Published in final edited form as:
Nat Struct Mol Biol. 2019 October 01; 26(10): 858–862. doi:10.1038/s41594-019-0312-0.

RNA modifying enzymes and their function in a chromatin context

Konstantinos Tzelepis[1], **Oliver Rausch**[2], **Tony Kouzarides**[1,*]

[1]The Gurdon Institute and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK

[2]Storm Therapeutics Ltd, Babraham Research Campus, Cambridge CB22 3AT, UK

Abstract

During the last years, exciting research has connected specific RNA modifications to chromatin, providing evidence for co-transcriptional deposition and function in gene regulation. Here, we review new insights gained from studying the co-transcriptional roles of RNA modifications, and their influence in normal and disease contexts. We also discuss how the availability of novel technical approaches could raise the translational potential of targeting RNA-modifying enzymes for the treatment of disease.

Regulation of chromatin plasticity has an established role in the control of gene expression and pivotal functions in normal and disease contexts. During the last decade, targeting of the epigenetic machinery emerged as a promising new therapeutic strategy, with a rising number of small molecules targeting a wide range of epigenetic regulators in pre-clinical or clinical development[1]. These molecules selectively inhibit various chromatin-associated proteins, including histone methyltransferases such as MLL, EZH2 and PRMT1[2–5], bromodomain and extra-terminal family members such as BRD4[6,7] as well as histone demethylases or deacetylases including LSD1, members of the HDAC family and KDM4C[4,8,9]. Recent efforts have connected several RNA binding proteins (RBPs) and long noncoding RNAs (lncRNAs) to chromatin regulation, suggesting that RNA acts as an important link between chromatin-associated proteins and transcriptional regulators[10,11]. Furthermore, a number of long noncoding RNAs (lncRNAs) have been proposed to function in binding and modulating the activity of chromatin modifiers[12–14]. One of these is the lncRNA Xist, which has a fundamental role in the inactivation of one X chromosome in female mammalian cells via recruitment of epigenetic and transcriptional repressors[15,16]. Interestingly, Xist was recently shown to alter chromatin accessibility via a mechanism involving its binding to the chromatin remodeller BRG1, an event that leads to expulsion of the SWI/SNF complex from the X chromosome[17].

*Correspondence: t.kouzarides@gurdon.cam.ac.uk.

Competing interests

T.K. is a co-founder of Abcam Plc and Storm Therapeutics Ltd, Cambridge, UK. O.R. is an employee of Storm Therapeutics Ltd, Cambridge, UK.

Europe PMC Funders Author Manuscripts

Current advances in the field of post-transcriptional regulation of gene expression have revealed that the cellular RNA modifications, termed the 'epitranscriptome', are an appealing equivalent to DNA and histone modifications, despite clear differences such as copy number and half-life[18–20]. Although vastly underexplored, mainly due to the limited availability of detection systems and reagents with sufficient sensitivity and specificity, there are, to date, more than 140 identified RNA modifications, the majority so far detected in noncoding RNAs (rRNA and tRNA). Despite the urgent need of unbiased and quantitative approaches to interrogate RNA modifications, a number of recent technological advances have aided the molecular understanding of selected RNA modifications. These technologies, including widely used RIP and CLIP-based approaches, utilise modification-specific antibodies and/or chemical reagents followed by high-throughput DNA sequencing to identify modifications on RNA nucleotides[22–31]. Evidently, publications illustrating the context-specific contributions of modifications have been biased towards highly abundant modifications such as N^6-methyladenosine (m^6A), 5-methylcytosine (m^5C) and pseudouridine (Ψ) for which specific analytical reagents are available[22–24, 28, 32–34]. Promising recent developments such as sequence-contextual analysis of RNA modifications by mass spectrometry (MS), based on the adaptation of methodologies used for proteomics, open up new opportunities to accurately identify and quantify RNA modifications. However, these approaches are hampered by the lack of fast bioinformatic search algorithms and are currently also limited to the analysis of abundant RNA species[35–37].

During the last 5 years, several studies showed a clear connection between changes in the epitranscriptome and disease phenotypes. In particular, a number of RNA modifying enzymes have been shown to have a role in the development and maintenance of different types of cancer, predominantly dependent on their catalytic activity[38–42]. These findings suggest that targeting the catalytic pocket of those enzymes could be a promising therapeutic avenue for various devastating diseases. Moreover, the latest major advances in gene editing and recessive screening have provided genome-wide and unbiased evidence for the essential nature of a large number of putative RNA-modifying enzymes further highlighting the therapeutic potential of these targets[43–47].

In this Perspective, we provide a brief overview of the latest discoveries pertaining disease - associated RNA modifications, with a focus on the underlying regulatory mechanisms and their biological effects in the context of chromatin. We also discuss the emerging potential for targeting epitranscriptomic mechanisms for the treatment of disease.

RNA modifications and the chromatin connection

In-depth characterisation of chromatin function over the last two decades has established the epigenome as a dynamic layer of information, involving chromatin modifying or remodelling enzymes and modification dependent chromatin interactors[38,48,49,50]. Interestingly, a number of investigations have revealed unforeseen mechanistic links between chromatin and other enzymatically active proteins including kinases and metabolic enzymes, making the epigenetic landscape even more diverse[51–54]. One such example is a novel nuclear function of JAK2, which was shown to phosphorylate histone 3 tyrosine 41 (H3Y41) leading to downstream effects in normal and malignant hemopoiesis[51]. In line

with these observations, several studies have recently highlighted the possibility that RNA modifying enzymes and their co-factors could efficiently direct gene regulation through co-transcriptional mechanisms (Table 1). These studies suggest that RNA enzymes can be targeted to DNA by chromatin proteins[55], chromatin modifications[56] and by sequence specific DNA binding transcription factors[57]. The functional consequence of this recruitment of RNA enzymes to site of transcription is not yet well understood, but one common theme is the regulation of the subsequent translation of the target RNAs in the cytoplasm[42,57].

N^6-Methyladenosine (m^{6}A)

m^{6}A, discovered back in 1974, is the most abundant eukaryotic mRNA modification, with the majority of mRNAs containing approximately three modified residues[58–60]. Catalysis of m^{6}A is mediated by a methyltransferase complex consisting of METTL3 and METTL14, together with their cofactors WTAP, RBM15, RBM15B, ZC3H13, VIRMA and HAKAI[61–66]. Moreover, the formation of the METTL3-METTL14 complex has been shown to be negatively affected by the interaction between the transcription factor ZFP217 and METTL3[67]. Very recently, METTL16 was proposed as another m^{6}A RNA methyltransferase, modifying the 3′-UTR of specific mRNAs such as *MAT2A*. METTL16 was also suggested to target important noncoding RNAs including the spliceosomal component U6 small nuclear RNA, which pairs with the 5’ splice sites of pre-mRNAs during splicing[68,69]. m^{6}A has been strongly linked to alterations of mRNA translation, mRNA degradation, nuclear-cytoplasmic mRNA export and splicing[34,70–74]. The publication of the crystal structure of the METTL3/METTL14 complex revealed that METTL3 exclusively acts as the catalytic subunit of the complex, while METTL14 mostly functions as a structural scaffold with roles in complex maintenance and substrate binding[75–77]. Analysis of the METTL3/METTL14 binding sites suggested that this protein complex targets m^{6}A predominantly in coding sequences and 3’ UTRs, with a considerable distribution bias to the latter. In addition, a substantial portion of m^{6}A is present on intronic sequences, indicating possible co-transcriptional functions of the modification[78].

Indeed, there is compelling evidence of co-transcriptional involvement of key members of the m^{6}A methyltransferase complex. Initially, m^{6}A was shown to act as a link between transcription and translation through the interaction of RNAPII and METTL3[79]. The authors demonstrated that RNAPII and its dynamics effect the deposition of m^{6}A, with suboptimal transcription leading to enhanced appearance of m^{6}A on mRNAs and reduced translation. METTL3 was further reported to have a pivotal role in the UV-mediated DNA damage response[80]. Upon UV-mediated DNA damage, DNA polymerase κ (Pol κ) and METTL3 colocalise to UV-induced DNA damage sites in order to facilitate repair and cell survival, a function strictly dependent on METTL3 enzymatic activity. The mechanism of METTL3 recruitment to DNA, however, remained unknown. Further connections of m^{6}A to chromatin were shown using chromatin immunoprecipitation assays and sequencing (ChIP-seq). One of the studies demonstrated that METTL3 has a preferential enrichment at the 3’ end of protein-coding genes, reflecting the canonical m^{6}A distribution on mRNAs[71]. The authors further showed that following acute temperature stress, METTL3 together with DGCR8 localises to selected chromatin regions, where they co-transcriptionally mark mRNAs for RNA degradation. Another study reported that in acute myeloid leukemia (AML),

METTL3, independently from METTL14, localizes to transcriptional start sites (TSSs) of cancer-related genes and induces m^6A modification within the coding region of the associated mRNAs, thereby boosting their translation by alleviating ribosomal stalling[57]. Recruitment of METTL3 to TSSs is facilitated by the CAATT-box binding protein CEBPZ and mediates the translational efficiency of oncogenic factors like SP1 and SP2, creating a novel cancer pathway with appealing druggable potential. Remarkably, analysis of METTL3 and METTL14 ChIP-seq datasets in human AML cells revealed minimal genomic overlap suggesting separate roles for the two METTLs on chromatin.

The conceptual role of m^6A in a chromatin context was further highlighted by a study demonstrating an interaction between the transcription factor SMAD2/3 and the METTL3-METTL14-WTAP complex in embryonic stem cells[81]. This interaction promotes co-transcriptional binding of the m^6A methyltransferase complex to selected transcripts mainly involved in pathways regulating early cell fate decisions, thus marking them for fast-track downregulation upon differentiation. Intriguingly, histone H3 trimethylated at Lys36 (H3K36me3), a marker for transcription elongation, was found to directly promote global deposition of m^6A[56]. Specifically, METTL14 was shown to preferential bind H3K36me3, thus recruiting the METTL3-METTL14-WTAP complex to adjacent RNAPII, triggering subsequent m^6A modification of the nascent RNA.

Collectively, there is considerable evidence supporting a chromatin-associated function of m^6A, regulating the fate of nascent mRNA substrates in a context-dependent manner (Table 1). We anticipate that further studies will not only provide evidence for new roles of m^6A but also dissect how these novel functions are related to chromatin regulation.

Other RNA modifications

Despite the progress in understanding the functions of m^6A, there is relatively little known about other RNA modifications, although this gap narrowing, also revealing connections to chromatin (Table 1). 5-methylcytosine (m^5C) modification of RNA was identified several decades ago[82,83]. m^5C is an abundant modification found in both DNA and RNA, and several RNA modifying enzymes have been identified. The majority of the known m^5C methyltransferases belong to the highly conserved NOL1/NOP2/Sun (NSUN) domain containing family[84,85]. NSUN2 had originally been identified as a tRNA m^5C methyltransferase, but it is also responsible for m^5C methylation in several mRNAs and lncRNAs[24,86,87]. Furthermore, the export factor Aly/REF was shown to recognize m^5C and control the export of bound mRNA transcripts in an NSUN2-dependent mechanism[87]. m^5C methylation of tRNAs by NSUN2 has been linked to the generation of small tRNA fragments, regulation of protein synthesis and the stress response, as well as normal development and carcinogenesis[41,88]. It was also reported that m^5C on RNA appears to be increased in circulating tumour cells of patients with lung cancer further indicating a potential role in carcinogenesis[88]. However, a co-transcriptional impact of m^5C was not suggested until recently, despite the established presence of the modification on mRNAs. Pioneering data now demonstrated that the catalytic activity of NSUN1 promotes formation of a distinct active chromatin structure at nascent sites of transcription, through interactions

with RNA polymerase II and BRD4[55]. Future efforts will likely uncover additional co-transcriptional mechanisms behind the catalytic function of m^5C methyltransferases.

The conversion of adenosine nucleosides to inosine (A-to-I), orchestrated by the ADAR family of enzymes, is one of the most prevailing form of post-transcriptional RNA modification. Recent efforts have identified important functions of ADAR enzymes, but there is still uncertainty regarding the functional and regulatory role of A-to-I editing events per se[89–91]. It is also unclear how exactly ADARs recognise a specific A-to-I RNA editing site and whether recognition occurs co-transcriptionally. Importantly, however, A-to-I editing of double stranded RNAs leads to heterochromatic gene silencing through a mechanism involving Vigilin and its high affinity for inosine-containing RNAs[92].

The catalytic activity of MePCE has been tightly linked to O-methylation of the 5′ triphosphate on the gamma position of RNA molecules (5′-P**γ**)[93,94]. MePCE inhibits the degradation of the small nuclear RNA 7SK either through direct binding or by 5′-P**γ**-methyl capping[95]. Reduction of MePCE negatively affects migration and invasion of human breast cancer cells suggesting that this methyltransferase plays a role in tumorigenesis[96]. Besides, MePCE loss modulates the expression of oncogenic pathways associated with breast cancer via a mechanism involving RNAPII, indicating a possible chromatin association. Interestingly, results from *in vitro* assays have illustrated that MePCE preferentially binds to the tail of histone H4, an interaction mediated by the C terminal of its S-adenosyl-methionine-binding domain (SAM-BD)[96]. BCDIN3D, a homolog of MePCE has been previously connected to dimethylation of the 5′-phosphate on specific microRNA precursors, which blocks Dicerdependent processing[39]. To date, there is however no evidence suggesting a co-transcriptional function of BCDIN3D.

One of the most evolutionarily conserved modifications on eukaryotic mRNA is the 5′ m^7G cap. The m^7G RNA modification operates as a distinctive molecular module that facilitates functions such as pre-mRNA splicing and nuclear export as well as cap-dependent protein synthesis. Cap-related m^7G methylation is mediated by the methyltransferase RNMT[97]. Conversely, deposition of m^7G on tRNAs and rRNAs is mediated by the methyltransferase METTL1, in association with its co-factor WDR4[98]. METTL1 has been shown to be critical for the growth of several cancer types[43,47,57] and it is well-known that mutations in WDR4 are causing microcephalic primordial dwarfism in humans[99]. Recent evidence suggested that reduction of m^7G on tRNAs due to loss of METTL1 leads to global effects on tRNA function as well as defects in embryonic stem cell self-renewal and differentiation[100]. Moreover, two studies recently revealed that METTL1-mediated m^7G also occurs internally in miRNAs and mRNAs, despite the general dogma that the modification in mRNAs is limited to the cap[101,102]. m^7 G modification of miRNAs was suggested to increase miRNA maturation and processing by disruption of the inhibitory secondary structure of G-quadruplexes present in numerous miRNA precursors[101]. It was additionally demonstrated that the catalytic activity of METTL1 was associated with suppression of lung cancer cell migration, indicating a possible tumour suppressive role[101]. Furthermore, loss of internal m^7G on mRNAs by METTL1 knockdown was shown to affect mRNA translation of m^7G-modified transcripts[102]. The identification of m^7G on miRNA and mRNA is expanding the functional repertoire of the gene regulation control by the epitranscriptome. It will be

interesting now to investigate whether there are links between m^7G modification of target RNAs and chromatin.

Concluding remarks and future perspectives

Recent discoveries in epitranscriptomics have revealed novel functions for RNA modifications in nearly every class of cellular RNA, some with strong links to disease. To date, there are however only few studies illustrating epigenetic–epitranscriptomic regulatory feedback loops and synchronization between these two regulatory layers. Work in this area is evidently at an early stage. Nevertheless, the observed interplay between specific RNA modifications and chromatin suggests promising therapeutic opportunities. Indeed, in various cancer types, there is remarkable overlap between druggable targets or pathways regulated by both chromatin and RNA modifications. For instance, the control of oncogenic proteins like MYC, BCL2 and BRD4 has been linked to m^6A-mediated mechanisms, supporting the therapeutic promise of targeting the catalytic function of METTL3 as an anticancer approach[42,57,103]. Intriguingly, BRD4 is functionally involved in an NSUN1 and m^5C-dependent co-transcriptional mechanism that regulates the response of cancer cells to 5-Azacitidine[55]. These kinds of studies draw a translational path for future efforts in identifying effective combinatorial therapies using clinically approved (or under clinical development) “epigenetic” drugs coupled with small-molecules inhibitors of selected RNA modifying enzymes.

Pharmacological targeting of key RNA modifying enzymes is anticipated and we envisage that it will be beneficial in several diseases. While RNA methyltransferase inhibitors have not yet been described, inhibitors for the m^6A demethylase FTO have recently shown encouraging anticancer effects in a preclinical study[104]. However, given that individual RNA modifications have been shown to mediate contrasting phenotypes in different tissue types[57,101], it will also be crucial to assess the potential caveats and general effects of such therapies. A strong paradigm is m^6A, which was shown to either promote or disrupt the development and/or maintenance of malignant phenotypes dependent on the tumor type[42,105–108]. Similarly, loss of METTL1-mediated m^7G is promoting migration and invasion in lung cancer[101] while it appears to be essential for acute myeloid leukemia cells[57].

An important avenue to probe therapeutic and functional interactions between druggable chromatin targets and modifiers of RNA are CRISPR sensitisation screening platforms in clinically relevant disease models. Such powerful genetic approaches have the potential to unearth synthetic lethal interactions that could provide patient stratification routes and link RNA modifications with an improved response to existing therapies or evasion of drug resistance. In that regard, a recent publication presented compelling evidence that RNA modifying enzymes responsible for the mcm^5s^2U modification on the wobble U position in tRNA anticodons are key mediators of resistance to BRAF inhibitors observed in $BRAF^{V600E}$-melanomas[109].

As the field of epitranscriptomics develops, we anticipate novel chromatin associations. New and improved detection technologies could pave the way for the exploration of

Europe PMC Funders Author Manuscripts

other, currently uncharacterised RNA modification. In addition, the development of specific pharmacological inhibitors of RNA modifying enzymes will revolutionise research in this field and open exciting new approaches for the treatment of cancer and other diseases.

References

1. Xhemalce B, Dawson MA, Bannister AJ. Histone Modifications. Meyers RA. Reviews in Cell Biology and Molecular Medicine. 2011; doi: 10.1002/3527600906.mcb.201100004
2. Grembecka J, et al. Menin-MLL inhibitors reverse oncogenic activity of MLL fusion proteins in leukemia. Nat Chem Biol. 2012; 8: 277–284. DOI: 10.1038/nchembio.773 [PubMed: 22286128]
3. McCabe MT, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature. 2012; 492: 108–112. DOI: 10.1038/nature11606 [PubMed: 23051747]
4. Cheung N, et al. Targeting Aberrant Epigenetic Networks Mediated by PRMT1 and KDM4C in Acute Myeloid Leukemia. Cancer Cell. 2016; 29: 32–48. DOI: 10.1016/j.ccell.2015.12.007 [PubMed: 26766589]
5. Hu H, Qian K, Ho MC, Zheng YG. Small Molecule Inhibitors of Protein Arginine Methyltransferases. Expert Opin Investig Drugs. 2016; 25: 335–358. DOI: 10.1517/13543784.2016.1144747
6. Zuber J, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature. 2011; 478: 524–528. DOI: 10.1038/nature10334 [PubMed: 21814200]
7. Dawson MA, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature. 2011; 478: 529–533. DOI: 10.1038/nature10509 [PubMed: 21964340]
8. Schenk T, et al. Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia. Nat Med. 2012; 18: 605–611. DOI: 10.1038/nm.2661 [PubMed: 22406747]
9. Eckschlager T, Plch J, Stiborova M, Hrabeta J. Histone Deacetylase Inhibitors as Anticancer Drugs. Int J Mol Sci. 2017; 18 doi: 10.3390/ijms18071414
10. Nickerson JA, Krochmalnic G, Wan KM, Penman S. Chromatin architecture and nuclear RNA. Proc Natl Acad Sci U S A. 1989; 86: 177–181. DOI: 10.1073/pnas.86.1.177 [PubMed: 2911567]
11. Bernstein E, Allis CD. RNA meets chromatin. Genes Dev. 2005; 19: 1635–1655. DOI: 10.1101/gad.1324305 [PubMed: 16024654]
12. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol Biol. 2013; 20: 300–307. DOI: 10.1038/nsmb.2480 [PubMed: 23463315]
13. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2012; 81: 145–166. DOI: 10.1146/annurev-biochem-051410-092902 [PubMed: 22663078]
14. Tsai MC, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science. 2010; 329: 689–693. DOI: 10.1126/science.1192002 [PubMed: 20616235]
15. Chu C, et al. Systematic discovery of Xist RNA binding proteins. Cell. 2015; 161: 404–416. DOI: 10.1016/j.cell.2015.03.025 [PubMed: 25843628]
16. McHugh CA, et al. The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. Nature. 2015; 521: 232–236. DOI: 10.1038/nature14443 [PubMed: 25915022]
17. Jegu T, et al. Xist RNA antagonizes the SWI/SNF chromatin remodeler BRG1 on the inactive X chromosome. Nat Struct Mol Biol. 2019; 26: 96–109. DOI: 10.1038/s41594-018-0176-8 [PubMed: 30664740]
18. Frye M, Jaffrey SR, Pan T, Rechavi G, Suzuki T. RNA modifications: what have we learned and whereare we headed? Nat Rev Genet. 2016; 17: 365–372. DOI: 10.1038/nrg.2016.47 [PubMed: 27140282]
19. Saletore Y, et al. The birth of the Epitranscriptome: deciphering the function of RNA modifications. Genome Biol. 2012; 13: 175. doi: 10.1186/gb-2012-13-10-175 [PubMed: 23113984]
20. Schwartz S. Cracking the epitranscriptome. RNA. 2016; 22: 169–174. DOI: 10.1261/rna.054502.115 [PubMed: 26787305]

21. Machnicka MA, et al. MODOMICS: a database of RNA modification pathways--2013 update. Nucleic Acids Res. 2013; 41: D262–267. DOI: 10.1093/nar/gks1007 [PubMed: 23118484]
22. Dominissini D, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature. 2012; 485: 201–206. DOI: 10.1038/nature11112 [PubMed: 22575960]
23. Meyer KD, et al. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell. 2012; 149: 1635–1646. DOI: 10.1016/j.cell.2012.05.003 [PubMed: 22608085]
24. Khoddami V, Cairns BR. Identification of direct targets and modified bases of RNA cytosine methyltransferases. Nat Biotechnol. 2013; 31: 458–464. DOI: 10.1038/nbt.2566 [PubMed: 23604283]
25. Khoddami V, Cairns BR. Transcriptome-wide target profiling of RNA cytosine methyltransferases using the mechanism-based enrichment procedure Aza-IP. Nat Protoc. 2014; 9: 337–361. DOI: 10.1038/nprot.2014.014 [PubMed: 24434802]
26. Khoddami V, et al. Transcriptome-wide profiling of multiple RNA modifications simultaneously at single-base resolution. Proc Natl Acad Sci U S A. 2019; 116: 6784–6789. DOI: 10.1073/pnas.1817334116 [PubMed: 30872485]
27. Linder B, et al. Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods. 2015; 12: 767–772. DOI: 10.1038/nmeth.3453 [PubMed: 26121403]
28. Chen K, et al. High-resolution N(6) -methyladenosine (m(6) A) map using photocrosslinking-assisted m(6) A sequencing. Angew Chem Int Ed Engl. 2015; 54: 1587–1590. DOI: 10.1002/anie.201410647 [PubMed: 25491922]
29. Garcia-Campos MA, et al. Deciphering the "m(6)A Code" via AntibodyIndependent Quantitative Profiling. Cell. 2019; 178: 731–747. e716 doi: 10.1016/j.cell.2019.06.013 [PubMed: 31257032]
30. Zhang Z, et al. Single-base mapping of m(6)A by an antibody-independent method. Sci Adv. 2019; 5 eaax0250 doi: 10.1126/sciadv.aax0250 [PubMed: 31281898]
31. Clark WC, Evans ME, Dominissini D, Zheng G, Pan T. tRNA base methylation identification and quantification via high-throughput sequencing. RNA. 2016; 22: 1771–1784. DOI: 10.1261/rna.056531.116 [PubMed: 27613580]
32. Edelheit S, Schwartz S, Mumbach MR, Wurtzel O, Sorek R. Transcriptomewide mapping of 5-methylcytidine RNA modifications in bacteria, archaea, and yeast reveals m5C within archaeal mRNAs. PLoS Genet. 2013; 9 e1003602 doi: 10.1371/journal.pgen.1003602 [PubMed: 23825970]
33. Carlile TM, et al. Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature. 2014; 515: 143–146. DOI: 10.1038/nature13802 [PubMed: 25192136]
34. Schwartz S, et al. Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. Cell. 2014; 159: 148–162. DOI: 10.1016/j.cell.2014.08.028 [PubMed: 25219674]
35. Cao X, Limbach PA. Enhanced detection of post-transcriptional modifications using a mass-exclusion list strategy for RNA modification mapping by LC-MS/MS. Anal Chem. 2015; 87: 8433–8440. DOI: 10.1021/acs.analchem.5b01826 [PubMed: 26176336]
36. Wetzel C, Limbach PA. Mass spectrometry of modified RNAs: recent developments. Analyst. 2016; 141: 16–23. DOI: 10.1039/c5an01797a [PubMed: 26501195]
37. Ross R, Cao X, Yu N, Limbach PA. Sequence mapping of transfer RNA chemical modifications by liquid chromatography tandem mass spectrometry. Methods. 2016; 107: 73–78. DOI: 10.1016/j.ymeth.2016.03.016 [PubMed: 27033178]
38. Montanaro L, et al. Dyskerin expression influences the level of ribosomal RNA pseudo-uridylation and telomerase RNA component in human breast cancer. J Pathol. 2006; 210: 10–18. DOI: 10.1002/path.2023 [PubMed: 16841302]
39. Xhemalce B, Robson SC, Kouzarides T. Human RNA methyltransferase BCDIN3D regulates microRNA processing. Cell. 2012; 151: 278–288. DOI: 10.1016/j.cell.2012.08.041 [PubMed: 23063121]
40. Jiang Q, et al. ADAR1 promotes malignant progenitor reprogrammingin chronic myeloid leukemia. Proc Natl Acad Sci U S A. 2013; 110: 1041–1046. DOI: 10.1073/pnas.1213021110 [PubMed: 23275297]

41. Blanco S, et al. Stem cell function and stress response are controlled by protein synthesis. Nature. 2016; 534: 335–340. DOI: 10.1038/nature18282 [PubMed: 27306184]
42. Vu LP, et al. The N(6)-methyladenosine (m(6)A)-forming enzyme METTL3 controls myeloiddifferentiation of normal hematopoietic and leukemia cells. Nat Med. 2017; 23: 1369–1376. DOI: 10.1038/nm.4416 [PubMed: 28920958]
43. Hart T, et al. High-Resolution CRISPR Screens Reveal Fitness Genes and GenotypeSpecific Cancer Liabilities. Cell. 2015; 163: 1515–1526. DOI: 10.1016/j.cell.2015.11.015 [PubMed: 26627737]
44. Steinhart Z, et al. Genome-wide CRISPR screens reveal a Wnt-FZD5 signaling circuit as a druggable vulnerability of RNF43-mutant pancreatic tumors. Nat Med. 2017; 23: 60–68. DOI: 10.1038/nm.4219 [PubMed: 27869803]
45. Tzelepis K, et al. A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and Therapeutic Targets in Acute Myeloid Leukemia. Cell Rep. 2016; 17: 1193–1205. DOI: 10.1016/j.celrep.2016.09.079 [PubMed: 27760321]
46. Wang T, et al. Gene Essentiality Profiling Reveals Gene Networks and Synthetic Lethal Interactions with Oncogenic Ras. Cell. 2017; 168: 890–903. e815 doi: 10.1016/j.cell.2017.01.013 [PubMed: 28162770]
47. Behan FM, et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature. 2019; 568: 511–516. DOI: 10.1038/s41586-019-1103-9 [PubMed: 30971826]
48. Tessarz P, Kouzarides T. Histone core modifications regulating nucleosome structure and dynamics. Nat Rev Mol Cell Biol. 2014; 15: 703–708. DOI: 10.1038/nrm3890 [PubMed: 25315270]
49. Dawson MA, Kouzarides T, Huntly BJ. Targeting epigenetic readers in cancer. N Engl J Med. 2012; 367: 647–657. DOI: 10.1056/NEJMra1112635 [PubMed: 22894577]
50. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M. Epigenetic protein families: a new frontier for drug discovery. Nat Rev Drug Discov. 2012; 11: 384–400. DOI: 10.1038/nrd3674 [PubMed: 22498752]
51. Dawson MA, et al. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. Nature. 2009; 461: 819–822. DOI: 10.1038/nature08448 [PubMed: 19783980]
52. Li X, Egervari G, Wang Y, Berger SL, Lu Z. Regulation of chromatin and gene expression by metabolic enzymes and metabolites. Nat Rev Mol Cell Biol. 2018; 19: 563–578. DOI: 10.1038/s41580-018-0029-7 [PubMed: 29930302]
53. Mews P, et al. Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. Nature. 2017; 546: 381–386. DOI: 10.1038/nature22405 [PubMed: 28562591]
54. Katoh Y, et al. Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoprotein. Mol Cell. 2011; 41: 554–566. DOI: 10.1016/j.molcel.2011.02.018 [PubMed: 21362551]
55. Cheng JX, et al. RNA cytosine methylation and methyltransferases mediate chromatin organization and 5-azacytidine response and resistance in leukaemia. Nat Commun. 2018; 9: 1163. doi: 10.1038/s41467-018-03513-4 [PubMed: 29563491]
56. Huang H, et al. Histone H3 trimethylation at lysine 36 guides m(6)A RNA modification co-transcriptionally. Nature. 2019; 567: 414–419. DOI: 10.1038/s41586-019-1016-7 [PubMed: 30867593]
57. Barbieri I, et al. Promoter-bound METTL3 maintains myeloid leukaemiaby m(6)A-dependent translation control. Nature. 2017; 552: 126–131. DOI: 10.1038/nature24678 [PubMed: 29186125]
58. Rottman F, Shatkin AJ, Perry RP. Sequences containing methylated nucleotides at the 5' termini ofmessenger RNAs: possible implications forprocessing. Cell. 1974; 3: 197–199. [PubMed: 4373171]
59. Narayan P, Rottman FM. An in vitro system for accurate methylation of internal adenosine residues in messenger RNA. Science. 1988; 242: 1159–1162. DOI: 10.1126/science.3187541 [PubMed: 3187541]
60. Csepany T, Lin A, Baldick CJ Jr, Beemon K. Sequence specificity of mRNA N6-adenosine methyltransferase. J Biol Chem. 1990; 265: 20117–20122. [PubMed: 2173695]

61. Wang Y, et al. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol. 2014; 16: 191–198. DOI: 10.1038/ncb2902 [PubMed: 24394384]
62. Liu J, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol. 2014; 10: 93–95. DOI: 10.1038/nchembio.1432 [PubMed: 24316715]
63. Patil DP, et al. m(6)A RNA methylation promotes XIST-mediated transcriptional repression. Nature. 2016; 537: 369–373. DOI: 10.1038/nature19342 [PubMed: 27602518]
64. Knuckles P, Buhler M. Adenosine methylation as a molecular imprint defining the fate of RNA. FEBS Lett. 2018; 592: 2845–2859. DOI: 10.1002/1873-3468.13107 [PubMed: 29782652]
65. Yue Y, et al. VIRMA mediates preferential m(6)A mRNA methylation in 3'UTR and near stop codon and associates with alternative polyadenylation. Cell Discov. 2018; 4: 10. doi: 10.1038/s41421-018-0019-0 [PubMed: 29507755]
66. Wen J, et al. Zc3h13 Regulates Nuclear RNA m(6)A Methylation and Mouse Embryonic StemCell Self-Renewal. Mol Cell. 2018; 69: 1028–1038. e1026 doi: 10.1016/j.molcel.2018.02.015 [PubMed: 29547716]
67. Aguilo F, et al. Coordination of m(6)A mRNA Methylation and Gene Transcription by ZFP217Regulates Pluripotency and Reprogramming. Cell Stem Cell. 2015; 17: 689–704. DOI: 10.1016/j.stem.2015.09.005 [PubMed: 26526723]
68. Pendleton KE, et al. The U6 snRNA m(6)A Methyltransferase METTL16 Regulates SAM Synthetase Intron Retention. Cell. 2017; 169: 824–835. e814 doi: 10.1016/j.cell.2017.05.003 [PubMed: 28525753]
69. Warda AS, et al. Human METTL16 is a N(6)-methyladenosine (m(6)A) methyltransferase that targets pre-mRNAs and various non-coding RNAs. EMBO Rep. 2017; 18: 2004–2014. DOI: 10.15252/embr.201744940 [PubMed: 29051200]
70. Geula S, et al. Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. Science. 2015; 347: 1002–1006. DOI: 10.1126/science.1261417 [PubMed: 25569111]
71. Knuckles P, et al. RNA fate determination through cotranscriptional adenosine methylation and microprocessor binding. Nat Struct Mol Biol. 2017; 24: 561–569. DOI: 10.1038/nsmb.3419 [PubMed: 28581511]
72. Wang X, et al. N(6)-methyladenosine Modulates Messenger RNA Translation Efficiency. Cell. 2015; 161: 1388–1399. DOI: 10.1016/j.cell.2015.05.014 [PubMed: 26046440]
73. Alarcon CR, et al. HNRNPA2B1 Is a Mediator of m(6)A-Dependent Nuclear RNA Processing Events. Cell. 2015; 162: 1299–1308. DOI: 10.1016/j.cell.2015.08.011 [PubMed: 26321680]
74. Huang H, et al. Recognition of RNA N(6)-methyladenosine by IGF2BP proteins enhances mRNA stability and translation. Nat Cell Biol. 2018; 20: 285–295. DOI: 10.1038/s41556-018-0045-z [PubMed: 29476152]
75. Sledz P, Jinek M. Structural insights into the molecular mechanism of the m(6)A writer complex. Elife. 2016; 5 doi: 10.7554/eLife.18434
76. Wang X, et al. Structural basis of N(6)-adenosine methylation by the METTL3-METTL14 complex. Nature. 2016; 534: 575–578. DOI: 10.1038/nature18298 [PubMed: 27281194]
77. Wang P, Doxtader KA, Nam Y. Structural Basis for Cooperative Function of Mettl3 and Mettl14 Methyltransferases. Mol Cell. 2016; 63: 306–317. DOI: 10.1016/j.molcel.2016.05.041 [PubMed: 27373337]
78. Ping XL, et al. Mammalian WTAP is a regulatory subunit of the RNA N6-methyladenosine methyltransferase. Cell Res. 2014; 24: 177–189. DOI: 10.1038/cr.2014.3 [PubMed: 24407421]
79. Slobodin B, et al. Transcription Impacts the Efficiency of mRNA Translation via Co-transcriptional N6-adenosine Methylation. Cell. 2017; 169: 326–337. e312 doi: 10.1016/j.cell.2017.03.031 [PubMed: 28388414]
80. Xiang Y, et al. RNA m(6)A methylation regulates the ultraviolet-induced DNA damage response. Nature. 2017; 543: 573–576. DOI: 10.1038/nature21671 [PubMed: 28297716]

81. Bertero A, et al. The SMAD2/3 interactome reveals that TGFbeta controls m(6)A mRNA methylation in pluripotency. Nature. 2018; 555: 256–259. DOI: 10.1038/nature25784 [PubMed: 29489750]
82. Desrosiers R, Friderici K, Rottman F. Identification of methylated nucleosides in messenger RNA from Novikoffhepatoma cells. Proc Natl Acad Sci U S A. 1974; 71: 3971–3975. DOI: 10.1073/pnas.71.10.3971 [PubMed: 4372599]
83. Dubin DT, Taylor RH. The methylation state of poly A-containing messenger RNA from cultured hamster cells. Nucleic Acids Res. 1975; 2: 1653–1668. DOI: 10.1093/nar/2.10.1653 [PubMed: 1187339]
84. Motorin Y, Lyko F, Helm M. 5-methylcytosine in RNA: detection, enzymatic formation and biological functions. Nucleic Acids Res. 2010; 38: 1415–1430. DOI: 10.1093/nar/gkp1117 [PubMed: 20007150]
85. Schaefer M, et al. RNA methylation by Dnmt2 protects transfer RNAs against stress-inducedcleavage. Genes Dev. 2010; 24: 1590–1595. DOI: 10.1101/gad.586710 [PubMed: 20679393]
86. Hussain S, et al. NSun2-mediated cytosine-5 methylation of vault noncoding RNA determinesits processing into regulatory small RNAs. Cell Rep. 2013; 4: 255–261. DOI: 10.1016/j.celrep.2013.06.029 [PubMed: 23871666]
87. Yang X, et al. 5-methylcytosine promotes mRNA export - NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. Cell Res. 2017; 27: 606–625. DOI: 10.1038/cr.2017.55 [PubMed: 28418038]
88. Frye M, et al. Genomic gain of 5p15 leads to over-expression of Misu (NSUN2) in breastcancer. Cancer Lett. 2010; 289: 71–80. DOI: 10.1016/j.canlet.2009.08.004 [PubMed: 19740597]
89. Fumagalli D, et al. Principles Governing A-to-I RNA Editing in the Breast Cancer Transcriptome. Cell Rep. 2015; 13: 277–289. DOI: 10.1016/j.celrep.2015.09.032 [PubMed: 26440892]
90. Rosenthal JJ. The emerging role of RNA editing in plasticity. J Exp Biol. 2015; 218: 1812–1821. DOI: 10.1242/jeb.119065 [PubMed: 26085659]
91. Zipeto MA, et al. ADAR1 Activation Drives Leukemia Stem Cell Self-Renewal by Impairing Let-7 Biogenesis. Cell Stem Cell. 2016; 19: 177–191. DOI: 10.1016/j.stem.2016.05.004 [PubMed: 27292188]
92. Wang Q, Zhang Z, Blackwell K, Carmichael GG. Vigilins bind to promiscuously A-to-I-edited RNAs and are involved in the formation of heterochromatin. Curr Biol. 2005; 15: 384–391. DOI: 10.1016/j.cub.2005.01.046 [PubMed: 15723802]
93. Jeronimo C, et al. Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. Mol Cell. 2007; 27: 262–274. DOI: 10.1016/j.molcel.2007.06.027 [PubMed: 17643375]
94. Shuman S. Transcriptional networking cap-tures the 7SK RNA 5'-gamma-methyltransferase. Mol Cell. 2007; 27: 517–519. DOI: 10.1016/j.molcel.2007.08.001 [PubMed: 17707222]
95. Xue Y, Yang Z, Chen R, Zhou Q. A capping-independent function of MePCE in stabilizing 7SK snRNA and facilitating the assembly of 7SK snRNP. Nucleic Acids Res. 2010; 38: 360–369. DOI: 10.1093/nar/gkp977 [PubMed: 19906723]
96. Shelton SB, et al. Crosstalk between the RNA Methylation and Histone-Binding Activities ofMePCE Regulates P-TEFb Activation on Chromatin. Cell Rep. 2018; 22: 1374–1383. DOI: 10.1016/j.celrep.2018.01.028 [PubMed: 29425494]
97. Aregger M, Cowling VH. Regulation of mRNA capping in the cell cycle. RNA Biol. 2017; 14: 11–14. DOI: 10.1080/15476286.2016.1251540 [PubMed: 27791484]
98. Alexandrov A, Martzen MR, Phizicky EM. Two proteins that form a complex are required for 7-methylguanosine modification of yeast tRNA. RNA. 2002; 8: 1253–1266. DOI: 10.1017/s1355838202024019 [PubMed: 12403464]
99. Shaheen R, et al. Mutation in WDR4 impairs tRNA m(7)G46 methylation and causes a distinctform of microcephalic primordial dwarfism. Genome Biol. 2015; 16: 210. doi: 10.1186/s13059-015-0779-x [PubMed: 26416026]

100. Lin S, et al. Mettl1/Wdr4-Mediated m(7)G tRNA Methylome Is Required for Normal mRNA Translation and Embryonic Stem Cell Self-Renewal and Differentiation. Mol Cell. 2018; 71: 244–255. e245 doi: 10.1016/j.molcel.2018.06.001 [PubMed: 29983320]

101. Pandolfini L, et al. METTL1 Promotes let-7 MicroRNA Processing via m7GMethylation. Mol Cell. 2019; 74: 1278–1290. e1279 doi: 10.1016/j.molcel.2019.03.040 [PubMed: 31031083]

102. Zhang LS, et al. Transcriptome-wide Mapping of Internal N(7)-Methylguanosine Methylome in Mammalian mRNA. Mol Cell. 2019; 74: 1304–1316. e1308 doi: 10.1016/j.molcel.2019.03.036 [PubMed: 31031084]

103. Choe J, et al. mRNA circularization by METTL3-eIF3h enhances translation and promotes oncogenesis. Nature. 2018; 561: 556–560. DOI: 10.1038/s41586-018-0538-8 [PubMed: 30232453]

104. Huang Y, et al. Small-Molecule Targeting of Oncogenic FTO Demethylase in Acute Myeloid Leukemia. Cancer Cell. 2019; 35: 677–691. e610 doi: 10.1016/j.ccell.2019.03.006 [PubMed: 30991027]

105. Lin S, Choe J, Du P, Triboulet R, Gregory RI. The m(6)A Methyltransferase METTL3 Promotes Translation in Human CancerCells. Mol Cell. 2016; 62: 335–345. DOI: 10.1016/j.molcel.2016.03.021 [PubMed: 27117702]

106. Ma JZ, et al. METTL14 suppresses the metastatic potential of hepatocellular carcinomaby modulating N(6) -methyladenosine-dependent primary MicroRNA processing. Hepatology. 2017; 65: 529–543. DOI: 10.1002/hep.28885 [PubMed: 27774652]

107. Su R, et al. R-2HG Exhibits Anti-tumor Activity by Targeting FTO/m(6)A/MYC/CEBPA Signaling. Cell. 2018; 172: 90–105. e123 doi: 10.1016/j.cell.2017.11.031 [PubMed: 29249359]

108. Han D, et al. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. Nature. 2019; 566: 270–274. DOI: 10.1038/s41586-019-0916-x [PubMed: 30728504]

109. Rapino F, et al. Codon-specific translation reprogramming promotesresistance to targetedtherapy. Nature. 2018; 558: 605–609. DOI: 10.1038/s41586-018-0243-7 [PubMed: 29925953]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1
Overview of RNA modifications with chromatin-associated function

RNA Modification	RNA Enzyme or co-factor	Chromatin-associated Function	Reference
m^6A	METTL3	METTL3 and DNA polymerase κ (Pol κ) co-localise to UV-induced DNA damage sites and facilitate DNA repair and cell survival	73
m^6A	METTL3	METTL3 and DGCR8 co-localise on chromatin following triggering of acute temperature stress, where they co-transcriptionally m^6A-modify mRNAs for RNA degradation	66
m^6A	METTL3	METTL3 is recruited by CEBPZ to transcriptional start sites (TSSs) of active genes and induces m^6A modification within the coding region of the relevant mRNAs, leading to efficient mRNA translation by block of ribosomal stalling	54
m^6A	METTL3/ METTL14/ WTAP	The interaction between SMAD2/3 and the METTL3-METTL14-WTAP complex promotes co-transcriptional binding of the m^6A methyltransferase complex on selected mRNA and prevents their downregulation upon differentiation and cell control	74
m^6A	METTL3/ METTL14/ WTAP	METTL14 dictates the binding of the m6A methyltransferase complex (METTL3, METTL14 and WTAP) to RNA polymerase II and the m^6A marking of actively transcribed nascent RNAs via its preferential binding to H3K36me3	53
m^5C	NSUN1	NSUN1 regulates the formation of a distinct active chromatin structure at nascent RNAs via its interaction with RNA polymerase II and BRD4	52
A-to-I	ADAR	A-to-I editing of double stranded RNAs via ADAR leads to gene silencing of heterochromatin through a mechanism involving Vigilin and its high affinity for inosine-containing RNAs	85
5′ phosphomethylation	MEPCE	MePCE preferentially binds to the tail of histone H4, functioning as a P-TEFb activator at specific genes important for cellular identity, while this histone binding interrupts MePCE's RNA methyltransferase activity toward 7SK	89