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REVIEW

Understanding m^{6}A Function Through Uncovering the Diversity Roles of YTH Domain-Containing Proteins

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Abstract
N^6-methyladenosine (m^{6}A) is the most abundant—internal modification of eukaryotic mRNA. m^{6}A can be installed and removed by specific enzymes. The “writer,” “eraser,” and “reader” of m^{6}A modification have been reported. These discoveries facilitate our understanding of the functional significance of m^{6}A. m^{6}A plays an essential role in diverse biological processes by recruiting the corresponding YTH domain-containing proteins, as well as recruiting additional translation initiation factors. Here, we provide an update on the various aspects of YTH domain-containing proteins, including an introduction to the YTH domain, the categories, distribution in cells, and biological roles of YTH proteins. Then we focus on the mechanisms that YTH proteins recognize m^{6}A and mediate the fate of methylated-RNAs in eukaryotic cells.

Keywords YTH domain · m^{6}A binding proteins · m^{6}A · RNA modifications · Epigenetics

Introduction

N^6-methyladenosine (m^{6}A) is the most abundant and widely conserved internal modification of message RNA [1, 2], which was discovered in a large-scale of RNAs in 1970s [3–5]. However, due to the technical limitations, the function of m^{6}A has not been intensively studied. In 2011, the discovery of the m^{6}A demethylase FTO defines m^{6}A modification as a dynamic process [6]. It revives the interest of mRNA/lncRNA methylation field. In 2012, the novel m^{6}A profiling analysis, which is based on m^{6}A specific antibody-mediated capture and next-generation sequencing, advanced our understandings of the methylated transcriptome in mammals [7, 8]. These results reignited research into the mechanisms of m^{6}A-mediated functions. Subsequent studies show that m^{6}A contributes to diverse aspects of gene regulation [9], cell renewal and differentiation [10], and gene stability maintenance [11] by recruiting specific proteins [1]. The m^{6}A modification performs diverse functions through binding to specific proteins. It is feasible that m^{6}A mRNA modification executes its functions through two major methods: destabilize local RNA structure to block or induce protein–RNA interactions, or directly recruit specific proteins to mRNA therefore induce subsequent reactions. m^{6}A recruits translation initiation factor eIF3 [12] and YTH domain-containing proteins which include YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2 [13, 14]. Studies reveal that YTH domain-containing proteins combine to m^{6}A transcript and determine its subsequent preference. Here, we review the various capabilities of YTH domain-containing proteins in regulating m^{6}A in transcriptome.

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m^{6}A is a Dynamic and Reversible Modification in RNA

m^{6}A methylation was firstly reported in 1974 [15]. It tends to be regularly distributed over the coding sequences (CDS),

3′-untranslated region (3′ UTR), stop codons, and the long exon regions of the mRNA [7, 8, 13, 16]. These modified regions have a high degree of conservation in human and mice [7, 8, 13]. Early studies used a liquid chromatography technique to reveal the m^{6}A content, which m^{6}A accounted for 0.2–0.6% of the total adenosines content in the mRNA [17]. It was also found that the modified regions tended to contain "RRACH" or "DRACH" (with D = G, A, or U; R = G or A; and H = C, A, or U) consensus motif [4, 18]. As other epigenetic modifications, m^{6}A also has a dynamic and reversible mechanism, which could be established by the METTL3–METTL14–WTAP methyltransferase complex (writers of methylation) [18, 19] and removed by demethylases (erasers) including FTO [6] and ALKBH5 (Fig. 1) [20]. A multiprotein methyltransferase complex methylates m^{6}A on transcriptome [21–23]. METTL3 is firstly identified as a *S*-adenosyl-L-methionine (SAM)-binding component and processes catalytic functions of the methyltransferase complex [23]. Although METTL14 is a homologue gene of METTL3 [17], only METTL3 can bind to SAM, whereas METTL14 binds to the RNA substrate and regulates the activity of METTL3 [18, 24, 25]. Deficiency of METTL3 or METTL14 both lead to depletion of m^{6}A on mRNA [18, 26, 27]. WTAP also cooperates with METTL3–METTL14 core complex [18, 19] and facilitates complex translocation to nuclear speckles [19]. FTO and ALKBH5 are identified as demethylases and they both belong to the α-ketoglutarate-dependent dioxygenase family [6, 20, 28]. The activity of FTO and ALKBH5 demethylases is likely transcript-specific. Only certain transcripts are actively demethylated by these enzymes [29, 30]. Deficiency of FTO in HeLa and 293FT cells enhances total m^{6}A levels in transcriptome, and overexpression of FTO reduces m^{6}A levels on RNA [6]. Equally, knockdown of ALKBH5 on human cell lines turns to increased total m^{6}A levels.

In the last few years, numerous literatures showed the significant roles of m^{6}A in biological processes. It impacts the pluripotency of embryonic stem cells (ESCs) [26, 27, 31], cancer [32, 33], circadian rhythm [34–36], virus replication [37, 38], immunity [39, 40], and sex determination [41, 42]. m^{6}A modification influences the fate of mRNA in cells by affecting RNA stability, mRNA translation, and post-transcriptional processing [9]. However, the underlying mechanisms of m^{6}A functions still are a riddle. Besides, m^{6}A can destabilize local RNA structure [43] and promote proteins to binding sites adjacent to m^{6}A. Another influential effect of m^{6}A is to recruit precise binding proteins to transcriptome. These proteins recognize m^{6}A through a distinct YTH domain. Here, we review the various functions of YTH domain-containing proteins about mediating the results of m^{6}A in mRNA.

YTH Proteins Recognize m^{6}A Through YTH Domain

Initially, yeast two-hybrid screening technique has been used for exploring functions of RA301 which is an RNA-binding protein. Furthermore, researchers cloned a new RNA-binding protein, designated YT521-B, for RA301 [44]. YT521-B is the first discovered YTH protein which acts as a novel candidate for RNA splicing-related proteins [45]. Studies show that YT521-B homology is typical and abundant in the eukaryotes and plants, termed YT homology (YTH) domain. Studies reveal that YTH domains are formed with six β strands and four or five α helices [46]. These six β strands form a β barrel, with the α helices wrapped against the β strands to stabilize the hydrophobic activation core [46]. The approximately 140 amino acid domain shows remarkable conservation across the species which includes 14 invariant and 19 highly conserved residues. The aromatic residues in the β-sheets of the YTH domain are homologous with the RNA-binding RRM (RNA recognition motif) domain, indicating that the YTH domain probably serves as the m^{6}A-binding module. The RRM β-sheet domain has a crucial function on binding to RNA [47–49]. To recognize the binding mechanism of YTH domain, subsequent studies have explored the molecular basis of YTH

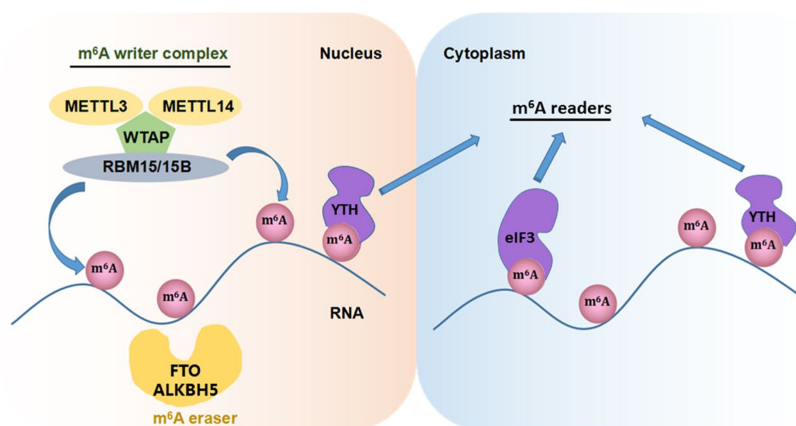

Fig. 1 The regulation process of the m^{6}A writer proteins (m^{6}A methyltransferase complex METTL3, METTL14, WTAP RBM15), eraser proteins (ALKBH5, FTO), and reader proteins (YTH domain-containing proteins). *METTL3* methyltransferase like 3, *METTL14* methyltransferase like 14, *WTAP* Wilms' tumor 1 associated protein, *ALKBH5* AlkB homolog 5 RNA demethylase, *FTO* fat mass and obesity-associated protein, *YTH* YT521-B homology

domain. The aromatic cage of YTH domain is pivotal in recognizing m^{6}A modification. The residues of tyrosine (Y260) and tryptophan (W200) in the protein sandwich the m^{6}A group. The methyl group is pointed toward tryptophan (W254) which forms the base of the cage. N^6 amino group utilizes its remaining hydrogen atom to bond the Ser201 main-chain carbonyl oxygen. The interactions between m^{6}A and aromatic cage can make a combination of m^{6}A and YTH domain-containing proteins and simultaneously prevent YTH domain from binding double methylated adenines [50, 51]. The amino acids of the cage are highly conserved in YTH proteins across diverse organisms. The affinity of YTH domain with m^{6}A is controversial. Some studies showed the affinity is in the range of 100 and 300 nM [46, 50, 52], while other studies showed an affinity of between 1 and 3 μM [51, 53]. According to the non-redundant protein–RNA-binding benchmark dataset, unlike the specific RNA-binding interactions, the non-methylated RNA generally perform a weaker binding affinity about fivefold to tenfold, which is in a low nanomolar range [54]. It suggests that although the YTH domain binds to RNA primarily through m^{6}A site, it also can bind RNA through the presence of other modifications. Is it possible for the YTH domain to interact with other methylation modifications of adenosine, for example, N^6,2′-*O*-dimethyladenosine (m^{6}A$_m$), which is also one of the prevalent modifications? m^{6}A$_m$ is a reversible modification that influences cellular mRNAs fate of stability. It sits at the 5′ end of mRNAs, the first encoded nucleotide adjacent to the 7-methylguanosine cap [55, 56]. YTHDF1, YTHDF2, and YTHDF3 could show a specific recognition of the 2′-OH with a side chain asparagine, and YTHDC1 mediates this function through a distinct asparagine. Thus, these YTH domains unlikely bind to m^{6}A$_m$ [46, 50, 53]. The structural and CLIP studies suggest that the YTH domain does not bind to other modified adenosines, such as m^{1}A, or m$^{6\prime6}$A [57]. Recent studies employ a quantitative proteomics method, identifying YTHDF1-3 and YTHDC1, besides YTHDC2, directly bind to m^{1}A in RNA [58, 59]. This is consistent with previously published data, which reveal transcriptome-wide colocalization of YTH domain-containing proteins and the m^{1}A sites in HeLa cells [59, 60]. Researchers have found that Trp432 which is conserved in the hydrophobic pocket of the YTH domain in YTHDF2 is necessary for recognition of m^{1}A [58].

Diversity of m^{6}A-binding proteins

The YTH domain exists in 174 different proteins and is conserved in the eukaryotic species. YTH domain-containing proteins have three categories: YTHDF family proteins, YTHDC1 and YTHDC2 protein. The YTHDF family proteins have more similarities in amino acids identity, whereas YTHDC1 and YTHDC2 are not homologous to YTHDF family except for the YTH domain and family name [41, 42]. YTHDF family proteins include three classic proteins which share high gene homology and amino acids composition upon their entire structure: YTHDF1, YTHDF2, and YTHDF3. The YTHDF family proteins contain a C-terminal YTH domain in the gene structure and a simple region of approximately 350 amino acids, which lacks specific modular protein domain and includes some P/Q/N-rich patches [61]. Recent studies have also examined YTHDC2 homologs across species [61]. YTHDC2 includes R3H, DEXDc, ankyrin repeats (ANK), HELICc, HA2, and OB-fold domains beside a YTH domain. YTH proteins show different distribution over cells, among which YTHDF family proteins and YTHDC2 are widely distributed in the cytoplasm, while YTHDC1 is abundant in nucleus. YTH proteins have a function in enhancing the translation efficiency and promoting mRNA degradation [13, 62–64]. YTHDC1 mainly distributes in the nucleus, and it relates RNA post-transcriptional splicing and chromosome modification [9, 57].

With the exception of YTH domain-containing proteins, eukaryotic initiation factor 3 (eIF3) is also binding to the m^{6}A [65]. Study shows that eIF3 facilitates eIF4-independent translation initiation through a m^{6}A-mediated cap-independent manner in the 5′ UTRs [65]. Besides, HNRNPA2B1, a RBP containing RRM domain, could also bind to m^{6}A-rich sites in the mRNA [66–68]. The recent study reveals that insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs; including IGF2BP1/2/3) could target mRNA transcripts through recognizing the m^{6}A site and acts as a m^{6}A-binding protein [69].

Several YTH domain-containing proteins are expressed in plants [70]. The YTH domain-containing proteins, ECT proteins, part of EVOLUTIONARILY CONSERVED C-TERMINAL REGION1-11 (ECT1-11) family proteins in *Arabidopsis*, are revealed as m^{6}A reader proteins. The mRNA-protein interactome study indicates that YTH domain of several ECT family proteins has the capacity to bind to m^{6}A site of mRNA in vivo [71]. The homology modeling strongly indicates the existence of intact m^{6}A binding sites in ECT2 and ECT3 [72]. Researchers have developed the formaldehyde cross-linking and immunoprecipitation method to recognize that the 3′ UTRs of target genes are enriching ECT2 binding sites and ECT2 leads to the recognition of a plant-specific m^{6}A motif, URUAY (R = G > A, Y = U > A, where the majority [over 90%] is UGUAY) [71]. Studies suggest that the location of ECT2 causes dual roles in modulating 3′ UTR processing in the nucleus and thereby facilitating mRNA stability in the cytoplasm sequently [71, 72].

Biological Functions of YTH Proteins

The YTH proteins, as the “reader” of m^{6}A, target to a specific m^{6}A site via a methylation-dependent manner. By recruiting diverse “readers” to bind m^{6}A sites, the YTH domain-containing proteins, no less than other RBPs, contribute to biological process in many aspects.

Overexpression of YTHDF1 could enhance the HIV-1 replication [37]. Scholars precisely describe the m^{6}A editing site map on the HIV-1 genome showing that the m^{6}A sites cluster in the HIV 3′-untranslated region (3′ UTR). YTHDF proteins bind to m^{6}A sites to enhance the HIV-1 replication [73]. YTHDF family protein-mediated inhibition of HIV-1 infection is due to the negative regulation on HIV-1 post-entry infection. YTHDF family proteins and HIV-1 Gag make a complex with RNA in cells. YTHDF1-3 blocks the viral reverse transcription by degrading the HIV-1 Gag RNA in infected cells [73]. YTHDF family proteins affect the HIV virus self-replication and reverse transcription process, thus affecting the HIV virus infection [37]. Recent studies show that YTHDF1 is associated with various tumor behaviors and cancer stages. YTHDF2 is also thought to play roles in acute myeloid leukemia [74]. Researchers utilized 3′ rapid amplification of cDNA ends method and panhandle polymerase chain reaction to identify YTHDF2 as a novel RUNX1 translocation partner gene in the three acute myeloid leukemia (AML) patients [74]. The functions of YTHDF2 in the AML disease need to be further researched. Heat shock stress is an important issue, which related to many aspects of animal health, breeding and development, and production performance. The nuclear YTHDF2 preserves 5′ UTR methylation upon heat shock stress-induced transcripts by limiting the m^{6}A demethylase FTO from demethylation [75]. The increased m^{6}A content promotes cap-independent translational initiation and provides a mechanism underlying the selective mRNA translation under the heat shock response situation [75]. In our group, we find that YTHDF1 plays important roles in adipogenesis. Our study shows that the ratio of m^{6}A/A in the layer of backfat (LB) is prominently higher in Landrace (lean models) than that in Jinhua (fat models). Patatin-like phospholipase domain containing 2 (PNPLA2) is one of the unique m^{6}A peak genes in Landrace LB. The result shows that overexpression of YTHDF1 increases PNPLA2 expression [76], further inhibiting the adipogenesis. YTHDF family proteins also affect the cell cycle. We find EGCG could increase the expression of YTHDF2 and lead to the down-regulation of protein levels of CCNA2 and CDK2. It means that YTHDF2 plays an important role in cell cycle [77]. In addition, YTHDF2 accelerates the clearance of over one-third of maternal-to-zygotic transition (MZT) related transcript during the early life of embryos [78]. Deficiency of YTHDF2 in zebrafish embryos hinders zygotic genome expression which fails to initiate timely MZT, cell cycle, and delays the development of larval life [78]. These results highlight the vital roles of m^{6}A readers in animal development.

The recent accompanying features find that several ECT family proteins are the m^{6}A readers of *Arabidopsis thaliana* and the m^{6}A binding function of ECT family proteins is required for the development of *Arabidopsis morphology* [72, 79, 80]. Studies indicate that one of the ECT family proteins, ECT2, is a m^{6}A reader protein [79]. ECT2 is highly expressed in rapidly developing tissues and has a function on trichome morphogenesis [79]. Consistent research demonstrates that ECT2, ECT3, and ECT4 are strongly expressed at leaf formation sites in the young seedlings shoot apex and the division zone of developing leave [72]. Mutation of ECT4 enhances the delayed leaf emergence and mutation of ECT2 and ECT3 causes the defection of leaf morphology [72], In addition, ECT2 is required for normal branching of trichome through controlling ploidy levels. These indicate the m^{6}A-YTH regulatory module play a vital role in the plant organogenesis.

YTHDC2 also influences the development of mammals. Study shows that YTHDC2 knockout mice have significantly smaller testes and ovaries than other littermates, which means YTHDC2 plays an important role in motivating early spermatogenesis [81]. In addition, YTHDC2 also relates to cell proliferation. The target genes of YTHDC2 are involved in meiosis-related classifications [81]. The MEIOC might interact with YTHDC2 for controlling the germline transition into meiosis [82]. In addition, YTHDC1 also acts on the herpes simplex virus (HSV), by affecting its infection process [83]. The results show that YTHDC1 responds to HSV-1 infection and the modulation depends on the expression of the viral E3 ubiquitin-ligase ICP0 [83]. Removing an arginine-rich region at the C-terminus of the YTHDC1 impacts its RNA-binding ability. Some researchers have figured out that the truncation of YTHDC1 is a potential candidate cancer driver mutation in Jurkat cells [84]. YTHDC2 is a member of the DExD/H-box family which belongs to RNA helicases. Knockdown of YTHDC2 reduces protein expression hypoxia-inducible factor-1 alpha (HIF-1α) and other metastasis genes, which inhibit metastasis of colon tumor cells in turn [64]. It suggests that YTHDC2 is a potential diagnostic marker and a target gene of colon cancer. It conduces to colon tumor metastasis by promoting translation of HIF-1α [64].

The recent study shows that YTH domain-containing protein, Mmi1, could sequestrate meiotic-related transcripts from the translation machinery in the fission yeast *Schizosaccharomyces pombe* [85]. Mmi1 tethers the specific mRNA to the nuclear foci during vegetative proliferation in order to prevent mistimed expression of meiotic proteins [85].

YTH Proteins Mediate Intracellular Biological Functions

YTH domain-containing proteins participate in extensive post-transcriptional regulation through directly binding to m^{6}A site. As we know, YTHDF family proteins are mainly distributed among cytoplasm, suggesting that its function may be related to RNA in the cytoplasm, as they play an important role in translation and degradation of RNA [14, 55, 75]. Some researchers have found that YTHDF1 could specifically recognize and bind to m^{6}A among 3′ UTR. It could recruit the 43S pre-initiation complex to begin translation processes [12, 65]. eIF3 is a multiprotein complex which could stimulate the eukaryotic translational initiation. The study reveals that YTHDF1 could promote ribosome binding on its target mRNAs and directly interact with initiation factors to promote translation, such like eIF3 [13]. YTHDF1 could be spatially close to translational initiation sites through eIF4G which looks like a bridge. During the classic YTHDF1-mediated translation initiation, eIF4G binds both poly (A)-binding protein and cap-binding protein eIF4E to form a "closed-loop" structure [13]. If eIF4G-mediated loop structure is interrupted to form, the stimulation of YTHDF1 on mRNAs translation would be weaker. These integrated results propose a mechanism of how YTHDF1 promotes m^{6}A-dependent translation of mRNA. Recently, YTHDF3 is also discovered to promote translation [37]. YTHDF3 facilitates the translation of methylated mRNA through interacting with 40S and 60S ribosome subunits by cooperating with YTHDF1 [12, 86]. But we still need a clear mechanism of YTHDF1 and YTHDF3 in translational enhancement process.

On the contrary, m^{6}A is selectively recognized by the YTHDF2 protein, which promotes mRNA degradation. The YTH domain position in C-terminal of YTHDF2 selectively binds to m^{6}A-containing mRNAs. Whereas the N-terminal domain of YTHDF2 is intended to localize the YTHDF2-mRNA complex to P-body, which is the RNA decay site in cells [14, 87]. Other researchers suggest that YTHDF2 mediates mRNA degradation by generating mRNA deadenylation subsequently translocating to P-bodies [57]. The N-terminus of YTHDF2 recruits CCR4-NOT transcription complex subunit 1 (CNOT1), a component of the CCR4-NOT deadenylation complex, promoting deadenylation of mRNA [57]. In addition, researchers find that YTHDF family proteins recruit CCR4–NOT and enhance the deadenylation of mRNA [57]. In contrast, other researches find that that YTHDF1 has effects on mRNA stability [13]. In consideration of the different functions of YTHDF1 and YTHDF2, the researchers have found that YTHDF1 binds to RNA earlier than YTHDF2 during the mRNA life cycle, suggesting that two proteins act a cooperation to mediate translation efficiency of transcripts which are requiring a transient and dynamic control [86]. In this regulating model, YTHDF3 also plays a vital role, helping regulate the mRNAs fate by manipulating YTHDF1 and YTHDF2 [86]. Knockdown of YTHDF3 reduces the RNA-binding specificity of both YTHDF1 and YTHDF2 [86].

YTHDF family proteins have a dynamic interaction function in the cytosol: when m^{6}A-contained mRNAs are exported to the cytoplasm from cell nucleus, it is immediately recognized by the YTHDF3 or YTHDF3-YTHDF1 complex in order to enhance the translation efficiency and then eventually bound by YTHDF2 to decay site. YTHDF3 influences the functions of YTHDF1 and YTHDF2 and mRNA translation efficiency or mRNA stability [86, 88]. Translation and degradation are two reverse fates of mRNA, suggesting a complicated dynamic network of the overall functions of m^{6}A on mRNAs. This machinery achieves a fast response with adequate gene expression and could keep a steady protein quantity meanwhile reduce the noise of gene expression process. Recent studies show that neither of the YTHDF family proteins performs only single function, and all the three proteins have similar properties in terms of translation and degradation of mRNAs. A model that accurately characterizes the respective functions and interaction network of the three proteins is needed.

Recent studies demonstrate the subcellular localization of YTH domain-containing protein ECT2 in *Arabidopsis*. ECT is present in both the nucleus and cytoplasm which indicates that ECT plays dual roles on pre-mRNA processing in nucleus and mRNA metabolism in cytoplasm [79]. Researchers have found that ECT2 may specifically bind to m^{6}A-containing poly(A), subsequently recruiting the polyadenylation machinery to process alternative polyadenylation and 3′ UTR processing [79]. Besides, ECT2 may facilitate m^{6}A-mediated mRNA stability in the cytoplasm [79].

YTHDC1 was firstly discovered as a splicing tool for exon inclusion [89]. It is in the nucleus and the binding site is almost entirely coincident with the m^{6}A site, indicating that it functions on nuclear mRNA processing and nuclear-localized RNAs. YTHDC1 regulates mRNA splicing through recruiting pre-mRNA splicing factors SRSF3 (SRp20) and repressing SRSF10 binding site to promote the exon inclusion [9]. The expression of SRSF3 is 30-fold higher than SRSF10 in endogenous condition, which means the exon inclusion measure by YTHDC1 and SRSF3 is probably predominant [9]. The binding ability is based on existence of m^{6}A locus [9]. However, the evidence of m^{6}A splicing regulation is unclear. The first in-depth analysis of altered splicing finds that knockdown METTL3 induced < 100 m^{6}A contained-exons has been alternatively spliced [7]. Further experiments reveal that METTL3 might be involved in the splicing of mRNAs and 0.5% of the exons has been found alternatively spliced in METTL3$^{-/-}$ mouse embryonic stem cells [7, 27, 90].

Besides, YTHDC1 also mediates the nuclear non-coding RNA X-inactive specific transcript (XIST). XIST mediates the transcriptional silencing of genes on the X chromosome by recruiting chromatin-modifying factors and coating the X chromosome [91]. XIST is highly methylated with almost 78 m^{6}A in human cells [57]. The highly enriched m^{6}A throughout XIST offers the possibility about the recruitment of the nuclear m^{6}A-binding protein YTHDC1. XIST interacts with gene-silencing proteins to achieve the silencing of X chromosome gene transcription during development of female mammalian. XIST binds to YTHDC1 therefore activates gene-silencing mechanisms [57, 91]. YTHDC1 recognizes m^{6}A residues of XIST preferentially and is probably required for XIST functions. But how YTHDC1 binds to XIST resulting in gene silencing remains indistinct. Loss of m^{6}A or YTHDC1 equally rescues XIST-mediated gene silencing [57].

In contrast to the other YTH domain-containing proteins, YTHDC2 has several undefined domains [92]. Recent studies reveal that apart from YTH domain, the ankyrin repeats of YTHDC2 regulate an RNA-independent interaction with the 5′–3′ exoribonuclease XRN1. Researchers have uncovered that YTHDC2 cooperates with the small ribosomal subunit in the close proximity sites of entrance and exitance of mRNA [92].

A major problem is whether these proteins have independent functions or their functions overlap with each other. Several studies report the debatable conclusions, and find that YTHDF1, YTHDF2, and YTHDF3 maybe in a similar category. Their similar sequence is an important reason to prove that three proteins have similar functions: YTHDF1, YTHDF2, and YTHDF3 do not contain diverse modules which might grant unique property to each protein. In the meantime, the m^{6}A-mediate functions of YTHDC2 have not been completely and clearly found either. By CLIP data, YTHDC2 does not exhibit clear binding to m^{6}A [57], thus, YTHDC2 does not show single binding mode toward m^{6}A. YTHDC2 might bind m^{6}A under unique condition or in certain cell type. Tumor necrosis factor-alpha induces YTHDC2 to regulate cell type [82, 93], and increase the translation efficiency of interaction to hypoxia-inducible factor-lα (HIF1-α) by spreading its 5′-UTR region through the YTHDC2 RNA helicase domain [64]. Whether these processes are mediated by m^{6}A is unclear.

Remark and Outlook

In the past few years, although > 100 RNA modifications have been discovered in organisms, their distinct roles need further exploration. m^{6}A mediates the extensive functions of eukaryotic RNAs. The YTH-contained proteins are relying on the YTH domain to recognize and bind m^{6}A, thereby regulate the translational efficiency or the other fates of mRNAs. From the current research, the crystal structure and the protein conformation of YTH domain have been studied clearly. Few studies focus on the functions of YTH domain-containing proteins. It is controversial that the specific function of the YTHDF proteins is unitary or not. YTH proteins regulate the fate of gene expression by the specific control methods, which still need further studies to prove. YTHDF2 has higher expression than YTHDF1 or YTHDF3, which means using YTH proteins knockdown experiment is hampered to exhibit a significant effect as the compensation of loss YTHDF2 do. It indicates that knockout all three YTHDF proteins from cells and construct one of YTHDF proteins exogenous expression at the same time may offer the ability on finding out definite function of these proteins.

Although YTHDC1 and YTHDC2 have similar names, their genetic structures are quite different. YTHDC2 functions are still not clear and need to be further explored. YTHDC1 is the only m^{6}A-binding protein localized in the nucleus which means it plays an important role in the regulation of RNA fate in the nucleus. Recent studies investigate several parts of complicated modules of YTHDC1 and reveal that these modules modulate the interaction between mRNA with YTHDC1 [92]. But the remaining modules which functions are still waiting to be explored. In addition, whether the functions of YTHDC2 are mediated by m^{6}A-dependent process is completely unknown.

While studying the function of YTH proteins, it is important to investigate whether m^{6}A has other reader-mediated mechanisms in cells and verify the function of YTH domain exerted through m^{6}A. The corresponding effect of m^{6}A can be studied by YTH proteins knockout cells.

And more remarkably, the biological functions of YTH domain-containing proteins are becoming a hotspot. In the past several years, researchers have focused on the capacity of YTH domain-containing protein on animal growth, reproduction, and disease. And recent studies explore the novel function of YTH domain-containing proteins on regulating the development of plants. This will provide the new thinking on prospective researches. In addition, researchers have revealed that m^{6}A modification influences the reprogram of Naïve T cells for proliferation and differentiation [39]. Recent studies indicate the potential relationship between m^{6}A with systemic lupus erythematosus [40]. These studies demonstrate that m^{6}A modification acts as a critical regulator of immune cell homeostasis through mediating the gene expression of immune-related signaling pathway. However, whether YTH domain-containing proteins participate in the immune processes is still unknown. These findings suggest that YTH domain-containing proteins might act as regulators of immune-related signaling pathway through a m^{6}A-dependent manner.

YTHDF family proteins are regulated diversely by particular signals need to be further explored.

Phosphoproteomic experiments show existing mass phosphorylation sites among the P/Q/N-rich regions and adjacent to the YTH domain of the YTHDF family proteins [94]. Phosphorylation could add phosphate (PO_4) to a non-polar R group of amino acid residue and change hydrophobic portion into a polar hydrophilic portion in order to alter the structure of the protein which could prevent proteins from granules [95]. Besides phosphorylation, YTHDF proteins have found to be myristoylated [96]. It suggests that post-translational modifications are likely to regulate YTHDF family proteins clustering. At present, there are no research findings that signaling pathways could modulate m^6A. Therefore, whether YTH domain is regulated by some signaling pathways would advance our learning of m^6A-mediated regulation and processes.

Numerous studies show that m^6A plays important roles in various diseases including viral infections and cancers [37, 56, 63, 73, 97–102]. It attracts attention to the study of m^6A readers [64, 83, 84]. Future study can focus on screening out the target genes of YTH proteins, to explore which are related to YTH proteins. This provides an advance for further studies of the biological functions of YTH proteins.

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