

Review

Reading m⁶A in the Transcriptome:
m⁶A-Binding ProteinsDeepak P. Patil,^{1,2} Brian F. Pickering,^{1,2} and Samie R. Jaffrey^{1,*}

N⁶-Methyladenosine (m⁶A) is the most prevalent post-transcriptional modification of eukaryotic mRNA and long noncoding RNA. m⁶A mediates its effects primarily by recruiting proteins, including the multiprotein eukaryotic initiation factor 3 complex and a set of proteins that contain the YTH domain. Here we describe the mechanisms by which YTH domain-containing proteins bind m⁶A and influence the fate of m⁶A-containing RNA in mammalian cells. We discuss the diverse, and occasionally contradictory, functions ascribed to these proteins and the emerging concepts that are influencing our understanding of these proteins and their effects on the epitranscriptome.

m⁶A and the Epitranscriptomic Code

N⁶-Methyladenosine (m⁶A) is the most prevalent post-transcriptional modification in eukaryotic RNAs (Box 1). It was first discovered and proposed to be a potential regulator of mRNA processing in cells in the 1970s [1,2]. Subsequent studies in yeast showed that m⁶A levels are dynamic, for example, induced upon yeast sporulation, and deletion of the m⁶A-synthesizing enzyme inhibited sporulation in yeast and arrested seed development [3,4]. Thus, m⁶A was observed to be a dynamic modification with important roles in physiologic processes. However, m⁶A could only be measured in poly(A) RNA preparations, and not individual transcripts, which prevented an understanding of whether m⁶A was selectively located on specific mRNAs or if it was a nonspecific mark in the transcriptome. The development of epitranscriptomic mapping methods for m⁶A [5,6] provided the critical technologies needed to specifically link m⁶A to specific mRNAs and to address the hypotheses that m⁶A is a regulator of mRNA biology that was initially put forward more than 40 years ago.

m⁶A has many functions in cells, with cell differentiation showing the clearest dependence on m⁶A [7–9]. m⁶A affects cancer progression [10,11], circadian rhythm [12], neuronal function and sex determination in *Drosophila* [13–15], and X-chromosome inactivation (XCI) [16]. m⁶A mediates its physiologic effects by influencing the fate of mRNA in cells. One function of m⁶A is to destabilize local RNA structure [17] and thereby increase the accessibility of proteins to binding sites adjacent to m⁶A. A more influential effect of m⁶A is to recruit specific proteins to mRNA. m⁶A recruits the translation initiation factor eukaryotic initiation factor 3 (eIF3) [18] and proteins containing a YTH (YT521-B homology) domain [6,19]. m⁶A-dependent recruitment of eIF3 enables mRNAs to be translated in the absence of eIF4E [18], the canonical cap-binding protein. This interaction is limited to mRNAs with m⁶A in the 5'-untranslated region (5'-UTR), and may be particularly important in stress or disease states in which eIF4E is inhibited [20]. It remains unknown exactly how eIF3 recognizes m⁶A and which protein domains are necessary for this interaction to occur. In contrast to eIF3, YTH proteins recognize m⁶A through a specific well-characterized YTH domain.

Trends

m⁶A is the most abundant mRNA modification. The YTH domain specifically recognizes m⁶A and is conserved from yeast to humans.

The first function assigned to a YTH protein was destabilization of mRNAs by YTHDF2. Since then YTH proteins have been linked to both mRNA degradation and protein translation (YTHDF1 and YTHDF3), and regulate splicing and chromatin modification (YTHDC1).

m⁶A has been mapped in multiple viruses and binding of YTH proteins was shown to either help or inhibit viral replication. Nuclear replicating viruses are helped by YTH proteins, while cytoplasmic replicating viruses are inhibited.

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Box 1. m⁶A Writers, Readers, and Erasers

Adenosines in cellular RNAs can be chemically modified by the addition of a methyl group at the N6 position of the adenine base generating m⁶A. The m⁶A writer complex is a multiprotein complex composed of METTL3, METTL14, WTAP, and RBM15/RBM15B [16,63,82,83]. METTL3 is the catalytic subunit that transfers a methyl group from S-adenosylmethionine (SAM) to an adenosine in RNA [84,85]. m⁶A is found in a DRACH consensus sequence, where D = A/G/U, R = A/G, and H = A/C/U. m⁶A can be removed from select mRNAs by ALKBH5, which is found predominantly in the nucleus and is the only known m⁶A 'eraser' [86]. Multiple m⁶A reader proteins exist in both the nucleus and cytoplasm. Of the known m⁶A reader proteins, most contain a YTH domain that specifically recognizes m⁶A versus A. The m⁶A reader YTHDC1 is predominantly found in the nucleus, while YTHDF1, YTHDF2, YTHDF3, and YTHDC2 are primarily cytoplasmic. In addition to YTH proteins, eukaryotic initiation factor 3 (eIF3) serves as a reader of m⁶A and promotes cap-independent translation upon induction of cellular stress [18] (Figure 1).

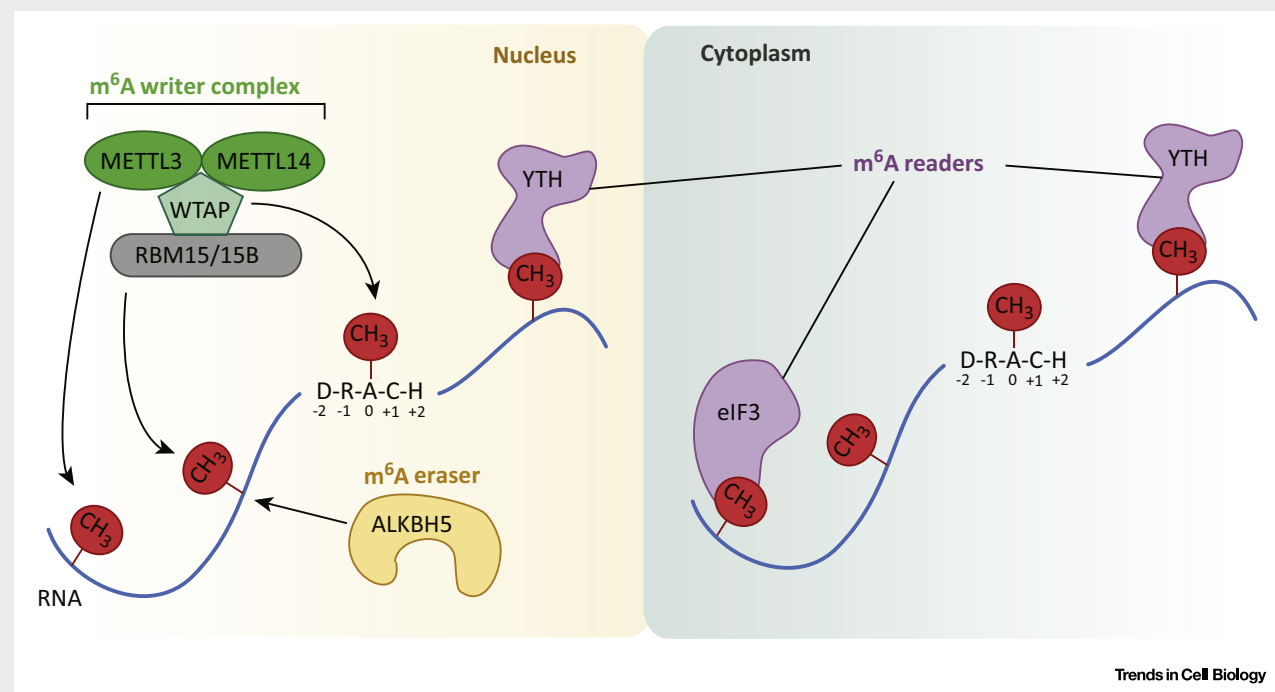


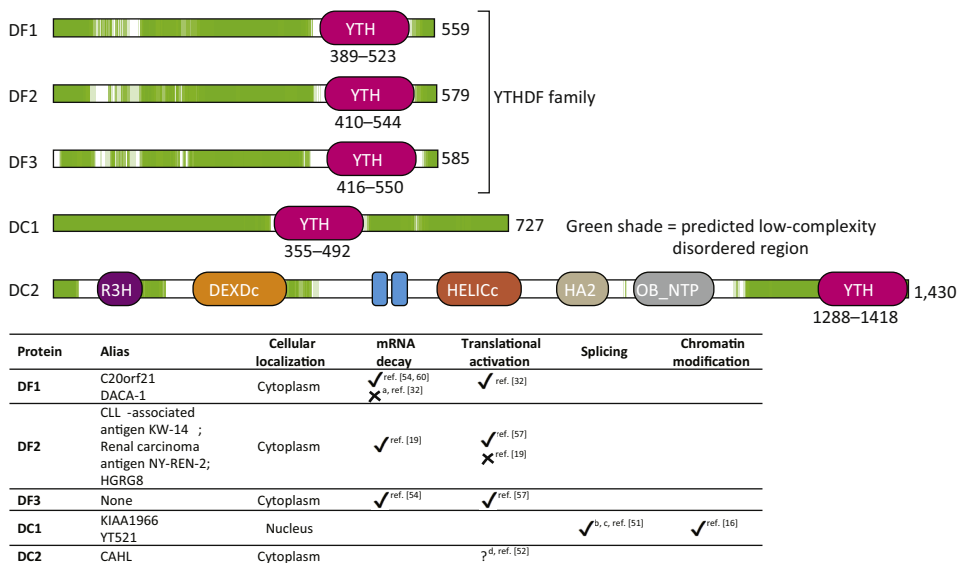
Figure 1. Components of the m⁶A Writer, Eraser, and Reader Complexes that Regulate mRNA.

Here we review the diverse functions of YTH domain-containing proteins in mediating the effects of m⁶A in mRNA.

Diversity of YTH Domain-Containing Proteins

The first YTH protein was discovered in a yeast two-hybrid screen for binding partners of transformer-2 protein homolog (TRA-2 β), a spliceosomal complex component [21,22]. The recovered protein was designated YT521-B. The YTH domain was discovered in a Basic Local Alignment Search Tool (BLAST) analysis that revealed an approximately 140 amino acid domain that was especially highly conserved among YT521-B homologs [23]. BLAST searches using just this domain revealed YT521-B homologs as well as other proteins that contained this domain. Proteins containing this YT521-B homology or YTH domain were observed across various organisms, including plants and yeast. The YTH domain was predicted to be a novel RNA-binding domain based on homology with the RNA-binding RRM (RNA recognition motif) domain [23].

Vertebrate YTH domain-containing proteins can be classified into three categories: YTHDF (YTH domain-containing family protein) family, YTHDC1 (YTH domain-containing protein 1, also called DC1), and YTHDC2 (YTH domain-containing protein 2, also called DC2) (Figure 1). Aside



^aRef. 32 reports DF1 may not be directly involved in RNA decay.

^bRef. 51 suggests m⁶A-dependent splicing events are rare.

^cRef. 13-15 report the role of *Drosophila* homolog of DC1 in splicing.

^dFound to promote translation of HIF-1 α but unclear if m⁶A-dependent.

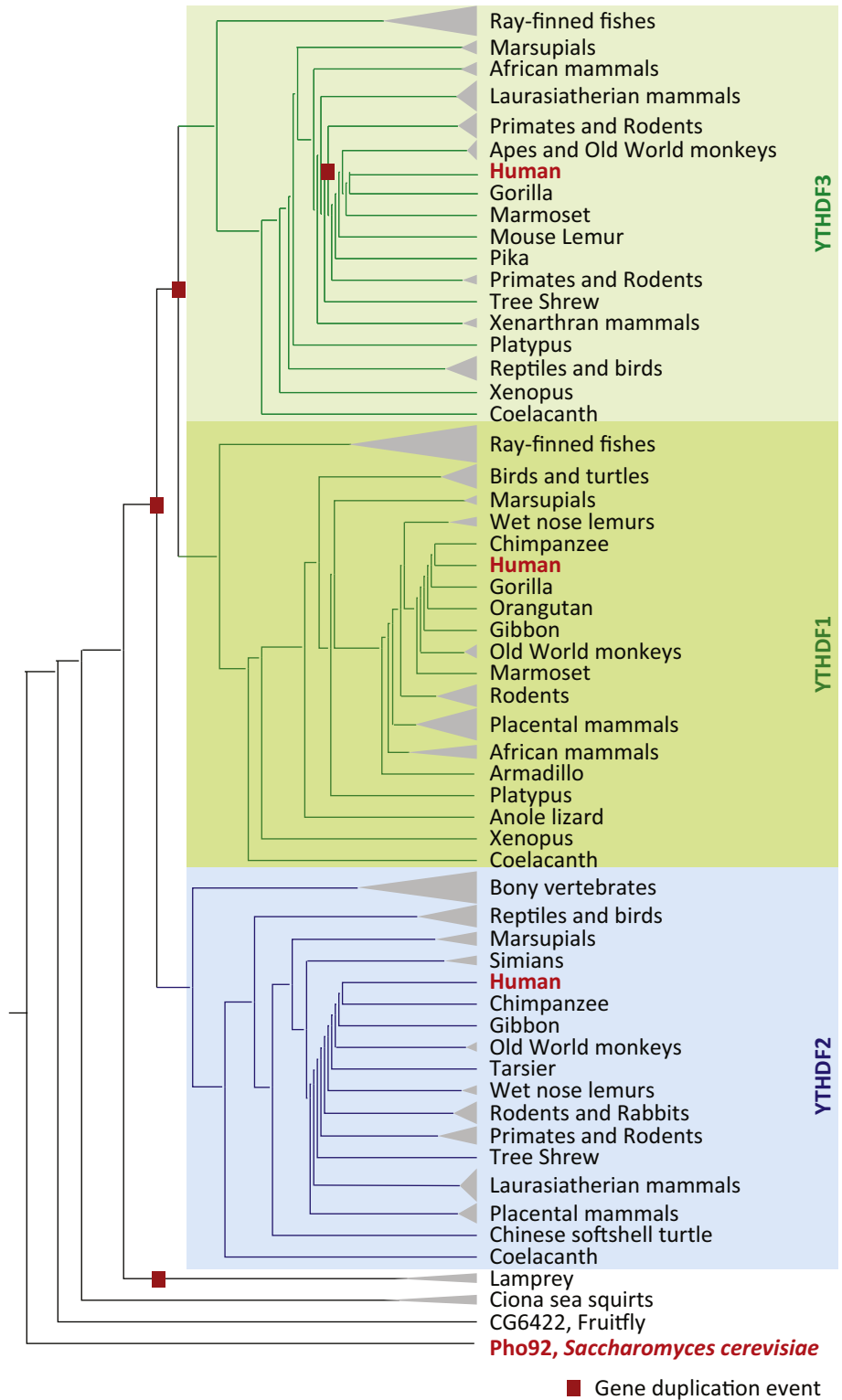
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Figure 1. Domain Structure and Functions of Human YTH Proteins. Schematic representation of domains and disordered regions of human YTH proteins: DF1, DF2, DF3, DC1, and DC2. DF1, DF2, and DF3 together make the YTHDF (DF) family. DC2 and the DF family proteins have a C-terminal YTH domain (pink), while DC1 has an internal YTH domain. All the proteins have low-complexity disordered regions (green). Disordered regions were identified using D2P2 database tools [80]. Additional domains in DC2 include R3H, DEXDc, ankyrin repeats (ANK), HELICc, HA2, and OB-fold domains. Protein length (in amino acids) is indicated next to each protein schematic. Functions of the human YTH proteins along with their alias names and major cellular localization are also indicated in a table (bottom panel). See also [13–16,19,36,51,52,62,69,70,72]. Abbreviations: HIF-1 α , hypoxia-inducible factor-1 α .

from their conserved YTH domain, DC1 and DC2 are unrelated to other YTH domain-containing proteins based on amino acid sequence, size, and overall domain organization. Therefore, DC1 and DC2 are not paralogs and do not comprise a family despite their name. By contrast, the YTHDF family comprises three paralogs that share high amino acid identity over their entire length: YTHDF1, YTHDF2, and YTHDF3 (also called DF1, DF2, and DF3, respectively; Figure 2). DF proteins possess a C-terminal YTH domain and a low-complexity region of approximately 350 amino acids, which lacks a recognizable modular protein domain and contains several P/Q/N-rich patches (Figure 1).

Compared to vertebrates, *Drosophila* has one homolog of the DF family and one homolog of DC1. *Caenorhabditis elegans* completely lacks YTH proteins, while *Arabidopsis* has 12 genes that encode YTH domain-containing proteins. Outside the YTH domains, these *Arabidopsis* proteins lack homology with human YTH proteins. Because of differences in the types of proteins that contain YTH domains in these organisms, the fate of m⁶A-containing mRNA may be different in plants compared to mammals.

Recent studies have also examined DC2 homologs across species [24]. Although DC2 orthologs are readily detected in multicellular organisms, several organisms have DC2 orthologs in which the YTH domain was lost. In mammals, DC2 proteins contain a YTH domain except for the platypus DC2 ortholog. Similarly, some birds, amphibians, and reptiles do not contain a YTH domain in their DC2 homologs [24]. *Drosophila* and nematodes also lack a YTH



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domain in their DC2 orthologs. Taken together, these data suggest that DC2 evolved to function in a YTH-independent manner in some organisms.

YTH Domains: Some Bind m⁶A, Some Do Not

Initially, *in vitro* assays [6] and subsequent binding experiments using recombinantly expressed YTH domain demonstrated that the YTH domain binds RNA in an m⁶A-dependent manner, albeit with low affinity [25–27]. YTH domains have been reported to bind to m⁶A with affinities between 100 and 300 nM [25,28–30]; however, some studies have also reported affinities between 1 and 3 μM [26,27]. Nonmethylated RNAs typically show about fivefold to tenfold weaker binding. Unlike most specific RNA-binding interactions, which have affinities in the low nanomolar range [31], the weak binding affinity suggests that the YTH domain does not form a stable complex with an mRNA target on its own but requires additional interactions by their low-complexity region to bind.

m⁶A Recognition by the YTH Domain

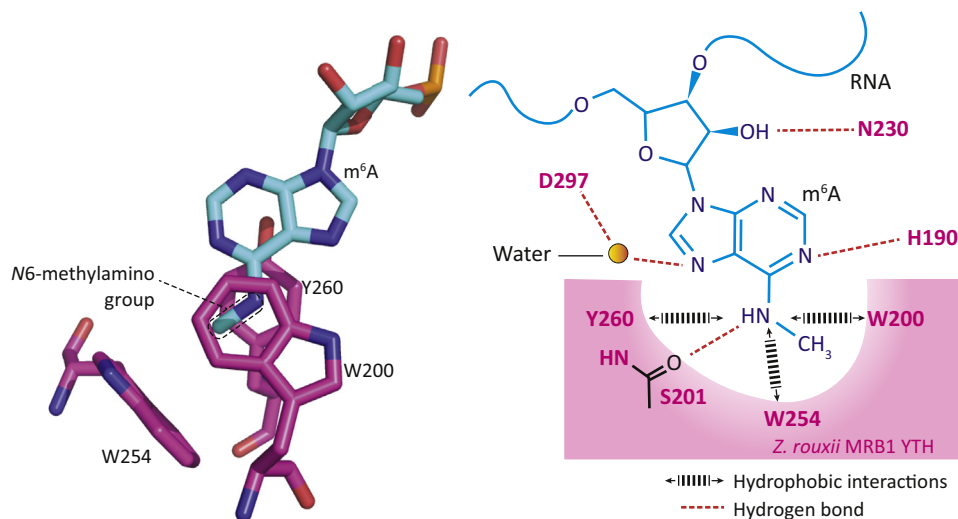
YTH domains in DF proteins and DC1 employ a conserved mechanism for recognizing m⁶A. The structure of a YTH domain bound to m⁶A was described in three independent reports [25,28,29]. One of the reported structures was of methylated RNA-binding protein 1 (MRB1), a *Zygosaccharomyces rouxii* YTH domain-containing protein [25]. Two other studies reported the structure of human DC1 in complex with an m⁶A-containing RNA [28,29]. Structures of DC1 [28,30], DF2 [26,27], and DF1 [30] have since been reported.

In MRB1, the methyl moiety of m⁶A is recognized by an aromatic cage formed from a tyrosine and two tryptophans (Figure 3) [25]. The adenine base does not form stacking interactions with any of the aromatic amino acids forming the cage. The DF1 and DF2 crystal structures show an aromatic cage comprising three tryptophans similarly recognizing the N6-methyl group (Figure 3). The cage-forming amino acids are highly conserved in YTH domains across various organisms (Figure 4). Methyl recognition by the YTH domain is different than methyl recognition by methyl-lysine-binding proteins, such as tudor and chromodomains [32,33]. Methyl-lysine recognition involves the methylammonium (i.e., positively charged methyl-lysine side chain) forming cation–pi interactions with an aromatic cage [34]. However, m⁶A is not cationic. Thus, m⁶A is unlikely to have the same binding strength as methyl lysine to its cognate binding protein. Indeed, heterochromatin protein 1 (HP1) chromo domains show an approximately tenfold increased binding selectivity toward the methylated lysine [35], while m⁶A binds with between fivefold and tenfold selectivity over the nonmethylated base.

The crystal structures show that the YTH domain does not have selective interactions with bases besides the 6-methyladenine. Instead, the YTH domain has selective recognition of phosphate and sugars in each YTH domain at the –2, –1, +1, and +2 positions (Box 1 and Figure 4) [25,28].

Transcriptome-wide binding studies of overexpressed [19,36] or endogenous YTH proteins [16] using cross-linking and immunoprecipitation (CLIP) methods demonstrated that most mammalian YTH proteins bind to the m⁶A motif in RNA. Indeed, analysis of the binding

Figure 2. Human YTHDF Proteins Are Paralogs of Each Other. Shown is a species-aware phylogenetic gene tree of YTHDF family genes across the indicated organisms or group of organisms. This tree was constructed using the Ensembl Gene Orthology and Paralogy prediction pipeline [81]. Gene duplication events (red squares) and YTHDF1, YTHDF2, and YTHDF3 gene clades are also indicated.



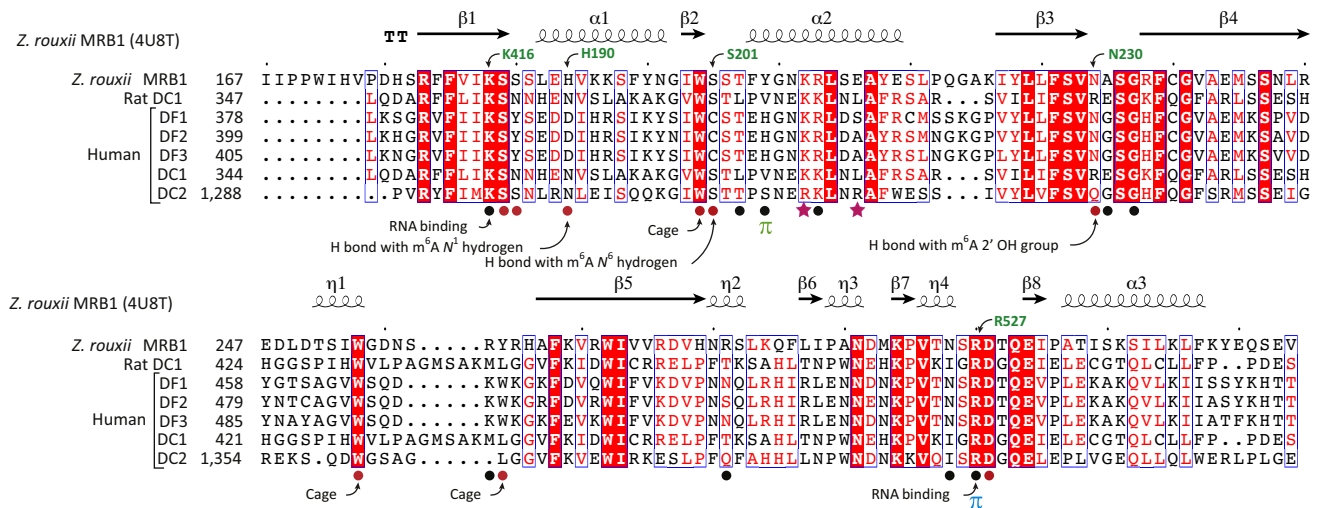
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Figure 3. A Hydrophobic Aromatic Cage in the YTH Domain Recognizes m⁶A. Shown here is the organization of m⁶A (blue) in the WWY-type aromatic cage (pink) of *Zygosaccharomyces rouxii* MRB1 YTH domain. Tyrosine (Y260) and tryptophan (W200) residues in the protein sandwich the 6-methyladenine group. This positions the methyl group pointing to another tryptophan (W254), which forms the base of the cage. Notably, the purine ring of the m⁶A residue is not involved in this hydrophobic interaction. The amino acid positions in the domain are highly conserved across species. The YTHDF family of proteins has a WWW cage, and YTHDC1 and YTHDC2 have a WWL-type cage. Abbreviations: m⁶A, N⁶-methyladenosine; MRB1, methylated RNA-binding protein 1.

properties of endogenous DF proteins showed nearly identical and complete overlap in their binding sites throughout the transcriptome. The vast majority of DF-binding sites are located in the consensus site for m⁶A (Box 1) [37], and the distribution of DF protein-binding sites in the transcriptome – referred to as a metagene – precisely mimics the distribution of m⁶A on mRNA [16]. Overall, this supports the idea that no m⁶A site is uniquely bound by one DF protein compared to others. By contrast, an earlier study argued that DF1 and DF2 bind to distinct sites, with an overlap of only approximately 60% of their mapped sites [36]. This was unexpected since the DF proteins are nearly identical and show identical binding specificity toward m⁶A RNA [30]. The likely explanation for the discrepancy is how the RNA-binding sites were identified from the CLIP data. The earlier studies used a bioinformatics approach in which sites were called based on whether the CLIP data showed signals above a specific threshold [36]. Subthreshold sites were not called. This approach results in stochastic calling efficiencies for sites that are near the threshold. However, the more recent study focused on m⁶A sites rather than on DF sites [16]. At each m⁶A site, read densities for each DF protein were compared, revealing that each m⁶A in the transcriptome has a highly similar ratio of DF1, DF2, and DF3 reads [16]. Thus, based on this analysis, it is clear that no m⁶A site is uniquely bound by one of the DF proteins compared to the others.

Unlike the DF proteins, DC1-binding sites show nearly complete overlap with m⁶A sites in nuclear RNAs, such as X-inactive specific transcript (*XIST*) and metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*). Thus, DC1 appears to be the nuclear reader, while the DF proteins bind predominantly to m⁶A in cytoplasmic mRNAs.

Individual-nucleotide-resolution CLIP (iCLIP) analysis of DF1, DF2, DF3, and DC1 shows binding to the 10 putative m⁶A residues in a C-m⁶A-G motif formed by methyltransferase like



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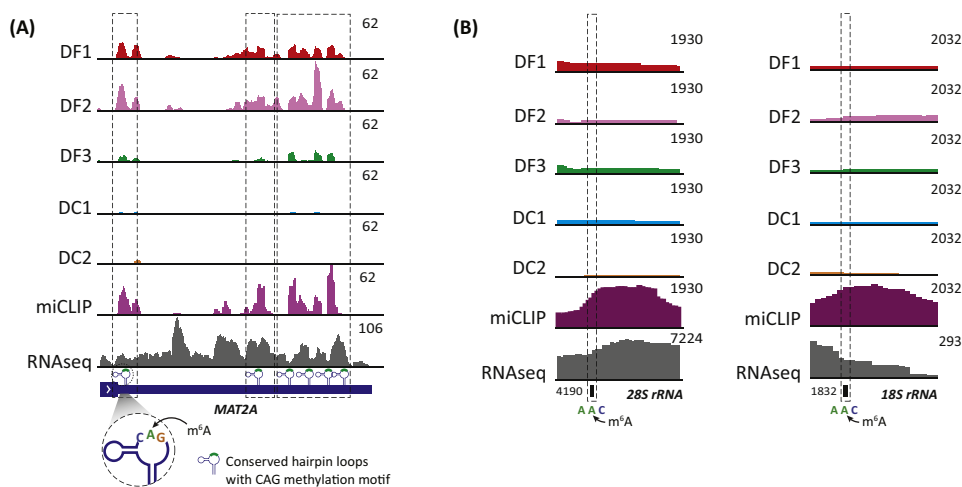
Figure 4. YTH Domains Share a Conserved Mode of Binding m⁶A RNA. Amino acid sequence alignment of the YTH domains from the indicated organisms is shown here. Secondary structural elements in the YTH domain from *Zygosaccharomyces rouxii* MRB1 (PDB: 4U8T) are shown above the sequence alignment. Amino acid positions in individual protein are also indicated next to the protein names. Amino acids participating in recognition of different parts of m⁶A RNA are indicated with filled shapes below the sequence alignment. Above the alignments: TT, strict β-turns; arrows, β-strands; coils, α-helices. In the alignments: red shade with white characters, identical amino acids; red characters, similar amino acids; blue frame, marks block of sequence with similarity. Below the alignments: closed red circles, residues making contact with bases other than m⁶A in *Z. rouxii* MRB1; closed black circles, residues making contact with the m⁶A base in *Z. rouxii* MRB1; green π, π-stacking Y205 in *Z. rouxii* MRB1 with G upstream of the m⁶A nucleotide; not conserved in human DC2; blue π, π-stacking R296 in *Z. rouxii* MRB1 with the C on the 3' side of the m⁶A nucleotide; closed stars, R1318 and R1322 in human DC2 form an increased positive surface charge than in human DC1. Amino acids involved in cage formation, RNA binding, and hydrogen bond (H bond) formation are also indicated. Abbreviations: m⁶A, N⁶-methyladenosine; MRB1, methylated RNA-binding protein 1.

16 (METTL16) in the *MAT2A* mRNA [38] (Figure 5A). Notably, previous studies of the binding affinity of the YTH domain to m⁶A-containing RNAs did not include RNAs in the C-m⁶A-G motif. However, examination of an m⁶A-containing RNA in which the m⁶A was present in a C-m⁶A-C motif [29,30] revealed that the presence of C preceding the m⁶A did not reduce binding affinity. Because of this, the YTH domain likely recognizes m⁶A generated by both methyltransferase like 3 (METTL3) and METTL16, despite different sequence contexts of these m⁶A residues. In addition, DC2 does not show clear binding to m⁶A based on CLIP studies [16]. Most m⁶A sites in mRNA lack a corresponding DC2 iCLIP signal [16]. Thus, DC2 does not exhibit simple binding toward m⁶A. It is possible that DC2 binds m⁶A under unique circumstances or in certain cell types.

Do the YTH proteins bind m⁶A in 18S rRNA and 28S rRNA? These m⁶A are in an A-m⁶A-C sequence [39]. Nevertheless, there is an absence of YTH iCLIP reads at m⁶A in these RNAs, indicating that these residues do not bind YTH proteins (Figure 5B). The recently determined structure of human ribosomes show that each m⁶A residue in both 18S and 28S rRNA is buried within the subunit [40]. Thus, the lack of YTH binding may reflect the inaccessibility of these m⁶A residues.

Binding to Other Methylated Forms of Adenosine?

A highly prevalent modified form of adenosine is N⁶,2'-O-dimethyladenosine (m⁶A_m) [41]. m⁶A_m is found in 30–40% of all transcripts in vertebrate mRNA, adjacent to the m⁷G cap at the first encoded position of mRNAs [41]. Notably, m⁶A_m markedly enhances mRNA stability and can be demethylated to 2'-O-methyladenosine (A_m) by RNA demethylase fat mass and obesity-



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Figure 5. Recognition of m^6A in Human *MAT2A* mRNA and lack of recognition in rRNAs. (A) YTH domain proteins recognize m^6A in CAG motif. 3'-UTR of *MAT2A* mRNA contains m^6A in an evolutionarily conserved CAG motif in U6-like hairpin loops. Shown here is a gene model of *MAT2A* 3'-UTR (dark blue), m^6A miCLIP tag profile (purple track), and iCLIP tag-based binding profiles of human DF1, DF2, DF3, DC1, and DC2 proteins. Broken boxes mark the overlapping DF1, DF2, DF3, and DC1 peaks and the methylated regions on *MAT2A* 3'-UTR. The green shaded portion on the hairpin loops marks the N^6 -methyladenosine containing CAG motif (zoomed-in view). (B) YTH proteins do not bind m^6A on rRNAs. The highly prevalent m^6A -containing 28S and 18S rRNAs each have one m^6A modification in a AAC sequence motif. Shown is the location of m^6A in 28S rRNA (left) and 18S rRNA (right), along with m^6A peaks (miCLIP, purple), and the iCLIP binding profile of endogenous human YTH proteins. The absence of a peak in the YTH iCLIP reads shows that none of the five YTH proteins bind at the m^6A site on either 28S or 18S rRNA. Abbreviations: iCLIP, Individual-nucleotide-resolution cross-linking and immunoprecipitation; m^6A , N^6 -methyladenosine; miCLIP, m^6A individual-nucleotide-resolution cross-linking and immunoprecipitation; UTR, untranslated region.

related protein (FTO) [42]. FTO was initially thought to demethylate m^6A [43], but recent studies show that FTO has essentially no activity toward m^6A *in vivo* [42].

Could YTH domain proteins bind m^6A_m ? DF1, DF2, and DF3 show specific recognition of the 2'-OH with a side chain asparagine (N230 in MRB1) through a hydrogen bond (Figure 4) [25,27,30], and in DC1 a distinct asparagine (N363 in human DC1) mediates this function. Thus, these YTH domains are unlikely to bind to m^6A_m .

Besides m^6A and m^6A_m , adenosine can be methylated to N^1 -methyladenosine (m^1A) and N^6 , N^6 -dimethyladenosine ($m^{6,6}A$). A global mapping study of m^1A proposed that mammalian mRNAs contain at least 7000 m^1A residues [44,45], but more recent analysis shows that just seven cellular mRNAs contain m^1A [46]. Nevertheless, the YTH domain is unlikely to mediate recognition of m^1A because methylation of the N1 atom of the adenine ring would block hydrogen-bonding interactions that depend on the nonmethylated nitrogen. The YTH domain proteins also do not bind $m^{6,6}A$, a modification in the 18S rRNA, based on CLIP data [16]. YTH proteins form interactions with the N^6 hydrogen on m^6A , which is not present in $m^{6,6}A$ (Figure 3). Taken together, the structural and CLIP studies suggest that the YTH domain does not bind other modified adenosines, such as m^6A_m , m^1A , or $m^{6,6}A$.

A YTH Domain That Does Not Bind m^6A

The YTH domain in the *Schizosaccharomyces pombe* protein Mmi1 does not show m^6A -enhanced binding despite possessing an aromatic cage. The YTH domain binds a

Key Figure

Multiple Functions of YTH Proteins

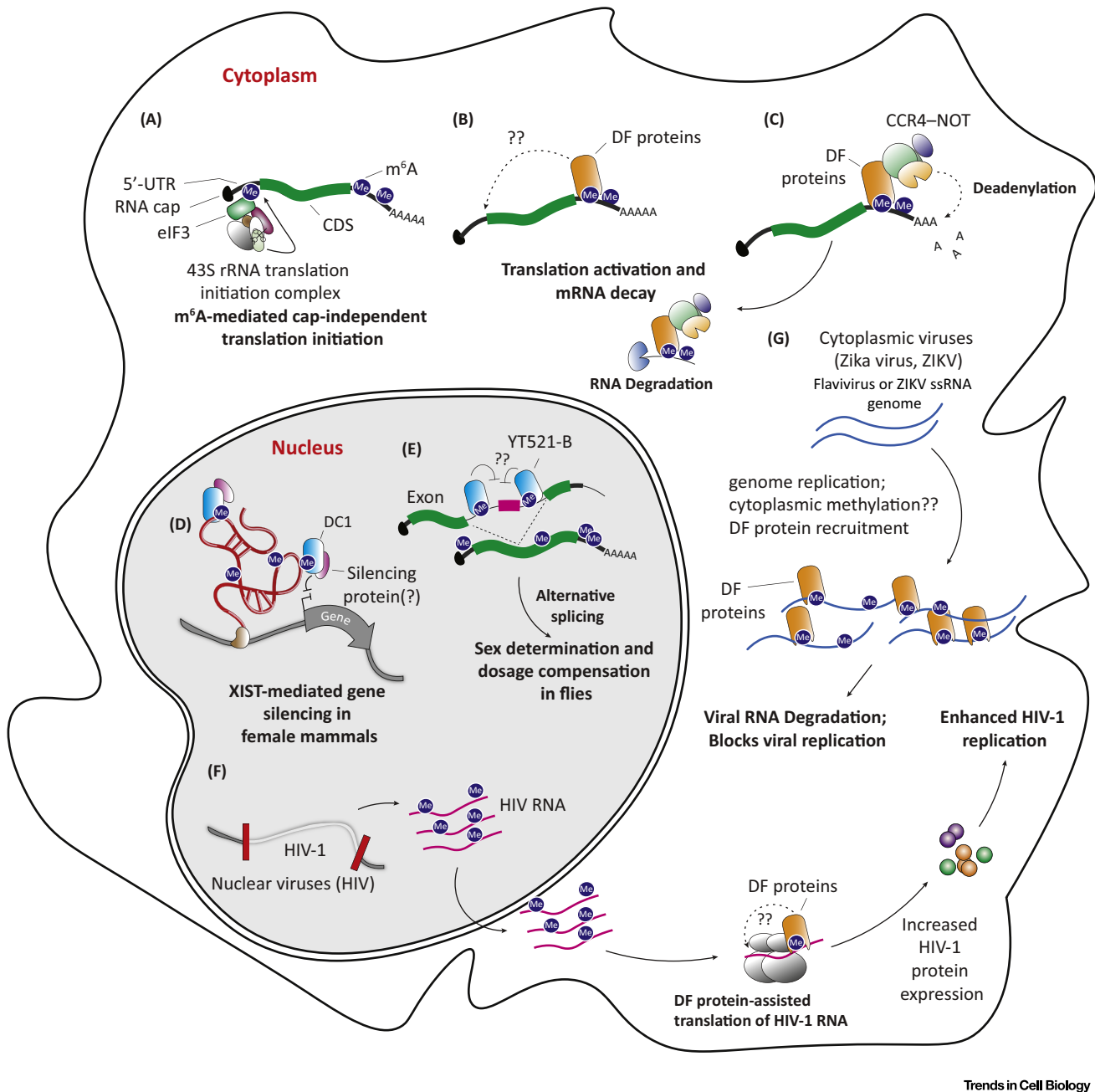


Figure 6. (A) Cap-independent translation mediated by m⁶A and eIF3. A subset of transcripts contains m⁶As in their 5'-UTR that can be recognized by eIF3. Binding of eIF3 recruits the 43S translation initiation complex leading to cap-independent translation. (B) YTHDF proteins regulate translation of transcripts by binding to m⁶A in the 3'-UTR and recruiting eIF3. It is proposed that the mRNA circularizes to bring eIF3 in proximity to the cap to initiate translation. (C) YTHDF proteins promote mRNA decay. The C-terminal YTH domain binds m⁶A on transcripts and the N-terminal disordered region recruits CNOT1, a component of the CCR4-NOT complex. Following

(Figure legend continued on the bottom of the next page.)

nonmethylated RNA motif (UUAAAC), also known as the determinant of selective removal (DSR) motif [47]. However, DSR sequences with m⁶A do not show enhanced binding, and in some cases, display worse binding compared to the nonmethylated RNA. Structural studies of Mmi1 bound to the DSR RNA revealed that the RNA binds a different surface than the canonical RNA-binding surface seen in the other YTH proteins [47]. Thus, the aromatic cage is completely unoccupied when the DSR RNA is bound to the Mmi1 YTH domain. Thus, this YTH domain appears to bind nonmethylated RNAs despite the presence of an aromatic cage.

DF Family: Redundant or Different Functions?

DF proteins are enriched in the cytoplasm [19,36,48–50], suggesting that they affect the fates of mRNA in the cytoplasm, such as mRNA stability and translation (Figure 6, Key Figure). A major question is whether YTHDF proteins have different or redundant functions. Early studies of DF1 and DF2 showed that these proteins have highly distinct functions in promoting translation and mRNA instability, respectively [19,36]. However, other studies have come to the opposite conclusion, and found that DF1, DF2, and DF3 function in a similar manner [51,52]. The finding that these proteins have similar functions is consistent based on their sequence: DF1, DF2, and DF3 do not contain different modules that might confer unique properties to each protein. In addition, lower organisms such as *Drosophila* express a single DF-like YTH protein, CG6422, rather than separate homologs for DF1, DF2, and DF3 [13], consistent with the idea that there is a single conserved function for these proteins.

mRNA Stability

DF2 was originally implicated in mediating m⁶A-associated mRNA instability, as knockdown of DF2 stabilized mRNAs that contained DF2-binding sites [19]. This appears to involve P-bodies as DF2 colocalization with P-bodies through its N-terminal P/Q/N-rich low-complexity region [19]. However, other studies did not find DF2 in immunoprecipitates of GW182, a core component of P-bodies [52]. Moreover, none of the YTHDF proteins were identified in a comprehensive analysis of proteins enriched in P-bodies [87]. It is likely that the association of DF2 with P-bodies is transient, which accounts for the difficulty in detecting DF2 in these bodies. Others have suggested that DF2 mediates mRNA degradation by first triggering mRNA deadenylation, followed by subsequent translocation to P-bodies [52]. Indeed, the N terminus of DF2 was shown to bind to CCR4–NOT transcription complex subunit 1 (CNOT1), a component of the CCR4–NOT deadenylation complex, leading to deadenylation [52]. Notably, this study found that all DF proteins recruit CCR4–NOT and promote mRNA deadenylation [52]. This contrasts with earlier studies, which had argued that DF1 has minimal effects on mRNA stability [36].

Although DF2 appears to contribute to mRNA stability, other m⁶A readers may also contribute to m⁶A-mediated mRNA instability. To address this, it will be critical to determine the extent to which m⁶A regulates mRNA stability in cells depleted of DF proteins.

deadenylation by CCR4–NOT1 transcripts are decapped and degraded. (D) YTHDC1 is necessary for XCI. *XIST* is a heavily methylated noncoding RNA bound by DC1. In the absence of m⁶A or DC1 XCI is impaired. (E) YTHDC1 (YT-521B in *Drosophila*) binding to m⁶A is necessary for X chromosome dosage compensation and sex determination in flies. In female flies, the introns flanking the second exon of the *sex-lethal* (*Sxl*) transcript contain m⁶A and binding of DC1 promotes skipping of the second exon and production of full-length *Sxl* protein. (F) Nuclear replicating viruses, such as HIV, have highly conserved m⁶A sites that actively recruit DF proteins to promote translation of viral proteins. (G) Cytoplasmic replicating viruses, such as Zika virus, are methylated in the cytoplasm through an unknown METTL3-dependent mechanism and bound by the DF proteins. Binding of DF proteins to cytoplasmic virus transcripts confines the transcripts to lipid droplets in liver cells and inhibits viral replication. Abbreviations: CNOT1, CCR4–NOT transcription complex subunit 1; eIF3, eukaryotic initiation factor 3; m⁶A, N⁶-methyladenosine; METTL3, methyltransferase like 3; UTR, untranslated region; XCI, X-chromosome inactivation; *XIST*, X-inactive specific transcript.

Translational Activation

Until recently, DF1 was the only DF protein expected to regulate translation. DF1 enhanced translational efficiency in mRNAs that are normally bound by DF1 [36] by direct binding of DF1 to eIF3. Since DF1-binding sites, like m⁶A, are primarily around the stop codon and 3'-UTR, this model suggests that eIF3 is recruited near mRNA stop codons. Previous studies showed that eIF3-induced translation activation occurs by recruitment of eIF3 to 5'-UTRs, for example by viral sequences [53] and other translation-promoting elements [18,54], but it remains unclear how this noncanonical stop codon recruitment of eIF3 would promote translation.

Recently, other DF proteins were shown to promote translation [51]. DF proteins that were tethered to a reporter RNA resulted in enhanced translation of the reporter [51]. This is partially supported in another study that showed that DF3 exhibits both translation enhancement and mRNA degradation upon binding its targets [48,50]. Thus, DF3 was proposed to have a combination of the features of DF1 and DF2. As with DF1, a clear mechanism by which binding leads to translation is unclear and is needed to fully understand the role of DF1 and DF3 in translational enhancement.

A common theme in these more recent studies is that all three DF proteins share a similar, rather than distinct function. Each of these proteins has been shown to promote translation and each can induce RNA degradation. It will be important to definitively determine if this more recent model of redundant function explains the DF proteins or if the original concept of distinct translation and mRNA destabilization functions for DF1 and DF2 is accurate.

m⁶A: Proviral or Antiviral?

m⁶A was found in simian virus 40, Rous sarcoma virus, adenovirus-2, herpes simplex virus type-1, avian sarcoma virus B77, and influenza virus viral RNA since the 1970s [55–60]. However, the function of m⁶A in these viruses has been a mystery. In the case of HIV it appears that the virus is actively utilizing the host RNA methylation machinery for its replication. Loss of the m⁶A writing complex inhibits HIV replication [61,62]. HIV recruits DF proteins to methylation sites and this enhances viral replication [51]. How DF proteins enhance viral replication remains unknown, but it seems to be inconsistent with the destabilizing function of the DF proteins on host cell-encoded mRNAs.

RNA viruses that replicate in the cytosol can also contain m⁶A. This is unexpected since METTL3 is classically thought to be nuclear [63], and methylation is thought to occur cotranscriptionally [64]. Nevertheless, various cytoplasmic RNA viruses exhibit m⁶A in their viral genome, including Zika virus, and other flaviviruses including dengue virus, West Nile virus, yellow fever virus, and hepatitis C virus [65,66]. Unlike in HIV where m⁶A seems to promote viral replication, m⁶A in these viruses seems to suppress replication. In the case of hepatitis C virus, loss of m⁶A or the DF proteins enhanced the production of viral particles [66]. The mechanism of m⁶A-mediated suppression of viral replication does not appear to involve m⁶A effects on RNA stability or translation. Instead, hepatitis C virus may require the formation of lipid droplets and that localization of the DF proteins to the droplets impairs the formation of viral particles [66].

YTHDC1

Unlike DF proteins, DC1-binding sites show nearly complete overlap with m⁶A sites in nuclear RNAs, such as *XIST* and *MALAT1*. Thus, DC1 influences nuclear processing and functions of nuclear-localized RNAs.

m⁶A-Directed Regulator of Splicing

DC1 was originally characterized as a regulator of splicing by promoting exon inclusion [67]. The YTH domain was found to be essential for this effect [68]. Based on our current understanding that the YTH domain binds m⁶A, this finding indicates that the effect of DC1 on splicing is dependent on m⁶A [67]. Recent studies indicate that DC1 recruits serine/arginine-rich splicing factor 3 (SRSF3) to promote exon inclusion [68]. However, evidence for m⁶A in regulation of splicing is limited. The first in-depth analysis of altered splicing discovered that less than 100 exons containing an m⁶A site are alternatively spliced upon knockdown of METTL3 [6]. Further examination of m⁶A-induced splicing was analyzed in *Mettl3*^{-/-} mouse embryonic stem cells, revealing that less than 0.5% of all exons exhibit alternative splicing and of those less than 100 contain an m⁶A site [7,69].

While m⁶A may not globally affect splicing in mammalian cells, m⁶A regulates sex-specific gene expression by regulating splicing of *sex-lethal* (*Sxl*) transcript in *Drosophila* [13–15]. Males express an *Sxl* transcript that includes an additional exon that contains a stop codon, thus preventing *Sxl* protein synthesis. In females, m⁶A in *Sxl* recruits YT521-B (the *Drosophila* DC1 ortholog), resulting in skipping of the exon and production of a transcript that encodes full-length *Sxl* protein. Loss of m⁶A results in impaired exon skipping in *Sxl*, which leads to a male bias in the progeny. Overexpression of YT521-B is lethal to males [14]. Presumably, this is because overexpression of YT521-B results in femalelike splicing of *Sxl*, which inhibits translation of male-specific lethal 2 (*msl-2*) leading to improper X-chromosome dosage compensation in males. Thus, m⁶A acts to directly regulate specific splicing events through YT521-B in *Drosophila*.

Regulating the Epigenome through Modulation of the Epitranscriptome

DC1 mediates the action of the nuclear noncoding RNA *XIST*. *XIST* has an essential role in XCI, a process that occurs in female mammalian cells. One X chromosome is randomly silenced in a process regulated by *XIST*, which coats the X chromosome and recruits chromatin-modifying factors that silence the chromosome [70]. *XIST* contains at least 76 m⁶A residues, and depletion of m⁶A from embryonic stem cells prevents *XIST* from inducing X chromosome gene silencing [16]. Of all the YTH proteins, DC1 preferentially binds to m⁶A sites in *XIST*, which is consistent with the nuclear localization of DC1. Loss of DC1 prevents *XIST*-mediated X chromosome gene silencing [16]. XCI can be restored in m⁶A-deficient cells by artificially tethering DC1 to *XIST* [16]. Thus, the primary role of m⁶A in XCI is to enable DC1 recruitment to *XIST*.

Concluding Remarks

Although the structure of the YTH domains is well understood, the functions of the YTH proteins remain unclear (see Outstanding Questions). In the case of DF proteins, a major question is whether these proteins are functionally redundant, or if they have different functions. Interpretation of experiments using knockdowns of DF proteins is hampered because DF2 is often more highly expressed than DF1 or DF3. Therefore, loss of DF1 or DF3 may not produce an effect similar to DF2 loss due to compensation. Deletion of all three DF proteins from cells followed by exogenous expression of one DF protein at a time may shed light on the ability of these proteins to perform redundant functions.

In addition, a clear m⁶A-dependent function for DC2 has yet to be described. DC2 is localized to the cytoplasm and can be induced by tumor necrosis factor- α [24,71], raising the possibility that its function may be regulated in certain cell types through external stimuli. DC2 increased the translational efficiency of hypoxia-inducible factor-1 α (HIF1- α) by unwinding its 5'-UTR via

Outstanding Questions

Do the DF proteins have distinct or redundant functions? Independent studies report conflicting findings. These discrepancies need to be addressed with careful experimental design.

What is the role of post-translational modifications in controlling YTH proteins?

Does DC1 regulate chromatin modification globally through interacting with noncoding RNAs?

What is the function of DC2?

Are there other m⁶A readers?

the DC2 RNA helicase domain [72], but whether this is an m⁶A-dependent process remains unknown.

Relatively little information is also available on the regulation of YTH proteins. It is possible that DF proteins may be regulated differently based on the tissue type or in response to particular signals. Phosphoproteomic studies show numerous phosphorylation sites throughout the P/Q/N-rich region of the DF proteins and adjacent to the YTH domain [73]. Phosphorylation is known to prevent proteins from forming granules [74]. Therefore, it is possible that post-translational modifications regulate DF-mediated clustering interactions involved in liquid–liquid phase separation or granule formation. In addition to phosphorylation, DF proteins were found to be myristoylated in a proteomic study of myristoylated proteins [75]. However, it remains to be determined whether there are external or internal stimuli that lead to altered post-translational modifications. If so, how do these post-translational modifications alter YTH protein interaction with other proteins or mRNA, or affect YTH localization? Currently, there are no known signaling pathways that modulate m⁶A. Therefore, any discovery of altered YTH regulation following such signals would advance our understanding of how m⁶A is regulated and what processes m⁶A regulates.

An important question is whether there are other mechanisms or readers by which m⁶A mediates its effects in cells, and the degree to which the actions of m⁶A can be ascribed to YTH proteins and eIF3. It will be important to examine whether m⁶A can still influence mRNA in cells deficient in YTH proteins.

An exciting yet unexplored area is the recent evidence that the epitranscriptome can regulate the epigenome based on studies of m⁶A in *XIST* [16]. The functions of m⁶A in *XIST* are mediated by its binding to DC1. If DC1 is indeed a general regulator of noncoding RNAs, then m⁶A may have a general role in regulating chromatin marks.

m⁶A has a demonstrated importance in a number of diseases including viral infections and various cancers [51,61,62,65,66,76–79]. This has garnered interest in developing small-molecule inhibitors that target the m⁶A writer complex. However, another option that can be explored is pharmacologic targeting of the YTH proteins. The YTH domain forms a unique tryptophan cage necessary for the recognition and binding of m⁶As. Given the unique m⁶A is recognized by a well-defined binding pocket, this site may be suitable for small-molecule inhibitors that would compete with m⁶A RNA and negate the effects of YTH proteins. Therefore, better understanding of the m⁶A-binding proteins can reveal novel targets that can be used for therapeutic manipulation of the epitranscriptome.

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References

1. Perry, R.P. and Kelley, D.E. (1974) Existence of methylated messenger RNA in mouse L cells. *Cell* 1, 37–42
2. Desrosiers, R. et al. (1974) Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. *Proc. Natl. Acad. Sci. U. S. A.* 71, 3971–3975
3. Clancy, M.J. et al. (2002) Induction of sporulation in *Saccharomyces cerevisiae* leads to the formation of N⁶-methyladenosine in mRNA: a potential mechanism for the activity of the *IME4* gene. *Nucleic Acids Res.* 30, 4509–4518
4. Zhong, S. et al. (2008) MTA is an *Arabidopsis* messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. *Plant Cell* 20, 1278–1288
5. Meyer, K.D. et al. (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. *Cell* 149, 1635–1646
6. Dominissini, D. et al. (2012) Topology of the human and mouse m⁶A RNA methylomes revealed by m⁶A-seq. *Nature* 485, 201–206

7. Geula, S. *et al.* (2015) Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. *Science* 347, 1002–1006
8. Batista, P.J. *et al.* (2014) m(6)A RNA modification controls cell fate transition in mammalian embryonic stem cells. *Cell Stem Cell* 15, 707–719
9. Wang, Y. *et al.* (2014) N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. *Nat. Cell Biol.* 16, 191–198
10. Jaffrey, S.R. and Kharas, M.G. (2017) Emerging links between m6A and misregulated mRNA methylation in cancer. *Genome Med.* 9, 2
11. Vu, L.P. *et al.* (2017) The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloid differentiation of normal hematopoietic and leukemia cells. *Nat. Med.* Published online September 18, 2017. <http://dx.doi.org/10.1038/nm.4416>
12. Fustin, J.M. *et al.* (2013) RNA-methylation-dependent RNA processing controls the speed of the circadian clock. *Cell* 155, 793–806
13. Kan, L. *et al.* (2017) The m6A pathway facilitates sex determination in *Drosophila*. *Nat. Commun.* 8, 15737
14. Haussmann, I.U. *et al.* (2016) m6A potentiates Sxl alternative pre-mRNA splicing for robust *Drosophila* sex determination. *Nature* 540, 301–304
15. Lence, T. *et al.* (2016) m6A modulates neuronal functions and sex determination in *Drosophila*. *Nature* 540, 242–247
16. Patil, D.P. *et al.* (2016) m(6)A RNA methylation promotes XIST-mediated transcriptional repression. *Nature* 537, 369–373
17. Roost, C. *et al.* (2015) Structure and thermodynamics of N6-methyladenosine in RNA: a spring-loaded base modification. *J. Am. Chem. Soc.* 137, 2107–2115
18. Meyer, K.D. *et al.* (2015) 5' UTR m(6)A promotes cap-independent translation. *Cell* 163, 999–1010
19. Wang, X. *et al.* (2014) N6-Methyladenosine-dependent regulation of messenger RNA stability. *Nature* 505, 117–120
20. Musa, J. *et al.* (2016) Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1): a master regulator of mRNA translation involved in tumorigenesis. *Oncogene* 35, 4675–4688
21. Imai, Y. *et al.* (1998) Cloning of a gene, YT521, for a novel RNA splicing-related protein induced by hypoxia/reoxygenation. *Brain Res. Mol. Brain Res.* 53, 33–40
22. Hartmann, A.M. *et al.* (1999) The interaction and colocalization of Sam68 with the splicing-associated factor YT521-B in nuclear dots is regulated by the Src family kinase p59(fyn). *Mol. Biol. Cell* 10, 3909–3926
23. Stoilov, P. *et al.* (2002) YTH: a new domain in nuclear proteins. *Trends Biochem. Sci.* 27, 495–497
24. Jain, D. *et al.* (2017) ketu mutant mice uncover an essential meiotic function for the ancient, putative RNA helicase YTHDC2. *bioRxiv* Published online August 6, 2017. <http://dx.doi.org/10.1101/171827>
25. Luo, S. and Tong, L. (2014) Molecular basis for the recognition of methylated adenines in RNA by the eukaryotic YTH domain. *Proc. Natl. Acad. Sci. U. S. A.* 111, 13834–13839
26. Zhu, T. *et al.* (2014) Crystal structure of the YTH domain of YTHDF2 reveals mechanism for recognition of N6-methyladenosine. *Cell Res.* 24, 1493–1496
27. Li, F. *et al.* (2014) Structure of the YTH domain of human YTHDF2 in complex with an m(6)A mononucleotide reveals an aromatic cage for m(6)A recognition. *Cell Res.* 24, 1490–1492
28. Theler, D. *et al.* (2014) Solution structure of the YTH domain in complex with N6-methyladenosine RNA: a reader of methylated RNA. *Nucleic Acids Res.* 42, 13911–13919
29. Xu, C. *et al.* (2014) Structural basis for selective binding of m6A RNA by the YTHDC1 YTH domain. *Nat. Chem. Biol.* 10, 927–929
30. Xu, C. *et al.* (2015) Structural basis for the discriminative recognition of N6-methyladenosine RNA by the human YT521-B homology domain family of proteins. *J. Biol. Chem.* 290, 24902–24913
31. Yang, X. *et al.* (2013) The dataset for protein-RNA binding affinity. *Protein Sci.* 22, 1808–1811
32. Qin, S. and Min, J. (2014) Structure and function of the nucleosome-binding PWWP domain. *Trends Biochem. Sci.* 39, 536–547
33. Yap, K.L. and Zhou, M.M. (2011) Structure and mechanisms of lysine methylation recognition by the chromodomain in gene transcription. *Biochemistry* 50, 1966–1980
34. Beaver, J.E. and Waters, M.L. (2016) Molecular recognition of Lys and Arg methylation. *ACS Chem. Biol.* 11, 643–653
35. Hughes, R.M. *et al.* (2007) Recognition of trimethyllysine by a chromodomain is not driven by the hydrophobic effect. *Proc. Natl. Acad. Sci. U. S. A.* 104, 11184–11188
36. Wang, X. *et al.* (2015) N(6)-Methyladenosine modulates messenger RNA translation efficiency. *Cell* 161, 1388–1399
37. Linder, B. *et al.* (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. *Nat. Methods* 12, 767–772
38. Pendleton, K.E. *et al.* (2017) The U6 snRNA m6A methyltransferase METTL16 regulates SAM synthetase intron retention. *Cell* 169, 824–835.e14
39. Piekna-Przybylska, D. (2008) The 3D rRNA modification maps database: with interactive tools for ribosome analysis. *Nucleic Acids Res.* 36, D178–D183
40. Khatter, H. *et al.* (2015) Structure of the human 80S ribosome. *Nature* 520, 640–645
41. Wei, C. *et al.* (1975) N6, O2'-Dimethyladenosine a novel methylated ribonucleoside next to the 5' terminal of animal cell and virus mRNAs. *Nature* 257, 251–253
42. Mauer, J. *et al.* (2017) Reversible methylation of m6Am in the 5' cap controls mRNA stability. *Nature* 541, 371–375
43. Jia, G. *et al.* (2011) N6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. *Nat. Chem. Biol.* 7, 885–887
44. Dominissini, D. *et al.* (2016) The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. *Nature* 530, 441–446
45. Li, X. *et al.* (2016) Transcriptome-wide mapping reveals reversible and dynamic N(1)-methyladenosine methylome. *Nat. Chem. Biol.* 12, 311–316
46. Safra, M. *et al.* (2017) The m1A landscape on cytosolic and mitochondrial RNA at single base resolution. *Nature* Published online October 25, 2017. <http://dx.doi.org/10.1038/nature24456>
47. Wang, C. *et al.* (2016) A novel RNA-binding mode of the YTH domain reveals the mechanism for recognition of determinant of selective removal by Mmi1. *Nucleic Acids Res.* 44, 969–982
48. Li, A. *et al.* (2017) Cytoplasmic m6A reader YTHDF3 promotes mRNA translation. *Cell Res.* 27, 444–447
49. Zhou, J. *et al.* (2015) Dynamic m(6)A mRNA methylation directs translational control of heat shock response. *Nature* 526, 591–954
50. Shi, H. *et al.* (2017) YTHDF3 facilitates translation and decay of N6-methyladenosine-modified RNA. *Cell Res.* 27, 315–328
51. Kennedy, E.M. *et al.* (2016) Posttranscriptional m(6)A editing of HIV-1 mRNAs enhances viral gene expression. *Elife* 19, 675–685
52. Du, H. *et al.* (2016) YTHDF2 destabilizes m(6)A-containing RNA through direct recruitment of the CCR4-NOT deadenylase complex. *Nat. Commun.* 7, 12626
53. Jackson, R.J. *et al.* (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat. Rev. Mol. Cell Biol.* 11, 113–127
54. Lee, A.S. *et al.* (2015) eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. *Nature* 522, 111–114
55. Canaani, D. *et al.* (1979) Identification and mapping of N6-methyladenosine containing sequences in simian virus 40 RNA. *Nucleic Acids Res.* 6, 2879–2899

56. Kane, S.E. and Beemon, K. (1985) Precise localization of m6A in Rous sarcoma virus RNA reveals clustering of methylation sites: implications for RNA processing. *Mol. Cell. Biol.* 5, 2298–2306
57. Sommer, S. *et al.* (1976) The methylation of adenovirus-specific nuclear and cytoplasmic RNA. *Nucleic Acids Res.* 3, 749–765
58. Moss, B. *et al.* (1977) 5'-Terminal and internal methylated nucleosides in herpes simplex virus type 1 mRNA. *J. Virol.* 23, 234–239
59. Dimock, K. and Stoltzfus, C.M. (1977) Sequence specificity of internal methylation in B77 avian sarcoma virus RNA subunits. *Biochemistry* 16, 471–478
60. Krug, R.M. *et al.* (1976) Influenza viral mRNA contains internal N6-methyladenosine and 5'-terminal 7-methylguanosine in cap structures. *J. Virol.* 20, 45–53
61. Tirumuru, N. *et al.* (2016) N(6)-Methyladenosine of HIV-1 RNA regulates viral infection and HIV-1 Gag protein expression. *Elife* 5, e15528
62. Lichinchi, G. *et al.* (2016) Dynamics of the human and viral m(6)A RNA methylomes during HIV-1 infection of T cells. *Nat. Microbiol.* 1, 16011
63. Bokar, J.A. *et al.* (1997) Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. *RNA* 3, 1233–1247
64. Slobodin, B. *et al.* (2017) Transcription impacts the efficiency of mRNA translation via co-transcriptional N6-adenosine methylation. *Cell* 169, 326–337.e12
65. Lichinchi, G. *et al.* (2016) Dynamics of human and viral RNA methylation during Zika virus infection. *Cell Host Microbe* 20, 666–673
66. Gokhale, N.S. *et al.* (2016) N6-Methyladenosine in Flaviviridae viral RNA genomes regulates infection. *Cell Host Microbe* 20, 654–665
67. Zhang, Z. *et al.* (2010) The YTH domain is a novel RNA binding domain. *J. Biol. Chem.* 285, 14701–14710
68. Xiao, W. *et al.* (2016) Nuclear m(6)A reader YTHDC1 regulates mRNA splicing. *Mol. Cell* 61, 507–519
69. Ke, S. *et al.* (2017) m6A mRNA modifications are deposited in nascent pre-mRNA and are not required for splicing but do specify cytoplasmic turnover. *Genes Dev.* 31, 990–1006
70. Engreitz, J.M. *et al.* (2013) The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome. *Science* 341, 1237973
71. Tanabe, A. *et al.* (2014) Transcriptional machinery of TNF-alpha-inducible YTH domain containing 2 (YTHDC2) gene. *Gene* 535, 24–32
72. Tanabe, A. *et al.* (2016) RNA helicase YTHDC2 promotes cancer metastasis via the enhancement of the efficiency by which HIF-1alpha mRNA is translated. *Cancer Lett.* 376, 34–42
73. Hornbeck, P.V. *et al.* (2015) PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res.* 43, D512–D520
74. Kato, M. *et al.* (2012) Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. *Cell* 149, 753–767
75. Thinon, E. *et al.* (2014) Global profiling of co- and post-translationally N-myristoylated proteomes in human cells. *Nat. Commun.* 5, 4919
76. Dixit, D. *et al.* (2017) Messenger RNA methylation regulates glioblastoma tumorigenesis. *Cancer Cell* 31, 474–475
77. Zhang, S. *et al.* (2017) m6A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program. *Cancer Cell* 31, 591–606.e6
78. Zhang, C. *et al.* (2016) Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells. *Oncotarget* 7, 64527–64542
79. Kwok, C.T. *et al.* (2017) Genetic alterations of m6A regulators predict poorer survival in acute myeloid leukemia. *J. Hematol. Oncol.* 10, 39
80. Oates, M.E. *et al.* (2013) D(2)P(2): database of disordered protein predictions. *Nucleic Acids Res.* 41, D508–D516
81. Vilella, A.J. *et al.* (2009) EnsemblCompara GeneTrees: complete, duplication-aware phylogenetic trees in vertebrates. *Genome Res.* 19, 327–335
82. Liu, J. *et al.* (2014) A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat. Chem. Biol.* 10, 93–95
83. Schwartz, S. *et al.* (2014) Perturbation of m6A writers reveals two distinct classes of mRNA methylation at internal and 5' sites. *Cell Rep.* 8, 284–296
84. Wang, X. *et al.* (2016) Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. *Nature* 534, 575–578
85. Wang, P. *et al.* (2016) Structural basis for cooperative function of Mettl3 and Mettl14 methyltransferases. *Mol. Cell* 63, 306–317
86. Zheng, G. *et al.* (2013) ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. *Mol. Cell* 49, 18–29
87. Hubstenberger, A. *et al.* (2017) P-body purification reveals the condensation of repressed mRNA regulons. *Mol. Cell* 68, 87144–157.e5