNRNPA2B1 depletion reduced the binding of endogenous DGCR8 with HNRNPA2B1-dependent pri-miRNA substrates. Thus, HNRNPA2B1 promotes processing of METTL3-dependent miRNAs [18].

The "Erasers" of m^{6}A Modification

The discovery of two demethylases, FTO and ALKBH5, implies that m^{6}A modification is dynamic and reversible [10, 20] (Fig. 3). FTO is the first enzyme identified to demethylate RNA 3-methyluridine (m^{3}U) and m^{6}A [57]. It is also a potent regulator of nuclear mRNA processing events such as alternative splicing and 3′ end mRNA processing [58]. FTO belongs to the nonheme Fe(II)- and α-KG-dependent dioxygenase AlkB family proteins [10]. It efficiently induces demethylation of m^{6}A at neutral pH in vitro, and the amount of m^{6}A in cellular mRNAs is affected by the oxidation activity of FTO in vivo. *FTO* knockout mouse displays increased m^{6}A, altered dopaminergic neurotransmission, lean body mass, and changes in the metabolic rate [59, 60]. Interestingly, recent studies

have shown that FTO preferentially catalyzes demethylation of m^6A_m rather than m^6A, and reduces the stability of m^6A_m mRNAs [61] (Fig. 4). The catalytic constant (k_{cat}) for FTO toward m^6A_m is at least 20 times higher than m^6A, and the catalytic efficiency of FTO is approximately 100-fold higher toward m^6A_m than m^6A. Notably, the 2′-O-methyl substituent, which distinguishes m^6A_m from m^6A, is also important for the demethylation activity of FTO [62]. These studies imply that FTO is one of the main demethylases in m^6A, and has an optimal effect on m^6A_m.

Furthermore, *ALKBH5* knockdown results in subtle increases in m^6A on cellular poly(A) mRNAs, and *ALKBH5* overexpression has an opposite effect, suggesting that ALKBH5 might serve as another demethylase and regulate m^6A in mRNAs [20] (Fig. 3). Different from FTO demethylation on m^6A_m, ALKBH5 has no activity toward m^6A_m and appears specific to m^6A [61]. The demethylation activity of ALKBH5 significantly affects mRNA export and RNA metabolism as well as the assembly of mRNA processing factors in nuclear speckles. In addition, DEAD-box RNA helicases (DDX3) can modulate demethylation of mRNAs and miRNAs through binding to ALKBH5 and plays essential roles in key biological processes such as cell cycle, stress response, apoptosis, and RNA metabolism [63].

Functions of m^6A Modification

Functions of m^6A in biological processes have been highly appreciated [10]. m^6A modification plays crucial roles in embryonic stem cell maintenance and differentiation [11, 64–66], development and maintenance of acute myeloid leukemia (AML) and self-renewal of leukemia stem/initiation cells (LSCs/LICs) [67], circadian rhythm modification and dosage compensation [68, 69], heat shock response [46], meiotic progression [70, 71], DNA ultraviolet (UV) damage [72], *XIST*-mediated gene silencing [34], and human diseases.

m^6A Regulates Transcription and Translation

The major role of m^6A is posttranscriptional fine-tuning of gene expression. Opposite to miRNA regulation, which normally silences gene expression, m^6A provides a fast track to maximize gene expression at the posttranscriptional regulatory level. m^6A can also reduce gene expression at specific developmental stages or in specific cell types [73]. Silencing the m^6A methyltransferase has a significant impact on changing alternative splicing patterns and gene expression, particularly in the p53 signaling pathway and apoptosis [25, 32, 74].

The m^6A formation on *XIST*, as well as on cellular mRNAs, is mediated by RBM15 and RBM15B, which form the m^6A-methylation complex and recruit them to specific sites on RNAs. This results in the methylation of adenosine nucleotides in adjacent m^6A consensus motifs, which is essential to maintain *XIST*-mediated gene silencing [34].

Protein translation typically begins with the recruitment of the 43S ribosomal complex to the 5′ cap of mRNAs by a cap-binding complex. Interestingly, m^6A modification of mRNAs is co-transcriptional and depends upon the dynamics of the transcribing RNA polymerase II (RNAPII). Suboptimal transcription rates lead to elevated m^6A content, which may result in reduced translation [75]. Moreover, some transcripts are translated in a cap-independent manner. In response to heat shock stress, certain adenosines are preferentially methylated within the 5′UTR of newly transcribed mRNAs. The dynamic 5′UTR methylation is generated by stress-induced nuclear localization of YTHDF2. The nuclear YTHDF2 preserves 5′UTR methylation of stress-induced transcripts by limiting FTO demethylation. The increased formation of m^6A in 5′UTR promotes cap-independent translation initiation, which provides a mechanism for selective translation of mRNAs under heat shock stress [46]. Additionally, a single site m^6A modification in the 5′UTR enables translation initiation independent of the 5′end m^7G cap [17, 46]. Therefore, m^6A modification is essential for proper RNA transcription and protein translation.

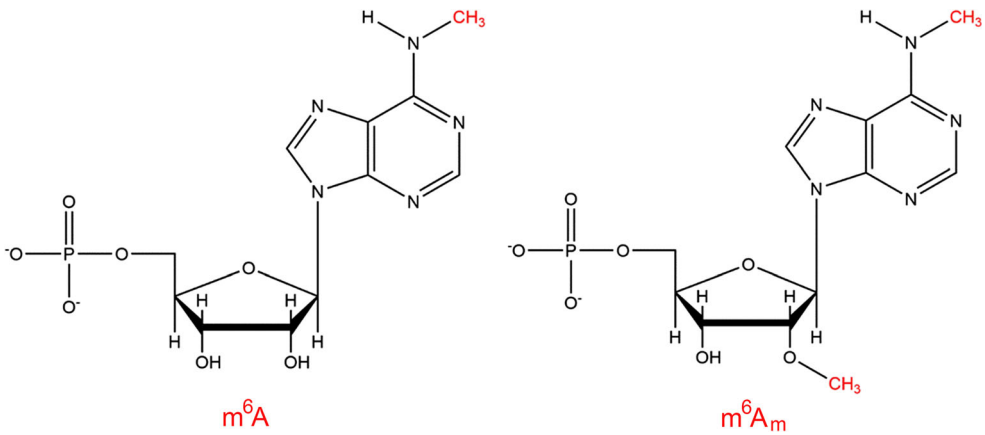

Fig. 4 m^6A and m^6A_m are two substrates catalyzed by FTO. There is one more 2′-O-methyl modification on the ribose moiety of the nucleotide in m^6A_m than m^6A. FTO displays a higher efficiency in demethylation of m^6A_m than m^6A and reduces the stability of m^6A_m mRNAs

m^{6}A Controls Cell Behaviors

Studies have shown that m^{6}A methylation of mRNAs controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathway [76]. m^{6}A plays important roles in inducible mRNA degradation of suppressor of cytokine signaling (SOCS) genes in response to IL-7 signaling in order to reprogram naïve T cells for proliferation and differentiation, as demonstrated by generating conditional knockout mice of the m^{6}A writer gene *METTL3* in CD4$^+$ T cells [76]. In an adoptive transfer model of lymphopenic mouse, naïve *METTL3*-deficient T cells fail to undergo homeostatic expansion and remain in the naïve state for up to 12 weeks.

In addition, m^{6}A modulates specification of hematopoietic stem cells and progenitor cells [77]. *ALKBH5*-deficient male mice have shown increased m^{6}A levels in mRNAs, resulting in aberrant apoptosis and further impaired fertility [70].

m^{6}A Regulates Physiological Conditions in Different Species

Methylation of m^{6}A exists in genomes of many species and has a close relationship with the biological functions of an organism. Methyltransferase has been shown to affect the meiosis process of *yeast*. In *yeast*, the core RNA methyltransferase consists of three components that form a MIS complex: Mum2 (orthologous to mammalian WTAP), Ime4 (orthologous to mammalian METTL3), and Slz1 (MIS) [78]. This MIS complex functions in starvation pathways. Prolonged starvation leads to lineage restriction, for instance, cells exiting meiotic prophase are committed to complete sporulation even if nutrients are restored. Lineage restriction during this time is, in part, dependent on the activation of mRNA methyltransferase Ime4. Mutations in any of the three genes that comprise the core RNA methyltransferase are not lethal in *yeast*. However, mutations in each of the three protein components lead to impaired meiosis [78, 79].

The modification of m^{6}A is required for embryogenesis in *Arabidopsis thaliana* [8]. *Arabidopsis thaliana* mRNAs contain m^{6}As that are similar to those of animal cells' mRNAs. Inactivation of the writer protein METLL3 results in failure of progressive transition from the globular stage during embryonic development [80].

During *zebrafish* embryogenesis, m^{6}A determines the endothelial-to-hematopoietic transition (EHT) to specify the earliest hematopoietic stem/progenitor cells (HSPCs) [81]. High m^{6}A mRNA methylation has been detected in embryos from 5 to 28 h postfertilization. m^{6}A modification in endothelial cells specifically regulates HSPC generation through inhibiting endothelial Notch signaling.

Furthermore, some corresponding homologs, such as inducer of meiosis 4 (Ime4, homolog for METTL3), karyogamy protein 4 (KAR4, homolog for METTL14), Female-lethal (2)d (Fl(2)d) and virilizer (Vir), have been identified in *Drosophila* [82]. Zinc finger CCCH domain-containing protein 13 (Zc3h13) and Fl(2)d-associated complex component (Flacc) have been identified as novel interactors of the m^{6}A methyltransferase complex in *Drosophila* and mice [38, 83]. Fl(2)d and Vir are required for sex-dependent regulation of alternative splicing of the sex determination factor Sex lethal (Sxl). In addition, *Drosophila Ime4*-null mutants, in which m^{6}A is absent in their mRNAs, remain viable, though flightless, and show a sex bias toward maleness [82].

m^{6}A Modification in Neural Development and Human Diseases

m^{6}A modification has been shown to play important roles in the development of the nervous system by using mouse genetic and cell culture approaches. Dysregulation of m^{6}A is also associated with human disorders.

m^{6}A Modification Regulates Neural Development

The cerebral cortex is constructed by different projection neurons, which emerge from a pool of neural stem cells and neural progenitors divided at the ventricular zone (VZ) [84]. Developmental perturbations of birth date, location, and connectivity of newborn neurons can cause neurological and psychiatric disorders. *METTL14* deletion in the central nervous system caused prolonged cortical neurogenesis into postnatal stages [85]. Decreasing m^{6}A levels using either *METTL14* knockout or *METTL3* knockdown resulted in protracted cell-cycle progression of cortical neural progenitors and reduced differentiation of radial glia cells (RGCs) [85].

Moreover, m^{6}A-seq of human fetal forebrains and human brain organoids has revealed conserved and unique m^{6}A landscape features compared to those in mouse embryonic forebrains [85]. Gene ontology (GO) analysis of m^{6}A-tagged transcripts has shown enrichment of human genes related to neurogenesis, neuronal differentiation, and development. Disease ontology analysis has also identified some m^{6}A-tagged genes related to mental disorders, mental retardation, schizophrenia, and bipolar disorder [85]. These analyses imply the importance of m^{6}A modification in human brain development and neurological disorders.

In addition, studies have shown high expression of FTO in several brain regions in mice [86, 87] and its association with nutritional regulation [88]. *FTO*-deficient mice display retardation of postnatal growth, reduced IGF-1 concentrations, increased energy expenditure, and altered locomotor activity [60]. These phenotypes are very similar to the major characteristics of mice lacking dopamine receptor type 2 (D2R) [89]. D2R is a crucial signaling pathway regulating learning,

reward behavior, motor functions, and feeding [90, 91]. Analyses of global m^{6}A modification in the midbrain and striatum of *FTO*-deficient mice have revealed increased adenosine methylation in a subset of mRNAs important for neuronal signaling, including the dopaminergic (DA) signaling pathway [59]. Moreover, FTO has been shown to regulate demethylation of specific mRNAs in vivo, and this activity is related to the control of DA transmission [59].

Another interesting finding has revealed that Fl(2)d protein is distributed throughout the entire eye-antennal imaginal disc in *Drosophila* [92]. Mutations of Fl(2)d lead to perturbations in retinal development. In addition, a biochemical complex consisting of Sine Oculis (So) and Fl(2)d has been identified using *yeast* two-hybrid screens and co-immunoprecipitation assays and plays a role in the developing eye in *Drosophila* [92].

Human Diseases Are Associated with m^{6}A Modification

Dysregulation of m^{6}A modifications has been shown to be related to human diseases. *FTO* gene mutations cause growth retardation, developmental delay, and facial dysmorphism in patients [10, 93]. Variants of the *FTO* gene have been linked to attention deficit disorder in humans [60, 94]. These variants were initially identified as the strongest genetic factor known to predispose humans to nonmonogenic obesity [95, 96]. However, it has been proposed that there is no direct connection between the obesity-associated variants and FTO expression or function. The link between the *FTO* gene and obesity may be from regulatory elements for the adjacent iroquois homeobox 3 (IRX3) gene within the *FTO* gene, rather than a function of the FTO protein [97]. Besides, studies have shown that the obesity-associated variants are also associated with nonobesity phenotypes, such as reduced brain volume in healthy elderlies [96], and are inversely correlated with alcohol consumption and risk for alcohol dependence [98].

Consistent with defects in humans, *FTO* knockout mice also display severe growth retardation [60]. Mechanistically, altered m^{6}A modifications are likely the cause of defects in humans and mice. For example, m^{6}A modifications are present in over 4500 mRNAs in the mouse brain, and *FTO* overexpression in cultured cells leads to reduced amounts of m^{6}A on mRNAs [10, 25, 26].

The TGFβ pathway has an essential role in embryonic development, organ homeostasis, tissue repair, and disease pathogenesis. These diverse effects are mediated through the intracellular effectors SMAD2 and SMAD3 (hereafter SMAD2/3). SMAD2/3 promotes binding of the m^{6}A methyltransferase complex to a subset of transcripts involved in early cell fate decisions. This mechanism destabilizes specific SMAD2/3 transcriptional targets, including the pluripotency factor gene NANOG, priming them for rapid downregulation upon differentiation to enable a timely exit from pluripotency [99].

m^{6}A has an extremely important role in the occurrence and development of cancer, and a significant number of cancers have been related to m^{6}A [100–105]. For example, m^{6}A affects the invasiveness and enrichment of cancer cells [105, 106]. Mutations and/or copy number variations of m^{6}A regulatory genes are strongly associated with the presence of TP53 mutations in AML patients. Alterations in m^{6}A regulatory genes confer a lower survival rate in patients with AML. Therefore, genetic alterations of m^{6}A regulatory genes may cooperate with TP53 and/or its regulators/downstream targets in the pathogenesis and maintenance of AML [107]. Moreover, METTL14 is highly expressed in AML cells and is downregulated during myeloid differentiation. METTL14 exerts its oncogenic role by regulating its mRNA targets through m^{6}A modification [67].

Immunostaining of YTHDF1 displayed its expression associated with various malignant tumor behaviors, such as depth, lymph node metastasis, and more advanced cancer stages in colorectal cancer [108]. Studying upstream of the YTHDF1 gene indicated that an oncogenic transcription factor c-Myc is associated with YTHDF1 in both expression and chromatin immunoprecipitation [108]. In addition, ALKBH5 is highly expressed in glioblastoma stem-like cells (GSCs). Silencing ALKBH5 suppresses the proliferation of patient-derived GSCs [20, 109]. Exposure of breast cancer cells to hypoxia has been found to stimulate hypoxia-inducible factor (HIF)-1α- and HIF-2α-dependent expression of ALKBH5 [106].

Furthermore, m^{6}A is closely related with degenerative and neurodevelopmental diseases [22, 59, 110, 111]. FTO is critically involved in insulin defect-related Alzheimer's disease (AD), and conditional knockout of *FTO* in neurons reduces the cognitive deficits in AD mice [112]. Defective insulin signaling in diabetes and obesity in humans and mice activates the FTO in the brain tissues. Lentivirus-mediated knockdown of FTO reduces the phosphorylation of Tau protein, whereas overexpression of FTO promotes the level of phosphorylated Tau in neurons [112]. A mechanistic study has demonstrated that FTO activates the phosphorylation of Tau in mammalian targets in a rapamycin (mTOR)-dependent manner [112].

Moreover, a survey of Chinese Han people has shown that the *ALKBH5* gene is associated with the risk of major depressive disorder (MDD) [110]. Even though how m^{6}A modifications affect human health is still unclear, its importance to biological functions is increasingly appreciated.

Conclusions and Perspectives

Posttranscriptional modifications of RNAs, in particular methylation of RNAs, have drawn significant attention due to their broad functions in regulating gene expression, cell behaviors, and physiological conditions in many species, including humans. Because of the successful identification of several

key molecules in the m^{6}A biological processing pathway, such as METTL3 and FTO, m^{6}A-related regulatory mechanism is becoming a major focus. However, detailed molecular mechanisms of m^{6}A modifications remain unclear.

The biological significance of the m^{6}A positions in mRNAs requires further exploration. Studies have shown that FTO does not affect all m^{6}A modifications on mRNAs, suggesting a preferential regulation of FTO in a subset of RNAs. It is also unknown why there is a selective m^{6}A modification, and how it controls gene expression. Moreover, molecular regulations of m^{6}A modifications in specific cell types at different developmental stages and in distinct tissues and organs need to be further investigated. The correlation of specific m^{6}A modification with certain biological processes and functions in the nervous system requires vigorous examination. In particular, how m^{6}A modification regulates normal physiological conditions and modulates human diseases needs to be further understood.

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