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Division of labor in epitranscriptomics: What have we learnt from the structures of eukaryotic and viral multimeric RNA methyltransferases?

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Division of labor in epitranscriptomics :

What have we learnt from the structures of eukaryotic and viral ? multimeric RNA methyltransferases.

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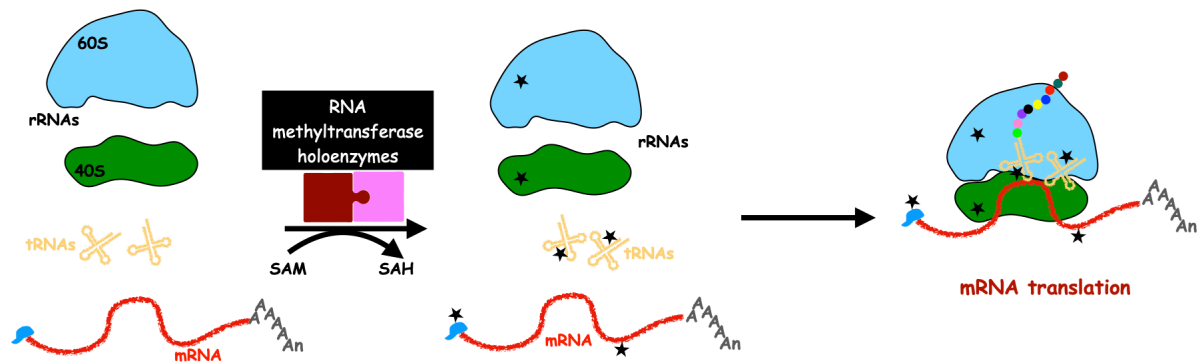
Marc GRAILLE*, ORCID ID : 0000-0002-7853-5852, Laboratoire de Biologie Structurale de la Cellule (BIOC), CNRS, Ecole polytechnique, IP Paris, F-91128 Palaiseau Cedex, France, marc.graille@polytechnique.edu, no conflict of interest to declare.

Abstract

The translation of an mRNA template into the corresponding protein is a highly complex and regulated choreography performed by ribosomes, tRNAs and translation factors. Most RNAs involved in this process are decorated by multiple chemical modifications (known as epitranscriptomic marks) contributing to the efficiency, the fidelity and the regulation of the mRNA translation process. Many of these epitranscriptomic marks are written by holoenzymes made of a catalytic subunit associated with an activating subunit. These holoenzymes play critical roles in cell development. Indeed, several mutations being identified in the genes encoding for those proteins are linked to human pathologies such as cancers and intellectual disorders for instance. This review describes the structural and functional properties of RNA methyl-

transferase holoenzymes, which when mutated often result in brain development pathologies. It illustrates how structurally different activating subunits contribute to the catalytic activity of these holoenzymes through common mechanistic trends that most likely apply to other classes of holoenzymes.

Graphical/Visual Abstract and Caption



Caption : The catalytic subunits (wine red) of several RNA methyltransferases acting on factors involved in protein synthesis require a tight partnership with activating subunits (pink) to be functional. Methyl groups are shown as black stars.

1. INTRODUCTION

Protein synthesis is a highly complex process regulated at the transcriptional and translational levels to rapidly modulate protein production and to allow cells to adapt during the cell cycle. The RNAs (tRNAs, mRNAs and rRNAs) involved in translation are subject to multiple and various chemical modifications (known as epitranscriptomic marks), which participate to the efficiency, the fidelity and the regulation of the translation process. The importance of these post-transcriptional modifications has long been underestimated due to the absence of obvious phenotypes upon inactivation of many of the genes encoding for RNA modification enzymes in various model systems (*E. coli*, *S. cerevisiae* or human cells). However, it is now clear that they constitute an important, complex and tunable layer in the regulation of gene expression. The recent development and constant improvements of highly sensitive methods (next-generation sequencing, ribosome profiling, mass spectrometry, ...) as well as genome-wide association studies on patients, have revived interest for epitranscriptomic marks. The RNA modifications and their writing machines are now

clearly considered as key factors in the development of various human organs or in disease etiology (Barbieri & Kouzarides, 2020; Madugalle, Meyer, Wang & Bredy, 2020; Sarin & Leidel, 2014; Torres, Batlle & Ribas de Pouplana, 2014). In particular, neurological development emerges as highly sensitive to translation defects as demonstrated by the increasing number of mutations identified in RNA modification genes in patients suffering from intellectual disabilities (IDs, (Angelova et al., 2018; Arrondel et al., 2019; Braun et al., 2017; Livneh et al., 2020; Madugalle, Meyer, Wang & Bredy, 2020; Sarin & Leidel, 2014; Torres, Batlle & Ribas de Pouplana, 2014)). Most of these modifications are catalyzed by evolutionarily conserved enzymes that have gained in complexity from mitochondria and bacteria (mostly stand-alone enzymes) to eukaryotes (multi-protein complexes), the situation in archaea being intermediate depending on the considered enzymes. For instance, the universally conserved t⁶A (N⁶-threonyl-carbamoyl) tRNA modification present at position 37 of nearly all tRNAs decoding ANN codons is catalyzed by the sole Tcs4/Qri7 protein in mitochondria, by three proteins (TsaB, TsaD and TsaE) in bacteria and by the KEOPS complex in archaea (4 subunits) and eukarya (5 subunits; (Thiaville, Iwata-Reuyl & de Crecy-Lagard, 2014)). Other examples illustrating the importance of RNA modifications are the convergent evolution of structurally different methyltransferases (MTases) to form the same modified nucleotide on a specific tRNA position (Kimura, 2021). This is indeed the case for the bacterial TrmD, a member of the SPOUT trefoil knotted fold family (for SpoU-TrmD), and for the eukaryotic and archaeal class I MTases Trm5, which all catalyze formation of m¹G³⁷ (Goto-Ito, Ito & Yokoyama, 2017). The same is true for the bacterial TrmL SPOUT MTase and for the eukaryotic FTSJ1/WDR6 complex, which both catalyze the S-adenosyl-L-methionine (SAM) dependent formation of Nm at position 34 in tRNA^{Leu} (Guy et al., 2012; Pang, Deng, Wang & Xie, 2017). Even more striking is the case of the m⁵U₅₄ (or ribothymidine) tRNA modification. In eukaryotes and many Gram-negative bacteria, this modification is formed by the class I SAM-dependent MTases Trm2 and TrmA, respectively (Nordlund, Johansson, von Pawel-Rammingen & Bystrom, 2000) while in Gram-positive bacteria and mycoplasma species, it is generated by the flavin-dependent TrmFO enzyme, which uses methylene-tetrahydrofolate as methyl donor group (Urbonavicius, Skouloubris, Myllykallio & Grosjean, 2005; Yamagami et al., 2012).

Methylation is the major RNA modification and is found both on the base and the sugar moieties of the nucleotides, where it is written by methyltransferases (MTases). These can be either stand-alone enzymes, RNA-guided enzymes or holoenzymes (*i.e.* multi-protein complexes composed at least of a catalytic subunit and an activating subunit). This review specifically focuses on the structural and functional properties of eukaryotic and viral holoenzyme MTases of known three-dimensional structures. Hence, the C/D box RNA-guided MTase, which is involved in site-specific 2'O-methylation events of rRNAs or small nuclear or nucleolar RNAs (sn(o)RNAs), will not be discussed but information on this enzyme can be found in excellent recent reviews (Hofler & Carlomagno, 2020; Yu, Zhao & Li, 2018).

2. CLASS I SAM-DEPENDENT METHYLTRANSFERASES :

It is estimated that MTases represent 1% of the human proteome and that one-third of these enzymes are associated with pathologies such as cancers and IDs (Petrossian & Clarke, 2011). These proteins use various methyl donors such as SAM or methylenetetrahydrofolate derivatives but SAM is by far the most common one as it is used by around 90% of the MTases (Fenwick & Ealick, 2018). Based on their three-dimensional structures, the MTases have been divided into at least fifteen classes, among which eleven are SAM-dependent MTases with the class I representing more than two-third of total MTases identified so far (Fenwick & Ealick, 2018). As all the MTases discussed in this review belong to the class I family (Table 1), this section will briefly describe its structural hallmarks.

Class I SAM-dependent MTases transfer the methyl group from SAM onto a substrate, thereby releasing the methylated product and S-adenosyl-homocysteine (SAH). They adopt a Rossmann fold found in many proteins interacting with nucleoside cofactors (SAM, FAD and NAD). In MTases, this fold consists of a central seven-stranded β -sheet (strands order 3214576 with strand β 7 running antiparallel to the other ones; Fig. 1A-B). This β -sheet is surrounded by 3 helices (α Z, α A, α B) on one side and at least 2 helices (α D and α E) on the other side. Variations in this fold such as the presence of an additional helix (α C) between strands β 3 and β 4, the

Table 1 : The eukaryotic MTase holoenzymes discussed in this review.

<i>Homo sapiens</i>	<i>S. cerevisiae</i>	COG ^a	Role	Major substrate	Modification	PDB codes ^b
METTL3	Ime4	COG4725	Catalytic subunit	mRNA	m ⁶ A	5IL1 ; 5IL2; 5ILO; 5TEY; 5K7U; 5K7W; 5K7M; 5L6D; 5L6E
METTL14	Kar4 ^c	COG4725	Activating subunit			
RNMT	Abd1	COG2226	Catalytic subunit	mRNA 5'cap	m ⁷ G	3EPP
RAM	-	-	Activating subunit			
TRMT61A	Trm61/ Gcd14	COG2519	Catalytic subunit	tRNA	m ¹ A	5CCX ; 5ERG; 5EQJ; 5CD1; 5CCB
TRMT6	Trm6/ Gcd10	COG2519	Activating subunit			
METTL1	Trm8	COG0220	Catalytic subunit	tRNA	m ⁷ G	2VDU
WDR4	Trm82	COG2319	Activating subunit			
FTSJ1	Trm7	COG0293	Catalytic subunit	tRNA	Cm32 or Nm34	6JP6 ; 6JPL
THADA	Trm732	COG5543	Activating subunit		Cm32	-
WDR6	Trm734/ Rtt10	COG5635	Activating subunit		Nm34	6JP6 ; 6JPL
TRMT112	Trm112	COG2835	Activating subunit	tRNAs and rRNAs		2J6A
ALKBH8	Trm9	COG2226	Catalytic subunit	tRNA	mc(h)m ⁵ U	5CM2
TRMT11	Trm11	COG1041	Catalytic subunit	tRNA	m ² G	6ZXW
BUD23/ WBSCR22	Bud23	COG2230	Catalytic subunit	rRNA	m ⁷ G	4QTT ; 4QTU
METTL5	-	COG2263	Catalytic subunit	rRNA	m ⁶ A	6H2U ; 6H2V
hTrm9L/ TRMT9B	-	COG2226	Catalytic subunit	?	?	-
HEMK2/ N6AMT1/ KMT9	Mtq2	COG2890	Catalytic subunit	Proteins	N-5-methyl- glutamine and N-methyl- lysine	3Q87 ; 6KMS; 6KMR; 6K0X; 6H1D; 6H1E

^a Cluster of Orthologous Groups of proteins.

^b PDB codes used to generate figures are indicated in bold.

^c Inferred from bioinformatics analyses (Bujnicki, Feder, Radlinska & Blumenthal, 2002).

presence of N- or C-terminal extensions or long internal insertions (mostly between strand $\beta 5$ and helix αE) have been observed (Fig. 1B). Two motifs located at the C-terminal end of the β -sheet play critical roles for SAM binding (Fig. 1). First, a relatively well conserved [D/E]XGXGXG motif (known as motif I) present in the loop connecting strand $\beta 1$ and helix αA interacts with its L-methionine moiety. Second, a conserved acidic residue (Asp or Glu) located at the C-terminal end of strand $\beta 2$ forms hydrogen bonds with its ribose 2' and 3' hydroxyl groups. Finally, most of the time, a conserved motif serving as an active site signature for a group of MTases modifying a specific type of chemical group, is found in the loop connecting strand $\beta 4$ to helix αD . This signature plays an important role for enzymatic activity as it contributes to the optimal orientation of the atom to be methylated relative to the SAM methyl group to be transferred.

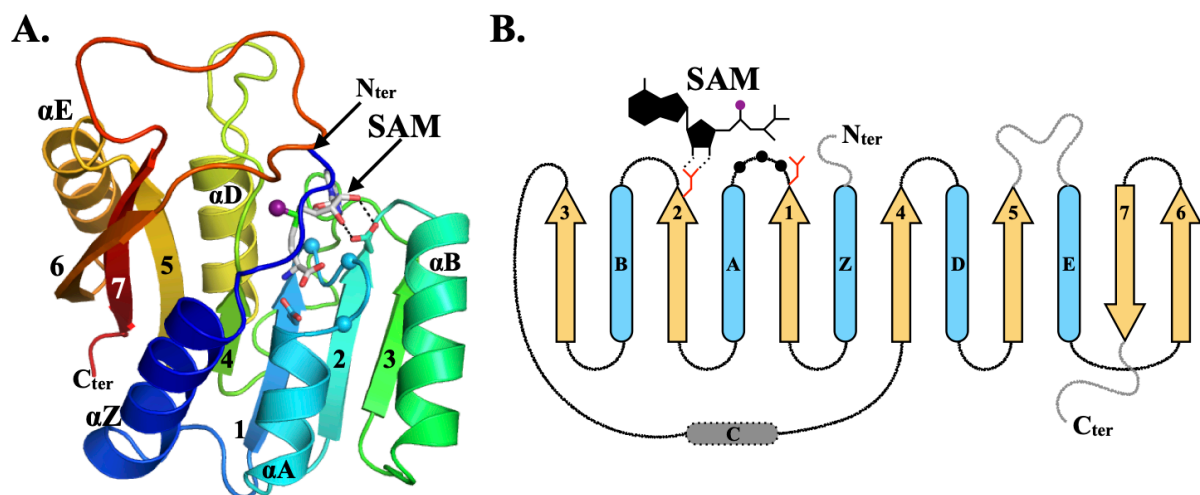


Figure 1 : The class I SAM-dependent MTase fold.

A. Ribbon representation of this fold colored from its N-terminus (blue) to its C-terminus (red). The SAM molecule is shown as grey sticks with the methyl group to be transferred highlighted by a purple sphere. The Ca atoms of the three glycine residues from motif I are shown as blue spheres. The side chains from the two conserved acidic residues interacting with the SAM cofactor are shown as sticks. This figure was generated using the coordinates of the crystal structure of human METTL5 (van Tran et al., 2019). With the exception of Fig. 4D, all figures representing three dimensional structures of protein complexes have been generated using the PYMOL[®] software version 1.7.2.2 Schrödinger, LLC (<http://www.pymol.org/>).

B. Topology diagram of the class I SAM-dependent MTase fold. Helices and strands are colored in light blue and light orange, respectively. Regions where insertions have been observed in various structures of MTase domains adopting this fold are colored in grey. The three glycine residues from motif I are highlighted as black spheres. The methyl group to be transferred onto the substrate is depicted as a purple sphere.

For the sake of clarity, when describing the interfaces between the MTases and their activators, the following terms will refer to the sides of the MTase domain interacting with its partner. The N- and C-faces correspond to the MTase regions formed by helices αZ , αA , αB or αD and αE , respectively. The $\beta 3$ and $\beta 6$ sides refer to the solvent accessible areas formed by these strands on the conventional Rossmann fold while the active site side corresponds to the region located at the C-terminal end of the β -sheet that binds the SAM cofactor and the substrate.

3. THE MAJOR m⁶A MRNA MTASE WRITER : A « MACE » TO PUSH A SMALL PIN.

The multimeric RNA MTase currently under the spotlights, is undoubtedly the eukaryotic METTL3-METTL14 complex, which deposits a methyl group on the nitrogen at position 6 of the adenine to form N⁶-methyladenosine (m⁶A) on mRNAs but also on long noncoding RNAs (Fig. 2A; Table 1; (He & He, 2021)). This complex is responsible for the deposition of more than 99% of the m⁶As detected on mRNAs (Geula et al., 2015). The m⁶A marks are written co-transcriptionally most probably through the recruitment of METTL3 to chromatin by the transcription machinery (H. Huang et al., 2019; Knuckles et al., 2017; Slobodin et al., 2017; Tzelepis, Rausch & Kouzarides, 2019). They are mostly clustered in the neighborhood of the stop codon (end of coding sequence or beginning of 3' UTR). Although m⁶A is found within a DRACH consensus sequence (where D, R and H stand for G/A/U, purine and A/U, respectively; the methylated A is underlined; (Dominissini et al., 2012; Meyer et al., 2012; Schwartz et al., 2013)), only a small subset of these signatures are methylated. This suggests that yet unknown cis- or trans-acting signals are responsible for the specific modification of some of these signatures. The m⁶As are enriched in mRNAs regulating cell development and cell fate decision, which are characterized by

relatively short lifetimes while they are largely absent from highly stable transcripts encoding for house-keeping proteins (Schwartz et al., 2014).

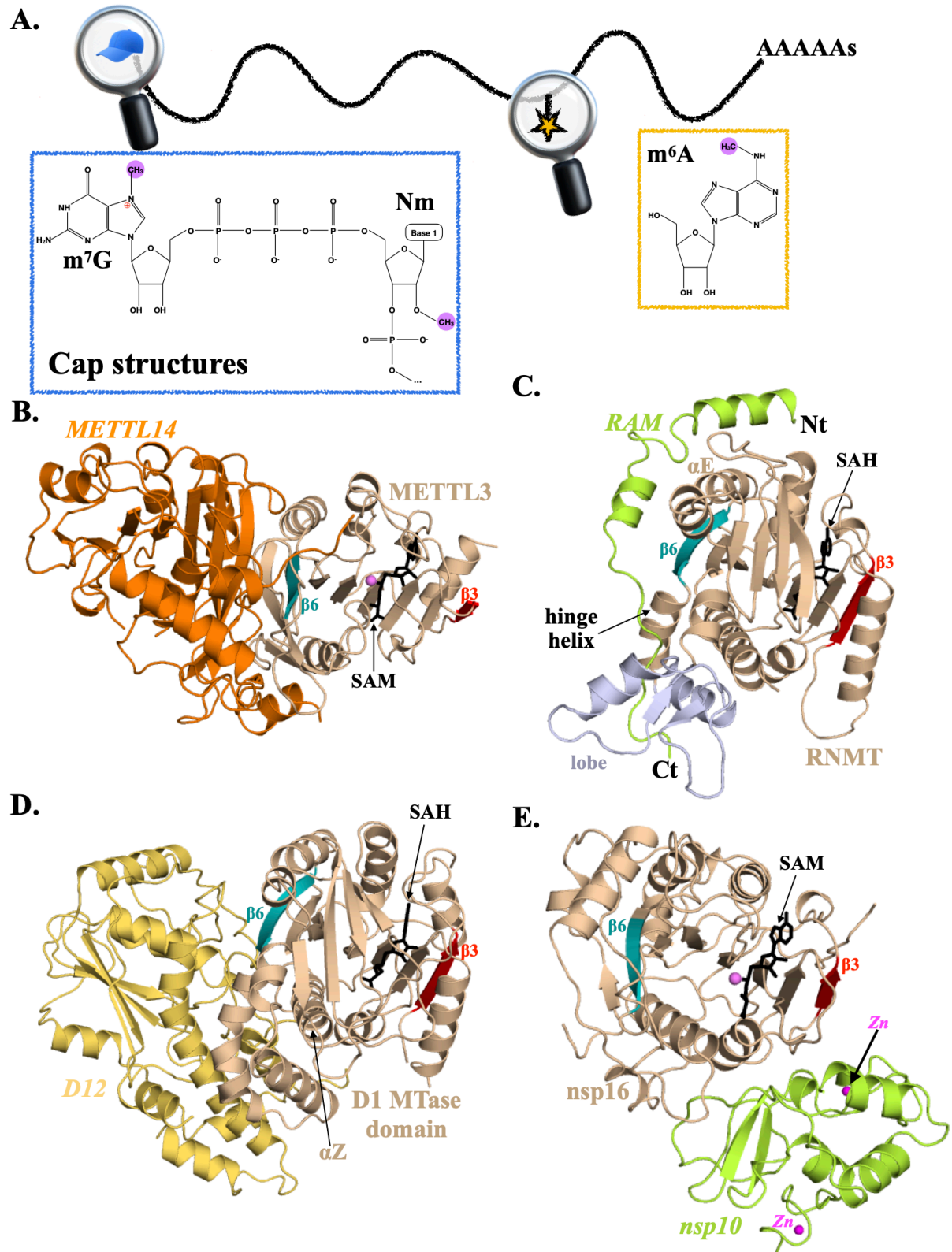


Figure 2 : The mRNA cap and m⁶A methyltransferases.

A. Schematic representation of a eukaryotic mRNA showing the locations of the mRNA marks discussed in this review. Insets : Chemical structures of the 5' cap and m⁶A modifications. Note that the N7-methylation of guanine ring introduces a positive charge (red sign) on the ring.

B. Complex formed between the MTase domains from human METTL3 and METTL14 (orange) proteins. In all figure panels, the active MTase domain is shown in light brown, strands β 3 and β 6 from the MTase domain are colored in red and blue, respectively. The activating subunits are labeled in italics. The SAM or SAH cofactors are shown as black sticks. The methyl group to be transferred from SAM to the product is highlighted by a purple sphere. Labels related to activating subunits are in italics.

C. The RNMT m⁷G cap MTase bound to RAM (green). The RNMT lobe domain is in light blue.

D. The m⁷G cap MTase D1-D12 complex from *Vaccinia virus*. The D12 subunit is shown in yellow. This figure was generated using the 2VDW PDB code.

E. The SARS-Cov 2'OH cap MTase complex formed by nsp16 and nsp10 (green) subunits. The zinc atoms bound to nsp10 are shown as magenta spheres. This figure was generated using the 3R24 PDB code.

The recognition of these m⁶A marks by various proteins dubbed readers will affect mRNA processing and fate (splicing, decay and translation) and thereby various important cellular processes (stem cell differentiation, circadian clock, stress response, sex determination, ... for more information, read (Meyer & Jaffrey, 2017; Patil, Pickering & Jaffrey, 2018; Roignant & Soller, 2017; Wu et al., 2017; Zaccara, Ries & Jaffrey, 2019)). Hence, defects in m⁶A deposition and recognition emerge as key players in the development of pathologies such as for instance neurological disorders or cancers. *Drosophila melanogaster* flies lacking m⁶A are viable but flightless, have reduced fertility and suffer from neurological and sex determination defects (Hausmann et al., 2016; Lence et al., 2016). METTL3-dependent m⁶A modifications also promote miRNA processing thereby contributing to tumor growth metastasis (Alarcon et al., 2015; Chen et al., 2020), play roles in XIST-mediated gene silencing (Patil et al., 2016) and in pluripotent and differentiation of mouse embryonic

stem cells (Yang et al., 2018). Finally, when present near the 5' end of a mRNA, m⁶A can also enhance its cap-independent translation through the recruitment of eIF3 translation initiation complex (Meyer et al., 2015).

In the METTL3-METTL14 complex, both proteins form a tight heterodimer, where METTL3 is the catalytic subunit while, despite the presence of a MTase domain, METTL14 is enzymatically inert (Scholler et al., 2018; X. Wang et al., 2016). The METTL3-METTL14 complex is active as an m⁶A RNA MTase *in vitro*, yet it requires the assistance of many additional proteins *in vivo*, forming large complexes of more than 1 MDa (Bokar et al., 1994). These proteins fulfill different roles, which are being progressively unraveled. Their importance is for instance supported by the apoptosis of HeLa cells upon depletion of METTL3 (Bokar, 2005). The currently known human METTL3-METTL14 partners are WTAP, VIRMA (also known as or *aka* KIAA1429), ZC3H13 (*aka* KIAA0853), HAKAI, RBM15 and its paralog RBM15B (the names of the orthologous proteins from *Arabidopsis thaliana*, fruit fly and baker's yeast can be found in (Garcias Morales & Reyes, 2020)). WTAP, an essential component of this complex, plays a central role together with ZC3H13 in the sub-cellular localization of the METTL3-METTL14 complex to nuclear speckles, which are particularly enriched in pre-mRNA splicing factors (Ping et al., 2014; Wen et al., 2018). WTAP also stabilizes the METTL3-METTL14 complex, interacts with the METTL3 N-terminal region and contributes to RNA binding (Ping et al., 2014; Scholler et al., 2018). The RBM15/RBM15B paralogs interact with U-rich regions immediately adjacent to the m⁶A sites and hence most likely recruit this complex in the proximity of the m⁶A consensus sites (Patil et al., 2016). VIRMA seems to be involved in the preferential deposition of m⁶As in the vicinity of stop codons and in the 3' UTRs while the role of the E3 ubiquitin protein ligase HAKAI in m⁶A deposition remains to be established (Yue et al., 2018).

In the yeast *S. cerevisiae*, IME4, the orthologue of METTL3 gene, is strongly expressed during sporulation and stationary phase (Clancy, Shambaugh, Timpte & Bokar, 2002; Gasch et al., 2000), and accordingly, m⁶A is considerably enriched in mRNAs during meiosis. The Ime4 enzymatic activity is essential for its key role in promoting meiosis and sporulation (Agarwala, Blitzblau, Hochwagen & Fink, 2012; Clancy, Shambaugh, Timpte & Bokar, 2002). Together with the proteins Mum2 (the

fungal WTAP orthologue) and Slz1 (which has no obvious orthologue in metazoan), Ime4 forms the MIS complex (for Mum2-Ime4-Slz1 complex, (Agarwala, Blitzblau, Hochwagen & Fink, 2012)). Mum2 is more important for m⁶A formation than Slz1 but this latter plays a predominant role in addressing the MIS complex to the nucleolus where m⁶As are formed on mRNAs (Schwartz et al., 2013).

The three concomitantly determined crystal structures of the minimal human METTL3-METTL14 core complex formed by the MTase domains from both proteins, clarified their respective roles (Sledz & Jinek, 2016; Wang, Doxtader & Nam, 2016; X. Wang et al., 2016). Both MTase domains adopt a Rossmann fold but due to a circular permutation, their topology significantly differs from the classical one. The fact that both METTL3 and METTL14 MTase domains share the same topology, suggests that they arose from a gene duplication event. The METTL3 active site is strongly evolutionarily conserved and harbors the DPPW active site motif. Such motif is also found in bacterial DNA m⁶A MTases and is assumed to coordinate the adenine to be methylated within the enzymes' active site (Bujnicki, Feder, Radlinska & Blumenthal, 2002). The corresponding region from METTL14 is much less conserved, partly occluded by bulky side chains and consequently, does not bind SAM. Hence, METTL3 is the catalytic subunit while METTL14 has no MTase activity (Scholler et al., 2018; X. Wang et al., 2016). Although METTL3 is able to interact with SAM and harbors the DPPW signature, it is catalytically inactive alone but the sole addition of METTL14 to METTL3 is sufficient to restore enzymatic activity *in vitro*. Both MTase domains interact together through an extensive interface highly enriched in hydrophobic residues and formed by their β 6 sides (Fig. 2B), rationalizing the reciprocal stabilization of both partners *in vivo* (Wang et al., 2014). A composite positively charged and strongly conserved groove is formed at the interface between both MTase domains nearby the METTL3 SAM binding pocket and it most likely corresponds to the RNA substrate binding site. Interestingly, point mutations of METTL3 (Y406C) and METTL14 (R298P) residues located in the vicinity of the active site or at the METTL3-METTL14 interface have been identified in various cancers. These mutations significantly reduce (R298P) or abrogate (Y406C) the *in vitro* enzymatic activity of this complex (Wang, Doxtader & Nam, 2016). The METTL3-METTL14 complex formed by the sole MTase domains has a low intrinsic *in vitro* enzymatic activity as other regions from each subunit contribute to the MTase activity of this com-

plex (Scholler et al., 2018; Sledz & Jinek, 2016). Indeed, the two tandem zinc finger domains at the N-terminus of the METTL3 MTase domain are required for RNA MTase activity probably through a role in RNA binding (J. Huang et al., 2019; Wang, Doxtader & Nam, 2016).

In summary, METTL14 stabilizes the METTL3 catalytic subunit and directly contributes to methyl deposition on DRACH motifs but the other subunits of this complex play central and various roles in targeting this complex to a restricted subset of motifs within mRNAs and to the nuclear speckles for co-transcriptional methylation. Additional structural and functional studies are clearly needed to unravel the roles of those different subunits.

4. VIRAL AND EUKARYOTIC MRNA CAP METHYLTRANSFERASES

The 5' end of eukaryotic and viral mRNAs is protected from uncontrolled degradation by a 5' cap, which is also essential for mRNA translation. This cap consists in a m⁷G (N⁷-methylguanosine) linked by an inverted 5'-5' triphosphate bridge to the first transcribed nucleotide (Fig. 2A; (Shuman, 2015)). In eukaryotes and some viruses, the 5' cap is added upon the successive action of three enzymatic activities. The first step is catalyzed by a 5' triphosphatase, which generates a diphosphate group at the 5' extremity of the nascent mRNA. Then, an RNA guanylyltransferase adds a GMP molecule to form GpppN. Finally, a methyl group is grafted by an RNA guanine-7 MTase (RNMT) onto the nitrogen at position 7 of the terminal guanine ring to form m⁷G (Fig. 2A). The three enzymatic activities required to form this m⁷G cap (also known as cap-0) can be grouped in a single protein in viruses or spread on two to three proteins in eukaryotes (Shuman, 2002). The cap-0 structure can be further methylated on the ribose 2'-OH groups of the first (cap-1) and second transcribed nucleotides as well as on the N6 atom from the first transcribed mRNA nucleotide if this latter is an adenosine (Fig. 2A; (Akichika et al., 2019; Aregger & Cowling, 2017)). Alternatively, some viruses such as influenza virus or Bunyaviruses have evolved a machinery to snatch caps from eukaryotic mRNAs and transfer them onto viral ones (Olschewski, Cusack & Rosenthal, 2020).

In vertebrates, RNMT, the eukaryotic enzyme responsible for the last step of eukaryotic m⁷G cap formation is assisted in its function by the small protein RAM (for

RNMT-Activating Miniprotein; Table 1; (Gonatopoulos-Pournatzis, Dunn, Bounds & Cowling, 2011)). This latter is important for the cellular stability of RNMT and conversely. RAM also strongly enhances SAM and RNA binding to the MTase domain *in vitro* (Gonatopoulos-Pournatzis, Dunn, Bounds & Cowling, 2011; Varshney et al., 2016). RAM interacts with the class I SAM-dependent MTase domain from RNMT via its N-terminal half, which is sufficient for the activation. The crystal structure of the RNMT MTase domain bound to this RAM region has revealed that this latter folds as two α helices upon binding to RNMT (Fig. 2C; (Varshney et al., 2016)). RAM interacts with the C-face of the MTase domain as well as on a positively charged groove formed by an RNMT region known as lobe, and with the hinge α -helix connecting this lobe to the core of the MTase domain. The lobe is particularly important for RNMT enzymatic activity and is stabilized upon RAM binding. Interestingly, it is present only in proteins from organisms possessing RAM, arguing for the co-evolution of both elements in vertebrates (Varshney et al., 2016). The complex formation is mainly ruled by electrostatic interactions, but some hydrophobic residues are also engaged in the interface between both proteins. Intriguingly, although RAM does not interact in the vicinity of the RNMT SAM binding pocket, it stimulates its MTase activity and SAM binding through long-distance effects that most probably contribute to the optimal conformation of the enzyme active site (Varshney et al., 2016).

Another example of a heterodimeric cap MTase comes from the *Vaccinia* virus D1-D12 complex (De la Pena, Kyrieleis & Cusack, 2007). The large D1 subunit (97 kDa) is endowed with all three enzymatic activities necessary to generate the m⁷GpppN cap at the 5' end of viral mRNAs. However, the low intrinsic activity of its class I SAM-dependent MTase domain is strongly enhanced by the smaller D12 subunit (33 kDa; (Higman, Bourgeois & Niles, 1992; Mao & Shuman, 1994)). D12 increases the affinity of D1 for its SAM cofactor and the RNA substrate. It also slightly increases the k_{cat} of the enzyme (Schwer, Hausmann, Schneider & Shuman, 2006). Interestingly, the D12 subunit adopts the class I SAM-dependent MTase fold (De la Pena, Kyrieleis & Cusack, 2007), but its SAM binding site is degenerated, explaining why it has no enzymatic activity (Mao & Shuman, 1994). The D12 subunit displays strong structural similarities with ribose 2'-O-MTases, suggesting that it could have originally be involved in the methylation of the ribose 2'-OH group of the first transcribed nucleotide to generate the so-called cap1 (see Fig. 2A). During evolution, with

the acquisition of the VP39 gene encoding for the cap 2'-O-MTase, D12 active site may have lost its enzymatic activity (De la Pena, Kyrieleis & Cusack, 2007). The D12 subunit contacts mainly the N-face of the D1 subunit but also its $\beta 6$ side (Fig. 2D). The large interface between both subunits is mostly formed by hydrophobic and non-polar residues, rationalizing the stabilizing effect of D12 on D1 (De la Pena, Kyrieleis & Cusack, 2007). This interface is located at the opposite of the D1 active site indicating that as D12 does not participate directly to substrate binding and catalysis (De la Pena, Kyrieleis & Cusack, 2007). Yet, it contributes to the stabilization of helix αZ from the D1 subunit, which is involved in SAM binding, and hence most likely stimulates D1 via long range effects.

The last examples come from the nsp16-nsp10 cap MTase from several coronaviruses (SARS-Cov, MERS-Cov and SARS-Cov-2, (Chen et al., 2011; Decroly et al., 2011; Rosas-Lemus et al., 2020); PDB code : 5YN6). These complexes are responsible for the methylation of the 2'OH group from the ribose of the first RNA nucleotide (Fig. 2A), when this latter is an A, to generate cap-1. Cap-1 is particularly important for viral mRNAs through its role in translation stimulation and protection from RNA decay, but also for viral mRNAs to escape host immune recognition (Daffis et al., 2010). The nsp16 catalytic subunit requires the small zinc-binding protein nsp10 (15 kDa) to be active. From the crystal structure of this complex, nsp10 interacts with the N-face of nsp16 and in the vicinity of its active site (Fig. 2E). The center of this interface is formed by a hydrophobic core surrounded by hydrophilic interactions. Nsp10 directly contacts and stabilizes a long loop from nsp16 that interacts directly with SAM, thereby explaining why it is mandatory for the nsp16 MTase domain to bind SAM (Chen et al., 2011). Nsp10 further provides a positively charged area in the vicinity of nsp16 active site. This area is most probably responsible for the nsp10 RNA binding property, which is required for nsp16 to specifically interact with a capped m⁷GpppA-RNA (Chen et al., 2011). Contrary to many MTases discussed in this review, the nsp10 and nsp16 proteins do not interact tightly, suggesting that this complex is dynamic (Chen et al., 2011). Such property could be important to regulate the enzymatic activity of nsp16 during the virus life cycle. Interestingly, nsp10 also interacts with the m⁷G cap MTase nsp14 but does not influence its enzymatic activity (Bouvet et al., 2014).

These examples illustrate the various and complex strategies developed by eukaryotes and viruses to methylate the cap structures located at the 5' extremity of their mRNAs. Similarly to eukaryotic decapping enzyme Dcp2 (Charenton & Graille, 2018), these different complexes do not recognize the sole 5' cap structure but also other nucleotides on the mRNA body, most probably to strengthen the interaction with their substrate and/or to specifically modify the caps of a subset of mRNAs.

5. THE TRMT61A-TRMT6 M¹A₅₈ MTASE

The m¹A₅₈ tRNA modification is universally conserved and located in the TΨC loop (Fig. 3), where it forms a Hoogsteen base pair with T54 (Robertus et al., 1974). In archaea and bacteria, this modification is catalyzed by the homotetrameric Trm1 enzyme (Oerum, Degut, Barraud & Tisne, 2017). In eukarya, this modification is found in cytosolic and mitochondrial tRNAs. The latter are modified by TRMT61B protein, which similarly to bacterial enzymes, forms homotetramers (PDB code : 2B25; (Chujo & Suzuki, 2012)). This enzyme also catalyzes the formation of m¹A at position 947 of the 16S rRNA from the large subunit of the human mitochondrial ribosome (Bar-Yaacov et al., 2016). TRMT61B expression is altered in Alzheimer's disease, suggesting affected levels of mitochondrial tRNA and rRNA modifications in patients (Sekar et al., 2015). In cytoplasmic tRNAs, the m¹A₅₈ modification is catalyzed in the nucleus by the TRMT6/TRMT61A complex (proteins also known as Trm6/Gcd10 and Trm61/Gcd14 in budding yeast, respectively; Table 1; (Anderson, Phan & Hinnebusch, 2000; Ozanick, Krecic, Andersland & Anderson, 2005)). TRMT61A is the catalytic subunit of this holoenzyme but TRMT6 is mandatory for its enzymatic activity (Anderson, Phan & Hinnebusch, 2000). Both subunits are particularly important for the stability of initiator tRNA_i^{Met} in yeast and human but not of the other m¹A₅₈ containing tRNAs (23 out of the *S. cerevisiae* yeast 35 cytosolic tRNAs; (Anderson et al., 1998; Anderson, Phan & Hinnebusch, 2000; Calvo et al., 1999; Kim et al., 2010; Macari et al., 2016)). Indeed, hypomodified tRNA_i^{Met} lacking m¹A₅₈ are destabilized upon the action of a nuclear tRNA surveillance pathway (Kadaba et al., 2004; Liu et al., 2016; Macari et al., 2016; Ozanick et al., 2009). Consequently, the formation of the eIF2-GTP-tRNA_i^{Met} translation initiation complex is strongly reduced, leading to a concomitant decrease in translation initiation (Calvo et al., 1999; Liu et al., 2016). Interestingly, the siRNA-mediated knock-down of either *TRMT6* or *TRMT61A* genes in

glioma cells diminishes their proliferation and increases cell death (Macari et al., 2016). This makes this complex appealing for the development of cancer drugs. Finally, the TRMT61A-TRMT6 complex also introduces m¹A modifications within a small subset of human mRNAs in tRNA-like local structure (Safra et al., 2017). The presence of this modification reduces the expression levels of the corresponding proteins but further studies are required to grasp the role of these m¹A mRNA modifications.

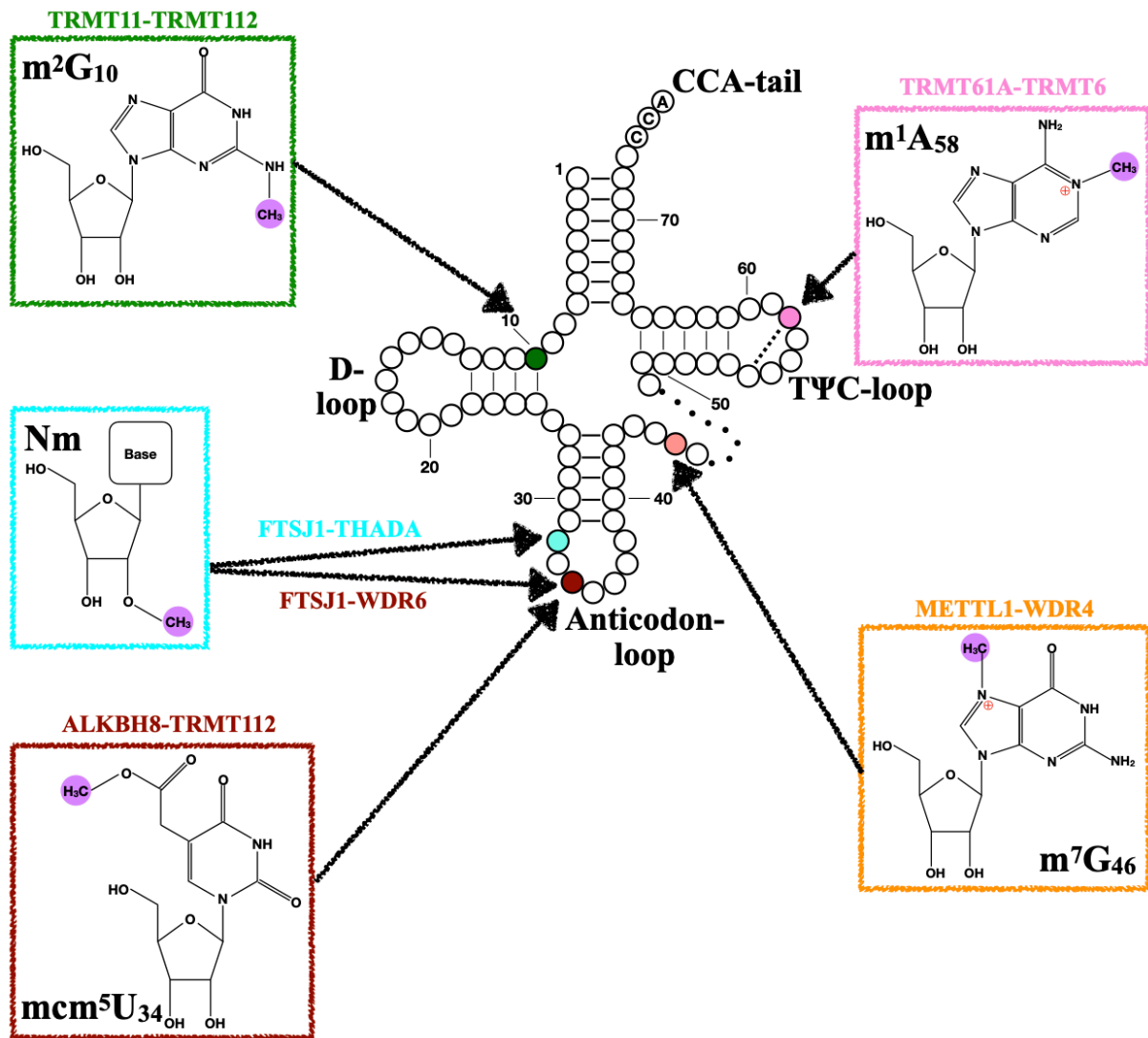


Figure 3 : Structure and location of tRNA modifications discussed in this review.

Clover-leaf representation of a tRNA with the location and chemical structures (insets) of discussed modifications. The variable loop is shown as a dotted line. Note that the N1-methylation of adenine ring and N7-methylation of guanine ring introduce

a positive charge (red sign) on those rings. The Hoogsteen base pair formed between m¹A₅₈ and T54 is shown as a dashed line.

Details on the molecular organization of TRMT61A-TRMT6 complex and on its tRNA binding property were recently obtained thanks to the crystal structures of the yeast complex (M. Wang et al., 2016) and of the human complex bound to tRNA₃^{Lys} (Finer-Moore et al., 2015). Both subunits adopt the same overall fold (despite only 15% sequence identity between both proteins) and are structurally similar to prokaryotic TrmI proteins. They are made of a small N-terminal β -barrel followed by the C-terminal class I MTase domain (Fig. 4A). TRMT6 proteins harbor an additional small α -helical domain inserted within the β -barrel. Similarly to METTL14, TRMT6 SAM binding pocket is occluded by large residues and hence, this subunit is enzymatically inactive (Anderson, Phan & Hinnebusch, 2000). The TRMT61A-TRMT6 complex is organized as a dimer of heterodimers (Fig. 4B; (Finer-Moore et al., 2015; M. Wang et al., 2016)), which is structurally similar to that observed for the bacterial and archaeal TrmI enzymes. In the heterodimer, the MTase domains from both subunits form the major interface area, with in particular, the very long strand β 6 from both domains associated in an anti-parallel manner (Fig. 4A). Additional contacts are also observed between the β -barrel domain from one subunit and the MTase domain from the other. A large proportion of this heterodimeric interface is composed of hydrophobic residues. In the heterotetramer, strands β 6 and β 7 form the heart to the interface between the four protomers and then connect them together (Fig. 4B). Additional hydrophilic but also hydrophobic interactions are present at the interface between the four molecules. Strikingly, a large number of contacts involved in the formation of tetramers are well conserved between prokaryotic and eukaryotic complexes, indicating a strong evolutionary pressure to maintain this assembly. To my knowledge, whether TRMT6 stabilizes TRMT61A *in vivo* or enhances SAM binding to this latter as shown for other holoenzymes, has not been clarified.

Further information regarding the tRNA binding mode came from the structure of the human TRMT61A-TRMT6 complex bound to tRNA₃^{Lys}, revealing that the active form of the enzyme is the heterotetramer (Finer-Moore et al., 2015). Indeed, the A₅₈ nucleotide fits snugly at the interface between both heterodimers, diving into the TRMT61A active site of one heterodimer while the anticodon stem-loop interacts with

the TRMT6 subunit from the second one (Fig. 4C). In addition, the TΨC loop is sandwiched at the interface between both heterodimers with the small α -helical domain from the TRMT6 N-terminal region contacting the phosphate groups from this loop. This large composite area forms a positively charged region ideal for tRNA binding (Fig. 4D; (Finer-Moore et al., 2015)). Upon binding to its tRNA substrate, TRMT61A-TRMT6 dissociates the otherwise tightly associated TΨC and D loops, allowing A₅₈ to enter into the TRMT61A active site upon a 20 Å movement. Such local reorganization of the tRNA structure is consistent with the higher incorporation of m¹A₅₈ in tRNA mutants locally affecting the inter-digitized structure formed between the D and TΨC loops (Grosjean, Edqvist, Straby & Giege, 1996). This structure, one of the rare of a complex between a tRNA MTase and its full-length substrate, further highlights the paucity of the hydrogen bonding network formed between the protein and the tRNA, thereby rationalizing that this enzyme can catalyze the formation of m¹A₅₈ in most tRNAs. This structure also suggests that one heterotetramer can simultaneously interact with two tRNA substrates as prokaryotic TrmI enzymes do (Barraud et al., 2008). However, another crystal form suggests that only one active site of the heterotetramer might be active at once. Thereby, the enzymatic activity could switch from one active site to the other one after each methylation event. One can also imagine that the methylation of one tRNA in the first active site could favor the optimal conformation of the second active site, of its tRNA substrate or of both. Binding of a tRNA to the opposite active site might also stimulate the release of the methylated tRNA from the other active site. Such allosteric behavior remains to be investigated in detail.

In conclusion, this complex, which is essential for the stability of eukaryotic tRNA_i^{Met}, most probably arose from gene duplication from a prokaryotic ancestor. This is one of the most structurally characterized complex thanks to the determination of the crystal structure of the human TRMT6-TRMT61-tRNA complex. However, these studies have not completely clarified the roles of the TRMT6 subunit on the TRMT61A catalytic subunit, in particular with respect to its *in vivo* stability and SAM binding property.

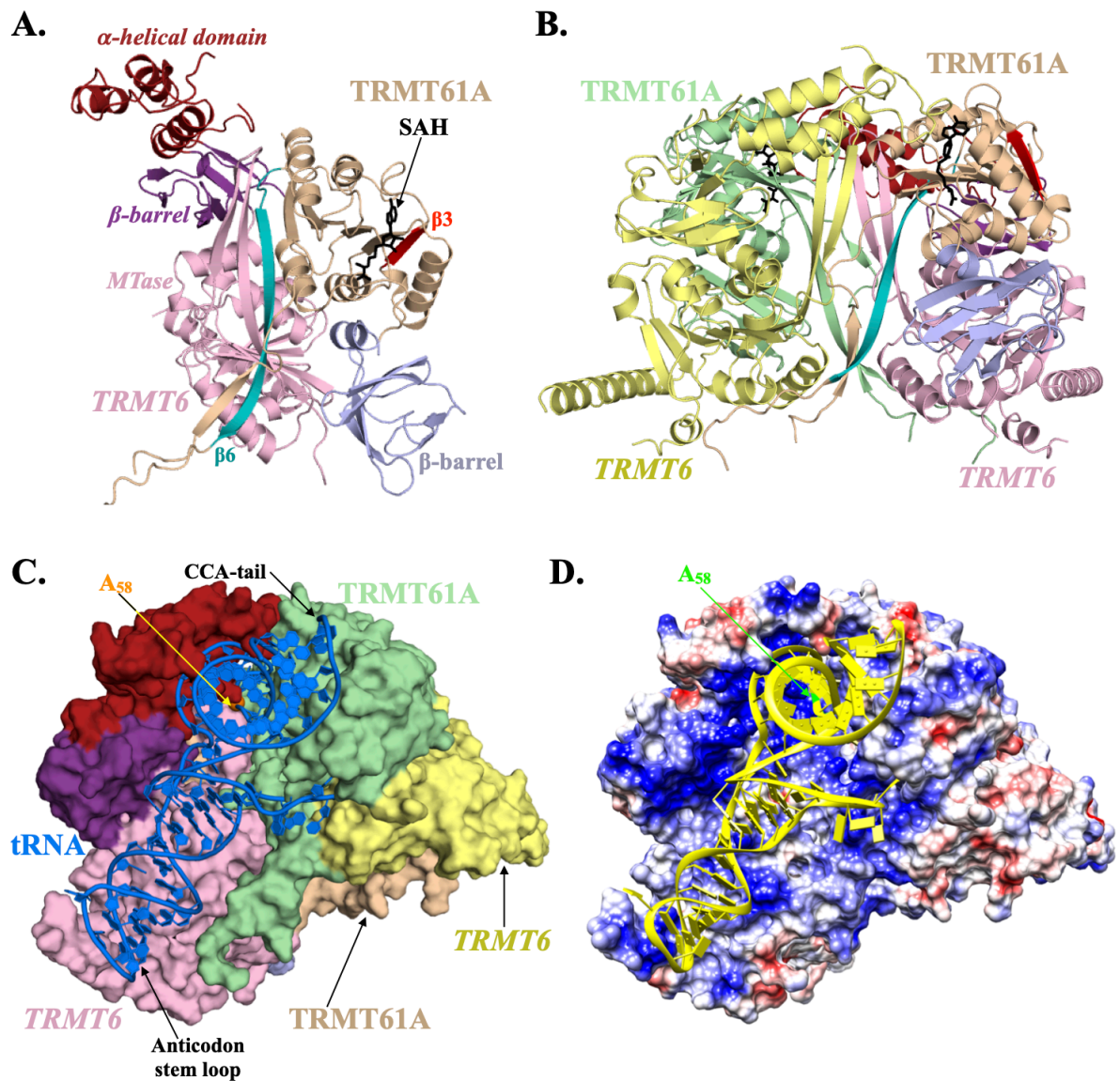


Figure 4 : The TRMT61A-TRMT6 m¹A₅₈ tRNA methyltransferase.

A. The human TRMT61A-TRMT6 heterodimeric complex. The TRMT6 MTase domain is colored pink. The β -barrels from TRMT61A and TRMT6 are colored in light blue and purple, respectively. The α -helical domain from TRMT6 is shown in wine red.

B. Representation of the human TRMT61A-TRMT6 heterotetrameric complex. The same color as for panel A is used for one heterodimer. For the second one, the TRMT61A and TRMT6 proteins are colored light green and yellow, respectively.

C. Surface representation of the human TRMT61A-TRMT6 heterotetrameric complex bound to one tRNA substrate (blue), showing that the tRNA binding site spans a large area contributed by subunits from both heterodimers. The same color code as for panel B is used. The A₅₈ tRNA position is shown as an orange sphere.

D. Electrostatic representation of the human TRMT61A-TRMT6 heterotetrameric complex bound to a tRNA substrate (yellow). The A₅₈ tRNA position is highlighted in green. This panel was generated using the CHIMERA software (Pettersen et al.,

2004). Positively charged ($10 \text{ k}_B\text{T}/e^-$) and negatively charged ($-10 \text{ k}_B\text{T}/e^-$) regions are colored in blue and red, respectively. The orientation is the same as in panel C.

6. THE TRM8-TRM82 m^7G_{46} TRNA MTASE

The m^7G modification found in the eukaryotic mRNA caps is also present in bacterial and eukaryotic tRNAs, almost always at position 46 (m^7G_{46} ; Fig. 3). It is found in 12 out of 34 cytosolic tRNAs from yeast and 21 tRNAs in mouse embryonic stem cells (Boccaletto et al., 2018; Lin et al., 2018; Machnicka, Olchowik, Grosjean & Bujnicki, 2014).

This tRNA modification is catalyzed by the stand-alone MTase TrmB in bacteria (De Bie et al., 2003) and by its METTL1 orthologue (Trm8 in *S. cerevisiae*) in eukaryotes. In these latter, the catalytic subunit METTL1 is only active as a complex with the WDR4 activating subunit (Trm82 in *S. cerevisiae* or Wuho/wh in metazoa; Table 1; (Alexandrov, Martzen & Phizicky, 2002)). In yeast, Trm82 strongly stabilizes Trm8 protein levels *in vivo* and considerably stimulates its enzymatic activity (Alexandrov, Grayhack & Phizicky, 2005; Alexandrov, Martzen & Phizicky, 2002). The m^7G_{46} tRNA modification is biologically important as the over-expression of bacterial TrmB or wild-type Trm8 but not of a Trm8 catalytic mutant rescues the yeast growth defect phenotypes observed in some conditions upon deletion of either TRM8, TRM82 or both genes (Alexandrov et al., 2006; Alexandrov, Grayhack & Phizicky, 2005). This modification is particularly important for tRNA stability as some hypomodified tRNAs lacking m^7G_{46} are deacylated and degraded in *S. cerevisiae* or *S. pombe* yeasts (Alexandrov et al., 2006; Chernyakov et al., 2008; De Zoysa & Phizicky, 2020). This relies on a rapid tRNA decay (RTD) mechanism implying the nuclear Rat1 and cytoplasmic Xrn1 5'-3' exonucleases and that is distinct from the pathway involved in the decay of tRNA_{Met} lacking m^1A_{58} . In both yeasts, the inactivation of the TRM8 gene results in the induction of the general amino acid control (GAAC) response. In the GAAC pathway, an increase of uncharged tRNAs (due to amino acid starvation or hypomodification) activates the GCN2 kinase, resulting in the phosphorylation of eIF2 α followed by a global translational repression. It also induces the derepression of the GCN4 transcription factor, which then activates the transcription of 10% of the

genes including many involved in amino acid biosynthesis (Hinnebusch, 2005). Surprisingly, the induction of the GAAC upon TRM8 deletion has opposite effects in *S. pombe* compared to *S. cerevisiae* (De Zoysa & Phizicky, 2020). Indeed, in *S. pombe*, this exacerbates growth defects as it is the case in *S. cerevisiae* cells lacking TRM7 gene ((Han, Guy, Kon & Phizicky, 2018), see below) while it rescues the growth defects of budding yeast cells lacking both TRM8 and TRM4 (forming m⁵C₄₉ in tRNAs) genes.

In mice, the deletion of WDR4 is early embryonic lethal (Cheng et al., 2016) while the METTL1-dependent m⁷G₄₆ tRNA modification is important for global translation (Lin et al., 2018; Zhang et al., 2019). Interestingly, genes with reduced translation efficiency upon METTL1 depletion are enriched in those involved in brain formation, such as neural lineage genes, which have a higher frequency of codons recognized by m⁷G₄₆-containing tRNAs. This is in line with the phenotypes described for patients with mutations in the WDR4 gene, who suffer from a form of primordial dwarfism accompanied by facial dysmorphism and brain malformation (microcephaly) or from the Galloway-Mowat syndrome (GAMOS; Table 2; (Braun et al., 2018; Chen et al., 2018; Shaheen et al., 2015; Trimouille et al., 2018)). Another category of genes down regulated at the protein level in cells depleted for METTL1 is involved in cell cycle progression, in line with the impaired G2/M transition and the slower proliferation of these cells (Lin et al., 2018). The human METTL1-WDR4 complex also catalyzes the formation of m⁷G within tRNA-like regions of mRNAs and METTL1 depletion reduces translation efficiency of these m⁷G-containing mRNAs (Zhang et al., 2019). METTL1 is involved in cancer progression (Tian et al., 2019). It also modifies a specific set of miRNAs such as let-7 or miR-149-3p, which are particularly important for the inhibition of cell migration (Liu et al., 2019; Pandolfini et al., 2019). These m⁷G marks affect the structure of local G-quadruplexes present within pri-miRNAs, most probably improving their processing in pre-miRNAs (Liu et al., 2019; Pandolfini et al., 2019). Interestingly, the colon cancer cells resistant to cisplatin chemotherapeutic drug have lower levels of METTL1 mRNA and protein as well as of miR-149-3p compared to the cisplatin sensitive cells. The over-expression of METTL1 increases the levels of miR-149-3p and renders these cells more sensitive to high dose of cisplatin, most probably through its role on the maturation of miR-149-3p (Liu et al., 2019). In parallel, the decreased expression of METTL1 in

colon cancer cells (HCT116) results in lower levels of let-7e miRNA, and thereby in higher cell migration (Liu et al., 2020). In drosophila, the inactivation of wh gene (*aka* *wuho*), the Trm82/WDR4 orthologue, results in sterile male and semi-sterile female (as this gene is located on the X-chromosome), but does not affect early embryogenesis (Wu, Hou & Hsieh, 2006). This is due to defects in oogenesis and arrested spermatogenesis. However, the potential role of tRNA modifications in this phenotypic outcome is currently unclear as the WH protein is also implicated in maintaining genome stability in mice and human cells (Cheng et al., 2016).

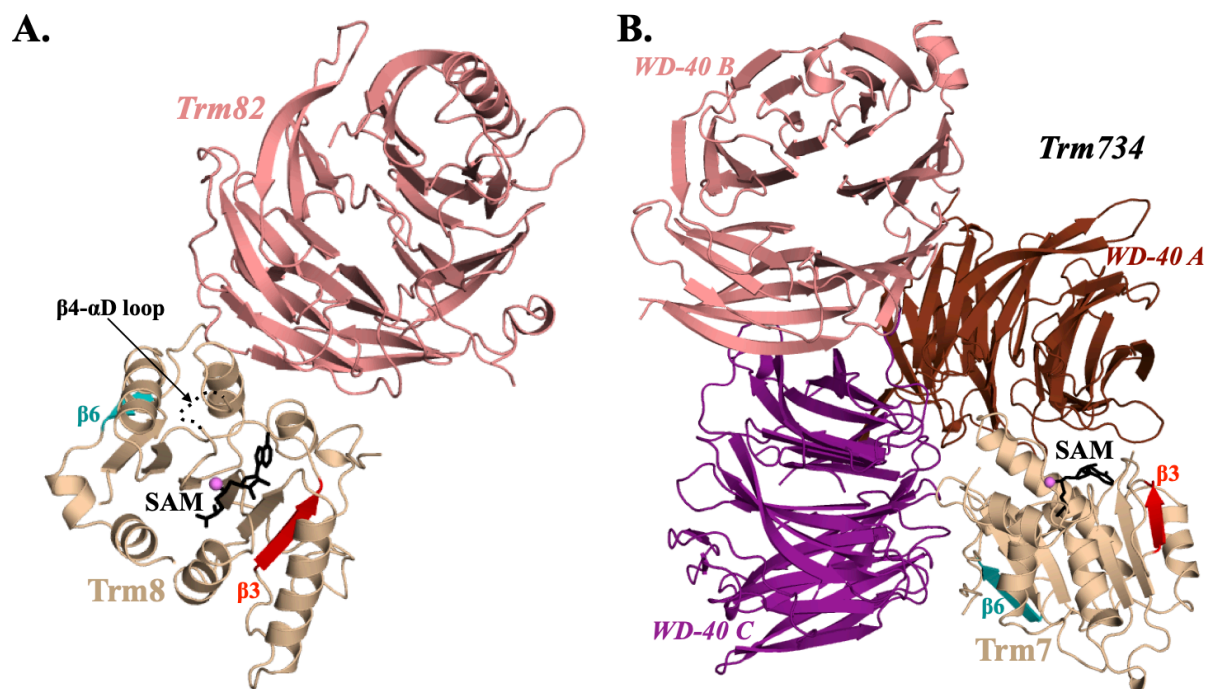


Figure 5 : tRNA methyltransferase subunits activated by WD-40 proteins.

A. The *S. cerevisiae* Trm8-Trm82 m^7G_{46} tRNA MTase bound to SAM.

B. The *S. cerevisiae* Trm7-Trm734 Nm_{34} tRNA MTase. The three WD-40 domains from Trm734 are shown in different colors.

Table 2 : Heterodimeric RNA methyltransferases associated with intellectual disorders.

Holoenzyme ^a	Subunit affected	Mutations	Major phenotypes	References
METTL1-WDR4	WDR4	Homozygous variant : NM_033661.4: c.509G>T; p.Arg170Leu	Microcephalic primordial dwarfism; intellectual disability; agenesis of corpus callosum; abnormal gyros pattern	Shaheen <i>et al.</i> , 2015
	WDR4	Homozygous variant : NM_018669.5: c.454-2A>C (obligatory splice site mutation)	Growth deficiency; microcephaly; dwarfism; intellectual disability; proteinuria and nephrotic syndrome	Braun <i>et al.</i> , 2018
	WDR4	Heterozygous variants : NM_033661.4: c491A>C; p.Asp164Ala & NM_033661.4: c940dupC;p.Leu314Profs*16	Microcephalic primordial dwarfism; intellectual disability	Chen <i>et al.</i> , 2018
	WDR4	Heterozygous variants : NM_033661.4: c509G>A; p.Arg170Gln & NM_033661.4: c911_927dup;p.Gln310Glyfs*30	Microcephalic primordial dwarfism; intellectual disability	Trimouille <i>et al.</i> , 2018
FTSJ1-WDR6/THADA	FTSJ1	X-chromosome : NM_012280: c655G>A (splice site)	Nonsyndromic X-linked intellectual disability	Freude <i>et al.</i> , 2004
	FTSJ1	X-chromosome : NM_012280: c196C>T; Gly65*		
	FTSJ1	X-chromosome : NM_012280: IVS2+1delG (splice site)		
	FTSJ1	X-chromosome : NM_012280: IVS3-2A>G; 49* (acceptor consensus splice site)		
	FTSJ1	Microdeletion of the FTSJ1 gene		Froyen <i>et al.</i> , 2004
FTSJ1	X-chromosome : NM_012280: c.571+1G>A	Takano <i>et al.</i> , 2008		
TRMT112-ALKBH8	ALKBH8	NM_001301010.1: c.1794delC; Trp599Glyfs*19	Mild dysmorphism; macrocephaly; attention deficit hyperactivity disorder; congenital heart disease; prune belly syndrome	Monies <i>et al.</i> , 2019

Holoenzyme ^a	Subunit affected	Mutations	Major phenotypes	References
	ALKBH8	NM_001301010.1: c.1660C>T; Arg554*	Mild dysmorphism; attention deficit hyperactivity disorder	
<u>TRMT112-</u> <u>METTL5</u>	METTL5	NM_014168.2: c.182G>A; p.-Gly61Asp	Microcephaly; intellectual disability	Hu <i>et al.</i> , 2019
	METTL5	NM_014168.2: c.344_345del-GA; p.Arg115Asnfs*19		Richard <i>et al.</i> , 2019
	METTL5	NM_014168.2: c.571_572del-IAA; p.Lys191Valfs*10		
<u>METTL2A/</u> <u>B-</u> <u>DALRD3</u>	DALRD3	NM_001276405.1: c.1251C>A; p.Tyr417*	Severe intellectual disability; subtle facial dysmorphism	Lentini <i>et al.</i> , 2020

^a The catalytic subunit is underlined

The crystal structures of *S. cerevisiae* Trm8 catalytic subunit alone or in complex with Trm82 have brought useful information regarding the influence of Trm82 on the *in vivo* activity of Trm8 (Fig. 5A; (Leulliot et al., 2008)). Trm82, which is made of a seven-bladed β -propeller WD-40 domain, interacts mostly with the C-face of the Trm8 MTase domain. The interface is rich in hydrophobic residues and hence, those hydrophobic residues are masked from the solvent upon complex formation. This most likely explains the stabilizing effect of Trm82 on Trm8 *in vivo* (Alexandrov, Grayhack & Phizicky, 2005). Interestingly, based on the structure of the yeast complex, the WDR4 positions mutated in patients suffering from primordial dwarfism (D164A, R170L or R170Q; (Chen et al., 2018; Shaheen et al., 2015; Trimouille et al., 2018)) are all predicted to be located at the WDR4-METTL1 interface. They most likely affect complex formation and then the *in vivo* stability of METTL1. Close comparison of these structures suggests that Trm82 likely activates Trm8 by molding its active site (Leulliot et al., 2008). Indeed, the loop β 4- α D, which contains a strictly conserved Arg very important for *E. coli* TrmB MTase activity (Purta et al., 2005), is not defined in the structure of Trm8 alone due to intrinsic flexibility. Upon Trm82 binding, this loop partially folds to adopt the same conformation as in the structure of *Bacillus subtilis* TrmB (Zegers et al., 2006). These structures together with additional biochemical approaches tend to indicate that Trm82 does not influence SAM binding and might not be necessary for tRNA binding, rationalizing the absence of Trm82 orthologues in bacteria and of cross-link between tRNA and Trm82 (Alexandrov, Grayhack & Phizicky, 2005). Further studies will be required on the human METTL1-WDR4 complex as the crystal structure of the yeast complex does not explain for instance how the PKB α -dependent phosphorylation of METTL1 on Ser27 (the equivalent residue is not visible in the yeast structure) strongly inhibits its enzymatic activity without affecting the interaction with WDR4 (Cartlidge et al., 2005).

7. TRM7/FTSJ1 : A DUAL-SPECIFICITY ENZYME

2'O-methylation (Nm) is a widespread RNA modification frequently present in rRNAs, sn(o)RNAs, where they are most of the time catalyzed by the C/D box RNA-guided multimeric MTase (Hofler & Carlomagno, 2020). In tRNAs, this modification is mostly deposited by stand-alone proteins, such as the fungal Trm7 MTase and its metazoan ortholog FTSJ1 (Table 1). These enzymes are responsible for the forma-

tion of Cm₃₂ and Nm₃₄ in the anticodon loop of some tRNAs (Fig. 3), thereby rigidifying this local structure and strengthening the codon-anticodon interaction during decoding (Guy & Phizicky, 2015; Li et al., 2020; Pintard et al., 2002). The Nm₃₄ modification plays a prominent role in translation efficiency and fidelity in *E. coli* (Sato et al., 2000), while it protects tRNAs from endonucleolytic cleavage in human cells and fruit flies (Angelova et al., 2020; Vitali & Kiss, 2019).

The Trm7/FTSJ1 proteins are strongly conserved within eukaryotes and are composed of a single MTase domain followed by a C-terminal extension of little to no functional importance (Hirata et al., 2019). The deletion of *TRM7* gene in *S. cerevisiae* and *S. pombe* yeasts severely impacts growth (Guy & Phizicky, 2015; Pintard et al., 2002) while defects (splicing errors, premature stop codons, single point mutants or frameshift) in human *FTSJ1* gene are associated with neurodevelopmental disorders known as non-syndromic X-linked intellectual disability (NSXLID; Table 2; (Freude et al., 2004; Froyen et al., 2007; Guy et al., 2015; Ramser et al., 2004; Takano et al., 2008)). Furthermore, in neuroblastoma cells and Ehrlich tumors, a large proportion of tRNA^{Phe} is devoid of Cm₃₂ and Gm₃₄ (see below; (Kuchino et al., 1982)), arguing in favor of a more critical role in brain than in other organs. This is in agreement with the much higher fetal expression levels of FTSJ1 mRNA in brain than in other organs (Freude et al., 2004). Homozygous male or female mice deficient in FTSJ1 expression are less sensitive to pain, have affected learning capacities, defects in metabolism, hematology and muscle strength, phenotypes also shared by human patients suffering from NSXLID (Jensen et al., 2019). Human FTSJ1 is also down-regulated in lung cancer cells compared to in normal lung cells and so Nm is in tRNAs (He et al., 2020). The expression level of FTSJ1 in those cells is directly correlated with the suppression of cell proliferation, the inhibition of cell migration and the promotion of apoptosis rates, indicating that this tRNA modification enzyme could function as a tumor suppressor gene. In human cells, the silencing of FTSJ1 results in reduced expression of p21, an inhibitor of cyclin-dependent kinase with tumor suppressor functions (Holzer et al., 2019). This effect is independent of the role of FTSJ1 on tRNA^{Phe} modification but could occur through rRNA methylation as the yeast Trm7 was shown to be involved in the formation of Nm in 18S and 25S rRNAs (Chou et al., 2017). These modifications occur within rRNA regions adopting conformations similar to the tRNA anticodon arm and one of these modifications re-

quired Trm734 (Chou et al., 2017). In fruit-fly, a specialization of tRNA Nm MTase enzymes occurred with the emergence of two FTSJ1 orthologues sharing 63% sequence identity : CG7009 and CG5220, which are responsible for the formation of Nm₃₄ and Cm₃₂, respectively (Angelova et al., 2020). The mutation of one of these genes does not affect fly viability or fertility but results in various phenotypes such as the dysfunction of small RNA pathways (si-, pi- and mi-RNAs) and an increased sensitivity to the infection by RNA viruses. The single mutants both display general locomotion troubles while the double mutant exhibits measurable reductions of weight and size as well as in the lifespan (Angelova et al., 2020).

Most phenotypic outcomes observed in mice and humans likely result from affected protein synthesis and mirror recently described effects due to mutations in amino acyl tRNA synthetases (Musante et al., 2017). Indeed, the deletion of Trm7 in budding yeast results in a significant reduction in protein synthesis (reduction of 70%) and a higher sensitivity to paromomycin (Pintard et al., 2002). In fission and budding yeasts, the deletion of TRM7 gene leads to a subtle but specific increase in the levels of uncharged tRNA^{Phe} (a tRNA containing both Cm₃₂ and Gm₃₄ modifications), leading to the activation of the GAAC response (Han, Guy, Kon & Phizicky, 2018). In FTSJ1 KO human cells, the translation efficiency of the UUU codon was recently shown to be reduced. This could rationalize the ID phenotype of patients with defective FTSJ1 proteins as about 40% of genes enriched in this codon are involved in neurodevelopment process and nervous system or brain function (Li et al., 2020). Considering that the Gm₃₄-dependent wybutosine (yW₃₇ in yeast) or peroxywybutosine (o2yW₃₇ in human) modifications at position 37 of tRNA^{Phe} are important for the maintenance of the correct reading frame (Waas, Druzina, Hanan & Schimmel, 2007), the Trm7/FTSJ1 enzymatic activity appears as important for the accuracy of mRNA decoding. The importance of Nm₃₄ in decoding fidelity is also highlighted by the recent identification of 2,6-diaminopurine as an inhibitor of metazoan FTSJ1 (Trzaska et al., 2020). Indeed, upon treatment with this molecule, the reduction of Cm₃₄ modification in tRNA^{Trp} (decoding the UGG codon) led to a specific increased read-through of UGA nonsense mutations in human cells and in a mouse model, accompanied by the insertion of a Trp residue, indicating that the Cm₃₄ modification reduces the competition between tRNA^{Trp} and release factor eRF1 during translation termination events on the UGA codon (Trzaska et al., 2020).

The dual-specificity of the Trm7/FTSJ1 proteins as Cm₃₂ and Nm₃₄ MTases relies on their ability to interact with two mandatory activators in a mutually exclusive manner (Table 1; (Guy et al., 2012)). On the one hand, in complex with Trm732 in yeasts or THADA in human, Trm7/FTSJ1 catalyzes the formation of Cm₃₂. Trm732 contains a DUF2428 domain and Armadillo repeats (Guy et al., 2012) but so far, no structural information on this protein is available. On the other hand, when Trm7/FTSJ1 associates with Trm734/Rtt10 (*S. cerevisiae*) or WDR6 (human), it catalyzes the formation of Nm₃₄. The crystal structure of the *S. cerevisiae* Trm7-Trm734 complex revealed that Trm734 is composed of three WD-40 repeat seven-bladed β -propeller domains (Hirata et al., 2019). Among those WD-40 domains, the first (WD-40 A) and third (WD-40 C) domains contribute equally to complex formation and mainly interact with the active site side of Trm7 MTase domain (Fig. 5B). The crucial role played by Trm734 in Trm7-dependent Nm₃₄ modification is likely due to its contribution to tRNA binding using a large positively charged area made by basic residues at the surface of the second WD-40 domain (WD-40 B) and also in the proximity of the SAM binding site. This region was proposed to interact with the tRNA D-arm to correctly orient the anticodon arm into the active site from Trm7 (Hirata et al., 2019). Future structural studies will have to determine more precisely the tRNA binding mode to this complex.

8. TRMT112 AND ITS MTASE PARTNERS : ON ALL FRONTS OF MRNA TRANSLATION

TRMT112 (Trm112 in *S. cerevisiae*) is a small protein present in the three domains of life. So far, the TRMT112 proteins have been mostly characterized in eukaryotes where they are very important for optimal growth in *S. cerevisiae* and in *A. thaliana* for instance (Bourgeois, Letoquart, van Tran & Graille, 2017; Hu et al., 2010; Mazauric et al., 2010). TRMT112 is an essential component of various MTase holoenzymes involved in the biogenesis of both ribosomal subunits, translation elongation and termination and hence TRMT112 is at the crossroad of the protein synthesis mechanism (Table 1). It interacts with 4 and 6 MTases in *S. cerevisiae* and in human, respectively. All these catalytic subunits belong to the SAM-dependent class I family of MTases and adopt the classical Rossmann fold. Most of the pioneer studies on TRMT112 network were performed using *S. cerevisiae* as model organism and then

replicated in human cells, demonstrating the strong conservation of the function of these enzymes across evolution. Recently, the TRMT112 interaction network has been characterized in archaea, revealing that the biochemical activities of four eukaryotic TRMT112-MTase complexes are conserved in archaea, thereby emphasizing on the strong similarities between eukaryotic and archaeal translation machineries (van Tran et al., 2019; van Tran et al., 2018; Wang et al., 2020).

A first MTase interacting with TRMT112 is BUD23 (also known as WBSCR22 in human and Bud23 in *S. cerevisiae*). This enzyme catalyzes the formation of m⁷G (Fig. 3) on 18S rRNAs at position 1575 in *S. cerevisiae* (Figaro et al., 2012; Letoquart et al., 2014; White et al., 2008) and 1636 in human, respectively (Haag, Kretschmer & Bohnsack, 2015; Ounap, Kasper, Kurg & Kurg, 2013; Zorbas et al., 2015). This modified nucleotide is at the crest between ribosomal tRNA P- and E-sites (Fig. 6A-B). Surprisingly, although BUD23 active site is evolutionary conserved, its enzymatic activity is not required for BUD23 function in yeast and human cells under laboratory conditions (Letoquart et al., 2014; White et al., 2008; Zorbas et al., 2015). However, the deletion of the BUD23 gene results in a ribosomal subunit imbalance due to 18S rRNA processing defects indicating that BUD23 is very important for the 40S biogenesis. This complex most probably plays a role as an RNA chaperone during this process, in line with its early recruitment to pre-ribosomes, while the m⁷G formation is a late event occurring on almost mature 40S subunits (Ameismeier, Cheng, Berninghausen & Beckmann, 2018; Letoquart et al., 2014). The precise role of BUD23 catalytic activity remains unknown but it could facilitate the release of the TRMT112-BUD23 complex or act as one of the numerous checkpoints occurring along the very complex mechanism of 40S biogenesis.

Another TRMT112 partner also involved in 18S rRNA modification is the human METTL5 protein, the long sought-after enzyme responsible for the formation of m⁶A at position 1832 of human 18S rRNA (Fig. 2A, 6A & 6C; (van Tran et al., 2019)), a modification identified in human 18S rRNA almost 40 years ago (Maden, 1986). In the mature 40S subunit, this modification is located in very close proximity from the mRNA P-site codon within the decoding center. Several recent studies on METTL5 revealed that this protein is important for pluripotency, global translation rate and differentiation in mouse embryonic stem cells (Ignatova et al., 2020; Xing et al., 2020),

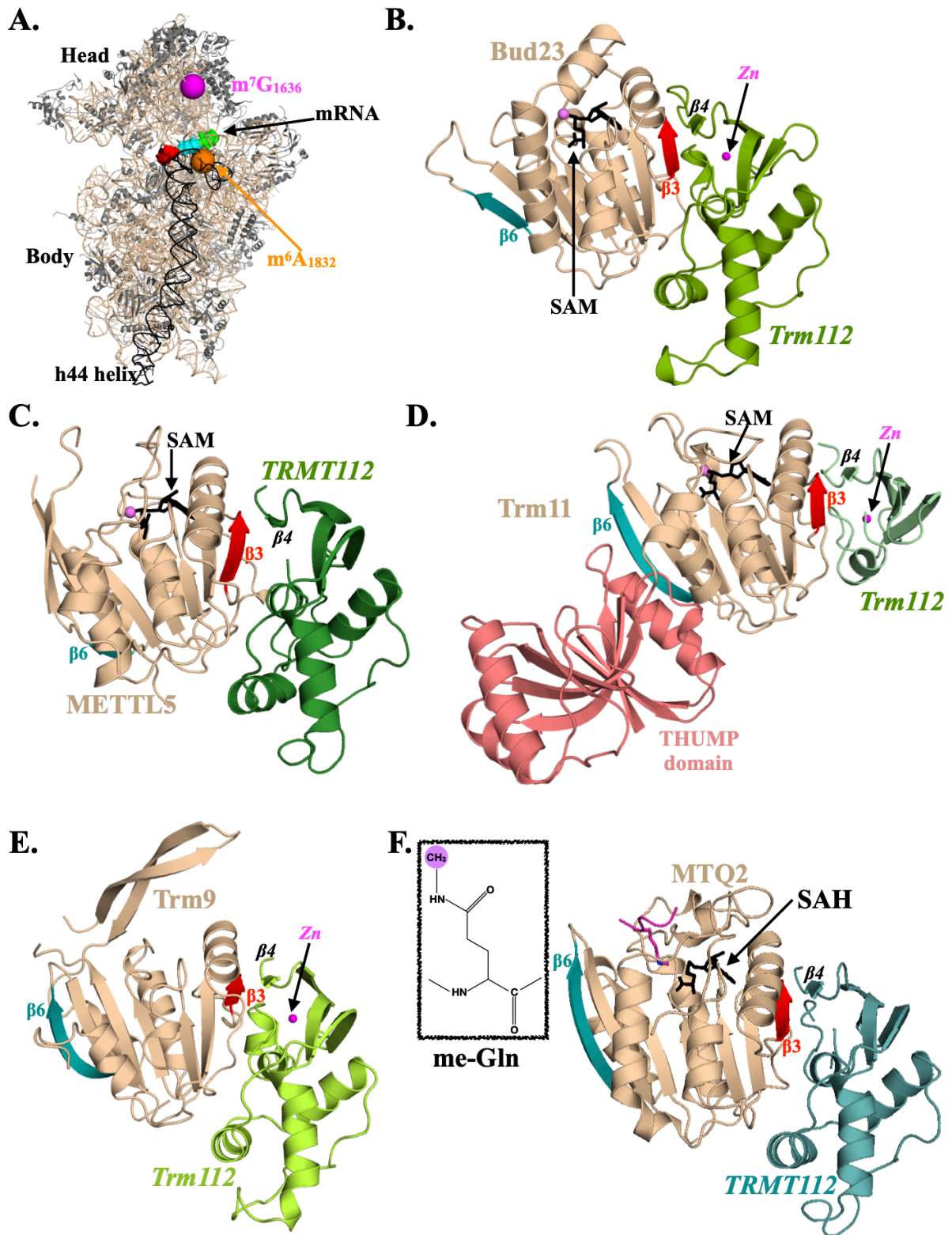


Figure 6 : TRMT112, a master modifier of translation actors.

A. Representation of the cryo-EM structure of the mature human 40S with nucleotides modified by the BUD23-TRMT112 and METTL5-TRMT112 complexes, high-

lighted as magenta and orange spheres, respectively. The 18S rRNA is shown in light brown while ribosomal proteins are in grey. The h44 helix, a structural hallmark of the small ribosomal subunit, is colored in black. The mRNA codons present in the A-, P- and E-sites are colored in red, cyan and green, respectively.

B. The *S. cerevisiae* Bud23-Trm112 complex bound to SAM (black sticks). The zinc atom bound to yeast Trm112 is shown as a magenta sphere. For the sake of clarity, the same orientation is used in all panels of this figure. TRMT112 elements are labeled in italics in all panels.

C. The human METTL5-TRMT112 complex bound to SAM (black sticks).

D. The *Archaeoglobus fulgidus* Trm11-Trm112 complex bound to SAM (black sticks). The zinc atom bound to *A. fulgidus* Trm112 is shown as a magenta sphere. The Trm11 THUMP domain is colored pink.

E. The *Yarrowia lipolytica* yeast Trm9-Trm112 complex. The zinc atom bound to Trm112 is shown as a magenta sphere.

F. The human HEMK2-TRMT112 complex bound to SAH (black sticks) and to a peptide from its histone H4 substrate (magenta, which Lys12 methylated shown as sticks). Inset : Chemical structure of N5-methyl-glutamine (me-Gln).

for stress resistance in *C. elegans* (Lieberman et al., 2020) and for normal walking behavior in *D. melanogaster* (Leismann et al., 2020). Very interestingly, although the METTL5 enzyme does not seem important for proper 18S rRNA processing and hence 40S biogenesis in human cells, mutations in this gene were identified in patients suffering from intellectual disorders (Table 2; (Hu et al., 2019; Richard et al., 2019)), clearly sustaining a crucial role for METTL5 in proper brain maturation. In addition, this enzyme regulates translation initiation, is up-regulated in breast cancer tissues and is necessary for growth of these cancer cells (Rong et al., 2020).

Two other TRMT112 partners modify tRNAs and hence contribute to mRNA decoding and translation elongation. Indeed, TRMT112 interacts with the tRNA MTase TRMT11 (Trm11 in *S. cerevisiae*), which catalyzes the formation of methyl-2-guanosine (m²G) at position 10 of many tRNAs (Fig. 3 & 6D ; (Bourgeois et al., 2017; Purushothaman, Bujnicki, Grosjean & Lapeyre, 2005)). TRMT11 proteins are composed of a N-terminal THUMP (for Thiouridine synthase, methyltransferase and pseudouri-

dine synthase) domain, which is involved in the recognition of the 3' CCA tail of the tRNA substrates (Neumann et al., 2014) followed by a MTase domain. The m²G₁₀ modification is assumed to contribute to tRNA stability and is very important for yeast growth under conditions where the formation of dimethyl-2-guanosine (m^{2,2}G) at position 26, which stacks onto m²G₁₀ in the structure of mature tRNAs, is affected. This suggests that both modifications play an important role for the tRNA tertiary structure (Purushothaman, Bujnicki, Grosjean & Lapeyre, 2005). The biological relevance of this modification at position 10 further comes from a study performed in *Thermococcus kodakarensis* archaeon where this gene turns out to be important for growth at very high temperature (Orita et al., 2019). The second tRNA MTase interacting with TRMT112 is ALKBH8 (Trm9 in *S. cerevisiae*), which catalyzes the methylation of the 5-carboxymethyluridine group (cm⁵U) formed by the Elongator (or ELP) complex at position 34 from tRNAs into 5-methoxycarbonylmethyl-uridine (mcm⁵U; Fig. 3 & 6E; (Chen, Huang, Anderson & Bystrom, 2011; Kalhor & Clarke, 2003; Mazauric et al., 2010)). This modification of the wobble position enhances decoding of AGA, CAA, GAA and AGG codons and is important for translation fidelity. It also prevents mispairing between tRNA^{Arg} and AGU or AGC Ser codons (Blanchet et al., 2018; Patil, Chan, et al., 2012). In yeast, Trm9 contributes to DNA damage response (Begley et al., 2007; Patil, Dyavaiah, et al., 2012) and protects cells from proteotoxic stress that can arise upon affected translation (Nedialkova & Leidel, 2015). ALKBH8 orthologues in metazoa and worms are more complex than yeast Trm9 as they possess two additional domains : an RNA-recognition motif (RRM), which is assumed to contribute to tRNA substrate recognition and an AlkB-like dioxygenase domain that catalyzes the hydroxylation of mcm⁵U into mchm⁵U ((S)-5-methoxycarbonylhydroxymethyluridine; (D. Fu et al., 2010; Y. Fu et al., 2010; Pastore et al., 2012; Songe-Moller et al., 2010; van den Born et al., 2011)). ALKBH8 is highly expressed in a variety of cancer cells and its silencing induces apoptosis, thereby suppressing tumor growth, angiogenesis and metastasis (Shimada et al., 2009). Finally, although mice lacking ALKBH8 (ALKBH8^{-/-}) appear normal (Songe-Moller et al., 2010), mutations in human ALKBH8 gene have been identified in patients suffering from intellectual disabilities associated with dysmorphism or macrocephaly (Table 2; (Monies et al., 2019)), emphasizing again on the functional importance of tRNA modifications for correct brain development. In human cells, TRMT112 also interacts with a second

Trm9 orthologue TRMT9B (or hTrm9L), a gene acting as a negative regulator of tumor growth but of yet unknown molecular function (Begley et al., 2013; Gu et al., 2018). It is noteworthy that the depletion of one of these two Trm9 orthologues renders human cancer cells sensitive either to anti-cancer drugs (ALKBH8; (D. Fu et al., 2010)) or to antibiotics (TRMT9B; (Begley et al., 2013)).

Finally, although MTQ2/HEMK2, the last MTase known to interact with TRMT112, is not an RNA but a protein MTase, it deserves attention as it is also acting on an essential component of the mRNA synthesis process (Figaro, Scrima, Buckingham & Heurgue-Hamard, 2008; Heurgue-Hamard et al., 2005; Heurgue-Hamard et al., 2006). Indeed, MTQ2 modifies the translation termination factor eRF1, a tRNA mimicry that recognizes the stop codons present in the ribosomal A-site. More precisely, the TRMT112-MTQ2 complex methylates the nitrogen atom from the Gln side chain of the universally conserved GGQ motif (Fig. 6F). This latter is critical for the release of the nascent proteins as it dives into the ribosome peptidyl transferase center and contributes to the coordination of the water molecule attacking the ester bond between the nascent peptide and the P-site tRNA. The methylation of this Gln side chain most probably stabilizes the correct orientation of the GGQ motif into the PTC by increasing van der Waals interaction with surrounding nucleotides (Li, Chang, Ward & Cate, 2020), similarly to its role in bacteria (Svidritskiy et al., 2019). The MTQ2 protein is also essential for the biogenesis of the 60S large ribosomal subunit in yeast (Lacoux et al., 2020). However, in contrast to Bud23, its catalytic activity is mandatory for this latter function. Whether this role occurs through the methylation of eRF1 or of a yet unknown substrate remains to be clarified. Finally, human MTQ2 also methylates histone H4 on Lys12 side chain nitrogen atom, thereby controlling proliferation of prostate cancer cells (Metzger et al., 2019). The MTQ2 gene is of utmost biological importance as its inactivation in mouse results in early embryonic lethality (Liu et al., 2010).

The small size of the TRMT112 proteins and their large number of MTase partners made TRMT112 a particularly intriguing factor. A combination of biochemical, structural and functional assays has dissected the organization of these complexes and revealed the role of TRMT112 as a crucial component of these various holoenzymes. First, the crystal structures of most TRMT112-MTase complexes have been solved (Fig. 6B-F), revealing that although these various MTases from the same or-

ganism share less than 25%, they all compete to interact in a very similar way with TRMT112 (Bourgeois, Letoquart, van Tran & Graille, 2017; Bourgeois et al., 2017; Letoquart et al., 2014; Letoquart et al., 2015; Liger et al., 2011; Metzger et al., 2019; van Tran et al., 2019; Wang et al., 2020). Indeed, the same region from their MTase domains, *i.e.* the $\beta 3$ side, is contacting the same region from TRMT112. In particular, strand $\beta 3$ from these MTase domains interacts in a parallel manner with strand $\beta 4$ from TRMT112 through a β -zipper to form an extended β -sheet made of the central β -sheets from both partners. As this β -zipper relies on the formation of hydrogen bonds between main chain atoms from both partners, its formation is less sensitive to amino acid sequence variation than to local structure. In addition, some specific electrostatic hot spots involving side chains from residues conserved among the different TRMT112 MTase partners are critical for the interaction between TRMT112 and these MTases (Bourgeois, Letoquart, van Tran & Graille, 2017). Finally, the formation of these complexes buries large hydrophobic areas at the surface of both partners and more specifically on MTases. This latter observation certainly rationalizes why TRMT112 stabilizes most of its MTase partners *in cellulo* (Figaro et al., 2012; Leetsi, Ounap, Abroi & Kurg, 2019; Ounap, Leetsi, Matsoo & Kurg, 2015; van Tran et al., 2019; Zorbas et al., 2015). Indeed, in the absence of TRMT112, the hydrophobic regions of these MTases are exposed to the solvent, most probably leading to their destabilization and to their elimination by the proteasome (Ounap, Leetsi, Matsoo & Kurg, 2015). Hence, these MTases are very unlikely to be present as stable proteins in the absence of their TRMT112 partner. From these studies, TRMT112 also turned out to be required for SAM binding by the MTase subunit (Bourgeois et al., 2017; Liger et al., 2011). Indeed, the loop connecting strands $\beta 3$ and $\beta 4$ from the MTases is directly sandwiched by the adenosine moiety of the SAM cofactor on one side and TRMT112 on the other side. Hence, in the absence of TRMT112, this loop is most probably too flexible to contribute to SAM binding while upon interaction with TRMT112, it is stabilized thereby stimulating SAM binding. Reciprocally, SAM binding seems to strengthen the interaction between TRMT112 and some MTases as shown for Trm9 or Mtq2 mutants unable to interact with SAM and that co-purify less efficiently with Trm112 in yeast (Lacoux et al., 2020; Liger et al., 2011). Finally, a conserved region from TRMT112 that comes into close proximity from the MTase active sites, contributes to substrate binding by at least yeast Mtq2

and Trm11 enzymes but not BUD23 according to the cryo-EM of the human BUD23-TRMT112 complex bound to a late 40S (Ameismeier, Cheng, Berninghausen & Beckmann, 2018; Bourgeois et al., 2017; Liger et al., 2011; Wang et al., 2020).

In summary, TRMT112 is crucial for the activity of numerous MTases modifying various components of the mRNA translation machinery. Hence, this single protein can act as a lever regulating simultaneously the cellular abundance of these different MTases. The up- or down-regulation of TRMT112 may rapidly affect the activity of all these MTase partners while the up- or down-regulation of one of TRMT112 MTase partner might also positively or negatively influence the activity of the other complexes, thereby potentially perturbing a delicate equilibrium.

9. COMMON THEMES IN RNA MTASE ACTIVATION

The different eukaryotic and viral MTase holoenzymes discussed in this review illustrate how various protein architectures can act as activators of the class I SAM-dependent MTase subunits. Indeed, three activators (TRMT6, METTL14 and vaccinia virus D12) adopt a class I MTase domain with a degenerated SAM binding site and hence have lost intrinsic catalytic activity. The METTL14 and TRMT6 activating subunits most probably arose from gene duplication events (Finer-Moore et al., 2015; Sledz & Jinek, 2016; M. Wang et al., 2016; Wang, Doxtader & Nam, 2016; X. Wang et al., 2016). This is striking for METTL14, which exhibits the same circular permutation in the β strands order as its METTL3 partner. This is also obvious for TRMT6 as the eukaryotic TRMT6-TRMT61A heterotetramer is structurally very similar to the prokaryotic TrmI homotetramers (Barraud et al., 2008; Finer-Moore et al., 2015; M. Wang et al., 2016). Based on its structural similarity with ribose 2'-OH cap MTases, the vaccinia virus D12 protein very likely originated from a 2'-OH ribose MTase present in an ancestral virus. Interestingly, these three proteins all interact with the β_6 side of the catalytic MTase domain but with different orientations relative to this domain (Fig. 2B, 2D & 4A). Two other activators (WDR4 and WDR6) are made of the same building block, *i.e.* a seven-bladed β -propeller (also known as WD-40 domain) present as one copy in WDR4 but as three consecutive domains in WDR6 (Hirata et al., 2019; Leulliot et al., 2008) but they interact with various regions of the MTase domain (Fig. 5). Two other activators (viral Nsp10 and TRMT112) are zinc-binding domains (although only TRMT112 proteins from some archaea and

most fungi still bind a zinc atom, while metazoan proteins do not; Fig. 2E & 6B-F). The last activator (RAM) folds as two extended α helices (Fig. 2C). These latter partners all bind to different regions of the catalytic MTase domain indicating that there is no specific region to which the activators should bind to fulfill their role. Beyond the structural diversity observed for the discussed holoenzymes, some common trends emerge to explain the crucial influence of the activating subunits. Indeed, most of these activators (METTL14, RAM, D12, WDR4 and TRMT112) stabilize the catalytic subunit *in vivo* by shielding from the solvent an hydrophobic region present at the surface of the MTase domain. Several activators (RAM, D12, Nsp10, WDR6 and TRMT112) also increase the affinity of the catalytic subunit for SAM while most (all but WDR4) contribute directly or indirectly to substrate binding. However, a more comprehensive view of the roles of these activators will require further structural studies of complexes formed by these holoenzymes with their substrates. Indeed, with the exception of the nsp10-nsp16 cap MTase bound to short cap analogues (Rosas-Lemus et al., 2020), of TRMT61A-TRMT6 complex with tRNA^{Lys} (Finer-Moore et al., 2015) and of the human BUD23-TRMT112 complex bound to a 40S undergoing late maturation events (Ameismeier, Cheng, Berninghausen & Beckmann, 2018), the structures of the other complexes could not be obtained in the presence of an RNA substrate. This is most probably due to the transient interaction between these enzymes and their RNA substrates but also to the intrinsic flexibility of the RNA molecules, all of which renders structural analyses of protein-RNA complexes very challenging.

The higher complexity observed for these eukaryotic enzymes very likely offers an exquisite manner to finely tune their activity but can also be considered as a double-edged sword as the more subunits, the higher the risk to induce pathologies due to mutations of one of these subunits. Among these different holoenzymes, two systems deserve special attention as they offer several levers to tune the levels of some specific post-transcriptional modifications. First, depending on its activating subunit (WDR6 or THADA), FTSJ1 will target two different tRNA positions (32 and 34 on various tRNAs). Second, TRMT112 interacts in a very similar way and in a competitive manner with at least six human class I MTases acting on various RNA or protein substrates and activate them (Fig. 6B-F). On the one hand, the reduced/enhanced expression of the central subunit (FTSJ1 or TRMT112) or its inactivation/stimula-

tion by post-translational modifications such as phosphorylation could concomitantly reduce/increase the levels of several epitranscriptomic marks. On the other hand, the variation in the expression level or activity of a single subunit (WDR6, THADA or any MTase interacting with TRMT112) could change the balance between the various modifications they contribute to, with yet unanticipated physiological consequences. For instance, patients with duplication of the normal FTSJ1 gene suffer from moderate mental impairment and mild dysmorphic features (Bonnet et al., 2006), indicating that the FTSJ1 levels in the brain need to be carefully controlled. To my knowledge, such regulatory circuits have still been poorly characterized but pioneer work performed on METTL1 has shown that its PKB α -dependent phosphorylation strongly inhibits its enzymatic activity (Cartlidge et al., 2005).

CONCLUSION

This review only focuses on viral and eukaryotic RNA MTase holoenzymes, whose three-dimensional structures have been solved. This is probably only the tip of the iceberg as many other RNA MTase complexes are likely awaiting to be discovered. Some might be less stable than those discussed in this review, making their identification more difficult. It is tempting to speculate that due to the cellular macromolecular crowding, several RNA modification enzymes are spatially very close and may even organize as more labile larger complexes or « modificosomes » that can be difficult to isolate (Grosjean, 2015; Ishitani et al., 2003). Other RNA modification complexes might also be cell-specific. The identification of new multimeric RNA MTases will likely emphasize on the large variety of structural solutions that have evolved to activate the MTase subunits. Indeed, THADA (Trm732 in yeast) is predicted to be an Armadillo-repeat protein, a scaffold not represented in the already determined crystal structures of holoenzymes discussed in this review. Recently, DALRD3, a protein predicted to fold as an amino-acyl tRNA synthetase, was shown to interact with and activate the METTL2A and METTL2B MTases to catalyze the formation of m³C₃₂ on some tRNA^{Arg} isoacceptors (Lentini et al., 2020; Xu et al., 2017). DALRD3 is enhancing binding of tRNA substrates by METTL2A/B subunits but does not affect their cellular stability contrary to several other activating subunits discussed here (Lentini et al., 2020). As for several other RNA modification enzymes, a DALRD3 variant has been identified in two brothers suffering from developmental

brain disorder (Table 2). This variant is characterized by the presence of a premature stop codon that leads to the destabilization of the DALRD3 protein and consequently, reduced levels of m³C₃₂ in tRNAs modified by the DALRD3-METTL2A/B complexes (Lentini et al., 2020). The NSUN4 RNA MTase responsible for the formation of m⁵C at position 911 from 12S rRNA of the mitochondrial small ribosomal subunit is also interacting with an accessory protein, *i.e.* MTERF4 (Camara et al., 2011; Metodiev et al., 2014). Although the structure of this complex has been solved (Spahr et al., 2012; Yakubovskaya et al., 2012), this case is not discussed in this review as NSUN4 is sufficient for the methylation of 12S rRNA. Indeed, its association with MTERF4 is only required for the joining of the small and large ribosomal subunits into functional ribosomes (Metodiev et al., 2014). Other cases of holoenzyme MTases will most probably emerge in the future as suggested by the recent description of the interaction network of several human METTL proteins (Ignatova et al., 2019). The molecular strategies discussed in this review illustrate the division of labor between activating and catalytic subunits in RNA MTases. Some of these properties probably apply to other holoenzymes such as the Dcp1-Dcp2 mRNA decapping enzyme (Charenton & Graille, 2018), the Tad2-Tad3 A₃₄-to-I₃₄ tRNA deaminase (Gerber & Keller, 1999), the KEOPS and elongator complexes responsible respectively for the t⁶A₃₇ and cm⁵U₃₄ tRNA modifications (Abbassi, Biela, Glatt & Lin, 2020; Krutyholowa, Zakrzewski & Glatt, 2019; Thiaville, Iwata-Reuyl & de Crecy-Lagard, 2014), the human THUMPD1-NAT10 (Tan1-Kre33 in yeast) ac⁴C₁₂ tRNA acetyltransferase (Sharma et al., 2015) or the Rtt109-Vps75 and Rtt109-Asf1 histone acetyltransferases (Kolonko et al., 2010; Lercher, Danilenko, Kirkpatrick & Carlomagno, 2018).

The understanding of the exact physiological roles of all these different RNA modifications is only emerging and will require further studies. In particular, for diseases associated to a less active variant of a precise RNA modification enzyme or of its activating partner, it remains to be established whether the pathology is directly due to the diminished level or the absence of the corresponding modification or to the fact that this modification is required for the addition of another more important modification. Indeed, it is well-known that the deposition of some tRNA modifications require the presence of other modifications due to the existence of modification circuits (Barraud et al., 2019). For instance, the synthesis of wybutosine at position 37

(yW₃₇) of yeast tRNAs or the corresponding peroxywybutosine (o2yW₃₇) modification in human tRNAs from m¹G₃₇ requires the presence of Cm₃₂ and Gm₃₄ in tRNA^{Phe} (Guy & Phizicky, 2015; Guy et al., 2012; Guy et al., 2015). One can also easily imagine that mutations severely affecting the expression, stability or function of several RNA modifications enzymes will have a dramatic effect on human development and hence will be lethal, thereby escaping identification by genome and whole exome sequencing approaches commonly used. Hence, it is of utmost importance to develop appropriate animal model systems to apprehend the crucial role of those enzymes in human physiology.

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