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The m^{6}A writer: Rise of a machine for growing tasks

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Table of contents:

ABSTRACT

The central dogma of molecular biology introduced by Crick describes a linear flow of information from DNA to mRNA to protein. Since then it has become evident that RNA undergoes several maturation steps such as capping, splicing, 3'-end processing and editing. Likewise, nucleotide modifications are common in mRNA and are present in all organisms impacting on the regulation of gene expression. The most abundant modification found in mRNA is N6-methyladenosine (m^6A). Deposition of m^6A is a nuclear process and is performed by a megadalton writer complex primarily on mRNAs, but also on microRNAs and lncRNAs. The m^6A methylosome is composed of the enzymatic core components METTL3 and METTL14, and several auxiliary proteins necessary for its correct positioning and functioning, which are WTAP, VIRMA, FLACC, RBM15 and HAKAI. The m^6A epimark is decoded by YTH domain containing reader proteins YTHDC and YTHDF, but METTLs can act as 'readers' as well. Eraser proteins, such as FTO and ALKBH5, can remove the methyl group.

Here we review recent progress on the role of m^6A in regulating gene expression in light of Crick's central dogma of molecular biology. In particular, we address the complexity of the writer complex from an evolutionary perspective to obtain insights into the mechanism of ancient m^6A methylation and its regulation.

INTRODUCTION

Francis Crick's central dogma of molecular biology describes gene expression as a linear flow of information contained in DNA, which is transcribed into RNA and subsequently exported from nucleus to cytoplasm where it is then translated into protein [1]. The dogma foresees steady state levels of gene expression as very little was known about regulation at the RNA level at that time. Meanwhile, it has become evident that eukaryotic primary transcripts maturate in several steps including 5'-end capping, splicing and 3'-end processing [2-5]. Many pre-mRNAs can also be alternatively spliced and/or polyadenylated, which violates the linear flow of information from DNA to protein suggested by Crick's central dogma.

In some transcripts single nucleotides can be modified by a process termed editing where adenosine or cytidine are deaminated to inosine or uridine, respectively [6-8]. RNA editing can directly affect the translation of coding transcripts as the inosine pairs with cytosine and uracil with adenine. A-to-I RNA editing has been implicated in central nervous system (CNS) functions, the onset of cancer, as well as the innate immunity responses to dsRNAs [6, 8].

For over 40 years, it has been known that individual nucleotides can be modified in mRNA. The most common modifications are N^6-methyladenosine (m^6A), pseudouridine (Ψ), 5-methylcytosine (m^5C), 5-hydroxymethylcytosine (hm^5C), and N^1-methyladenosine (m^1A) and 2'-O-ribose methylation [8]. These modifications do not alter the coding capacity of mRNA but affect some aspect of the regulation of the gene expression.

The most prevalent mRNA modification is m^6A, occurring at an estimated frequency of 3-5 sites per transcript in mammals [9, 10]. Recently, sites containing m^6A were mapped globally by pulldown and high-throughput sequencing approaches and were found distributed non-randomly in the mRNA. In fact, m^6A sites are enriched in a consensus sequence RRACH (R=G/A; H=A/C/U). Most m^6A sites are found in the 5' UTR, around the stop codon in the 3' UTR and in long exons, but are also present in ncRNAs and microRNAs [9, 11-15]. These mapping approaches used poly(A) mRNA, but m^6A is also present in introns. The exact levels of m^6A in introns, however, remains to be determined [14-16] (Figure 1).

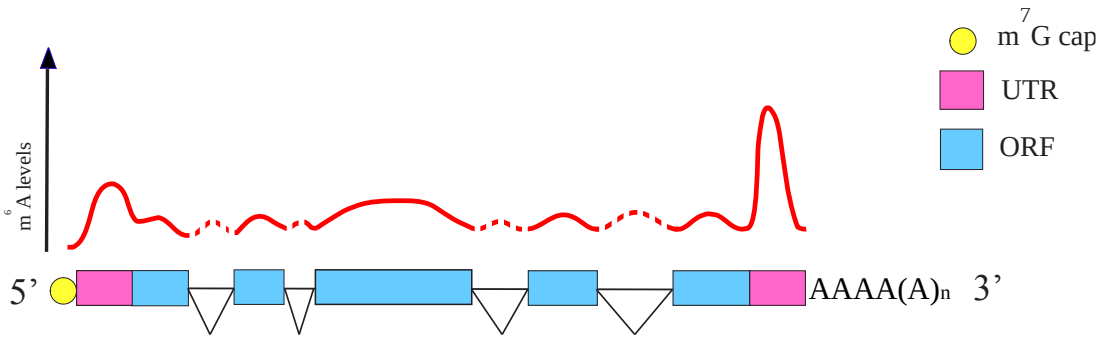

Figure 1. Distribution of m^{6}A sites in mRNA. The enrichment of m^{6}A modification is found in the 5' UTR, in large exons and in the proximity of the stop codon in the 3' UTR, but is also present in some introns.

The deposition of m^{6}A is performed by a ~900 kDa m^{6}A methylosome complex that contains two distinct methyltransferase enzymes, Methyltransferase-like protein 3 (METTL3) and METTL14, which together form the enzymatic core [17-19]. In addition, the complex contains the auxiliary proteins Wilms' tumour 1-associated protein (WTAP), a homolog of *Drosophila*'s Female-Lethal 2d (Fl(2)d), Virilizer like m^{6}A methyltransferase associated protein (VIRMA/KIA1429), RNA-binding protein 15 (RBM15), Fl(2)d-associated complex component (Flacc/KIAA0853) and Hakai [20-23]. Fl(2)d and Vir were first discovered in *Drosophila* for their role in sex determination and dosage compensation but are now recognised to be part of the m^{6}A methylosome[24, 25]. Later, the RBM15 ortholog Spenito (Nito) was also found to have roles in sex determination [26]. The m^{6}A methylosome colocalises to sites of transcription and because it is involved in *Sex lethal* (*Sxl*) splicing it is thought that most m^{6}A is deposited before splicing [15, 26].

The m^{6}A epimark is decoded by reader proteins that contain the YTH domain, which specifically recognise m^{6}A [27-29]. Furthermore, it has been suggested that eraser proteins FTO [30] and ALKHB5 are able to remove the methyl group [31].

The m^{6}A methylosome deposits m^{6}A and impacts on the linear flow of information proposed by Crick's central dogma of molecular biology. The m^{6}A modification impacts broadly on mRNA metabolism including splicing, polyadenylation, nuclear export, RNA stability and translation and is essential for many biological function [32, 33].

To obtain insights into the regulation of m^{6}A methylation and its effect on gene expression, we used evolutionary approaches to examine the emergence of a complex machinery in 64 eukaryotic species. This analysis defines a minimal m^{6}A methylosome present in the Last Eukaryotic Common Ancestor

(LECA) that consisted of a writer (METTL4 and/or METTL3/14), an auxiliary protein and a reader (YTH domain containing proteins) base. Gene duplications of an ancestral methyltransferase protein, together with recruitment of arising novel proteins such as WTAP, VIRMA, FLACC and HAKAI, led to the complexity of the m^{6}A machinery in animals and plants.

The enzymatic core of the m^{6}A methylosome

Structure and architecture of METTL3 and METTL14

RNA methyltransferases (RNMT) are a large family of diverse proteins with a conserved catalytic core that adopt a Rossman-fold, but otherwise differ in their architectures [17, 18, 34]. Nuclear extract from tissue culture cells and synthetic substrate RNA were used to purify a 900 kDa holoenzyme, as well as 200 kDa and 70 kDa sub-complexes with reduced catalytic activity. Since the 70 kDa fraction contained S-adenosylmethionine (SAM)-binding methyltransferase activity, it was initially termed MT-70 then later METTL3 [19, 35, 36].

METTL3 and METTL14 are very similar in their methyltransferase domain (MTD) and interact with each other by forming a heterodimer via this domain [37-39]. The N-terminal portion of METTL3 interacts with the WTAP N-terminus, and the region between the MTD and the N-terminus contains a nuclear localisation signal (NLS) [37]. Adjacent to the N-terminus of the MTD, METTL3 has a zinc finger domain (ZFD) composed of two CCCH-type zinc fingers. In contrast, METTL14 does not contain this ZFD, but it contains C-terminal arginine-glycine (RGG) repeats not present in METTL3 (Figure 2A). The secondary structures of the enzymatic core of METTL3 and METTL14 are conserved as shown by imposition of the crystal structures (PDB ID: 5TEY, Figure 2B).

Residues critical for METTL3 methyltransferase enzymatic activity are Asp377, Asp395, Asn539 and Glu532, identified from the crystal structure of METTL3-METTL14 bound to SAM [39] (Figure 2C). Interestingly, these residues are not conserved in METTL14 of metazoa as residue Asp395 is substituted with Asn, residue Asn539 with Thr or Ser, and residue Glu532 with His. In humans, METTL14 is inactive in *in vitro* assays [38-40]. In contrast, the catalytic core of METTL14 is likely active in most plants and fungi (Figure 2D, Supporting information 1).

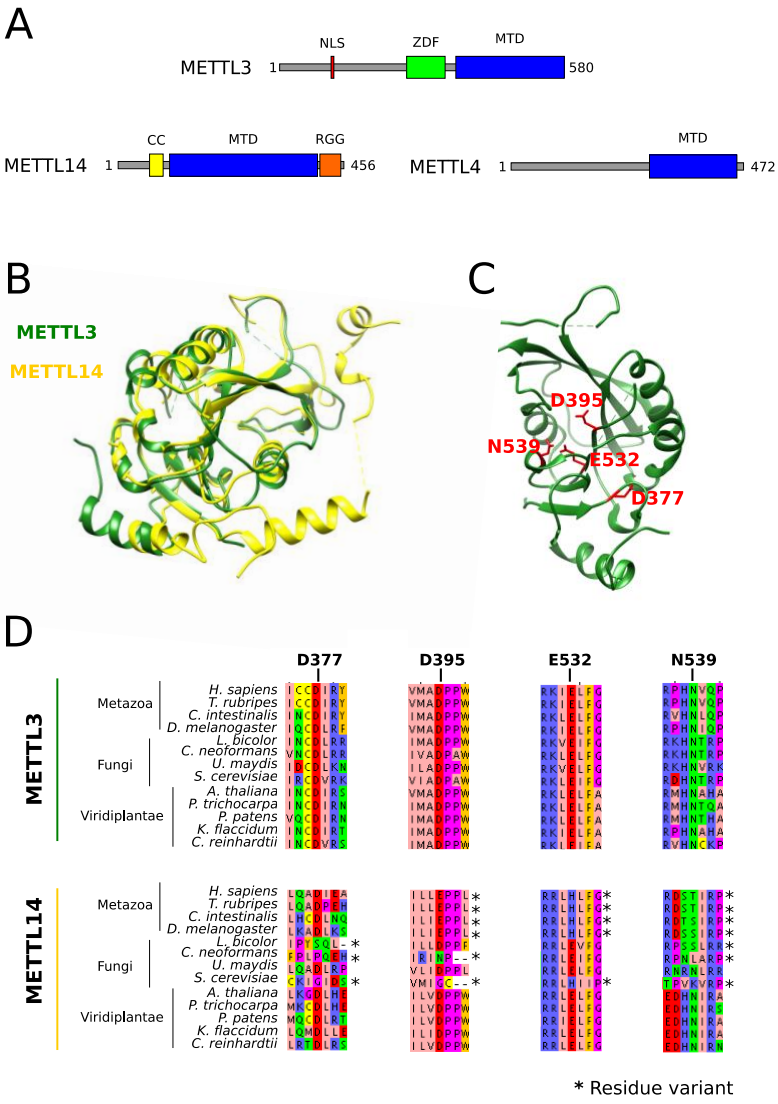

Figure 2. Eukaryotic MT70 methyltransferases. (A) Schematic of human METTL3, METTL14 and METTL4 protein structures with their domains indicated. METTL3 contains a nuclear localisation sequence (NLS, residues 105-109), a zinc finger domain (ZFD, residues 259-336) and a methyltransferase domain (MTD residues 358-580). METTL14 contains a coiled coil region (CC, residues 63-92), a methyltransferase domain (MTD residues 110-404) and C-terminal arginine-glycine repeats (RGG, residues 408-452). METTL4 contains a single methyltransferase domain (MTD residues 281-455). **(B)** Superimposition of the crystal structures of the enzymatic core of METTL3 (green) and METTL14 (yellow) (PDB ID: 5TEY). **(C)** Residues critical for METTL3 methyltransferase activity are highlighted in red in the crystal structure. **(D)** Alignment of MTase critical residues of METTL3 and METTL14 of metazoa, fungi and viridiplantae. Residue variants of METTL14 in regards to METTL3 are indicated with asterisks.

In animals, METTL14 enhances METTL3 methyltransferase activity by binding the mRNA and orientating the SAM methyl group for the reaction [23, 38-43]. The formation of a charged groove at the interface of METTL3 and METTL14 is essential for contact with the mRNA and for the catalytic activity. METTL3 catalytic activity is significantly reduced by SUMOylation of residues Lys177, Lys211, Lys212, and Lys215 without affecting its stability, assembly in the complex, or cellular localisation [44]. The two zinc fingers in METTL3 ZFD are responsible for the complex's specificity to the mRNA motif RRACH and METTL14 RGG repeats allow mRNA binding in a non-sequence-specific manner [37, 39, 45].

Roles of METTL3 and METTL14 in development and cancer

In animal and plants, a number of auxiliary components are required for m^6A deposition, and likely contribute to its specificity and regulation. An essential function of m^6A was first identified in *Saccharomyces cerevisiae* where METTL3 ortholog Inducer of meiosis 4 (Ime4) induces sporulation in response to nutritional stimuli [46].

A further role in sexual differentiation was found in *Drosophila*, where METTL3 plays a key role in the alternative splicing of Sxl, the master regulator of sex determination and dosage compensation [15].

In addition to the defect in sexual differentiation, METTL3 deficient flies are flightless and have defects in locomotion due to altered neuronal function, but survive to adulthood [15, 17, 26]. Loss of function of METTL14 and YTHDC1 phenocopies METTL3 mutant phenotypes [15, 26].

In vertebrates and plants, loss of METTL3 is lethal identifying key roles in early embryonic development [47]. Here, METTL3 is required for cell cycle progression, differentiation of embryonic stem cells (ECSs) and tissue differentiation [42, 48-52]. METTL3 also regulates the circadian clock, cell progression, maintenance of radial glial cells in neuronal differentiation and nervous system development, as well as learning and memory [51, 53-56].

Similarly, METTL14 plays an important role in maintaining the identity of neuronal populations and its depletion leads to prolonged cell cycle, abnormal embryo development, reduced functional axon regeneration in the peripheral nervous system and impairs motor learning in mice by disrupting striatal neuron properties [51, 57-59]. METTL3 and METT14 play important roles for murine spermatogonal stem cells (SSCs) differentiation and spermatogenesis at different developmental stages. Early inactivation of METTL3 or METTL14 in germ cells dramatically reduces global m^6A levels resulting in developmental

defects of SSCs and their excessive proliferation, which causes premature exhaustion and infertility. In later stages of spermatogenesis, METTL3 and METTL14 knockout mice show translational down-regulation of m^{6}A mRNAs that regulates spermatogenesis and are fertile, but have smaller testes [60]. Double knockout of METTL3 and METTL14 showed stronger defects in spermatogenesis [60].

Moreover, aberrant expression of METTL3 and METTL14 suggests that methylation has a critical role in tumorigenesis and cellular proliferation [61-63]. During development, m^{6}A is essential for cell identity maintenance and its dysregulation alters several regulatory pathways of gene expression, contributing in cancer development [63].

METTL3 promotes the translation of several oncogenes such as TAZ, EGFR, DNMT2a and MK2, and its overexpression in lung cancer cells results in increased cell proliferation, survival and invasion [64]. In fact, in lung cancer cells the miRNA mir33-a, which negatively regulates METTL3 expression, was found at low levels resulting in the overexpression of METTL3 [65].

In addition, the residue arginine 298 of METTL14 was found mutated to proline in endometrial cancer and reduced the methyltransferase activity of the m^{6}A methylosome [38]. In acute megakaryocytic leukaemia (AML), the down-regulation of the transcription factor SPI1 leads to the overexpression of METTL14, which in turn promotes cell proliferation and self-renewal [66]. In liver cancer, down-regulation of METTL14 significantly reduced m^{6}A deposition on pri-miR-126 resulting in metastasization of the tumour [67].

Ablation of either METTL3 or METTL14 mostly results in similar phenotypes suggesting their inter-dependence for m^{6}A methylation [15, 26, 51, 60]. In murine spermatogenesis, however, the single mutants have weaker phenotypes of the double mutants, arguing that METTL3 and METTL14 are active as individual MTases or have methylation independent functions [60]. Indeed, in addition to its methylation-dependent functions, METTL3 in the cytoplasm can bind the ribosome to promote translation of specific mRNAs independent of its methyltransferase activity and acts as reader protein [64].

Conservation of the enzymatic core among Eukaryotes

Given that m^{6}A has adopted many more roles in higher eukaryotes we anticipate that such complexity reflects the expansion of the m^{6}A methylosome complex components, readers, and erasers. To obtain insights in the functionality of the m^{6}A machinery, phylogenetic approaches were used to shed light on how higher eukaryotes evolved a complex m^{6}A methylosome from a single methyltransferase.

METTL3 and METTL14 methyltransferases are evolutionary conserved among eukaryotes and originated from bacterial DNA methyltransferases that can function as dimers [17, 18, 68]. A comprehensive evolutionary analysis on the components of the m^{6}A machinery in 64 eukaryotic organisms reveals that METTL3 and METTL14 are broadly conserved, although not present in some species, including model organisms *Caenorhabditis elegans*, *Dictyostelium discoideum* and pathogens *Trypanosoma brucei* and *Giardia intestinalis* (Figure 3).

Interestingly, METTL3 and METTL14 are always present together suggesting that the dimerization is essential for their function and possibly a remnant of bacterial DNA methyltransferases [69] (Figure 2). Based on the distribution of the methyltransferases among species, this analysis indicates that an ancestor methyltransferase gene was present in the LECA, which was a complex organism not very different from some extant eukaryotes [70].

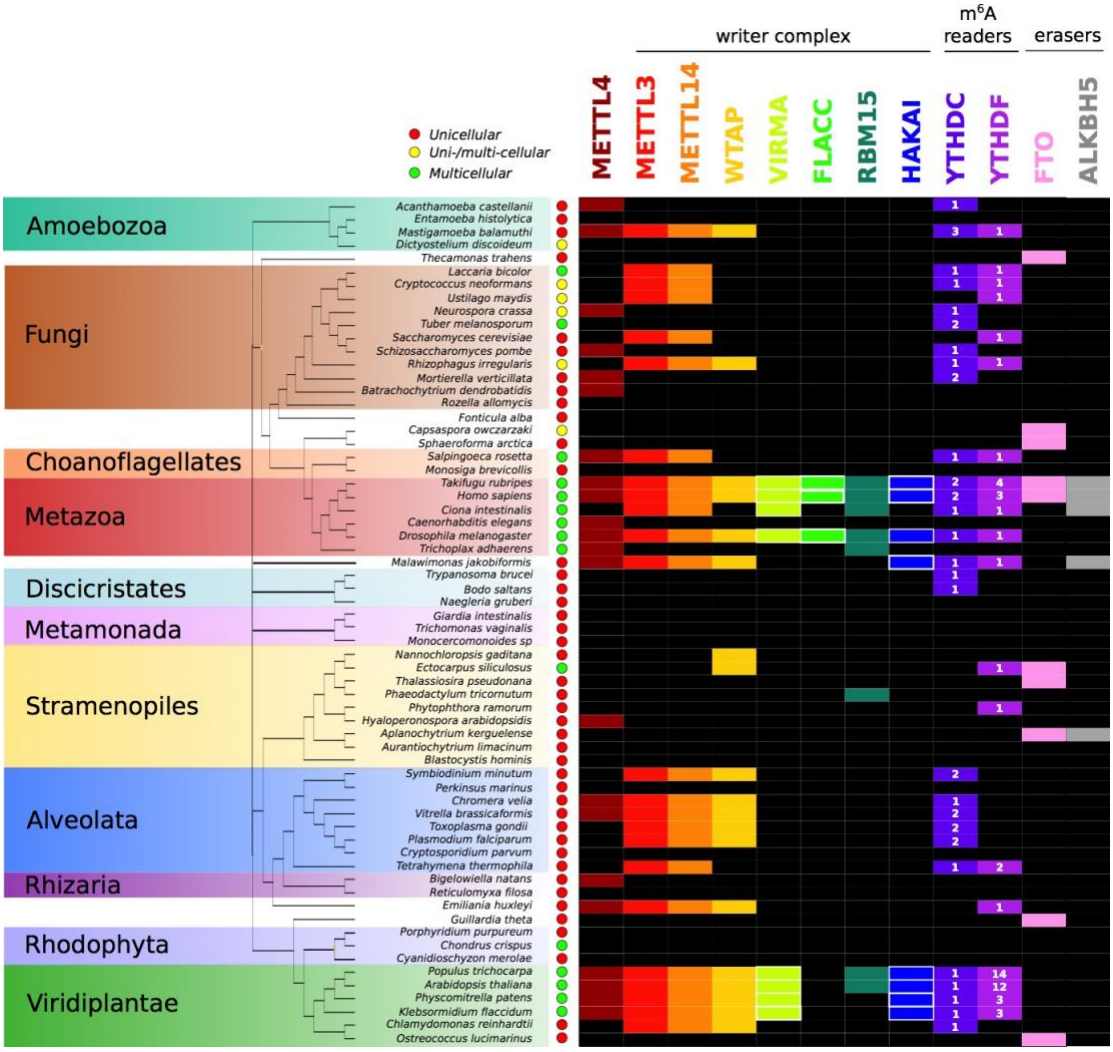

Figure 3. Conservation of the m^{6}A machinery among eukaryotes. The figure shows detected (coloured box) or not detected (black box) components of the writer complex, m^{6}A readers and erasers in 64 eukaryotic organisms from different groups, named on the left of the cladogram.

Sequences were retrieved using BLAST. Orthologs and paralogs were identified using orthoMCL, HHMscanner and manual inspection. Phylogeny was reconstructed using MAFFT and FastTree. Only sequences with alignment E-value less than 1E-12 were used. The number of YTHDC and YTHDF orthologs are indicated in their respective boxes. Proteins indicated with the white outline (VIRMA, FLACC and HAKAI) were not clustered together due to their high divergence, but were recovered by manual inspection and orthoMCL clustering.

METTL4

METTL4 is paralog of METTL3 and METTL14, and is the precursor of mRNA methyltransferases closely related to bacterial DNA methyltransferases [18]. Although METTL3 and METTL14 methylate mRNA, METTL4 was suggested to be a DNA methyltransferase [18]. Recent analyses of the specificity of the antibodies used to detect N6-methyladenine, however, revealed that 98% of all m^{6}A sites identified in DNA were false positive due to off-target binding of the antibodies and was also absent in mouse ESC as indicated by mass spectrometry [71, 72].

METTL4 is broadly conserved amongst eukaryotes. Some species including *Schizosaccharomyces pombe*, *Neurospora crassa*, *C. elegans,* and *Trichoplax adhaerens* have METTL4 but not METTL3 and METTL14. Interestingly, *Acanthamoeba castaellana*, *N. crassa* and *S. pombe* contain METTL4 and YTHDC reader proteins and whether in these organisms METTL4 act as RNA m^{6}A methyltransferase remains to be determined (Figure 3).

Whether species that have only METTL4 and not YTH domain containing proteins use readers is currently not known, but m^{6}A could also be used to perturb mRNA structure [73]. Some species such as *T. brucei*, *Bodo saltans*, *Tuber melanosporum* and *Phytophthora ramorum* lack methyltransferases but have YTH domain containing proteins.

In addition, in some species, such as *A. castellana*, *Thecamonas trahens*, *Naegleria gruberi*, *Trichomonas vaginalis*, *Reticulomyxa filose*, mRNA methyltransferase diversified three additional paralogs, but their function is currently unknown and they are not broadly conserved (Supporting Information 2) [17, 18]. Hence, the mRNA m^{6}A methyltransferases originated from a single gene that

underwent several duplications that led up to six m^{6}A methyltransferase clades during the eukaryotic evolutionary history.

METTL16

Although the METTL3-METTL14 writer complex is the major methyltransferase enzyme acting on polyadenylated mRNA, the methyltransferase METTL16 can also methylate mRNA in addition to U6 snRNA and various lncRNAs [74-77]. METTL16, however, belongs to a different methyltransferase protein family and is not paralog of METTL3, METTL4, and METTL14. Interestingly, METTL16 has been found to form homodimers [78, 79].

The auxiliary subunits of the m^{6}A methylosome: WTAP-VIRMA-FLACC -RBM15 -HAKAI

METTL3 and METTL14 comprise the core of the methylosome holoenzyme, which also includes the auxiliary proteins WTAP, VIRMA, FLACC, RBM15 and HAKAI. While METTL3-METTL14 mutants are viable in *Drosophila*, most of the auxiliary proteins are essential suggesting additional roles in cellular processes [20, 32, 80].

The predicted molecular weight of each component of the complex (METTL3: 64 kDa, METTL14: 52 kDa, WTAP: 44 kDa, VIRMA: 202 kDa, ZC3H13: 197 kDa, RBM15: 107 kDa, and HAKAI: 54.5 kDa) would make the overall molecular weight of ~720.5 kDa, which is smaller than the ~900 kDa complex purified by Bokar et al. [19], suggesting that either additional proteins may assemble in the complex, that some components are present as dimers, or that the complex has an elongated shape.

Fl(2)d/WTAP

Fl(2)d was first identified in *Drosophila* as a pioneer protein with no discernible motive for its involvement in sex determination and dosage compensation by regulating Sxl alternative splicing in females [25].

The mammalian ortholog, WTAP, is associated with the tumour suppressor gene Wilms' tumour 1 (WT1), which causes onset of paediatric nephroblastoma and cancers when induced [81]. Although WTAP is up-regulated in many tumours, including acute myeloid leukaemia (ALM), it is not directly related to carcinogenesis. Its oncogenic role is dependent on METTL3 levels and presence of a functional methyltransferase complex [82, 83].

In its N-terminus, WTAP has an NLS required for its localisation to nuclear speckles where it interacts with splicing factors, and coiled coils required for the interaction with METTL3 and METTL14 [37, 42]. WTAP stabilizes the interaction between METTL3 and METTL14 [22, 26, 81, 84].

Given its additional roles, Fl(2)d is essential in *Drosophila* and other organisms. WTAP is conserved among eukaryotes and its presence is correlated with the presence of METTL3 and METTL14. Interestingly, *S. cerevisiae* Ime4, the ortholog of METTL3, was found in complex with Muddled Meiosis 2 (Mum2) and Sporulation-specific Leucine Zipper 1 (Slz1) and this was termed the MIS complex [85]. An intact MIS complex is essential for the methyltransferase activity [85], but although Mum2 is considered an ortholog of WTAP, it is highly divergent (Figure 3). In *C. intestinalis*, *Tetrahymena thermophyla*, and most fungi, WTAP is absent but they may have other proteins involved in the assembly of the m^{6}A methylosome, similar to the MIS complex in *S. cerevisiae*. Some species, such as stramenopiles, have WTAP but do not have METTL3 and METTL14 methyltransferases, further supporting the idea that the protein has other roles (Figure 2).

Virilizer/VIRMA

Virilizer (Vir) was also identified in *Drosophila* due to its essential roles in sex determination and dosage compensation by regulating Sxl female specific splicing [24, 26, 86]. Vir lacks any known protein motifs, similar to Fl(2)d, but physically interacts with Fl(2)d [87].

The mammalian ortholog VIRMA was identified through its interaction with METTL3 [22, 23]. The VIRMA N-terminus binds the WTAP-METTL3-METTL14 complex via WTAP in an RNA-independent manner [88], but how VIRMA is required for m^{6}A methylation is unknown. VIRMA is present also in viridiplantae and animals [49], but these two groups of proteins are highly divergent (Figure 3).

Flacc/ZC3H13

Flacc (Fl(2)d-associated complex component)/ZC3H13 (zinc finger CCCH domain-containing protein 13) is another component of the methylosome complex and bridges Fl(2)d/WTAP and Nito/RBM15 in *Drosophila* and mice [20]. Like Fl(2)d, Flacc is also required for Sxl alternative splicing in sex determination and dosage compensation and is likely a constitutive component of the writer complex, as its depletion leads to reduced m^{6}A levels, splicing defects and phenotypes similar to METTL3 mutants [20, 89, 90].

FLACC does not seem to be a conserved protein among eukaryotes. In fact, the protein is animal specific and is present in humans and zebrafish with high similarity (Figure 3). *Drosophila* ortholog is highly divergent and does not contain zinc fingers, whereas other insects including *Apis mellifera* and *Bombyx mori* have long zinc fingers [20]. The lack of known protein motifs other than the zinc fingers in vertebrates suggests that FLACC might have a scaffolding function in the m^{6}A methylosome. Although a plant homologue could not be identified, we cannot exclude its presence because of the low complexity of FLACC.

Nito/RBM15

Spenito (Nito) was initially identified as an interacting partner of WTAP and was found to regulate of Sxl alternative splicing in *Drosophila* [22, 26, 91]. Likewise, co-immunoprecipitations analysis revealed that RBM15 interacts with METTL3 in a WTAP-dependent manner and that it is an essential component of the methyltransferase complex [92]. Nito's depletion dramatically affects m^{6}A levels and causes lethality in *Drosophila* and mice [26, 92].

RBM15 is member of the split end protein family (Spen). In its N-terminus, RBM15 has three RNA recognition motifs (RRM) characteristic of prototype RNA binding proteins. The C-terminus is characterised by the presence of a Spen paralogs and orthologs C-terminal (SPOC) domain, responsible for the interaction with other proteins such as the H3K4me3 histone methyltransferase Setd1b [92]. RBM15 likely acts as an adapter protein recruiting the m^{6}A methylosome to U-rich regions [92, 93].

RBM15 is a regulator of the m^{6}A levels of the long noncoding RNA (lncRNA) X-inactive specific transcript (*XIST*), which is required for dosage compensation in mammals, raising the question whether m^{6}A has broader roles in sex determination [92]. RBM15 is also involved in haematopoietic differentiation and its depletion has been linked to cardiovascular and spleen defects, as well as AML, but whether its oncogenic role is dependent on METTL3 is currently not known [94-96].

RBM15 is present in animals and plants and belongs to a big protein family containing RNA binding motifs, which could potentially be substituted with other RNA binding proteins (RBPs) (Figure 3).

HAKAI

Hakai was first identified through its interaction with WTAP and is present in plant and animals (Figure 3). In *Arabidopsis thaliana*, Hakai is an essential component of the m^{6}A methylosome. Its knockdown

leads to reduced m^{6}A levels and defects in embryonic development similar to the loss of other members of the m^{6}A methylosome [21].

Hakai, also known as Casitas B-lineage lymphoma-transforming sequence-like protein 1 (CBLL1), is an E3 ubiquitin ligase that regulates endocytosis of E-cadherin, and plays a crucial role in cell proliferation and tumorigenesis [97, 98].

Whether presence of Hakai in the viridiplantae *Populus trichocarpa*, *Physcomitrella patens*, *Klebsoridium flaccidum,* and in *Malawimonas jakobiformis* is linked to m^{6}A methylation remains to be determined.

Humans have an additional paralog of Hakai, the E3 ubiquitin-protein ligase ZNF645, which can play similar roles to HAKAI in the regulation of endocytosis, but whether is involved in m^{6}A methylation is currently not known [99].

READERS

The YT521-B homology (YTH) domain proteins were initially identified as splicing regulators based on their interaction with Sam68 [27, 100]. Later, YTH domain containing proteins were identified in mouse as m^{6}A mRNA binding proteins in pulldown experiments [9].

The crystal structures of human YTH domain revealed that residues Trp411, Trp465 and Trp470 form a buried hydrophobic aromatic cage where the 6-methylamino group is accommodated. This pocket is conserved in animals and plants and discriminates between m^{6}A and non-methylated mRNA with an increase in affinity of about 20-50 fold [15, 29, 73, 101, 102]. However, the YTH domain alone has a low affinity to mRNA and requires additional low-complexity regions of the full-length proteins, possibly together with other RBPs to stably bind the mRNA [29, 103-105].

The YTH domain containing protein family is a large group of reader proteins broadly conserved among eukaryotes, which categorises into two classes: the nuclear YTHDC proteins and the mainly cytoplasmic YTHDF proteins.

Drosophila has one YTHDC and one YTHDF protein, humans have two YTHDC proteins (YTHDC1 and YTHDC2) and three YTHDF proteins (YTHDF1, YTHDF2, YTHDF3), whereas the plant *A. thaliana* underwent multiple duplication events that led to the expansion of the protein family to 13 members [102]. In agreement with their essential role as m^{6}A reader, YTH domain containing proteins are present in organisms that have the methyltransferase sub-complex METTL3-METTL14-WTAP.

However, YTHDC proteins were also identified in species lacking METTL3 and METTL14, such as *T. brucei, B. saltans* and *T. melanosporum*, suggesting that these species either have other proteins that can methylate or YTHDC proteins are remnant of an ancient mechanism nowadays lost (Figure 2). Intriguingly, Mmi1, the *S. pombe* YTH domain containing protein has evolved a novel mode to bind in absence of m^6A mRNAs containing the determinant of selective removal (DSR) region, which re-localizes meiosis-specific transcripts to nuclear foci for degradation via the nuclear exosome [106].

Some fungi, including *S. pombe*, *N. crassa* and *Mortirella verticillate*, do not have METTL3 or METTL14, but do have METTL4 and YTHDC proteins. YTHDC proteins are broadly conserved, suggesting a more ancient and nuclear function of the YTH domain containing proteins.

YTHDC proteins

YTHDC1, previously known as YT521-B, was first identified as spicing regulator and, in the nucleus, accumulates in 5-20 dots termed YT bodies [100]. The YTH domain is located in the middle region of the protein. The N-terminus contains four NLSs, which are responsible for the nuclear localisation, and a glutamic acid-rich region. The C-terminus contains a glutamic acid/arginine-rich region and a proline-rich region responsible for the sub-localisation in YT bodies [100]. Nuclear sub-localisation is also dependant on Sam68 protein, although this interaction is regulated via tyrosine phosphorylation by $p59^{fyn}$ kinase [100, 107].

YTHDC1 in *Drosophila* is involved in sex determination and dosage compensation by regulating alternative splicing of Sxl. Specifically, it is thought that YTHDC1 enhances Sxl binding, rigorously controlling splicing in females possibly as an adaptive mechanism to compensate for tissue specific concentration differences of splicing factors [15, 26].

In humans, YTHDC1 also has a role in dosage compensation. Here it binds m^6A sites on *Xist* mRNA to promote inactivation of the X chromosome [92]. In addition, YTHDC1 interacts with splicing factors to regulate alternative splicing and nuclear export [108, 109].

YTHDC2 is a nucleo-cytoplasmic protein present only in mammals and is characterised by a helicase domain, ankyrin repeats, YTH domain and DUF1065 domain. YTHDC2 is highly expressed in testis and plays important roles in germ cell differentiation. For example, in mouse germ cells YTHDC2 regulates cellular transition from mitosis to meiosis for proper spermatocyte development in early spermatogenesis

[110]. Moreover, YTHDC2 regulates progression and completion of the meiotic program in mammalian germlines promoting mRNA decay [111, 112].

YTHDF proteins

YTHDF proteins, which contain the YTH domain at the C-terminus, are mainly cytoplasmic, but under stress conditions can re-localise to the nucleus [113]. In the N-terminus the proteins contain large low-complexity regions, but their functions remain to be determined [93].

Drosophila has one YTHDF protein, while vertebrates underwent duplication events that led to the emergence of three members YTHDF1, YTHDF2 and YTHDF3, thereby their functions have diverged to roles in translation, localisation and mRNA decay. YHTDF1 has mainly been associated with translation, while YTHDF2 primarily promotes mRNA decay. YTHDF3 works cooperatively with the other two proteins regulating both translation and mRNA decay. YTHDF1 is the closest relative to *Drosophila*'s YTHDF. METTL3 null flies have about equal number of up- and down-regulated genes suggesting that YTHDF does not have a major role in regulating mRNA stability in flies as in this case an increased number of up-regulated genes would be expected [15, 32]. In this organism, YTHDF may be primarily involved in regulating translation reminiscent of an ancient function.

YTHDF1 binds m^{6}A sites in the 3'UTR of mRNAs and mediates the recruitment of the translation machinery [58, 114]. In fact, upon injury in peripheral nerves YTHDF1 promotes local translation of retrograde signalling molecule mRNAs for rapid axonal regeneration [58]. In addition, under heat stress YTHDF1 stimulates the translation of heat shock proteins mRNAs in a cap-independent manner [114, 115]

In murine embryonic stem cells, pluripotency-promoting transcripts containing m^{6}A sites have shorter half-lives compared to the non-methylated mRNAs [48, 50]. YTHDF2 also stimulates mRNA decay negative regulators of neural development and its ablation results in altered neural stem/progenitor cell (NSPC) proliferation, unpaired neural differentiation, delayed cortical development and lethality at late stages of embryonic development [116].

In addition, YTHDF2 is required for degradation of maternal mRNAs, which is required for proper oocyte maturation and early zygotic development in mice [117]. Likewise, in zebrafish removal of maternal mRNAs is mediated by YTHDF2 during maternal to zygotic transition (MZT), but removal of YTHDF2 results only in developmental delay [118].

The plant YTHDF orthologs Evolutionarily conserved C-terminal region 2, 3 and 4 (ECT2/3/4) are essential to increase the stability of target m^{6}A containing mRNAs in the cytoplasm in protodermal cells for exit from mitosis and their transit in endoreduplication in early stages of trichrome development for correct timing of leaf formation and normal morphology [102, 119, 120].

In human cells, YTHDF3 promotes both translation and mRNA decay working cooperatively with the other two YTHDF proteins [121, 122].

Whether individual YTHDF proteins adopted regulation of distinct steps of mRNA processing during evolution or whether they recognise complement of specific targets and trigger differential RNA processes responses in a context dependent manner remains to be determined.

A reader function for METTLs

In addition to their role as methyltransferases, METTLs can be stalled at site of methylation acting as readers and thereby regulating the expression of target genes. Accordingly, METTL16 acts as a reader when SAM concentrations are low. Under these conditions METTL16 remains bound to SAM synthetase MAT2A mRNA stimulating transcript splicing and export, in turn increasing the availability of SAM. In contrast, when SAM concentrations are high, METTL16 methylates MAT2A mRNA and will be released leading to intron retention and nuclear mRNA decay [75].

Likewise, METTL3 can act as a reader and positively regulate translation independently of its catalytic activity and of YTH domain containing proteins, as shown by tethering to a subset of oncogenic mRNAs [64]. METTL3 binds specific m^{6}A sites in proximity of the stop codon and interacts directly with Eukaryotic Initiation Factor 3 subunit h (eIF3h) at the 5' UTR, which recruits other factors for translation initiation and promotes circularisation of the mRNA for ribosome-recycling [123].

ELAV1, eIF3, IGF2BPs, FMRP: additional readers

Apart from the YTH domain containing proteins, other RNA binding proteins such as Embryonic, Lethal, Abnormal vision, *Drosophila* Homolog-like 1 (ELAV1) also known as human antigen R (HuR), eIF3, Insulin-like Growth Factor 2 mRNA-Binding Proteins (IGF2BPs), and Fragile X Mental Retardation Protein (FMRP), have been suggested to read the m^{6}A sites. Although these proteins lack the pocket necessary to discriminate between the N6-methylated or non-methylated status of the adenosine, they might essentially support binding of YTH domain containing proteins.

ELAV1/HuR is member of the ELAV/Hu RNA-binding proteins and was significantly associated to m^{6}A sites in pulldown experiments from nuclear extracts [9]. ELAV is the closest relative of Sxl, which is a neuronal RNA binding protein in non-drosophilid flies [124]. Thereby, ELAV might support YTH proteins in the recognition of m^{6}A [9].

FMRP interacts with WTAP and recently has been suggested to bind m^{6}A sites by competing with YTHDF2 to regulate specific mRNAs turnover, revealing a link between m^{6}A and intellectual disability such as Fragile X syndrome and autism [22, 125, 126].

Moreover, in response to heat shock conditions eIF3 binds m^{6}A containing mRNAs in the 5'UTR to recruit the 43S ribosomal complex stimulating translation initiation in a cap-independent manner [115]. Ablation of METTL3 negatively affects translation efficiency of cellular mRNAs containing m^{6}A in their 5'UTRs such as Hsp70 [23, 115]. Likewise, depletion of FTO stimulates cap-independent translation initiation of Hsp70 in response to heat shock increasing m^{6}A levels in its 5'UTR [9, 115].

In addition, pulldown experiments from nuclear extract suggest that IGF2BPs are also able to bind m^{6}A containing mRNAs [127].

ERASERS

The non-heme Fe(II)- and α-KG-dependent dioxygenase ALKB family of proteins mediates repair of alkylated nucleotides in DNA and RNA [128]. The protein family includes fat mass and obesity-associated protein (FTO) and ALKBH5 that act as RNA m^{6}A demethylases.

FTO was first identified in genome-wide studies as directly correlated with diabetes and obesity [129]. In mice its overexpression increases body mass causing obesity, whereas its depletion leads to postnatal growth retardation, defects in dopaminergic signalling pathway in neurons, and reduced body mass [130, 131]. FTO is conserved among eukaryotes, but it is absent in many species (Figure 3). Initial studies showed that FTO acts on m^{6}A sites in the body of the mRNA, but FTO also targets N^{6}2'-O-dimethyladenosine (m^{6}Am) on the first nucleoside adjacent to the cap [132].

FTO was also found overexpressed in some cancers such as AML where down-regulates the expression of specific mRNAs necessary for normal haematopoiesis [133].

ALKBH5 is a nuclear protein that differs from the other members of the ALKB protein family as it binds single stranded nucleic acids [134]. Ablation of ALKBH5 in HeLa cells modestly increased m^{6}A levels, whereas overexpression reduced significantly m^{6}A levels [134]. In addition, ALKBH5 deficient mice have

increased m^{6}A levels and show aberrant spermatogenesis and males are infertile [134]. Despite being member of the same protein family as FTO, ALKBH5 cannot demethylate m^{6}Am at the cap [132]. ALKBH5 is not broadly conserved as it was only found in vertebrates, *C. intestinalis*, *M. jakobidiformis* and *A. kergulense* (Figure 3). However, as it belongs to a large protein family with broad functionality, other related proteins may compensate for its absence.

Role of m^{6}A in gene expression

Role of m^{6}A in splicing regulation

A role of m^{6}A in splicing was suggested by the co-localisation of METTL3 with splicing factors in nuclear speckles in HeLa cells [36, 37, 42, 81].

In *Drosophila*, m^{6}A is essential for alternative splicing of Sxl, which regulates sexual differentiation and prevents dosage compensation in females. In particular, m^{6}A has been mapped near Sxl binding sites adjacent to the alternatively spliced male exon. YTHDC binds m^{6}A and supports Sxl in inhibiting the inclusion of this exon, which contains a premature stop codon that prevents Sxl expression in males [15].

In flies, m^{6}A regulates the alternative splicing of about 2% of the genes and the majority of m^{6}A sites are located in 5'UTRs. Here, m^{6}A in this way reduces the number of upstream AUGs, which will up-regulate translation [15].

METTL3 localises to site of transcription suggesting that it could play a broader role in splicing regulation [15]. Deposition of m^{6}A on nascent mRNAs modulates splicing kinetics and efficiency. More than half of m^{6}A sites maps to introns and is associated with slower processing and alternative splicing, whereas a significant fraction of m^{6}A sites are found near splice site junctions in exons and is associated with fast splicing kinetics [16].

The effect of m^{6}A on alternative splicing is mediated by YTHDC1, which interacts with the splicing factor SRSF3 to increase its ability to bind RNA and promotes exon inclusion. In contrast, interaction with SRS10 reduces its RNA binding ability and stimulates exon exclusion re-positioning of hnRNPC on mRNAs to promote the inclusion of alternatively spliced exons [73].

In humans m^{6}A has been shown to regulate the alternative splicing of MyD88 during the lipopolysaccharide (LPS)-induced inflammatory response of dental pulp cells promoting the expression of inflammatory cytokines, the NF-κB and the MAPK signalling pathways [135].

Role of m^{6}A in the regulation of alternative polyadenylation (APA)

Alternative polyadenylation (APA) regulates the generation of isoforms differing in the 3'end of mRNA and impacts on mRNA stability, nuclear export, mRNA localisation and translation [2] .

VIRMA has been suggested to mediate the recruitment of the writer complex to the 3' UTR and interacts with the subunits of the cleavage factor CFI, CPSF5 and CPFS6, in an RNA-dependent manner [88]. For instance, YTHDC1 interacts with CPSF5 and the splicing factors SRSF3 and SRSF7, which couple splicing of the last exon and polyadenylation to nuclear export [136]. SRSF3 and SRSF7 regulate APA in opposing manner as depletion of SRF3 results in shorter 3' UTRs, whereas knockdown of SRSF7 leads to longer 5' UTRs [137].

In mice male germ cells knockout of ALKBH5 results in aberrant splicing and in the production of longer 3' UTRs [138]. In addition, depletion of m^{6}A in human adenocarcinoma lung cells is associated with the switching of alternative polyadenylation sites from distal to proximal resulting in shorter 3' UTRs in a subset of transcripts [139].

In contrast, m^{6}A sites in the last exon promote the use of proximal polyadenylation site resulting in shorter 3' UTR in human embryonic cells and B-cell lymphoblastoid cells [140].

These findings suggest that the effect of m^{6}A on APA in the choice between distal and proximal polyadenylation sites is likely context dependent and cell-type specific.

m^{6}A enhances nuclear export

Co-knockdown of WTAP and VIRMA results in block of the nuclear export for methylated mRNAs and m^{6}A is found significantly enriched in long internal exons [141].

The nuclear export of mRNAs involves the recruitment of several factors such as the TREX complex, the nuclear export factor 1:nuclear transport factor 2-like export factor 1 (NXF1:NXT1) heterodimer and most SR proteins . Adapter subunits of the TREX complex are responsible for the recruitment of NXF1:NXT1, which promotes the transport of the mRNA to the cytoplasm through the nuclear pore. ALYREF, the adapter subunit of the TREX complex, associates with the exon junction complex (EJC) to promote an efficient nuclear export [142].

However, long internal exons and 3' UTRs do not have an EJC in their vicinity. YTHDC1 can compensate for the absence of an EJC in these regions by binding nearby m^{6}A sites and mediate the recruitment of the TREX complex to promote nuclear export [141].

In fact, YTHDC1 interacts with the splicing factor SRSF3 and facilitates recruitment of NXF1 to mRNAs and contacts directly the TREX complex promoting the nuclear export [109, 141]. In addition, the m^{6}A methylosome component RBM15 directly interacts with NXF1 and its silencing reduces cytoplasmic m^{6}A containing mRNA levels [143].

Depletion of METTL3 negatively affects the nuclear export of specific clock factor mRNAs resulting in prolonged circadian rhythms [53]. In addition, ablation of ALKBH5 in mice increases m^{6}A levels in the nucleus. This mRNA export defects lead to infertility and compromised spermatogenesis [53, 134].

m^{6}A impacts on translation

Enrichment of m^{6}A was found in polysome-associated mRNAs and its presence increased translation efficiency of dihydrofolate reductase mRNAs by 1.5 fold compared to non-methylated transcripts [15, 114, 144, 145].

The effect of m^{6}A on translation efficiency is mediated by YTHDF1 that upon binding of m^{6}A interacts with the eukaryotic translation initiation factor eIF3 in the 5'UTR, which in turn recruit the 43S pre-initiation complex stimulating translation in a cap-independent manner [114, 115]. YTHDF3 interacts with YTHDF1 and has a synergistic effect on promoting translation by recruiting ribosomal proteins [121, 122]. In addition, METTL3 in the 3' UTR interacts with eIF3h bound to the translation start site to promote closed-loop conformation, stimulating translation through enhanced ribosome recycling [64, 123].

The m^{6}A modification plays a pivotal role in translation regulation in response to heat shock, which induces translation shut-down by decapping. In response to heat stress, heat shock protein mRNAs need to be translated and eIF3 is suggested to directly bind m^{6}A in the 5' UTR to promote translation initiation in a cap-independent manner [114, 115]. In this context, YTHDF2 re-localises to the nucleus where it binds specifically to m^{6}A sites in the 5' UTR of newly transcribed heat shock responsive mRNAs protecting transcripts from the FTO demethylase activity to stimulate cap-independent translation initiation [113].

Additionally, injuries in the nervous system stimulate local translation of mRNAs that regulate the axons regeneration upon YTHDF1 binding of m^{6}A sites [58].

In contrast, m^{6}A can also negatively impact on translation. In *Xenopus laevis,* oogenesis m^{6}A containing mRNAs show a decreased translation rate without changes in their stability [146]. Likewise, FTO is essential for stimulating local translation of mRNAs necessary for axon elongation as m^{6}A inhibits translation of specific mRNA in nervous system [147].

These findings illustrate that although m^{6}A affects protein synthesis by multiple mechanisms, their regulation depends on the cell type, development stage, and cellular context.

A role for m^{6}A in promoting mRNA decay

In mammalian embryonic stem cells, knock-down of METTL3 resulted in longer mRNA half-lives of a subset of transcripts [48, 50]. Likewise, YTH domain containing proteins play a pivotal role in mediating mRNA decay. Upon binding of m^{6}A sites, YTHDF2's residues 101-200 transiently interact with the CNOT1 subunit of the CCR4-NOT complex in P-bodies leading to the shortening of the mRNA poly(A) tail, which triggers mRNA degradation [43, 148].

In addition, YTHDC2 acts as an adaptor recruiting the cytoplasmic 5'-3' exonuclease Xrn1 via its ankyrin repeats on mRNA promoting rapid degradation [112, 149]. The YTHDC2 helicase activity is also essential for the decay of specific mitotic mRNAs for meiosis progression and completion meiotic program in mammalian germlines [111, 112]. Likewise, in zebrafish embryos, YTHDF2 is responsible for the decay of maternal mRNA containing m^{6}A facilitating the maternal-to-zygotic transition [118]. Moreover, in humans YTHDF3 interacts with YTHDF2 enhancing its positive regulatory effect on mRNA decay [121].

Role of m^{6}A in miRNA processing regulation

Apart from its presence on mRNA, m^{6}A is also deposited on miRNAs and plays roles in regulation of their expression [150]. Fluctuations of mRNA levels of the ribonuclease DROSHA, AGO2, and miRNA expression were found in human peripheral blood mononuclear cells (PBMCs). In young PBMCs, DROSHA and AGO2 mRNAs are enriched in m^{6}A sites and have high expression rates, whereas in old PBMCs reduced m^{6}A leads to a destabilisation of AGO2 mRNA. Fluctuations in miRNA expression levels are also observed between old and young PBMCs suggesting a negative impact of m^{6}A on miRNA expression by regulation of AGO2 mRNA decay during cellular senescence [151].

Upon acute heat shock, DGCR8 and METTL3 relocalise to stress responsive genes such as Hsp70 for stimulating mRNA degradation to control timing and magnitude of the response in mouse embryonic stem cells [152].

HRNPA2B1 recognises m^{6}A containing pri-miRNAs and interacts with the microRNA microprocessor complex protein DGCR8 that in turn recruits DROSHA for their processing [153]. The proapoptotic miRNA

miR-145 targets the 3'-UTR of YTHDF2 mRNA to suppress its expression, which result in a reduced mRNA degradation and increased m^{6}A levels [154].

The negative effect of miRNAs on mRNA translation occurs with delays as their turnover is slow and they remain in the cytoplasm exerting their function until they are degraded. In response to stimuli that require quick changes in translated transcripts, cells may use m^{6}A to evade miRNA-mediated repression. In fact, m^{6}A is deposited early on the transcript and can act instantly on site of transcription positively affecting translation, to antagonise to the role of miRNA.

Role of m^{6}A in lncRNA and its impact on transcriptional regulation

The m^{6}A modification is also found in lncRNAs such as the X-inactive specific transcript (XIST) involved in dosage compensation and the cancer-associated metastasis-associated lung adenocarcinoma transcript 1 (MALAT-1) [74, 155, 156]. The lncRNA XIST mediates gene silencing of the X-chromosome during female mammal development and XIST is highly decorated with m^{6}A [92]. Knockdown of RBM15 or METTL13 impairs X-inactivation indicating that m^{6}A is required for the function of XIST [92]. In fact, the methyltransferase complex targets specific sites of XIST promoting the deposition of m^{6}A. YTHDC1 reads the m^{6}A sites and in turn induces XIST-mediated gene silencing [92]. However, it remains to be determined how YTHDC1 mechanistically contributes to the repression of one of the X chromosomes. Interestingly, cross-linking analyses revealed that XIST also associates with METTL16, but the function of the METTL16-XIST complex is unknown [74].

In addition, the METTL16 homodimer interacts with the 3' region of MALAT-1 that adopts a triple helix secondary structure known as element for nuclear expression with a downstream A-rich tract (ENE+A) [74, 78, 79, 155]. A small amount of m^{6}A is found in the stem loop region of MALAT-1, but not in the ENE+A structure [155]. Whether MALAT1 is a substrate of the methyltransferase activity of METTL16 and the function of the METTL16-MALAT1 ENE+A complex remains to be determined.

During stem cells differentiation, m^{6}A has an indirect effect on transcription as it affects the stability of core factor Oct4 [48]. In contrast to the role of m^{6}A in silencing transcription of the inactive X chromosome via XIST, m^{6}A has also been found to have a direct and positive role on transcription. In fact, in AML METTL3 localises at the promoter of active genes via CEBPZ to facilitate transcription by altering chromatin structure [157]. Similarly, promoter-associated lncRNAs, such as Promoter upstream transcripts (PROMPTs), which impact gene expression, could be targets of m^{6}A methyltransferase activity of

METTLs [158]. Additionally, a slow RNA polymerase II leads to an increase of m^{6}A, possibly because the methylosome has more time to recognise its substrates [159].

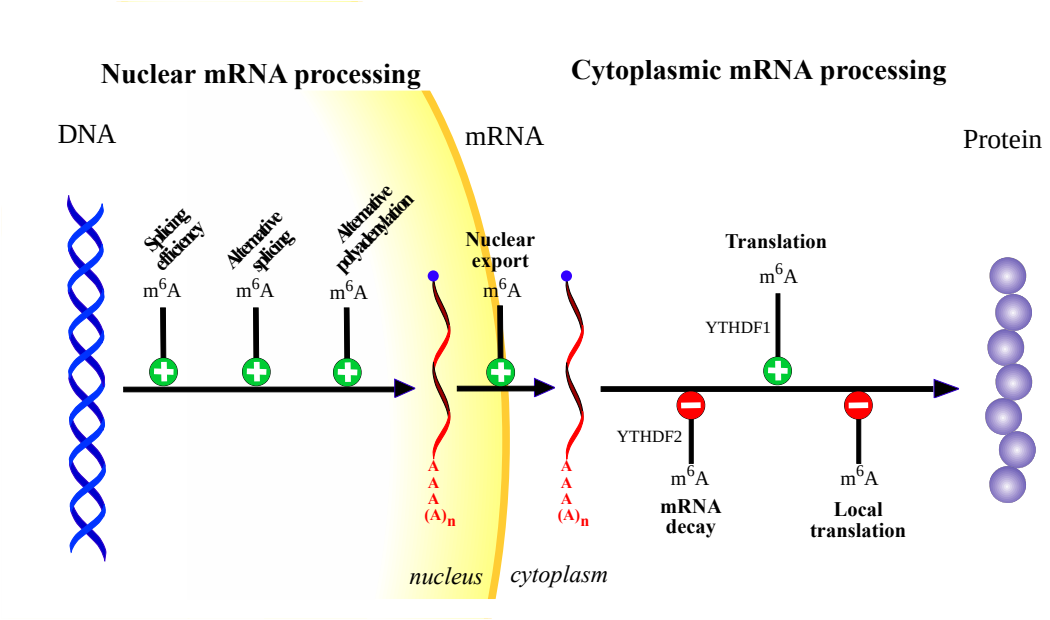

Figure 4. Impact of m^{6}A on the regulation of the gene expression. The m^{6}A modification positively regulates splicing efficiency, alternative splicing and polyadenylation during mRNA maturation, and promotes its nuclear export. In the cytoplasm m^{6}A sites are read by YTHDF1 enhancing translation, while YTHDF2 re-localises transcripts to P-bodies for mRNA degradation. Local translation of specific mRNAs is inhibited by m^{6}A methylation.

Concluding remarks

The central dogma of molecular biology introduced by Crick proposed that DNA is transcribed into mRNA in the nucleus and once exported to the cytoplasm is translated into proteins [1]. The model attributed little regulation to RNA, viewed only as a passenger to bring the genetic information to the cytoplasm. In the last decade it has become clear that m^{6}A contributes to the regulation of gene expression, impacting on all aspects of mRNA maturation, nuclear export, decay, and translation (Figure 4).

The deposition of m^{6}A is performed by a megadalton methylosome [36]. The enzymatic core of the complex is composed by the two methyltransferases METTL3 and METTL14. The methyltransferases are highly conserved in their MTD domain, but METTL14 seems catalytically inactive in animals, due to changes in key residues critical for the methyltransferase activity. In plants, however, METTL14 is likely active

because these residues are the same as METTL3 (Figure 2). The main readers of m^{6}A are the YTH domain containing proteins, but METTL16 and METTL3 can also act as readers in the cytosol.

An exhaustive phylogenetic analysis of all the component of the m^{6}A machinery points out that the methyltransferases METTL3 and METTL14 are always present, mostly together with WTAP. In addition, the presence of METTL4, in conjunction with readers in species lacking paralogs of METTL3 and METTL14 raises the question whether this protein can act as m^{6}A mRNA methyltransferase (Figure 3).

Moreover, the auxiliary components of the complex VIRMA, FLACC, RBM15, and HAKAI are not broadly conserved and are highly divergent. Interestingly, humans have evolved an additional paralog of HAKAI, the E3 ubiquitin-protein ligase ZNF645, which has similar roles in the regulation of endocytosis, but whether it is involved in m^{6}A methylation remains to be determined. VIRMA and HAKAI are highly divergent between plants and animals and not present in lower eukaryotes. However, the emergence of these proteins in both taxa could represent a case of convergent evolution as occurred for the origin of parental genomic imprinting [160].

The high conservation of the m^{6}A machinery between very distant organisms suggests that m^{6}A methylation was a regulatory mechanism present in the Last Eukaryotic Common Ancestor (LECA) and it has been mostly maintained among eukaryotes. The ancestral m^{6}A machinery was simpler than the extant one and was likely composed by one methyltransferase ancestor protein, one auxiliary ancient WTAP protein, and one ancestral YTH domain. The ancestral m^{6}A machinery evolved in the extant complex methyltransferase machinery recruiting additional proteins, multiple readers, and erasers that work together to regulate gene expression. Whether the m^{6}A machinery increased its complexity in higher eukaryotes to target specific mRNAs or to trigger specific cellular processes in response to stimuli remains to be determined.

ASSOCIATED CONTENT

Supporting Information 1. Alignment of METTL3 and METTL14 orthologs.

Supporting Information 2. Phylogenetic tree of the MT70 domain containing protein family.

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