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Reversible RNA adenosine methylation in biological regulation

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Abstract

N^6-methyladenosine (m^6A) is a ubiquitous modification in messenger RNA (mRNA) and other RNAs across most eukaryotes. For many years, however, the exact functions of m^6A were not clearly understood. The discovery that the fat mass and obesity associated protein (FTO) is an m^6A demethylase indicates that this modification is reversible and dynamically regulated, suggesting it has regulatory roles. In addition, it has been shown that m^6A affects cell fate decisions in yeast and plant development. Recent affinity-based m^6A profiling in mouse and human cells further showed that this modification is a widespread mark in coding and non-coding RNA transcripts and is likely dynamically regulated throughout developmental processes. Therefore, reversible RNA methylation, analogous to reversible DNA and histone modifications, may affect gene expression and cell fate decisions by modulating multiple RNA-related cellular pathways, which potentially provides rapid responses to various cellular and environmental signals, including energy and nutrient availability in mammals.

RNA modifications

RNA plays a central role in multiple cellular processes, including functioning as a carrier of genetic information, catalyzing and regulating biochemical reactions, serving as an adapter molecule in protein synthesis, and providing a structural scaffold in subcellular organelles [1]. To create more diversity to fulfill all the different functions of RNAs, numerous modifications are added to the four canonical bases of the nascent precursor RNA transcripts. More than 100 post-transcriptionally modified ribonucleosides have been identified in almost all types of RNA [2]. In addition, recent evidence has shown that RNA modifications are very dynamic [3,4], which may increase the functional complexity and diversity of RNA and suggests some of these modifications participate in the regulation of gene expression at the post-transcriptional level.

In eukaryotic mRNA, different types of methylation modifications have been documented. Generally demonstrated as $m^7G(5')ppp(5')N_{1(m)}pN_{2(m)}pNpNpN\ldots$, mRNA includes N^7-methylguanosine (m^7G) at the 5′ cap structure [5–7], N^6-methyl-2′-*O*-methyladenosine (m^6A_m) at the first position of the 5′ terminus (N_1) and internal positions [8,9], 2′-*O*-methylated nucleosides (N_m) on the first two starting positions at the 5′ terminus (N_1 and N_2) and in internal sequences [8–10], internal 5-methylcytosine (m^5C) [11], and internal N^6-methyladenosine (m^6A) [8,9,12] (Figure 1). Among these, only the modifications on the cap have been well studied: m^7G is involved in mRNA processing such as translation initiation, mRNA transport, splicing, and degradation [13,14]; and 2′-*O*-methylation within the cap

structure of mRNA is thought to provide a molecular signature for host cells to distinguish self-mRNAs from non-self RNAs through sensory proteins in viral/mammalian systems [15,16]. By contrast, despite being discovered decades ago, the function of the most prevalent internal modification of eukaryotic mRNA, m^{6}A [17], has remained unclear. The recent discovery of the fat mass and obesity-associated (FTO) protein as an m^{6}A-demethylase demonstrates that m^{6}A is a reversible and dynamic RNA modification that may impact mammalian energy homeostasis [18]. In this review we briefly introduce m^{6}A in mRNA and the enzymes involved in the methylation and demethylation processes, describe recent results obtained from the m^{6}A methylome, and discuss potential biological roles of this dynamic modification.

m^{6}A methylation

m^{6}A was first found in mammalian mRNA in the mid 1970s (Figure 2). It has been detected in mRNA isolated from eukaryotes including mammals [6,8,9,12,19], plants [20,21], flies [22], and yeast in the meiotic state [23], as well as in viral RNAs that replicate in the nucleus, such as influenza virus and Rous Sarcoma virus (RSV) [24, 25]. The abundance of m^{6}A has been shown to be ~0.1%–0.4% of total adenosine residues in cellular mRNA [6,12], and the average content of m^{6}A has been estimated to be 3–5 residues per mammalian mRNA [9], and 1–15 per RSV viral RNAs [25]. m^{6}A methylation has been shown to preferentially occur at the consensus sequence RRACH (where R represents purine, A is m^{6}A site, and H is a nonguanine base) [8,9]. m^{6}A is also found in introns, which indicates this modification is formed prior to mRNA splicing [26]. However, methylation at a specific site is non-stoichiometric, and only a portion of these consensus sequences are methylated in mRNA; the percentage is ~20–90% in RSV and ~20% in the mRNA encoding bovine prolactin (bPRL) [25, 27, 28].

m^{6}A is formed during processing of the nascent pre-mRNA by N^6-adenosine methyltransferase. To date, the only component of the methyltransferase complex identified is the 70kD SAM (*S*-adenosylmethionine)-binding subunit MT-A70 (also known as METTL3) [29,30]. Although the rest of the complex has not been characterized, the mechanism of the methyl transfer is most likely the conserved S_N2 type reaction from SAM to the amino group of adenosine, based on the high similarity of the catalytic domain of MT-A70 to those of the other methyltransferase enzymes (Figure 3) [31]. MT-A70 co-localizes with nuclear speckles in HeLa cells, which indicates that the methylation may happen co-transcriptionally. Loss of MT-A70 does not lead to cell cycle arrest, but appears to cause cell death, highlighting its critical role in mammalian cells [17].

Because the presence of a methyl group at the N^6 position of adenosine does not alter its Watson-Crick base-pairing property, m^{6}A cannot be mapped by reverse transcription-based methods, which hindered the functional study of m^{6}A in mRNA [32–35]. So far, only thirteen m^{6}A base-resolution sites from RSV viral RNA and one m^{6}A site from the cellular mRNA bPRL have been mapped despite the fact that this modification has been known for more than 30 years [25–28]. Two approaches were applied to probe the biological function of m^{6}A [36–38]. The first was to construct an expression system with RSV or bPRL sequences and then mutate the m^{6}A sites within them to study its effect on mRNA biogenesis. Although little new insight was gained, one interesting observation was that the mutation of the only m^{6}A site in the bPRL sequence led to a new methylation site in an adjacent consensus sequence that was not normally methylated. The second method used general methylation inhibitors (*S*-tubericidinylhomocysteine or cycloleucine) in human HeLa cells [37] or mouse sarcoma cells [38], and the results suggest that these chemicals affect the efficiency of pre-mRNA splicing or transport of mRNA from the nucleus to the cytoplasm; however, they inhibit both m^{6}A and cap-associated 2′-*O*-methyl formation as

well as many other biological methylation events, making it impossible to distinguish the effects of just loss of m^6A. It was reported that methylated dihydrofolate reductase transcript was translated 20% more efficiently than the non-methylated transcript in an *in vitro* translation assay, indicating that the presence of m^6A in mRNA may affect translation efficiency [38].

Additional clues into the function of this modification have come from the recent identification of several putative m^6A-binding proteins [32], suggesting that methylation of RNA may modulate the affinity of certain RNA-binding proteins to mRNA and thus likely impact aspects of mRNA metabolism. Because m^6A is enriched within 3′-UTRs that contain miRNA-binding sites (discussed below), miRNAs may affect the methylation levels of their target transcripts [33]. However, further experimental investigations are required to validate these hypotheses.

m⁶A RNA methylomes in mammals

New insight into the role of RNA methylation has come from the recent development of affinity-based m^6A profiling, which has revealed the transcriptome-wide maps of m^6A distributions in human [32] and mouse cells [33]. This technology was enabled by advances in high-throughput sequencing combined with the m^6A antibody that was successfully developed and used to investigate m^6A modifications in snRNAs in HeLa cells [39]. In this new method, mRNA isolated from mouse liver or brain or human cell lines was chemically fragmented into approximately 100 nt long stretches, followed by m^6A-antibody-based immunoprecipitation. The captured methylated RNA fragments were then subjected to high-throughput sequencing, providing the first initial view of the human and mouse m^6A modification landscape in a transcriptome-wide manner.

These studies identified m^6A in more than 7,000 mammalian mRNA transcripts and 300 non-coding RNAs (ncRNAs), indicating that m^6A is a wide-spread modification. The data revealed a preference for m^6A deposition around stop codons, in 3′UTRs, and within long internal exons, and that the m^6A sites are highly conserved between human and mouse [29,30]. Many of these modifications appear to be quite stable, as 70–95% of m^6A peak positions remained constant under various treatments such as ultra-violet radiation, heat shock, hepatocyte growth factor, and interferon-γ, but some of them were stimulus-dependent and dynamically modulated by interferon-γ treatment [32]. The m^6A modification also exhibits tissue-specific regulation and is markedly increased throughout brain development [33]. Although m^6A is not enriched at splice junctions [33], knockdown of MT-A70 (the SAM binding subunit of the putative mRNA N^6-adenosine methyltransferase complex) affects gene expression and alternative splicing patterns as well as modulates *p*53 signaling and induction of apoptosis [17,32]. In general, the RRACH consensus sequence was confirmed, but the previous suggestion of the preferential occurrence of m^6A within the loop of a stem-loop structure was not supported, as no strong secondary structures were found in regions harboring newly identified m^6A peaks [29]. However, due to the lack of single-base resolution, we still cannot rule out the possibility that other m^6A-containing motifs may exist in mRNA and ncRNA and are installed by other N^6-adenosine methyltransferases. It should be noted that the m^6A sites in human rRNA and U6 small nuclear RNA do not match the RRACH consensus sequences found in mRNA [40].

m⁶A in other organisms

In the yeast *Saccharomyces cerevisiae*, m^6A is not detectable in mRNA from mitotic log phase cells; however, during meiosis, m^6A can be easily detected in the mRNAs. The ratio of m^6A to A is 1.0% in the poly(A) RNA isolated from cells growing for 3 h in sporulation

medium [41]. Ime4, the homolog of MT-A70 in *S. cerevisiae*, is required for m^6A modification and has a role in the initiation and nutritional control of meiosis [23,41,42]. The antisense ncRNA *RME2* (Regulator of Meiosis 2) prevents *IME4* gene expression by blocking the elongation of the full-length *IME4* transcript, whereas in diploid cells, the a1-α2 complex represses the transcription of *RME2*, thus allowing *IME4* induction during meiosis [43,44]. During meiosis, *IME4* mRNA expression increases to a significant level by 6 h and decreases substantially to near baseline levels by 24 h after yeast cells are transferred to sporulation medium [23]. Targets for methylation include the mRNA of Ime4 itself, together with the transcripts encoding Ime1 and Ime2, two key regulators of meiosis [41]. In a recent study [45], two meiotic proteins, Mum2 and Slz1, were identified as Ime4 interacting partners, and it was found that these proteins are required for m^6A formation. In addition, the study revealed that the m^6A level increases when yeast cells enter meiosis in nutrient-poor liquid medium, and that the m^6A level drops rapidly when nutrients are returned and cells begin foraging and undergo pseudohyphal growth. These observations indicate that this RNA methylation controls cell fate and the initiation of meiosis in yeast.

m^6A was found in the mRNA of the monocot plants maize [20], wheat [46], and oat [47] more than thirty years ago. The potential function of m^6A has been studied relatively more thoroughly in the model organism *Arabidopsis thaliana* [21,48]. *Arabidopsis* mRNA contains a similar amount of m^6A to that harbored in mammalian cells, with a ratio of m^6A to A of 1.5% from 2-week-old seedlings. Similar to the enrichment of m^6A in the 3′-UTR in mammals, m^6A in plants is shown to be located at the 3′ end of transcripts in a region 100–150 bp before the poly(A) tail [48]. m^6A content varies in different tissues, and a higher ratio of m^6A to A was found in the flower buds relative to the roots and leaves, which correlates with MTA (a homolog of human MT-A70 and yeast Ime4, encoded by At4g10760) expression levels [21]. MTA expression is also strongly associated with dividing tissues, suggesting that RNA methylation is involved in cell division. In agreement with this, inactivation of MTA in *Arabidopsis* leads to failure of the developing embryo to progress past the globular stage, indicative of cell division defects, and the arrested seeds from the *MTA* knockout line lack m^6A-containing mRNAs [21]. Further insight into the role of m^6A in plants has come from studying a mutant with decreased levels of MTA expression. These plants exhibit decreased m^6A levels and abnormal growth with reduced apical dominance, abnormal organ definition, and an increased number of trichome branches [48]. Microarray data from plants with reduced m^6A levels showed that a significant portion of the down-regulated mRNAs are involved in transport or targeted transport, and most of the up-regulated genes are associated with stress and stimuli responses [48]. These data indicate that this modification may be a dynamic response to environmental cues, which is particularly important for plants. Interestingly, MTA was also found to interact and colocalize with the splicing factor FIP37 in splicing speckle-like nuclear compartments in plants [21]. *Arabidopsis* FIP37 is a homolog of yeast Mum2, *Drosophila* female-lethal-2-D (FL(2)D), and human wilms' tumor 1-associating protein (WTAP). Both FL(2)D and WTAP are suggested to be associated with the spliceosome. *Drosophila* FL(2)D is required for the accumulation of correctly spliced forms of two critical sexual determination gene (*sex lethal* and *transformer*) [49,50], and human WTAP interacts with wilms' tumour1 (WT1) protein, one isoform of which (+KTS WT1) binds to RNA and is incorporated into spliceosomes [51,52]. The interaction of MTA with splicing factors in plants suggests a role for m^6A in regulating RNA splicing, and moreover, that this may be conserved in flies and humans.

The internal m^6A modification in *Drosophila* mRNA was first discovered in 1978 [22]. A study in 2011 focused on *Dm ime4,* the *Drosophila* homolog of MT-A70, but did not evaluate the levels of m^6A in wild-type and *Dm ime4* knockout flies [53]. *Dm ime4* is expressed in ovaries and testes and localizes to soma and germ-line cells in ovarioles. The levels of Notch signaling are reduced in follicle cells of *Dm ime4* mutants, which

phenotypically lead to fused egg chambers with follicle-cell defects. A complete deletion of *Dm ime4* is lethal, suggesting that m^6A is essential for viability and participates in Notch signaling during egg chamber development. The studies of m^6A in these other organisms further point to significant involvement of this prevalent modification in various aspects of mRNA metabolism.

The m^6A demethylases—FTO and ALKBH5 proteins

Several genome-wide association studies (GWAS) showed a strong association of single nucleotide polymorphisms (SNPs) in the fat mass and obesity-associated (*FTO*) gene with body mass index (BMI) and risk of obesity and type 2 diabetes in multiple populations [54–56]. The trend from these studies has continued, and *FTO* has recently been linked with the phenotypic variability of BMI [57]. Mice overexpressing *Fto* display increased food intake and increased adiposity in a dose-dependent manner [58], in agreement with the dose-dependent effects of FTO in adiposity observed in humans [54]. Mice lacking *Fto* show increased postnatal lethality, postnatal growth retardation, reduced fat mass, lower body weight, increased energy expenditure, and a relative increase in food intake [59–61]. Deletion of *Fto* in the brain recapitulates the reduced body weight of *Fto* nulls, indicating that *Fto* functions in the central nervous system to regulate postnatal energy homeostasis [61]. GWAS have also shown that common variants in the *FTO* gene have been linked with glucose metabolism [62–64], neurological diseases including depression [65] and Alzheimer's disease [66], cardiovascular disease [64], cancer [67,68], and end-stage renal disease [69]. In addition, a loss-of-function homozygous mutation (R316Q) in the *FTO* gene typically leads to postnatal growth retardation, microcephaly, psychomotor delay, facial dysmorphism, certain brain malformations, cardiac abnormalities, and cleft palate. In all cases, lethality usually occurred before the age of 3 years [70].

Despite the strong genetic association between FTO and body weight observed by GWAS, little was known about the molecular mechanism leading to the phenotypes. FTO is a homolog of the non-heme Fe(II)/α-ketoglutarate (α-KG)-dependent AlkB family dioxygenases, which typically catalyze demethlyation/hydroxylation, and has been shown to oxidatively demethylate m^3T in single-stranded DNA (ssDNA) and m^3U in single-stranded RNA (ssRNA) *in vitro* [71,72], although the observed activity is exceedingly low compared to those of the other AlkB family proteins. The arginine at position 316 is one of the α-KG ligands, and the R316Q mutant causes reduced FTO catalytic activity *in vitro*. The phenotypes associated with this mutation described above are direct evidence that the enzymatic activity of FTO is critical. However, because m^3T is very rare in mammalian genomic DNA, and m^3U only exists in a deeply buried position in folded rRNA, they are unlikely to be the physiological substrates of FTO. The preference of FTO for ssDNA/ssRNA can be explained by the presence of an extra loop covering one side of the conserved jelly-roll motif, which selectively competes with the complementary strand of the DNA/RNA duplex [73]. In a surprising discovery, FTO was found to be an m^6A RNA demethylase [18].

FTO can efficiently convert m^6A to adenosine in synthetic ssRNA and ssDNA with much higher activity compared to m^3U at neutral pH *in vitro* (Figure 3a). m^6A is the most prevalent modification in mRNA of eukaryotes, but is undetectable in genomic DNA in higher eukaryotes [74], suggesting that the physiological substrate of FTO is RNA, not DNA. To test this, FTO was either knocked down or overexpressed in two human cell lines, HeLa and 293FT. mRNA was then isolated by using poly(dT) beads followed by an rRNA depletion step, and m^6A was quantified by three different methods: LC-MS/MS, 2D-TLC, and m^6A-antibody-based dot-blot, respectively. All results showed that m^6A in mRNA is subject to FTO-mediated demethylation inside these cells, confirming it as a physiological

substrate of FTO. It was further found that FTO is partially co-localized with nuclear splicing speckle factors (SART1 and SC35) and RNA polymerase II (phosphorylated at Ser2), but not with markers for other nuclear subregions such as telomeres, replication sites, Cajal bodies, cleavage bodies, or P-bodies, which indicated that FTO is involved in the processing of nascent transcribed mRNA [18].

The discovery that FTO is an mRNA m^6A demethylase added RNA modification to the list of reversible and dynamic modifications, similar to DNA methylation and histone modifications, but exerting its functions post-transcriptionally. It further suggests physiological roles of m^6A in human biological processes, in particular energy homeostasis regulation. Additional m^6A demethylases may exist and play functional roles in biological regulation. Indeed, another human m^6A demethylase was recently identified in mammals that has significant effects on spermatogenesis [75]. ALKBH5 (alkB, alkylation repair homolog 5) is a homologue of FTO [76]. Its m^6A demethylation activity impacts total RNA synthesis and mRNA (polyA-tailed RNA) export inside mammalian cells. *Alkbh5*-knockout male mice exhibited increased m^6A in mRNA and impaired fertility resulting from apoptosis that affects meiotic metaphase-stage spermatocytes. The discovery of this second m^6A demethylase for mRNA and ncRNA reinforces the notion that this modification plays dynamic regulatory roles in fundamental process.

To date, FTO homologs have been found in vertebrates (from fish to mammals) and marine algae (from unicellular photosynthetic picoplankton to multicellular seaweed), but not in invertebrate animals, fungi, plants, bacteria, or archaea [77]. Although ALKBH5 is also only conserved in vertebrates [76], it is not unlikely that m^6A demethylases other than FTO and ALKBH5 exist in other organisms.

Perspectives on the role of reversible RNA methylation

There is growing evidence that adenosine methylation in RNA (mRNA and ncRNAs) plays important regulatory roles, and that mis-regulation ultimately affects physiologic processes in mammals. These data support the hypothesis that dynamic/reversible RNA modifications may function analogously to reversible DNA and protein modifications, heralding the arrival of “RNA epigenetics” [3,4].

The dynamics of m^6A can be temporally and spatially modulated in different manners depending on the localization and concentration of methyltransferase/demethylase enzymes and RNA substrates. The majority of FTO localizes in nuclear speckles, where methyltransferases reside. Because adenosine methylation is non-stoichiometric, the methylated/unmethylated ratio at specific adenosine sites on a given mRNA can be tuned by differentially expressing methyltransferase/demethylase enzymes as well as by regulating the subcellular localization of both the transcript and the enzymes. Apart from the equilibrium model, a potential “irreversible” mechanism may exist through the generation of short-lived intermediates during demethylation (Ye Fu, Guifang Jia, Chuan He, *et al.* unpublished), which prevents direct re-methylation shortly after oxidation of m^6A by FTO; the formation of fully demethylated adenosine from the oxidation intermediate could be delayed until mRNA is transported out of speckles (Figure 4a). Additionally, certain RNAs may be transported to concentrated FTO loci, possibly via a specific sequence recognized by an RNA-binding protein that also interacts with FTO or FTO partner proteins. This would result in greater demethylation of those targeted RNAs.

The comparison between RNA methylation and other epigenetic marks also extends to the enzymes and proteins involved: the methyltransferases and demethylases are “writers” and “erasers”, and the m^6A modification on RNA can be recognized by “readers”, m^6A-specific binding proteins [32] that recognize the m^6A modification and affect pre-mRNA splicing,

mRNA transport from nuclear to cytoplasm, translation, mRNA turnover, mRNA stability or sub-cellular localization [78] (Figure 4b). m^6A might also be involved in siRNA or miRNA pathways as a reversible chemical handle. As mentioned above, a significant portion of m^6A occurs in the 3′UTR of the transcripts, which correlates with miRNA-binding sites. About two thirds of 3′UTRs that contain m^6A peaks also contain at least one predicted miRNA binding site, and the most highly expressed miRNAs have a significantly greater percentage of target transcripts that contain m^6A [33]. Because m^6A is not exactly on the miRNA binding sites but near them, the presence of m^6A in 3′UTRs of target transcripts might recruit specific binding proteins to weaken or enhance the subsequent interactions of the transcripts with the miRNA (Figure 4b).

Over 90% of the human genome is likely to be transcribed, of which only 2% (around 20,000 genes) is translated into proteins and the remaining 98% are ncRNAs, including tens of thousands of long noncoding RNAs (lncRNAs). Although the majority of lncRNAs have yet to be characterized, some have been found to serve key regulatory roles [79, 80]. An emerging theme from multiple model systems is that lncRNAs form extensive networks of ribonucleoprotein (RNP) complexes with numerous chromatin regulators and subsequently target these enzymatic activities to appropriate locations in the genome [80]. The finding that m^6A is also widespread in lncRNAs [33] establishes a possible connection between RNA modification and chromatin regulations. The potential link between m^6A to the function of lncRNA is an intriguing area to explore. Regulation at the RNA level could allow for rapid response to signaling and stimuli and might be heritable, in particular if the methylation status of certain lncRNA or mRNA affects histone and/or DNA modifications.

Interestingly, the human methyltransferase BCDIN3D has been shown to methylate the 5′ phosphate of a miRNA precursor, pre-miR-145, and prevent its processing by Dicer to produce the mature miRNA product [81]. It would be interesting to see whether this methylation can be reversed to re-activate the methyl-trapped pre-miRNA as another example of reversible methylation on RNA. Thus, pre-miRNAs, and possibly even pre-tRNAs and pre-rRNAs, could be subjected to similar reversible methylation, which may affect maturation and transport of these RNAs and add additional layers of complexity and potential regulation mechanisms.

Concluding remarks

Although the exact mechanism and function of m^6A in mRNA and ncRNA remain obscure, it is certain that this reversible adenosine methylation has important regulatory roles across eukaryotes. The discovery of at least two m^6A mRNA demethylases strongly indicates that this reversible modification has dynamic roles in fundamental biological regulation. It is highly likely that other methyltransferases and demethylases also exist. Investigations of these writers and erasers will help to establish related cellular regulatory pathways. For example, characterization of m^6A distribution within the wild-type and *FTO* mutant cells will help reveal the mechanisms of FTO-based regulation. In addition, exploration of m^6A-binding proteins is essential to the understanding of the functional roles of the m^6A modification. The ability to perform transcriptome-wide profiling of m^6A is a significant step forward and has provided new insight into the biology of RNA methylation [32,33]. The next challenge is to develop single-base resolution sequencing, which could quantify the relative abundance of m^6A at each modification site and greatly expand our understanding of RNA epigenetics.

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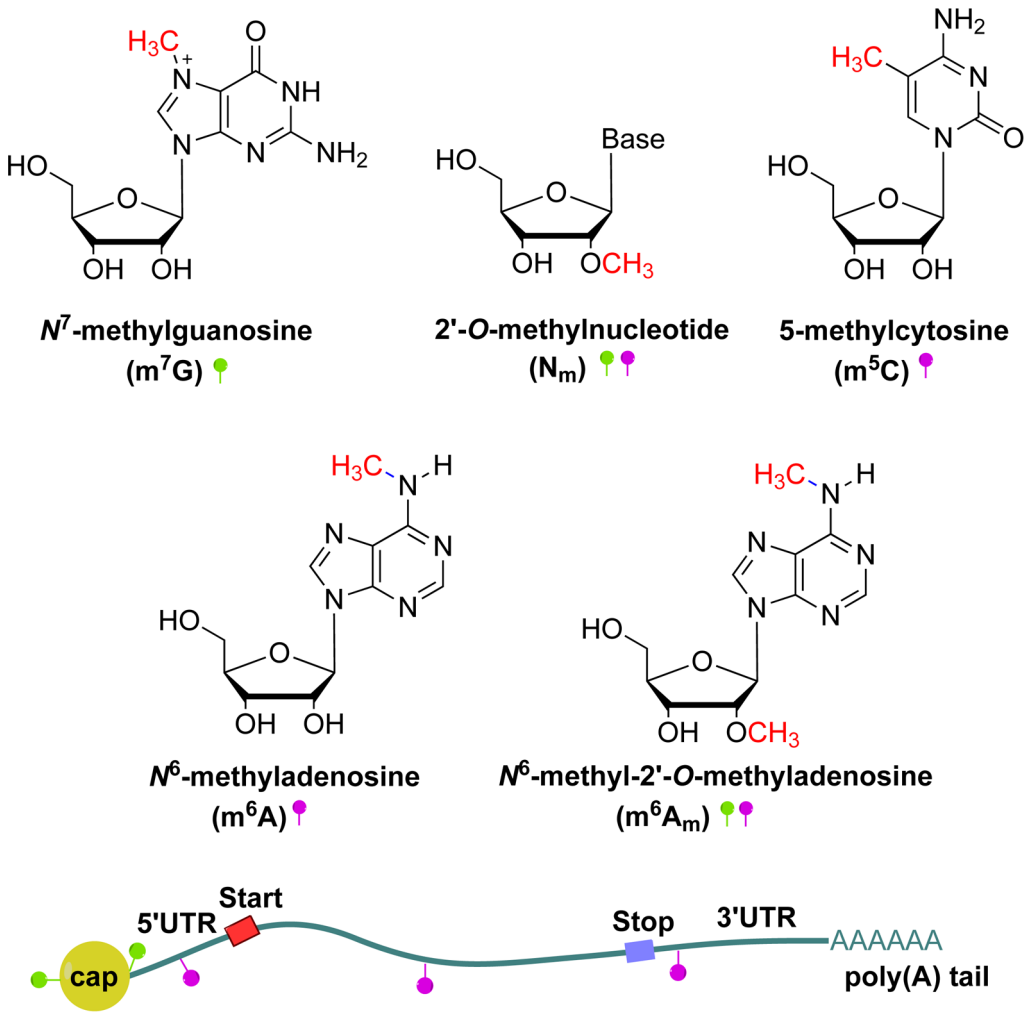

Figure 1. Modifications in eukaryote mRNA

The structure and localization of RNA modifications in mRNA are shown. m^7G is at the 5′ terminus of the cap structure, m^6A_m at the first position of the 5′ terminus and internal positions, 2′-*O*-methylated N_m on the first two starting positions of the 5′ terminus and internal sequence, and m^5C and m^6A are internal mRNA modifications (green ball indicates the modifications located at the cap, pink ball indicates internal modifications).

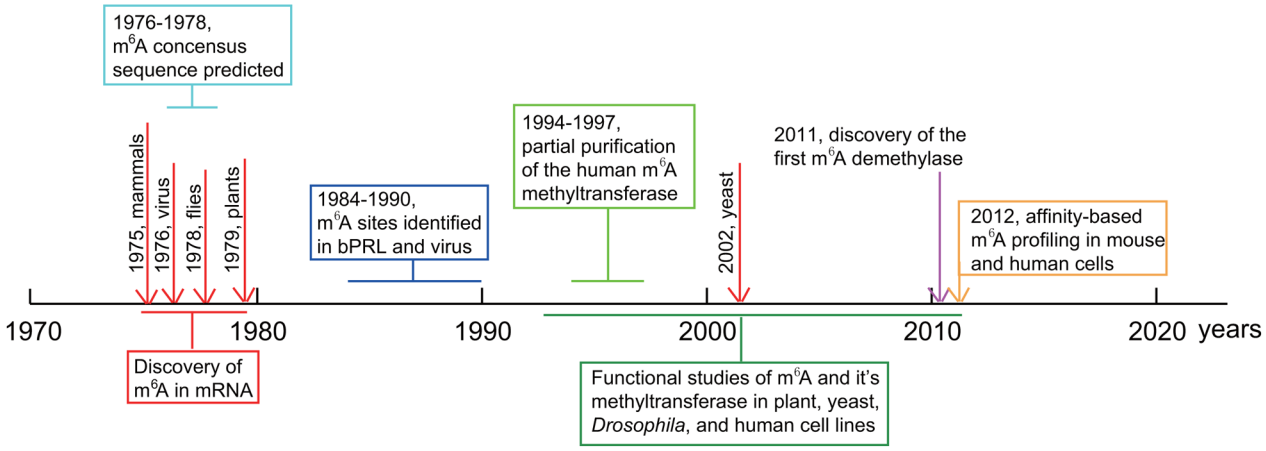

Figure 2.
The history of the research on m^{6}A in mRNA.

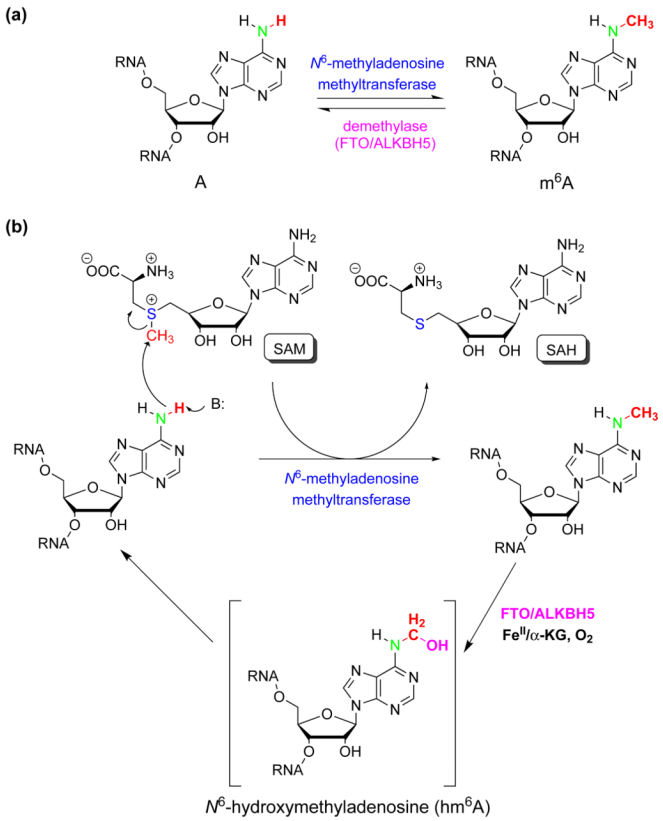

Figure 3. The reversible adenosine methylation reaction in mRNA and non-coding RNA

(**a**) The reversible m^{6}A methylation catalyzed by *N*^{6}-adenosine methyltransferase and demethylases (FTO and ALKBH5). (**b**) The proposed S_N2 methylation reaction of *N*^{6}-adenosine and the oxidative demethylation through a proposed *N*^{6}-hydroxymethyladenosine intermediate. SAM: *S*-adenosylmethionine, SAH: *S*-adenosyl-L-homocysteine.

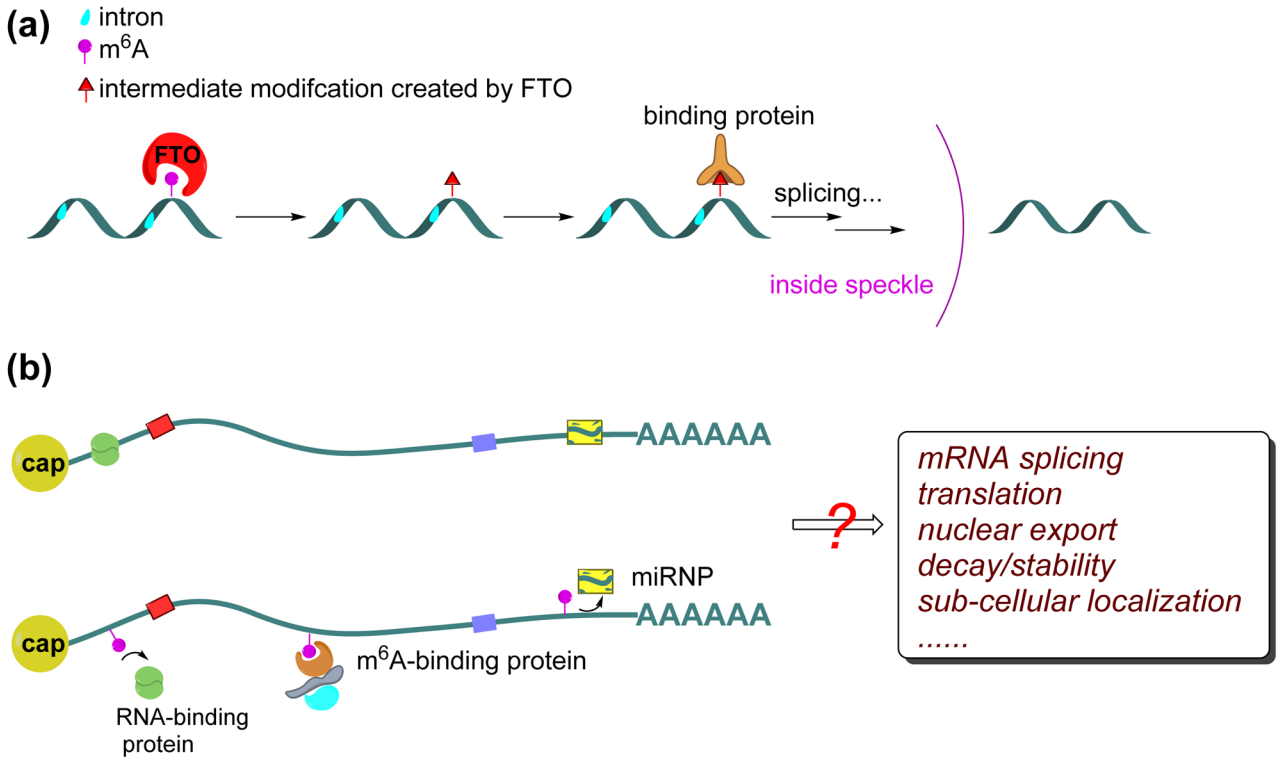

Figure 4. Proposed functions of reversible m^{6}A methylation

(**a**) The intermediate modification created by FTO could be recognized by a specific RNA-binding protein to impact RNA metabolism. If this interaction occurred in the nuclear speckle, where FTO is localized, it might protect the modification until it was exported from the nuclear speckle (**b**) The m^{6}A methylation may be recognized by specific m^{6}A-binding proteins, or preclude the binding by certain RNA-binding proteins that preferably bind to unmethylated RNA. The presence of m^{6}A can therefore impact various aspects of RNA metabolism such as pre-mRNA splicing, mRNA transport from nuclear to cytoplasm, translation, mRNA turnover, mRNA stability or sub-cellular localization. In addition, the presence of m^{6}A in the 3′UTR of mRNA might weaken or enhance mRNA association with miRNA (yellow box) via modulation of protein-RNA recognitions.