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## Put the Pedal to the METTL1: Adding Internal m<sup>7</sup>G Increases mRNA Translation Efficiency and Augments miRNA Processing

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Complementary papers by Zhang, Liu, and colleagues (Zhang et al., 2019) and Pandolfini, Barbieri, and colleagues (Pandolfini et al., 2019) develop new sequencing techniques that reveal that METTL1 N7-methylates internal guanosines in mRNAs and miRNAs to increase translation efficiency and miRNA processing, respectively.

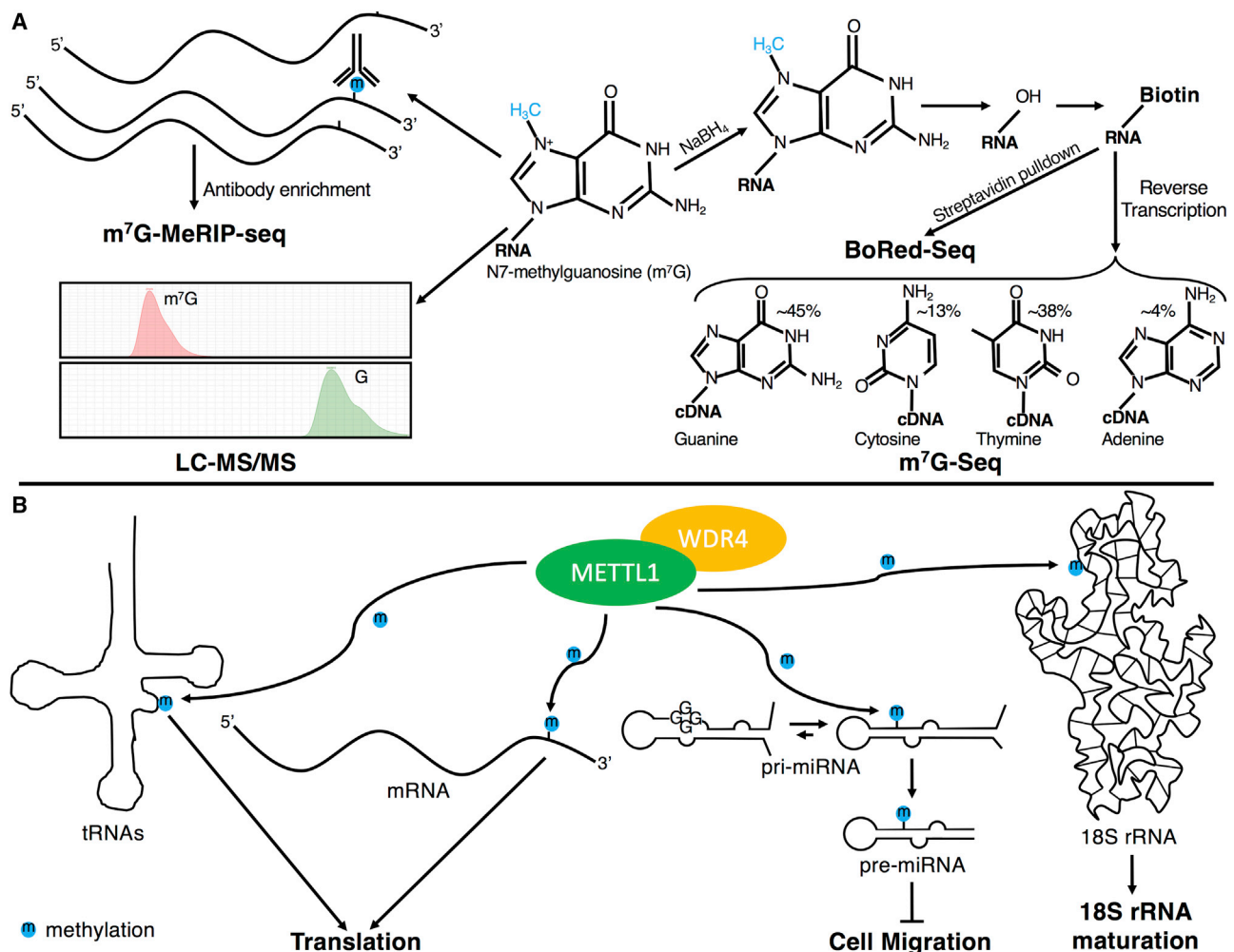
More than 100 distinct post-transcriptional chemical modifications have been identified in cellular RNAs of all kingdoms of life. They constitute the “epitranscriptome,” and the identification of these residues as well as the enzymes that add, remove, and recognize the modified bases has revealed added complexity that controls virtually every aspect of RNA processing. Most of these modifications were initially identified and characterized in ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs). Presumably due to the low relative concentration, modifications to other types of RNAs have remained sparse and poorly characterized. In recent years, however, the development of more sensitive detection techniques has allowed the identification and transcriptome-wide mapping of several modifications within mRNAs and small RNAs. The fast growing list includes N7-methylguanosine (m<sup>7</sup>G) on the cap of eukaryotic and viral mRNAs, which

helps to direct translation, splicing, and nuclear export and to prevent degradation; the abundant and well-studied N6-methyladenosine (m<sup>6</sup>A), with critical roles in almost every step of mRNA biogenesis and microRNA (miRNA) processing; the highly similar N6, 2'-O-dimethyladenosine (m<sup>6</sup>Am), which occurs at the adenosine adjacent to the m<sup>7</sup>G cap and may affect translation and stability; N1-methyladenosine (m<sup>1</sup>A), which regulates translation; pseudouridine ( $\Psi$ ), which regulates translation efficiency and splicing; N4-acetylcytidine (a<sup>4</sup>C), which promotes translation; C5-methylcytosine (m<sup>5</sup>C), which promotes mRNA export, and its oxidized form, C5-hydroxymethylcytosine (hm<sup>5</sup>C); 5'-methylphosphate (5'mP), which inhibits miRNA processing; and methylation of 2'-hydroxyl of the ribose sugar of cap-adjacent nucleosides (Am, Cm, Gm, and Um), which regulates translation efficiency and self-distinction during innate immunity (Roundtree et al., 2017).

A pair of papers in this issue of *Molecular Cell* (Pandolfini et al., 2019; Zhang et al., 2019) identify that m<sup>7</sup>G is not restricted to the caps of mRNAs but occurs internally in mRNAs and miRNAs. It's known that m<sup>7</sup>G occurs on mRNA caps of eukaryotes and viral mRNAs as well as within tRNAs and 18S rRNAs, where it is thought to impact mRNA translation (Lin et al., 2018) and rRNA biogenesis, respectively. METTL1, together with its cofactor WDR4, has been identified as the enzyme responsible for adding m<sup>7</sup>G to tRNAs and rRNAs (Alexandrov et al., 2002). Mutations in WDR4 in humans have been proposed to cause microcephalic primordial dwarfism (Shahen et al., 2015), while METTL1 has been shown to play a role in the growth of acute myeloid leukemia cells (Barbieri et al., 2017) and embryonic stem cell self-renewal and differentiation (Lin et al., 2018).

The authors perform m<sup>7</sup>G-methyl RNA immunoprecipitation followed by sequencing (MeRIP-seq) and orthogonal





**Figure 1. METTL1 Internally Methylates N7-Guanosine on tRNAs, 18S rRNA, mRNA, and miRNA as Assessed by Sequencing and LC-MS/MS**  
 (A) Methods to detect m<sup>7</sup>G in RNA.  
 (B) METTL1 internally methylates mRNA and miRNA in addition to its canonical substrates, tRNAs and 18S rRNA.

sequencing techniques using sodium borohydride (NaBH<sub>4</sub>) to specifically reduce m<sup>7</sup>G, followed by depurination by low pH to remove the base and biotinylation of the resulting abasic site, either to enable pull-down of m<sup>7</sup>G-modified RNAs (Pandolfini et al., 2019) or to perform reverse transcription to induce mutation of the guanosine site (Zhang et al., 2019) and identify internal m<sup>7</sup>G within miRNAs and mRNAs, respectively (Figure 1A). Both papers perform elegant adaptations to the existing m<sup>7</sup>G mapping technique used for tRNAs and rRNAs (Zueva et al., 1985). Each technique has its own limitations: with the biotin pull-down, nucleotide resolution is not possible, and the induced mutation technique only converts a per-

centage of m<sup>7</sup>G nucleosides to alternative bases. However, both groups complement these sequencing techniques with MeRIP-seq and additional biochemical validations. Both groups identify METTL1, the known N7-guanosine methyltransferase, as the enzyme responsible for m<sup>7</sup>G in miRNAs and mRNAs. The mRNA m<sup>7</sup>Gs were mapped at single-nucleotide resolution and suggest a function of internal m<sup>7</sup>G in increasing translation efficiency (Zhang et al., 2019). The miRNA m<sup>7</sup>Gs are proposed to augment miRNA processing by disrupting the inhibitory secondary structure of G-quadruplexes found in several primary miRNA (pri-miRNA) transcripts (Pandolfini et al., 2019) (Figure 1B). However, METTL1's known effects on

tRNA and rRNA methylation and therefore on translation (Lin et al., 2018) make it difficult to discern the specific effects of m<sup>7</sup>G on miRNAs or mRNAs. Both groups perform knockdowns of METTL1 and reproduce previously reported decreases in tRNA m<sup>7</sup>G. As this decrease has a modest effect on ribosome profiling (Pandolfini et al., 2019), Kouzarides and colleagues advance that their observed phenotypes are specific to miRNA methylation. To complement this, they perform *ex vivo* experiments replacing the m<sup>7</sup>G residue they identified on *let-7e-5p* miRNA with the structurally similar 7-deaza-guanosine (DAG) and observe that this increased the efficiency of processing of DAG-modified *let-7e* primary hairpin

compared to WT *let-7e* primary hairpin in a METTL1-dependent manner. He and colleagues observe a decrease in translation of m<sup>7</sup>G-modified transcripts upon knock-down of *METTL1* (Zhang et al., 2019) and propose that these effects are specific to decreases in mRNA methylation rather than tRNA methylation due to the similar frequency of m<sup>7</sup>G-modified tRNA codons in the target N7-guanosine-methylated mRNAs and control unmethylated mRNAs. Both groups demonstrated that METTL1/WDR4 complexes had the capacity to methylate mRNA and miRNA *in vitro*. To ensure the specificity and directness of the reported effects on miRNA and mRNA function, it will be important in future experiments to perform *in vitro* methylation assays on target miRNAs or mRNAs and transfect these RNAs into *METTL1*-depleted cells.

Using LC-MS/MS analysis of decapped polyA-selected mRNAs of different human and mouse cell lines, Zhang, Liu, and colleagues found ~0.02%–0.05% of total guanines were N7 methylated. Mapping of m<sup>7</sup>G revealed enrichment at the 3' UTR, while motif analysis revealed GA- and GG-enriched sequence motifs. m<sup>7</sup>G-seq in two human cell lines identified more than 90 high-confidence conserved sites that are frequently methylated (more than 20%), while biotin pull-down enrichment yielded around 800 conserved sites, which are less frequently methylated. It will be interesting to determine whether these levels and locations of m<sup>7</sup>G change in response to environmental manipulations, whether structural similarities exist between tRNA and rRNA m<sup>7</sup>G locations and the methylated mRNA residues, and whether these modifications are similar in different tissues across evolution. It will also be interesting to determine whether m<sup>7</sup>G affects other epitranscriptome modifications.

While both groups observed decreases in tRNA methylation upon *METTL1* knock-down, neither group observed a complete elimination of m<sup>7</sup>G. This could reflect incomplete knockdown or the presence of additional N7-guanosine methyltransferases. Zhang, Liu, and colleagues (Zhang et al., 2019) identified multiple

m<sup>7</sup>G motifs in mRNAs, which would suggest that the enzyme is promiscuous or that multiple enzymes exist. METTL1 has been shown to be phosphorylated by AKT, which inhibits its enzymatic activity (Cartlidge et al., 2005). This and other modifications of METTL1 could provide a mode by which METTL1 targets could be specified. Future studies examining additional binding partners of METTL1, how METTL1 itself is regulated, and how target RNAs are selected should prove interesting.

Methylation can affect binding partners, stability, or structure of its target RNAs. m<sup>7</sup>G's positive charge could affect RNA structure or interactions via its electrostatic and steric effects. Through a series of elegant experiments, Pandolfini, Barbieri, and colleagues show that N7-guanosine methylation has no effect on the levels of *let-7e* and *miR-125a* primary transcripts but affects the processing of these miRNAs, which causes a decrease at the levels of precursor and mature forms of *let-7e-5p* and *miR-125a-5p* (Pandolfini et al., 2019). The m<sup>7</sup>G residues occur within G-quadruplexes. Since m<sup>7</sup>G does not cause increased depurination in RNA or impair Watson-Crick base complementarity (Kriek and Emmelot, 1964), the authors demonstrate that this methylation affects non-canonical base pairing, which leads to changes in the stability of the secondary structure G-quadruplex. Replacing the m<sup>7</sup>G base with DAG affects hairpin formation, presumably through G-quadruplex disruption, and augments miRNA processing by disrupting the inhibitory G-quadruplex structure within the pri-miRNA. In this context, internal m<sup>7</sup>G modifies secondary structure rather than regulating the binding of interactors as the m<sup>7</sup>G within the mRNA cap does. Future mechanistic studies will be needed to address whether internal mRNA m<sup>7</sup>Gs act to change the secondary structure of mRNAs or provide a platform for the recruitment of m<sup>7</sup>G binding proteins. However, it is clear that the newly found existence of m<sup>7</sup>G in mRNAs and miRNAs expands the epitranscriptome modifications and provides additional modes in the control of gene

expression. It will be interesting in future studies to examine whether internal m<sup>7</sup>G in mRNAs and miRNAs are dynamic and reversible, if enzymes other than METTL1 can write this mark, what the detailed mechanisms involved in m<sup>7</sup>G actions are, and what biological pathways are most affected by changes in m<sup>7</sup>G levels in normal conditions or disease.

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