

Minireview

m⁶A in the Signal Transduction Network

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In response to environmental changes, signaling pathways rewire gene expression programs through transcription factors. Epigenetic modification of the transcribed RNA can be another layer of gene expression regulation. N⁶-adenosine methylation (m⁶A) is one of the most common modifications on mRNA. It is a reversible chemical mark catalyzed by the enzymes that deposit and remove methyl groups. m⁶A recruits effector proteins that determine the fate of mRNAs through changes in splicing, cellular localization, stability, and translation efficiency. Emerging evidence shows that key signal transduction pathways including TGFβ (transforming growth factor-β), ERK (extracellular signal-regulated kinase), and mTORC1 (mechanistic target of rapamycin complex 1) regulate downstream gene expression through m⁶A processing. Conversely, m⁶A can modulate the activity of signal transduction networks via m⁶A modification of signaling pathway genes or by acting as a ligand for receptors. In this review, we discuss the current understanding of the crosstalk between m⁶A and signaling pathways and its implication for biological systems.

Keywords: ERK, mTOR, N⁶-methyladenosine, RNA modification, signaling, TGFβ

INTRODUCTION

N⁶-methyladenosine (m⁶A) is a methylation modification of adenosine on RNA. m⁶A is evolutionarily conserved, ranging from yeasts, plants, insects to mammals (Meyer and Jaffrey,

2017; Yue et al., 2019). In mammalian cells, m⁶A is detected on 0.1%-1% of adenosines in mRNA with an average of 2-3 sites per transcript (Perry et al., 1975). Transcriptome-wide sequencing revealed that m⁶A occurs in the consensus motif DRACH (D = A, G, or U; R = A or G; A* = m⁶A-modified A; H = A, C, or U) (Dominissini et al., 2012; Meyer et al., 2012). Considering that DRACH appears once every ~57 nucleotides in mRNA, many transcripts have the potential to be modified with m⁶A. Nevertheless, only 20%-30% of coding genes are methylated in cells (Dominissini et al., 2012; Meyer et al., 2012), indicating a specific site selection mechanism of m⁶A modification.

Indeed, cells tightly regulate m⁶A modification using specialized enzymes, m⁶A writers and erasers. The m⁶A-modified RNAs then recruit m⁶A-binding proteins (readers) that guide these RNAs for RNA biogenesis processes such as pre-mRNA splicing, nuclear export, stabilization, degradation, and translation. Aberrant m⁶A modifications by overactivation or suppression of these enzymes lead to human diseases such as cancer, diabetes, and neurological disorders. There are comprehensive review papers about the molecular functions of m⁶A enzymes (Meyer and Jaffrey, 2017; Wiener and Schwartz, 2021; Zaccara et al., 2019) and their pathophysiological functions (Barbieri and Kouzarides, 2020; He and He, 2021; Huang et al., 2020; Kasowitz et al., 2018). In this review, we will focus on how the signal transduction pathways, which play key roles in diverse physiological and pathological conditions, coordinate cellular processes through m⁶A. Given that m⁶A also controls signaling pathways through RNA modification or acting as a ligand, understanding the crosstalk be-

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tween signal transduction networks and m⁶A RNA processing will provide us insights into the complex biological systems.

THE m⁶A PROCESSING PROTEINS: WRITER, ERASER, AND READER

The m⁶A methyltransferase (writer) consists of the enzymatic m⁶A-methyltransferase like (METTL) complex (MAC) and the scaffolding MAC-associated complex (MACOM) (Fig. 1). METTL3 is a catalytic core of MAC, which methylates target mRNAs on the adenosine of DRACH sequence (Bokar et al., 1994; Dominissini et al., 2012; Meyer et al., 2012). METTL14 acts as a scaffolding protein of MAC by recognizing the substrate RNA and interacting with Wilms' tumor 1-associating protein (WTAP) of MACOM (Bujnicki et al., 2002; Liu et al., 2014; Ping et al., 2014). MACOM consists of several adaptor proteins including WTAP, VIRMA (vir-like m⁶A methyltransferase associated), RBM15 (RNA-binding motif protein 15),

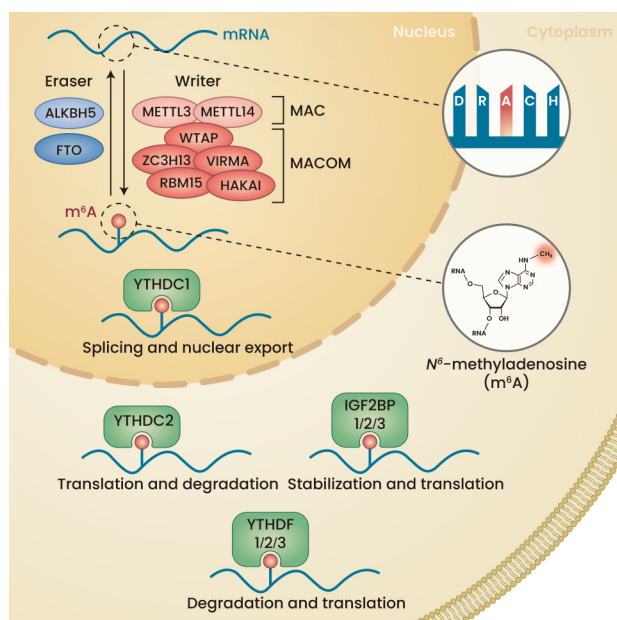


Fig. 1. Key players of the m⁶A RNA modification process. The deposition of m⁶A on mRNA is mediated by the writer complex which consists of m⁶A-METTL complex (MAC) and scaffolding MAC-associated complex (MACOM). MAC includes METTL3, a catalytic core protein, and METTL14, a scaffolding protein, which methylates adenosine in the consensus motif (DRACH, D = A, G, or U; R = A or G; A = m⁶A-modified A; H = A, C, or U). MACOM consists of adaptor proteins including Wilms' tumor 1-associated protein (WTAP), VIRMA (vir-like m⁶A methyltransferase associated), RBM15 (RNA-binding motif protein 15), HAKAI, and ZC3H13 (zinc finger CCCH domain-containing protein 13). FTO and ALKBH5 demethylate m⁶A (erasers). The m⁶A binding proteins (readers) include YT521-B homology (YTH) and insulin-like growth factor-2 mRNA binding protein (IGF2BP) family proteins, which determine the fate of m⁶A-methylated mRNA such as splicing, nuclear export, stability, and translation. The chemical structure of m⁶A is shown in the circle.

HAKAI, and ZC3H13 (zinc finger CCCH domain-containing protein 13) (Knuckles et al., 2018; Patil et al., 2016; Růžička et al., 2017; Śledź and Jinek, 2016; Wang et al., 2021; Yue et al., 2018). The MACOM complex does not have catalytic activity, but it is necessary for efficient m⁶A methylation by recruiting RNA substrates and stabilizing the MAC-MACOM complex in the nucleus and nuclear speckles.

Similar to DNA and histone methylations, m⁶A RNA methylation is a reversible process regulated by the demethylase enzymes (erasers): Fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5) (Jia et al., 2011; Zheng et al., 2013) (Fig. 1). They belong to AlkB homolog iron(II) and α KG-dependent dioxygenases, which include nine proteins with different substrate preferences toward single-stranded (ss) or double-stranded (ds) DNA and RNA substrates (Guengerich, 2015). Interestingly, although FTO and ALKBH5 exhibit comparable catalytic activities for m⁶A demethylation on ssRNA, the reaction steps are quite different. While ALKBH5 directly converts m⁶A to adenosine, FTO produces two intermediates N⁶-hydroxymethyladenosine (hm⁶A) and N⁶-formyladenosine (fm⁶A) during the demethylation process (Chen et al., 2014; Fu et al., 2013). This could be one of the reasons why FTO can demethylate another m⁶A-related modification, N⁶,2'-O-dimethyladenosine (m⁶A_m) (Mauer et al., 2017; Zhang et al., 2019). In contrast to the tissue-specific function of ALKBH5 in testes (Zheng et al., 2013), FTO is expressed in most tissues and involved in various human diseases including diabetes, obesity, and several cancers (Hirayama et al., 2020; Losman et al., 2020; Song et al., 2019; Zhao et al., 2014). Therefore, the substrate pools and specificity of FTO may dynamically change depending on the cellular context. Studies illuminating the predominant substrate of FTO in specific tissues and pathophysiological conditions will be needed.

The m⁶A binding proteins that determine the fates of m⁶A-methylated mRNA are classified as readers, which include several proteins such as YT521-B homology (YTH) and insulin-like growth factor-2 mRNA-binding protein (IGF2BP) family proteins (Fig. 1). As a common function, YTHDF1/2/3 promote the degradation of m⁶A-containing mRNAs (Lee et al., 2020; Patil et al., 2018; Wang et al., 2014; Zaccara and Jaffrey, 2020). YTHDF1 and YTHDF3 facilitate protein translation of m⁶A-methylated mRNAs by promoting ribosome assembly (Li et al., 2017; Shi et al., 2017; Wang et al., 2015). YTHDF2 undergoes liquid-liquid phase separation with mRNAs containing multiple m⁶A residues (Ries et al., 2019; Wang et al., 2014). Similar to YTHDF1 and YTHDF3, YTHDC2 induces degradation of m⁶A-modified mRNAs while enhancing their translation efficiency (Hsu et al., 2017; Mao et al., 2019; Tanabe et al., 2016; Wojtas et al., 2017; Zhou et al., 2021). The last YTH family protein, YTHDC1, facilitates pre-mRNA splicing and nuclear export of m⁶A-modified mRNAs (Kasowitz et al., 2018; Roundtree et al., 2017a; 2017b; Xiao et al., 2016; Xu et al., 2014). In contrast to the YTH family proteins, IGF2BP family proteins increase both the stability and translation efficiency of m⁶A-modified mRNAs, maximizing the expression of m⁶A-modified genes (Huang et al., 2018).

TRANSFORMING GROWTH FACTOR- β (TGF β) SIGNALING REWIRES GENE EXPRESSION PROGRAM THROUGH m⁶A MODIFICATION

TGF β signaling pathway plays an essential role in cell fate decisions, including pluripotency maintenance, differentiation, senescence, apoptosis, and tumorigenesis (Derynck and Zhang, 2003). TGF β family proteins are ligands for TGF β receptors (TGF β R), which includes TGF β , nodal, activin, bone morphogenetic protein (BMP), and growth differentiation factor (GDF) (Derynck and Zhang, 2003; Zhang et al., 2017). The activated TGF β R phosphorylates downstream signaling proteins, the receptor-regulated SMADs (R-SMADs). Each TGF β family ligand activates distinct R-SMADs. For example, BMP and GDF promote the phosphorylation of SMAD1, SMAD5, and SMAD8 (Hata and Chen, 2016). On the other hand, TGF β , nodal, and activin promote SMAD2 and SMAD3 phosphorylation (Hata and Chen, 2016). The phosphorylated R-SMADs form a heterodimer such as SMAD2-SMAD3 (SMAD2/3), which subsequently binds with a common binding partner SMAD4 (co-SMAD). The SMAD complex then moves into the nucleus and associates with transcription factors and chromatin remodeling proteins to induce transcription of target genes (Derynck and Zhang, 2003; Hata and Chen, 2016) (Fig. 2).

In addition to their well-established role in transcription, a

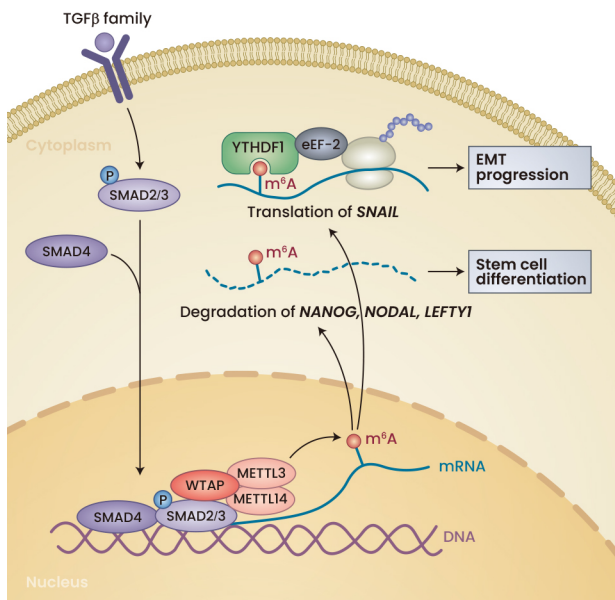


Fig. 2. TGF β controls gene expression through m⁶A modification. Upon TGF β stimulation, SMAD2/3 interact with METTL3, METTL14, and WTAP, to induce m⁶A methylation and degradation of pluripotency genes for differentiation of embryonic stem cells. On the other hand, in cancer cells, TGF β induces *SNAIL* mRNA methylation during EMT. The methylated *SNAIL* mRNA binds with YTHDF1, which induces *SNAIL* translation through interaction with a translation elongation factor eEF-2 (eukaryotic elongation factor-2).

novel function of TGF β -SMAD signaling has been revealed in m⁶A modification (Fig. 2). TGF β ligands activin and nodal maintain embryonic stem cell (ESC) stemness through SMAD2/3-dependent pluripotency gene expression (James et al., 2005). On the other hand, considering the rapid differentiation of ESCs upon TGF β withdrawal, ESCs would have also developed some mechanisms for such a rapid, efficient transition. From interactome analysis of SMAD2/3, Bertero et al. (2018) found that the phosphorylated SMAD2/3 interacts with the m⁶A writer complex, METTL3, METTL14, and WTAP. m⁶A methylation of SMAD2/3 target genes, *NANOG*, *NODAL*, and *LEFTY1*, leads to degradation of these transcripts, thereby inducing ESC differentiation. In line with their findings, *Mettl3* knockout induces prolonged *Nanog* expression and impaired differentiation in ESCs (Batista et al., 2014; Geula et al., 2015).

Interestingly, SMAD2/3 do not directly control the activity of m⁶A writer complex (Bertero et al., 2018). Instead, SMAD2/3 induce m⁶A methylation of its target genes by recruiting the m⁶A writer complex to the active transcription sites (Fig. 2). In another study, Huang et al. (2019) showed that trimethylation of histone H3 at Lys36 (H3K36me3) recruits m⁶A writer complex to the active transcription elongation sites. It may seem odd that transcription factors and elongation markers recruit m⁶A enzymes to label newly transcribed mRNAs with m⁶A for degradation. However, this priming system would be most efficient when a timely cell fate transition is required. For example, in early development of zebrafish embryos, maternal mRNAs are marked with m⁶A and degraded by the YTHDF2 reader protein during the maternal-to-zygotic transition (Zhao et al., 2017).

TGF β signaling also triggers m⁶A modification (EMT) of cancer cells (Fig. 2). TGF β treatment increases m⁶A levels in diverse cancer cells, including cervical, liver, breast, and lung cancers. Particularly, TGF β induces methylation of *SNAIL* mRNA, an important transcription factor in EMT. The methylated *SNAIL* mRNA binds to an m⁶A reader protein, YTHDF1, which then induces translation of *SNAIL* by recruiting eEF-2 (eukaryotic elongation factor-2). Interestingly, METTL3 depletion stabilized *SNAIL* mRNA, implying that m⁶A modification promotes its degradation while inducing translation (Lin et al., 2019). Further investigation is needed to examine whether these opposite effects of m⁶A on *SNAIL* mRNA fates are mediated solely by YTHDF1 or through other m⁶A reader proteins that are activated by TGF β signaling.

REGULATION OF m⁶A PROCESSING ENZYMES BY EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK)

ERK is a member of the mitogen-activated protein kinases (MAPKs) family (Roberts and Der, 2007). MAPK pathway is a phosphorylation cascade composed of GTPase-activated kinase (MAPKKK) that phosphorylates and activates an intermediate kinase (MAPKK), which in turn phosphorylates and activates the effector kinase (MAPK) (Lavoie et al., 2020). In the ERK MAPK pathway, the epidermal growth factor (EGF) binds to the receptor tyrosine kinase, EGF receptor (EGFR), to activate RAS GTPase (Boriack-Sjodin et al., 1998). Then the

GTP-loaded RAS promotes the kinase activity of RAF (MAPKKK), which is followed by MEK (MAPKK) activation (Lavoie and Therrien, 2015). Finally, MEK activates ERK (MAPK) that controls a wide range of cellular processes by phosphorylating downstream target proteins (Wee and Wang, 2017). Because of its key role in regulating cell proliferation, survival, and differentiation, ERK signaling is one of the frequently activated signaling pathways in human cancers (Davies et al., 2002; Li et al., 2018).

The activity of RNA processing enzymes such as RNA polymerase, splicing enzymes, and translation factors are often regulated by phosphorylation (Nosella and Forman-Kay, 2021; Thapar, 2015). In a recent paper, Sun et al. (2020) provided direct evidence for the phosphorylation-dependent regulation of m⁶A processing enzymes. To find a new regulator of m⁶A modification, the authors performed a CRISPR knockout screen using a GFP reporter system that contains m⁶A modification site on a circular RNA sequence. Once the GFP RNA is assembled by back splicing of the circular RNA (Yang et al., 2017), m⁶A methylation on that RNA drives translation and expression of GFP. From the screen, several genes in the MAPK signaling pathway were identified (Sun et al., 2020). Mechanistically, it turns out that the effector protein of MAPK signaling pathway, ERK, phosphorylates m⁶A writer proteins METTL3 and WTAP. The ERK-dependent phosphorylation of METTL3 and WTAP strengthened their interaction; however, ERK did not influence the binding between METTL3 and METTL14 (Sun et al., 2020). The association between METTL3 and METTL14 is strong and not affected by other phosphorylation events either, including a serine residue of METTL14 that forms a salt bridge with METTL3 (Schöller et al., 2018; Wang et al., 2016).

Even though the binding of METTL3-METTL14 was not regulated by ERK-dependent phosphorylation, ERK controlled the activity of MAC complex through METTL3 stabilization (Sun et al., 2020) (Fig. 3). While the non-phosphorylated METTL3 is degraded by ubiquitination, the phosphorylated METTL3 recruits ubiquitin-specific protease 5 (USP5) which removes ubiquitin from METTL3. From the m⁶A-GFP reporter CRISPR screen, Sun et al. (2020) also found several E3 ubiquitin ligases that decrease m⁶A levels. Knockdown of ubiquitin ligase candidates *SPOP*, *ANAPC1*, or *TRIM28* restored METTL3 expression. Depletion of *SPOP* or *ANAPC1* decreased K11 and K48 ubiquitination of METTL3, the ubiquitination sites targeted by USP5 (Sun et al., 2020). In contrast to the negative effect of *TRIM28* on m⁶A modification on the m⁶A-GFP reporter, *TRIM28* did not affect global m⁶A levels (Yue et al., 2018). Considering that *TRIM28* was identified as an interacting protein of the m⁶A writer complex, *TRIM28* may regulate m⁶A modification of specific target genes by localizing the writer complex to the target transcripts like MACOM complex proteins.

In addition to m⁶A writers, ERK-dependent regulation of the m⁶A reader protein is reported (Fig. 3). Fang et al. (2021) found that ERK phosphorylation status correlates with YTHDF2 expression level in the glioblastoma tissues. Upon EGF stimulation or EGFR overexpression, ERK phosphorylates YTHDF2 to induce stabilization of YTHDF2. The stabilized YTHDF2 degrades m⁶A-modified *liver X receptor alpha* (*LXRA*)

and *human immunodeficiency virus type 1 enhancer binding protein 2* (*HIVEP2*) genes, which elevates cholesterol uptake and proliferation of glioblastoma cells (Fang et al., 2021).

Under stress conditions, such as heat shock, m⁶A modification of mRNA is globally increased (Meyer et al., 2015; Ries et al., 2019; Zhou et al., 2015). Yu et al. (2021) found that m⁶A rapidly accumulates upon reactive oxygen species (ROS) stress, within five minutes. Such rapid response indicates that ROS may directly influence the activity of m⁶A enzymes. Surprisingly, ERK signaling plays a crucial role in this stress response by promoting sumoylation of m⁶A eraser, ALKBH5 (Fig. 3). In hematopoietic stem and progenitor cells, ROS-induced ERK and another MAPK, JNK (c-Jun N-terminal kinase), phosphorylate ALKBH5. ALKBH5 phosphorylation promotes its interaction with UBC9, a SUMO E2 conjugating enzyme, and disassociates ALKBH5 from the desumoylase SENP1. Consequently, the increased sumoylation on ALKBH5 prevents its binding with the substrate mRNAs and leads to the elevation of m⁶A levels on mRNAs (Yu et al., 2021). Among the m⁶A readers, the mRNA stabilizing reader, IGF2BP (Huang et al., 2018), plays a major role in ROS-ERK-ALKBH5-dependent gene expression regulation. In response to ROS, IGF2BP1/2/3 stabilize mRNA of *FA core complex association protein 20* (*FAAP20*) and *alpha-thalassemia/mental retardation X-linked* (*ATRX*), which are critical enzymes for DNA repair under oxidative stress (Yu et al., 2021). These seminal studies show that ERK signaling pathway insistently controls m⁶A modification process through the regulation of writers, readers, and erasers, to rewire gene expression programs in diverse physiological and pathological conditions.

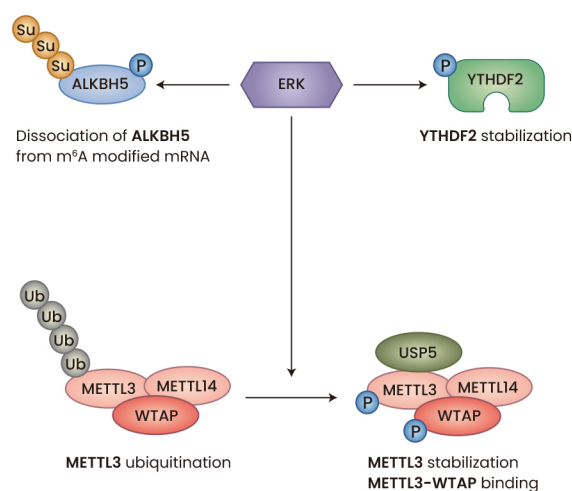


Fig. 3. Dynamic regulation of m⁶A enzymes by ERK. METTL3 phosphorylation by ERK inhibits its degradation by recruiting ubiquitin-specific protease 5 (USP5). ERK-mediated METTL3 phosphorylation also enhances the interaction between METTL3 and WTAP (bottom panel). ERK stabilizes YTHDF2 through phosphorylation (right panel). ALKBH5 phosphorylation by ERK sustains its sumoylation and induces dissociation of ALKBH5 from m⁶A-modified mRNA (left panel).

ACTIVATION OF m⁶A WRITER COMPLEX BY MECHANISTIC TARGET OF RAPAMYCIN COMPLEX 1 (mTORC1) SIGNALING

mTORC1 is a serine/threonine kinase that promotes anabolic process including synthesis of proteins, nucleic acids, and lipids (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014). In response to extracellular stimuli such as growth factors and nutrients, PI3K-Akt initiate the signaling cascade that activates mTORC1. Akt inhibits tuberous sclerosis complex (TSC) 1/2, a GTPase activating protein that inhibits mTORC1-activating small GTPase Rheb (Saxton and Sabatini, 2017; Shimobayashi and Hall, 2014). To turn on the gene expression program, mTORC1 activates RNA processes from transcription, splicing, to translation through its downstream proteins including ribosomal protein S6 kinase (S6K), SRPK (serine/arginine-rich protein specific kinase), eIF4B (eukaryotic translation initiation factor 4B), and 4E-BP (eIF4E-binding protein) (Lee et al., 2017; Ma and Blenis, 2009).

mTORC1's role in promoting m⁶A RNA modification has also been elucidated. Cho et al. (2021) and Villa et al. (2021) found that mTORC1 increases expression of the MACOM component, WTAP (Fig. 4). mTORC1/S6K enhances WTAP translation through eIF4A/4B-dependent unwinding of secondary structure in WTAP's 5'-untranslated region. The elevation of WTAP expression enhances m⁶A methyltransferase activity (Cho et al., 2021; Villa et al., 2021). In another paper, Tang et al. (2021) found that mTORC1-mediated activation of the chaperonin protein, chaperonin containing tailless complex polypeptide 1 (CCT) complex, stabilizes the MAC component proteins, METTL3 and METTL14 (Fig. 4). In addition to regulating enzyme expression, mTORC1 promotes m⁶A modification by increasing S-adenosylmethionine (SAM) level (Villa et al., 2021) (Fig. 4). SAM is a methyl donor for m⁶A, whose increase can stimulate the activity of methyltransferase enzymes (Bokar et al., 1997; Kim and Lee, 2021; Tuck, 1992). mTORC1 induces SAM synthesis through cMyc-dependent expression of MAT2A (methionine adenosyltransferase 2A) (Villa et al., 2021). SAM has been shown to induce condensation of METTL3 through liquid-liquid phase separation and promotes the association of MAC with WTAP at nuclear speckles (Han et al., 2022). Therefore, induction of SAM levels could be another way of increasing the activity of m⁶A methyltransferase complex by mTORC1. Further studies are necessary to elucidate how mTORC1-dependent expression and localization changes of MAC (METTL3 and METTL14) and MACOM (WTAP) proteins coordinate m⁶A processing in physiological and disease conditions.

Surprisingly, mTORC1-induced m⁶A modification induced degradation of mRNAs, which seems to be the opposite of canonical mTORC1 function in promoting macromolecule synthesis. However, those mRNAs methylated by mTORC1 include cell growth-suppressing genes such as cMyc suppressor and autophagy machinery (Cho et al., 2021; Tang et al., 2021). For example, mTORC1 induces methylation of *MAX dimerization protein 2 (MXD2)*, which is followed by YTHDF2/3-mediated degradation of *MXD2* mRNA (Cho et al., 2021). MXD2 is a cMyc inhibitor that competes with cMyc for binding with a transcription activator MAX (Mathsyaraja

et al., 2019; Schreiber-Agus et al., 1995). The decreased MXD2 expression results in cMyc activation, which induces the proliferation of cancer cells derived from diverse tissues including kidney, breast, lung, and colon (Cho et al., 2021). These findings demonstrate a complex interplay of mTORC1, cMyc, and m⁶A signals in tumorigenesis.

REGULATION OF CELLULAR SIGNALING BY m⁶A

In the signal transduction cascade, feedback inhibition of upstream signaling by the downstream components plays crucial role in preventing overactivation of signal transduction cascade (Mendoza et al., 2011). Such negative feedback loops are often hijacked by cancers to promote cell proliferation and survival. From a genome-wide sequencing study of endometrial cancers, Liu et al. (2018) found that *METTL14* is frequently mutated in cancers with a predominant mutation of arginine 298 to proline. The arginine 298 locates in the RNA binding domain of METTL14 and mutation of this residue decreases m⁶A methylation activity of the MAC complex (Śledź and Jinek, 2016; Wang et al., 2016). In the endometrial cancers that do not contain METTL14 loss-of-function mutations, the expression of METTL3 is decreased, indicating that decreasing the activity of MAC either by decreasing METTL3 expression or through METTL14 mutation promotes

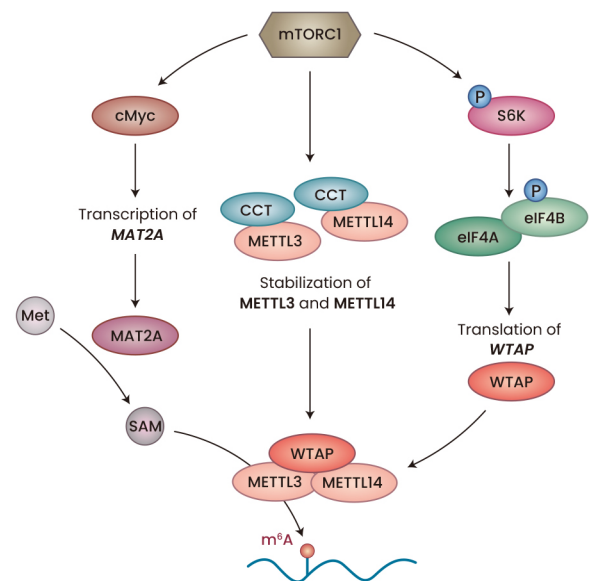


Fig. 4. mTORC1 activates the activity of m⁶A writer complex. mTORC1 activates the m⁶A writer complex in three ways. mTORC1-mediated activation of chaperonin protein, chaperonin containing tailless complex polypeptide 1 (CCT) complex, stabilize METTL3 and METTL14 (middle panel). mTORC1 induces WTAP expression through eukaryotic initiation factor 4A (eIF4A)/4B-dependent translation (right panel). mTORC1 also stimulates S-adenosylmethionine (SAM) synthesis through cMyc-mediated upregulation of MAT2A (methionine adenosyltransferase 2A) (left panel).

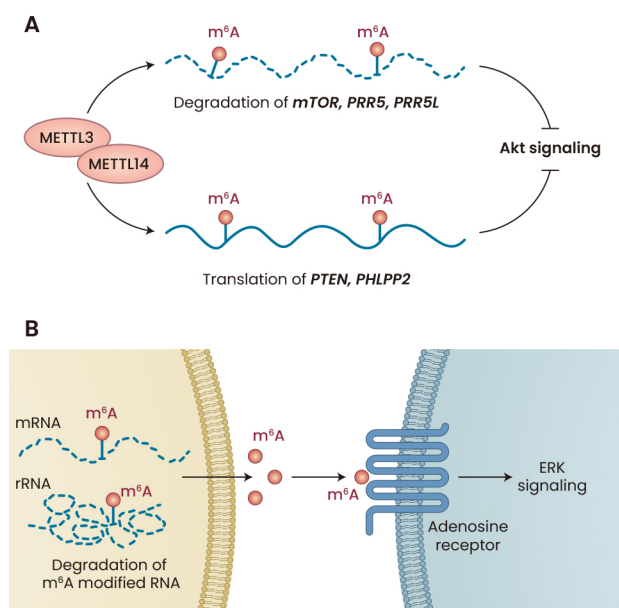


Fig. 5. Regulation of signal transduction by m⁶A. (A) m⁶A modification suppresses Akt signaling by destabilizing mRNAs of Akt activators *mTOR*, *proline rich protein 5* (*PRR5*), and *PRR5-like* (*PRR5L*) (top panel), while increasing translational efficiency of Akt suppressors *phosphatase and tensin homolog* (*PTEN*) and *PH domain leucine rich repeat protein phosphatase 2* (*PHLPP2*) (bottom panel). (B) Upon cytototoxic stress, m⁶A is generated by RNA degradation and binds to the G-protein coupled receptor, adenosine receptor, which in turn activates ERK signaling.

endometrial cancers (Liu et al., 2018). Transcriptome-wide m⁶A sequencing of endometrial tumors revealed that m⁶A modification is decreased in the group of genes that regulate Akt activity. Interestingly, the decreased m⁶A modification of Akt phosphorylation-inducing genes, such as *mTOR*, *proline rich protein 5* (*PRR5*), and *PRR5-like* (*PRR5L*), led to stabilization of those transcripts. In contrast, the decreased m⁶A modification of Akt phosphatase, *PH domain leucine rich repeat protein phosphatase 2* (*PHLPP2*), reduced translation of *PHLPP2*. Together, these changes increase Akt phosphorylation and thus activate Akt downstream signaling for proliferation of endometrial cancer cells (Liu et al., 2018) (Fig. 5A). In another study, using a reverse phase protein microarray assay (RPPA), Vu et al. (2017) found that the activity of Akt signaling pathway components is increased by *METTL3* knockdown in leukemia cells. Specifically, the decreased m⁶A modification of *phosphatase and tensin homolog* (*PTEN*) mRNA, a negative regulator of Akt, decrease *PTEN* translation; and, the decreased *PTEN* expression lead to activation of Akt signaling in *METTL3* knockdown cells (Vu et al., 2017) (Fig. 5A).

In addition to the gene expression regulation, an unexpected function of m⁶A has been uncovered as an extracellular signaling molecule. Considering the existence of G-protein coupled receptors (GPCR) that are activated by nucleotide ligands such as adenosine receptor (AR) (Borea et al., 2015),

Ogawa et al. (2021) performed a screen to identify a new nucleotide ligand for ARs. From the screen, several adenosine derivatives including 1-methyladenosine (m¹A), m⁶A, and m⁶A_m activated adenosine A3 receptor (A3R), with m⁶A being the most potent activator. In fact, m⁶A activated A3R approximately 10-fold higher than adenosine, with EC₅₀ (half maximal effective concentration) of 10 nM in contrast to that of adenosine being 100 nM. The ligand binding domain of A3R has hydrophobic amino acids including valine, leucine, and isoleucine, which could form van der Waals interactions with the methyl group on m⁶A. In contrast, adenosine would be less stable in the ligand binding pocket due to the lack of those intermolecular interactions. Indeed, when the ligand binding pocket of other ARs was mutated to contain those hydrophobic amino acids, they were also activated by m⁶A (Ogawa et al., 2021).

When m⁶A was treated onto A3R-expressing cells, the AR downstream signals such as ERK and intracellular calcium transport were induced, which was abolished by AR antagonist. Upon cytotoxic stresses such as ROS that activate AR signaling, m⁶A was produced in cells by lysosomal degradation of mRNA and rRNA to initiate the m⁶A-AR-ERK signaling pathway (Fig. 5B) (Ogawa et al., 2021). Although Ogawa et al. (2021) tested the activity of only single nucleoside m⁶A molecules, it is possible that m⁶A-containing oligonucleotides can also act as a ligand for GPCRs. The RNase T2 ribonucleases in the lysosome generate both mono- and oligo-nucleotides (Fujiwara et al., 2017) which can be secreted outside of the cells. It will be exciting to dissect the potential roles of m⁶A-containing nucleic acids as receptor-binding signaling molecules in various developmental and disease processes.

CONCLUDING REMARKS

The regulation of m⁶A modification by multiple signaling pathways demonstrates cells' abilities to dynamically determine their fate by rewiring gene expression via post-transcriptional gene modifications beyond the gene transcription level. Although research has begun to identify the effects of individual signaling pathways on m⁶A processing, there remains open questions regarding the potential for crosstalk between interwoven signaling pathways. For example, phosphorylation of *METTL3* by ERK signaling induces m⁶A methylation of pluripotency genes such as *Nanog*, *Klf2*, *Sox2*, and *Lefty1*, which results in degradation of these transcripts and mouse ESC differentiation (Sun et al., 2020). In another study, Bertero et al. (2018) observed that upon TGFβ stimulation, the transcription factors SMAD2/3 bind with *METTL3* to promote m⁶A modification and degradation of pluripotency genes including *NANOG* and *LEFTY1*. Considering that ERK-mediated *METTL3* phosphorylation strengthens its interaction with WTAP and USP5 (Sun et al., 2020), the phosphorylated *METTL3* could also interact with other proteins. Future work will be needed to elucidate whether *METTL3* phosphorylation induces its interaction with SMAD2/3 and triggers m⁶A modification of SMAD2/3 target genes, which could be the nexus between TGFβ and ERK signals for stem cell differentiation. Building a comprehensive signaling map for the m⁶A-dependent gene expression program will provide

us further insights into understanding the complex biological networks in human health and diseases.

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AUTHOR CONTRIBUTIONS

K.-H.J., C.R.H., and G.L. researched data for the review. C.R.H. made substantial contributions to the m⁶A processing enzyme section and figures. K.-H.J. made substantial contributions to the rest of the review. K.-H.J., C.R.H., and G.L. wrote, reviewed, and edited the manuscript.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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