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Review

A fly view on the roles and mechanisms of the m^{6}A mRNA modification and its players

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Key words

RNA modifications, m^{6}A, *Drosophila*, splicing, neurogenesis, *Sex lethal*

Abstract

RNA modifications are an emerging layer of posttranscriptional gene regulation in eukaryotes. *N*^{6}-methyladenosine (m^{6}A) is amongst the most abundant modifications in messenger RNAs (mRNAs) that was shown to influence many physiological processes from yeast to mammals. Like DNA methylation, m^{6}A in mRNA is dynamically regulated. A conserved methyltransferase complex catalyzes the deposition of the methyl group on adenosine, which can be removed by specific classes of demethylases. Furthermore, YTH-domain containing proteins can recognize this modification to mediate m^{6}A-dependent activities. Here we review the functions and mechanisms of the main m^{6}A players with a particular focus on *Drosophila melanogaster*.

Introduction

Epigenetic modifications regulate gene expression in response to changes in environmental cues. While the effect of DNA and chromatin modifications has been well studied, the role of RNA modifications on gene expression during organismal development and human disease is only starting to be unveiled. More than 100 modifications have so far been discovered; most of them were found on highly abundant RNAs such as transfer and ribosomal RNAs (*1*, *2*). One of the most prevalent modifications on mRNA is N^6-methlyadenosine (m^6A). Two independent groups developed a few years ago a method called MeRIP-seq (methylated RNA immunoprecipitation sequencing) enabling for the first time a global mapping of m^6A to the transcriptome (*3*, *4*). These studies revealed the presence of m^6A in more than ten thousand transcripts of mRNAs and long noncoding RNAs with enrichments around stop codons, in the 3' untranslated regions (UTRs) and within long internal exons as well as in alternatively spliced ones (*3*, *4*). A consensus sequence for m^6A was derived from these and subsequent genome-wide studies, which consists of RRACH where R represents purine and H a non-guanine base (*3-5*).

m^6A formation is catalyzed by a methyltransferase complex, composed of METTL3, METTL14 and WTAP (*6-10*) (Figure 1). METTL3 catalyzes S-adenosyl methionine (SAM)-mediated transfer of methyl group to N^6-position of adenosine base, while METTL14 is thought to be catalytically inactive with a structural role for facilitating METTL3 activity (*11-13*). WTAP, instead, appears essential to stabilize the interaction between the two METTL proteins (*7*). The three components of the core complex localize in nuclear speckles and recognize the previously derived consensus sequence RRACH (*6*, *14*). However, since only a subset of sites is methylated *in vivo*, additional subunits of the complex, such as KIAA1429 and RBM15, have been proposed to function in guiding the methylation to targeted sites (*15*).

The m^6A modification was shown to modulate several physiological processes by regulating many aspects of mRNA processing, including splicing, mRNA decay and translation (for a recent review see (*16, 17*)). Most of these functions are mediated by members of the YTH-domain family of proteins, which specifically recognize modified adenosines and serve as m^6A readers (*3, 18, 19*). Vertebrates have five and plants have thirteen members of YTH domain proteins, while only two members of the YTH domain family proteins exist in *Drosophila*; the nuclear YT521-B and the yet uncharacterized cytoplasmic protein CG6422 (*20-22*). Both *Drosophila* proteins share high homology to their human counterparts in the YTH region, but low degree of similarities are observed outside the YTH domain.

m^6A is dynamically regulated in mammals as it can be reverted by oxidative demethylation via the activity of two demethylases, FTO and ALKBH5. Their differential specificity towards m^6A may rely on distinct temporal and spatial expression (*23, 24*) as well as on the sequence context surrounding the methylated adenosine (*25*). Accordingly, FTO has recently been shown to act primarily on N6-methylated adenosine that is introduced at the first position after the cap (*26*).

Our recent work uncovers the *in vivo* roles of m^6A in *Drosophila melanogaster* and identifies several major regulators. Some of these regulators bear distinct functions, suggesting that they also have m^6A-independent roles. Here we summarize our current knowledge about the m^6A players in *Drosophila*, and compare their functions with homologs in other species.

The m^6A methyltransferase complex

- **Ime4**

The corresponding homologue of METTL3 (or MTA in plants) in *Drosophila* is IME4 (Inducer of meiosis 4 in yeast) (Figure 2), which was the first subunit of the methyltransferase complex shown to bind S-Adenosyl methionine (SAM) and to transfer the methyl group from the SAM donor to N^6-position of adenosine (*27*). A phylogenetic analysis of METTL3 revealed that

many species ranging from yeast to human contain a conserved protein (*28*), which plays essential roles in development, but apparently is absent in fission yeast *S. pombe* and *C. elegans* (20). Sporulation is affected in budding yeast *S. cerevisae* that lack Ime4 (*29*) and abnormal growth and seed development is observed in *A. thaliana* upon loss of MTA (*30, 31*) (Table 1). Knock out of METTL3 prevents naïve embryonic stem cells to differentiate and leads to early embryonic lethality in mice (*32*). In zebrafish, METTL3 is enriched in brain regions and its depletion leads to increased apoptosis in embryos (*8*). In contrast, *Drosophila Ime4* knock out flies survive to adulthood, but are flightless (*21, 22*). In addition, they display severe locomotion defects in orientation, walking speed and activity due to impaired neuronal functions. Also, defects in oogenesis due to altered Notch signaling have been reported (28). Intriguingly, female *Ime4* mutant flies show altered splicing of *Sxl*, the master regulator of sex determination and dosage compensation in flies. In the soma, Sxl determines female physiognomy through regulation of alternative splicing of *transformer* (*tra*) and prevents dosage compensation in females by inhibition of male-specific lethal 2 (msl-2) (*33*). In addition, Sxl is also required to initiate germ cell differentiation, but this pathway is independent of the sex determination pathway mediated by Tra. Consistently, genetic interaction between *Ime4* and *Sxl* mutants show increased female lethality during development due to compromised dosage compensation and sexual transformations in the absence of msl-2. Furthermore, female germ cell differentiation defects were observed revealing a fine-tuning function of the m^6A modification in the sex determination pathway. Initial links to the sex determination pathway have further been indicated by the interaction of Arabidopsis MTA with Fip37, the homologue of Fl(2)d in flies, previously identified to play a role in *Drosophila* sex determination (*30*) (see below).

Ime4 is a 68 kilodalton (kDa) protein with a nuclear localization signal. Ime4 co-localizes extensively with RNA Pol II on *Drosophila* polytene chromosomes suggesting global

co-transcriptional deposition of m^6A (*21*). However, m^6A levels in *Drosophila* mRNAs measured by dot blot and LC-MS/MS analyses are low, indicating that more methyl groups might initially be incorporated but then be removed by intron removal or demethylases. Alternatively, the methyltransferase complex, despite being widely present on chromosomes, might be active only under certain conditions. MeRIP-seq analysis with mRNA from *Drosophila* S2 embryonic cells also points towards lower m^6A levels compared to vertebrates as around one thousand putative m^6A sites were identified in *Drosophila* compared to at least ten thousands sites identified in various vertebrate cells (*3, 4, 22*). Whether m^6A modification is more abundant and plays more prominent roles in *Drosophila* neuronal cells remains to be investigated. Nevertheless the consensus RRACH was present in most of the m^6A peaks, and enrichment near start and stop codons was also observed, suggesting conserved functions and regulatory mechanisms.

Intriguingly, a novel function for mammalian METTL3, independent of its m^6A catalytic activity, was recently found. A fraction of METTL3 localizes in the cytoplasm and was shown to promote translation of a subset of RNAs containing m^6A peaks in 3'UTRs by interaction with the eIF3b subunit of the translation initiation complex (*34*). Whether *Drosophila* Ime4 has a similar role in the cytoplasm remains to be investigated.

- **Mettl14**

The corresponding homologue of METTL14 in *Drosophila* is Mettl14 (Figure 2). Mettl14 is an essential component of the methyltransferase complex and contains, like IME4, a catalytic domain (*7-10*). However, recent structural studies revealed that steric constraints from side groups near the putative SAM binding pocket prevent METTL14 to accommodate SAM and is therefore catalytically inactive (*11-13*). Rather, METTL14 was shown to stabilize the interaction between the methyltransferase complex and RNA by forming a charged grove at the interface of METTL3 and METTL14 for RNA accommodation (*11-13*). These studies therefore

indicate that METTL3 requires METTL14 for its activity. *Drosophila* Mettl14 shares a 62 % identity with human ortholog and sequence comparison shows that side chains, which prevent accommodation of SAM in METTL14 are conserved. Both proteins lack aromatic residues that interact with acceptor adenine as well as residues that enable formation of hydrogen bonds with SAM in METTL3 protein. Loss of function of *Mettl14* in *Drosophila* is reminiscent to the loss of function of *Ime4*, suggesting that they act together (*21, 22*) (Table 1). Furthermore, quantitative mass spectrometry analysis revealed an interaction at a 1 to 1 ratio and show that the stability of both proteins depends on each other (*22*). Interestingly, the ortholog of METTL14 in yeast, Kar4, can bind DNA and possesses transcriptional activity (*35*). Whether this function is related to m^6A methylation and whether similar functions linked to transcription exist for METTL14 in other species is currently unknown.

- **Fl(2)d**

FEMALE-Lethal(2)D is the ortholog of mammalian WTAP (Wilm's tumor 1 associated protein), a nuclear protein that was found to interact with splicing factors and other proteins involved in RNA processing (*36*) (Figure 2). Its localization to nuclear speckles depends on the presence of BCLAF1 and THRAP3 (24). WTAP was initially found in a yeast two-hybrid screen to identify interactors of Wilms` tumor-1 protein (WT1) (*14*). *WT1* encodes several protein isoforms that can either interact with DNA and acts in transcription, or binds RNA and co-localizes with splicing factors (*37*). Intriguingly, isoforms binding to RNA are required for sex determination in mice, since male mutants lacking these isoforms undergo sex reversal due to reduced levels of sex-determining region Y (SRY) protein (*38*). Whether m^6A and WTAP are involved in this function remain to be addressed. WTAP knock out mice show embryonic lethality and defects in cell cycle progression (*36, 39*) (Table 1). *Drosophila* Fl(2)d also colocalizes with a number of splicing factors in the nucleus and regulates m^6A levels (*22, 40*). Accordingly, its expression pattern strictly correlates with the level of m^6A during development,

supporting the notion that, in *Drosophila,* m^6A metabolism is primarily dependent on the presence of a functional methyltransferase complex, and less so from potential demethylases (see below). Its depletion strongly compromises the interaction between METTL3 and METTL14 (*22*).

Fl(2)d was among the first proteins identified to be required for sex-specific alternative splicing of *Sxl* and *tra* (*41, 42*). In contrast to *Ime4* and *Mettl14*, *fl(2)d* is essential during development and analysis of sexual mosaics showed male somatic transformations in females, which is also observed in transheterozygous *Ime4, sxl* female mutants made viable by the lack of msl-2 (17). Lethality of *fl(2)d* mutant females thus suggests other roles, independent of its activity within the methyltransferase complex. In line with these observations, depletion of WTAP in zebrafish also causes more severe developmental defects compared to the loss of METTL3 (*8*). Furthermore, gel filtration experiments indicate that human WTAP co-fractions with METTL3 and METTL14 at a size of 300 kDa, but is also present at a higher molecular weight, supporting its association in distinct complexes (*7*).

fl(2)d encodes for two isoforms generated via alternative 5'splice site selection in the 5'UTR. A long isoform contains an N-terminal histidine and glutamine rich region, found in many transcription factors (*43*). This isoform interacts with Sine Oculis (So) to control retinal development (*44*). Interestingly, *fl(2)d* splicing is regulated via m^6A located near the proximal splice site. Depletion of methyltransferase complex components leads to increased usage of the distal splice site and formation of the long protein isoform, but whether this impacts on m^6A methylosome activity is currently unknown (*22*).

- **Virilizer**

Virlizer (Vir) is the ortholog of KIAA1429, which was found in a mass spectrometry-based approach as an interacting protein of the core components of the methyltransferase complex (Figure 2). Its depletion severely reduces m^6A levels on mRNA (*9, 22*). Vir is a large nuclear

protein of 1854 amino acids, and like Fl(2)d has essential functions as null mutants are lethal. Like Fl(2)d, Vir is also required for female specific alternative splicing of *Sxl* (*45, 46*) (Table 1). In *vir* female mutants, ectopic expression of Sxl is sufficient to rescue female lethality. The precise role of Vir and its human homologue in the context of m^6A biogenesis is currently unknown.

- **Spenito**

Spenito (Nito) has two orthologs in mammals: RBM15 and RBM15b (Figure 2). Mice lacking RBM15 die at embryonic day 9.5 and display defects in heart, spleen, vasculature as well as in hematopoiesis, B-cell and megakaryocyte differentiation (*47-49*) (Table 1). A well-characterized chromosomal aberration involving RBM15 and Megakaryoblastic leukemia 1 (MKL1) is associated with acute megakaryoblastic leukemia (*50*), demonstrating also the pivotal role of RBM15 in cancer. In *Drosophila*, Nito promotes Wingless signaling (*51*) and its overexpression in the eye leads to defects in photoreceptor development (*52*), while its depletion in ovaries results in stem cell tumor appearance (*53*). The loss of Nito affects *Sxl* splicing and gives rise to male somatic transformations, which is in agreement with the role of other m^6A components in sex determination (*53*). Furthermore, Nito interacts with subunits of the methyltransferase complex and its depletion drastically decreases m^6A levels (*22*). Interestingly, RBM15 was also recently found to regulate m^6A levels in human cells and to control X-chromosome inactivation for dosage compensation in female cells via m^6A-methylation of *XIST*, which in turn promotes transcriptional repression of the inactive X chromosome (*15*). RBM15 binds near m^6A sites on *XIST* mRNA and on other transcripts and is predicted to recruit the methyltransferase complex to its target transcripts. RBM15 interacts with RNA directly via its RRM domains and was also shown to interact with the Setd1b protein, an H3K4me3 histone methlyltransferase via its SPOC domain. The importance of the individual motif for RBM15/Nito function in regards to m^6A activity is currently unknown.

Demethylases

In vertebrates, methylation of adenosine is reversible due to the activity of two demethylases, namely FTO and ALKBH5 (*24, 54*). FTO demethylates m^6A through N^6-hydroxymethyladenosine (hm^6A) and N^6-formyladenosine (f^6A) intermediates (*55, 56*). A recent study showed that FTO preferentially acts on N6,2'-O-dimethyladenosine (m6Am) modification adjacent to mRNA cap, which in turn negatively affects mRNA stability (*26*). FTO loss of function leads to postnatal growth retardation, altered locomotor activity, defects of signaling in dopaminergic neurons and reduced fat mass (*23, 57-62*). Likewise, over-expression of FTO results in obesity (*63*). Another m^6A demethylase, ALKBH5, was later found to play a role in male fertility (*24*). In contrast to vertebrates, the specificity of m^6A occupancy in yeast seems to be determined by the restricted expression of the methyltransferase complex (*64*). Similarly, alignment of FTO and ALKBH5 nucleotide sequences to the *Drosophila* genome failed to identify homologs in flies. FTO appearance is concomitant to the vertebrate clade, with the exception of homologs present in diverse marine eukaryotic algae (*65*). Despite the fact that ALKBH5 is also absent, additional members of the ALKBH family that localize into the cytoplasm are present. However, depletion of these candidates, either individually or in combination, has no consequence on the m^6A/A ratio (Lence et al, unpublished data), indicating that these factors are not functional in flies in regards to their ability to demethylate m^6A on mRNA. Additional studies will be necessary to address whether other unknown demethylases are required to fine-tune m^6A levels in this organism.

m^6A binding YTH proteins

- **YT521-B**

YT521-B is the closest ortholog of YTHDC1 (Figure 2). The YTH domain was initially found as an RNA binding domain recognizing the hexanucleotide GCAUAC sequence, based on *in*

vitro SELEX experiments (*18*). More recently, proteins of the YTH-domain family were recognized as specific binders of m^6A RNA modification (*3*). A number of crystal structures revealed the mechanism of this binding by the hydrophobic pocket and aromatic residues (*19, 66-68*). A 50-fold increase in binding to methylated in comparison to non methylated residues was observed (*19*). YTHDC1 is localized in the nucleus and is involved in splicing regulation via m^6A-in long exons. This mechanism involves the YTHDC1-mediated recruitment of the splicing regulator SRSF3 and the exclusion of SRSF10 (*69*). YTHDC1 was also recently shown to induce X chromosome inactivation in human via binding to m^6A on *Xist* RNA (*15*). The precise mechanism of YTHDC1 in this process is currently unclear. Interactome studies indicate its association with members of Polycomb group complexes, suggesting that YTHDC1, via its ability to recognize m^6A, may facilitate the binding of gene-silencing proteins to *Xist* RNA. The sub-nuclear distribution of YTHDC1 is controlled via its association with the KH-domain containing Sam68 protein and this interaction is abolished upon YTHDC1 phosphorylation by $p59^{fyn}$ kinase (*70, 71*). In *Drosophila*, YT521-B is enriched in the embryonic neuroectoderm and in heads of adult flies (*22*). It localizes to the nucleus and specifically binds m^6A-modified transcripts (*21, 22*). In particular, YT521-B assists Sxl in repressing inclusion of the male-specific alternative exon by binding to nearby intronic m^6A sites (*21*). Consistent with the observation in mammals, *Drosophila* YT521-B regulates most of m^6A-dependent splicing events (about 60 to 70 % overlap with Ime4). Intron retention and alternative splicing in 5'UTRs are overrepresented. This regulation influences the number of upstream AUGs in 5'UTRs, suggesting that m^6A-regulated alternative splicing affects translation. Furthermore, YT521-B appears to be the main mediator of m^6A-dependent processes *in vivo*, as flies lacking a functional YT521-B resemble the phenotypes observed in mutants for methyltransferase complex components (*21, 22*). Using SILAC-based proteomic analysis of YT521-B, a number of potential interactors were found, which includes many

predicted mRNA binding proteins, such as the KH-domain containing Quaking related-family proteins, Hrb27C, Syp, Imp and others. The relevance of these interactions, however, awaits further validations.

- **CG6422**

The closest vertebrate ortholog of CG6422 is YTHDF2 (Figure 2). YTHDF2 is a cytoplasmic protein that belongs to the YTH-domain containing family of proteins. It was identified as an m^6A binding protein in a pull-down experiment with a methylated probe (*3*). Its role in mRNA decay was the first study providing a functional mechanism of m^6A modification in the mRNA life cycle (*72*). YTHDF2 binds m^6A predominantly in the 3'UTR of mRNAs via its C-terminally located YTH domain. Its N-terminal Glutamine/Proline-rich region interacts directly with CNOT1, a component of the CCR4-NOT deadenylase complex, guiding methylated mRNAs to processing bodies (*73*). This function appears important in human embryonic cells to degrade mRNA encoding pluripotency factors, eventually allowing for differentiation (*32*, *74*). Likewise, in zebrafish, YTHDF2 is required for maternal mRNA clearance during the maternal to zygotic transition (*75*). Interestingly, YTHDF2 was shown to re-localize to the nucleus under stress conditions and to specifically bind m^6A in the 5'UTRs of the newly transcribed mRNA. This binding protects m^6A sites from FTO-mediated demethylation, which in turn enhances cap-independent translation of heat shock responsive transcripts (*56*). Further m^6A can also serve on its own to direct cap-independent translation. eIF3 was shown to directly bind m^6A in the 5'UTR of mRNA upon UV-irradiation or heat-shock conditions, allowing the recruitment of the 43S pre-initiation complex independently of the m^7G cap modification (*76*). Hence, translation of stress-induced transcripts is enabled by this mechanism when translation of other cellular transcripts is shut down. YTHDF1 is another cytoplasmic YTH-domain containing protein in vertebrates that binds m^6A around stop codons and in the 3'UTR. YTHDF1 was shown to enhance protein production by direct interaction with eIF3 (*76*).

Recently, a third protein, YTHDF3, was shown to act cooperatively with YTHDF1 and YTHDF2, to promote translation and mRNA decay, respectively (*77, 78*). In *Drosophila*, CG6422 localizes in the cytoplasm but does not re-localize to the nucleus upon heat shock or under UV-irradiation (Lence et al, unpublished data). Consistent also with a possible role in the maternal to zygotic transition its expression is high in the first two hours of embryogenesis and decreases during development (*22*). Its function in the context of m^6A and potential roles in translation and /or mRNA decay await further investigation.

Conclusion and future directions

With the advance of novel techniques, m^6A was found on thousands of mRNA sites in several species. Several recent studies demonstrated its role in nearly all aspects of mRNA processing, via a group of YTH-domain family proteins, but also by altering the binding of some RNA interacting proteins to their recognition sites via m^6A-mediated changes in RNA secondary structure or "RNA switches" (*10, 79*). The players that catalyze, remove and recognize the modification are conserved across evolution, although with exceptions.

While the precise molecular function of the core methyltransferase complex, including METTL3, METTL14 and WTAP is almost solved; the role of the other co-factors remains less understood. KIAA1429 and its fly homolog Vir, interact with other components of the methlyltransferase complex and regulate m^6A levels, but their molecular functions are currently unknown. RBM15 binds near methylated sites and its absence prevents recruitment of the methlytransferase complex to its targeted sites. While this model provides an elegant explanation on why only a subset of m^6A sites is methylated, it remains to be explored whether RBM15 is sufficient to recruit the complex and to induce m^6A or whether other players are also involved? Intriguingly, RBM15 was shown to interact with chromatin binding proteins via its SPOC domain and has the ability to influence histone marks. Whether RBM15 provides a link between methylated RNA and the chromatin state is therefore an attractive possibility. In fact,

it is likely that m^{6}A deposition happens co-transcriptionally, as m^{6}A regulates splicing and several sites were found in introns (*3*, *21*). Thus, future research will reveal the dynamics of m^{6}A deposition and its distribution in relation with transcription and chromatin features.

Loss of components of the *Drosophila* methyltransferase leads to neuronal and sex determination defects, but how m^{6}A precisely regulates these processes remains to be determined. It will be interesting to examine whether other m^{6}A functions could be revealed upon stress conditions or in a sensitive context when the dosage of important players involved in specific physiological processes is reduced? Intriguingly, the loss of Ime4 and Mettl14 give rise to milder phenotypes compared to the depletion of the other complex components such as Nito, Fl(2)d and Vir. This suggests m^{6}A-independent functions for these subunits, or else, they may work with distinct m^{6}A methyltransferases.

Once the RNA is methylated it can be recognized by a different set of reader proteins and/or the methylation can be removed by the activity of demethylases. Several questions on how selectivity is achieved remain open. Likely, competition exists at individual m^{6}A sites for various proteins to bind and protect the m^{6}A or to remove it. In addition, binding of other RNA binding proteins such as hnRNPC or ELAV/Hu family proteins might be regulated by m^{6}A RNA switches and/or concomitant binding of YTH proteins (*3*, *79*, *80*). Therefore, sequence context for m^{6}A sites is a key to which proteins will bind and which regulatory program will be initiated; as for example, distinct members of the YTHDF proteins can initiate translation or mRNA decay. Overall, the fate of each m^{6}A-modified mRNA should take into consideration the "place and time" including the m^{6}A position within mRNA, the sequence context, the expression levels of m^{6}A regulators as well as the cell type and its developmental stage. Therefore, we foresee that the study of single gene reporters will bring additional mechanistic insights into these questions.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

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Table 1: Components of the m^{6}A methyltransferase complex and their biological roles

METTL3		
Human	METTL3	• METTL3 KD leads to circadian clock period elongation (*81*)
		• METTL3 promotes translation independently of its catalytic activity (*34*)
		• METTL3 KD prevents differentiation of hESC (*74*)
Mouse	Mettl3	• Mettl3 KO in naïve mESC leads to hyper naïve ground state, while in primed mESC boost cell differentiation (*32*)
		• Mettl3 KO leads to embryonic lethality (*32*)
Zebrafish	METTL3	• Morpholino depletion leads to developmental defects during embryogenesis (*8*)
A. thaliana	MTA	• MTA disruption results in embryonic lethality (*30*)
		• MTA reduction leads to various developmental and organ definition defects (*31*)
S. cerevisiae	Ime4	• Ime4 is required for sporulation and meiosis (*64, 82*)
D. melanogaster	Ime4	• Ime4 inactivation leads to defects during oogenesis (*83*)
		• Ime4 KO affects fly locomotion due to impaired neuronal functions (*21, 22*)
		• Ime4 regulates splicing of *Sxl* and fine tunes sex determination (*21, 22*)
METTL14		
Human	METTL14	• Structural component of the methyltransferase complex (*7, 8, 11-13*)
S. cerevisiae	Kar4	• Transcriptional activator required for karyogamy (*35, 84*)
D. melanogaster	Mettl14	• Mettl14 knock out affects fly locomotion due to impaired neuronal functions (*22*)

		• Mettl14 regulates splicing of *Sxl* and fine tunes sex determination (*21, 22*)
WTAP		
Human	WTAP	• Structural component of the mehyltransferase complex required for METTL3-METTL14 stabilization (*7, 8*)
Mouse	WTAP	• WTAP KO results in early embryonic lethality (*39*)
Zebrafish	WTAP	• *WTAP* morpholinos display defects in head and brain development (*8*)
S. cerevisiae	Mum2	• Mum2 is required for meiotic mRNA methylation as part of MIS complex (Mum2, Ime4, Slz1) (*82*)
D. melanogaster	Fl(2)d	• Fl(2)D is required for splicing of *Sxl* and its KO leads to embryonic lethality (*41*)
		• Fl(2)d controls retinal development (*44*)
		• Structural component of the methyltransferase complex, required for Ime4-Mettl14 stabilization (*22*)
RBM15, RBM15B		
Human	RBM15, RBM15B	• RBM15 fusion with MKL1 is associated with acute megakaryoblastic leukemia (*50*)
		• RBM15 and RBM15B are components of the methyltransferase complex, responsible for complex recruitment to targeted sites (*15*)
Mouse	RBM15, RBM15B	• Loss of RBM15 leads to embryonic lethality (*47*)
		• RBM15 controls hematopoiesis, B-cell and megakaryocyte differentiation (*47-49*)
D. melanogaster	Nito	• Nito regulates wingless signaling and photoreceptor development (*51, 52*)
		• Nito is required for splicing of *Sxl* (*53*)

		• Component of the methyltransferase complex (*22*)

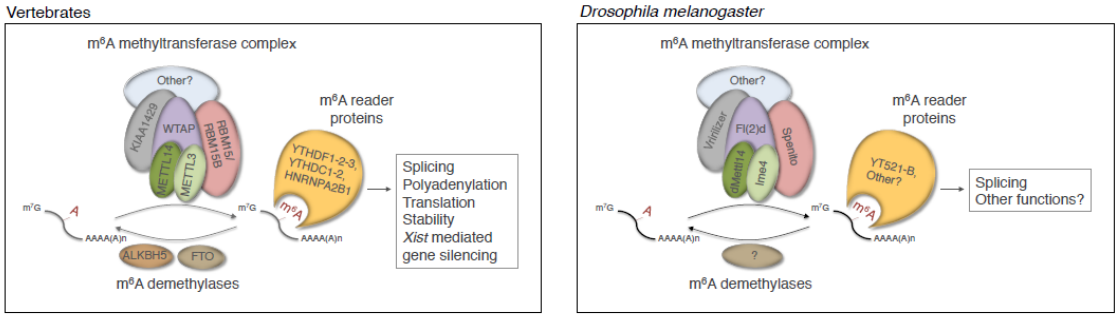

Figure 1: m^{6}A mRNA pathway in vertebrates and *Drosophila melanogaster*. The m^{6}A methyltransferase complex is composed of five factors. In *Drosophila*, the methyltransferase complex controls neural development and sex determination via its nuclear reader YT521-B. The precise mechanisms of Virilizer and its vertebrate homolog remain to be identified. No demethlyase has been identified so far in *Drosophila*.

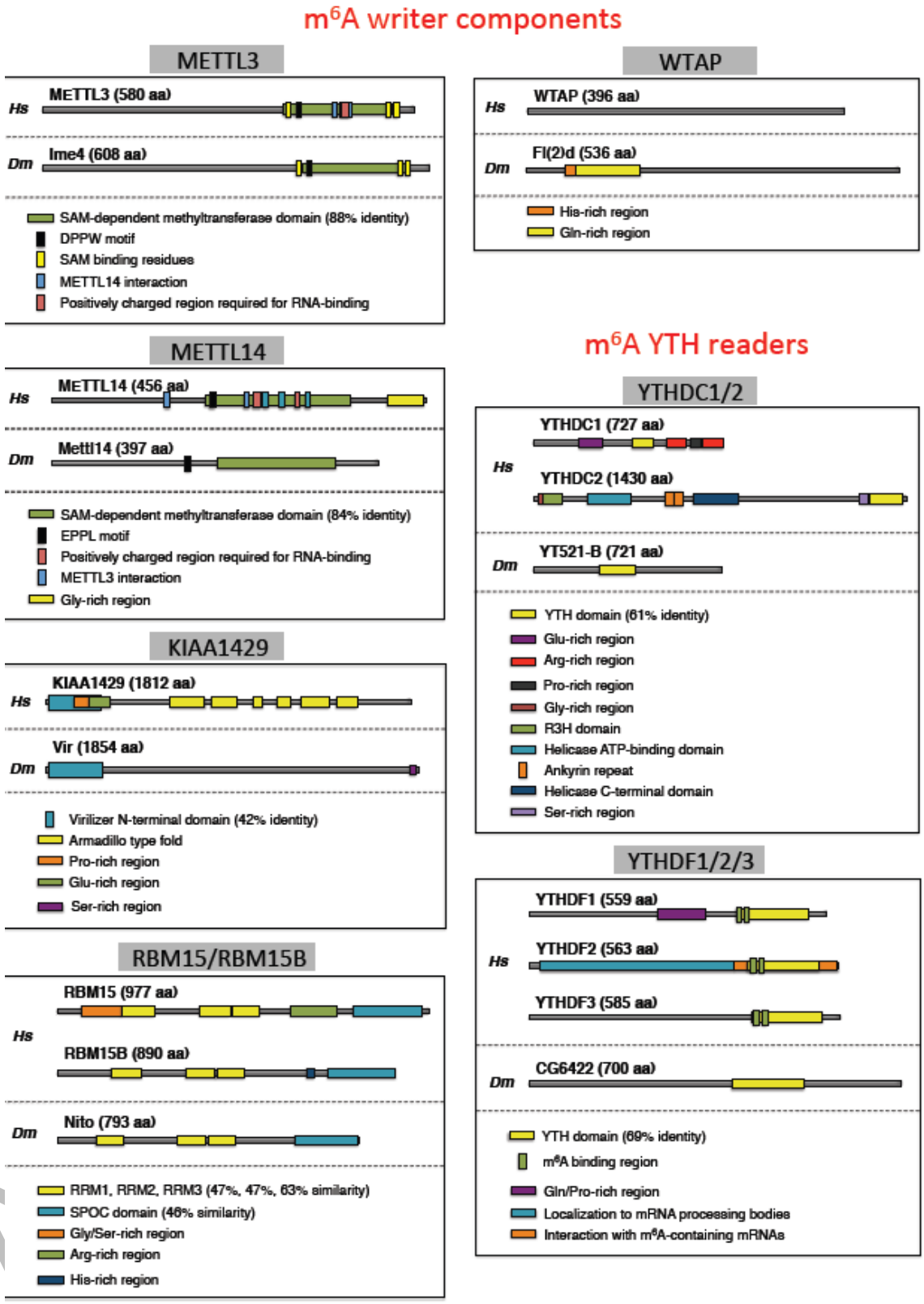

Figure 2: Domain-structure comparison of m^{6}A writers and readers between *Drosophila* and Human. Comparison of the different proteins was based on uniprot (www.uniprot.org). Homology between similar domains was analyzed via protein BLAST from NCBI. Individual

domains of Spenito were compared with RBM15; YT521-B with YTHDC1 and CG6422 with YTHDF2.