RNA epigenetics

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Mammalian messenger RNA (mRNA) and long noncoding RNA (lncRNA) contain tens of thousands of posttranscriptional chemical modifications. Among these, the N^6-methyl-adenosine (m^6A) modification is the most abundant and can be removed by specific mammalian enzymes. m^6A modification is recognized by families of RNA binding proteins that affect many aspects of mRNA function. mRNA/lncRNA modification represents another layer of epigenetic regulation of gene expression, analogous to DNA methylation and histone modification. (Translational Research 2015;165:28–35)

Abbreviations: lncRNA = long noncoding RNA; m^1A = N^1-methyl-A; m^1G = N^1-methyl-G; m^5C = 5-methyl cytosine; m^6A = N^6-methyl adenosine; METTL14 = Methyltransferaselike 14; mRNA = Messenger RNA; Nm = 2′-*O*-methyl nucleotides; Ψ = pseudouridine; RT = reverse transcriptase; tRNA = transfer RNA

More than 100 types of posttranscriptional modifications have been identified in cellular RNA, starting during the 1950s. For example, the human ribosomal RNA contains more than 200 modifications consisting of 3 major types[1]: ~100 2′-*O*-methyl nucleotides (Nm), ~100 pseudouridines (Ψ), and ~10 base methylations (eg, 5-methyl cytosine [m^5C]). Each human transfer RNA (tRNA) contains, on average, 14 modifications consisting of various base methylations, Ψ, Nm, and chemically elaborate, modified wobble bases that require catalysis by multiple enzymes.[2,3] Ribosomal RNA modifications are generally used as quality control checkpoints in ribosome assembly.[4] tRNA modifications outside the anticodon loop are generally used to maintain tRNA stability or to modulate tRNA folding, whereas modifications in the anticodon loop are generally used to tune decoding capacity and to control decoding accuracy.[5]

Up until 2 years ago, internal modifications in messenger RNA (mRNA) and long noncoding RNA (lncRNA) were very much neglected. Discovered during the 1970s,[6-9] the most abundant internal mRNA/lncRNA modification is made of N^6-methyl adenosine (m^6A), present, on average, in more than 3 sites per mRNA molecule[10-13] (Fig 1, *A*). Other types of modifications, such as m^5C or Nm, have also been indicated to occur internally in mRNA[9,14] (Fig 1, *B* and *C*), and many m^5C modification sites have now been identified.[15,16] A common feature of these modifications is that their presence cannot be detected by the commonly used reverse transcriptases in complementary DNA synthesis. It was, therefore, extremely difficult to map these modifications at single-nucleotide resolution. Global m^6A modification was shown to be important functionally because siRNA knockdown of a known human m^6A methyltransferase (METTL3) led to apoptosis in cell culture.[17] Suggested functions for m^6A modification include effects on mRNA splicing, transport, stability, and immune tolerance.[17,18]

Interest in mRNA/lncRNA modification was revived in 2011 upon the discovery that m^6A modification is

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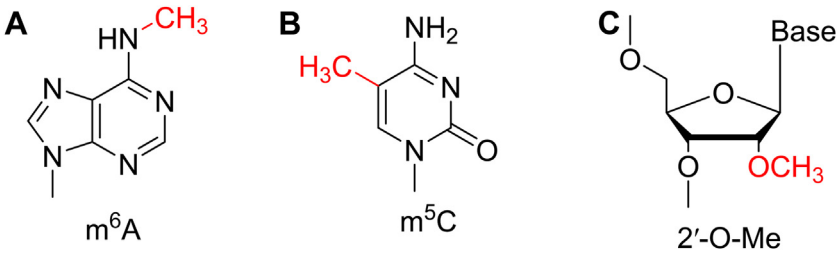

Fig 1. Chemical structure of internal messenger RNA/long noncoding RNA modifications. (**A**) N^6-methyl-adenosine (m^6A). (**B**) 5-Methyl cytosine (m^5C). (**C**) 2′-*O*-methyl nucleotides (2′-*O*-Me).

the cellular substrate for the human enzyme FTO.[19] *FTO* belongs to a family of human genes that are homologous to the *Escherichia coli* AlkB protein, which catalyzes oxidative reversal of methylated DNA and RNA bases.[20,21] In genomewide association studies, the human *FTO* gene is associated greatly with diabetes and obesity in the human population.[22,23] *FTO* knockout mice are much leaner than the wild-type mice, presumably as a result of perturbations in controlling cellular metabolism.[24] The discovery of FTO acting on m^6A in mRNA/lncRNA indicates that m^6A modification is subject to sophisticated cellular control.

The discovery of this first RNA demodification enzyme also highlights the idea that RNA modifications may act as epigenetic markers and controls akin to DNA methylation and histone modification.[25,26] Three groups of proteins are needed for epigenetic control that maintains specific modification patterns in cell type- and cell state-dependent manners. "Writers" catalyze chemical modifications at specific sites, "erasers" remove modifications at specific sites, and "readers" recognize the modified sites in DNA or histones (Fig 2, *A*). For m^6A in mRNA/lncRNA, members in all 3 groups of proteins have now been found in mammalian cells (Fig 2, *B*). However, the current list of these proteins likely represents just the beginning. In particular, the number of reader proteins that recognize m^6A modified mRNA/lncRNA sites will certainly expand greatly in the coming years. As of today, only the m^6A modification has been shown to exhibit all signatures of epigenetic regulation. This review therefore focuses on m^6A modifications in mRNA/lncRNA, with an emphasis on its effect on human health and disease.

TECHNIQUES USED TO STUDY m^6A IN mRNA/lncRNA

A prerequisite for mRNA/lncRNA transcriptome studies is the copying of RNA into complementary DNA (cDNA) by reverse transcriptase (RT). m^6A modification does not affect Watson-Crick base pairing, and it behaves like an unmodified adenosine for the commonly used RTs. A widely applied method for m^6A study is to use immunoprecipitation with a commercial m^6A antibody followed by high-throughput sequencing (m^6A-seq or MeRIP-seq[27,28]). The mRNA/lncRNA mixture is first fragmented chemically to produce suitable-size RNA segments for deep sequencing and to increase the resolution of m^6A detection. The fragmented RNA is split in 2. One is used for m^6A antibody immunoprecipitation to enrich RNA segments that contain m^6A; the other is used as the reference. The location of m^6A modification is obtained by comparing the sequencing read profiles of both samples. This method could identify readily tens of thousands of candidate m^6A modification sites in mammalian mRNA/lncRNA at an average resolution of ~100 nucleotides.[27,28] Studies before the advent of high-throughput sequencing have determined a consensus sequence for mammalian m^6A modification consisting of RRACH (R = A, G; H = A, C, U; m^6A site underlined[13]). Indeed, this consensus sequence is present in a majority of m^6A/MeRIP-seq peaks. Peaks without this consensus sequence are likely m^6A antibody binding artifacts, as demonstrated in a yeast m^6A study.[29]

To map transcriptomewide m^6A sites at or near single-nucleotide resolution, a combination of high-coverage sequencing and bioinformatics was used in the yeast m^6A study for ~1300 m^6A sites.[29] This approach may not be readily applicable to mammalian RNA, in which the number of m^6A sites is at least 1 order of magnitude greater and the context of m^6A modification is much more diverse. It was shown recently that the human immunodeficiency virus RT is sensitive to the presence of m^6A in RNA using the single-molecule real-time sequencing method by Pacific Biosciences.[30] *Thermus thermophilus* DNA polymerase I can work as an RT in the presence of Mn^{2+}; this RT activity is sensitive to the presence of m^6A modification in the RNA template.[31] It remains to be seen whether these particular RT activities will be developed further for high-resolution, transcriptome-wide identification of m^6A sites.

Liu et al[32] developed a low-throughput method that can determine directly the presence and the modification

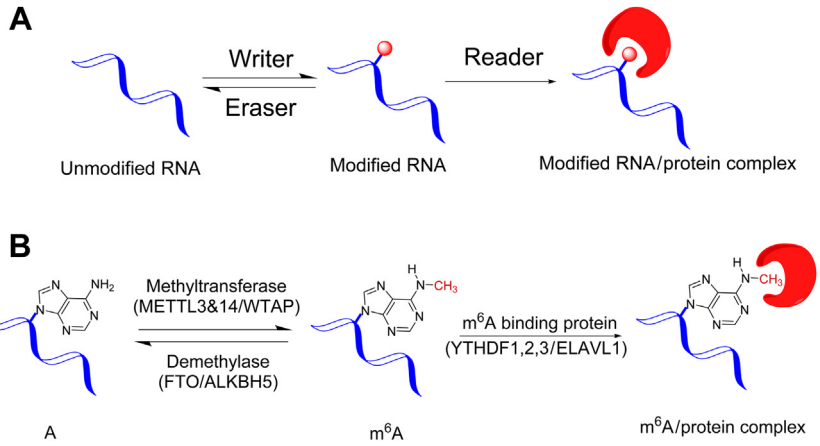

Fig 2. RNA epigenetic marking and control requires 3 groups of proteins. (**A**) Schematic plot for general RNA modification. Writer = modification enzymes; Eraser = demodification enzymes; Reader = RNA binding proteins that recognize specific sites in the modified messenger RNA/long noncoding RNA. (**B**) Schematic plot for the N^6-methyl-adenosine (m^6A) modification.

fraction of a candidate m^6A site at single-nucleotide resolution (called SCARLET). The SCARLET method starts with total $polyA^+$ RNA. Hybridization of a specific 2′-*O*-Me-2′-deoxy oligonucleotide enables a single, site-specific cut by RNase H at the 5′ of the candidate site, which is first identified from the m^6A/MeRIP-seq data. The cut site is radiolabeled with ^{32}P, followed by targeted ligation with a long, single-stranded DNA oligo. The sample is then digested with ribonucleases to completion; the only remaining nucleic acid is the ^{32}P-labeled candidate adenosine nucleotide linked to the DNA oligo. This ^{32}P-labeled product is purified on denaturing gels and digested with another nuclease to obtain 2 ^{32}P-labeled products—5′p-A and 5′p-m^6A—which are separated by thin-layer chromatography and visualized by phosphorimaging. SCARLET can detect not only the presence of m^6A, but also it determines the modification fraction of that site, which has not been possible using m^6A antibody-based techniques.

m^6A WRITERS

The first m^6A methyltransferase identified is the protein encoded by the *METTL3* gene.[33] This gene is conserved from mammals to yeast. Knockdown or deletion of METTL3 led to a wide range of phenotypes, such as apoptosis in human cell lines, viability in plants and *Drosophila*, and sporulation defects in yeast.[17,34-36]

Recent studies discovered another human methyltransferaselike 14 (METTL14) protein that can also catalyze m^6A RNA methylation, and the METTL14 protein forms a stable heterodimer complex with METTL3.[37] Both METTL3 and METTL14 belong to the same methyltransferase superfamily; they are 43% identical in their primary sequences. Knockdown of either METTL3 or METTL14 leads to a marked decrease of m^6A content in mRNA and causes mouse embryonic stem cells to lose their self-renewal capability.[38] Both METTL3 and METTL14 are catalytically active *in vitro* in the methylation of single-stranded RNA oligo substrates. These results indicate that both proteins are catalytic subunits of the complex. Each enzyme may methylate a distinct and overlapping set of m^6A sites.

The METTL3-METTL14 core complex has been found to interact with WTAP.[37,39] WTAP is a protein known to be involved in mRNA splicing.[40] siRNA knockdown of WTAP also leads to a significant decrease of m^6A content, but the WTAP protein itself does not show any methyltransferase activity *in vitro*.[37] These results indicate that WTAP acts as an accessory protein that may be needed to enhance the methyltransferase selectivity or for subnuclear localization of the methyltransferases.[37,39]

m^6A ERASERS

The first m^6A eraser identified was the protein encoded by the *FTO* gene.[19] FTO belongs to the family of Fe^{2+}-α-ketoglutarate dependent dioxygenases and removes the methyl group of m^6A through successive oxidation[41] (Fig 3, *A*). FTO overexpression led to a ~15%–20% reduction of m^6A content, whereas siRNA knockdown of FTO led to an ~20% increase of m^6A content in human cell lines. FTO reaction generates 2 intermediate products—N^6-hydroxymethyladenosine and N^6-formyladenosine—that are stable for several hours in the mammalian cell.[41] These intermediates

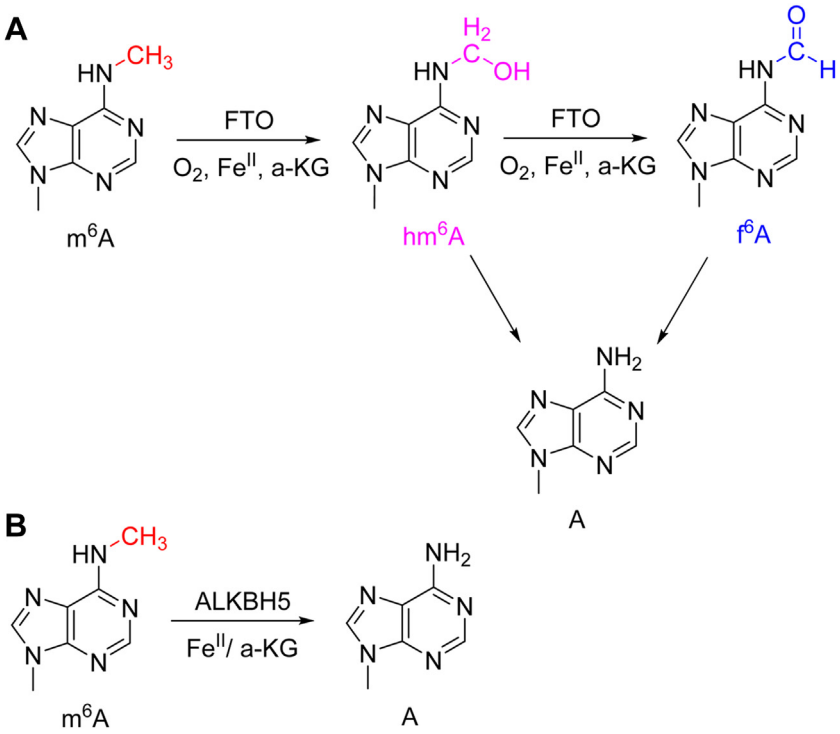

Fig 3. Reaction mechanism of N^6-methyl-adenosine (m^6A) erasers. (**A**) The FTO reaction generates 2 intermediates that are stable for several hours before decomposition and reversal to adenosine. (**B**) The ALKBH5 reaction removes the methyl group from m^6A directly.

may be used to recruit specific proteins that recognize this particular chemical feature.

Another m^6A eraser known to date is the protein encoded by the ALKBH5 gene.[42] ALKBH5 belongs to the same protein family as FTO. Other members in this family that have known cellular substrates include ALKBH2 for DNA methylation repair, ALKBH3 for RNA methylation repair, and ALKBH8 for hydroxylation of a specific human $tRNA^{Gly}$.[43,44] ALKBH5 removes the methyl group of m^6A directly, without the accumulation of any detectable intermediate product[42] (Fig 3, *B*).

m^6A READERS

Numerous mammalian proteins were found to bind preferentially a synthetic RNA oligo derived from Rous sarcoma virus genomic RNA with or without a specific m^6A residue as bait.[27] The 3 proteins detected with greatest confidence include ELAVL1, YTHDF2, and YTHDF3. ELAVL1 or HuR (human antigen R) belongs to the ELAVL family of RNA-binding proteins that contains several RNA-binding domains, and bind *cis*-acting AU-rich elements selectively located in the 3′UTR regions of mRNA.[45] ELAVL1 is known to stabilize AU-rich element-containing mRNAs,[46,47] and RNA-ELAVL1 interactions have been shown to regulate the stability of many mRNAs in embryonic stem cells in an m^6A-dependent way.[38] Both the YTHDF2 and YTHDF3 proteins belong to a superfamily of RNA-binding proteins containing the YTH domain.[48] The YTH domain is conserved in eukaryotes and is particularly abundant in plant genomes. Except for YTHDF2, additional studies are needed to validate whether these proteins are authentic m^6A readers *in vivo*.

A more recent study has shown the YTHDF2 protein recognizes directly m^6A-modified RNA *in vitro* and *in vivo*.[49] The YTHDF2 protein binds a single-stranded RNA oligo containing a single m^6A with an ~15-fold greater affinity for the modified RNA *in vitro*. YTHDF3 and another member of this family, YTHDF1, are also shown to prefer this m^6A-containing RNA oligo by 5–20-fold *in vitro*. In human cell lines, YTHDF2 binds more than 3000 cellular RNA targets; most are located in mRNAs. YTHDF2 competes directly with ribosomes for translatable mRNA molecules in the cytoplasm. YTHDF2 binding results in mRNA localization to mRNA decay sites such as processing bodies. YTHDF2 is basically a triage factor for m^6A-containing mRNA. When sufficient amounts of ribosomes are available, these mRNAs are bound by the ribosome for active translation, and a decreasing amount of available ribosomes enables their binding to

YTHDF2 protein for targeted relocalization and degradation.

The YTHDF2 protein is made of 2 functional domains. One binds the m^{6}A-modified RNA directly; the other is required for mRNA localization within the cytoplasm.[49] Among YTHDF1–3, the RNA-binding domain is highly conserved, but the conservation of the other domain is far less pronounced. Therefore, these YTHDF proteins may affect mRNA metabolism in different ways, although they can all interact with m^{6}A-containing RNAs in a similar manner.

Because tens of thousands of m^{6}A sites have already been found, other m^{6}A reader proteins are certain to exist. Other m^{6}A-binding proteins may interact directly with the modified adenosine, such as YTHDF2, or they may sense m^{6}A-modified RNA indirectly through changes in the RNA structure.[50] The biologic function of m^{6}A modification is executed through the reader proteins. As more m^{6}A readers are identified, we anticipate a rapid growth in our understanding of how m^{6}A affects all aspects of mRNA and lncRNA function.

m^{6}A MODIFICATION IN HUMAN HEALTH AND DISEASE

The current knowledge of the writer, eraser, and readers of m^{6}A modification is summarized in Table I. m^{6}A modification regulates a variety of biologic processes and has been linked to numerous human diseases. Both m^{6}A methyltransferases (METTL3 and METTL14) are crucial for cell development, and their depletion causes cell death. Diseases associated with METTL3 include prostatitis and Aicardi syndrome, whereas diseases associated with METTL14 include alcohol dependence and alcoholism. The WTAP protein, known to affect mRNA splicing, is associated with Wilms' tumor, kidney cancer, and other ailments. Variants within introns of the m^{6}A demethylase gene *FTO* have been indicated to increase risk for obesity and diabetes, although recent studies suggest that these variants within *FTO* introns form long-range functional connections with the homeobox gene *IRX3* at the DNA level.[51] Another identified m^{6}A demethylase, ALKBH5, has been reported to affect Smith-Magenis syndrome, hypoxia, and others. Aside from the still-unclear biologic role of YTHDF3, the 3 other identified m^{6}A readers (YTHDF1, YTHDF2, and ELAVL1) are related to mRNA stability. Diseases associated with the m^{6}A readers include cancer, leukemia, hepatitis, Alzheimer's disease, arthritis, prostatitis, hypoxia, pancreatitis, and others.

RNA EPIGENETICS BEYOND m^{6}A IN mRNA/lncRNA

Most RNA methylations can be, in principle, reversed by another enzyme. In practice, the most likely

Table I. Human diseases associated with genes involved in m^{6}A modification

Gene	Function	Phenotype	Associated diseases	References
METTL3	Methyltransferase	Apoptosis, development	Prostatitis, Aicardi syndrome	Wang et al,[38] Bujnicki et al,[58] and Niu et al[59] Bujnicki
METTL14	Methyltransferase	Apoptosis, development	Alcohol dependence, alcoholism	Wang et al[38] and Niu et al[59]
WTAP	Scaffolding or localization	mRNA splicing	Wilms' tumor, hypospadias, sarcoma, malignant mesothelioma, synovial sarcoma	Zhong et al,[34] Niu et al,[59] Ortega et al,[60] Utsch et al,[61] Su et al,[62] and Jin[63]
FTO	Demethylase	Cellular energy homeostasis	Obesity, diabetes, polycystic ovary syndrome, heart attack, cancer, alcoholism, mental disorders, cataract, hepatitis	Jia et al,[19] Zeggini et al,[64] Kalnina et al,[65] Akilzhanova et al,[66] Reddy et al,[67] Karra et al,[68] Lin et al,[69] and Wang et al[70]
ALKBH5	Demethylase	Male fertility	Hypoxia, Smith-Magenis syndrome	Zheng et al[42] and Thalhammer et al[71]
YTHDF1	m^{6}A binding	mRNA stability	Pancreatic cancer, pancreatitis, dermatomyositis	Wang et al[49]
YTHDF2	m^{6}A binding	mRNA stability	Leukemia, renal cell carcinoma, breast cancer	Dominissini et al,[27] Wang et al,[49] Cardelli et al,[72] and Heiliger et al[73]
YTHDF3	m^{6}A binding	N/A	N/A	Dominissini et al[27] and Wang et al[49]
ELAVL1	m^{6}A binding	Apoptosis, mRNA stability	Cancer, leukemia, hepatitis, anoxia, Alzheimer's disease, arthritis, endothelilitis, prostatitis, hypoxia, laryngitis, keratoconus, pancreatitis	Dominissini et al,[27] Wang et al,[38] Wang et al,[74] Li et al,[75] Lee et al,[76] Yang et al,[77] Zhou et al,[78] Yang et al,[79] and Pang et al[80]

Abbreviations: m^{6}A, N^6-methyl-adenosine; mRNA, messenger RNA; N/A, no available information.
Disease information from ref. 81.

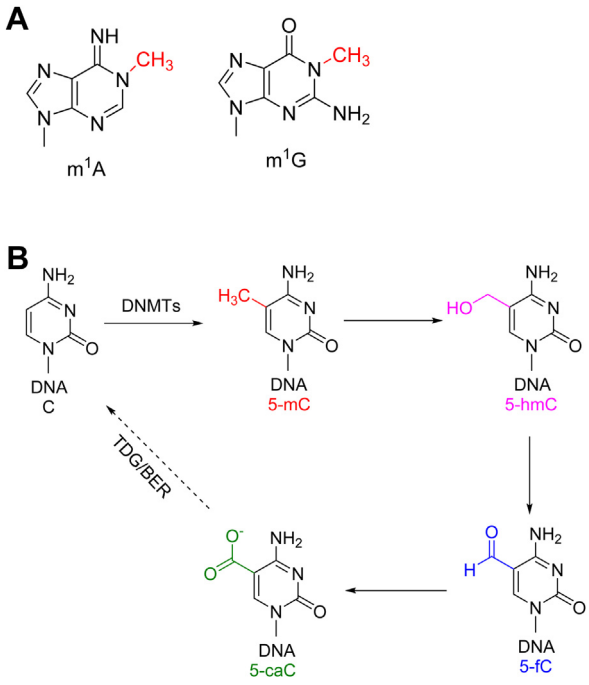

Fig 4. Other potentially reversible RNA methylations. (**A**) N^1-methyl-A (m^1A) and N^1-methyl-G (m^1G). (**B**) DNA 5-methyl cytosine (m^5C) oxidative reversal pathway.

candidates for reversal include m^5C in mRNA and in tRNA,[16] N^1-methyl-A (m^1A) in tRNA and N^1-methyl-G (m^1G) in tRNA (Fig 4, *A*). m^5C is the epigenetic marker of DNA and it can be reversed by the Tet1/Tet2 enzymes and the DNA repair enzyme thymine-DNA glycosylase (Fig 4, *B*).[52] A similar oxidative reversal pathway may also work for m^5C modification in RNA, although the actual enzymes catalyzing such reactions in RNA have not yet been identified. m^1A is present in almost every tRNA species in eukaryotes; it is required for the stability of some tRNAs.[53,54] The *E. coli* AlkB enzyme reversed m^1A in RNA when m^1A modification was introduced by chemical methylation agents.[55] An AlkB-like enzyme in a human cell may therefore potentially reverse endogenous m^1A in some tRNAs to control their stability. m^1G is present in about half of tRNA species in eukaryotes; it is needed for accurate decoding or for tRNA stability.[56,57] No natural enzyme is yet known that reverses m^1G with high efficiency; however, m^1G should be readily reversible based on its chemical feature.

Finding reader proteins for these other methylations, however, may be far more challenging. To our knowledge, an RNA-binding protein that recognizes m^5C-modified mRNA directly has not yet been identified. For tRNAs, these modifications are generally used to control decoding accuracy and efficiency, and to confer stability. It is unclear whether any reader protein is needed for their direct recognition. For now, m^6A modification is, clearly, the prominent marker of RNA epigenetics.

CONCLUDING REMARKS

Although mRNA modification has been known since the 1970s, its functional importance in mRNA metabolism and its effect on human biology have not been studied extensively in the past. The discovery of the m^6A eraser protein FTO in 2011 links m^6A modification in mRNA/lncRNA directly to human health and disease. Subsequent studies show that the m^6A modification is connected to many aspects of human biology. The field of RNA epigenetics is still in its infancy. We look forward to many exciting discoveries in the coming years.

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Conflicts of Interest: All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

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REFERENCES

1. Piekna-Przybylska D, Decatur WA, Fournier MJ. The 3D rRNA modification maps database: with interactive tools for ribosome analysis. Nucl Acids Res 2008;36:D178–83.
2. El Yacoubi B, Bailly M, de Crecy-Lagard V. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. Annu Rev Genet 2012;46:69–95.
3. Grosjean H, de Crecy-Lagard V, Marck C. Deciphering synonymous codons in the three domains of life: co-evolution with specific tRNA modification enzymes. FEBS Lett 2009;584:252–64.
4. Song X, Nazar RN. Modification of rRNA as a "quality control mechanism" in ribosome biogenesis. FEBS Lett 2002;523:182–6.
5. Agris PF. Decoding the genome: a modified view. Nucl Acids Res 2004;32:223–38.
6. Desrosiers R, Friderici K, Rottman F. Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc Natl Acad Sci U S A 1974;71:3971–5.
7. Adams JM, Cory S. Modified nucleosides and bizarre 5′-termini in mouse myeloma mRNA. Nature 1975;255:28–33.
8. Wei CM, Gershowitz A, Moss B. 5′-Terminal and internal methylated nucleotide sequences in HeLa cell mRNA. Biochemistry 1976;15:397–401.
9. Perry RP, Kelley DE, Friderici K, Rottman F. The methylated constituents of L cell messenger RNA: evidence for an unusual cluster at the 5′terminus. Cell 1975;4:387–94.
10. Wei C, Gershowitz A, Moss B. N6, O2′-dimethyladenosine a novel methylated ribonucleoside next to the 5′terminal of animal cell and virus mRNAs. Nature 1975;257:251–3.
11. Narayan P, Rottman FM. An *in vitro* system for accurate methylation of internal adenosine residues in messenger RNA. Science 1988;242:1159–62.
12. Horowitz S, Horowitz A, Nilsen TW, Munns TW, Rottman FM. Mapping of N6-methyladenosine residues in bovine prolactin mRNA. Proc Natl Acad Sci U S A 1984;81:5667–71.

13. Harper JE, Miceli SM, Roberts RJ, Manley JL. Sequence specificity of the human mRNA N6-adenosine methylase *in vitro*. Nucl Acids Res 1990;18:5735–41.
14. Dubin DT, Taylor RH. The methylation state of poly A-containing messenger RNA from cultured hamster cells. Nucl Acids Res 1975;2:1653–68.
15. Schaefer M, Pollex T, Hanna K, Lyko F. RNA cytosine methylation analysis by bisulfite sequencing. Nucl Acids Res 2009;37:e12.
16. Squires JE, Patel HR, Nousch M, et al. Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. Nucl Acids Res 2012;40:5023–33.
17. Bokar JA. The biosynthesis and functional roles of methylated nucleosides in eukaryotic mRNA. In: Grosjean H, ed. Fine-tuning of RNA functions by modification and editing. Berlin: Springer-Verlag, 2005:141–78.
18. Kariko K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 2005;23:165–75.
19. Jia G, Fu Y, Zhao X, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011;7:885–7.
20. Trewick SC, Henshaw TF, Hausinger RP, Lindahl T, Sedgwick B. Oxidative demethylation by *Escherichia coli* AlkB directly reverts DNA base damage. Nature 2002;419:174–8.
21. Delaney JC, Essigmann JM. Mutagenesis, genotoxicity, and repair of 1-methyladenine, 3-alkylcytosines, 1-methylguanine, and 3-methylthymine in alkB *Escherichia coli*. Proc Natl Acad Sci U S A 2004;101:14051–6.
22. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–94.
23. Gerken T, Girard CA, Tung YC, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007;318:1469–72.
24. Church C, Lee S, Bagg EA, et al. A mouse model for the metabolic effects of the human fat mass and obesity associated FTO gene. PLoS Genet 2009;5:e1000599.
25. He C. Grand challenge commentary: RNA epigenetics? Nat Chem Biol 2010;6:863–5.
26. Yi C, Pan T. Cellular dynamics of RNA modification. Acc Chem Res 2011;44:1380–8.
27. Dominissini D, Moshitch-Moshkovitz S, Schwartz S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 2012;485:201–6.
28. Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3′UTRs and near stop codons. Cell 2012;149:1635–46.
29. Schwartz S, Agarwala SD, Mumbach MR, et al. High-resolution mapping reveals a conserved, widespread, dynamic mRNA methylation program in yeast meiosis. Cell 2013;155:1409–21.
30. Vilfan ID, Tsai YC, Clark TA, et al. Analysis of RNA base modification and structural rearrangement by single-molecule real-time detection of reverse transcription. J Nanobiotechnol 2013;11:8.
31. Harcourt EM, Ehrenschwender T, Batista PJ, Chang HY, Kool ET. Identification of a selective polymerase enables detection of n(6)-methyladenosine in RNA. J Am Chem Soc 2013;135:19079–82.
32. Liu N, Parisien M, Dai Q, Zheng G, He C, Pan T. Probing N6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. RNA 2013;19:1848–56.
33. Bokar JA, Shambaugh ME, Polayes D, Matera AG, Rottman FM. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA 1997;3:1233–47.
34. Zhong S, Li H, Bodi Z, et al. MTA is an Arabidopsis messenger RNA adenosine methylase and interacts with a homolog of a sex-specific splicing factor. Plant Cell 2008;20:1278–88.
35. Clancy MJ, Shambaugh ME, Timpte CS, Bokar JA. Induction of sporulation in *Saccharomyces cerevisiae* leads to the formation of N6-methyladenosine in mRNA: a potential mechanism for the activity of the IME4 gene. Nucl Acids Res 2002;30:4509–18.
36. Hongay CF, Orr-Weaver TL. *Drosophila* Inducer of MEiosis 4 (IME4) is required for Notch signaling during oogenesis. Proc Natl Acad Sci U S A 2011;108:14855–60.
37. Liu J, Yue Y, Han D, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N-adenosine methylation. Nat Chem Biol 2014;10:93–5.
38. Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z, Zhao JC. N-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol 2014;16:191–8.
39. Ping XL, Sun BF, Wang L, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res 2014;24:177–89.
40. Horiuchi K, Umetani M, Minami T, et al. Wilms' tumor 1-associating protein regulates G2/M transition through stabilization of cyclin A2 mRNA. Proc Natl Acad Sci U S A 2006;103:17278–83.
41. Fu Y, Jia G, Pang X, et al. FTO-mediated formation of N6-hydroxymethyladenosine and N4-formyladenosine in mammalian RNA. Nat Commun 2013;4:1798.
42. Zheng G, Dahl JA, Niu Y, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 2012;49:18–29.
43. Yang CG, Yi C, Duguid EM, et al. Crystal structures of DNA/RNA repair enzymes AlkB and ABH2 bound to dsDNA. Nature 2008;452:961–5.
44. van den Born E, Vagbo CB, Songe-Moller L, et al. ALKBH8-mediated formation of a novel diastereomeric pair of wobble nucleosides in mammalian tRNA. Nat Commun 2011;2:172.
45. Ma WJ, Cheng S, Campbell C, Wright A, Furneaux H. Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. J Biol Chem 1996;271:8144–51.
46. Kedde M, Agami R. Interplay between microRNAs and RNA-binding proteins determines developmental processes. Cell Cycle 2008;7:899–903.
47. Kundu P, Fabian MR, Sonenberg N, Bhattacharyya SN, Filipowicz W. HuR protein attenuates miRNA-mediated repression by promoting miRISC dissociation from the target RNA. Nucl Acids Res 2012;40:5088–100.
48. Stoilov P, Rafalska I, Stamm S. YTH: a new domain in nuclear proteins. Trends Biochem Sci 2002;27:495–7.
49. Wang X, Lu Z, Gomez A, et al. N6-methyladenosine-dependent regulation of messenger RNA stability. Nature 2014;505:117–20.
50. Pan T. N6-methyl-adenosine modification in messenger and long non-coding RNA. Trends Biochem Sci 2013;38:204–9.
51. Smemo S, Tena JJ, Kim KH, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature 2014;507:371–5.
52. Ito S, Shen L, Dai Q, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science 2011;333:1300–3.

53. Anderson J, Phan L, Hinnebusch AG. The Gcd10p/Gcd14p complex is the essential two-subunit tRNA(1-methyladenosine) methyltransferase of *Saccharomyces cerevisiae*. Proc Natl Acad Sci U S A 2000;97:5173–8.
54. Saikia M, Fu Y, Pavon-Eternod M, He CA, Pan T. Genome-wide analysis of N-1-methyl-adenosine modification in human tRNAs. RNA 2010;16:1317–27.
55. Ougland R, Zhang CM, Liiv A, et al. AlkB restores the biological function of mRNA and tRNA inactivated by chemical methylation. Mol Cell 2004;16:107–16.
56. Bjork GR, Wikstrom PM, Bystrom AS. Prevention of translational frameshifting by the modified nucleoside 1-methylguanosine. Science 1989;244:986–9.
57. Jackman JE, Montange RK, Malik HS, Phizicky EM. Identification of the yeast gene encoding the tRNA m1G methyltransferase responsible for modification at position 9. RNA 2003;9:574–85.
58. Bujnicki JM, Feder M, Radlinska M, Blumenthal RM. Structure prediction and phylogenetic analysis of a functionally diverse family of proteins homologous to the MT-A70 subunit of the human mRNA:m(6)A methyltransferase. J Mol Evol 2002;55:431–44.
59. Niu Y, Zhao X, Wu YS, Li MM, Wang XJ, Yang YG. N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function. Genomics Proteomics Bioinformatics 2013;11:8–17.
60. Ortega A, Niksic M, Bachi A, et al. Biochemical function of female-lethal (2)D/Wilms' tumor suppressor-1-associated proteins in alternative pre-mRNA splicing. J Biol Chem 2003;278:3040–7.
61. Utsch B, Kaya A, Ozburun A, Lentze MJ, Albers N, Ludwig M. Exclusion of WTAP and HOXA13 as candidate genes for isolated hypospadias. Scand J Urol Nephrol 2003;37:498–501.
62. Su J, Li SJ, Chen ZH, et al. Evaluation of podocyte lesion in patients with diabetic nephropathy: Wilms' tumor-1 protein used as a podocyte marker. Diabetes Res Clin Pract 2010;87:167–75.
63. Jin DI, Lee SW, Han ME, et al. Expression and roles of Wilms' tumor 1-associating protein in glioblastoma. Cancer Sci 2012;103:2102–9.
64. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 2007;316:1336–41.
65. Kalnina I, Zaharenko L, Vaivade I, et al. Polymorphisms in FTO and near TMEM18 associate with type 2 diabetes and predispose to younger age at diagnosis of diabetes. Gene 2013;527:462–8.
66. Akilzhanova A, Nurkina Z, Momynaliev K, et al. Genetic profile and determinants of homocysteine levels in Kazakhstan patients with breast cancer. Anticancer Res 2013;33:4049–59.
67. Reddy SM, Sadim M, Li J, et al. Clinical and genetic predictors of weight gain in patients diagnosed with breast cancer. Br J Cancer 2013;109:872–81.
68. Karra E, O'Daly OG, Choudhury AI, et al. A link between FTO, ghrelin, and impaired brain food-cue responsivity. J Clin Invest 2013;123:3539–51.
69. Lin Y, Ueda J, Yagyu K, et al. Association between variations in the fat mass and obesity-associated gene and pancreatic cancer risk: a case-control study in Japan. BMC Cancer 2013;13:337.
70. Wang L, Yu Q, Xiong Y, et al. Variant rs1421085 in the FTO gene contribute childhood obesity in Chinese children aged 3–6 years. Obes Res Clin Pract 2013;7:e1–88.
71. Thalhammer A, Bencokova Z, Poole R, et al. Human AlkB homologue 5 is a nuclear 2-oxoglutarate dependent oxygenase and a direct target of hypoxia-inducible factor 1alpha (HIF-1alpha). PLoS One 2011;6:e16210.
72. Cardelli M, Marchegiani F, Cavallone L, et al. A polymorphism of the YTHDF2 gene (1p35) located in an Alu-rich genomic domain is associated with human longevity. J Gerontol A Biol Sci Med Sci 2006;61:547–56.
73. Heiliger KJ, Hess J, Vitagliano D, et al. Novel candidate genes of thyroid tumourigenesis identified in Trk-T1 transgenic mice. Endocr Rel Cancer 2012;19:409–21.
74. Wang W, Furneaux H, Cheng H, et al. HuR regulates p21 mRNA stabilization by UV light. Mol Cell Biol 2000;20:760–9.
75. Li H, Park S, Kilburn B, et al. Lipopolysaccharide-induced methylation of HuR, an mRNA-stabilizing protein, by CARM1: coactivator-associated arginine methyltransferase. J Biol Chem 2002;277:44623–30.
76. Lee SJ, Lee AW, Kang CS, et al. Clinicopathological implications of human papillomavirus (HPV) L1 capsid protein immunoreactivity in HPV16-positive cervical cytology. Int J Med Sci 2014;11:80–6.
77. Yang H, Zheng Y, Li TW, et al. Methionine adenosyltransferase 2B, HuR, and sirtuin 1 protein cross-talk impacts on the effect of resveratrol on apoptosis and growth in liver cancer cells. J Biol Chem 2013;288:23161–70.
78. Zhu Z, Wang B, Bi J, et al. Cytoplasmic HuR expression correlates with P-gp, HER-2 positivity, and poor outcome in breast cancer. Tumour Biol 2013;34:2299–308.
79. Yang F, Miao L, Mei Y, Wu M. Retinoic acid-induced HOXA5 expression is co-regulated by HuR and miR-130a. Cell Signal 2013;25:1476–85.
80. Pang L, Tian H, Chang N, et al. Loss of CARM1 is linked to reduced HuR function in replicative senescence. BMC Mol Biol 2013;14:15.
81. http://www.malacards.org/.