The detection and functions of RNA modification m^{6}A based on m^{6}A writers and erasers

Received for publication, January 4, 2021, and in revised form, July 12, 2021 Published, Papers in Press, July 16, 2021,
https://doi.org/10.1016/j.jbc.2021.100973

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Edited by Karin Musier-Forsyth

N^6-methyladenosine (m^{6}A) is the most frequent chemical modification in eukaryotic mRNA and is known to participate in a variety of physiological processes, including cancer progression and viral infection. The reversible and dynamic m^{6}A modification is installed by m^{6}A methyltransferase (writer) enzymes and erased by m^{6}A demethylase (eraser) enzymes. m^{6}A modification recognized by m^{6}A binding proteins (readers) regulates RNA processing and metabolism, leading to downstream biological effects such as promotion of stability and translation or increased degradation. The m^{6}A writers and erasers determine the abundance of m^{6}A modifications and play decisive roles in its distribution and function. In this review, we focused on m^{6}A writers and erasers and present an overview on their known functions and enzymatic molecular mechanisms, showing how they recognize substrates and install or remove m^{6}A modifications. We also summarize the current applications of m^{6}A writers and erasers for m^{6}A detection and highlight the merits and drawbacks of these available methods. Lastly, we describe the biological functions of m^{6}A in cancers and viral infection based on research of m^{6}A writers and erasers and introduce new assays for m^{6}A functionality *via* programmable m^{6}A editing tools.

RNA plays a central role in central dogma of molecular biology and is responsible for transmitting the genetic information encoded by DNA into functional proteins. There are different types of RNA molecules that are involved in the regulation of several biological processes, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), microRNA (miRNA), long noncoding RNA (lncRNA), *etc.* Analogous to DNA or histone modifications (1, 2), RNA molecules contain numerous (more than 150) chemical modifications (3). Among them, N^7-methylguanosine (m^{7}G), N^6-methyladenosine (m^{6}A), 5-methylcytosine (m^{5}C), N^1-methyladenosine (m^{1}A), pseudouridine (Ψ), and 2′-*O*-methylation (N$_m$), cap N^6,2′-*O*-dimethyladenosine (m^{6}A$_m$), N^4-acetylcytidine (Ac^{4}C) have been identified in mammalian mRNA (4, 5). All RNA modifications are installed by endogenous "writer" enzymes, such as methyltransferase. Some RNA methylation modifications can be reversibly removed by "eraser" demethylases and recognized by "reader" RNA modification binding proteins for regulation of RNA processing and metabolism (6, 7). The fast-expanding research of RNA modifications is termed as epitranscriptomics.

m^{6}A is the most abundant internal chemical modification in mRNA and comprises approximately 0.1 to 0.4% of all adenosines, and there are about 3 to 5 m^{6}A in each mRNA on average, mainly composed of G(m^{6}A)C (70%) or A(m^{6}A)C (30%) and located nearby stop codon and 3′ untranslated region (3′ UTR) (8, 9). The existence of m^{6}A was first reported in mRNA from mammalian cells in the 1970s (10), demonstrating a unique distribution of methylated nucleosides in mRNA different from rRNA and tRNA, which possess complex base-methylnucleoside patterns. In 1990s, the m^{6}A multisubunit complex was first identified to be responsible for the addition of methyl group onto adenosine from nuclear extracts of HeLa cells (11). However, the biological functions of m^{6}A have been rarely reported due to the limitation of advanced detection technology.

The widespread study of m^{6}A was reignited after the discovery of the first m^{6}A demethylase in 2011—the fat obesity-associated protein (FTO) (12), which indicates that m^{6}A is a dynamically reversible RNA modification similar to 5-methylcytosine (5 mC) in DNA (13). Development of antibody-based transcriptomic m^{6}A sequencing methods paved the way for m^{6}A functional study (14–17). Since then, m^{6}A-related proteins have been vastly studied and successively identified, including the subunits of m^{6}A methyltransferase complex, demethylases, and binding proteins, which are also termed as m^{6}A writers, erasers, and readers, respectively (18–24). The dynamic m^{6}A modifications are cotranscriptionally written by METTL3-METTL14 core methyltransferase complex and erased by two m^{6}A demethylases, FTO and ALKBH5 (12, 18, 25). The methyl group on m^{6}A is recognized by m^{6}A readers such as YTH domain family proteins and IGF2BP1-3 to regulate RNA processing, including transcription, splicing, nuclear export, stability, and translation (19, 21, 26–30). The discovery of these m^{6}A machineries has facilitated the understanding of the m^{6}A biological functions in physiological processes (Fig. 1).

The development of m^{6}A detection technology is of great importance for studying the biological functions of m^{6}A. Mass spectrometry technology can identify changes in the overall

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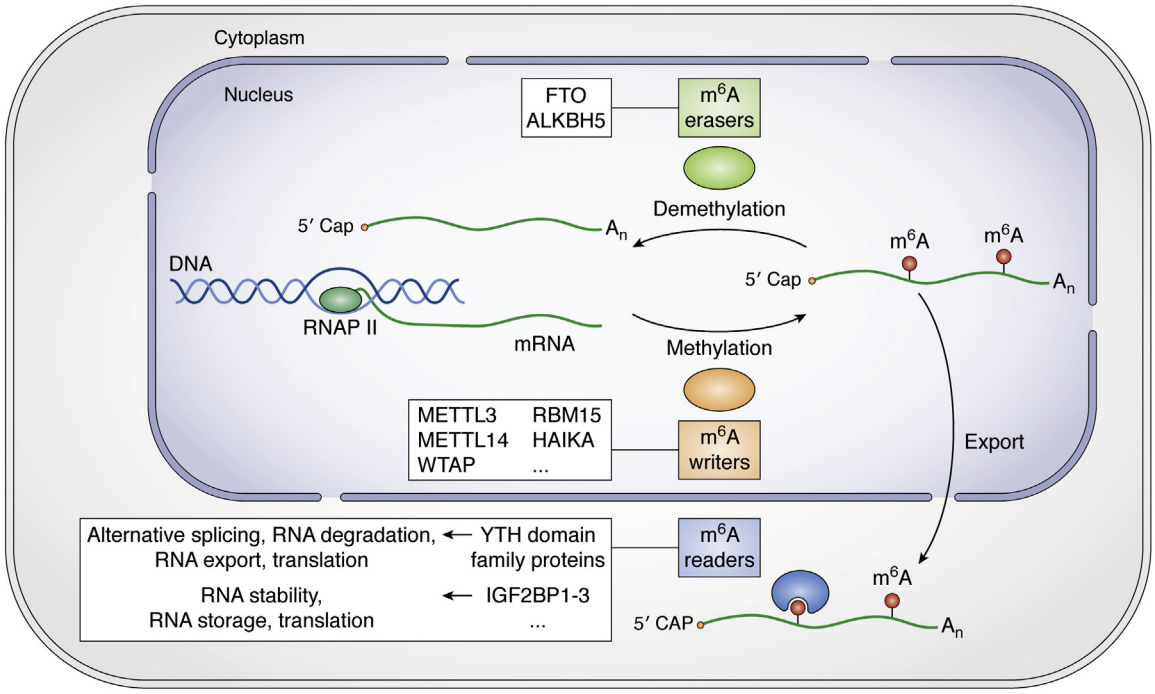

Figure 1. Scheme overview of m^{6}A machinery in mammals. m^{6}A is deposited and removed by m^{6}A methyltransferase complex (writers) and demethylases (erasers) in the nucleus respectively to realize dynamic regulation. m^{6}A-binding proteins (m^{6}A readers) can recognize and bind m^{6}A-modified mRNA to perform various biological functions such as increasing translation efficiency and degrading RNA.

content of m^{6}A in cells, and m^{6}A antibody-based high-throughput sequencing methods help us determine the distribution of m^{6}A on transcripts (31, 32). However, antibody-based detection methods have poor specificity and low resolution, which hinders in-depth research on m^{6}A (33). To overcome the shortcomings of antibody-based methods, m^{6}A writers and erasers have been used to develop new m^{6}A detection methods (34–38). Moreover, the research on the biological functions of m^{6}A has been smoothly transitioned from the fundamental identification of m^{6}A writers, erasers, and readers and investigation of m^{6}A functions in RNA fate to its roles in physiological and pathological processes, including embryonic development, neurogenesis, and various diseases (19, 21, 39, 40). The basic mechanisms of the impact of differential expression of m^{6}A writers and erasers in the occurrence of cancer and other diseases have been initially investigated from transcriptome-wide range, explaining the physiological functions of m^{6}A in certain diseases (41–48). However, the reliability and completeness of the results still need to be improved with more precise methods. In the past 2 years, the combination of m^{6}A writers and erasers with CRISPR/Cas technology has achieved the programmable m^{6}A editing, which provides benign verifying and regulating tools for deeply studying the functions of m^{6}A (49–55).

In this review, we mainly focus on m^{6}A writers and erasers. We will summarize the identification and enzymatic molecular mechanisms of m^{6}A writers and erasers and compare m^{6}A writers- and erasers-assisted m^{6}A detection methods with traditional antibody-based methods. m^{6}A writers and erasers regulate plenty of physiological and pathological processes, and here we will elaborate the recent findings about the regulatory roles of m^{6}A writers and erasers in pathological processes, especially in viral infection and cancer progression. Finally, we will introduce the approaches for programmable m^{6}A editing developed through the conjugation of m^{6}A writers or erasers with CRISPR/Cas system, which could be widely used for m^{6}A functional study.

m^{6}A writers and erasers

m^{6}A writers

The m^{6}A methyltransferase complex for installing the majority of mRNA m^{6}A modifications

m^{6}A methyltransferase complex was first purified and characterized from the nuclear extracts of HeLa cells in 1990s (11). The first catalytic subunit METTL3 was identified in 1997 (56). METTL3 contains an S-adenosyl methionine (SAM) binding domain and a DPPW motif (Asn-Pro-Pro-Trp) for transferring the methyl group from the SAM to the N6 position of the targeted adenosine (Fig. 2, *A* and *B*). Later, METTL14 was identified as the second core component of the mRNA m^{6}A methyltransferase complex, sharing around 43% sequence identity with METTL3 based on sequence alignment analysis (57, 58). Biochemical studies show that METTL14 and METTL3 form a heterodimer and exhibit higher m^{6}A installation activity than METTL3 alone (58). The crystal structure analysis indicates that only METTL3 can bind SAM, and METTL14 structurally supports METTL3 by providing an RNA binding scaffold, which substantially enhances its methylation efficiency as a result (59–61) (Fig. 2*A*).

METTL3 and METTL14 are key core subunits of m^{6}A methyltransferase complex and play pivotal roles in different

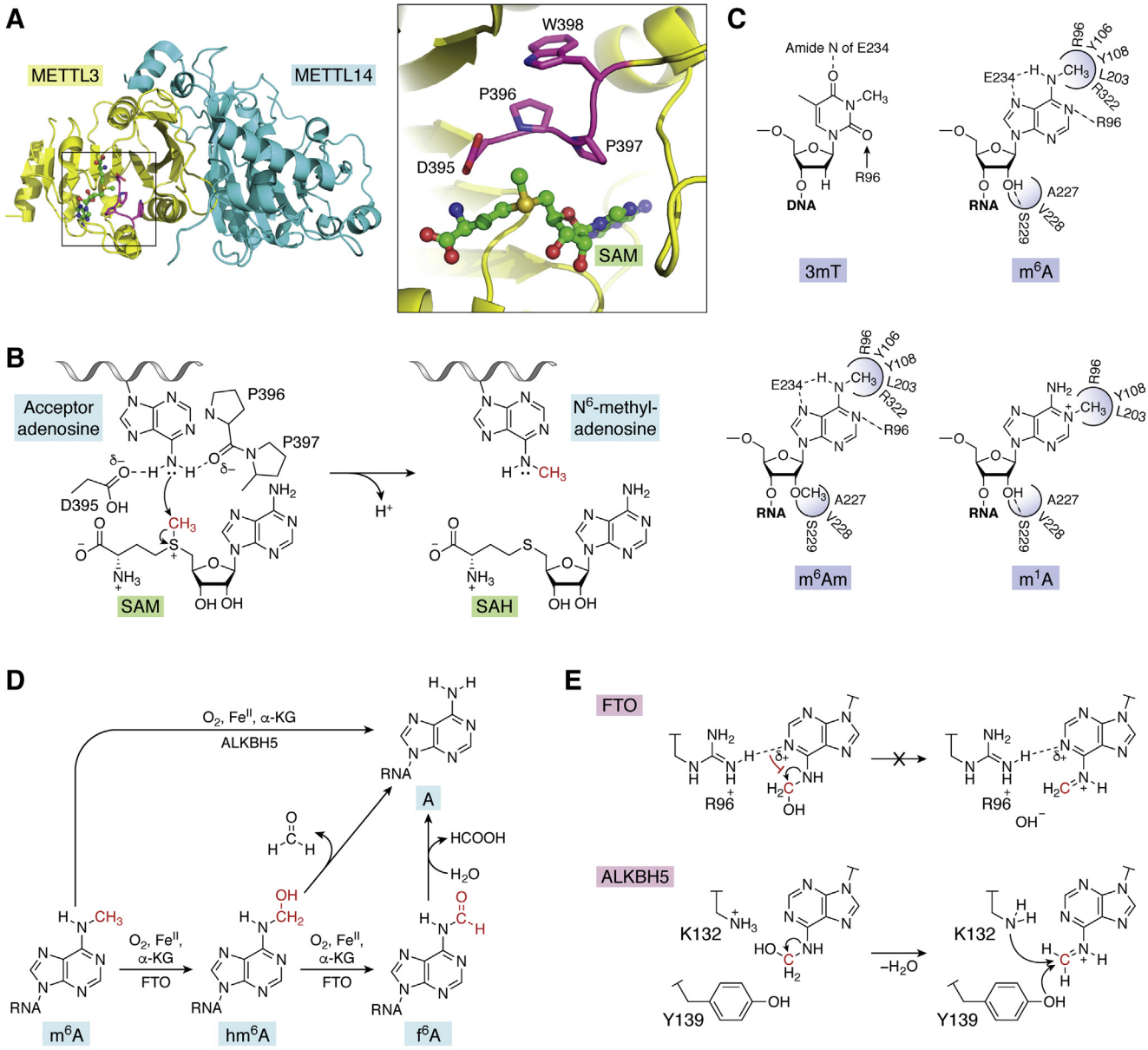

Figure 2. The working mechanisms of m^{6}A writers and erasers. *A*, crystal analysis indicates that METTL3 possesses an SAM-binding pocket and DPPL motif for m^{6}A deposition and can form a stable heterodimer with METTL14. *B*, proposed mechanism of methyl transfer catalyzed by METTL3. *C*, proposed mechanisms of FTO toward different substrates. *D*, differential oxidation steps of FTO and ALKBH5 toward m^{6}A. *E*, differential behavior of FTO and ALKBH5 in the nucleobase recognition region.

biological processes, including embryonic development and neurogenesis (39, 40). METTL3$^{-/-}$ mice exhibit early embryonic lethality (62), and the depletion of DmIME4 (METTL3 homolog) leads to gametogenic defects in *Drosophila* (63). Knockout of METTL14 and METTL3 in embryonic mouse brains can both prolong the cell cycle of radial glia cells and extend cortical neurogenesis into postnatal stages in an m^{6}A-dependent manner (40).

Wilms' tumor 1-associating protein (WTAP) has been identified as the third subunit of mRNA m^{6}A methyltransferase complex and classified as a splicing factor that binds to the Wilms' tumor 1 protein in mammals that plays a key role in embryonic development (64, 65). While WTAP has no catalytical activity toward RNA targets, it can assist the METTL3-METTL14 heterodimer to locate in nuclear speckles and facilitate m^{6}A deposition (66). Virilizar was first found to be involved in sex determination in *Drosophila* (67). VIRMA, a mammal homolog of the *Drosophila* Virilizar, was found to interact with METTL3 *via* its N-terminus and characterized as a subunit of m^{6}A methyltransferase complex (22). More recently, VIRMA was verified to associate with alternative polyadenylation *via* preferentially mediating m^{6}A deposition on 3′ UTRs near the stop codon (68).

Before WTAP was identified as the subunit of mRNA m^{6}A methyltransferase complex, an early co-immunoprecipitation study had indicated that WTAP can form a novel protein complex with VIRMA, HAKAI, RBM15, ZC3H13, Bcl-2-associated transcription factor 1 (BCLAF1), and thyroid

hormone receptor-associated protein 3 (THRAP3) (23). Inspired by the result, VIRMA, HAKAI, RBM15, and ZC3H13 now have been characterized as subunits of mRNA m^{6}A methyltransferase complex (22, 24, 69, 70). HAKAI, an E3 ubiquitin ligase, was first identified as a subunit of m^{6}A methyltransferase complex in *Arabidopsis* (24). The knockdown of *HAKAI* in mammalian cells leads to a reduction of 23% in m^{6}A abundance, while its disruption in *Arabidopsis* leads to a more moderate decrease in m^{6}A levels with no obvious phenotypical consequence (24). RNA-binding motif protein 15 (RBM15) as a subunit of m^{6}A methyltransferase complex allows the formation of m^{6}A in long noncoding RNA X-inactive specific transcript (*XIST*) and mRNA transcripts. The knockdown of *RBM15* impairs *XIST*-mediated gene silencing in a manner that depends on YTH domain containing 1 (YTHDC1) (69). The zinc-finger protein ZC3H13 plays an important role in regulating m^{6}A formation in the nucleus. The knockdown of *ZC3H13* results in the translocation of WTAP, HAKAI, and VIRMA to the cytoplasm, which dramatically reduces m^{6}A level by 60 to 70% and affects the self-renewal of mESCs (70).

Other m^{6}A writers

Apart from the mRNA m^{6}A methyltransferase complex, several other methyltransferases are able to add m^{6}A in other RNA types. For example, ZCCHC4 installs m6A at A4220 position of 28S rRNA, and knockdown of *ZCCHC4* leads to the downregulation of translation and inhibits cell proliferation (71). METTL5, an 18S rRNA m^{6}A methyltransferase, can form a heterodimer with tRNA methyltransferase homolog 112 (TRMT112) to gain metabolic stability through the formation of a parallel β-zipper between main chain atoms (72). METTL16 is a U6 spliceosomal small nuclear RNA (snRNA) m^{6}A methyltransferase and also specifically deposits m^{6}A at an intron of SAM synthetase *MAT2A* that carries an evolutionarily conserved U6 m^{6}A motif UACm^{6}AGAGAA (73). The m^{6}A on *MAT2A* pre-mRNA deposited by METTL16 leads to *MTA2A* intron retention and nuclear degradation, consequently regulating intracellular SAM homeostasis and mouse early embryonic development (73–75). Although depletion of human *METTL16* increases lots of m^{6}A methylation sites in mRNA (74), recent finding revealed that the increased m^{6}A sites lacking the UACm^{6}AGAGAA motif were mediated by the reduced intercellular SAM level (75), suggesting that mammalian METTL16 only directly methylates mRNA containing the UACm^{6}AGAGAA motifs.

m^{6}A erasers

Early genome-wide association studies have shown that FTO influences human obesity and energy homeostasis (76–79). The overexpression of FTO in mice leads to an increased food intake and obesity, and the knockout of FTO in mice leads to significant reductions in body mass and growth retardation (80–82). Patients with loss-of-function mutation R316Q in FTO (R316Q mutation abolishes FTO enzyme function) have a severe polymalformation syndrome (80), which shows that the demethylation activity of FTO is required for normal developments of central nervous and cardiovascular systems in human. FTO is initially regarded as a demethylase that oxidatively demethylates N^3-methylthymidine (3 mT) in ssDNA and N^3-methyluridine (m^{3}U) in ssRNA (83, 84). However, DNA modification 3 mT and RNA modification m^{3}U are extremely rare *in vivo*, suggesting that these two modifications are not the physiological substrates of FTO for the striking phenotypes observed in *FTO*-deficient mice or patients (81, 82). Later, FTO was identified as an m^{6}A demethylase in nuclear RNA (12), uncovering the reversibility of RNA modification. The m^{6}A demethylation of FTO regulates adipogenesis through m^{6}A-mediated alternative splicing of Runt-related transcription factor 1 (*RUNX1T1*), an adipogenesis-related transcription factor, which provides a novel mechanism explaining obesity (85, 86).

FTO can also demethylate other RNA methylation modifications such as cap m^{6}A$_m$ and m^{1}A with varying efficiency and its subcellular localization affects the substrates specificity (87–89). In the nucleus, FTO preferentially demethylates internal m^{6}A in poly(A) RNA, m^{6}A and m^{6}A$_m$ in snRNA, and m^{1}A in tRNA; in the cytoplasm, FTO demethylates both internal m^{6}A and cap m^{6}A$_m$ in poly(A) and m^{1}A in tRNA (88). The crystal structure of FTO bound to 6mA-modified ssDNA reveals the molecular basis of the recognition and catalytic demethylation of FTO toward different substrates (90) (Fig. 2*C*), demonstrating that N^6-methyladenine is the most favorable nucleobase substrate of FTO. FTO uses the ligands R96 and E234 to form three H bonds with the N1, N6, and N7 atoms of the 6 mA purine ring for locking the 6 mA base in the catalytic pocket and utilizes the side chains of R96, Y106, Y108, L203, and R322 ligands to form a hydrophobic pocket for stabilization of the N^6-methyl group at the orientation of Fe(II) and α-ketoglutarate (α-KG) for oxidation (Fig. 2*C*). Compared with the recognition of FTO toward m^{6}A, the weak H bond between the amide nitrogen of E234 and O4 atom of 3 mT and the loss of H bond between the purine ring of m^{1}A with both E234 and R96 may explain why FTO exhibits slower enzymatic activity for 3 mT and m^{1}A than for m^{6}A (90, 91).

Splicing factor proline and glutamine-rich (SFPQ) is a multifunctional nuclear protein that participates in several cellular activities, including RNA transport, apoptosis, and DNA repair (92). *Via* the genetically encoded and site-specific photo-cross-linking strategy, SFPQ is identified to directly interact with FTO and to facilitate its choice of specific RNAs for FTO-mediated m^{6}A demethylation (93), which implies the possibility that protein–protein interactions are another regulatory factor affecting FTO's substrates preference.

ALKBH5 is the second identified m^{6}A demethylase that oxidatively demethylates m^{6}A in mRNA both *in vitro* and *in vivo* (18). Similar to METTL3, ALKBH5 colocalizes with nuclear speckles and influences mRNA processing, which ultimately affects mRNA export and metabolism (18, 94). The knockdown or overexpression of *ALKBH5* can increase or decrease mRNA m^{6}A abundance, respectively. The *ALKBH5*-deficient male mice exhibit compromised spermatogenesis (18). Unlike FTO, ALKBH5 demethylases m^{6}A highly specifically with no other substrates found so far.

Although ALKBH5 and FTO both belong to the Fe(II)/α-KG-dependent dioxygenase AlkB family, their catalytical mechanisms for m^{6}A demethylation are different. FTO performs twice oxidation reactions to oxidize m^{6}A to N^6-hydroxymethyladenosine (hm^{6}A) quickly and to further oxidize hm^{6}A to N^6-formyladenosine (f^{6}A) slowly. The intermediates hm^{6}A and f^{6}A are not stable (∼3 h at neutral pH) and automatically form adenosine by releasing formaldehyde and formic acid, respectively (95) (Fig. 2*D*). Distinct from FTO, ALKBH5 catalyzes a direct m^{6}A-to-A transformation with rapid formaldehyde release (95, 96) (Fig. 2*D*). Specifically, the hydrogen bond between R96 of FTO and N1 atom of purine ring imbues the nucleobase with a partial positive charge and inductively and mesomerically lowers the highest-occupied molecular orbital of the N6 lone pair, thereby disfavoring Schiff base formation and forming hm^{6}A as the product. However, K132 and Y139 of ALKBH5 in the nucleobase recognition region facilitate the formation of a covalent intermediate *via* elimination of H_2O (96) (Fig. 2*E*). The proposed mechanism could explain the difference of catalytical oxidation between FTO and ALKBH5. However, it is still unclear about the catalytical oxidation mechanism how FTO recognizes hm^{6}A to perform the second round oxidation.

The applications of m^{6}A writers and erasers in m^{6}A detection

It is of prime importance to identify the amount and distribution of m^{6}A in RNA molecules in order to understand its biological functions. The development of mass spectrometric techniques and high-throughput sequencing has facilitated the successive development of novel m^{6}A detection approaches, providing useful and valuable tools for studying the physiological functions of m^{6}A (97, 98). m^{6}A antibody-based m^{6}A sequencing (MeRIP) as the first generation of m^{6}A sequencing method provides the transcriptome-wide m^{6}A distribution information and quickens the functional study of m^{6}A (14, 15). However, MeRIP method relies on the specificity of m^{6}A antibody, and its resolution is quite low (at least 200 nt) (33). To overcome these shortcomings, more advanced methods are urgently required to study the role of m^{6}A in living organisms. In recent years, with the in-depth study of the enzymatic mechanisms of m^{6}A writers and erasers, they have been applied to develop new m^{6}A detection methods (34–38) (Fig. 3).

Antibody-based m^{6}A sequencing methods

The most common strategy for transcriptome-wide detection of RNA modifications is RNA immunoprecipitation (RIP) with specific antibodies of RNA modifications coupled with high-throughput sequencing. m^{6}A-seq (also termed MeRIP-seq) utilizes commercial anti-m^{6}A antibody to incubate with fragmented mRNA and enriches the m^{6}A-modified fragments for high-throughput sequencing (14, 15) (Fig. 3*A*). In this way, m^{6}A-seq or MeRIP-seq provides the information of both m^{6}A-modified genes and m^{6}A locations with a resolution around 200 nt.

In order to refine m^{6}A locus more precisely, inspired by PAR-CLIP and iCLIP methods (99, 100), m^{6}A-seq was modified into two similar methods: photo-cross-linking-assisted m^{6}A sequencing strategy (PA-m^{6}A-seq) and m^{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP) (16, 17). PA-m^{6}A-seq metabolically incorporates 4-thiouridine (4SU) into mRNA and covalently cross-links 4SU with nearby aromatic amino acid residues of m^{6}A antibody upon 365 nm UV irradiation (16). This technique vastly improves m^{6}A resolution up to around 23 nt, but can only be used in cells due to the requirement of 4SU metabolism. miCLIP exploits 254 nm irradiation to cross-link mRNA with m^{6}A antibody and introduces nucleotide mismatches or truncation signatures near m^{6}A sites during reverse transcription (17). Although miCLIP successfully achieves a desired single-base resolution of m^{6}A mapping at a transcriptome-wide level, the number of identified m^{6}A sites by miCLIP is limited due to the low cross-linking efficiency.

Although the aforementioned m^{6}A antibody-based m^{6}A sequencing methods are widely applied, these methods have some unavoidable drawbacks. Commercial m^{6}A antibodies from different resources show differences in affinity toward m^{6}A, and m^{6}A antibodies can also recognize non-m^{6}A specific sequences and m^{6}A$_m$, which leads to undesirably high false-positive rates (101). Furthermore, the low reproducibility (30%–60%) was observed across MeRIP-seq datasets in the same cell types or even between biological replicates (16, 33). Together, it is extremely necessary and urgently needed to develop more accurate and precise m^{6}A detection methods.

m^{6}A writer or eraser-based m^{6}A detection methods

m^{6}A antibody-based profiling has displayed moderate effectiveness but with limitations in degree of accuracy and precision. m^{6}A writers and erasers can directly and highly specifically interact with m^{6}A, which can be used for overcoming the shortcomings rising from antibody-based methods.

m^{6}A-REF-seq and MAZTER-seq

MazF is an m^{6}A-sensitive endoribonuclease that cuts the ACA motif but not the m^{6}ACA sequence (102). Taken of this feature, two similar methods, MAZTER-seq and m^{6}A-REF-seq, have been developed (34, 35), which respectively exploit MazF to parallelly digest mRNAs isolated from control *versus* m^{6}A writer knockout cells or RNA treated with FTO demethylation reaction *versus* without FTO treatment (Fig. 3*B*). The m^{6}A removal by either *in vivo* knockout of m^{6}A methyltransferase or *in vitro* FTO demethylation reaction becomes the key step to determine the specific site of m^{6}A by comparing the cutting reads at m^{6}A sites between paired samples. Both methods provide highly accurate and precise information of m^{6}A distribution at single-base resolution; however, they can only identify ∼16 to 25% m^{6}A sites because of the restrictions of MazF specifically recognizing ACA motif.

m^{6}A-label-seq

Similar to other methylation enzymes, the methyl group donor SAM acts as a cosubstrate of the m^{6}A methyltransferase complex and is responsible for transferring a methyl group to

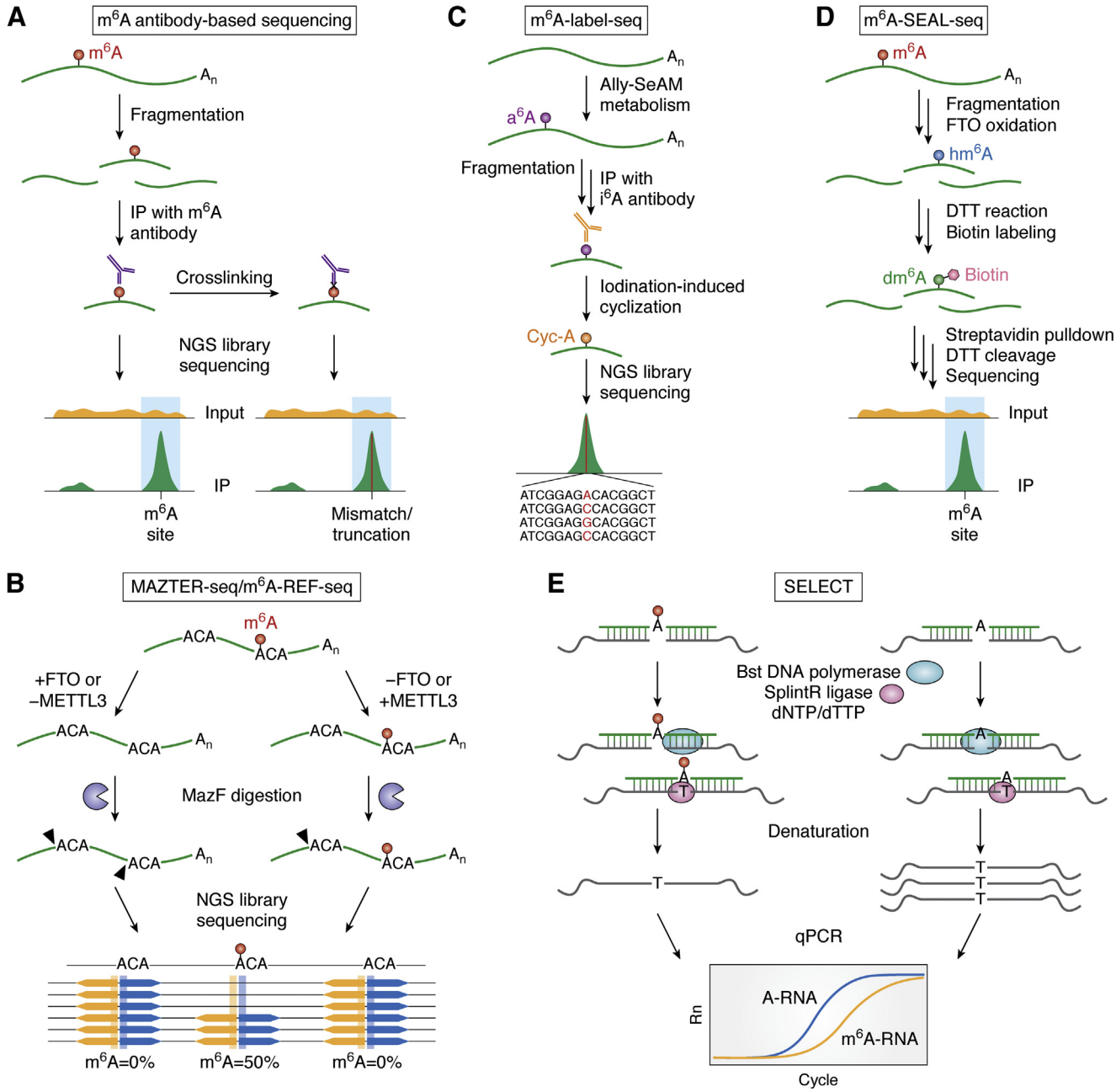

Figure 3. The workflow of m^{6}A detection methods assisted by m^{6}A writers or erasers. *A*, MeRIP-seq/miCLIP/PA-m^{6}A-seq, immunoprecipitation with m^{6}A-specific antibody. *B*, MAZTER-seq/m^{6}A-REF-seq, FTO-assisted differential digestion toward m^{6}A and A. *C*, m^{6}A-label-seq, metabolic labeling with N^6-allyladenosine through methyltransferase as m^{6}A putative sites. *D*, m^{6}A-SEAL-seq, immunoprecipitation with biotin labeling to hm^{6}A produced by FTO-assisted oxidation. *E*, SELECT, FTO assisted single-base elongation and ligation with PCR amplification for quantification.

the N6-position of specific adenosines. A metabolic labeling method was developed to detect mRNA m^{6}A from transcriptome-wide range at single-base resolution (m^{6}A-label-seq) (36). This approach metabolically modifies adenosine into *N*^{6}-allyladenosine (a^{6}A) at the supposed m^{6}A-generating sites by leveraging Se-allyl-l-selenohomocysteine, which forms allyl-SAM (substituting the methyl group on SAM with an allyl) in cells. The produced a^{6}A modifications in mRNA are enriched by antibody and further performed iodination-induced cyclization. The cyclized a^{6}A can induce base misincorporation during reverse transcription and thus provides the transcriptome-wide m^{6}A location at single-base resolution (Fig. 3*C*). However, m^{6}A-label-seq requires the metabolism of Se-allyl-l-selenohomocysteine and only can be used in cellular system.

m^{6}A-SEAL-seq

FTO can oxidize m^{6}A twice to generate hm^{6}A and f^{6}A as intermediate modifications with a half-life of ∼3 h in an aqueous solution under physiological relevant conditions (95). Recently, a FTO-assisted m^{6}A selective chemical labeling

method, termed m^{6}A-SEAL-seq, utilizes a thiol addition reaction to selectively label the m^{6}A oxidation production, hm^{6}A, for m^{6}A detection (37). In this process, m^{6}A modification in fragmented mRNA is first oxidized by FTO into an unstable hm^{6}A, which further reacts with dithiothreitol (DTT) to form stable N^6-dithiolsitolmethyladenosine (dm^{6}A). The free sulfhydryl group on dm^{6}A can be biotinylated for enrichment and sequencing subsequently. m^{6}A-SEAL-seq is an antibody-free and chemically covalent cross-linking method for m^{6}A detection with ∼200 nt resolution (Fig. 3*D*). The validation of m^{6}A-SEAL identified m^{6}A sites revealed that m^{6}A-SEAL-seq has a greater specificity and sensitivity than other available methods, but it needs to be improved to single-base resolution in the future.

SELECT

It is highly desirable to develop m^{6}A locus detection method at the single gene level in order to perform studies of m^{6}A biological functions. Site-specific cleavage and radioactive labeling of the modified nucleotides followed by ligation-assisted extraction and thin-layer chromatography (SCARLET) are some of the most reliable techniques to detect the presence and modification fraction of m^{6}A (103). Briefly, the RNase H cleaves the 5′ of the specific site in a candidate sequence by a complementary 2′-OMe/2′-H chimeric oligonucleotide, followed by radiolabeling with 32P and splinted-ligation of ssDNA. After digesting with RNase T1/A and nuclease, it is possible to distinguish and quantify P1, 32P-A, and 32P-m^{6}A through thin-layer chromatography to indicate the m^{6}A modification status at the candidate sites. SCARLET is the first method to achieve a precise determination of the location and modification proportion of m^{6}A in mRNA or lncRNA. However, it should be noted that the time-consuming and radiolabeling properties make this approach difficult and far from ideal for extensive applications.

A single-base elongation- and ligation-based qPCR amplification method (SELECT) can perfectly quantify m^{6}A locus and fraction at a specific site in mRNA and lncRNA (38) (Fig. 3*E*), which is easy, convenient, and free of radiolabeling. Specifically, SELECT takes advantage of the fact that m^{6}A blocks the single-base elongation activity of DNA polymerase and the nick ligation efficiency of ligases, which influence the formation of intact complementary DNA targeting candidate RNA sequences and ultimately result in a discrepant number of qPCR cycles between m^{6}A and non-m^{6}A samples. Assisted by FTO-mediated m^{6}A demethylation, SELECT can easily distinguish m^{6}A sites in mRNA or lncRNA through comparing the qPCR cycles between total RNA treated with FTO demethylation reaction and without FTO treatment.

The newly developed m^{6}A writers- and erasers-assisted m^{6}A detection methods should be further improved. MAZTER-seq and m^{6}A-REF-seq lose most of the m^{6}A sites (34, 35), which urges us to find new endoribonuclease capable of recognizing more universal sequence motifs while retaining the m^{6}A sensitivity. In m^{6}A-label-seq, the low labeling yield and moderate cellular stress induced by methionine analog result in the loss of endogenous m^{6}A sites and the change of nascent RNA methylation status (36). Currently it can only be used in cells due to the requirement of metabolism of methionine analog, and seeking a methyltransferase that can transfer the allyl group onto m^{6}A sites *in vitro* would provide a good strategy for m^{6}A sequencing. The introduction of DTT into methyl group on N6 position in m^{6}A-SEAL-seq has no obvious effect on base paring between adenosine and thymine, and thus no mutation or stop signal occurs during reverse transcription, which makes it fail to provide precise distribution of m^{6}A (37). m^{6}A-SEAL-seq needs to be further modified to introduce mismatches or truncations during reverse transcription for achieving the single-base resolution.

The functions of m^{6}A based on m^{6}A writers and erasers

m^{6}A modifications have shown significant effects on many aspects of physiological processes including regulating the circadian rhythm, spermatogenesis, stem cell differentiation, viral infection, cancer progression, and so on (39, 104–107). Different m^{6}A readers bind m^{6}A modifications to regulate mRNA processing and metabolism (19, 21, 26–30). The METTL3-METTL14-core complex and FTO/ALKBH5 have decisive impacts on m^{6}A deposition and are thus crucial for m^{6}A-dependent biological functions. The aberrant expression of writers and erasers influences a series of physiological activities (108–111). Here we focus on the functions of m^{6}A writers and erasers in two major biological events, viral infection, and cancer progression, in which m^{6}A were reported to play vital roles and the biological functions of m^{6}A have undergone widespread and elaborate investigations, and we also introduce newly developed m^{6}A writers- and erasers-assisted techniques for deeply and rigorously studying and verifying the physiological functions of m^{6}A.

Viral infection

m^{6}A multidirectionally influences the interactions between RNA virus and host. The host m^{6}A writers and erasers determine the deposition of m^{6}A in viral RNA and affect viral RNA replication and virus proliferation. In host cells, m^{6}A on transcripts related to antiviral pathways also affects the host's antiviral ability (112). (Fig. 4*A*).

Although there is no reported m^{6}A machinery gene in virus genomic RNA (gRNA), viral RNA has been known to contain m^{6}A modification since the 1970s (113–116). m^{6}A is a conserved regulatory modification in viral RNA across the flaviviridae family, whose RNA replicates exclusively in the cytoplasm (117). The host m^{6}A writers and erasers are responsible for the installation and removal of m^{6}A in virus RNA, and the host m^{6}A readers recognize m^{6}A of virus RNA for regulation of viral RNA metabolism. Both Hepatitis C virus (HCV) and Zika virus (ZIKV) contain m^{6}A modifications and are manipulated by host m^{6}A machinery (m^{6}A writer, eraser, and reader) (41, 42) (Fig. 4*A*). Knockdown of host m^{6}A writers or erasers can respectively promote or inhibit infectious particle production of HCV and ZIKV during viral infection, indicating m^{6}A negatively regulates ZIKV or HCV infection.

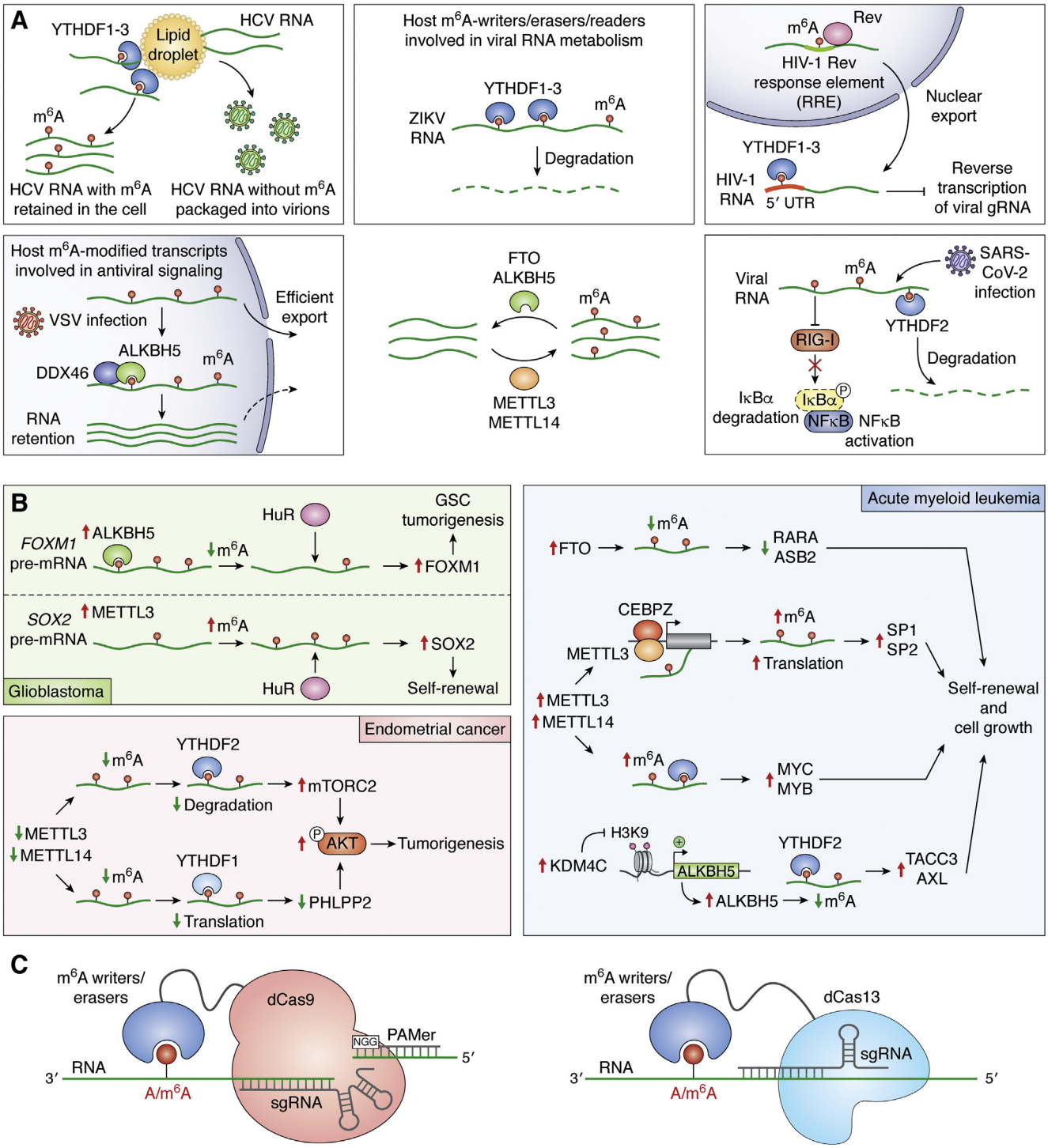

Figure 4. The function research of m^{6}A modification by m^{6}A writers and erasers. m^{6}A writers and erasers play crucial roles in various diseases. *A*, in viral infection, m^{6}A writers and erasers can regulate the m^{6}A level of both viral RNA itself and host antiviral RNAs, affecting viral RNA replication and viral particle production. *B*, in cancers, m^{6}A writers or eraser can act on the mRNA of oncogenes or tumor suppressor genes, thereby affecting the expression of related genes and tumorigenesis. *C*, function research of m^{6}A modification *via* site-specific regulation of m^{6}A by CRISPR/Cas conjugated with m^{6}A writers or erasers. The main components of m^{6}A editing system include m^{6}A writers or erasers, linker, programmable RNA-binding proteins such as dCas9 or dCas13, and sgRNA. When fused with dCas9 or dCas13, m^{6}A writers or erasers can edit specific A or m^{6}A site under the guidance of sgRNA to realize the conversion between A and m^{6}A.

Further studies reveal that host m^{6}A reader proteins YTHDF1-3 are able to relocalize at lipid droplets, viral assembly sites, under HCV infection and bind HCV RNAs to reduce HCV particle production (41). Differently, the functions of m^{6}A in ZIKV particle assembly occurs *via* YTHDF-mediated ZIKV RNA degradation (Fig. 4*A*).

In HIV-1, 14 m^{6}A methylation peaks have been identified in its coding and noncoding regions of HIV-1 gRNA, and 56

human transcripts are specifically methylated in HIV-1-infected T cells. Knockdown of host m^{6}A writers or erasers decreases or increases HIV-1 replication by affecting the m^{6}A methylation of HIV-1 Rev response element (RRE) RNA and the subsequent export of viral RNA. The m^{6}A in HIV-1 RRE RNA enhances the binding of HIV-1 Rev and facilities the nuclear export of HIV-1 RNA (118). Other studies reveal that in a postentry step of HIV-1 infection, host m^{6}A writers can install m^{6}A in HIV-1 RNAs in the cytoplasm and, subsequently, host YTHDF1-3 bind m^{6}A-modified HIV-1 genomic RNA (gRNA), which reduces reverse transcription of HIV-1 gRNA into proviral dsDNA (119, 120) (Fig. 4*A*). These studies provide a new therapeutic thought for treatment of AIDS.

The m^{6}A modifications on viral RNAs installed by host m^{6}A writers can also serve as a molecular marker for host to distinguish self and non-self RNA in innate immune response (121). The host utilizes several pattern-recognition receptors to sense foreign RNAs, such as retinoic-acid-inducible gene I-like receptors (RIG-I) and melanoma differentiation-associated gene 5 (MDA5). Both of them can sense non-self-viral RNA species and activate mitochondrial antiviral signaling, leading to the expression of type I and III interferons (IFNs) and antiviral response (122). RNA modification 2′-*O*-methylation in viral RNA can escape the RNA sensor MDA5 (123). Similarly, m^{6}A can also be hijacked by virus to escape the host RIG-I sensing machinery. The results from human metapneumovirus (HMPV), Hepatitis B virus (HBV), and HCV indicate that m^{6}A in these viral RNA decreases the binding of RIG-I and promotes virus replication while m^{6}A deficiency in these viral RNA increases RIG-I binding and the expression of type I IFNs (43).

Studies of m^{6}A writers and erasers indicate that m^{6}A can also directly regulate gene expression of host genes involved in antiviral immune signaling. Upon vesicular stomatitis virus (VSV) infection, DEAD-box helicase 46 (DDX46) recruits m^{6}A demethylase ALKBH5 onto its binding transcripts encoding antiviral proteins MAVS, TRAF3, and TRAF6 for m^{6}A demethylation, which leads to the nuclear retention of these transcripts and inhibits the production of IFN-I (44) (Fig. 4*A*). Besides, *Alkbh5*-deficient mice display viral resistance through the regulation of metabolite itaconate, an important immune-activated metabolic enzyme, which is required for the effective infection of macrophages and epithelial cells by virus *via* an IFN-I independent mechanism (124, 125). α-KG dehydrogenase (OGDH) participates in tricarboxylic acid (TCA) cycle, which influences the production of itaconate, and m^{6}A modification in *OGDH* mRNA is the target of ALKBH5. The depletion of *ALKBH5* increases more m^{6}A in *OGDH* mRNA and decreases *OGDH* expression through m^{6}A-mediated mRNA degradation, thereby reducing the production of itaconate and inhibiting viral replication (126). Additionally, heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPA2B1) as an m^{6}A-binding protein plays regulatory roles in initiation and enhancement of the innate immune response to DNA virus herpes simplex virus-1 (HSV-1) and interacts with FTO. Upon HSV-1 infection, hnRNPA2B1 breaks the interaction with FTO to retain more m^{6}A in *CGAS*, *IFI16*, and *STING* mRNA and promotes nuclear exports and the translation of these transcripts, therefore enhancing STING-dependent cytoplasmic antiviral signaling (45). These findings demonstrate that m^{6}A on the host transcripts related to antiviral immune pathway can affect the stability of the transcripts, thereby regulating gene expression and antiviral ability of the host.

The Coronavirus Disease 2019 (COVID-19) has become a pandemic in the world since December 2019, which is caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) (127). SARS-CoV-2 has a 30 kb-length single-stranded, positive-sense genomic RNA (128). After the entry of SARS-CoV-2, the full-length and subgenomic negative-sense RNAs are synthesized from the positive-sense gRNA and serve as templates for progeny viral RNA synthesis and mRNA synthesis, respectively. Recent findings suggest that the m^{6}A modification plays regulatory role in SARS-CoV-2 infection. The host m^{6}A writer complex containing METTL3/METTL14 key subunits installs m^{6}A in SARS-CoV-2 gRNA and the negative-sense RNA (129). SARS-CoV-2 infection leads to the nuclear subcellular localization of the host m^{6}A writer METTL14 and eraser ALKBH5 into the cytoplasm in Huh7 cells. The replication of SARS-CoV-2 increases upon knockdown of m^{6}A writer *METTL3*, *METTL14*, and m^{6}A reader YTH domain factor 2 (YTHDF2), but decreases after knockdown of m^{6}A eraser *ALKBH5*, showing that m^{6}A inhibits SARS-CoV-2 replication (129).

The m^{6}A modification also regulates host cell innate immune responses during SARS-CoV-2 infection. RIG-I interacts with SARS-CoV-2 gRNA, but not m^{6}A-modified SARS-CoV-2 gRNA. Knockdown of *METTL3* reduces m^{6}A methylation in SARS-CoV-2 gRNA and increases RIG-I binding to SARS-CoV-2 gRNA, thereby triggering the downstream innate immune signaling pathway and inflammatory gene expression (Fig. 4*A*). Consistently, the patients with severe COVID-19 exhibit the decreased METTL3 expression levels and induced inflammatory genes expression (130). Collectively, these results suggest that m^{6}A plays dual functions in SARS-CoV-2 infection: the host uses m^{6}A writer to methylate SARS-CoV-2 gRNA for degradation through YTHDF2-mediated mRNA decay; however, m^{6}A is also hijacked by SARS-CoV-2 to escape the host RIG-I sensing machinery.

Cancer progression

In different types of cancer, the aberrant expression of m^{6}A writers or erasers results in a significant change of m^{6}A levels in cancer cells and thus influences oncogenesis, including cell adhesion, proliferation, invasion, and apoptosis (46–48).

In glioblastoma (GBM), stem cells are considered as a new promising therapy target (131). ALKBH5 is highly expressed in glioblastoma stem cells (GSCs) and is required for their proliferation and tumorigenesis, and knockdown of *ALKBH5* inhibits the proliferation of patient-derived GSCs. ALKBH5 demethylates m^{6}A-modified *FOXM1* pre-mRNA, a key cell-cycle molecule that is required for the transition between G1/S and G2/M during cell

division (132). The m^{6}A demethylation on *FOXM1* by ALKBH5 affects the interaction between *FOXM1* pre-mRNA and HuR, a nuclear RNA binding protein, and subsequently stabilizes FOXM1 expression levels (133) (Fig. 4*B*). Moreover, METTL3 is also elevated in GSCs and attenuated during differentiation. Specifically, METTL3 increases the m^{6}A methylation of the transcription factor *SOX2* mRNA, which controls a number of genes involved in embryonic development, and enhances its stability by recruiting HuR and promotes the maintenance and radio-resistance of GSCs (134) (Fig. 4*B*). However, in these two studies, HuR plays opposite binding property, preferentially binds m^{6}A-unmodified and -modified RNA substrates, respectively, which depends on the distance between the HuR-binding site and m^{6}A site. In addition, METTL3 alters A-to-I and C-to-U RNA editing events by posttranscriptionally regulating the RNA editing enzymes ADAR and APOBEC3A and manipulates m^{6}A modification on lncRNAs in GSCs. The silencing of METTL3 boosts several aberrant alternative splicing events, which indicates that METTL3 is able to determine several steps in RNA processing and to fine-tune the expressions of genes involved in the oncogenic pathway in GSCs (135).

In some subtypes of acute myeloid leukemia (AML), m^{6}A writers and erasers are typically upregulated and the inactivation of the m^{6}A machinery proteins results in restricted cell proliferation. FTO is highly expressed in AMLs with t(11q23)/MLL rearrangements, t(15;17)/PML-RARA, FLT3-ITD, and/or NPM1 mutations (136). The highly expressed FTO reduces m^{6}A in mRNA transcripts ankyrin repeat and SOCS box containing 2 (*ASB2*) and retinoic acid receptor alpha (*RARA*) and subsequently decreases the transcript expression levels of *ASB2* and *RARA*, thereby enhancing leukemic oncogene-mediated cell transformation and leukemogenesis and inhibiting all-trans-retinoic acid (ATRA)-induced AML cell differentiation (136) (Fig. 4*B*). However, m^{6}A readers YTHDF1 and YTHDF2 have no obvious effect on the regulation of *ASB2* and *RARA*, suggesting that the reader that promotes the mRNA stability of FTO target transcripts in AML has yet to be identified. The oncogenic role of the demethylation of FTO in AML is further confirmed by small-molecule inhibitor FB23-2 selectively targeting FTO demethylation activity (137). METTL3 and METTL14 display their oncogenic roles by promoting the expression of oncogenes such as *MYB* and *MYC* through m^{6}A modification (138, 139). Additionally, METTL3 binds to promoters for m^{6}A methylation and functions as an essential gene for leukemia growth (140). Mechanism study reveals that METTL3 alone is recruited to bind promoters of specific genes (*e.g.*, SP1) by CAATT-box binding protein CEBPZ and subsequently installs m^{6}A modifications within the coding region of these specific genes, thereby enhancing translation of these mRNAs by relieving ribosome stalling at GAN codons (N = A, U, C or G) (140) (Fig. 4*B*). Although CEBPZ can recruit METTL3 and guide it to specific transcripts for m^{6}A deposition, it does not belong to m^{6}A methyltransferase complex.

In addition, ALKBH5 selectively promotes tumorigenesis and cancer stem cell self-renewal in AML by influencing the stability of target mRNA in YTHDF2-dependent manner, including *TACC3*, *AXL*, and *KDM4C* transcripts (141, 142). KDM4C, an H3K9me3 demethylase, can regulate ALKBH5 expression by increasing the chromatin accessibility toward ALKBH5, which triggers the subsequent interaction between ALKBH5 and the target mRNA (Fig. 4*B*). METTL3, METTL14, FTO, and ALKBH5 are all upregulated in AML and promote cell growth, which seems a little contradictory. However, it is reasonable considering that the distinguishing downstream targets of these m^{6}A machinery proteins in different cancer context or different subtypes of AML determine the different directions of cell fate.

m^{6}A writers or erasers are highly expressed in some cancers and promote cell proliferation and tumorigenesis. Nevertheless, in certain cancer cells, either m^{6}A writers or erasers are downregulated and play an important role during oncogenesis (143). For example, in endometrial cancer, METTL3 and METTL14 are either downregulated or mutated, which consequently reduces m^{6}A abundance. The decreased expression of the negative AKT regulator PHLPP2 and increased expression of the positive AKT regulator mTORC2 mediating by YTHDF2 and YTHDF1 respectively lead to the activation of the AKT pathway, which results in increased proliferation and tumorigenicity (144) (Fig. 4*B*). Furthermore, in pancreatic cancer, ALKBH5 is downregulated and inhibits cell proliferation or motility by mediating Wnt signaling through the demethylation of Wnt inhibitory factor 1 (*WIF-1*) and decreasing the methylation and expression of the lncRNA *KCNK15-AS1* (145, 146). In general, m^{6}A modifications on different tumor suppressor genes or oncogenes are affected by either m^{6}A writers or erasers and recognized by diversified m^{6}A readers for gene regulation.

Based on the studies of m^{6}A writers and erasers in cancer progression and viral infection, m^{6}A displays complexity in regulation the occurrence of diseases. The basic functional mechanism of m^{6}A modification in diseases is that the significant change of specific m^{6}A on disease-related transcripts affects gene regulation through m^{6}A readers-mediated RNA processing and metabolism, thereby executing corresponding downstream functions. However, the current studies on the biological functions of m^{6}A are still not completely elucidated. Although the research on viral infection and cancer progression has reached single-transcript and single-base level, some reverse verification methods are still needed to prove the authenticity of the results.

Programmable m^{6}A editing based on m^{6}A writers/erasers-conjugated CRISPR/Cas system

Current evidence suggests that the specific m^{6}A content is extremely important for maintaining normal physiological processes in mammals. In cancer, the higher or lower expression of m^{6}A methyltransferase and demethylase can either promote or suppress tumorigenesis by affecting the expression of specific oncogenes or tumor suppressor genes. Therefore, the regulation of m^{6}A modification at specific sites is of great significance for deeply studying the biological functions associated with m^{6}A and to promote effective therapeutic strategies for cancer and other diseases. In recent 2 years, the combination of the CRISPR/Cas system and m^{6}A

writers or erasers has successfully achieved site-specific m^{6}A editing in single transcript (49–55).

The CRISPR/Cas system is a natural immune mechanism that allows for bacteria to resist viral infection (147). It is now regarded the most powerful gene editing tool that is able to achieve simple knockout of almost all genes in eukaryotes (148, 149). Due to the excellent ability to target genomic DNA, the catalytically inactive CRISPR/Cas as a guide system is combined with other proteins and applied in single-base editing, chromatin engineering, cell imaging, and epigenetic editing (59, 150–152). For example, the CRISPR/Cas9 system is able to mediate epigenetic editing by fusing catalytically dead Cas9 (dCas9) to the corresponding epigenetic modification enzymes, such as DNMT3A for 5 mC methylation, TET1 for 5 mC demethylation, or LSD1 for histone modification H3K4me2 demethylation (59, 153).

To create diverse ability on targeting RNA, two kinds of CRISPR/Cas systems have been developed to allow cutting target RNAs. CRISPR/Cas9 system was modified to specifically recognize and cut RNA by adding a protospacer adjacent motif (PAM)-presenting antisense oligonucleotide (PAMmer), known as RNA-guided Cas9 (RCas9) (154). Cas13, a type Ⅳ CRISPR/Cas system protein, assembles with crispr RNA (crRNA) and forms a crRNA-guided RNA targeting effector complex to directly cut RNA (155). Similarly, catalytically dead RCas9 (dRCas9) and Cas13 (dCas13) have been used as RNA-targeting tools to image RNA and to identify interaction proteins of RNA (156, 157). Recently, combining with m^{6}A writers or erasers, these two systems are successfully applied into programming RNA modification (Fig. 4*C*).

The fusion of dRCas9 with either MTase domain of METTL3 and METTL14 or with m^{6}A demethylases ALKBH5 or FTO forms engineered m^{6}A writers or erasers to install or delete m^{6}A at specific sites in transcripts. Site-specific addition of m^{6}A at 5′ UTR or 3′ UTR in mRNA regulates downstream translation or mRNA stability, and site-specific removal of m^{6}A in lncRNA *MALAT1* affects RNA structure to regulate the binding affinity of MALAT1-interaction protein HNRNPC (49). Furthermore, the single catalytical domain of METTL3 fused with dRCas9 also installs site-specific m^{6}A efficiently and has been used to write m^{6}A onto the 3′ UTR of *CDCP1* transcript in order to promote mRNA translation and bladder cancer development (50).

RCas9 system needs additional artificially synthesized PAMmer to target RNA, which makes the RCas9 system more complicated and incompatible with some delivery strategies, such as viral infection. The Cas13 protein, which directly targets RNA with a guide RNA, is able to avoid these drawbacks and provides a new tool for RNA editing. In particular, dCas13 fused to the METTL3 protein lacking its zinc finger domain is able to target single RNA and install m^{6}A modification. The dCas13-METTL3 m^{6}A editing system can be fused with either a nuclear localization signal sequence or a nuclear export signal sequence to edit specific m^{6}A sites in nuclear RNA or cytoplasmic mature transcripts (51). The engineered m^{6}A erasers are also developed through the combination of dCas13 with m^{6}A demethylases. The fusion of dCas13 and ALKBH5 (dm^{6}ACRISPR) is used to demethylate specific m^{6}A sites on *EGFR* and *MYC* transcripts to inhibit the proliferation of cancer cells (52), and the fusion of dCas13 and FTO can also remove m^{6}A in nuclear RNA *LINE-1* in order to affect transcription (53).

SunTag system can realize the recruitment of multiple protein copies to a polypeptide scaffold (158). dCas13 coupled with the SunTag system acts as another type of m^{6}A eraser tool, termed as TRADES. Specifically, dCas13 fused with ten copies of GCN4 peptides can recruit multiple scFv-FTO or ALKBH5 fusion proteins to demethylate the target m^{6}A, which increases the demethylation window (54). Furthermore, photoactivatable m^{6}A editing tools have also been developed using the blue-light-inducible heterodimer proteins CIBN and CRY2PHR. In this system, CIBN and CRY2PHR are respectively fused to dCas13 and m^{6}A writer or erasers. Under blue light, CIBN and CRY2PHR form a heterodimer to recruit m^{6}A writer or erasers at the dCas13-guided regions for m^{6}A editing (55).

Site-specific regulation of m^{6}A has achieved precise m^{6}A editing in cells. Compared with rough overexpression of m^{6}A writers or erasers, the utility of CRISPR/Cas system provides better targeting ability for the addition or removal of m^{6}A on specific sites and avoids the interference of global m^{6}A, which make the results more reliable when studying the biological functions of m^{6}A. However, in order to achieve the precise control of m^{6}A editing and solve the off-target effect rising from CRISPR/Cas system itself, more accurate tools are needed for comprehensively and deeply studying the physiological functions of m^{6}A and treating diseases.

Conclusions and perspectives

The m^{6}A modification in RNA participates in various biological processes. The normal performance of these biological processes is closely related to the levels of m^{6}A and their correct regulation. Therefore, studying the distribution patterns of m^{6}A in cells is of great significance for the exploration of the biological functions associated with m^{6}A. The emergence of m^{6}A-specific antibody provides an effective method for studying the transcriptome-wide distribution of m^{6}A. However, due to a high false-positive rate, poor reproducibility and low resolution, it is imperative to develop more accurate m^{6}A detection techniques. The in-depth exploration of the mechanisms of m^{6}A methyltransferase and demethylase has allowed to develop new m^{6}A sequencing methods assisted by m^{6}A writers and erasers, which greatly improved the authenticity and sensitivity of m^{6}A detection. However, all available methods have not fully met the needs for quantification of m^{6}A fraction, single-base resolution, and low amount of RNA (from nanograms of RNA to single cell) at the same time.

In recent years, the mechanistic role of m^{6}A in different diseases (*e.g.*, cancer) has been gradually explained. Many cancers are caused by the abnormal expression of m^{6}A writers or erasers in cells, which varies the m^{6}A fractions of some specific transcripts and subsequently affects gene expression, leading to the occurrence and development of diseases. Therefore, adjusting the level of m^{6}A on specific transcripts

might facilitate the maintenance of homeostasis in transcripts and impact biological functions. The development of engineered m^{6}A editing tools assisted by RNA guider dRCas9 or dCas13 provides an alternative method for site-specific m^{6}A regulation, which can be applied for m^{6}A functional study and potential tools for treatment of diseases.

Unlike the direct regulation of RNA expression through knockdown or overexpression, the regulation of m^{6}A in specific transcripts can allow for the posttranscriptional regulation of RNA. The function of m^{6}A is far from simple and is determined by the m^{6}A-binding proteins nearby (159). m^{6}A can be regarded as a multifunctional button that determines the fate of RNA molecules. By regulating the levels of m^{6}A in the transcript, organisms can make corresponding stress responses under different physiological conditions and achieve the autonomous regulation of transcript structure and expression to be able to adapt to changes in the environment.

Acknowledgments—This work was supported by Beijing Natural Science Foundation (Z200010), the National Natural Science Foundation of China (nos. 21822702, 92053109, and 21820102008), and the National Basic Research Program of China (2019YFA0802201 and 2017YFA0505201).

Author contributions—W. Z and Y. Q. writing–original draft; G. J. supervision; G. J. funding acquisition; G. J. writing–review and editing.

Conflict of interest—The authors have declared no conflicts of interest for this article.

Abbreviations—The abbreviations used are: 3′ UTR, 3′ untranslated region; 5 mC, 5-methylcytosine; α-KG, α-ketoglutarate; Ac^{4}C, N^4-acetylcytidine; AML, acute myeloid leukemia; dm^{6}A, N^6-dithiolsitolmethyladenosine; DTT, dithiothreitol; FTO, fat obesity-associated protein; GSC, glioblastoma stem cell; HCV, Hepatitis C virus; IFN, interferon; m^{6}A, N^6-methyladenosine; miCLIP, m^{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation; miRNA, microRNA; mRNA, messenger RNA; OGDH, α-KG dehydrogenase; PAM, protospacer adjacent motif; RIG-I, retinoic-acid-inducible gene I; RIP, RNA immunoprecipitation; rRNA, ribosomal RNA; SAM, S-adenosyl methionine; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFPQ, splicing factor proline and glutamine-rich; snRNA, small nuclear RNA; tRNA, transfer RNA; WTAP, Wilms' tumor 1-associating protein; XIST, X-inactive specific transcript; ZIKV, Zika virus.

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