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N6-methyladenosine Regulates Host Responses to Viral Infection

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Abstract

Recent discoveries have revealed that during viral infection the presence of the RNA modification *N6*-methyladenosine (m^6A) on viral and cellular RNAs has profound impacts on infection outcome. While m^6A directly regulates many viral RNA processes, its effects on cellular RNAs and pathways during infection have only recently begun to be elucidated. Disentangling the effects of m^6A on viral and host RNAs remains a challenge for the field. m^6A has been found to regulate host responses such as viral RNA sensing, cytokine responses, and immune cell functions. We highlight recent findings describing how m^6A modulates host responses to viral infection and discuss future directions that will lead to a synergistic understanding of the processes by which m^6A regulates viral infection.

Keywords

N6-methyladenosine; m^6A; RNA sensing; Interferon (IFN); Cytokines

m^6A modulates host responses to viral infection

During viral infection, the molecular processes of host cells are altered as viruses co-opt, usurp, or inhibit cellular machinery to facilitate their replication. Recent studies have revealed that chemical modification of RNA is an example of a category of host processes that viruses can exploit. In particular, the role of the RNA modification **m^6A** (see Glossary) during viral infection has generated a great deal of interest. While m^6A was first discovered on viral RNA in the 1970s [1–4], its functional roles in viral processes were only revealed more recently. m^6A can regulate many aspects of RNA biology [5], including RNA structure, splicing, stability, localization, and translation [6–10], thus its effects on viral RNAs are diverse (reviewed in [11]). In addition to these effects, m^6A has been shown to regulate cellular responses to viral infection, which is the focus of this review. m^6A is

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deposited on mRNA primarily by a cellular complex of ‘writer’ enzymes, composed of **methyltransferase like 3** (**METTL3**) and **METTL14** (METTL3/14) [12] and other accessory proteins, such as **WTAP**, **VIRMA**, **ZC3H13**, and **RBM15/RBM15B** [13–17]. Additional enzymes such as **METTL16**, **ZCCHC4**, and **METTL5** can catalyze the addition of m^6A, mainly on non-coding RNA species [18–22]. m^6A is the most abundant chemical modification to eukaryotic mRNA, and its effects on RNA metabolism are mediated by m^6A ‘reader’ proteins, such as the YTH domain proteins (**YTHDF1**, **YTHDF2**, **YTHDF3**, **YTHDC1**, **YTHDC2**), and others [23]. Additionally, m^6A can be removed from RNA by demethylase ‘eraser’ proteins including **FTO** and **ALKBH5** [9, 24]. Because m^6A can affect both viral and host processes, its regulatory effects on viral infection are complex. A more complete understanding of the effects of m^6A at the virus-host interface will require additional understanding of its effects on the cellular response to infection.

Viruses are equipped with strategies to manipulate host gene expression and cellular processes in ways that promote their replication. Meanwhile, host cells modulate their own processes in response to infection to limit viral replication. First, host cells utilize **pattern recognition receptors** (**PRRs**) to detect **pathogen associated molecular patterns** (**PAMPs**) and initiate innate immune responses [25]. Signaling molecules called cytokines can then be produced to transmit innate immune signals for the expression of antiviral genes that directly limit viral infection and to orchestrate functional adaptive immune responses [26]. Many viruses have also developed mechanisms to inhibit host innate immune responses [27]. Therefore, the interplay between viruses and their host cells is intricate and complex, resulting in cells undergoing many dynamic changes during infection. m^6A regulates many biological processes, including stress responses such as the integrated stress response, heat shock, and UV damage [28–30], and therefore likely regulates these or other stress responses important for viral infection. Recent research has shed light on the regulatory roles of m^6A on host responses to viral infection, including detection of viral RNA, innate immune pathways, stress response pathways, and metabolism; however, many intriguing questions in this field have yet to be explored. This review highlights recent discoveries describing the mechanisms by which m^6A regulates host processes during viral infection and some of the most pressing questions for future research.

Sensing of m^6A-modified RNAs

Recognition of viral nucleic acids is an important cellular surveillance strategy, and the ability to distinguish foreign RNAs from host, or ‘self’, RNAs, is crucial for viral detection. Additionally, protection of self RNAs from detection by PRRs is essential to avoid autoimmune disease [31]. RNA modifications are known to serve as molecular signatures that can distinguish self and non-self RNAs, and roles for m^6A in regulating PRR sensing of RNAs are now beginning to emerge. Other RNA modifications with well-established roles in shielding self RNAs from PRRs include the 7-methylguanosine cap (also known as cap0) and 2’-O-methylation of the first (cap1) and second (cap2). The cytosolic PRR **RIG-I** detects uncapped RNAs with 5’- tri- or diphosphate moieties that are generally found on viral RNA genomes or as viral replication intermediates [32–34]. 2’-O-methylation of the first transcribed nucleotide is also crucial for avoiding recognition by RIG-I [35, 36]. After

binding non-self RNA, RIG-I induces signaling through the **MAVS** pathway that results in the production of type I and III **interferons** (**IFNs**) to activate antiviral responses [37]. The RNA binding protein Interferon Induced Protein With Tetratricopeptide Repeats 1 (IFIT1) can also detect RNAs that lack cap1 2'-O-methylation and can sequester these RNAs to inhibit their translation [38–40]. Thus, these RNA modifications play essential roles in distinguishing self from non-self RNAs within the cytosol of cells. Mimicry or co-option of capping and 2'-O-methylation processes by viruses to avoid detection by PRRs also demonstrates the importance of these modifications as key determinants of self [38, 41].

The role of m^6A in modulating recognition of RNA substrates by PRRs is now beginning to be uncovered (Figure 1). For example, certain **Toll-like receptors** (**TLRs**) have been found to respond differently to RNAs derived from different organisms [42]. Human total RNA only weakly stimulates the Tumor Necrosis Factor alpha (TNF-α) response downstream of TLRs, while bacterial total RNA is a potent stimulator. Interestingly, tRNA from humans or bacteria does not strongly activate TNF-α. Given that tRNA species and human mRNAs are highly post-transcriptionally modified, these data suggested that these modifications may suppress TLR recognition of RNA. Indeed, early studies revealed that in vitro-transcribed RNAs containing modified nucleosides are less potent activators of TLRs than their unmodified counterparts, and m^6A is particularly effective at inhibiting TLR activation [42]. Similarly, in vitro-transcribed polyU/UC RNA from hepatitis C virus (HCV), which is a strong RIG-I ligand, binds poorly to RIG-I when modified by m^6A [43]. These studies suggest a role for m^6A in shielding RNA species from detection by PRRs. An additional study found that m^6A modification of human circular RNAs is necessary to inhibit their recognition by RIG-I, and this study proposes a model in which the m^6A reader protein YTHDF2 sequesters circular RNAs away from RIG-I [44]. While YTHDF2 is generally known for its role in facilitating the degradation of its m^6A-modified target RNAs, it has also been linked to mRNA localization and phase separation [8, 45], which could explain its role in circular RNA sequestration. However, the immunogenicity of unmodified circular RNA is controversial, and one study suggests that contaminating linear RNA may instead cause activation of RNA sensors [46]. Nevertheless, these studies suggest multiple roles for m^6A in prevention of aberrant innate immune activation in response to endogenous RNAs. Additionally, the m^6A writer Mettl3 was found to be essential for suppression of endogenous long double stranded RNA levels in murine hematopoietic stem cells. These RNA species activate cellular sensors such as **MDA5**, which specifically detects long double stranded RNAs and, like RIG-I, also signals through MAVS, as well as the Oligoadenylate Synthetase-Ribonuclease L (OAS-RNase L) and Protein Kinase-R-Eukaryotic Initiation Factor 2 alpha (PKR-eIF2α) pathways [37, 47, 48]. Thus, Mettl3 deletion resulted in aberrant upregulation of **interferon-stimulated genes** (**ISGs**) and failure of hematopoietic stem cells to differentiate [49]. The mechanisms by which METTL3 and m^6A suppress endogenous double stranded RNA levels are not yet clear, although m^6A could enable recognition by a reader protein such as YTHDF2 and subsequent degradation of these RNAs, or m^6A could directly modulate the RNA structures to prevent recognition by the described cellular sensors. m^6A-mediated effects on RNA structures have been previously described [6, 50], and these effects may be sufficient to interrupt long dsRNA structures and inhibit MDA5 recognition. Together these studies implicate m^6A as an important molecular

signature that can contribute to protecting self RNAs from innate immune sensing through sequestration by m^{6}A reader proteins, preventing RNA binding protein interactions, direct structural changes, or other mechanisms that have not yet been identified. Interestingly, while m^{6}A is a very prevalent modification on mRNA [51], not all mRNAs are m^{6}A-modified, demonstrating that m^{6}A modification must not be fully required for inhibition of host mRNA recognition by PRRs. Perhaps, in these contexts, other RNA modifications or structures serve redundant roles in inhibiting PRR sensing. Indeed, in vitro transcribed RNAs, for example, likely lack other internal modifications in addition to m^{6}A and may not be bound by the same repertoire of RNA binding proteins as endogenous RNAs after transfection into cells. Thus, determining the specific contexts in which m^{6}A is important for inhibiting PRR sensing will be crucial for understanding its importance as a signature of self RNA.

Supporting the role of m^{6}A in preventing sensing of RNAs by PRRs, recent studies have found evidence that viruses use m^{6}A to protect their RNA from recognition by PRRs. One study found that both the genome, antigenome, and mRNAs of the negative-sense, single-stranded RNA virus human metapneumovirus (HMPV) are m^{6}A-modified [52]. By depleting m^{6}A-related proteins, this study showed that m^{6}A has a proviral effect for HMPV, likely through its effects on both host and viral RNAs. Abrogation of m^{6}A sites in the HMPV genome resulted in viral mutants that induced more type I IFN and whose replication was attenuated. Interestingly, IFN induction by these mutants was dependent on RIG-I and appeared to be mediated by RIG-I specifically recognizing the m^{6}A-deficient genome and anti-genome, rather than the viral mRNAs. While it is not yet clear how m^{6}A inhibits RIG-I binding to HMPV RNA, it is possible that m^{6}A also inhibits RIG-I oligomerization along the RNA, thus preventing downstream signaling [52]. This study provides evidence of a virus co-opting m^{6}A modification to mask its RNA from cellular PRRs. An additional report suggests that both hepatitis B virus (HBV) and HCV, which have been shown to contain m^{6}A, may utilize similar strategies to avoid innate immune detection [53]. m^{6}A on HBV RNA was previously found to enhance reverse transcription and destabilize HBV transcripts [54], while we showed m^{6}A on HCV inhibits packaging of its RNA genome [55]. A recent study tested the effect of m^{6}A on the RNA of these viruses using in vitro transcribed viral RNA containing mutations at putative m^{6}A sites and found that these m^{6}A sites potentially inhibited RIG-I recognition of HBV and HCV RNA [53]. However, further work to ensure that the putative m^{6}A sites mutated in these studies are indeed modified following transfection of the in vitro transcribed RNAs will be of importance. This may also help to reconcile other differences with published work that shows that m^{6}A has antiviral roles during infection [54, 55].

The ability of m^{6}A to serve as an additional feature beyond the m^{7}G cap and 2’-O-methylation to mark cellular RNAs as self is an exciting function that the field is just beginning to understand. Future work detailing the mechanisms by which m^{6}A on viral RNA inhibits activation of RIG-I and other RNA sensors will contribute to our understanding of the functions of m^{6}A during viral infection, and also provide valuable information for designing attenuated vaccines, or for delivery of RNA therapeutics [56–58]. Additionally, whether m^{6}A modification in certain structural or sequence contexts on viral RNA could actually serve as a molecular signature to recruit innate immune surveillance

proteins will be interesting to explore further. Indeed, m^{6}A-induced structural alterations in RNA have been shown to regulate RNA binding protein interactions [6, 59, 60]. Additionally, a recent report suggested that, in IFN-stimulated cells, the antiviral protein Interferon-Stimulated Exonuclease Gene 20 (ISG20) can specifically recognize an m^{6}A-modified site in HBV RNA, perhaps through interaction with YTHDF2, and facilitate degradation of this RNA [61]. Other innate immune effector proteins are also known to recognize specific features of RNAs, such as IFIT1 which inhibits cap0 RNA translation [39, 40], or ZAP, which recognizes CG dinucleotides within viral RNA [62]. Therefore, there are many interesting possibilities to explore regarding the roles of m^{6}A for RNA recognition by innate immune surveillance proteins.

Cytokine production and responses

After detection of foreign nucleic acids or other components of viruses, signaling pathways are activated by PRRs that detect specific PAMPs and drive the production of cytokines, such as IFNs, which initiate antiviral responses and orchestrate the adaptive immune response [25]. For example, as mentioned in the previous section, detection of viral RNA by RIG-I or MDA5 activates the MAVS pathway and a signaling cascade that activates proteins such as Tank Binding Kinase 1 (TBK1), Tumor Necrosis Factor Receptor-Associated Factor (TRAF) proteins (TRAF2, TRAF3, and TRAF6), and the transcription factors Interferon Regulatory Factors 3 and 7 (IRF3 and IRF7) [63]. Interestingly, m^{6}A appears to play a role in the MAVS pathway by regulating the production of several of the signaling molecules in the pathway (Figure 1). In murine macrophages, it was found that *Mavs*, *Traf3*, and *Traf6* transcripts are m^{6}A-modified, and that DEAD-Box Helicase 46 (DDX46) can bind these mRNAs after viral infection to recruit the m^{6}A eraser ALKBH5, which demethylates these transcripts. Following demethylation, *Mavs*, *Traf3*, and *Traf6* mRNAs are increasingly retained in the nucleus, dampening signaling and production of IFNs [64]. These studies suggest that m^{6}A can promote antiviral signaling pathways by regulating the expression of signaling molecules.

m^{6}A may also be involved in regulation of other PAMP signaling pathways, such as the response to lipopolysaccharide (LPS) (Figure 1). A recent study found that the m^{6}A reader protein YTHDF2 inhibits the inflammatory response to LPS, which signals through TLR4 to activate Nuclear Factor Kappa B (NF-κB) and Mitogen-Activated Protein Kinase (MAPK) signaling [65–67]. However, it is unclear whether this YTHDF2-mediated regulation is dependent on m^{6}A. Additional evidence of a role for m^{6}A in LPS signaling was found in dental pulp cells, in which METTL3 depletion led to increased expression of a particular isoform of **MyD88**, a signaling molecule in the TLR4 pathway (Figure 1) [68]. This short isoform of MyD88 acts as a dominant-negative regulator of TLR4/MyD88 signaling, and therefore led to decreased activation of NF-κB and MAPK signaling [68]. Therefore, it appears METTL3 may modulate MyD88 splicing to promote the LPS-induced inflammatory response. While m^{6}A can regulate the splicing of certain transcripts [69], it is not yet clear whether *MyD88* mRNA is m^{6}A-modified or whether METTL3 has other trans-regulatory effects on the transcript. Taken together, these studies do seem to suggest roles for m^{6}A in the LPS response. In addition to its recently discovered roles in viral RNA and LPS-driven innate immune signaling pathways, m^{6}A likely has additional roles in other signaling

responses that stimulate induction of cytokines and inflammatory responses. Therefore, future explorations in these areas will be invaluable for understanding the role of m^{6}A in inflammatory conditions and autoimmune disease.

In addition to regulating innate immune signaling pathways, m^{6}A has recently been found to directly regulate the production of the important antiviral cytokine IFN-β [70, 71] (Figure 2). Following infection by human cytomegalovirus (HCMV), the expression of m^{6}A writers, erasers, and reader proteins was found to increase substantially. Interestingly, m^{6}A profiling revealed that after innate immune activation, the *IFNB1* transcript was m^{6}A-modified and that this methylation decreased its half-life [70]. These results suggest that m^{6}A suppresses IFN-β production, which could be exploited by viruses by inducing m^{6}A modification of *IFNB1* mRNA. In further support of this hypothesis, an additional study found that HCMV replication was decreased in METTL3 depleted cells due to enhanced expression of IFN-β [71]. This study found that YTHDF2 binds to m^{6}A-modified *IFNB1* mRNA to facilitate its degradation [71]. Thus, these two studies converged on the idea that m^{6}A dampens IFN-β production and contributes to turnover of this proinflammatory cytokine. This regulatory feature of m^{6}A may be important for controlling inflammatory conditions and autoimmunity, which have been linked to excessive IFN production [72]. Additionally, the apparent ability of HCMV to exploit this control of IFN-β expression to facilitate its replication by increasing m^{6}A modification of the *IFNB1* transcript and thus decreasing its production is an exciting discovery. Additional m^{6}A-mediated viral strategies for inhibiting IFN induction pathways also appear to exist. For example, HBV infection leads to increased m^{6}A modification of the *PTEN* transcript, which encodes Phosphatase And Tensin Homolog, a positive regulator of IRF3 nuclear translocation [73]. *PTEN* mRNA is destabilized by m^{6}A, thus increased m^{6}A levels on *PTEN* mRNA during HBV infection lead to less PTEN expression and less IFN-β production [74]. It is likely that other viruses also influence the production of IFN-β or other cytokines by manipulating m^{6}A modification on the transcripts of cytokines or molecules that regulate their production, and this will be an interesting avenue for future research.

As m^{6}A has been found to regulate the pathways that lead to cytokine production and the transcripts of cytokines themselves, a role for m^{6}A in cellular response pathways induced by cytokines is an interesting area to explore. We recently discovered a role for m^{6}A in the response to type I IFN [75] (Figure 2). While the transcript levels of ISGs were not regulated by METTL3/14 after IFN stimulation, suggesting m^{6}A does not regulate the **JAK-STAT signaling pathway** that leads to transcriptional activation of ISGs [76], we did find that METTL3/14 enhances the translation of a subset of m^{6}A modified ISGs, including many with known antiviral functions. Importantly, depletion of METTL3/14, which decreased antiviral effector ISG expression, led to a higher percentage of IFN pretreated cells becoming infected with vesicular stomatitis virus (VSV) [75]. These results suggest that m^{6}A enhances the antiviral effects of type I IFN, thus establishing a role for m^{6}A in the type I IFN response. An additional study in mouse macrophages found that the m^{6}A reader protein Ythdf3 indirectly regulates the transcription of ISGs by promoting the translation of *Foxo3*, which encodes Forkhead Box O3, which represses transcription of a subset of ISGs [77]. Surprisingly, Ythdf3 regulation of Foxo3 occurred independently of METTL3-

mediated m^{6}A modification. This study elucidated an interesting role for Ythdf3 in regulating the type I IFN response, although these studies have yet to be replicated in human cells. In addition to these findings, future research describing how m^{6}A regulates responses to other cytokines will be an important avenue, especially as these results would help to inform how m^{6}A regulates the cross-talk between the innate and adaptive immune responses.

Immune cell activation and function

Cytokines produced during viral infection recruit immune cells and influence their maturation and activation. As m^{6}A can control cytokine production, as seen for IFN-β, it likely also regulates the communication between virus-infected cells and immune cells, although this specific type of regulation has yet to be described. However, roles have recently been described for m^{6}A in regulating immune cell function (Figure 3). Dendritic cells (DCs) are a class of antigen presenting cells with important roles in linking innate and adaptive immune responses. m^{6}A has now been shown to regulate DC maturation [78]. Using murine DCs, it was found that Mettl3 promoted DC maturation in a manner dependent on its m^{6}A catalytic activity, likely through its promotion of the translation of the m^{6}A-modified transcripts of Cluster of Differentiation 40 and 80 (*CD40*, *CD80*), and *Tirap*. TIR Domain Containing Adaptor Protein (Tirap) is a signaling protein in the TLR4/MyD88 pathway [67], and thus its expression is important for TLR4 signaling and downstream DC activation, whereas CD40 and CD80 are co-stimulatory molecules important for T cell activation [79]. Importantly, DCs lacking Mettl3 are deficient in their ability to promote T cell proliferation, demonstrating the importance of m^{6}A in the maturation and function of DCs [78]. While the relevance of these findings have not been explored in the context of viral infection, many viruses also stimulate the MyD88 pathway through Tirap [80]. Additionally, it is known that DCs are potent producers of cytokines like type I IFNs in response to viral infection and are crucial for initiation of adaptive immune responses, as they activate naïve T cells [81]. Therefore, these findings provide some insight into potential roles of m^{6}A in linking the innate and adaptive immune responses to control viral infection.

Interestingly, m^{6}A also controls the homeostasis and differentiation of naïve T cells [82] (Figure 3). Recent work found that naïve T cells from conditional Mettl3 knockout mice are deficient in their ability to proliferate and differentiate into effector T cells. The model of T cell differentiation used in this study activates JAK1/STAT5 signaling [83], and in Mettl3 knockout T cells, this signaling was impaired, likely due to increased abundance of the transcripts and proteins of key suppressors of this pathway, Suppressor of Cytokine Signaling 1 and 3 (Socs1 and Socs3), and Cytokine-Inducible SH2-Containing Protein (Cish). These transcripts were all m^{6}A-modified and lost m^{6}A in Mettl3 knockout naïve T cells, which led to their stabilization [82]. Additionally, m^{6}A was found to regulate CD4+ regulatory T cells, which are important for controlling inflammation, likely through similar mechanisms [84]. An additional role for METTL3/14 and m^{6}A in the CD8+ T cell response to melanoma tumors has also recently been reported. In murine colorectal carcinoma models, Mettl3/14 depletion stabilizes the transcripts of Signal Transducer and Activator of Transcription 1 (*Stat1*) and *Irf1* and increases the sensitivity of tumor cells to IFN-γ treatment, leading to growth inhibition of these cells [85]. These results suggest a role for

m^{6}A in STAT1-mediated signaling pathways through destabilization of the *STAT1* transcript, although Mettl3 and m^{6}A appear to stabilize the *Stat1* transcript in mouse macrophages [86], thus more work will be required to determine the cell type-specific effects of m^{6}A on STAT1 signaling pathways. Together, these results elucidate that m^{6}A has a role in regulating T cell homeostasis and cytotoxic T cell functions.

While the functions of m^{6}A in immune cells have not been well studied during viral infection, these studies clearly demonstrate its importance in the normal function and activation of immune cells, which are crucial for viral clearance. Additionally, these results demonstrate how understanding the transcript-specific roles of m^{6}A in immune pathway regulation and control of immune cell functions will be useful for the development of future immunoregulatory therapies. As m^{6}A is known to play important roles in stem cell fate decisions [87], cell development and maturation appears to be an important general biological function of m^{6}A. Therefore, the discovery of additional roles of m^{6}A in DCs and T cells, as well as other immune cell subsets, such as macrophages, natural killer cells, and B cells will be of great importance for our overall understanding of the roles of m^{6}A in immunity.

Stress responses and metabolism

In addition to induction of immune responses, viral infection can induce cellular stress responses and can influence cellular metabolism [88]. As m^{6}A can regulate many cellular pathways, including stress responses, its roles in infection-induced pathways will be important to understand. Indeed, many studies have found that diverse viral infections shape the m^{6}A distribution within the host transcriptome [89–93]. However, in-depth functional validation of these m^{6}A changes has been rare, and recent research suggests that some of these findings may be worth revisiting, as gene expression changes can influence m^{6}A peak calling [51]. We recently profiled changes induced to the m^{6}A epitranscriptome during *Flaviviridae* infection using rigorous analyses and investigated functional roles for some of these changes [93]. Of the viruses studied (dengue virus, Zika virus, West Nile virus, and HCV), each induced alterations to m^{6}A modifications on certain transcripts, and some of these alterations were common across all viruses. Among the genes whose m^{6}A status changed during infection by all of these viruses was *RIOK3*, a transcript that gained m^{6}A during infection, which encodes RIO Kinase 3, a serine/threonine kinase that may regulate antiviral signaling. Interestingly, innate immune signaling driven by the transcription factor IRF3 was found to be important for the gain of m^{6}A following infection, and m^{6}A modification increased *RIOK3* translation. The m^{6}A status of *CIRBP*, which encodes Cold Inducible RNA Binding Protein, a stress-induced RNA binding protein, also changed in response to infection, although this transcript lost m^{6}A and consequently was increasingly alternatively spliced to its short isoform. Importantly, ER stress-inducing treatment was sufficient to induce the loss of m^{6}A on *CIRBP*, and *Flaviviridae* infection is known to induce ER stress responses [94]. While the precise mechanisms by which m^{6}A status changes during infection are not clear, these data suggest that activation of host cell pathways during infection can influence the m^{6}A status of individual transcripts. Additionally, many genes with m^{6}A alterations were found to be capable of regulating *Flaviviridae* infection, including RIOK3 and CIRBP. These results point to functional roles for changes to the m^{6}A landscape

during viral infection, and set the stage for further investigation of the mechanisms responsible for m^{6}A alterations [93].

Some possible mechanisms by which viral infection could induce changes to the m^{6}A landscape include differential transcription rates of m^{6}A-modified genes, changes in the expression, localization, or function of METTL3/14 or other RNA binding proteins involved in m^{6}A targeting, or similar changes to m^{6}A demethylase proteins like FTO or ALKBH5. Indeed, multiple viruses have been found to perturb the expression of the m^{6}A machinery. These include HCMV, which increases the abundance of the m^{6}A machinery [70, 71], or enterovirus 71, which increases the expression of METTL3 and METTL14 and changes the subcellular localization of reader, writer, and eraser proteins [95]. These alterations to the m^{6}A machinery may benefit viruses by allowing modification of their RNAs or by influencing the m^{6}A profile of the infected host cell. Interestingly, a recent study found that during VSV infection in mice, demethylation at a specific arginine residue in Alkbh5 impairs its m^{6}A demethylase activity [96]. Alkbh5 deficiency in macrophages resulted in perturbations to cellular metabolism. In particular, Oxoglutarate Dehydrogenase (Ogdh), an enzyme involved in the citric acid cycle, was found to be strongly downregulated in these cells, as Alkbh5 normally demethylates the Ogdh transcript, which increases its stability and expression. Ogdh deficiency, in turn, was found to decrease the abundance of the metabolite itaconate, which was capable of promoting VSV replication [96]. Therefore, these results point to demethylation of Alkbh5 as a means of controlling Ogdh expression, which in turn regulates the production of itaconate. Importantly, this study identified a mechanism by which the m^{6}A-modification of a cellular RNA can change in response to viral infection. Determining whether this interesting cellular response is specific to VSV infection, or relevant for other viruses, will be an important future step. Additionally, these results should set the stage for additional discoveries of mechanisms by which viral infection influences the cellular m^{6}A landscape. Such discoveries will be of utmost importance for our understanding of how viruses manipulate m^{6}A distribution for their benefit and how host cells utilize alteration of m^{6}A to restrict viral replication.

Concluding Remarks

Our understanding of the functional roles of m^{6}A in modulating host processes during viral infection is rapidly expanding, and these discoveries will also broaden our understanding of m^{6}A biology. Because of the diverse, transcript-specific effects of m^{6}A that can affect both viral and host RNAs, m^{6}A regulates viral infection in complex ways (see Outstanding Questions). In order to achieve a more synergistic understanding of the mechanisms by which m^{6}A and its related cellular machinery regulate viral infection, future research must continue to address the transcript-specific and position-specific roles of m^{6}A in regulation of cellular pathways in response to individual, as well as pan-viral infection. Additional mechanistic understanding of how m^{6}A regulates RNA sensing by PRRs, diverse cytokine production and responses, stress responses, immune cell biology, and cross-talk between the innate and adaptive immune system will be of great interest. Tissue- and cell type-specific m^{6}A machinery knockout animal models will likely be very useful in gaining a better understanding of the roles of m^{6}A in immune responses during viral infection. Finally, understanding whether and how viruses manipulate the m^{6}A machinery and abundance or

position of m^{6}A in the host transcriptome will inform our understanding of the role of m^{6}A at the virus-host interface and also elucidate potential m^{6}A-based therapies for viral infection or immunopathies.

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Glossary

ALKBH5
AlkB Homolog 5; a protein with m^{6}A demethylase activity

FTO
Fat Mass And Obesity-Associated Protein; a protein with m^{6}A demethylase activity

Interferon (IFN)
a family of proteins released from cells that induce interferon-stimulated genes to restrict viral replication

Interferon-Stimulated Gene (ISG)
a class of genes whose transcription can be stimulated by interferons.

JAK-STAT signaling pathway
Janus Kinase-Signal Transducer and Activator of Transcription; a pathway activated by various cytokines to induce transcriptional responses

m^{6}A
N6-methyladenosine; an adenosine residue containing a methyl group at its N6 position

MAVS
Mitochondrial Antiviral Signaling; a mitochondria-localized adaptor protein that interacts with RIG-I or MDA5 to form a platform for the interaction of signaling proteins to stimulate IFN production

MDA5
Melanoma Differentiation-Associated Protein 5; a cytosolic RNA helicase that recognizes long double stranded RNA

Methyltransferase Like 3 (METTL3)
the catalytic enzyme subunit of the m^{6}A methyltransferase complex

METTL5
Methyltransferase Like 5; a protein with m^{6}A methyltransferase ability that is responsible for m^{6}A deposition on 18S rRNA

METTL14

Methyltransferase Like 14; a protein that forms a heterodimer with METTL3 and is essential for m^{6}A deposition

METTL16
Methyltransferase Like 16; a protein with m^{6}A methyltransferase ability that is responsible for U6 snRNA m^{6}A deposition

MyD88
Myeloid Differentiation Primary Response 88; a signaling protein involved in activation of TLR-driven pathways

Pathogen-Associated Molecular Pattern (PAMP)
a molecular pattern that can be used to distinguish pathogens from their host cells

Pattern Recognition Receptor (PRR)
a cellular protein that recognizes pattern-associated or damage-associated molecular patterns

RBM15/RBM15B
RNA Binding Motif Protein 15 and RNA Binding Motif Protein 15B; accessory proteins in the m^{6}A methyltransferase complex

RIG-I
Retinoic Acid-Inducible Gene-I; a cytosolic RNA helicase that recognizes uncapped RNAs with 5’-tri or diphosphate moieties

Toll-Like Receptors (TLR)
membrane-spanning receptors that recognize structurally conserved molecules derived from microbes

VIRMA
Vir Like M6A Methyltransferase Associated; an accessory protein in the m^{6}A methyltransferase complex

WTAP
Wilms Tumor 1 Associated Protein; an accessory protein in the m^{6}A methyltransferase complex

YTHDC1
YTH Domain-Containing Protein 1; an m^{6}A reader protein involved in regulation of splicing

YTHDC2
YTH Domain-Containing Protein 2; an m^{6}A reader protein that can promote both translation and decay of m^{6}A-modified RNAs

YTHDF1
YTH Domain Family 1. An m^{6}A reader protein, primarily thought to promote translation of m^{6}A-modified RNAs

YTHDF2

YTH Domain Family 2. An m^6A reader protein, primarily thought to promote decay of m^6A-modified RNAs

YTHDF3
YTH Domain Family 3. An m^6A reader protein that can promote mRNA translation or decay through interactions with YTHDF1 and YTHDF2

ZC3H13
Zinc Finger CCCH-Type Containing 13. An accessory protein in the m^6A methyltransferase complex

ZCCHC4
Zinc Finger CCHC-Type Containing 4. A protein with m^6A methyltransferase ability that is responsible for m^6A deposition on 28S rRNA

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Outstanding Questions

- By what mechanisms does m^6A modification of host and viral RNAs inhibit activation of RNA sensors like RIG-I? Could m^6A modification of viral RNA recruit antiviral effector proteins?
- What roles does m^6A play in the production and response to cytokines beyond IFNs?
- How does m^6A regulate the cross-talk between the innate and adaptive immune responses?
- In addition to dendritic cells and T cells, what other immune cells are regulated by m^6A, and how does it modulate their development and effector processes?
- What changes to the host RNA landscape are induced by diverse viral infections, and how do these changes modulate cellular responses to infection?
- How do viruses manipulate m^6A modification of host transcripts to benefit their replication?

Highlights

- The transcript-specific effects of N6-methyladenosine (m^6A) exert control over host response pathways during viral infection.
- m^6A may serve as a molecular signature to regulate interactions between viral or host RNAs with antiviral RNA binding proteins.
- Host responses such as cytokine production, cytokine signaling, and ER stress are regulated by m^6A.
- Immune cell activation pathways are modulated by m^6A.

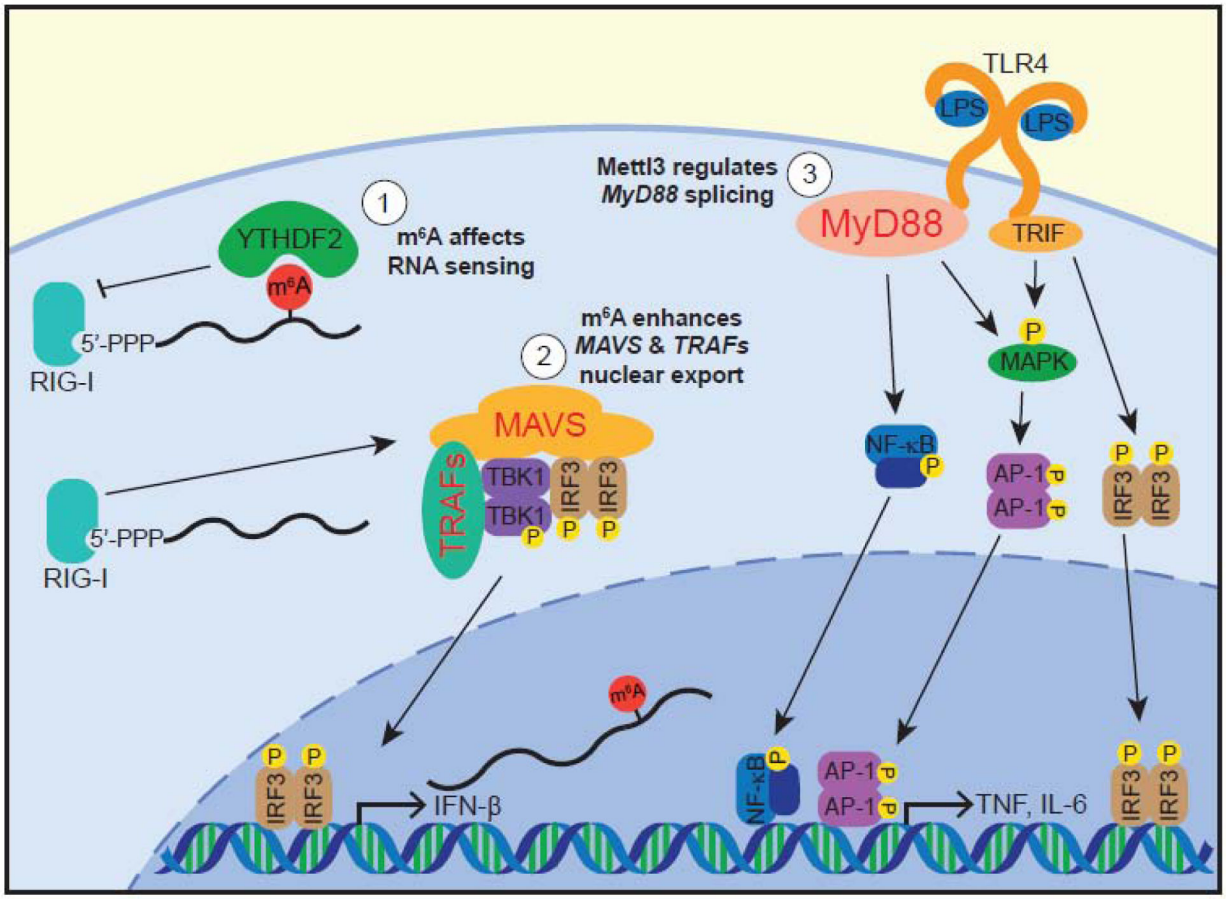

Figure 1. m^{6}A regulates innate immune signaling pathways.

m^{6}A regulates the induction of cytokine responses through multiple mechanisms. 1) m^{6}A, perhaps via interactions with the YTHDF2 protein, inhibits detection of RNAs by RIG-I, whose role is to sense foreign RNAs and activate the MAVS signaling pathway [42–44, 49, 52, 53]. 2) m^{6}A promotes the expression of MAVS and TRAFs by promoting the nuclear export of their mRNAs [64]. This induces signaling of the MAVS pathway, which activates the transcription factor IRF3 through the interaction of MAVS, TRAFs, and the kinase TBK1. Activated IRF3, along with NF-κB, induces transcription at the IFN-β promoter [37]. 3) METTL3 regulates MyD88 isoform usage in response to LPS stimulation of the TLR4 pathway [68]. TLR4 signals through proteins such as MyD88, MAPKs, and TRIF to activate the transcription factors NF-κB, AP-1, and IRF3. These transcription factors induce the production of proinflammatory cytokines like TNF, IL-6, and IFN-β [67]. YTHDF2 also regulates the TLR4 pathway [65] (not shown). Proteins whose expression are known to be regulated by m^{6}A or m^{6}A-related enzymes are shown in red text.

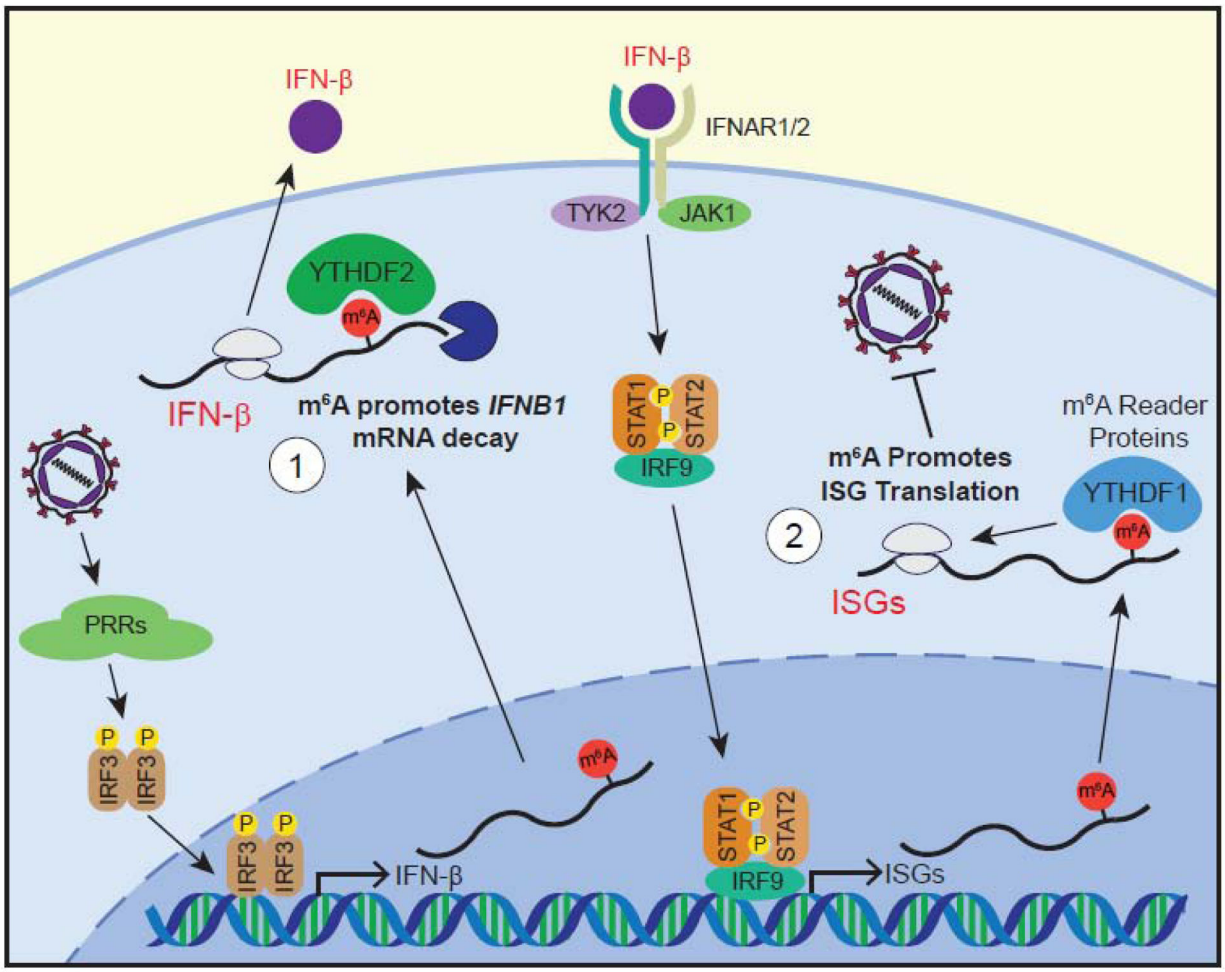

Figure 2. m^{6}A regulates IFN-β and the type I IFN response.

1) Following viral detection by PRRs and the activation of signaling pathways such as those described above, type I IFNs are produced [37]. The *IFNB1* transcript is m^{6}A modified, and m^{6}A recruits the reader protein YTHDF2 to facilitate *IFNB1* degradation, dampening the production of IFN-β [70, 71]. 2) IFN-β activates signaling through the JAK-STAT pathway, activating JAK1 and TYK2 kinases, which phosphorylate STAT1 and STAT2, which associate with IRF9, forming a transcription factor complex that drives the transcription of ISGs [72]. Many ISGs are m^{6}A-modified, and m^{6}A enhances the translation of a subset of these ISGs. This translation enhancement by m^{6}A and reader proteins like YTHDF1 promotes the antiviral effects of the type I IFN response [75]. Proteins whose expression are known to be regulated by m^{6}A or m^{6}A-related enzymes are shown in red text.

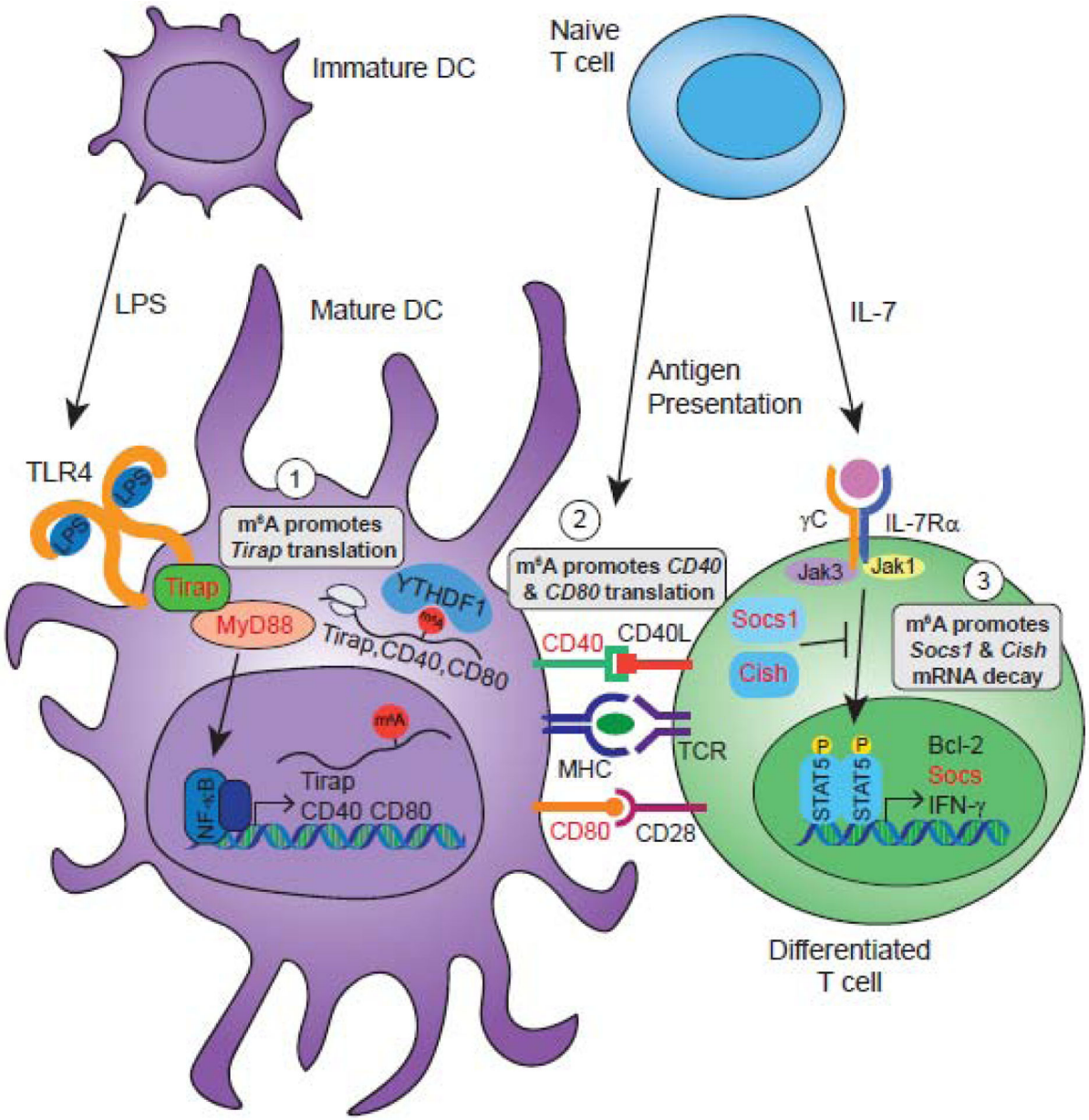

Figure 3. m^{6}A regulates immune cell function.

1) LPS treatment of murine DCs induces signaling through the TLR4/MyD88 pathway to activate the transcription factor NF-κB, which drives transcription of its target genes and DC maturation [67, 81]. m^{6}A regulates DC maturation by promoting the translation of Tirap, which encodes an important adaptor protein for TLR4/MyD88 interaction [78]. m^{6}A may also regulate MyD88 splicing [68]. 2) After activation, mature DCs can present antigens to T cells to influence their differentiation. This process involves MHC presentation of antigen peptides and interaction with T cell receptors (TCR), as well the interaction of co-stimulatory molecules such as CD40 and CD80 [81]. m^{6}A contributes to this process by promoting the translation of CD40 and CD80 mRNAs [78]. 3) T cells can also be activated by cytokines like IL-7. IL-7 signals through its receptor composed of IL-7Rα and the

common gamma chain (γC), activating JAK1 and JAK3, which induce the activation and homodimerization of STAT5, inducing cytokines like IFN-γ and genes involved in T cell survival and differentiation [83]. Additionally, this pathway induces suppressor of cytokine signaling (SOCS) proteins, such as SOCS1 and SOCS3, as well as Cish, all of which repress activation of the IL-7 pathway. The mRNA of Socs1, Socs3, and Cish are m^6A-modified, and m^6A destabilizes their transcripts, thus promoting the IL-7 pathway and T cell survival and differentiation [82]. Molecules whose expression are known to be regulated by m^6A or m^6A-related enzymes are shown in red text.