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# RNA Modification in the Immune System

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## Abstract

Characterization of RNA modifications has identified their distribution features and molecular functions. Dynamic changes in RNA modification on various forms of RNA are essential for the development and function of the immune system. In this review, we discuss the value of innovative RNA modification profiling technologies to uncover the function of these diverse, dynamic RNA modifications in various immune cells within healthy and diseased contexts. Further, we explore our current understanding of the mechanisms whereby aberrant RNA modifications modulate the immune milieu of the tumor microenvironment and point out outstanding research questions.

## 1. INTRODUCTION

RNA modifications are chemical marks to the bases or ribose sugar in RNA molecules. So far, more than 150 distinct modifications have been identified. The first discovered RNA modification, pseudouridine (Ψ), was reported as early as the 1950s (1). It was not until the last decade that researchers began to understand the broad biological impacts of these modifications. For example, the diverse impacts of $N^6$-methyladenosine ($m^6A$), the best-characterized mRNA modification, were revealed in 2011 (2–4). Subsequently, the role of $m^6A$ in regulating the fate of both coding RNA (mRNA) and noncoding RNAs (miRNA, tRNA, etc.) has been widely studied. Though the majority of our mechanistic understanding of how RNA modification functions in immunity comes from studies on $m^6A$, we will also consider research on other modifications, including but not limited to $m^5C$, $m^1A$, and Ψ (5, 6).

In this review, we discuss how RNA modifications control the immune response both in normal physiological processes and in various diseases. By summarizing the current understanding of how RNA modification affects multiple aspects of the RNA life cycle, as well as the development of state-of-the-art RNA modification sequencing methods that have ignited research on immune cells, we address the indispensable role of RNA modification in the immune system, including the development of immune cells and the regulation of innate and adaptive immune responses. Finally, we emphasize the roles of dysregulated RNA modification during antiviral and antitumor immune responses.

## 2. THE ROLE OF RNA MODIFICATIONS IN GENE EXPRESSION REGULATION

RNA modifications are regulated by three classes of proteins: writers, erasers, and readers, which add, remove, and bind the modified bases. Rapid progress in our understanding of the RNA modification machinery components, particularly $m^6A$, has revealed their critical roles in fine-tuning the immune response. In this section we introduce how RNA modifications regulate the expression and function of RNAs. We focus on the $m^6A$ machinery since the regulation and function of other RNA modifications remain open questions that have been reviewed by others (5–7).

### 2.1. Writer, Eraser, and Reader

$m^6A$ is added to mRNA by a writer complex composed of multiple subunits. METTL3 is the primary nuclear methyltransferase that adds $m^6A$ to mRNA cotranscriptionally (7a, 7b). METTL14, a homolog of METTL3, is also required for $m^6A$ formation in cells (8, 9). While METTL14 itself has no methyltransferase activity due to the lack of a SAM (*S*-adenosylmethionine)-binding domain, METTL14 binds to METTL3 and forms a heterodimer that stabilizes METTL3 and promotes its catalytic activity (10–12). The METTL3-METTL14 heterodimer is responsible for catalyzing the majority of $m^6A$ in mRNA (13, 14). Wilms tumor 1–associating protein (WTAP), tethering METTL3-METTL14 to transcription sites, promotes RNA binding and is required for $m^6A$ methyltransferase activity in vivo (15, 16). Further research has characterized the other accessory factors of the METTL3-METTL14 complex, including VIRMA (17), ZC3H13 (18), RBM15/15B (19), and HAKAI (20). In addition to METTL3, three other enzymes, METTL16, METTL5, and ZCCHC4, have been identified as eukaryotic $m^6A$ methyltransferases that are responsible for adding $m^6A$ on U6 small nuclear RNAs (snRNAs) (21), 18S ribosomal RNA (rRNA) (22) and 28S rRNA (23), respectively.

The $m^6A$ erasers are demethylases that convert $m^6A$ into adenosine. Two $m^6A$ erasers, FTO and ALKBH5, have been identified (2, 24). The expression patterns of these two $m^6A$ erasers are distinct among different tissues and cell types, indicating that FTO and ALKBH5 participate

in different biological pathways. In addition, FTO is also reported to demethylate other RNA modifications, including $N^6$,2′-*O*-dimethyladenosine ($m^6A_m$) and $m^1A$ (25, 26).

RNA $m^6A$ affects the fate of mRNA mainly by recruiting its reader protein. Multiple reader proteins have been identified with different functions, adding to the complexity of the $m^6A$ function. The primary $m^6A$ readers are YTH family proteins. These proteins, including YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2, all contain an $m^6A$-binding pocket in the YTH domain (27). YTHDF2, which was the first reader identified, promotes the degradation of its cytoplasmic binding targets by recruiting the CCR4-NOT complex (28). Two other YTH family proteins, YTHDF1 and YTHDF3, which promote translation efficiency of target mRNAs, were identified as cytoplasmic $m^6A$ reader proteins in later studies (29–29b). The nuclear $m^6A$ reader YTHDC1 was found to affect mRNA fate in multiple ways, including mRNA splicing, nuclear export, and RNA degradation (30–32). Another reader, YTHDC2, localizes in both the nucleus and cytosol (33), affecting both mRNA decay and translation during spermatogenesis (33a). Besides the YTH domain–containing readers, dozens of proteins have also been identified to preferentially bind $m^6A$ modified RNA, including IGF2BP1–3 (34), heterogeneous nuclear ribonucleoproteins (35–37), FMR1 (38), and eIF3 (eukaryotic translation initiation factor 3) (39). Further characterization of the regulatory functions of this expanding list of $m^6A$ readers will be critical for understanding the way $m^6A$ impacts the immune response and other biological processes.

## 2.2. RNA $m^6A$ Regulates RNA Life Cycle

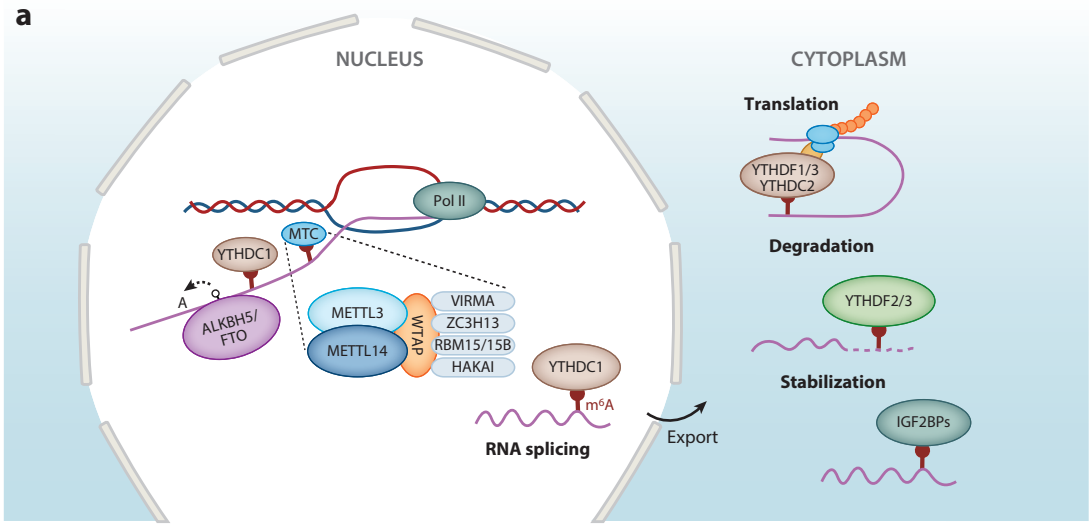
The functional consequences of RNA methylation mainly depend on the binding by the specific reader and cellular localization. It includes almost all aspects of the mRNA life cycle (**Figure 1**). The regulation starts at mRNA transcription, then the $m^6A$ reader in the nucleus regulates mRNA splicing (40), mRNA structure (37), and the decay of specific transcripts (30). After exporting the $m^6A$-marked mRNA to the cytoplasm (32), the readers in the cytoplasm affect the degradation (28), stability (34), and translation of the $m^6A$-containing mRNAs (29).

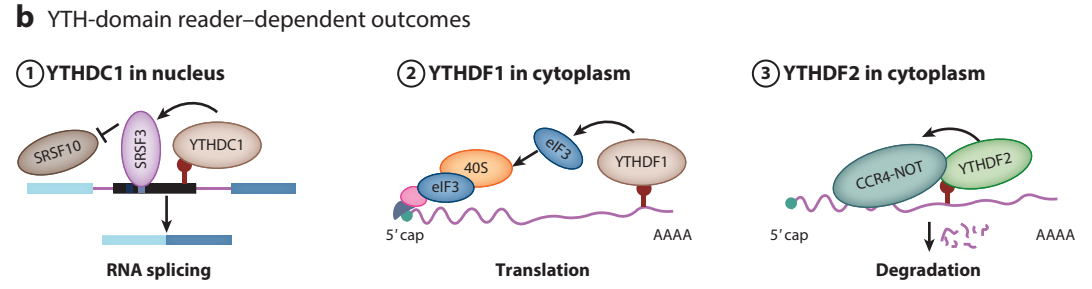
In the cytoplasm, YTHDF2 promotes degradation of its targets by localizing the YTHDF2-binding mRNA from the translatable pool to cellular mRNA decay sites (such as P-bodies) (28) and recruiting the CCR4-NOT deadenylase complex, thereby shortening the half-lives of $m^6A$-containing mRNAs (41). Another reader, YTHDF1, promotes the translation efficiency of $m^6A$-modified mRNAs by recruiting eIF3 (29), a major component of the translation initiation complex. Notably, two studies revealed additional mechanisms through which $m^6A$ enhances translation without binding of an $m^6A$ reader. The first found that eIF3 could directly bind $m^6A$ sites in the 5′ UTR (untranslated region) (39). The second found that METTL3, the $m^6A$ methyltransferase, remains bound to the $m^6A$-modified transcript in the cytoplasm, where METTL3 can target $m^6A$-marked transcripts and recruit eIF3 to promote translation (42). These findings highlight that $m^6A$ can function independently of YTH-domain reader proteins. Although the evidence is limited, a potential mechanism is that the methyltransferase (43) or demethylase might regulate the metabolism of $m^6A$-modified transcripts by recruiting RNA-binding proteins (RBPs) that deposit nearby $m^6A$ sites and RBPs consequently function to affect multiple aspects of the RNA life cycle. Considering that the spatial distribution of $m^6A$ readers dictates their accessibility toward $m^6A$-marked transcripts and that this distribution would translocate in response to certain stimuli, such a hypothesis will remain unconvincing until the molecular basis for differential cellular localization and the corresponding interaction between partners are further clarified.

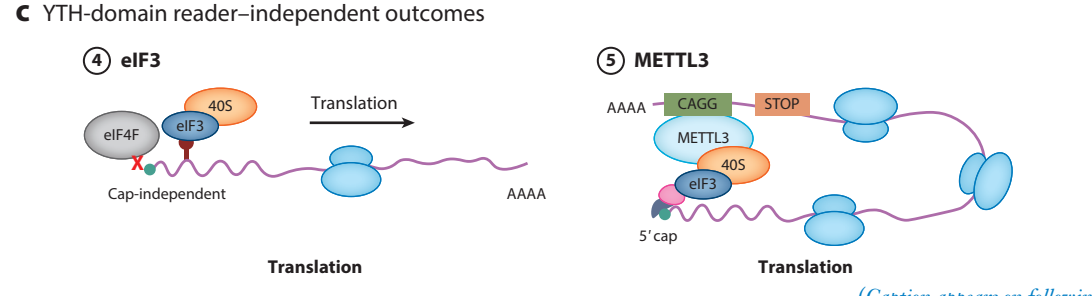
Another reader, YTHDC1, is considered the major $m^6A$ reader in the nucleus. The first identified function of YTHDC1 is regulating mRNA splicing in HeLa cells, through recruiting pre-mRNA splicing factor SRSF3 while blocking SRSF10 (31). Another report also finds that in *Drosophila*, YT521-B (the homolog of YTHDC1) affects the splicing of *Sxl*, thus regulating sex

determination (44). The binding of YTHDC1-SRSF3 enhances export of the m$^6$A-modified mRNA to the cytoplasm since SRSF3 is a key component of the NXF1 mRNA export pathway (32).

The previous model for m$^6$A function mainly relies on m$^6$A reader proteins together with other RBPs to execute specific functions, such as SRSF3 for splicing, CCR4-NOT for decay, and eIF3 for translation. Recent advances suggest another mechanism of action: the interaction between reader proteins in the YTH family and m$^6$A-modified mRNA promotes the formation of phase-separated, membrane-less granules in cells (45). YTH proteins contain a large




(*Caption appears on following page*)

**Figure 1** (*Figure appears on preceding page*)

m$^6$A affects multiple aspects of RNA life. (*a*) The m$^6$A modification is added to mRNA cotranscriptionally by the writer complex MTC in the nucleus. MTC is composed of methyltransferase METTL3 in complex with METTL14, and a set of accessory proteins (e.g., VIRMA, ZC3H13, RBM15/15B, and HAKAI). m$^6$A can be removed by two erasers, ALKBH5 and FTO. In the nucleus, YTHDC1 binds to m$^6$A-marked transcripts, affecting mRNA splicing and export. Upon export to the cytoplasm, m$^6$A-marked transcripts could be captured and regulated by cytoplasmic readers, affecting posttranscriptional processes, including mRNA translation, degradation, and stabilization. (*b*,*c*) m$^6$A affects the fate of mRNA mainly by recruiting its reader proteins. Representative molecular mechanisms that are dependent (*b*) or independent (*c*) of YTH-domain reader proteins are shown. ① YTHDC1 regulates mRNA splicing through recruiting pre-mRNA splicing factor SRSF3 and blocks SRSF10. ② YTHDF1 promotes mRNA translation efficiency by recruiting eIF3. ③ YTHDF2 promotes mRNA degradation by recruiting CCR4-NOT deadenylase complex. ④ m$^6$A residue within the 5′ UTR directly binds eIF3 and promotes cap-independent translation. ⑤ m$^6$A writer METTL3 binds to m$^6$A-marked transcripts and recruits eIF3 to promote translation. Abbreviations: eIF3, eukaryotic translation initiation factor 3; MTC, methyltransferase complex; pol II, polymerase II. Figure adapted from images created with BioRender.com.

intrinsically disordered region (IDR) that is enriched with proline and glutamine and promotes liquid-liquid phase separation (LLPS) (45). YTHDF1, YTHDF2, and YTHDF3 have all been shown to undergo LLPS and form RNA-protein condensates in the cell cytoplasm (46). Those m$^6$A-RNA-containing condensates then selectively partition into specific liquid condensates such as stress granules and P-bodies. This finding is in line with a previous observation that YTHDF2 colocalizes with P-bodies (28). This partitioning behavior may provide m$^6$A-modified mRNA with a higher chance of being compartmentalized into specific condensates in the cytosol. Given that condensates are dynamically formed and dissolved within a short time frame, the characteristics of m$^6$A-RNA-containing condensates thought to contribute to cellular function are still under investigation.

Chromatin-associated regulatory RNAs (carRNAs), including enhancer RNA (eRNA), promoter-associated RNA (paRNA), and RNA transcripts from repeat elements, are characterized by high levels of m$^6$A modification (47) (**Figure 2**). These m$^6$A-carRNAs can be recognized by YTHDC1 and degraded, further inhibiting nearby gene transcription (47). In addition, m$^6$A-marked RNA transcripts from repeat elements such as LINE1 and IAP (48, 49) that are recognized by YTHDC1 potentiate the formation of heterochromatin and suppress the expression of repeat transcripts. Nevertheless, a recent study reported that by binding with m$^6$A-modified eRNA, YTHDC1 facilitates the formation of transcriptional activator condensates, enhancing gene transcription (50). These data support that m$^6$A in eRNAs promotes gene expression through a mechanism involving YTHDC1. Another study revealed that YTHDC1 and an m$^6$A writer complex are recruited to gene promoters and promote the polymerase II pause release (51). Considering that the functions of different types of m$^6$A-carRNA are distinct and context dependent, further investigation into the molecular mechanisms mediating the cross talk between m$^6$A-carRNA and epigenetic regulators is needed.

## 3. DEVELOPMENT OF HIGH-THROUGHPUT SEQUENCING METHODS TO MAP RNA MODIFICATIONS

### 3.1. Principles of Sequencing Methods for RNA Modification Profiling

The advent of high-throughput sequencing technologies has been driving advances in understanding the distribution patterns and functions of various RNA modifications. In general, there are two fundamental strategies to identify modified RNA bases at a transcriptome-wide scale. The first is using a modification-specific antibody or chemical label to capture modified RNA fragments. An example of this strategy is MeRIP-seq (methylated RNA immunoprecipitation sequencing) for profiling m$^6$A (3, 4). These methods enrich signals around the modified site in the sequencing data. The second strategy is to use an enzyme-assisted reaction or a specific chemical reaction

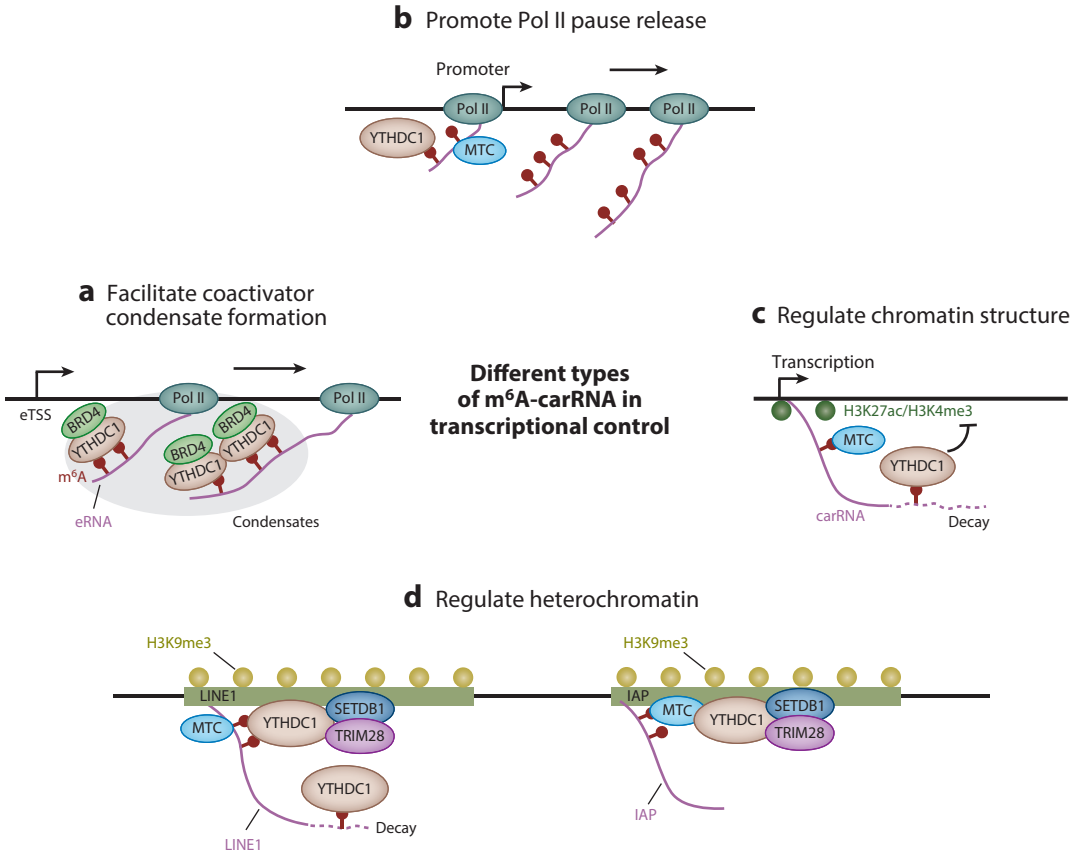



**Figure 2**

The functions of different types of m$^6$A-carRNA in transcriptional control. (*a*) m$^6$A-eRNA is transcribed by polymerase II from the enhancer transcriptional start site. m$^6$A-eRNA recruits the nuclear m$^6$A reader YTHDC1 to phase separate into liquid-like condensates, and the m$^6$A-eRNA/YTHDC1 condensate facilitates the formation of the BRD4 coactivator condensate to enhance gene expression. (*b*) The m$^6$A MTC and the nuclear reader YTHDC1 are recruited to gene promoters in an RNA- and transcription-dependent manner, enhancing the release of polymerase II toward the gene body. (*c*) MTC-mediated m$^6$A modification on carRNA, including paRNA and eRNA, is recognized by YTHDC1. The MTC-YTHDC1 axis promotes the decay of m$^6$A-modified carRNAs and reduces local chromatin accessibility, accompanied by decreased active histone marks H3K4me3 and H3K27ac. (*d*) METTL3 predominantly localizes to the genomic loci of repeats IAP and LINE1 and methylates the nascent repeat transcripts. These repeat transcripts can be recognized by YTHDC1, which interacts with METTL3 and subsequently promotes the association of METTL3 with chromatin. METTL3 interacts physically with H3K9me3 methyltransferase SETDB1 and its cofactor TRIM28 to form a transcriptionally repressive environment covering the repeats' genomic loci. Abbreviations: carRNA, chromatin-associated regulatory RNA; eRNA, enhancer RNA; eTSS, enhancer transcriptional start site; IAP, intracisternal A-particle; MTC, methyltransferase complex; paRNA, promoter-associated RNA; pol II, polymerase II.

on the modified bases, thus making them "visible" after reverse transcription. These reactions introduce base substitutions, deletions, or truncations (either before or after the modified bases). Pseudo-seq for Ψ and Bis-seq (bisulfite sequencing) for m$^5$C profiling are examples of this second strategy (52, 53).

### 3.2. Antibody-Based Sequencing Methods to Detect RNA Modifications

RNA immunoprecipitation (RIP) was the first method developed for profiling RNA modification at the transcriptome level. By using anti-m$^6$A antibody to capture m$^6$A-modified RNA

fragments, RIP-seq provides a global view of the $m^6A$ distribution pattern. Studies using this method have revealed that $m^6A$ is highly enriched near stop codons and in 3′ UTRs with a consensus GAC/AAC motif (3, 4). Note that RIP can only locate the position of $m^6A$ at a resolution of 100–200 nucleotides. To further improve the resolution, photo-induced cross-linking was combined with RIP to generate specific substitutions or a truncation signature around modification sites during reverse transcription, which could generate an $m^6A$ map at individual-nucleotide resolution [e.g., PA-$m^6A$-seq (photo-cross-linking-assisted $m^6A$ sequencing) (54) and miCLIP ($m^6A$ individual-nucleotide-resolution cross-linking and immunoprecipitation) (55)].

Both the original RIP-seq and related cross-linking-based sequencing methods rely on highly specific antibodies to recognize and immunoprecipitate modified RNA. In principle, these approaches could easily be transferred to other modifications (i.e., beyond $m^6A$), provided that an antibody recognizing the relevant modified base is available. Antibody-based sequencing has been applied to study modifications other than $m^6A$, including $m^1A$ (56, 57), $ac^4C$, and $m^7G$ (58–60). Recent work found that the $m^1A$ antibody employed undergoes cross-reactivity with the $m^7G$-cap, which may lead to false positives at the 5′ UTR region of transcripts (61). Although such cross-reactivity has also been detected for the $m^6A$ antibodies, the signal-to-noise ratio is considered acceptable (5), as the abundance of $m^6A$ is high.

### 3.3. State-of-the-Art Methods for Detecting and Profiling RNA Modifications

Although MeRIP- and miCLIP-based methods have been widely accepted and have yielded useful insights about the $m^6A$ distribution pattern and regulatory mechanisms, they have several disadvantages and need to be improved in many aspects. The first disadvantage is that these methods cannot be used for quantification of the modification ratio. To overcome this and enable quantification of the $m^6A$ ratio on mRNA, $m^6A$-LAIC-seq (61a) ($m^6A$-level and isoform-characterization sequencing) modifies the original MeRIP-seq method by isolating both $m^6A$-positive and $m^6A$-negative post-RIP fractions and sequences full-length transcripts. These steps support the quantification of $m^6A$ levels for all isoforms of transcripts for each gene. Two recently developed methods, MAZTER-seq and $m^6A$-REF-seq ($m^6A$-sensitive RNA-endoribonuclease-facilitated sequencing), use the MazF RNase enzyme that selectively cleaves RNA at unmethylated ACA motifs but not their $m^6A$ methylated counterparts. These methods can achieve quantitative detection of 16–25% of all $m^6A$ methylated sites at single-base resolution (62, 63). Another strategy is adding synthetic modification-free RNA molecules to RNA modification sequencing methods as an internal reference for quantification. Incorporating the internal reference in three representative modification sequencing methods (MeRIP-seq and MAZTER-Seq for $m^6A$; Bis-seq for $m^5C$) effectively eliminates false positives caused by sequence context and RNA secondary structure (64).

A second limitation faced when using antibody-based RNA modification profiling methods is poor sensitivity. Chemical-assisted labeling approaches have been widely used to improve the sensitivity and resolution of sequencing methods for multiple RNA and DNA modifications (65–67). Because the binding affinity of biotin-streptavidin is one of the strongest known noncovalent interactions, the $m^6A$ seal ($m^6A$ selective chemical labeling) method introduced a biotin tag selectively to modified bases, which can dramatically enhance enrichment efficiency (68).

A third disadvantage of antibody-based profiling methods is that these methods cannot reveal the location of a modified site with single-base resolution. Considering the fact that the chemical reactions used to label modification sites can induce mutation during cDNA reverse transcription, several methods employ such mutation-based concepts to develop $m^6A$ profiling methods to successfully achieve single-base resolution (69, 70). Conceptually similar approaches employing chemical labeling methods have also been used to profile Ψ modification at single-base resolution (53, 71). The development of novel sequencing methods with enhanced sensitivity continuously

advances our understanding of where and how RNA modifications work. For example, a recent study used Pseudo-seq to analyze Ψ modifications in human HepG2 cells, revealing that Ψ modifications are unexpectedly present on chromatin-associated pre-mRNAs and might be involved in the regulation of alternative pre-mRNA splicing and 3′ end processing (72).

One of the relatively recent milestones reached in the development of novel sequencing technologies is single-cell omics. Single-cell sequencing methods have been applied to dissect the complex ecosystem by profiling the immune landscapes in tumor microenvironments. For RNA modification, a method called DART-seq (deamination adjacent to RNA modification target sequencing) successfully monitors $m^6A$ modification at the single-cell level (73). DART-seq was originally developed in 2019 (73a); it uses cytidine deaminase APOBEC1 fused with the $m^6A$-binding YTH domain to induce a cytidine-to-uridine mutation at sites adjacent to $m^6A$-modified bases. In 2022, the same research group optimized the method, achieving single-cell resolution (73). By analyzing $m^6A$ in individual human HEK 293T cells, they identified differentially methylated mRNAs throughout the cell cycle. Moreover, researchers were able to define cellular subpopulations on the basis of their specific RNA methylation signatures. A notable limitation of single-cell DART-seq is that it requires overexpression of the APOBEC1-YTH fusion protein in cells, which prevents its application to human clinical samples. Thus, an enzyme-free, single-cell method for profiling RNA modifications that does not require genetic manipulation of primary cells is necessary to accurately assess the variety of RNA modifications occurring in different cellular states.

### 3.4. Guidance for Selecting Suitable Sequencing Method(s) for Immunology Studies

Recent experimental technologies based on RNA modifications have been successfully used to discover novel molecules and pathways that regulate immunological processes. Here we highlight several approaches that have benefitted studies investigating the regulatory landscapes of RNA modifications in immune cells. These studies, which serve as an essential step in achieving marker-informed sorting of cell population(s) of interest, require that the experimental sampling methods used reflect the context of the particular immune response being studied. Sorting is typically followed by profiling dynamic changes of modification(s) of interest (e.g., using mass spectrometry) while simultaneously monitoring relevant cellular phenotypes. It bears emphasizing that companion experiments that rationally modulate candidate regulators—including for example putative writer and eraser enzymes using CRISPR/Cas9 screening systems or conditional-knockout models—can yield a dynamic profile of how a given component of the epitranscriptomic machinery exerts its regulatory impacts.

Global profiling of RNA modifications can be performed to identify RNAs enriched for a particular modification, and the populations of these differentially modified RNAs can be compared under suitable cellular perturbations under both biological and medical contexts. For example, a study combining genetic perturbations of the $m^6A$ writer METTL14 quantification and global profiling of RNA $m^6A$ methylation on transcripts isolated from tumor-associated macrophages (TAMs) revealed a subset of TAMs that are explicitly regulated by METTL14-$m^6A$; that study proposed a mechanism wherein $m^6A$ drives a TAM phenotypic transition specifically within the tumor microenvironment (74).

In most scenarios characterized to date, the impact of an RNA modification in cells is mediated by reader proteins. Researchers can capture the binding sites of readers using, for example, RIP-seq or CLIP-seq methods and are also able to monitor the impact of modifications on specific steps of the RNA life cycle using various sequencing methods: e.g., Gro-seq (global run-on sequencing) for transcription, Ribo-seq (ribosome sequencing) for translation, or SLAM-seq [thiol(SH)-linked alkylation for the metabolic sequencing of RNA] for degradation. One study used RIP-seq of the

known m$^6$A reader YTHDF1 and ribosome profiling to evaluate the effect of YTHDF1 on its targets (75). With this approach—supported by follow-up validation in knockout mice—the authors demonstrated that YTHDF1 increases the translation efficacy of a group of m$^6$A-marked transcripts involved in immune evasion (75). Integrating additional types of output reflecting the RNA life cycle relies on several approaches shown to be effective by several immunological studies, including s4U-seq for RNA decay (76) and TT-seq (transient transcriptome sequencing) for nascent RNA production (50).

Recent explosive growth in the number of single-cell-resolution techniques for profiling diverse cellular processes (e.g., nascent RNA, RNA decay, and translation) is certain to further deepen our understanding of the apparently widespread functional impacts of RNA modification in diverse immune subsets both during normal development and in disease.

## 4. THE ROLE OF RNA MODIFICATIONS IN THE IMMUNE SYSTEM

### 4.1. RNA Modifications During Immune System Development

Hematopoiesis is a precisely orchestrated process in which hematopoietic stem cells (HSCs) (which are conceptualized as being at the top of the hematopoietic hierarchy) give rise to all cells comprising mammalian blood (77, 78). Given that hematopoiesis is tightly regulated by both transcriptional and epigenetic programs, HSC lineage commitment has been proposed as an informative model for studying the function of various epigenetic regulators (79). RNA modifications have recently been shown to affect the maintenance of the HSC pool and to direct lineage specification during hematopoiesis. RNA modifications have been detected in hematopoietic stem/progenitor cells (HSPCs) during embryogenesis, and as development proceeds, HSCs arise from the arterial endothelium in a process known as the endothelial-to-hematopoietic transition (80, 81). This process produces hundreds of blood progenitors, a portion of which develop into definitive HSCs. Several studies have revealed that in both zebrafish and mice, deletion of *Mettl3* or *Ythdf2* in arterial endothelial cells triggers the continuous activation of Notch signaling by reducing YTHDF2-mediated *Notch1a* mRNA decay. Such activation of Notch signaling blocks the endothelial-to-hematopoietic transition, thus repressing generation of the earliest HSCs (82, 83).

m$^6$A has been implicated in controlling multilineage hematopoiesis. *Mx1*-Cre-driven *Mettl3* deletion in adult hematopoietic cells leads to deficiencies in their reconstitution activity, reflected by the accumulation of dividing HSCs and defects in HSC differentiation (84, 85). A recent study employed single-cell RNA sequencing and showed that accumulated *Mettl3*-deficient HSCs represent a blocked immunophenotypic stem cell–like cell population (85). These cells have reduced self-renewal in vivo and fail to differentiate. METTL14, which heterodimerizes with METTL3 in the methyltransferase complex, is highly expressed in normal HSPCs, whereas its expression is repressed during myeloid differentiation (86). Inhibition of METTL14 promotes myeloid differentiation of HSPCs (86). Similarly, biased differentiation toward the myeloid lineage was observed upon knockdown of METTL3 in human cord blood CD34$^+$ HSPCs (87).

The hematopoiesis failure of *Mettl3*-deficient HSCs can be attributed to *Myc*. *Mettl3*-deficient HSCs fail to express MYC, while forced expression of *Myc* rescues differentiation defects of *Mettl3*-deficient HSCs (85). Another plausible explanation for hematopoiesis failure in *Mettl3*-deficient HSCs is related to double-stranded RNA (dsRNA)-triggered innate sensing. *Vav*-Cre-mediated deletion of *Mettl3* in murine fetal HSCs promotes the formation of dsRNAs, which has been linked to aberrant activation of an innate immune response involving multiple RNA-sensing pathways (including OAS-RNase L, PKR-eIF2a, MDA5, and RIG-I) in HSPCs (88). The resulting inflammatory response is deleterious to fetal liver HSPC proliferation and differentiation (88). Notably, m$^6$A-mediated mechanisms that protect against dsRNA-mediated

innate immune responses are not restricted to fetal hematopoiesis: An increase in dsRNA and an attendant innate immune response have also been observed in intestinal epithelial cells (IECs) of *Mettl3* knockout mice (89).

Other types of RNA modifications that affect translation have been implicated in HSC fate commitment. One study revealed that nucleophosmin (NPM1) regulates 2′-*O*-methylation on rRNA during hematopoiesis, showing that *Npm1* inactivation in adult HSCs results in hematopoietic failure (90). Human primary HSPCs are particularly sensitive to alterations in Ψ-driven posttranscriptional programs. For example, mutations in the Ψ synthase gene *DKC1* reduce the amount of Ψ residues on 18S rRNA and abolish the capacity of HSCs to generate mature myeloid and erythroid cells in in vitro differentiation assays (91). In an alternative scenario, HSPCs express high levels of pseudouridine synthase 7 (PUS7), and silencing of PUS7 interferes with PUS7-tRNA interactions, leading to increased protein synthesis and a severe defect in HSPC differentiation (92, 93). Modifications of tRNA by RNA methyltransferase DNMT2 have also been shown to serve a function during hematopoiesis (94). *Dnmt2* knockout mice with reduced protein translation fidelity exhibit a cell-autonomous defect in HSC differentiation. These studies provide genetic evidence connecting an aberrant epitranscriptome with impaired hematopoiesis. Further investigation is needed to uncover how these RNA modifications affect specific biomolecular mechanisms controlling stemness during hematopoiesis.

It is well accepted that HSCs are strongly affected by inflammatory cues (95). Proinflammatory signals are thought to induce enhanced myeloid differentiation and impair HSC reconstitution potential (96–98). YTHDF2 has been shown to be induced by inflammation in HSCs, where it functions as a repressor to counteract inflammatory responses that can be detrimental to the long-term integrity of HSCs (99). Although knockout of YTHDF2 shortly after the emergence of HSCs results in HSC expansion (99–101), HSCs from aged *Ythdf2* conditional knockout mice failed to efficiently reconstitute short- and long-term multilineage hematopoiesis upon transplantation and displayed a myeloid bias (99). The mechanisms through which YTHDF2 is induced by inflammation, and their involvement in protecting HSCs during aging, remain elusive and merit further investigation.

## 4.2. RNA Modifications in Macrophages

Macrophages are ubiquitously distributed immune sentinel cells known to respond to diverse stimuli (e.g., pathogens and tissue damage) (102, 103). Upon exposure to pathogen-associated molecular patterns (PAMPs) and contextual cytokines, pattern recognition receptors (PRRs) and cytokine receptors located on resting macrophages can be activated to trigger stimulus-specific transcriptional modules. Macrophages are known to reprogram their transcriptional modules on-demand, based on the integration of epigenetic and transcriptional regulatory mechanisms (104). The reprogramming process in macrophages is highly regulated, and host misregulation of context-dependent transcriptional modules leads to altered immune function in diseases involving aberrant cytokine conditioning, such as inflammatory bowel disease (105). Recent studies addressing the roles of RNA modification in regulating macrophage reprogramming have expanded our understanding of the context-dependent transcriptional changes in macrophages.

Several studies have demonstrated that $m^6A$ affects the mRNA half-life of negative-feedback regulators during macrophage reprograming and controls the amplitude of stimulus-dependent gene expression. For example, *Irakm*, an essential negative regulator of the TLR (Toll-like receptor)-NF-κB signaling pathway, was revealed to be target of METTL3 in macrophages (106). METTL3 deficiency led to the loss of $m^6A$ modification on *Irakm* transcripts, which slowed down its degradation, resulting in a higher level of IRAKM, which ultimately suppresses TLR signaling. A study on metabolic inflammatory diseases has found that *Mettl3* and other genes encoding

the m$^6$A machinery are upregulated in macrophages. Loss of METTL3 in macrophages stabilizes m$^6$A-marked *Ddit4* (DNA Damage Inducible Transcript 4) transcripts in response to metabolic endoplasmic reticulum stress and TNF-α stimulation. The increased DDIT4 in METTL3 knockout macrophages acts as a negative regulator to reduce mTOR and NF-κB signaling activity (107).

Under macrophage polarization, METTL3 restricts the amplitude of stimulus-dependent gene expression by promoting translation of the negative regulator SPRED2 (108). Another study identified that METTL14 targets *Socs1*, showing that m$^6$A enhances the translation of SOCS1, thereby negatively regulating TLR4/NF-κB signaling activity (109). Understanding RNA modification–mediated molecular mechanisms that give rise to each stimulus-specific response in macrophages may help delineate how the misregulation of RNA modification machinery results in autoinflammatory diseases.

### 4.3. RNA Modifications in Dendritic Cells

Dendritic cells (DCs) are the major antigen-presenting cells responsible for taking up and processing antigens from pathogens or tumors and presenting them to T cells to initiate adaptive immune responses. Research on DCs cells has revealed that METTL3-mediated mRNA m$^6$A methylation promotes DC activation and function via YTHDF1-dependent protein translation of TLR4 signaling adaptor TIRAP, strengthening TLR4/NF-κB signaling–induced cytokine production (110). In the context of tumors, YTHDF1 promotes the translation of cathepsins restricting the cross-presentation capacity of DCs, leading to an impaired downstream antitumor CD8$^+$ T cell response (75). Another study revealed that CCR7 stimulation can induce the demethylation of m$^6$A on long noncoding RNA *lnc-Dpf3* to accelerate RNA degradation. Silencing of *Ythdf2* could increase the expression of *lnc-Dpf3*, thereby preventing CCR7-mediated DC migration (111). Since different subtypes of DCs are programmed to respond differently to the same challenge, future studies into m$^6$A modification in DCs should take their specialization into consideration.

### 4.4. RNA Modifications in Natural Killer Cells

METTL3 is upregulated in natural killer (NK) cells in the presence of cytokines IL-15 and IL-10, whereas it can be downregulated by TGF-β treatment. The decrease in METTL3 protein expression is accompanied by impaired expression of effector molecules and receptors (112). NK cells have also been shown to constitutively express YTHDF2 at the highest level when compared to other readers, and YTHDF2 expression can be further upregulated in NK cells during IL-15-driven activation (113). At steady state, *Mettl3* deficiency and *Ythdf2* deficiency prevent terminal maturation of NK cells, reduce NK cell numbers in peripheral organs, and inhibit the expression of effector function–associated molecules on NK cells. Additionally, the METTL3-YTHDF2 axis is required for IL-15-mediated NK cell survival, proliferation, and effector functions (113). Transcriptome-wide identification of YTHDF2 targets revealed that *Tardbp*, a cell cycle negative regulator, is destabilized by YTHDF2, contributing to NK cell proliferation (113). In another study *Ptpn11*, identified as a direct target of METTL3, was downregulated in METTL3-deficient NK cells, which might ultimately lead to their impaired responsiveness to IL-15 (114).

### 4.5. RNA Modifications in T Cells

Upon recognition of a cognate antigen, quiescent naive T cells undergo substantial rewiring of their transcriptomes and rapidly differentiate into highly proliferative effector T cells (115). RNA modification has been shown to direct this transcriptome rewiring during T cell activation and differentiation. The initial exploration of the function of RNA modification in immune cells focused on CD4$^+$ T cells. Deletion of the m$^6$A writer METTL3 from CD4$^+$ T cells led

to disruption of their homeostatic proliferation and prevented naive T cells from differentiating into effector cells. In *Mettl3*-deficient naive $CD4^+$ T cells, mRNAs encoding the JAK (Janus kinase)-STAT (signal transducer and activator of transcription) signaling–inhibitory proteins (SOCS1, SOCS3, and CISH) increased the prolonged mRNA half-life, which consequently inhibited STAT5 activation induced by IL-7 and suppressed IL-7-mediated T cell homeostatic proliferation (76). When T cells transition from naive to activated states, they ramp up ribosome biogenesis and translation capacity to meet the demand for rapid cell division. RNMT, the $m^7G$-cap methyltransferase that specifically regulates ribosome production, functions as a mediator of T cell activation. Knockout of *Rnmt* in $CD4^+$ T cells leads to impaired ribosome synthesis, reduced translation rates, and proliferation failure (116). In addition, an investigation into the dynamics of tRNA modification following T cell receptor (TCR) stimulation revealed that the levels of two types of tRNA modifications, wybutosine and $ms^2t6A$, which govern translation fidelity and frameshift prevention, are reduced dramatically during peak T cell proliferation (117).

Upon activation, $CD4^+$ T cells differentiate into distinct subsets of effector T helper (Th) cells, including Th1, Th2, Th17, and T follicular helper (Tfh) cell subsets. The generation of these subsets is differentially regulated by cytokines and transcription factors. Emerging evidence suggests that RNA modification is also involved in promoting subset differentiation and in immunopathology such as autoimmunity. For example, knockout of writer METTL3 in $CD4^+$ T cells destabilizes *Tcf7* (a Tfh cell regulator) transcripts, ultimately leading to compromised activation of Tfh transcriptional programs (118). METTL3 knockout $CD4^+$ T cells exhibit defects in differentiation into Tfh cells and display elevated rates of apoptosis during infection with lymphocytic choriomeningitis virus. One study demonstrated that the knockout of the eraser enzyme ALKBH5 from T cells reduces the severity of T cell–mediated colitis and experimental autoimmune encephalomyelitis (EAE); the lack of functional ALKBH5 prevented the removal of $m^6A$ from *Cxcl2* and *Ifng* transcripts, thus destabilizing the cellular populations of these mRNA molecules (119). It remains unclear why ALKBH5 and METTL3 show a similar impact on $CD4^+$ T cell–mediated autoimmunity, but it is possibly because these two molecules fine-tune T cell activity through temporal-sequential ordering.

Two additional studies focusing on $Foxp3^+$ regulatory T cells (Tregs) revealed that specific deletion of METTL3 in Tregs leads to increased levels of mRNAs for *Socs* family genes (*Cish*, *Socs1*, *Socs2*, *Socs3*, *Asb2*), thereby suppressing IL-2–STAT5 signaling activity and ultimately reducing the suppressive function of Tregs (120). Deletion of METTL14, the catalytic partner of METTL3, in T cells also impairs the differentiation of naive T cells into induced Tregs (121).

## 5. RNA MODIFICATION IN ANTIVIRAL IMMUNE RESPONSES

Viral RNAs are described as having specific RNA modifications (122), and these internal modifications control either the stability or translation of viral genes (123–126). In host cells, RNA modifications deposited on viral RNAs could mask cytoplasmic viral RNAs to avoid recognition by host innate sensing pathways, including TLRs and RIG-I-like receptors (127, 128). For instance, some viruses have evolved to encode their own viral 2′-*O*-methyltransferase and hijack host-derived 2′-*O*-methyltransferase to methylate viral RNA caps, resulting in the subversion of host innate sensing and preventing induction of type I interferons (129–131). In this section, we focus on recent advances in $m^6A$-mediated immune evasion from innate sensing.

### 5.1. RNA Modification of Viral RNA

The presence of $m^6A$ modifications on viral RNA was first demonstrated in the 1970s (132, 133). Recent studies have used sequencing-based methods for transcriptome-wide $m^6A$ mapping and

have shown how specific m$^6$A sites on viral RNA can regulate the viral life cycle (134, 135). Importantly, no viral genomes examined to date encode an m$^6$A methyltransferase (136). The assumption has thus been that the m$^6$A detected on viral RNA must be deposited by host methyltransferases. Notably, viral infection has been shown to upregulate the expression of methyltransferases and enhance the catalytic activity of methylation machinery (137–139). Viral infection has also been shown to enhance the translocation of METTL3 from the nucleus to the cytoplasm (139). These alterations of host methyltransferases are thought to allow viral nucleotides to acquire m$^6$A methylation as a means of mimicking host RNA, thus suppressing type I interferon production and preventing the activation of host innate immunity (128).

Structurally, we know that m$^6$A modification on viral RNA can disrupt the conformation of duplex structures, which attenuate binding between viral RNA and host dsRNA sensors such as RIG-I (139). Additionally, YTHDF2 and YTHDF3 occupying the viral m$^6$A site also block recognition of viral RNA by RIG-I (140, 141). In contrast, mutating m$^6$A sites on viruses or overexpressing demethylase in host cells can generate m$^6$A-deficient recombinant viruses and virion RNAs that trigger RIG-I-dependent type I interferon production (140–144). Thus, viruses use the host m$^6$A methylation machinery for innate immune evasion. It therefore follows that rational modulation of m$^6$A on viral RNAs represents a potential approach for developing antiviral therapies.

## 5.2. m$^6$A of Host Cellular RNA

Viral infections not only influence viral RNA methylomes but also heavily impact the dynamics of cellular RNA methylomes in the host. The interferon pathway is the major target for m$^6$A methylation. m$^6$A writer proteins METTL3 and METTL14 and reader protein YTHDF2 are upregulated during infection and adversely regulate the type I interferon response in multiple ways (137). First, METTL3-METTL14-YTHDF2 accelerates the turnover rate of *IFNB* mRNAs and consequently facilitates viral propagation (137, 145). Also, METTL3 has additional strategies and targets for effectively suppressing host innate immune responses. For example, as a strategy to evade innate immunity, hepatitis B virus (HBV) induces METTL3 expression and increases m$^6$A modifications on transcripts of tumor suppressor PTEN, thereby contributing to its instability with a corresponding decrease in PTEN protein levels (146). PTEN can facilitate the nuclear import of IRF3 to trigger interferon synthesis. HBV infection might disrupt the production of interferon by decorating PTEN with m$^6$A.

The negative effect of antiviral innate immunity exerted by m$^6$A could be potentiated through its coordination with other RBPs. Multifunctional DEAD-box helicase 5 (DDX5), which is important in transcriptional regulation, is hijacked by diverse viruses to facilitate viral replication. DDX5 can recruit and interact with METTL3 via its P68HR domain to promote formation of the METTL3-METTL14 complex during vesicular stomatitis virus infection (147). In this study, m$^6$A-marked antiviral transcripts IKK$\gamma$ and p65 were degraded by YTHDF2 when bound by DDX5 and underwent increased m$^6$A modification, thus inhibiting the antiviral innate immune response.

Elevated m$^6$A modification during infection could be achieved by shutting down the expression of demethylase. For example, rotavirus infection in small bowel IECs downregulated expression of ALKBH5, which induced global m$^6$A modifications on mRNA transcripts (89). This study identified IRF7 as a master transcription factor triggering type I interferon– and type III interferon–dependent immune responses. IRF7 is deposited with m$^6$A on its mRNA. Conditional knockout of METTL3 in IECs increases *Irf7* mRNA stability by reducing its m$^6$A levels, thus enhancing the interferon response and protecting mice from rotavirus infection. Another study effectively showed that viral infection dampens the enzymatic activity of demethylase ALKBH5 in host cells by inducing a posttranslational modification of ALKBH5 protein (138).

Such impaired enzymatic activity leads to decreased expression of the metabolic enzyme OGDH (α-ketoglutarate dehydrogenase), which is responsible for the production of itaconate, a metabolite essential for inhibiting viral replication. ALKBH5-deficient mice show improved survival when compared to their wild-type littermates following viral infection. ALKBH5-mediated promotion of viral replication does not rely on the type I interferon pathway but relies instead on metabolic rewiring during cellular defense against infection. Thus, it reveals unique crosstalk of $m^6A$ RNA modification and metabolic processes.

The downstream regulation of type I interferon responses is also controlled by $m^6A$ modification. During antiviral immune responses, type I interferons initiate a signaling cascade through the JAK-STAT pathway, resulting in the transcription of thousands of interferon-stimulated genes (ISGs). The host manipulates these ISGs to restrain different steps of the viral life cycle, and $m^6A$, with its related proteins, can regulate ISG expression through a variety of mechanisms. The translation of a subset of ISGs, including IFITM1, is enhanced through interactions between the transcripts of $m^6A$-modified ISGs and the $m^6A$ reader YTHDF1 (148). Such posttranscriptional enhancement of ISG expression facilitates the establishment of an antiviral cellular state. The enzyme ADAR1, another ISG catalyzing adenosine-to-inosine editing, is $m^6A$ modified and recognized by YTHDF1 (149). Interferon-inducible ADAR1p150 is known to prevent global translational shutdown by inhibiting hyperactivation of PKR, a dsRNA sensor (150). YTHDF1 promotes the translation and enables the rapid expression of ADAR1p150 upon interferon stimulation, which consequently attenuates the dsRNA-sensing pathway. In contrast, another $m^6A$ reader, YTHDF3, has been shown to suppress ISG expression by promoting translation of FOXO3, which is known to negatively regulate ISG expression via suppression of IFNAR1 signaling (151). It is apparent that both virus and cellular transcripts contain $m^6A$ and are under the control of host-derived $m^6A$ regulators. Therefore, disentangling the regulatory effects of $m^6A$ on viral and host RNAs has been challenging, and the development of a precision editing tool to enable site-specific control of viral versus host epitranscriptome may speed up the process.

## 6. ABERRANT RNA MODIFICATION REGULATION IN THE ANTITUMOR IMMUNE RESPONSE

Accumulating evidence supports the notion that RNA modifications, including $m^6A$, are an additional mechanistic layer of regulation that serves important oncogenic and tumor-suppressing roles in different cancer types. Intrinsic tumor signaling along with environmental stimuli can drive aberrant expression and activity of multiple $m^6A$ regulators, resulting in altered modifications of cancer-associated transcripts. The regulatory mechanisms that control the onset and progression of cancer are comprehensively discussed in a number of other publications (152, 153). There is a growing appreciation for the fact that aberrant RNA modification is an important mechanism in both dictating the immune context of the tumor microenvironment and controlling immune evasion. We discuss recent insights into how tumor cells evade immune surveillance by co-opting RNA modification programs found in both tumor cells (**Figure 3**) and host immune cells (**Figure 4**).

### 6.1. Immune Evasion Mediated by Tumor-Intrinsic RNA Modification

Studies into the mechanisms of immune evasion employed by tumors have highlighted the following: (*a*) Tumor cells can influence T cell responses by altering the expression levels of immune checkpoint molecules, (*b*) tumor cells can prevent T cell infiltration and recruit immunosuppressive cells into the tumor, and (*c*) tumor cells can develop resistance to T cell–mediated recognition and killing (154, 155).

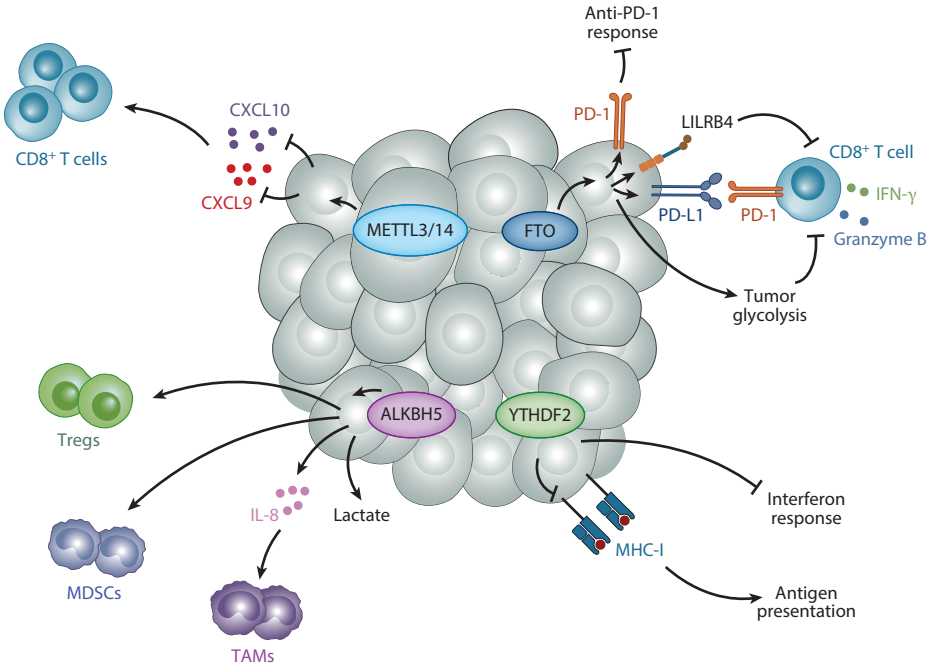



**Figure 3**

Immune evasion mediated by tumor-intrinsic RNA modification. m$^6$A writers METTL3 and METTL14 in tumor cells inhibit the expression of chemokines CXCL9 and CXCL10 and induce immune exclusion. m$^6$A eraser FTO enhances the expression of PD-1, PD-L1, and LILRB4 on tumor cells and potentiates the glycolysis capacity of tumor cells, which restricts the activation and effector states of CD8$^+$ T cells. m$^6$A eraser ALKBH5 increases the generation of lactate and the expression of IL-8 in tumor cells. IL-8 mediates the recruitment of TAMs into the tumor. ALKBH5 in tumor cells also increases the infiltration of MDSCs and Tregs into the tumor microenvironment. m$^6$A reader YTHDF2 attenuates the interferon signaling and downstream expression of MHC-I in tumor cells. Abbreviations: MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; Treg, regulatory T cell. Figure adapted from images created with BioRender.com.

**6.1.1. RNA modifications regulate the expression of immune checkpoint molecules by tumor cells.** Upregulated PD-L1 is able to engage with the immunological checkpoint receptor PD-1 on T cells to suppress their cytokine secretion, cytotoxic activity, and proliferation, a condition known as T cell dysfunction (154). The significance of tumor-intrinsic RNA modification in modulating PD-L1 expression is becoming increasingly clear. For example, in acute myeloid leukemia, deletion or inhibition of demethylase FTO by small-molecule inhibitors suppresses the expression of immune checkpoint molecules PD-L1 and LILRB4, resulting in the sensitization of tumor cells to T cell–mediated cytotoxicity (156). Conversely, tumor-intrinsic PD-1 expression on melanoma cells is positively regulated by FTO (157). When exposed to metabolic stress or starvation, melanoma cells upregulate FTO, whereas knockdown of FTO in melanoma cells increases m$^6$A deposition on PD-1, CXCR4, and SOX10, thereby accelerating the decay of their mRNAs through YTHDF2 and preventing tumor growth (157). Along with FTO, another demethylase, ALKBH5, has been shown to increase PD-L1 expression by tumor cells. Knockout of ALKBH5 in cancer cells increases the level of m$^6$A found at the 3′ UTR of PD-L1 mRNA and accelerates its degradation in a YTHDF2-dependent manner, thereby supporting T cell–mediated antitumor immunity (158).

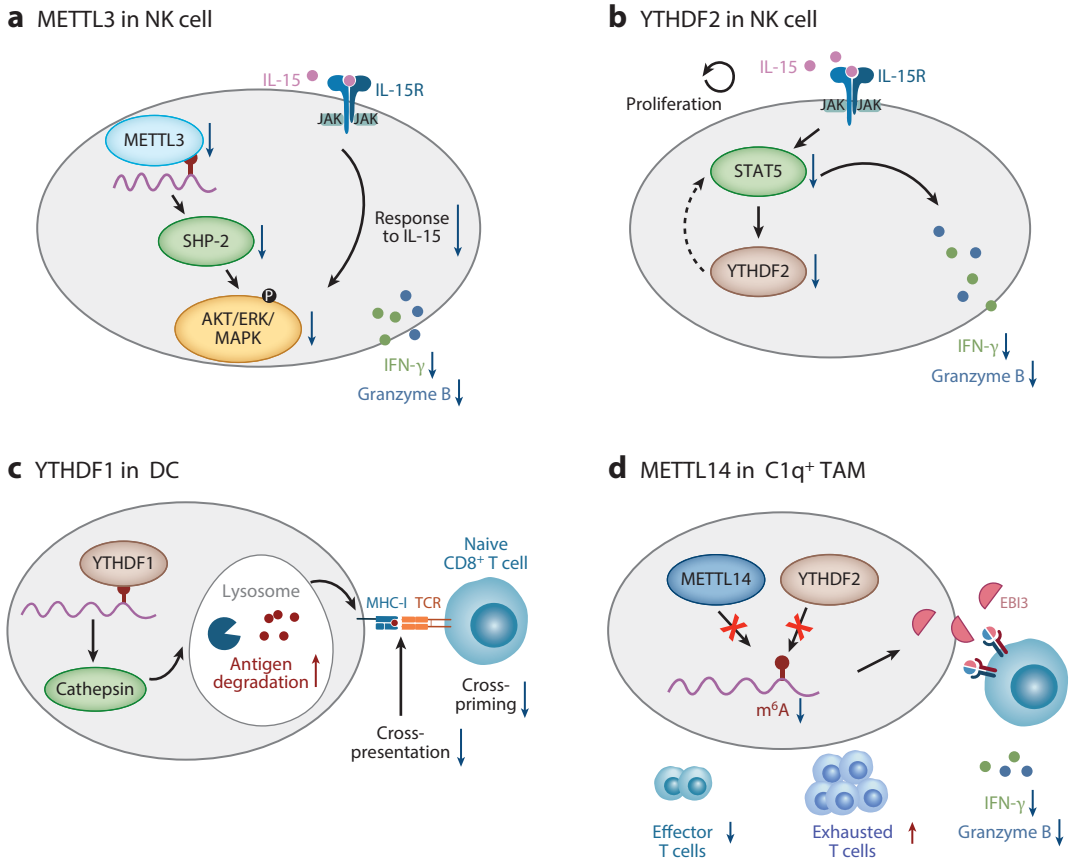



**Figure 4**

Dysregulation of RNA modification in tumor-infiltrating immune cells. (*a*) METTL3 is downregulated in tumor-infiltrating NK cells. SHP-2 is m$^6$A-modified and its protein expression is decreased in METTL3-deficient NK cells. Reduced SHP-2 activity suppresses the activation of the MAPK-ERK signaling pathway, thereby abrogating the responsiveness of NK cells to IL-15 and inhibiting the antitumor function of NK cells in the tumor microenvironment. (*b*) YTHDF2 promotes the function of NK cells by forming a STAT5-YTHDF2 positive feedback loop downstream of IL-15 stimulation, and YTHDF2 deficiency in NK cells impairs their antitumor ability. (*c*) When a DC migrates into the tumor, it takes up tumor-derived antigens and cross-presents them to CD8$^+$ T cells. YTHDF1 in a DC limits its cross-presentation capacity and results in poor activation and infiltration of CD8$^+$ T cells into the tumor microenvironment. (*d*) Tumor-infiltrating CD8$^+$ T cell cross talk with a C1q$^+$ TAM. Loss of METTL14 or YTHDF2 in C1q$^+$ TAMs promotes their immunosuppressive function through the production of EBI3, thus directing CD8$^+$ T cell differentiation into an exhausted phenotype. Abbreviations: DC, dendritic cell; NK, natural killer; TAM, tumor-associated macrophage; TCR, T cell receptor. Figure adapted from images created with BioRender.com.

**6.1.2. RNA modifications regulate chemokine production in tumor cells.** The accumulation of antitumorigenic immune cells, such as effector T cells and NK cells, at the invasive margin of the primary tumor can be beneficial for the desired response to immunotherapy. Effector T cells and NK cells express CXC receptor 3 (CXCR3), which is the receptor for the potent T cell–attracting chemokines CXC ligand 9 (CXCL9) and CXCL10, and this expression of CXCR3 enables them to migrate into tumors in response to the presence of these chemokines (159). However, various cancer-intrinsic factors shape the chemokine milieu within the tumor microenvironment, which silences the expression of CXCL9 and CXCL10 to hinder infiltration by CXCR3$^+$ effector cells into the tumor, resulting in immune escape (160). Recent data suggest that

the defect in chemokine production may result from tumor-induced modulation of RNA modification machinery that controls the expression of chemokines. For instance, m$^6$A writers METTL3 and METTL14 in tumor cells have been shown to repress the expression of CXCL9 and CXCL10 (161). *Mettl3* or *Mettl14* knockout tumors show elevated secretion of CXCL9 and CXCL10 thought to be the result of elevated expression of *Stat1* and *Irf1*. mRNA transcripts of *Stat1* and *Irf1* are stabilized by the decrease in m$^6$A enrichment upon *Mettl3* knockout, thereby recruiting CD8$^+$ T cells and NK cells into the tumor microenvironment. Such an increase in cytotoxic cell migration in vivo allows for tumor control and improves responsiveness to immunotherapies (161). In addition, PUS7-mediated tRNA pseudouridylation promotes glioblastoma stem cell tumorigenesis and represses expression of CXCL10 (162). This suggests that the reprogramming of RNA modification can remove the epigenetic repression of chemokines for T cell and NK cell trafficking and may be synergistic with current T cell–based therapies to improve therapeutic efficacy.

Another strategy for T cell exclusion that tumors utilize to evade immune surveillance is recruiting tumor-associated Gr1$^+$CD11b$^+$ myeloid-derived suppressor cells (MDSCs) and TAMs of immunosuppressive functions (163). m$^6$A eraser ALKBH5, which is transcriptionally induced under hypoxic conditions in human glioblastoma, has been shown to facilitate the expression of chemokine CXCL8/IL8 (163). IL-8 acts as a chemoattractant cytokine for TAM recruitment and immunosuppression. ALKBH5 knockout tumors show decreased TAMs and increased CD8$^+$ T cell infiltration. Further depletion of ALKBH5 in tumor cells abolishes the infiltration of MDSCs (164). CRISPR-mediated knockout of ALKBH5 in two poorly immunogenic murine tumor cell lines reduced the stability of *Mct4/Slc16a3*, thus downregulating the lactate levels and the infiltration of MDSCs into tumors, resulting in sensitization of the tumor to immunotherapies (164). These data demonstrate that tumor-derived RNA modifications interfere with T cell infiltration via establishment of an immunosuppressive tumor microenvironment. We are only now beginning to understand the RNA modification–mediated molecular pathways that regulate immune cell trafficking to the tumor microenvironment. How specific T cell subsets and myeloid compositions are recruited into tumors when interfering with the RNA modification program remains unknown. A better understanding of these processes may provide more insight into what the next steps in developing combinational therapy might be.

**6.1.3. RNA modifications trigger tumor resistance to cytotoxic T cell–mediated killing.** Cytotoxic T cells are major effectors of tumor immunity due to their ability to recognize and eliminate transformed cells after TCR recognition of tumor antigens bound to MHC-I proteins. When MHC-I expression and antigen presentation by tumor cells are reduced, T cell–mediated cytotoxicity becomes less effective. Such immune evasion by tumor cells might be driven in part by RNA modification reprogramming in tumor cells, which decreases tumor cells' vulnerability to T cell–mediated attack. It has been reported that YY1-mediated transcription and chromatin interactions in glioblastoma stem cells triggers m$^6$A modification programs. Inhibition of YY1-CDK9 complex in glioblastoma stem cells decreases total m$^6$A levels by suppressing the expression of METTL3 and YTHDF2, which induces interferon signaling and downstream MHC-I expression to augment the efficacy of immunotherapy (165). Moreover, inactivation of FTO in tumor cells also sensitizes tumor cells to T cell–mediated killing. Although FTO has no effect on the expression of MHC-I molecules and antigen presentation capacity in tumor cells, the glycolytic activity of tumor cells is impaired in *Fto* knockout tumors, eliminating the metabolic barrier for T cell activation and enhancing the recognition of tumor cells (166).

## 6.2. Dysregulation of RNA Modification in Tumor-Infiltrating Immune Cells

The alterations of the epitranscriptome in immune cells have been studied across several innate cell populations within the tumor microenvironment, including NK cells, DCs, and myeloid cells.

**6.2.1. Dysregulated RNA modification leads to NK cell dysfunction.** While tumor-intrinsic METTL3/14 inhibits the antitumor immune response, host cell–derived METTL3/14 acts in the opposite way to promote antitumor immune responses. METTL3 mRNA levels are decreased in tumor-infiltrating NK cells of hepatocellular carcinoma patients and ovarian cancer patients (112). Moreover, METTL3 mRNA expression is positively correlated with the module of the cytotoxic function of NK cells in different human tumors (112). METTL3 knockout NK cells exhibit lower cytotoxic activity than wild-type NK cells and fail to control tumor growth (112). Another group reveals a cell-intrinsic role of YTHDF2 in the regulation of NK cell antitumor immunity. YTHDF2 knockout in NK cells impairs the expression of IFN-$\gamma$ and granzyme B (113). In contrast to METTL3, YTHDF2 deficiency has little influence on the survival and proliferation of resting NK cells in vivo, yet YTHDF2 integrating into IL-15–STAT5 signaling is crucial in controlling proliferation and/or survival of activated NK cells triggered by IL-15 (113). It remains to be seen whether the loss of a STAT5-YTHDF2-driven positive feedback loop is a general obstacle to the persistence of other IL-15-responsive antitumor immune cells that exist within the tumor microenvironment, such as memory $CD8^+$ T cells.

**6.2.2. Dysregulated RNA modification leads to T cell dysfunction.** T cell dysfunction is a major barrier limiting the efficacy of cancer immunotherapies. The $m^6A$ reader YTHDF1 in DCs results in a suboptimal antitumor T cell response. Knockout of YTHDF1 enhances the antigen cross-presentation capacity of DCs by preserving the tumor neoantigen available for processing and consequently increasing the antitumor T cell response (75). Immunosuppressive myeloid cells engage in cross talk with tumor-infiltrating $CD8^+$ T cells and orchestrate T cell dysfunction, resulting in compromised antitumor responses and tumor escape from immune surveillance (155). The deterministic effects of RNA modification that drive the dysfunctional T cell have been elucidated (74, 106, 108). For example, METTL14-mediated epitranscriptomic regulation is reported to control the specification and function of TAMs (74). *Mettl14* depletion in a $C1q^+$ TAM subset abolishes the maintenance of progenitors of exhausted $CD8^+$ T cells and directs T cell differentiation toward the exhausted trajectory, consequently favoring the outgrowth of solid tumors. At the molecular level, altered $m^6A$ methylation leads to accumulation of downstream targets under the METTL14-YTHDF2 regulation axis, including *Ebi3* transcripts, thereby shaping T cell dysfunctions (74). Given that the phenotypic heterogeneity of myeloid cells within the tumor microenvironment has been reflected by their distinct transcriptome and plastic states, here arises a related question of whether their plastic functions are achieved through RNA modification programs. Since T cell dysfunction shows a continuum in transcriptional phenotypes, it is still under exploration whether an RNA modification program can drive the transcriptome's gradual switch to a dysfunctional phenotype. Ideally, these questions could be resolved with a toolbox of single-cell-based RNA modification sequencing methods.

## 7. FUTURE DIRECTIONS OF INTEREST

Although the impact of RNA modifications on immune responses is becoming ever more clear, several mechanistic gaps remain. The observed phenotype in cells where one of the RNA modification writers or readers has been genetically deleted is linked to a specific modified RNA as a candidate for downstream validation. In many cases, it is unclear whether such a specific target is directly recognized by writer or reader. We anticipate that the exciting development of RNA modification editors will enable us to perturb individual modified sites in the epitranscriptome of immune cells and help illuminate the causality between RNA modification and phenotypes (167–169).

Some clusters of coexpressed genes that are functionally coordinated are often marked and controlled by RNA modifications when immune cells are receiving certain stimuli to differentiate or switch their states. The selectivity and specificity of RNA-modifying machinery in marking distinct groups of transcripts still need to be addressed and could be examined in a framework of classical immunological systems with clear transcriptional regulatory networks such as $CD4^+$ T cell differentiation. The RNA modification machinery might rely on additional factors such as *cis*-regulatory elements enriched within these coexpressed genes to decide its selectivity. Furthermore, given the growing evidence for the cross talk of RNA modification of carRNA and epigenetic modifications in chromatin, studies investigating whether dynamic RNA modifications regulate the chromatin environment of immune cells in response to environmental stimuli are of great importance to this line of research.

While the alternation of the epitranscriptome in tumor and immune cells is known to play a role in antitumor immune responses, research into RNA modification dysregulation in tumor immunology is still in its infancy, in part because the types of cells being modulated by RNA modification have not been fully addressed. Careful dissection of RNA modification and its regulators in tumor cells versus immune cells will support the development of increasingly effective interventions (and combinations of interventions) aiming to modulate RNA modifications for therapeutic benefits. To this end, integrating the most cutting-edge single-cell scale and transcriptome-wide methods with RNA modification sequencing may be a powerful tool in the efforts to deepen our understanding of dysregulated RNA modification in the tumor microenvironment with dynamic complexity. The future development of potent inhibitors targeting RNA modification machinery as well as the application of RNA modification editors on immune cells are two promising strategies to broaden current treatment of immune-related diseases.

## DISCLOSURE STATEMENT

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