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Not immune to modification

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Competing interests

The authors declare no competing interests.

CYTOKINE RNA STABILITY

Not immune to modification

The N^6-methyladenosine (m^{6}A) RNA-modification pathway substantially affects the outcome of viral infection. Studies now show that m^{6}A modification of transcripts encoding type I interferons limits the duration of anti-viral signaling.

Charles R. Hesser and Britt A. Glaunsinger

The function and fate of RNA can be heavily influenced by chemical alterations to its sequence; more than 150 such modifications exist. Classic examples of this include modifications to the wobble position of the tRNA anticodon loop, which contribute to expanded coding potential, and to the 5′ cap structure of mRNA[1]. Over the past several years, a remarkable array of gene-regulatory phenotypes have been ascribed to modification of cellular mRNA by m^{6}A, the most abundant internal modification on these transcripts[1]. Published studies have shown that the cellular m^{6}A machinery can also influence the outcome of viral infection, at least in part by affecting expression of m^{6}A-modified viral RNA[2,3]. Two new complementary studies, by Winkler and colleagues in this issue of *Nature Immunology*[4] and by Rubio and colleagues in *Genes & Development*[5], reveal that the influence of the m^{6}A pathway also extends to immunological regulators by demonstrating that transcripts encoding type I interferons (IFN-α and IFN-β (IFN-α/β)) — pleotropic mediators of anti-viral immunity — are also targets of m^{6}A modification. Thus, inhibiting m^{6}A modification can alter the outcome of infection by affecting the fate of both viral mRNAs and host mRNAs that drive anti-viral responses.

Published studies have indicated that m^{6}A is an important component of host–pathogen interactions. For example, it has a critical role in regulating cellular transition states and maintaining T cell homeostasis[6]. A role for m^{6}A in recognition by the innate immune system has also been proposed, as m^{6}A-modified RNAs show reduced activation of Toll-like receptor pathways[7]. For these reasons, interest in the role of m^{6}A in infectious contexts has burgeoned. While numerous studies of viral infection have found enrichment for m^{6}A in viral transcripts, precisely how the m^{6}A pathway regulates viral life cycles remains an active area of investigation. For hepatitis C virus, which has an entirely cytoplasmic life cycle, m^{6}A restricts viral assembly[2]. For influenza A virus and SV40 polyomavirus, which replicate in the nucleus, m^{6}A instead promotes viral gene expression[8,9]. However, for several viruses with complex life cycles (human immunodeficiency virus, hepatitis B virus and Kaposi's sarcoma–associated herpesvirus), a combination of pro-viral effects and anti-viral effects has been proposed[2,3,10]. Furthermore, the extent to which these phenotypes result from the binding of reader proteins in cis to methylated viral RNAs versus their influencing host pathways involved in the viral life cycle has remained unclear.

Viral infection substantially changes the host transcriptome, and as m^{6}A has been proposed to have a role in 'transcriptome-turnover' events[1], its presence in transcripts encoding anti-viral cytokines could affect the innate immune response by accelerating their degradation. Indeed, anti-viral cytokines are extensively regulated post-transcriptionally and are characterized by short transcript half-lives. For example, AU-rich elements are present in 3′ untranslated regions of many cytokine-encoding mRNAs and have a critical role in mRNA decay. Of particular interest in viral infection, IFN-α/β products induce resistance to viral infection but also cause a variety of autoimmune diseases when their production is dysregulated[11]. After being secreted, they exert autocrine and paracrine effects, binding to the IFN-α receptor (IFNAR) and triggering a JAK–STAT signaling cascade that leads to the production of molecules encoded by interferon-stimulated genes (ISGs), which restrain viral replication via multiple mechanisms[11]. Thus, even subtle changes to the stability of transcripts encoding IFN-α/β could substantially affect the abundance of IFN-α/β proteins and the production of molecules encoded by ISGs. If m^{6}A modification of transcripts encoding IFN-α/β contributed substantially to their post-transcriptional decay, could this contribute to the pro-viral effects ascribed to the m^{6}A pathway during various viral infections?

Winkler and colleagues address that issue by focusing on the role of m^{6}A in host transcripts during infection with several human and mouse viruses, including human cytomegalovirus (HCMV), a large dsDNA virus that replicates in the nucleus[4]. Once installed, m^{6}A acts as a molecular 'beacon' to recruit selective methyl-RNA-binding proteins known as 'readers'. Depletion of either the catalytic subunit responsible for the installation of m^{6}A (METTL3) or the reader protein YTHDF2 results in stabilization of the *IFNB* transcript and increased induction of downstream ISGs during infection with HCMV. This phenotype also develops during infection with each of three other human viruses and one mouse virus, suggestive of an evolutionarily conserved mechanism in which the m^{6}A pathway promotes the decay of transcripts encoding IFN-α/β. In support of this hypothesis, the authors observe that m^{6}A modification of transcripts encoding IFN-α/β is conserved across several human and mouse cell types and that viral gene expression is not required for the increased abundance of *IFNB* transcripts

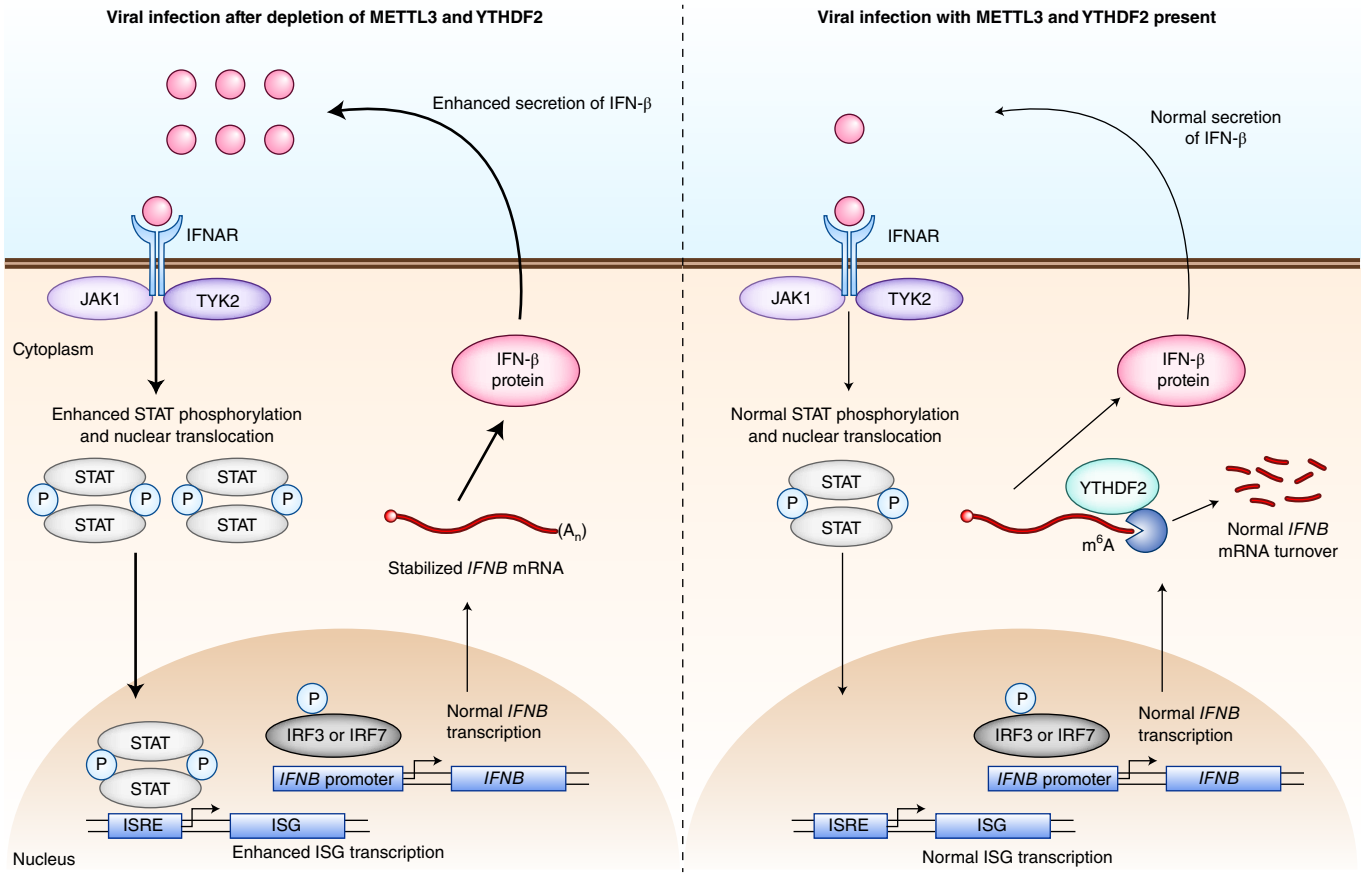

Fig. 1 | Depletion of METTL3 or YTHDF2 enhances *IFNB* stability. After viral infection, double-stranded DNA is sensed, which leads to the phosphorylation and nuclear translocation of the transcription factors IRF3 (interferon-regulatory factor 3) and IRF7, which activate transcription of *IFNB*. In METTL3-deficient cells, no changes occur in the expression or phosphorylation of IRF3 or IRF7 and there is no initial effect on the biogenesis of *IFNB* mRNA. Instead, increased stability of transcripts encoding IFN-α/β leads to an increase in mature secreted proteins, which bind to the cognate IFN-α receptor (IFNAR); this increases activation of the kinase JAK1 and tyrosine kinase TYK2 and recruits proteins of the STAT family of signal transducers and activators of transcription. Increased phosphorylation and nuclear translocation of STAT proteins then leads to a downstream increase in the expression of ISGs. ISRE, interferon-stimulated response element.

in cells depleted of METTL3. Furthermore, treatment with a selective chemical inhibitor of the kinases required for signaling downstream of IFNAR largely 'rescues' the virus-replication defect in METTL3- and YTHDF2-deficient cells. Finally, to confirm that m^{6}A modifications directly affect the stability of *IFNB* transcripts, the authors introduce synonymous mutations into the m^{6}A consensus motifs, which results in greater stability of the *IFNB* transcripts commensurate to that observed after depletion of METTL3. Thus, the authors propose that depletion of m^{6}A components impairs virion production mainly by extending the duration of IFN-α/β signaling (Fig. 1).

Rubio and colleagues similarly report regulation of the IFN-α/β response by the m^{6}A pathway[5]. In agreement with the study by Winkler and colleagues[4], they demonstrate that the m^{6}A pathway modulates the IFN-α/β response in the absence of viral gene expression, which indicates that a viral protein is not 'redirecting' the m^{6}A machinery away from viral transcripts to instead promote degradation of host transcripts. Both studies agree that *IFNB* mRNA is post-transcriptionally regulated by the m^{6}A pathway, although Rubio and colleagues also observe differences in *IFNB* transcription when this pathway is perturbed[5]. Specifically, depletion of the m^{6}A-methyltransferase subunit METTL14 increases *IFNB* transcription, while depletion of the m^{6}A-demethylase ALKBH5 has the opposite effect. Although determining how the m^{6}A pathway affects IFN-α/β induction requires further investigation, the increased abundance of IFN-α/β transcripts observed after depletion of METTL3 and/or METTL14 might help to explain published observations that the m^{6}A pathway promotes certain types of viral infection[8,9].

Modification of immunological regulators is only part of the effect of m^{6}A during infection, however, as viral RNAs are frequently modified and it has been reported that the m^{6}A pathway can exert pro-viral effects on these transcripts[8,9]. In these cases, it is possible that m^{6}A modification of viral transcripts can directly increase their stability, perhaps via alternative functions of the YTHDF proteins or the activity of additional m^{6}A readers. For example, the IGF2BP reader has been shown to promote the stability of m^{6}A-modified transcripts[12] (although this has yet to be explored in viral infection), and the reader YTHDC1 has been proposed to facilitate mRNA splicing and export from the nucleus[3] (including during viral infection). Furthermore, m^{6}A can promote both cap-dependent translation and cap-independent translation, which might also function in viral contexts[1,2]. Thus, the m^{6}A pathway probably influences the outcome of infection through cis-acting

effects on both viral transcripts and host anti-viral transcripts.

As m^{6}A modification is dynamic and its distribution is enriched or altered in certain cell types, an ongoing challenge is discriminating between direct effects of m^{6}A modifications and their indirect effects[1,3]. In DNA viruses, each phase of gene expression is dependent on the prior kinetic class of genes. Thus, when cells are depleted of writers and readers before infection, it is difficult to discern the relative effects on subsequent phases of the viral life cycle. While Winkler and colleagues attempt to minimize indirect effects by analyzing the distribution of m^{6}A on HCMV transcripts at relatively early time points post-infection[4], it remains possible that m^{6}A components directly affect viral late gene expression, as has been proposed during infection with SV40[9]. This possibility will be best resolved through the construction of viruses in which the m^{6}A consensus motifs are altered to contain synonymous mutations.

Overall, the observation that m^{6}A modification destabilizes transcripts encoding IFN-α/β provides important insight into the broad range of pro- and anti-viral phenotypes attributed to the m^{6}A pathway and reveals new questions to explore. For example, while the effect on the stability of transcripts encoding IFN-β is clear, ultimately it will be important to address the extent to which m^{6}A modifications influence the sensing of nucleic acids and induction of IFN-α/β. To this end, primary cells could be stimulated with a panel of nucleic acid agonists in the absence of m^{6}A machinery to determine whether effects on the transcription and stability of *IFNB* depend on the stimulus used. Furthermore, generating and infecting mice conditionally deficient in specific m^{6}A machinery could reveal whether cell type–specific differences in the distribution of m^{6}A affect viral tissue tropism. If the m^{6}A pathway broadly regulates the IFN-α/β response to multiple types of viral infection, it could serve as part of a conserved host response to tightly regulate the half-life of the transcripts encoding IFN-α/β, which sharpens the kinetics of induction and silencing to prevent deleterious autoimmune effects after viral replication has been curtailed. ❐

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Competing interests

The authors declare no competing interests.

IMMUNOMETABOLISM

Metabolic requirements for expanding and arming a clone army

A new target for controlling T cell responses adds to the list of key processes dependent on the synthesis of tetrahydrobiopterin, which is essential for neurotransmitter and nitric-oxide production and pain control.

Andrew L. Mellor and Lei Huang

T cells are the 'storm troopers' of the immune system, since they can kill other cells. Consequently, T cells must be licensed, through a tightly regulated process, to expand their populations and 'bear arms'[1]. A study in *Nature* by Cronin et al. now reveals a critical role for the enzyme cofactor tetrahydrobiopterin (BH4) in licensing T cell clones to proliferate and acquire 'weapons of destruction'[2]. These findings add to the extensive list of biological functions mediated by BH4, including the production of neurotransmitters and nitric oxide and pain control.

Circulating T cells are metabolically inert. After being activated, T cells proliferate rapidly and acquire effector functions, including, in the case of cytotoxic T cells, the ability to destroy target cells (Fig. 1). This licensing process has strict requirements for the coordinated delivery of activation signals from antigens, co-stimulatory ligands and pro-inflammatory cytokines[1]. These constraints ensure that potentially dangerous effector T cells are generated only as needed. Cronin et al. report that de novo, cell-intrinsic synthesis of BH4 is necessary for T cells to undergo clonal expansion[2]. The initial prompt is the observation that activated T cells express GTP cyclohydrolase I (GCH1), the first step in BH4 synthesis. The authors generate *Gch1*-deficient mice and find that activated T cells from these mice exhibit profound proliferative defects in vitro and in vivo, which confirms that T cell–intrinsic synthesis of BH4 is required for efficient T cell licensing. This proliferative defect is highly selective for T cells, as ablation of *Gch1* does not affect the development of T lymphocytes or B lymphocytes or the ability of B cells and regulatory T cells to acquire effector functions after being activated.

The potential clinical importance of BH4 emerges from mouse models of inflammatory colitis, allergy and autoimmune syndromes. In each model, inflammatory infiltrates and disease severity are substantially attenuated due to defective clonal expansion of T cells lacking *Gch1*.