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REVIEW

Three's a crowd – why did three N-terminal methyltransferases evolve for one job?

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ABSTRACT

N-terminal methylation of the α-amine group (Nα-methylation) is a post-translational modification (PTM) that was discovered over 40 years ago. Although it is not the most abundant of the Nα-PTMs, there are more than 300 predicted substrates of the three known mammalian Nα-methyltransferases, METTL11A and METTL11B (also known as NTMT1 and NTMT2, respectively) and METTL13. Of these ∼300 targets, the bulk are acted upon by METTL11A. Only one substrate is known to be Nα-methylated by METTL13, and METTL11B has no proven *in vivo* targets or predicted targets that are not also methylated by METTL11A. Given that METTL11A could clearly handle the entire substrate burden of Nα-methylation, it is unclear why three distinct Nα-methyltransferases have evolved. However, recent evidence suggests that many methyltransferases perform important biological functions outside of their catalytic activity, and the Nα-methyltransferases might be part of this emerging group. Here, we describe the distinct expression, localization and physiological roles of each Nα-methyltransferase, and compare these characteristics to other methyltransferases with non-catalytic functions, as well as to methyltransferases with both catalytic and non-catalytic functions, to give a better understanding of the global roles of these proteins. Based on these comparisons, we hypothesize that these three enzymes do not just have one common function but are actually performing three unique jobs in the cell.

KEY WORDS: METTL11A, METTL11B, METTL13, NTMT1, NTMT2, Methyltransferase

Introduction

Although N-terminal methylation of the α-amine group (Nα-methylation) was discovered many decades ago, its biochemical and biological roles are only recently being uncovered. Based on the N-terminal Ala/Pro/Ser-Pro-Lys sequence of known targets, it was predicted that all eukaryotic Nα-methylations could be attributed to the action of a single hypothetical enzyme, deemed the PK methyltransferase (Stock et al., 1987). However, it took over 20 additional years to identify METTL11A (also known as NRMT1 and NTMT1), from the methyltransferase-like family of seven-β-strand (7BS) methyltransferases (Fig. 1A), as the PK methyltransferase (Tooley et al., 2010; Webb et al., 2010), and it was not until this discovery that comprehensive analysis of Nα-methylation could begin. It is now predicted that over 300 proteins are Nα-methylated by METTL11A (Petkowski et al., 2012). This methylation regulates many aspects of protein biochemistry, including protein stability, protein–DNA interactions and protein–protein interactions, and as such, plays important roles in many biological processes, including mitosis, DNA damage repair and transcriptional regulation (Chen et al., 2007; Faughn et al., 2018; Nevitt et al., 2018). It also features prominently in oncogenesis and regulates many developmental processes (Bonsignore et al., 2015a, b; Catlin et al., 2021; Tooley et al., 2021; Zhang et al., 2021).

The second Nα-methyltransferase, METTL11B (also known as NRMT2 and NTMT2) (Fig. 1B), was identified through sequence homology with METTL11A (Petkowski et al., 2013; Webb et al., 2010), with 50% sequence identity and 75% sequence similarity (Petkowski et al., 2013). *In vitro*, METTL11B recognizes a similar Ala/Pro/Ser-Pro-Lys consensus sequence as METTL11A but differs in its catalytic activity. METTL11A is a distributive trimethylase, meaning it releases its substrate after each methyl group is placed, whereas METTL11B is primarily a monomethylase (Dong et al., 2018; Petkowski et al., 2013). *In vivo*, it is unclear whether METTL11B is active. The majority of Nα-methylated substrates are fully trimethylated (Chen et al., 2007), and the low amount of detected Nα-monomethylation could result from the distributive nature of METTL11A (Richardson et al., 2015). METTL11B also has low, tissue-specific expression, which points to a very specialized function (Petkowski et al., 2013). Currently, there are no verified *in vivo* substrates of METTL11B, no known METTL11B-specific substrates and no known loss-of-function phenotypes. Despite these undefined *in vivo* roles, METTL11B mutations are found in a variety of cancers, and its expression is altered during many developmental processes (Hong et al., 2020; Lin et al., 2022; Shields et al., 2017; Zhou et al., 2021), suggesting that it has biological importance.

The third Nα-methyltransferase that has been identified, METTL13 (also known as EEF1A-KNMT and FEAT) (Fig. 1C), is in the same family as METTL11A and METTL11B but more distantly related. METTL13 is not a PK methyltransferase, though both its S-adenosyl methionine (SAM)- and substrate-binding domains exhibit some structural similarity to those of METTL11A and METTL11B (Fig. 1D,E). METTL13 is a dual function methyltransferase with one known substrate, eukaryotic elongation factor 1α (eEF1A) (Jakobsson et al., 2018b). METTL13 can methylate both the Nα-amine of eEF1A, as well as the internal Lys55 (K55) residue (Jakobsson et al., 2018b). Loss of METTL13 results in codon-specific alterations in translation rate, which produces an overall decreased translational output (Jakobsson et al., 2018b; Liu et al., 2019). Altered translation rates have profound impacts on cancer growth, and as such METTL13 can act as both an oncogene and a tumor suppressor (Liu et al., 2019, 2021).

Given that, biochemically, Nα-methylation has the same function regardless of the enzyme that catalyzes it, it is unclear why three enzymes are designated for this modification, especially when one does the bulk of the work. METTL11A potentially has hundreds of substrates, whereas METTL13 only has one and METTL11B might

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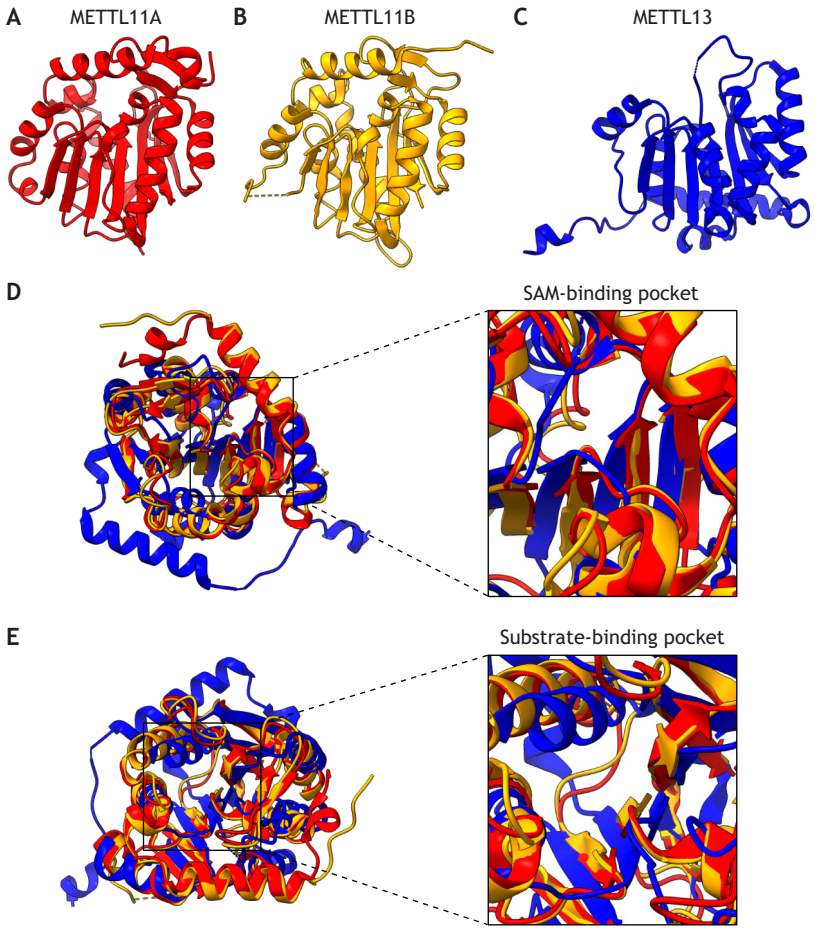

Fig. 1. Structure comparison of the three Nα-methyltransferases. (A) METTL11A is shown in red (PDB 2EX4), (B) METTL11B is shown in yellow (PDB 5UBB), and (C) the METTL13 C-terminal domain is shown in blue (PDB 5WCJ). (D) A merge of the three structures with a magnification showing the SAM-binding pocket. (E) A different view of the structures with a magnification showing the substrate-binding pocket. Though they have different substrate specificities, both the SAM- and substrate-binding pockets of METTL13 exhibit structural similarity to those of METTL11A and METTL11B.

not have any. These unequal substrate burdens suggest that the main function of METTL13 and METTL11B is not Nα-methylation. It is becoming more recognized that enzymes have functions beyond their catalytic activity in the methyltransferase field, not only among the METTL family, but among other DNA and protein methyltransferases as well. Here, we will compare the characteristics of the Nα-methyltransferases to other methyltransferases that perform both catalytic and non-catalytic functions to provide a more global understanding of the biological roles of the Nα-methyltransferases.

N-terminal methyltransferases

METTL11A

From the late 1970s to the late 1980s, the presence of di- or tri-methylation was reported on the N-terminus of several proteins, including *E. coli* ribosomal proteins (Dognin and Wittmann-Liebold, 1977, 1980; Lederer et al., 1977), *Crithidia oncopelti* cytochrome c557 (Pettigrew and Smith, 1977; Smith and Pettigrew, 1980), *Tetrahymena*, starfish *Asterias rubens*, and *Drosophila* histone H2B (Desrosiers and Tanguay, 1988; Martinage et al., 1985; Nomoto et al., 1982), as well as vertebrate myosin light chains (Henry et al., 1982). METTL11A, a 7BS Nα-methyltransferase, was identified as the responsible enzyme in 2010 by two independent groups that sought to characterize the Nα-methylation of human regulator of chromatin condensation 1 (RCC1) or yeast ribosomal proteins (Tooley et al., 2010; Webb et al., 2010). METTL11A was originally found to be a distributive trimethylase that methylated a canonical Ala/Pro/Ser-Pro-Lys (X-P-K) N-terminal consensus sequence after initiating methionine cleavage (Tooley et al., 2010). However, subsequent consensus sequence analysis identified an expanded non-canonical sequence that allows A/P/S/G/M in the first position, A/P/S/G/M/E/N/Q in the second position, and either K or R in the third position (Petkowski et al., 2012). Together, the canonical and non-canonical sequences predict over 300 METTL11A targets (Petkowski et al., 2012).

Biochemically, Nα-methylation has been shown to primarily regulate protein–nucleotide interactions, and accordingly, many METTL11A substrates play roles in chromatin organization, DNA damage repair, and transcriptional regulation (Cai et al., 2014; Chen et al., 2007; Conner et al., 2022; Dai et al., 2013; Nevitt et al., 2018; Sathyan et al., 2017) (Fig. 2). Nα-methylation of RCC1 is essential for its binding to mitotic DNA and establishing the Ran-GTP gradient (Chen et al., 2007). The centromere proteins A and B (CENP-A and -B) require Nα-methylation for recruitment and binding to the centromere, respectively (Dai et al., 2013; Sathyan et al., 2017). Nα-methylation of damaged DNA-binding protein 2 (DDB2) is required for its recruitment to cyclobutane pyrimidine dimers and efficient nucleotide excision repair (Cai et al., 2014), and both myosin light chain 9 (MYL9) and zinc fingers and homeoboxes 2 (ZHX2) require Nα-methylation for their roles in transcriptional regulation (Conner et al., 2022; Nevitt et al., 2018). Other verified targets include the ribosomal proteins L23a (RPL23A), L12a (RPL12A) and S25a (RPS25A) (Tooley et al., 2010; Webb et al., 2010). It is still unknown how Nα-methylation of the ribosomal proteins affects their function, though it is predicted it might regulate protein–RNA interactions.

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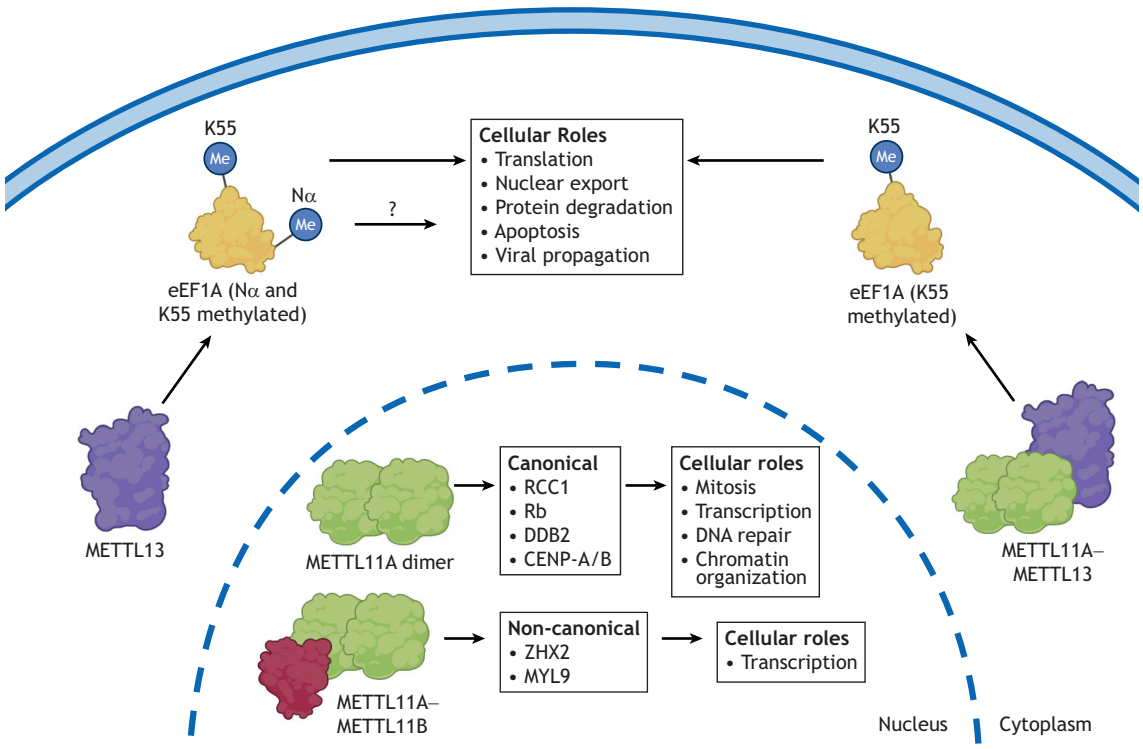

Fig. 2. Cellular context of the three Nα-methyltransferases. METTL11A alone is primarily a dimer that regulates processes reliant on protein–DNA interactions in the nucleus. Also in the nucleus, the METTL11A dimer can bind METTL11B, which activates METTL11A activity against non-canonical substrates. In the cytoplasm, METTL13 alone can methylate both the N-terminus and K55 of eEF1A. Although K55 methylation is known to promote translational output, the function of eEF1A Nα-methylation remains unknown. Also in the cytoplasm, METTL13 can bind METTL11A (predicted to also be a dimer here), which promotes K55 methylation and inhibits Nα-methylation.

METTL11A expression is ubiquitous across many different cell types and tissues (see Human Protein Atlas; https://www.proteinatlas.org/ENSG00000148335-NTMT1) (Fig. 3A,B). Within cells, it is found in both the nucleus and cytoplasm, although an enzymatic activity has only been observed in the nucleus (Petkowski et al., 2012). Transcription of METTL11A is regulated by cAMP responsive element-binding protein 1 (CREB1) and activated under conditions of serum starvation and during myoblast differentiation (Tooley et al., 2021). Loss of CREB1-mediated METTL11A expression in myoblasts results in their transdifferentiation to osteoblasts, implicating METTL11A as a regulator of stem cell differentiation (Tooley et al., 2021). METTL11A expression is also regulated by N^6-adenosine methylation (m^6A) (Bade et al., 2021). Depletion of METTL3, a subunit of the major m^6A writer complex, increases METTL11A protein levels, indicating that m^6A promotes the decay of METTL11A mRNA (Bade et al., 2021). Finally, METTL11A stability and activity are regulated through complex formation with the other Nα-methyltransferases. Heterotrimer formation of a METTL11A dimer and METTL11B monomer increases the half-life of METTL11A and trimethylation of its non-canonical substrates (Faughn et al., 2018). In contrast, complex formation with METTL13 decreases the activity of METTL11A on both its canonical and non-canonical substrates (Parker and Schaner Tooley, 2022 preprint).

Given the ubiquitous expression and localization of both METTL11A and its array of targets, it is not surprising that METTL11A has several different characterized roles in development and disease. METTL11A is necessary for proper development, as mice that lack METTL11A (*Nrmt1*$^{-/-}$) exhibit phenotypes associated with premature aging, including kyphosis, hair loss, gray fur, an impaired DNA damage response and neurodegeneration (Bonsignore et al., 2015b; Catlin et al., 2021). In the brain, loss of METTL11A specifically causes premature differentiation of the two postnatal neural stem cell niches, depleting these populations and causing severe neurodegeneration as the animals age (Catlin et al., 2021). As mentioned above, METTL11A also plays a role in muscle stem cell differentiation. Mouse myoblasts that are depleted of METTL11A do not express Pax7 and do not progress down the muscle differentiation pathway and instead exhibit characteristics of osteoblasts (Tooley et al., 2021).

METTL11A is also frequently mutated in human cancers and has both tumor suppressor and oncogene activity. In breast cancer cells, loss of METTL11A promotes oncogenic phenotypes, such as increased growth rate and invasiveness (Bonsignore et al., 2015a). In contrast, METTL11A promotes growth of colon cancer cells (Shields et al., 2017), and in cervical cancer, METTL11A overexpression promotes proliferation and migration through the transcription factor ELK3 (Zhang et al., 2021). A large multi-omics study found METTL11A to be overexpressed in several additional tumor types including lung adenocarcinoma, and this overexpression was correlated with poor prognosis (Campeanu et al., 2021). Work in the field now has focused on developing selective inhibitors of METTL11A, including inhibitors that target either the substrate-binding pocket (peptidomimetic) or the substrate-binding pocket and the cofactor (SAM)-binding pocket (bisubstrate) for treatment of cancers that exhibit METTL11A overexpression (Chen et al., 2020; Dong et al., 2022; Mackie et al., 2020).

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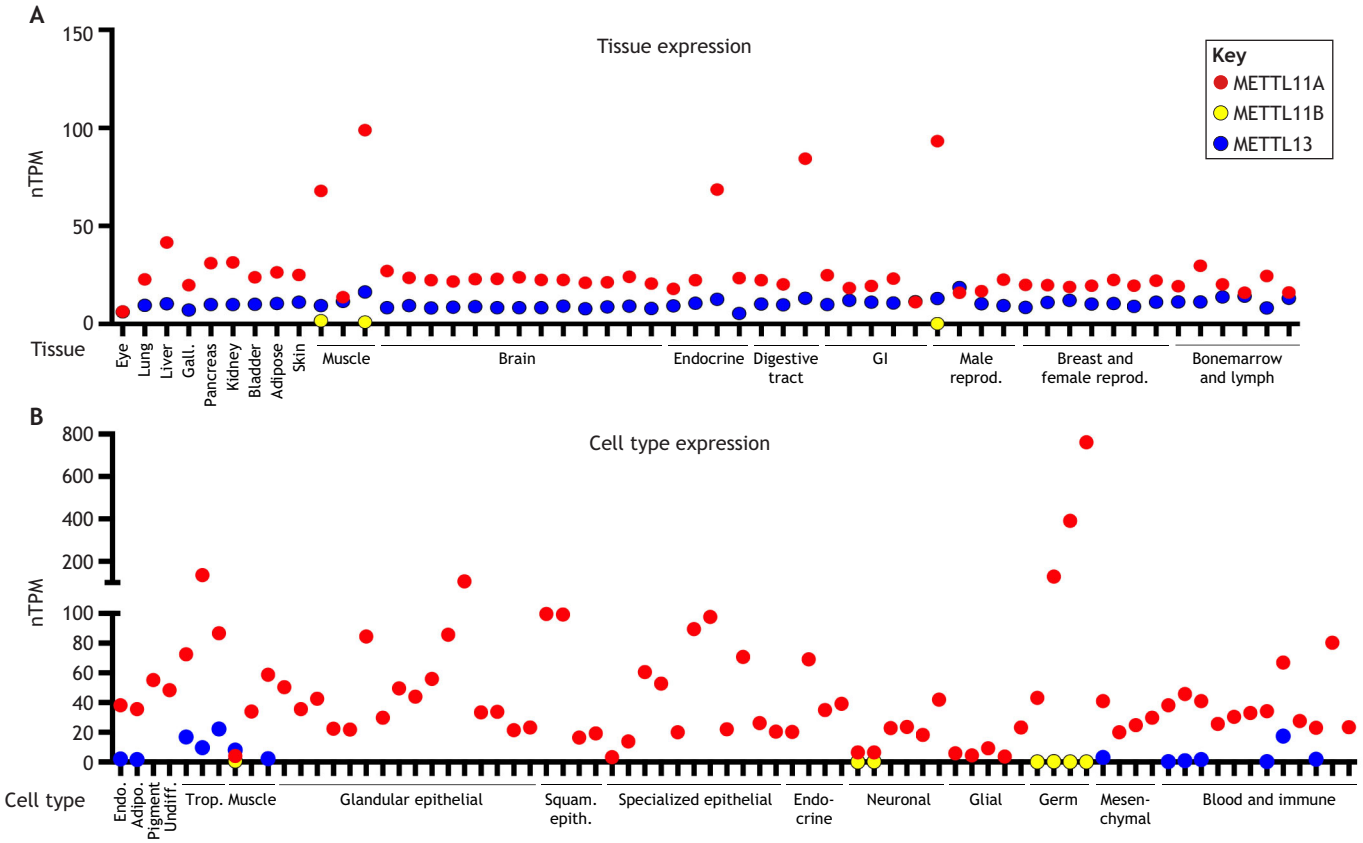

Fig. 3. Expression of the three Nα-methyltransferases. METTL11A, METTL11B, and METTL13 expression in human (A) tissue types and (B) cell types. Although only METTL11B (yellow) does not exhibit ubiquitous tissue expression, both METTL11B and METTL13 (blue) have cell-type-specific expression, with METTL11B expressed in neuronal, muscle and germline cells, and METTL13 primarily expressed in endothelial, trophoblastic, blood and immune cells. METTL11B is expressed in tissues and cell types where METTL11A expression is highest. The data were retrieved from the Human Protein Atlas (HPA, https://www.proteinatlas.org/ accessed on 28 September 2022). Values are the consensus transcript expression level reported as normalized transcripts per million (nTPM). Gall., gallbladder; reprod., reproductive system; GI, gastrointestinal tract; Endo., endothelial cells; Adipo., adipocytes; Undiff., undifferentiated cells; Trop., trophoblasts; Squam. Epith., squamous epithelial cells.

METTL11B

METTL11B was the second Nα-methyltransferase discovered based on its sequence similarity to METTL11A (Petkowski et al., 2013; Webb et al., 2010). Although METTL11B recognizes the same XPK consensus as METTL11A, it is primarily a monomethylase (with some evidence suggesting it is capable of di- or tri-methylation of GPK or PPK substrates) with much lower *in vitro* activity than METTL11A (Dong et al., 2018). In addition, no type of *in vivo* activity has been demonstrated for METTL11B, and no substrates that are exclusive to METTL11B have been found. METTL11B is localized to the nucleus, and its expression is highest in heart and skeletal muscle and the testes (Fig. 3A). Interestingly, METTL11B expression is often found in cells or tissues with high METTL11A expression (Fig. 3A,B). This has led to speculation that METTL11B is needed in tissues with a high substrate burden, such as skeletal muscle, which contains many myosins and ribosomal proteins (Petkowski et al., 2013). Although it has been shown that complex formation between METTL11A and METTL11B increases the half-life and methylation activity of METTL11A against non-canonical substrates (Fig. 2), the reverse is not true (Faughn et al., 2018). Catalytic activity of METTL11B is also not needed for the stabilization and activation of METTL11A, suggesting that METTL11B plays the role of a non-catalytic stabilizing protein for METTL11A (Faughn et al., 2018).

Similar to METTL11A, METTL11B mRNA is upregulated during myogenic and also osteoblastogenic and osteocytic differentiation (Hong et al., 2020). METTL11B also has its own distinct roles in development and disease separate from those of METTL11A. A genome wide association study (GWAS) of pig growth rate identified the METTL11B region as containing the most significantly associated SNPs (Horodyska et al., 2017), and three separate studies have identified *Mettl11b* mutation or methylation patterns to be significantly associated with atrial fibrillation or heart failure (Hong et al., 2021; Lin et al., 2022; Thorolfsdottir et al., 2017). Despite not having any confirmed exclusive *in vivo* substrates, METTL11B is highly mutated in many different cancer types (Catalogue of Somatic Mutations in Cancer, COSMIC, https://cancer.sanger.ac.uk/cosmic). High METTL11B expression correlates with worse prognosis in colon cancer (Zhou et al., 2021), and recently, machine learning was used to predict a network of genes involved in glioblastoma tumorigenesis that included METTL11B as a target of the Wnt/β-catenin pathway (Xiang et al., 2022).

METTL13

Early work to characterize PTMs on brine shrimp, rabbit and yeast eEF1A noted that its N-terminus was not susceptible to protease digestion (Cavallius et al., 1993; Dever et al., 1989; van Hemert et al., 1984), but the identity of the blocking modification was not

uncovered for another 30 years. In 2016, Nα-methylation of yeast eEF1A was identified, as well as the responsible enzyme, Efm7 (YLR285W) (Hamey et al., 2016). The human eEF1A Nα-methyltransferase, METTL13, was identified 2 years later (Jakobsson et al., 2018b). METTL13 has two distinct methyltransferase domains – the C-terminal domain that trimethylates the eEF1A N-terminus and an N-terminal domain that dimethylates K55 (Jakobsson et al., 2018b). Both the N- and C-terminal domains have the typical 7BS structure, but the C-terminal domain is more closely related to spermidine synthase (SpdS) than other 7BS methyltransferases (Jakobsson et al., 2018b). Through peptide assays, it was confirmed that the consensus sequence for Nα-methylation by METTL13 is M-[G/A/P]-[K/R/F/Y/Q/H]-[K/R/Q/H/I/L] after removal of the iMet (Jakobsson et al., 2018b). However, both the Nα and lysine methylation activities of METTL13 appear to be highly specific for eEF1A. No other methylation sites on any other proteins are disrupted by METTL13 loss *in vivo,* and no other substrates with the Nα-consensus sequence can be methylated by METTL13 *in vitro* (Jakobsson et al., 2018b). Although other minor, tissue-specific or developmental timepoint-specific substrates cannot be ruled out, these studies indicate that eEF1A is the primary substrate of METTL13.

The canonical role of eEF1A is to bind aminoacyl-tRNAs (aa-tRNAs) and facilitate their transport to the growing polypeptide chain at the A site of the ribosome during the elongation step of translation, through binding of GTP and release of GDP by the guanine exchange factor (GEF) eEF1B (Andersen et al., 2001). Domain I of eEF1A, which contains both the N-terminus and K55, is the domain responsible for GTP-binding and, along with domain II, interaction with eEF1B (Andersen et al., 2001). Methylation of eEF1A by METTL13 increases translational output (Liu et al., 2019), and knockout of METTL13 results in the codons for lysine and histidine being translated more slowly, whereas codons for alanine and tryptophan are translated faster (Jakobsson et al., 2018b). Mutation of K55 to an arginine residue (K55R) cannot rescue the protein synthesis defects seen with METTL13 loss, indicating that this methylation site plays a role in translational regulation (Liu et al., 2019). The functional role of eEF1A Nα-methylation remains unknown, though in addition to delivering aa-tRNAs to the ribosome, eEF1A has other roles in the cell, including nuclear export, protein degradation, apoptosis, and viral propagation (Mateyak and Kinzy, 2010), and Nα-methylation could be affecting any of these processes (Fig. 2).

METTL13 is primarily cytoplasmic, although it can be found in the nucleus (Li et al., 2016). It is ubiquitously expressed in all tissues but appears to only be expressed in certain cell types within those tissues with enrichment in endothelial, mesenchymal, blood and immune cells (Fig. 3A,B) (Human Protein Atlas; https://www.proteinatlas.org/ENSG00000010165-EEF1AKNMT). Several mechanisms for the regulation of METTL13 have been identified at the gene, mRNA and protein level. Hematopoietic-expressed and neurologic-expressed sequence 1-like (HN1L; also known as JPT2) can upregulate METTL13 expression by interaction with the transcription factor AP-2γ, which directly binds to the METTL13 promoter (Li et al., 2019). The microRNA miR-16 recognizes and binds to the 3′ untranslated region (UTR) of *Mettl13* mRNA, inhibiting its translation (Liang et al., 2015). METTL13 protein is also cleaved by caspase-3 during apoptosis, resulting in a C-terminal fragment with anti-apoptotic activity (Takahashi et al., 2011). In addition, METTL13 has been shown to interact with Myc, GAB1, SPROUTY2 and METTL11A (Liu et al., 2021; Parker and Schaner Tooley, 2022 preprint; Yousaf et al., 2018). Although the effects of its interactions with GAB1, SPROUTY2 and Myc remain to be identified, binding of METTL13 to METTL11A increases methylation at K55 and decreases Nα-methylation of eEF1A (Parker and Schaner Tooley, 2022 preprint).

During development, METTL13 is primarily expressed in the testis, brain and liver (Takahashi et al., 2011). In the testes, METTL13 is specifically expressed in fetal and adult Leydig cells (Li et al., 2018). Fetal Leydig cells secrete insulin like peptide 3 (INSL3), which acts on relaxin/insulin-like family peptide receptor 2 (RXFP2) in the gubernaculum ligament to cause swelling that is required for the first phase of testis descent (Bay and Andersson, 2011; Shima and Morohashi, 2017). Depletion of METTL13 correlates with increased activation of 5′ AMP-activated protein kinase (AMPK) and suppression of INSL3 expression in Leydig cells (Li et al., 2018). Deleting one *Mettl13* allele in male mice causes undescended testicles, defects in spermatogenesis and infertility, coupled with an absence of INSL3 protein in Leydig cells (Li et al., 2018). It is hypothesized that METTL13 enables normal testis migration by inhibiting the AMPK pathway and thus preventing a reduce in INSL3 secretion by Leydig cells (Li et al., 2018). Further studies are needed to determine what roles METTL13 has in the brain and liver. Also of note, METTL13 can act as a modifier to prevent deafness caused by a mutation in the GRB2-associated binding protein 1 (GAB1) gene (Yousaf et al., 2018). Individuals with the G116E mutation in GAB1 experience deafness, unless they also have the R544Q mutation in METTL13, which then protects against deafness and suggests these residues might be important for interaction (Yousaf et al., 2018).

Given its role in increasing translational output and the increased translational needs of cancer cells, it is not surprising that a large multi-omics study identified *METTL13* as the most mutated of all METTL genes in human cancers and found that METTL13 mutations were associated with unfavorable overall survival across different cancer types (Campeanu et al., 2021). In breast, head and neck squamous cell, and hepatocellular carcinomas, METTL13 is upregulated, and its expression is associated with poor prognosis (Elsemman et al., 2016; Li et al., 2019; Wang et al., 2017, 2021). There are also some studies indicating METTL13 acts as a tumor suppressor. In bladder cancer, METTL13 is underexpressed, and its downregulation is correlated with the development and progression of disease (Zhang et al., 2016). Overexpressing METTL13 in bladder cancer cells inhibits proliferation, migration, and invasion by reinstating the G1/S cell cycle checkpoint through downregulation of CDK6, CDK4 and CCND1 (Zhang et al., 2016). Similar to what is seen with bladder cancer, lower METTL13 expression in clear cell renal cell carcinoma (ccRCC) tissue is associated with poor prognosis, and METTL13 expression is negatively correlated with tumor grade and disease progression (Liu et al., 2021). As eEF1A K55 methylation is favorable to cancer growth (Liu et al., 2019), the ability of METTL13 to act as a tumor suppressor in certain tissues might be based on its Nα-methylation activity or non-catalytic roles.

‘Methyltransferases’ with only non-catalytic functions

There is a growing number of ‘methyltransferases’ that only have non-catalytic functions. These are often found in complex with other methyltransferases and regulate their activity. Some of these regulatory non-catalytic methyltransferases predominantly co-express with the methyltransferase they regulate, indicating constitutive regulation, while others exhibit unique expression patterns, indicating targeted regulation. Despite their lack of

catalytic activity, these methyltransferases are serving important biological roles. Below, we will discuss these roles for comparison with the Nα-methyltransferases.

PRMT1^{PRO}

Catalytically inactive enzyme paralogs (prozymes) have been most extensively studied in the human parasite *Trypanosoma brucei*. They were thought to be unique to the polyamine biosynthesis pathway (Willert et al., 2007), until a recent study showed that a predicted protein arginine methyltransferase (PRMT) in *T. brucei* is actually an inactive PRMT prozyme (Kafkova et al., 2017). Originally named *Tb*PRMT3, it was found to have no *in vitro* activity and to harbor mutations in conserved PRMT motifs (Kafkova et al., 2017). During polyamine synthesis in *T. brucei*, catalytically dead paralogs interact with weak paralogs to increase their activity (Willert et al., 2007). Accordingly, *Tb*PRMT3 was combined with the other *Tb*PRMT enzymes to look for cooperativity. Indeed, *Tb*PRMT3 was renamed *Tb*PRMT1^{PRO} after it was found that *Tb*PRMT3 activated the weak activity of *Tb*PRMT1 (Kafkova et al., 2017). *Tb*PRMT1^{PRO} activates *Tb*PRMT1 by providing a chaperone function; both *Tb*PRMT1^{PRO} and *Tb*PRMT1 are needed to adopt the highly conserved PRMT domain architecture and for substrate binding (Hashimoto et al., 2020). The *Tb*PRMT1 complex is necessary for the *T. brucei* starvation stress response, with *Tb*PRMT1^{PRO} mediating interaction with many metabolic proteins (Kafková et al., 2018). These were the first studies to demonstrate a methyltransferase enzyme–prozyme pair in *T. brucei* (Kafkova et al., 2017), and they also provide an example of a methyltransferase pair where both catalytically active and catalytically dead partners are needed for optimal activity.

DNMT3L

Although the term prozyme is most commonly used in *T. brucei*, complexes between active and inactive enzyme paralogs are also found in other species, such as the extensively studied mammalian DNMT3A–DNMT3B–DNMT3L DNA methyltransferase (DNMT) complex. DNMT3A and DNMT3B establish *de novo* methylation during mammalian development (Okano et al., 1999), whereas DNMT3L is an inactive paralog that lacks the essential motifs for enzyme activity (Aapola et al., 2000). Although it is catalytically inactive, DNMT3L stimulates cofactor binding and enzymatic activity of either DNMT3A or DNMT3B and also promotes the stability of DNMT3A (Hata et al., 2002; Veland et al., 2019). DNMT3A and DNMT3B are ubiquitously expressed in almost all tissues, whereas DNMT3L expression is very low and found primarily in the liver, kidney and testis (see Human Protein Atlas; Aapola et al., 2000). These data indicate that DNMT3L is not necessary for the function of DNMT3A or DNMT3B and provides targeted regulation. Accordingly, it has been shown that DNMT3B is capable of functioning without the activation of DNMT3L (Gao et al., 2022). Despite its low tissue-specific expression, DNMT3L does appear to have unique biological roles. Deletion of DNMT3L in male mice results in sterility due to improperly regulated paternal imprinting (Webster et al., 2005). In addition, DNMT3L specifically interacts with hepatitis B virus X protein (Hbx) and is involved in the progression of HBV-mediated hepatocellular carcinoma (Fan et al., 2016).

METTL14

Another well-studied mammalian complex of active and inactive methyltransferases is the METTL3–METTL14 complex (from the same family as METTL11A, METTL11B and METTL 13), which catalyzes m^{6}A, a modification that affects mRNA stability and subsequent translation (Wang et al., 2016b). Initially, METTL3 and METTL14 were identified individually as m^{6}A methyltransferases, and both were found to have methyltransferase activity *in vitro* (Liu et al., 2014). However, the crystal structure of the complex indicated that only METTL3 could bind SAM, indicating METTL14 was not catalytically active (Wang et al., 2016b). Subsequent biochemical assays demonstrated that, similar to what is seen with the PRMT1 complex in *T. brucei*, METTL3 is the primary catalytic core and METTL14 performs a structural role and serves as a substrate-binding platform (Wang et al., 2016b).

METTL3 and METTL14 have very similar tissue expression patterns, and even though METTL14 appears to only function non-catalytically, the consequences of its loss are equally severe as loss of METTL3. Both METTL3 and METTL14 have roles in stem cell and cancer biology (Tooley et al., 2022), and similar to what was seen with METTL3, loss of METTL14 results in embryonic stem cells that fail to differentiate and neural stem cells that fail to self-renew (Meng et al., 2019; Wang et al., 2018). Both proteins can also act as either tumor suppressors or oncogenes in various cancers (Shi et al., 2022), indicating that the catalytic and non-catalytic functions are equally important. Interestingly, even though METTL3 and METTL14 have similar expression patterns, their subcellular localization is not identical. Whereas METTL14 is only found in the nucleus and nuclear speckles (Zhang et al., 2020), where it is presumably carrying out m^{6}A methylation of RNA, METTL3 is found in the nucleus, nuclear speckles and cytoplasm (Lin et al., 2016; Zhang et al., 2020). In the cytoplasm, METTL3 interacts with the translation initiation machinery and promotes translation (Lin et al., 2016). Its ability to promote translation is independent of its catalytic activity (Lin et al., 2016), indicating that METTL3 has both catalytic and non-catalytic functions in translational regulation.

Methyltransferases with both catalytic and non-catalytic functions

In addition to METTL3, there are several other methyltransferases known to have both catalytic and non-catalytic functions. These functions can occur simultaneously in the same cell compartment (mediated through different protein domains) or can be cell compartment specific. Either way, the catalytic and non-catalytic functions frequently work together to regulate a common process. Below, we will discuss some known methyltransferases with both catalytic and non-catalytic functions for comparison with the Nα-methyltransferases.

METTL16

METTL16, an additional m^{6}A methyltransferase from the METTL family, has recently been found to have both catalytic and non-catalytic functions that regulate translation from both the nucleus and cytoplasm, respectively. Unlike METTL3 or METTL14, which have a broad substrate pool, METTL16 is only known to methylate a few distinct substrates, including *MAT2A* mRNA and U6 snRNA (Pendleton et al., 2017; Shima et al., 2017; Warda et al., 2017). *MAT2A* mRNA encodes for SAM synthetase, and its methylation by METTL16 regulates its abundance in response to SAM levels (Shima et al., 2017). m^{6}A methylation of U6 snRNA by METTL16 is hypothesized to weaken binding with the pre-mRNA, which allows for proper splicing and dissociation (Warda et al., 2017).

As m^{6}A methylation is primarily added co-transcriptionally in the nucleus, it was interesting to find that a large proportion of endogenous METTL16 actually localizes to the cytoplasm (Su et al., 2022). To determine whether cytoplasmic METTL16

could be interacting with ribosomes and affecting global translation efficiency, both wild-type (WT) and catalytically dead METTL16 were tethered to luciferase transcripts, and their translation efficiency measured. Interestingly, both proteins were able to significantly enhance luciferase protein expression (Su et al., 2022), indicating that regulating translation is also a non-catalytic function of METTL16. It was further shown that METTL16 directly interacts with eukaryotic initiation factors 3a and 3b (eIF3a and eIF3b) and ribosomal RNA (rRNA), promoting assembly of the translation initiation complex (Su et al., 2022). Although METTL16 has a small N-terminal RNA-binding domain and two vertebrate conserved regions (VCR1 and VCR2) in its C-terminal domain, it appears its methyltransferase domain is necessary for interaction with eIF3 and rRNA (Su et al., 2022), indicating that, like METTL3, localization regulates the catalytic and non-catalytic activities of METTL16.

DNMT1

DNMT1 is a mammalian maintenance methyltransferase that is responsible for the methylation of CpG islands during cell division. Methylation by DNMT1 in gene promoters represses expression, whereas methylation in gene bodies promotes expression (Mohan, 2022). DNMT1 has a conserved C-terminal catalytic domain and a large N-terminal domain that consists of a variety of regulatory motifs known to promote protein–protein interactions with other proteins involved in cell signaling, cell cycle and chromatin organization (Espada, 2012; Mohan, 2022). Interestingly, the N-terminal domain of DNMT1 alone can repress reporter gene expression, indicating that gene repression is not solely reliant on catalytic activity but could be regulated through these protein interactions (Espada, 2012; Mohan, 2022).

It had previously been hypothesized that gene promoters without a high density of CpG islands were silenced in a DNMT1-independent manner. However, new evidence suggests that repression at these sites could occur through a non-catalytic function of DNMT1. DNMT1 knockout in HCT116 human colon carcinoma cells results in increased expression of many downstream genes (Clements et al., 2012). However, unexpectedly, these increased transcription levels could be silenced by expression of both WT DNMT1 and a catalytically dead mutant, and in neither case, were promoter methylation levels restored (Clements et al., 2012). Both WT and catalytically dead DNMT1 interact with the histone demethylase LSD1 (also known as KDM1A), which removes activating histone methylation marks, and this interaction is required for LSD1 recruitment to promoters (Mohan, 2022). It is thought that the DNMT1-mediated repression seen in the absence of promoter methylation might be through this interaction with LSD1 (Mohan, 2022). DNMT1 can also promote gene expression by promoting methylation of the gene body, which is thought to be mediated by interaction with and sequestration of the SNAIL1 (also known as SNAI1)–HDAC1 repressive complex (Espada et al., 2011). These data clearly suggest that DNMT1 can perform catalytic and non-catalytic roles in gene regulation that are mediated through its different domains.

COMPASS

The histone H3 lysine 4 monomethylation (H3K4me1) mark is placed by the COMPASS-like family of methyltransferases, including Trithorax-related (Trr) in *Drosophila* and MLL3 and MLL4 (also known as KMT2C and KMT2B, respectively) in mammals (Herz et al., 2012). H3K4me1 is typically enriched in the body of actively transcribed genes and enhancers (Herz et al., 2012), indicating the COMPASS methyltransferases are needed for promotion of gene transcription. However, recent studies have found that catalytically deficient COMPASS mutants have milder phenotypes than expected. Knockout of Trr in *Drosophila* results in embryonic lethality, but this lethality can be rescued by catalytically deficient Trr mutants (Rickels et al., 2017). To determine whether a similar phenomenon exists in mammals, CRISPR-Cas9 editing was used to delete the methyltransferase domains from both MLL3 and MLL4 in mouse embryonic stem cells (mESCs). Complete removal of MLL3 and MLL4 from mESCs resulted in a decrease in their alkaline phosphatase activity (a marker of pluripotency) (Rickels et al., 2017). However, expression of mutants lacking the methyltransferase domains was able to rescue alkaline phosphatase activity (Rickels et al., 2017), indicating that MLL3 and MLL4 serve functions outside of H3K4 methylation.

The specific non-catalytic functions of the COMPASS methyltransferases are still unclear, although data suggest they might serve as scaffolds, regulating protein–protein or protein–DNA interactions. A small domain of Trr that binds and stabilizes the histone H3K27 demethylase UTX is sufficient for rescuing the viability of Trr-null mutants (Rickels et al., 2020). MLL4 is known to recruit p300 (also known as EP300) to enhancer sequences, but this is dependent on the protein itself and not the presence of H3K4me1 (Wang et al., 2016a). MLL3 and MLL4 are both primarily nuclear (van Nuland et al., 2013), indicating they are not performing cell compartment-specific functions. However, they do have a much-extended N-terminal domain as compared to other SET-domain containing methyltransferases, and their N-terminal domains contain a variety of interactions motifs, including plant homeodomains fingers (PHD) and high mobility group (HMG) boxes (Sze and Shilatifard, 2016), indicating that similar to what is seen for DNMT1, their catalytic and non-catalytic roles in gene regulation are mediated through different protein domains.

Perspectives

Similar to what is found for its family members METTL3 and METTL16, METTL11A is a methyltransferase that serves both catalytic and non-catalytic roles that are dependent on its cellular localization (Table 1) (Parker and Schaner Tooley, 2022 preprint). However, unlike the other methyltransferases discussed above, its catalytic and non-catalytic activities appear to serve different functions. Of the three Nα-methyltransferases, METTL11A performs the bulk of the enzymatic activity, indicating Nα-methylation is its primary role. We have recently shown that METTL11A also has a non-catalytic role in activating METTL13-mediated methylation of eEF1A K55 in the cytoplasm (Parker and Schaner Tooley, 2022 preprint). As K55 methylation of eEF1A promotes translation, this indicates a non-catalytic role for METTL11A in translational regulation. More comprehensively determining how Nα-methylation specifically affects the function of each substrate and linking METTL11A loss-of-function phenotypes to these substrates will better define its catalytic roles. Determining whether METTL11A has additional cytoplasmic interactors will better define its non-catalytic roles. Together, these experiments will establish whether METTL11A is a unique enzyme with catalytic and non-catalytic roles that serve different functions (Fig. 4).

Although METTL11B has been shown to have *in vitro* methylation activity, we predict it will ultimately be most similar to DNMT3L (Table 1) and primarily serve as a non-catalytic regulator of METTL11A in a targeted manner, perhaps specifically in tissues with a high Nα-methylation substrate burden.

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Table 1. Summary of discussed methyltransferases

Methyltransferase	*In vivo* catalytic activity	Regulation of non-catalytic role	Necessary for activity
METTL11A	Nα-trimethylation	Localization	Yes
METTL11B	Unknown	Expression	No
METTL13	Nα-trimethylation Lysine dimethylation	Domain	Yes
*Tb*PRMT^{PRO}	–	Constitutive	Yes
DNMT3L	–	Expression	No
METTL14	–	Constitutive	Yes
METTL3	N^6-adenosine methylation	Localization	Yes
METTL16	N^6-adenosine methylation	Localization	Yes
DNMT1	DNA methylation	Domain	Yes
COMPASS	Histone methylation	Domain	Yes

METTL11B does not appear to have any unique substrates that cannot be methylated by METTL11A, and its monomethylation activity is not needed for its activation of METTL11A (Faughn et al., 2018). Although the low expression and extreme tissue selectivity of METTL11B might point to limited biological relevance (Petkowski et al., 2013), its high mutation rate in cancers and accruing significance during development suggest otherwise (Hong et al., 2020, 2021; Horodyska et al., 2017; Lin et al., 2022; Thorolfsdottir et al., 2017; Xiang et al., 2022; Zhou et al., 2021). Given the high biological relevance of DNMT3L, we predict the non-catalytic role of METTL11B will turn out to be of equally high importance. This might be solely through its activation of METTL11A or through its regulation of other yet unknown enzymes. Confirming that METTL11B is indeed not catalytically active *in vivo* will be dependent on the generation of reagents to produce and monitor METTL11B loss, including proteolysis-targeting chimera strategies (PROTAC), which have recently been developed for METTL11A (Zhou et al., 2022) (Fig. 4).

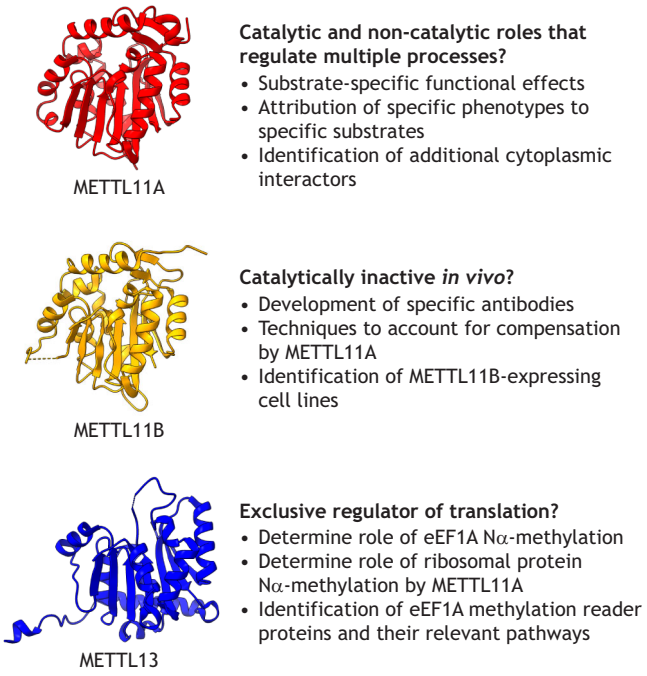

Fig. 4. Methyltransferase-specific questions and obstacles to overcome. We propose three unique functions for the three known Nα-methyltransferases. First, we hypothesize that Nα-methylation enzymatic activity is primarily carried out by METTL11A, although it is unique in that it has both catalytic and non-catalytic functions that serve different roles. Second, we propose that METTL11B is catalytically inactive and serves only to regulate METTL11A. Finally, we put forward the idea that METTL13 exclusively plays a role in translational control and has evolved to use Nα-methylation to this end. Obstacles to fully test these hypotheses are listed for each enzyme.

Finally, we predict that METTL13, like DNMT1 and the COMPASS enzymes (Table 1), is an enzyme whose catalytic and non-catalytic functions work coordinately to regulate translational control. eEF1A is the target of at least four other methyltransferases, METTL10, METTL21B, ECE2 and N6AMT2 (Jakobsson et al., 2018a), suggesting that eEF1A is a sufficiently important substrate to warrant extensive regulation, and both the catalytic and non-catalytic functions of METTL13 might be aimed to this end. We hypothesize that the main function of METTL13 is translational control through eEF1A and that its Nα-methylation activity has evolved as a way to enhance and finetune this control. The enriched expression of METTL13 in endothelial, trophoblastic, blood and immune cells (Fig. 3) supports this hypothesis, as these are cell types that require a large amount of translational control to deal with environmental signals (see Box 1) (Brant-Zawadzki et al., 2007; Kitroser et al., 2012; Piccirillo et al., 2014). Although the recently discovered regulatory effects of METTL11A binding on METTL13 activity also support this hypothesis, it is still unclear whether eEF1A Nα-methylation or reciprocal inhibition of METTL11A by METTL13 affect translational output. Determining the biological roles of eEF1A Nα-methylation by METTL13 and ribosomal protein Nα-methylation by METTL11A will help confirm this prediction by demonstrating whether all the catalytic and non-catalytic functions of METTL13 center on translational control. Identification of reader proteins that recognize eEF1A methylations will also help determine whether the roles of these PTMs are purely translational (Fig. 4).

Here, we have examined the three Nα-methyltransferases, other non-catalytic methyltransferases and methyltransferases with dual catalytic and non-catalytic functions, in order to better understand why mammals have evolved three Nα-methyltransferases to do a job that could easily be done by one. Based on this discussion, we propose that METTL11A evolved as the primary Nα-methyltransferase, although it is unique in that it has catalytic and non-catalytic roles that do not appear to coordinately regulate the same function. We also propose that METTL11B, whose catalytic activity was superfluent, evolved to instead regulate Nα-methylation through METTL11A. Finally, we hypothesize that the main function of METTL13 is translational control through eEF1A, and that both its catalytic and non-catalytic roles evolved to

Box 1. Translational control

Although control of gene expression commonly occurs at the level of transcription, it is also possible to selectively regulate protein synthesis from specific mRNA transcripts. This translational control of protein expression allows for a more rapid response to environmental signals and is useful for cell types that need to quickly alter their phenotypes according to these signals, including endothelial and immune cells (Brant-Zawadzki et al., 2007; Piccirillo et al., 2014). Both the efficiency and/or rate of protein synthesis can be selectively altered for mRNAs under translational control (Brant-Zawadzki et al., 2007). This is frequently accomplished through the translation initiation factors eIF2α and eIF4E. Phosphorylation of eIF2α generally inhibits translation, but it can also selectively upregulate translation of transcripts with open reading frames in their 5′ UTR (Trinh and Klann, 2013). Increased eIF4E activity results in the translation of transcripts with highly structured 5′ UTRs or those that are in complex with ribosome-binding proteins, which are otherwise inefficiently translated (Piccirillo et al., 2014). Although more rare, translational control can also be exerted during the elongation and termination phases. Altering the delivery rate of amino acids for specific codons can modulate the elongation rate and subsequent folding and expression of proteins with high representation of that codon (Hershey et al., 2012). Termination can be regulated for certain mRNAs that allow insertion of selenocysteine at UGA codons instead of terminating synthesis (Hershey et al., 2012). Proteins under translational control include those involved in promoting differentiation, inflammation, and angiogenesis is response to extracellular signals (Piccirillo et al., 2014).

enhance and finetune this function. Therefore, mammals have not evolved three enzymes to perform the same function, but rather one enzyme to place the modification, one enzyme to regulate the modification and one enzyme that uses the modification as a regulatory tool.

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Competing interests

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References

Aapola, U., Kawasaki, K., Scott, H. S., Ollila, J., Vihinen, M., Heino, M., Shintani, A., Kawasaki, K., Minoshima, S., Krohn, K. et al. (2000). Isolation and initial characterization of a novel zinc finger gene, DNMT3L, on 21q22.3, related to the cytosine-5-methyltransferase 3 gene family. *Genomics* **65**, 293-298. doi:10.1006/geno.2000.6168

Andersen, G. R., Valente, L., Pedersen, L., Kinzy, T. G. and Nyborg, J. (2001). Crystal structures of nucleotide exchange intermediates in the eEF1A-eEF1Bα complex. *Nat. Struct. Biol.* **8**, 531-534. doi:10.1038/88598

Bade, D., Cai, Q., Li, L., Yu, K., Dai, X., Miao, W. and Wang, Y. (2021). Modulation of N-terminal methyltransferase 1 by an N(6)-methyladenosine-based epitranscriptomic mechanism. *Biochem. Biophys. Res. Commun.* **546**, 54-58. doi:10.1016/j.bbrc.2021.01.088

Bay, K. and Andersson, A. M. (2011). Human testicular insulin-like factor 3: in relation to development, reproductive hormones and andrological disorders. *Int. J. Androl.* **34**, 97-109. doi:10.1111/j.1365-2605.2010.01074.x

Bonsignore, L. A., Butler, J. S., Klinge, C. M. and Schaner Tooley, C. E. (2015a). Loss of the N-terminal methyltransferase NRMT1 increases sensitivity to DNA damage and promotes mammary oncogenesis. *Oncotarget* **6**, 12248-12263. doi:10.18632/oncotarget.3653

Bonsignore, L. A., Tooley, J. G., Van Hoose, P. M., Wang, E., Cheng, A., Cole, M. P. and Schaner Tooley, C. E. (2015b). NRMT1 knockout mice exhibit phenotypes associated with impaired DNA repair and premature aging. *Mech. Ageing Dev.* **146-148**, 42-52. doi:10.1016/j.mad.2015.03.012

Brant-Zawadzki, P. B., Schmid, D. I., Jiang, H., Weyrich, A. S., Zimmerman, G. A. and Kraiss, L. W. (2007). Translational control in endothelial cells. *J. Vasc. Surg.* **45**, A8-A14. doi:10.1016/j.jvs.2007.02.033

Cai, Q., Fu, L., Wang, Z., Gan, N., Dai, X. and Wang, Y. (2014). alpha-N-methylation of damaged DNA-binding protein 2 (DDB2) and its function in nucleotide excision repair. *J. Biol. Chem.* **289**, 16046-16056. doi:10.1074/jbc.M114.558510

Campeanu, I. J., Jiang, Y., Liu, L., Pilecki, M., Najor, A., Cobani, E., Manning, M., Zhang, X. M. and Yang, Z. Q. (2021). Multi-omics integration of methyltransferase-like protein family reveals clinical outcomes and functional signatures in human cancer. *Sci. Rep.* **11**, 14784. doi:10.1038/s41598-021-94019-5

Catlin, J. P., Marziali, L. N., Rein, B., Yan, Z., Feltri, M. L. and Schaner Tooley, C. E. (2021). Age-related neurodegeneration and cognitive impairments of NRMT1 knockout mice are preceded by misregulation of RB and abnormal neural stem cell development. *Cell Death Dis.* **12**, 1014. doi:10.1038/s41419-021-04316-0

Cavallius, J., Zoll, W., Chakraburtty, K. and Merrick, W. C. (1993). Characterization of yeast EF-1α: non-conservation of post-translational modifications. *Biochim. Biophys. Acta* **1163**, 75-80. doi:10.1016/0167-4838(93)90281-U

Chen, T., Muratore, T. L., Schaner-Tooley, C. E., Shabanowitz, J., Hunt, D. F. and Macara, I. G. (2007). N-terminal α-methylation of RCC1 is necessary for stable chromatin association and normal mitosis. *Nat. Cell Biol.* **9**, 596-603. doi:10.1038/ncb1572

Chen, D., Dong, C., Dong, G., Srinivasan, K., Min, J., Noinaj, N. and Huang, R. (2020). Probing the plasticity in the active site of protein N-terminal methyltransferase 1 using bisubstrate analogues. *J. Med. Chem.* **63**, 8419-8431. doi:10.1021/acs.jmedchem.0c00770

Clements, E. G., Mohammad, H. P., Leadem, B. R., Easwaran, H., Cai, Y., Van Neste, L. and Baylin, S. B. (2012). DNMT1 modulates gene expression without its catalytic activity partially through its interactions with histone-modifying enzymes. *Nucleic Acids Res.* **40**, 4334-4346. doi:10.1093/nar/gks031

Conner, M. M., Parker, H. V., Falcone, D. R., Chung, G. and Schaner Tooley, C. E. (2022). Novel regulation of the transcription factor ZHX2 by N-terminal methylation. *Transcription* **13**, 1-15. doi:10.1080/21541264.2022.2079184

Dai, X., Otake, K., You, C., Cai, Q., Wang, Z., Masumoto, H. and Wang, Y. (2013). Identification of novel alpha-n-methylation of CENP-B that regulates its binding to the centromeric DNA. *J. Proteome Res.* **12**, 4167-4175. doi:10.1021/pr400498y

Desrosiers, R. and Tanguay, R. M. (1988). Methylation of Drosophila histones at proline, lysine, and arginine residues during heat shock. *J. Biol. Chem.* **263**, 4686-4692. doi:10.1016/S0021-9258(18)68837-4

Dever, T. E., Costello, C. E., Owens, C. L., Rosenberry, T. L. and Merrick, W. C. (1989). Location of seven post-translational modifications in rabbit elongation factor 1 alpha including dimethyllysine, trimethyllysine, and glycerylphosphorylethanolamine. *J. Biol. Chem.* **264**, 20518-20525. doi:10.1016/S0021-9258(19)47093-2

Dognin, M. J. and Wittmann-Liebold, B. (1977). The primary structure of L11, the most heavily methylated protein from Escherichia coli ribosomes. *FEBS Lett.* **84**, 342-346. doi:10.1016/0014-5793(77)80721-7

Dognin, M. J. and Wittmann-Liebold, B. (1980). Purification and primary structure determination of the N-terminal blocked protein, L11, from Escherichia coli ribosomes. *Eur. J. Biochem.* **112**, 131-151. doi:10.1111/j.1432-1033.1980.tb04995.x

Dong, C., Dong, G., Li, L., Zhu, L., Tempel, W., Liu, Y., Huang, R. and Min, J. (2018). An asparagine/glycine switch governs product specificity of human N-terminal methyltransferase NTMT2. *Commun. Biol.* **1**, 183. doi:10.1038/s42003-018-0196-2

Dong, G., Iyamu, I. D., Vilseck, J. Z., Chen, D. and Huang, R. (2022). Improved cell-potent and selective peptidomimetic inhibitors of protein N-terminal methyltransferase 1. *Molecules* **27**, 1381. doi:10.3390/molecules27041381

Elsemman, I. E., Mardinoglu, A., Shoaie, S., Soliman, T. H. and Nielsen, J. (2016). Systems biology analysis of hepatitis C virus infection reveals the role of copy number increases in regions of chromosome 1q in hepatocellular carcinoma metabolism. *Mol. Biosyst.* **12**, 1496-1506. doi:10.1039/C5MB00827A

Espada, J. (2012). Non-catalytic functions of DNMT1. *Epigenetics* **7**, 115-118. doi:10.4161/epi.7.2.18756

Espada, J., Peinado, H., Lopez-Serra, L., Setién, F., Lopez-Serra, P., Portela, A., Renart, J., Carrasco, E., Calvo, M., Juarranz, A. et al. (2011). Regulation of SNAIL1 and E-cadherin function by DNMT1 in a DNA methylation-independent context. *Nucleic Acids Res.* **39**, 9194-9205. doi:10.1093/nar/gkr658

Fan, H., Zhang, H., Pascuzzi, P. E. and Andrisani, O. (2016). Hepatitis B virus X protein induces EpCAM expression via active DNA demethylation directed by RelA in complex with EZH2 and TET2. *Oncogene* **35**, 715-726. doi:10.1038/onc.2015.122

Faughn, J. D., Dean, W. L. and Schaner Tooley, C. E. (2018). The N-terminal methyltransferase homologs NRMT1 and NRMT2 exhibit novel regulation of activity through heterotrimer formation. *Protein Sci.* **27**, 1585-1599. doi:10.1002/pro.3456

Gao, L., Guo, Y., Biswal, M., Lu, J., Yin, J., Fang, J., Chen, X., Shao, Z., Huang, M., Wang, Y. et al. (2022). Structure of DNMT3B homo-oligomer reveals vulnerability to impairment by ICF mutations. *Nat. Commun.* **13**, 4249. doi:10.1038/s41467-022-31933-w

Hamey, J. J., Winter, D. L., Yagoub, D., Overall, C. M., Hart-Smith, G. and Wilkins, M. R. (2016). Novel N-terminal and lysine methyltransferases that target translation elongation factor 1A in yeast and human. *Mol. Cell. Proteomics* **15**, 164-176. doi:10.1074/mcp.M115.052449

Hashimoto, H., Kafková, L., Raczkowski, A., Jordan, K. D., Read, L. K. and Debler, E. W. (2020). Structural basis of protein arginine methyltransferase activation by a catalytically dead homolog (Prozyme). *J. Mol. Biol.* **432**, 410-426. doi:10.1016/j.jmb.2019.11.002

Hata, K., Okano, M., Lei, H. and Li, E. (2002). Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* **129**, 1983-1993. doi:10.1242/dev.129.8.1983

Henry, G. D., Dalgarno, D. C., Marcus, G., Scott, M., Levine, B. A. and Trayer, I. P. (1982). The occurrence of alpha-N-trimethylalanine as the N-terminal amino acid of some myosin light chains. *FEBS Lett.* **144**, 11-15. doi:10.1016/0014-5793(82)80558-9

Hershey, J. W., Sonenberg, N. and Mathews, M. B. (2012). Principles of translational control: an overview. *Cold Spring Harb Perspect. Biol.* **4**, a011528. doi:10.1101/cshperspect.a011528

Herz, H. M., Mohan, M., Garruss, A. S., Liang, K., Takahashi, Y. H., Mickey, K., Voets, O., Verrijzer, C. P. and Shilatifard, A. (2012). Enhancer-associated H3K4 monomethylation by Trithorax-related, the Drosophila homolog of mammalian Mll3/Mll4. *Genes Dev.* **26**, 2604-2620. doi:10.1101/gad.201327.112

Hong, A. R., Kim, K., Lee, J. Y., Yang, J. Y., Kim, J. H., Shin, C. S. and Kim, S. W. (2020). Transformation of mature osteoblasts into bone lining cells and RNA sequencing-based transcriptome profiling of mouse bone during mechanical unloading. *Endocrinol. Metab (Seoul)* **35**, 456-469. doi:10.3803/EnM.2020.35.2.456

Hong, M., Ebana, Y., Shim, J., Choi, E. K., Lim, H. E., Hwang, I., Yu, H. T., Kim, T. H., Uhm, J. S., Joung, B. et al. (2021). Ethnic similarities in genetic polymorphisms associated with atrial fibrillation: far east Asian vs European populations. *Eur. J. Clin. Invest.* **51**, e13584. doi:10.1111/eci.13584

Horodyska, J., Hamill, R. M., Varley, P. F., Reyer, H. and Wimmers, K. (2017). Genome-wide association analysis and functional annotation of positional candidate genes for feed conversion efficiency and growth rate in pigs. *PLoS One* **12**, e0173482. doi:10.1371/journal.pone.0173482

Jakobsson, M. E., Małecki, J. and Falnes, P. (2018a). Regulation of eukaryotic elongation factor 1 alpha (eEF1A) by dynamic lysine methylation. *RNA Biol.* **15**, 314-319. doi:10.1080/15476286.2018.1440875

Jakobsson, M. E., Małecki, J. M., Halabelian, L., Nilges, B. S., Pinto, R., Kudithipudi, S., Munk, S., Davydova, E., Zuhairi, F. R., Arrowsmith, C. H. et al. (2018b). The dual methyltransferase METTL13 targets N terminus and Lys55 of eEF1A and modulates codon-specific translation rates. *Nat. Commun.* **9**, 3411. doi:10.1038/s41467-018-05646-y

Kafkova, L., Debler, E. W., Fisk, J. C., Jain, K., Clarke, S. G. and Read, L. K. (2017). The major protein arginine methyltransferase in Trypanosoma brucei functions as an Enzyme-Prozyme complex. *J. Biol. Chem.* **292**, 2089-2100. doi:10.1074/jbc.M116.757112

Kafková, L., Tu, C., Pazzo, K. L., Smith, K. P., Debler, E. W., Paul, K. S., Qu, J. and Read, L. K. (2018). Trypanosoma brucei PRMT1 is a nucleic acid binding protein with a role in energy metabolism and the starvation stress response. *mBio* **9**, a011528. doi:10.1128/mBio.02430-18

Kitroser, E., Pomeranz, M., Epstein Shochet, G., Fishman, A., Drucker, L., Sadeh-Mestechkin, D., Lishner, M. and Tartakover-Matalon, S. (2012). The involvement of eukaryotic translation initiation factor 4E in extravillous trophoblast cell function. *Placenta* **33**, 717-724. doi:10.1016/j.placenta.2012.06.004

Lederer, F., Alix, J. H. and Hayes, D. (1977). N-Trimethylalanine, a novel blocking group, found in E. coli ribosomal protein L11. *Biochem. Biophys. Res. Commun.* **77**, 470-480. doi:10.1016/S0006-291X(77)80004-1

Li, Y., Kobayashi, K., Mona, M. M., Satomi, C., Okano, S., Inoue, H., Tani, K. and Takahashi, A. (2016). Immunogenic FEAT protein circulates in the bloodstream of cancer patients. *J. Transl. Med.* **14**, 275. doi:10.1186/s12967-016-1034-2

Li, Y., Kobayashi, K., Murayama, K., Kawahara, K., Shima, Y., Suzuki, A., Tani, K. and Takahashi, A. (2018). FEAT enhances INSL3 expression in testicular Leydig cells. *Genes Cells* **23**, 952-962. doi:10.1111/gtc.12644

Li, L., Zheng, Y. L., Jiang, C., Fang, S., Zeng, T. T., Zhu, Y. H., Li, Y., Xie, D. and Guan, X. Y. (2019). HN1L-mediated transcriptional axis AP-2γ/METTL13/TCF3-ZEB1 drives tumor growth and metastasis in hepatocellular carcinoma. *Cell Death Differ.* **26**, 2268-2283. doi:10.1038/s41418-019-0301-1

Liang, H., Fu, Z., Jiang, X., Wang, N., Wang, F., Wang, X., Zhang, S., Wang, Y., Yan, X., Guan, W. X. et al. (2015). miR-16 promotes the apoptosis of human cancer cells by targeting FEAT. *BMC Cancer* **15**, 448. doi:10.1186/s12885-015-1458-8

Lin, S., Choe, J., Du, P., Triboulet, R. and Gregory, R. I. (2016). The m(6)A Methyltransferase METTL3 promotes translation in human cancer cells. *Mol. Cell* **62**, 335-345. doi:10.1016/j.molcel.2016.03.021

Lin, Z., Chang, J., Li, X., Wang, J., Wu, X., Liu, X., Zhu, Y. and Yu, X. Y. (2022). Association of DNA methylation and transcriptome reveals epigenetic etiology of heart failure. *Funct. Integr. Genomics* **22**, 89-112. doi:10.1007/s10142-021-00813-9

Liu, J., Yue, Y., Han, D., Wang, X., Fu, Y., Zhang, L., Jia, G., Yu, M., Lu, Z., Deng, X. et al. (2014). A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. *Nat. Chem. Biol.* **10**, 93-95. doi:10.1038/nchembio.1432

Liu, S., Hausmann, S., Carlson, S. M., Fuentes, M. E., Francis, J. W., Pillai, R., Lofgren, S. M., Hulea, L., Tandoc, K., Lu, J. et al. (2019). METTL13 methylation of eEF1A increases translational output to promote tumorigenesis. *Cell* **176**, 491-504.e21. doi:10.1016/j.cell.2018.11.038

Liu, Z., Sun, T., Piao, C., Zhang, Z. and Kong, C. (2021). METTL13 inhibits progression of clear cell renal cell carcinoma with repression on PI3K/AKT/mTOR/HIF-1α pathway and c-Myc expression. *J. Transl. Med.* **19**, 209. doi:10.1186/s12967-021-02879-2

Mackie, B. D., Chen, D., Dong, G., Dong, C., Parker, H., Schaner Tooley, C. E., Noinaj, N., Min, J. and Huang, R. (2020). Selective peptidomimetic inhibitors of NTMT1/2: rational design, synthesis, characterization, and crystallographic studies. *J. Med. Chem.* **63**, 9512-9522. doi:10.1021/acs.jmedchem.0c00689

Martinage, A., Briand, G., Van Dorsselaer, A., Turner, C. H. and Sautiere, P. (1985). Primary structure of histone H2B from gonads of the starfish Asterias rubens. Identification of an N-dimethylproline residue at the amino-terminal. *Eur. J. Biochem.* **147**, 351-359. doi:10.1111/j.1432-1033.1985.tb08757.x

Mateyak, M. K. and Kinzy, T. G. (2010). eEF1A: thinking outside the ribosome. *J. Biol. Chem.* **285**, 21209-21213. doi:10.1074/jbc.R110.113795

Meng, T.-G., Lu, X., Guo, L., Hou, G.-M., Ma, X.-S., Li, Q.-N., Huang, L., Fan, L.-H., Zhao, Z.-H., Ou, X.-H. et al. (2019). Mettl14 is required for mouse postimplantation development by facilitating epiblast maturation. *FASEB J.* **33**, 1179-1187. doi:10.1096/fj.201800719R

Mohan, K. N. (2022). DNMT1: catalytic and non-catalytic roles in different biological processes. *Epigenomics* **14**, 629-643. doi:10.2217/epi-2022-0035

Nevitt, C., Tooley, J. G. and Schaner Tooley, C. E. (2018). N-terminal acetylation and methylation differentially affect the function of MYL9. *Biochem. J.* **475**, 3201-3219. doi:10.1042/BCJ20180638

Nomoto, M., Kyogoku, Y. and Iwai, K. (1982). N-Trimethylalanine, a novel blocked N-terminal residue of Tetrahymena histone H2B. *J. Biochem.* **92**, 1675-1678. doi:10.1093/oxfordjournals.jbchem.a134096

Okano, M., Bell, D. W., Haber, D. A. and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* **99**, 247-257. doi:10.1016/S0092-8674(00)81656-6

Parker, H. V. and Schaner Tooley, C. E. (2022). Opposing regulation of METTL11A by its family members METTL11B and METTL13. *bioRxiv*, 2022.10.05.510978. doi:10.1101/2022.10.05.510978

Pendleton, K. E., Chen, B., Liu, K., Hunter, O. V., Xie, Y., Tu, B. P. and Conrad, N. K. (2017). The U6 snRNA m(6)A methyltransferase METTL16 regulates SAM synthetase intron retention. *Cell* **169**, 824-835.e14. doi:10.1016/j.cell.2017.05.003

Petkowski, J. J., Schaner Tooley, C. E., Anderson, L. C., Shumilin, I. A., Balsbaugh, J. L., Shabanowitz, J., Hunt, D. F., Minor, W. and Macara, I. G. (2012). Substrate specificity of mammalian N-terminal alpha-amino methyltransferase NRMT. *Biochemistry* **51**, 5942-5950. doi:10.1021/bi300278f

Petkowski, J. J., Bonsignore, L. A., Tooley, J. G., Wilkey, D. W., Merchant, M. L., Macara, I. G., Schaner, T. and Christine, E. (2013). NRMT2 is an N-terminal monomethylase that primes for its homologue NRMT1. *Biochem. J.* **456**, 453-462. doi:10.1042/BJ20131163

Pettigrew, G. W. and Smith, G. M. (1977). Novel N-terminal protein blocking group identified as dimethylproline. *Nature* **265**, 661-662. doi:10.1038/265661a0

Piccirillo, C. A., Bjur, E., Topisirovic, I., Sonenberg, N. and Larsson, O. (2014). Translational control of immune responses: from transcripts to translatomes. *Nat. Immunol.* **15**, 503-511. doi:10.1038/ni.2891

Richardson, S. L., Mao, Y., Zhang, G., Hanjra, P., Peterson, D. L. and Huang, R. (2015). Kinetic mechanism of protein N-terminal methyltransferase 1. *J. Biol. Chem.* **290**, 11601-11610. doi:10.1074/jbc.M114.626846

Rickels, R., Herz, H. M., Sze, C. C., Cao, K., Morgan, M. A., Collings, C. K., Gause, M., Takahashi, Y. H., Wang, L., Rendleman, E. J. et al. (2017). Histone H3K4 monomethylation catalyzed by Trr and mammalian COMPASS-like proteins at enhancers is dispensable for development and viability. *Nat. Genet.* **49**, 1647-1653. doi:10.1038/ng.3965

Rickels, R., Wang, L., Iwanaszko, M., Ozark, P. A., Morgan, M. A., Piunti, A., Khalatyan, N., Soliman, S. H. A., Rendleman, E. J., Savas, J. N. et al. (2020). A small UTX stabilization domain of Trr is conserved within mammalian MLL3-4/COMPASS and is sufficient to rescue loss of viability in null animals. *Genes Dev.* **34**, 1493-1502. doi:10.1101/gad.339762.120

Sathyan, K. M., Fachinetti, D. and Foltz, D. R. (2017). α-amino trimethylation of CENP-A by NRMT is required for full recruitment of the centromere. *Nat. Commun.* **8**, 14678. doi:10.1038/ncomms14678

Shi, B., Liu, W. W., Yang, K., Jiang, G. M. and Wang, H. (2022). The role, mechanism, and application of RNA methyltransferase METTL14 in gastrointestinal cancer. *Mol. Cancer* **21**, 163. doi:10.1186/s12943-022-01634-5

Shields, K. M., Tooley, J. G., Petkowski, J. J., Wilkey, D. W., Garbett, N. C., Merchant, M. L., Cheng, A. and Schaner Tooley, C. E. (2017). Select human cancer mutants of NRMT1 alter its catalytic activity and decrease N-terminal trimethylation. *Protein Sci.* **26**, 1639-1652. doi:10.1002/pro.3202

Shima, Y. and Morohashi, K. I. (2017). Leydig progenitor cells in fetal testis. *Mol. Cell. Endocrinol.* **445**, 55-64. doi:10.1016/j.mce.2016.12.006

Shima, H., Matsumoto, M., Ishigami, Y., Ebina, M., Muto, A., Sato, Y., Kumagai, S., Ochiai, K., Suzuki, T. and Igarashi, K. (2017). S-Adenosylmethionine synthesis is regulated by selective N(6)-adenosine methylation and mRNA degradation involving METTL16 and YTHDC1. *Cell Rep.* **21**, 3354-3363. doi:10.1016/j.celrep.2017.11.092

Smith, G. M. and Pettigrew, G. W. (1980). Identification of N,N-dimethylproline as the N-terminal blocking group of Crithidia oncopelti cytochrome c557. *Eur. J. Biochem.* **110**, 123-130. doi:10.1111/j.1432-1033.1980.tb04847.x

Stock, A., Clarke, S., Clarke, C. and Stock, J. (1987). N-terminal methylation of proteins: structure, function and specificity. *FEBS Lett.* **220**, 8-14. doi:10.1016/0014-5793(87)80866-9

Su, R., Dong, L., Li, Y., Gao, M., He, P. C., Liu, W., Wei, J., Zhao, Z., Gao, L., Han, L. et al. (2022). METTL16 exerts an m(6)A-independent function to facilitate translation and tumorigenesis. *Nat. Cell Biol.* **24**, 205-216. doi:10.1038/s41556-021-00835-2

Sze, C. C. and Shilatifard, A. (2016). MLL3/MLL4/COMPASS family on epigenetic regulation of enhancer function and cancer. *Cold Spring Harb Perspect. Med.* **6**, a026427. doi:10.1101/cshperspect.a026427

Takahashi, A., Tokita, H., Takahashi, K., Takeoka, T., Murayama, K., Tomotsune, D., Ohira, M., Iwamatsu, A., Ohara, K., Yazaki, K. et al. (2011). A novel potent tumour promoter aberrantly overexpressed in most human cancers. *Sci. Rep.* **1**, 15. doi:10.1038/srep00015

Thorolfsdottir, R. B., Sveinbjornsson, G., Sulem, P., Helgadottir, A., Gretarsdottir, S., Benonisdottir, S., Magnusdottir, A., Davidsson, O. B., Rajamani, S., Roden, D. M. et al. (2017). A Missense Variant in PLEC Increases Risk of Atrial Fibrillation. *J. Am. Coll. Cardiol.* **70**, 2157-2168. doi:10.1016/j.jacc.2017.09.005

Tooley, C. E., Petkowski, J. J., Muratore-Schroeder, T. L., Balsbaugh, J. L., Shabanowitz, J., Sabat, M., Minor, W., Hunt, D. F. and Macara, I. G. (2010). NRMT is an alpha-N-methyltransferase that methylates RCC1 and retinoblastoma protein. *Nature* **466**, 1125-1128. doi:10.1038/nature09343

Tooley, J. G., Catlin, J. P. and Schaner Tooley, C. E. (2021). CREB-mediated transcriptional activation of NRMT1 drives muscle differentiation. *Transcription* **12**, 72-88. doi:10.1080/21541264.2021.1963627

Tooley, J. G., Catlin, J. P. and Tooley, C. E. S. (2022). METTLing in stem cell and cancer biology. *Stem Cell Rev. Rep.* doi:10.1007/s12015-022-10444-7

Trinh, M. A. and Klann, E. (2013). Translational control by eIF2α kinases in long-lasting synaptic plasticity and long-term memory. *Neurobiol. Learn. Mem.* **105**, 93-99. doi:10.1016/j.nlm.2013.04.013

Van Hemert, F. J., Amons, R., Pluijms, W. J., Van Ormondt, H. and Möller, W. (1984). The primary structure of elongation factor EF-1 alpha from the brine shrimp Artemia. *EMBO J.* **3**, 1109-1113. doi:10.1002/j.1460-2075.1984.tb01937.x

Van Nuland, R., Smits, A. H., Pallaki, P., Jansen, P. W., Vermeulen, M. and Timmers, H. T. (2013). Quantitative dissection and stoichiometry determination of the human SET1/MLL histone methyltransferase complexes. *Mol. Cell. Biol.* **33**, 2067-2077. doi:10.1128/MCB.01742-12

Veland, N., Lu, Y., Hardikar, S., Gaddis, S., Zeng, Y., Liu, B., Estecio, M. R., Takata, Y., Lin, K., Tomida, M. W. et al. (2019). DNMT3L facilitates DNA methylation partly by maintaining DNMT3A stability in mouse embryonic stem cells. *Nucleic Acids Res.* **47**, 152-167. doi:10.1093/nar/gky947

Wang, C., Lee, J. E., Lai, B., Macfarlan, T. S., Xu, S., Zhuang, L., Liu, C., Peng, W. and Ge, K. (2016a). Enhancer priming by H3K4 methyltransferase MLL4 controls cell fate transition. *Proc. Natl. Acad. Sci. USA* **113**, 11871-11876. doi:10.1073/pnas.1606857113

Wang, P., Doxtader, K. A. and Nam, Y. (2016b). Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases. *Mol. Cell* **63**, 306-317. doi:10.1016/j.molcel.2016.05.041

Wang, S. M., Ye, M., Zhou, J., Ni, S. M. and Wei, Q. C. (2017). FEAT expression correlates with tumor size, PR status, HER2 expression, Ki67 index, and molecular subtype and predicts recurrence in breast cancer. *Neoplasma* **64**, 123-130. doi:10.4149/neo_2017_115

Wang, C. X., Cui, G. S., Liu, X., Xu, K., Wang, M., Zhang, X. X., Jiang, L. Y., Li, A., Yang, Y., Lai, W. Y. et al. (2018). METTL3-mediated m6A modification is required for cerebellar development. *PLoS Biol.* **16**, e2004880. doi:10.1371/journal.pbio.2004880

Wang, X., Li, K., Wan, Y., Chen, F., Cheng, M., Xiong, G., Wang, G., Chen, S., Chen, Z., Chen, J. et al. (2021). Methyltransferase like 13 mediates the translation of Snail in head and neck squamous cell carcinoma. *Int. J. Oral. Sci.* **13**, 26. doi:10.1038/s41368-021-00130-8

Warda, A. S., Kretschmer, J., Hackert, P., Lenz, C., Urlaub, H., Höbartner, C., Sloan, K. E. and Bohnsack, M. T. (2017). Human METTL16 is a N(6)-methyladenosine (m(6)A) methyltransferase that targets pre-mRNAs and various non-coding RNAs. *EMBO Rep.* **18**, 2004-2014. doi:10.15252/embr.201744940

Webb, K. J., Lipson, R. S., Al-Hadid, Q., Whitelegge, J. P. and Clarke, S. G. (2010). Identification of protein N-terminal methyltransferases in yeast and humans. *Biochemistry* **49**, 5225-5235. doi:10.1021/bi100428x

Webster, K. E., O'bryan, M. K., Fletcher, S., Crewther, P. E., Aapola, U., Craig, J., Harrison, D. K., Aung, H., Phutikanit, N., Lyle, R. et al. (2005). Meiotic and epigenetic defects in Dnmt3L-knockout mouse spermatogenesis. *Proc. Natl. Acad. Sci. USA* **102**, 4068-4073. doi:10.1073/pnas.0500702102

Willert, E. K., Fitzpatrick, R. and Phillips, M. A. (2007). Allosteric regulation of an essential trypanosome polyamine biosynthetic enzyme by a catalytically dead homolog. *Proc. Natl. Acad. Sci. USA* **104**, 8275-8280. doi:10.1073/pnas.0701111104

Xiang, C. X., Liu, X. G., Zhou, D. Q., Zhou, Y., Wang, X. and Chen, F. (2022). Identification of a glioma functional network from gene fitness data using machine learning. *J. Cell. Mol. Med.* **26**, 1253-1263. doi:10.1111/jcmm.17182

Yousaf, R., Ahmed, Z. M., Giese, A. P., Morell, R. J., Lagziel, A., Dabdoub, A., Wilcox, E. R., Riazuddin, S., Friedman, T. B. and Riazuddin, S. (2018). Modifier variant of METTL13 suppresses human GAB1-associated profound deafness. *J. Clin. Invest.* **128**, 1509-1522. doi:10.1172/JCI97350

Zhang, Z., Zhang, G., Kong, C., Zhan, B., Dong, X. and Man, X. (2016). METTL13 is downregulated in bladder carcinoma and suppresses cell proliferation, migration and invasion. *Sci. Rep.* **6**, 19261. doi:10.1038/srep19261

Zhang, J., Ao, Y., Zhang, Z., Mo, Y., Peng, L., Jiang, Y., Wang, Z. and Liu, B. (2020). Lamin A safeguards the m(6) A methylase METTL14 nuclear speckle reservoir to prevent cellular senescence. *Aging Cell* **19**, e13215. doi:10.1111/acel.13215

Zhang, J., Song, H., Chen, C., Chen, L., Dai, Y., Sun, P. H., Zou, C. and Wang, X. (2021). Methyltransferase-like protein 11A promotes migration of cervical cancer cells via up-regulating ELK3. *Pharmacol. Res.* **172**, 105814. doi:10.1016/j.phrs.2021.105814

Zhou, Y., Zhang, Y., Guo, R., Li, C. and Sun, N. (2021). Identification of methyltransferase-like protein 11B as a new prognostic biomarker for colorectal cancer through an analysis of The Cancer Genome Atlas. *J. Gastrointest. Oncol.* **12**, 2854-2871. doi:10.21037/jgo-21-781

Zhou, Q., Wu, W., Jia, K., Qi, G., Sun, X. S. and Li, P. (2022). Design and characterization of PROTAC degraders specific to protein N-terminal methyltransferase 1. *Eur. J. Med. Chem.* **244**, 114830. doi:10.1016/j.ejmech.2022.114830