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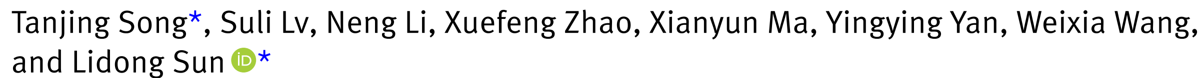
Review

Versatile functions of RNA m6A machinery on chromatin



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m6A, a conserved and abundant modification on RNA, regulates RNA processing and function. RNA m6A machinery, including writers, erasers, and readers of m6A, is indispensable for m6A installation and function. Intriguingly, recent studies have revealed that m6A machinery can be recruited to chromatin by pleiotropic factors, including nascent RNA, transcription factors, regulatory RNA, histone modifications, and epigenetic machinery. Consequently, recruitment of m6A machinery can directly regulate chromatin biology, such as transcription, DNA damage repair, and DNA recombination beyond installation of m6A on nascent mRNA. Here, we discuss recent evidence showing that m6A machinery is targeted to chromatin and the direct biological consequences along with the underlying mechanisms.

Keywords: m6A, chromatin, RNA methylation, histone modifications, epigenetics

Introduction

m6A RNA modification and m6A machinery: writers, readers, and erasers

m6A RNA modification is the most abundant modification on RNA among >100 modifications. m6A occurs on >25% mRNA and constitutes >0.1% of all adenines in RNA (Desrosiers et al., 1974; Wei et al., 1976; Wei and Moss, 1977; Dominissini et al., 2012; Meyer et al., 2012). m6A is installed mainly by an evolutionarily conserved multicomponent methyltransferase complex (MTC). MTC contains the catalytic subunit METTL3 (Bokar et al., 1997) and other auxiliary subunits such as METTL14 (Liu et al., 2014), WTAP (Liu et al., 2014; Schwartz et al., 2014), VIRMA (Schwartz et al., 2014; Yue et al., 2018), CBLL1 (Bawankar et al., 2021), and ZC3H13 (Knuckles et al., 2018; Wen et al., 2018). m6A can be removed by two enzymes, ALKBH5 (Zheng et al., 2013) and FTO (Jia et al., 2011). Essentially, all biological processes involving mRNA are reported to be affected by m6A, including polyadenylation, splicing, nuclear export, translation, and degradation (Zhao et al., 2017). m6A does not change the coding ability of mRNA. Instead, it mainly functions through a group of reader proteins. Well-established readers include the YTH domain-containing YTHDC1 (Xu et al., 2014), YTHDC2 (Hsu et al., 2017), YTHDF1 (Wang et al., 2014), YTHDF2 (Wang et al., 2014), and YTHDF3 (Shi et al., 2017), among which YTHDC1 is the only nuclear reader. Besides mRNA, m6A also occurs on and regulates non-coding RNAs, including transfer RNA, ribosomal RNA, and regulatory RNA such as enhancer RNA (eRNA), promoter antisense RNA, and long non-coding RNA (lncRNA) (Huang et al., 2020).

Divergence and stability of m6A modification on RNA

m6A distribution shows a high level of conservation across different species in the same tissues (Liu et al., 2020b), but the divergence is still quite significant. There is 20% consensus in shared m6A-modified mRNA across humans, mice, and rats (Zhang et al., 2019).

Albeit with conservation, m6A shows significant difference across different tissues (Liu et al., 2020b). Consistently, m6A distribution also changes significantly during development (Zhang et al., 2020b). Such divergence correlates with the regulatory roles of m6A.

The overall stability of m6A in somatic cells is high. As shown in HepG2 human liver cancer cells, ~75%–90% of m6A

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Table 1 MTC and YTHDC1 are recruited to chromatin.

Cell	m6A machinery	Method	Sites	Location	References
MOLM13	METTL3	ChIP–Seq	126 peaks	TSS	Barbieri et al. (2017)
MOLM13	METTL14	ChIP–Seq	119 peaks	TSS	Barbieri et al. (2017)
mES	Mettl3	ChIP–Seq	—	Heat-shock genes, 3′-UTR	Knuckles et al. (2017)
mES	Mettl3	ChIP	Smad2/3 targets	—	Bertero et al. (2018)
HepG2	HA-METTl14	ChIP–Seq	1973 peaks	65% in gene body	Huang et al. (2019a)
U2OS	METTL3	IF	—	Damage foci	Xiang et al. (2017); Zhang et al. (2020a)
U2OS	YTHDC1	IF	—	Damage foci	Zhang et al. (2020a)
mES	METTL3	ChIP–Seq	>8000 genes	Gene body	Li et al. (2020)
THP1	METTL3 and METTL14	ChIP	—	—	Wu et al. (2020)
MCF7	YTHDC1	ChIP–Seq	1760 sites	61.1% enhancer, 16.3% promoter	Lee et al. (2021)
mES	YTHDC1	ChIP–Seq	18525 peaks	TE (>50%)	Liu et al. (2021a)
mES	METTL3	ChIP–Seq	1928 peaks	TE	Xu et al. (2021b)
Embryonic body	METTL3	ChIP–Seq	3434 peaks	Promoter	Xu et al. (2021b)
HepG2	METTL3 and METTL14	ChIP	PTEN and GAPDH	—	Kim and Siddiqui (2021)
IMR90	METTL3	ChIP–Seq	SASP genes	TSS	Liu et al. (2021b)
IMR90	METTL14	ChIP–Seq	SASP genes	Enhancer	Liu et al. (2021b)

TSS, transcription start site; TE, transposable element.

distribution remains unchanged under different treatments. The rest of the m6A is subject to influence by stimuli (Dominissini et al., 2012). Nevertheless, the changes in m6A are still important for cellular response to extracellular stimuli. But until recently, it was unknown what controls gene-specific installation of m6A.

m6A RNA modification, conventionally perceived as post-transcriptional, is now increasingly recognized as a co-transcriptional event (Ke et al., 2017; Knuckles et al., 2017; Slobodin et al., 2017). Consistently, MTC interacts with RNAPII-CTD (Slobodin et al., 2017; Huang et al., 2019a). Intriguingly, recent studies have shown that m6A machinery, especially MTC and the nuclear m6A reader YTHDC1, can target to chromatin. Importantly, m6A machinery on chromatin can regulate chromatin biology beyond tagging nascent mRNA with m6A for later turnover.

m6A machinery is recruited to chromatin

Various methodologies, including chromatin immunoprecipitation followed by sequencing (ChIP–Seq), immunofluorescence (IF), and subcellular fractionation have shown that m6A machinery can localize to chromatin (Table 1). Depending on the cell type and the stimuli, m6A machinery can target to different subgenomic regions, such as enhancers, promoters, the gene body, retrotransposons, and DNA damage foci. The extent of recruitment of MTC or YTHDC1 to chromatin varies from report to report. Besides experimental variation, such phenomena indicate that chromosomal targeting of m6A machinery is subject to cell type- and stimulus-specific regulation. Indeed, Xu et al. (2021b) found a sharp increase in METTL3 targeting to a gene promoter during mouse embryonic stem (mES) cell differentiation. Another study shows that METTL3 is relocated to heat-shock genes upon heat shock (Knuckles et al., 2017). Multiple mechanisms have been revealed to promote chromosomal targeting of m6A machinery.

Nascent RNA and RNAPII

One contributing factor for chromosomal localization of m6A machinery is nascent RNA. The N-terminal CCCH zinc-finger domain in METTL3 is known to bind to RNA (Huang et al., 2019b). The nuclear m6A reader YTHDC1 can in turn be recruited to chromatin by m6A modification on nascent RNA. In addition, YTHDC1 can also bind to MTC, which may further increase MTC targeting to chromatin (Xu et al., 2021b). Besides hnRNA, m6A can occur on other nascent RNA species, including lncRNA, eRNA, and promoter antisense RNA, many of which may regulate gene activity *in cis*. Consistently, YTHDC1 can be recruited to chromatin by m6A on eRNA (Lee et al., 2021) and other non-coding RNA (Liu et al., 2020a; Yang et al., 2021).

Besides, MTC interacts with RNAPII (Slobodin et al., 2017; Huang et al., 2019a). Inhibiting transcription with α-amanitin dramatically decreases MTC localization on chromatin (Knuckles et al., 2017), indicating that RNAPII and nascent transcripts have a significant impact on targeting of MTC to chromatin.

Trans-acting regulatory RNA

Many regulatory RNAs function *in trans* (Liu et al., 2020a; Lee et al., 2021). Among them, lncRNA is one of the best-studied *trans*-acting regulatory RNAs with length >200 nucleotides. There are ∼20000 lncRNA genes in the human genome, giving rise to ∼30000 lncRNA transcripts (Ali and Grote, 2020). An important mechanism for lncRNA function is that lncRNAs can bind to chromatin and regulate gene transcription (Ali and Grote, 2020). Studies have found that hundreds to thousands of lncRNAs can be modified by m6A (Dominissini et al., 2012; Meyer et al., 2012; Zuo et al., 2021). Some m6A-modified lncRNAs have been shown to recruit YTHDC1 to chromatin at their target genes.

XIST is an lncRNA best known for its role in X-chromosome inactivation. XIST binds to X chromosome to be silenced and it is subject to m6A modification, which is recognized by YTHDC1, contributing to X-chromosome silencing (Patil et al., 2016).

Heat is an lncRNA identified recently in mice, which reins the stress response upon heat shock. Heat functions through directly binding to and inhibiting the heat-shock transcription factor HSF1. Interestingly, Heat is heavily modified by m6A and recruits YTHDC1 to chromatin for transcriptional silencing of heat-shock genes (Ji et al., 2021).

Transcript of retrotransposon LINE1 can function *in trans* as regulatory lncRNA during early embryonic development. LINE1 acts as an RNA scaffold that recruits Nucleolin and Kap1/Trim28 to regulate gene expression (Percharde et al., 2018). LINE1 is subject to m6A modification, which can be recognized by YTHDC1 in the nucleus. In turn, YTHDC1 regulates the LINE1–Nucleolin partnership as well as the chromatin recruitment of Kap1 (Chen et al., 2021).

Besides lncRNA, eRNA and promoter antisense upstream RNA (auRNA) are also important regulatory RNAs whose exact functional mechanisms remain elusive. eRNA and auRNA may function *in cis* or *in trans* (Li and Fu, 2019) and they can also recruit YTHDC1 to chromatin through m6A modification (Liu et al., 2020a; Lee et al., 2021).

Transcription factors

Several transcription factors are shown to interact with and recruit m6A machinery to chromatin.

METTL3 is found necessary for growth of acute myeloid leukemia cells. Mechanistically, METTL3 targets to hundreds of genomic loci in these cells. CEBPZ, a transcription factor, is necessary for METTL3 recruitment to seven gene targets (Barbieri et al., 2017). Interestingly, CEBPZ does not affect the mRNA level but the translation of its targets including SP1 as if regulating the m6A level of target genes is its major effect.

Another transcription factor that facilitates genomic targeting of METTL3 or METTL14 is NF-κB. Oncogene-induced senescence (OIS) is a pivotal mechanism for tumor suppression. OIS is associated with expression of inflammatory factors, known as senescence-associated secretory pathway (SASP). During RAS-induced OIS in human embryonic lung fibroblast IMR90 cells, METTL3 and METTL14 are found to relocate on the genome, where they interact with NF-κB and target NF-κB-regulated SASP genes. METTL3 mainly localizes to promoters whereas METTL14 mainly localizes to enhancers of these genes (Liu et al., 2021b). Interestingly, catalytically inactive METTL3 mimics the effect of the wild type, indicating that METTL3 and METTL14 function independently of m6A in this scenario.

Pathogens often hijack host mechanisms for their benefit. HBX, a critical virulent protein coded by the hepatitis B virus (HBV) genome, can interact with METTL3 and METTL14, increasing their recruitment to HBV minichromosome and m6A installation on HBV RNA. Besides, HBX promotes localization of METTL3 and METTL14 to promoters of host genes such as the tumor suppressor PTEN (Kim and Siddiqui, 2021), leading to destabilization of PTEN mRNA (Kim et al., 2021), which contributes to the pathogenesis related to HBV.

Besides the above transcription factors, SMAD2/3 downstream of TGFβ can interact with the METTL3/METTL14/WTAP complex (Bertero et al., 2018) and target mRNA of pluripotent genes for m6A-mediated degradation during human ES cell differentiation, although METTL3 targeting to chromatin was not detected in this case (Bertero et al., 2018).

Histone modifications and epigenetic machinery

Epigenetics studies the inheritable change in gene expression that is independent of DNA sequence change. Despite differences in the definition of epigenetics, in practice, DNA methylation and histone modifications have been the mainstay in epigenetic study (Cedar and Bergman, 2009). Just like m6A, histone modifications are also regulated by writers and erasers (Kooistra and Helin, 2012). Histone modifications, such as lysine methylation and acetylation, have pleiotropic effects on every process involving a genome, including transcription, DNA replication, and DNA damage repair (Suganuma and Workman, 2011). These modifications affect not only the local activity but also the higher-order structure of chromatin. One of the major mechanisms for histone modification functioning is through readers (Adams-Cioaba and Min, 2009). Recent studies have revealed exciting physical and functional interplay between epigenetic modifications/machinery and m6A/m6A machinery. For this section of the review, we will talk about one side of the story, i.e. histone modifications and epigenetic machinery can interact with m6A machinery and recruit it to chromatin.

Genomic studies have revealed correlation between m6A RNA modification and histone modifications. m6A tends to cohabit with some histone modifications, while being exclusive to others. ChIP–Seq peaks of METTL3 and METTL14 on chromatin in AML cells are enriched on promoters with bimodal H3K4me3 (Barbieri et al., 2017). In HepG2 cells, 69.2% of m6A peaks overlap with H3K36me3 (Huang et al., 2019a). Besides, m6A shows positive correlation with H4K20me1, H3K79me2, K3K9Ac, H3K4me1, H3K4me3, and H3K27Ac, while it correlates negatively with H2A.Z and H3K9me3 (Huang et al., 2019a). In addition, negative correlation between H3K9me2 and m6A on nascent RNA is reported in mES cells (Li et al., 2020). These studies generally show that m6A correlates positively with activating epigenetic markers, while it correlates negatively with repressive epigenetic markers in euchromatin regions. However, it is oversimplified to ascribe the reverse correlation between m6A and suppressive epigenetic markers to lack of transcription. First, m6A seems to preferentially correlate with some but not all active epigenetic markers. For example, a study on HepG2 cells shows correlation between m6A and H3K36me3 but not H3K27Ac (Huang et al., 2019a). Second, neither m6A nor epigenetic modifications distribute evenly in the same transcript or gene. Third, it was recently found that a certain level of transcription and m6A installation is actually necessary for efficient retrotransposon silencing and heterochromatin establishment (Liu et al., 2021a; Xu et al., 2021b).

What are the mechanisms underlying the coordination between m6A machinery and histone modifications? Several studies have revealed physical interaction between m6A machinery and epigenetic modifications/machinery (Table 2), which contributes to recruitment of m6A machinery to chromatin. H3K36me3 is known to be deposited in the gene body by SETD2 as RNAPII transcribes through the gene. Huang et al. (2019a) found genome-wide correlation between histone H3K36me3 and RNA m6A modification. Increasing H3K36me3

Table 2 Epigenetic modification/factors recruit m6A machinery to chromatin.

Interaction	Function	Reference
H3K36me3 and METTL14	Affect m6A deposition	Huang et al. (2019a)
KDM6B and METTL3/METTL14	Affect m6A deposition	Wu et al. (2020)
Spt6 and MTC	Affect m6A deposition	Akhtar et al. (2021)
TRIM28/SETDB1 and METTL3/YTHDC1	Promote YTHDC1 and METTL3 targeting	Xu et al. (2021b)

by overexpressing SETD2 can increase the m6A level, while decreasing H3K36me3 by overexpressing demethylase KDM4B has the opposite effect (Huang et al., 2019a). Mechanistically, METTL14, a core subunit of MTC, can directly bind to H3K36me3 (Huang et al., 2019a). In another study, Wu et al. (2020) observed a decrease in the global m6A level upon knocking down KDM6B, an H3K27me3 demethylase. Consistently, they found that m6A negatively correlates with H3K27me3 (Wu et al., 2020). Mechanistically, KDM6B can interact with METTL3 and METTL14 as detected by co-immunoprecipitation experiment, which implies that KDM6B may recruit MTC (Wu et al., 2020).

The mechanism for interplay between m6A and H3K9me3 at endogenous retrovirus elements (EREs) was identified recently in mES cells. ChIP–Seq studies found that METTL3, YTHDC1, and H3K9me3 are co-enriched at EREs (Chen et al., 2021; Liu et al., 2021a; Xu et al., 2021b). Such enrichment could have eluded earlier regular ChIP–Seq analysis pipelines, which typically focus on uniquely mapped reads. Xu et al. (2021b) showed that MTC colocalized with the histone H3K9me3 methyltransferase SETDB1 at EREs. Mechanistically, the interaction between METTL3/YTHDC1 and TRIM28/SETDB1 was identified. Abolishing SETDB1 decreases the targeting of METTL3 and YTHDC1 to EREs (Xu et al., 2021b), which supports that SETDB1 may facilitate recruitment of METTL3 and YTHDC1 to chromatin at EREs.

Besides histone-modifying enzymes and histone modifications, histone chaperones also play a key role in chromatin activity, which facilitates histone incorporation or removal from the nucleosome. Spt6, a histone chaperon in *Drosophila*, was shown to interact with MTC directly and recruit MTC to gene promoters (Akhtar et al., 2021). Consistently, Spt6 deletion decreases the m6A level.

m6A machinery regulates chromatin biology

For interplay between m6A machinery and chromatin, recruitment of m6A machinery by chromatin-bound RNA and protein factors is only one side of the story. m6A machinery can regulate chromatin modifications and activity. Recruitment of MTC to chromatin has a significant impact on m6A. Consistent with m6A installation being co-transcriptional, METTL3-bound gene loci have significantly increased the m6A level compared with those non-bound regions (Knuckles et al., 2017). Accordingly, chromatin-bound m6A machinery may regulate nascent mRNA fate or translation (Barbieri et al., 2017; Knuckles et al., 2017; Bertero et al., 2018; Huang et al., 2019a; Kim and Siddiqui, 2021). In addition, recruitment of YTHDC1 and MTC to chromatin also plays an important role in the function of nascent regulatory RNA such as eRNA (Liu et al., 2020a; Lee et al., 2021). Besides, recent studies show that m6A machinery on chromatin can also directly affect chromatin biology, such as gene transcription, DNA damage repair, and DNA recombination. Mechanistically, m6A machinery may facilitate the chromosomal recruitment of other proteins in these processes, including epigenetic factors, transcription factors, and damage repair proteins.

Regulation of transcription

m6A machinery on chromatin can either increase or decrease gene transcription. They may regulate transcription initiation as well as elongation (Akhtar et al., 2021) through different mechanisms. One mechanism is through nascent regulatory RNA. MTC may install m6A on nascent regulatory RNA, which could affect the fate and/or function of regulatory RNA and in turn regulate transcription (Lee et al., 2021; Xiong et al., 2021). Alternatively, m6A machinery may physically engage transcription-regulating proteins such as epigenetic machinery (Li et al., 2020; Chen et al., 2021; Lee et al., 2021; Liu et al., 2021a; Xu et al., 2021b), transcription factors (Liu et al., 2021b), and RNAPII (Liu et al., 2021b).

m6A machinery can promote transcription

m6A can promote transcription through regulating histone modifications. With a reporter construct, Li et al. (2020) showed that the m6A-containing reporter has a significantly decreased H3K9me2 level compared with the non-m6A counterpart. H3K9 dimethylation and trimethylation are well-known markers for gene repression. Li et al. (2020) performed m6A sequencing of nascent RNA in mES cells and found strong negative correlation between distributions of m6A and H3K9me2. Furthermore, the H3K9me2 level increased genome-widely in METTL3-mutant cells, while it decreased in mES and HEK293 cells upon loss of FTO. Mechanistically, YTHDC1, the nuclear m6A reader, can interact with and recruit KDM3B, a specific demethylase for H3K9. Artificial targeting of Cas13-YTHDC1 protein to nascent RNA led to local decrease in H3K9me2 (Li et al., 2020). This work showcases a paradigm where m6A on nascent mRNA can facilitate the change in chromatin and in turn increase transcription, which implicates an elegant model where gene status may be turned from inactive to active through a positive feedback involving m6A.

m6A may affect transcription elongation via RNAP-II. Akhtar et al. (2021) recently showed that recruitment of MTC promotes transcription elongation dependent on its m6A activity in *Drosophila*. Conversely, abolishing MTC increases promoter-proximal pause of RNAPII and inhibits transcription elongation.

Consistently, MTC-targeted genes statistically have higher expression than non-targeted genes (Akhtar et al., 2021). Moreover, Spt6, a histone chaperone regulating transcription elongation in *Drosophila*, can facilitate the recruitment of MTC. Spt6 interacts with Fl(2)d the homolog of mammalian WTA, a key component of MTC.

m6A machinery can promote transcription through m6A of eRNA (Li and Fu, 2019). eRNAs are typically short-lived. With a newly developed methodology termed MINT-seq, which metabolically labels nascent RNA for later capture (Lee et al., 2021; Xiong et al., 2021), Lee et al. (2021) identified abundant m6A on ∼20% of eRNAs in MCF7, HeLa, and K562 cells. They found that YTHDC1 is more commonly localized to the enhancer than the promoter, which is dependent on m6A of eRNA (Lee et al., 2021). m6A-modified eRNAs correlate with more active enhancers. Consistently, m6A can promote eRNA transcription but not stability, which is different from the conclusion of an earlier study (Liu et al., 2020a). A possible explanation for such contradiction is that nascent chromatin-associated RNA rather than steady-state RNA was isolated in this study. Lee et al. (2021) further examined the mechanism of eRNA m6A modification to promote transcription. They showed that YTHDC1 and eRNA form condensates partially overlapping with BRD4 condensates and contribute to BRD4 localization to the enhancer. In contrast, a YTHDC1 mutant lacking the phase-separating ability fails to recruit BRD4 or promote enhancer activity (Lee et al., 2021).

MTC components may also promote transcription independently of m6A. As mentioned earlier, Liu et al. (2021b) recently reported that METTL3 and METTL14 can target to promoters and enhancers of SASP genes during OIS induced by RAS oncogene in IMR90 cells, increasing the expression of SASP genes. Mechanistically, METTL3 facilitates NF-κB subunit p65 nuclear localization and targeting to SASP gene promoter. Consistently, cohabitation of METTL3 and NF-κB is seen at the promoter of SASP genes. Interestingly, this function of METTL3 is independent of its m6A catalytic activity, as an inactive METTL3 mutant can rescue the SASP gene expression pattern as well after the loss of endogenous METTL3 (Liu et al., 2021b). SASP has an important yet bipartite function during tumorigenesis, which is promoting neighboring cell growth *in vitro* and *in vivo* in immunocompromised mice while increasing the immunological eradication of the oncogene-expressing premalignant cells. Consistent with the role of METTL3/METTL14 in regulating SASP gene transcription, loss of METTL3 or METTL14 decreases both effects of OIS without affecting senescence itself (Liu et al., 2021b).

m6A machinery promotes gene silencing and heterochromatin formation

m6A can repress transcription through regulatory RNA. Liu et al. (2020a) isolated RNAs from chromatin fraction, referred to as chromatin-associated RNA (caRNA), many of which turned out to be regulatory RNA. They found that m6A decreases the stability of many caRNAs, which is dependent on YTHDC1. Conversely, the stability of these RNAs as well as global transcription is increased upon loss of METTL3 or YTHDC1. Consistently, loss of METTL3 or YTHDC1 increases the enrichment of H3K27Ac and P300, while decreasing the enrichment of JARID2, a component of the transcription-suppressing complex PRC2 (Liu et al., 2020a). LINE1 is among these caRNAs regulated by m6A modification. In mES cells, loss of METTL3 increases the LINE1 RNA level, which sabotages cell differentiation (Liu et al., 2020a). In addition, change in the m6A level of chromatin-associated regulatory RNA is also implicated in endometrial cancer progression (Liu et al., 2020a).

YTHDC1 may bind to m6A-modified lncRNAs and regulate their activity. Chen et al. (2021) found that YTHDC1 binds to the m6A-modified LINE1 transcript and is recruited to LINE1-targeted gene promoters. In turn, YTHDC1 is necessary for enrichment of TRIM28 and H3K9me3 at these regions (Chen et al., 2021). Loss of YTHDC1 in turn causes upregulation of dux, a protein critical for the 2C state during development. Consistently, loss of METTL3 or YTHDC1 causes transition of ES cells to a 2C-like state (Chen et al., 2021; Liu et al., 2021a). Besides LINE1, other lncRNAs can also be modified with m6A and recruit YTHDC1 to target chromatin, which contributes to the gene-regulatory function of lncRNA. XIST is a long-known lncRNA, which has a dominant role in initiating X-chromosome silencing in female mammals. XIST binds to the X chromosome to be silenced. In the past, XIST was shown to engage multiple histone-modifying enzymes to silence the X chromosome (Plath et al., 2003; Pintacuda et al., 2017). Consistently, depleting PRC2 or PRC1 abolishes normal X-chromosome silencing (Brockdorff, 2017). A recent study showed that XIST is modified with m6A (Patil et al., 2016) and the RNA-binding protein RBM15 is required to recruit MTC to XIST. Consequently, YTHDC1 is recruited to XIST and is necessary for X-chromosome silencing. To further corroborate the role of YTHDC1, artificially tethering YTHDC1 to XIST sufficiently rescues the role of XIST-m6A in X-chromosome silencing. Although it is not clear how YTHDC1 facilitates the silencing function of XIST, previous studies identified PRC1/2 and other histone-modifying enzymes as potential YTHDC1-interacting proteins (Patil et al., 2016). Besides, m6A machinery can also interact with SPEN, a transcriptional repressor binding to XIST (Dossin et al., 2020).

Heat is a lncRNA, which directly interacts with HSF1 and attenuates its transcriptional activity to tune down activation of the stress response during heat shock (Ji et al., 2021). Abolishing Heat promotes mouse embryonic fibroblast survival upon 45°C heat shock (Ji et al., 2021). Heat is modified with m6A, which then recruits YTHDC1 to stress-response gene promoters (Ji et al., 2021). Importantly, YTHDC1 is required for the transcription-silencing activity of Heat. Although the exact mechanism of how YTHDC1 facilitates gene repression is not shown, YTHDC1 can potentially interact with EZH2, a core component of the PRC2–histone H3K27 methyltransferase complex (Ji et al., 2021).

In addition, a recent study showed that m6A of non-coding RNA can influence the resolving of R-loop, promoting demethylation of H3K4 and local heterochromatin formation in *Arabidopsis*

Table 3 Interplay between m6A machinery and epigenetic machinery/histone modifications.

Interplay	Function	Reference
YTHDC1 and KDM3B	Affect global H3K9me2 deposition	Li et al. (2020)
YTHDC1 and BRD4	Affect global BRD4 targeting	Lee et al. (2021)
m6A and JARID2, H3K27Ac, P300	Affect JARID2, H3K27Ac, and P300 binding	Liu et al. (2020a)
m6A of eRNA and BRD4	Facilitate BRD4 condensates and gene activation; no effect on H3K27Ac	Lee et al. (2021)
YTHDC1 and SETDB1	Facilitate H3K9me3 at TEs and silence TEs	Liu et al. (2021a)
TRIM28/SETDB1 and METTL3/YTHDC1	Facilitate H3K9me3 at TEs, silence TEs, and promote YTHDC1 METTL3 targeting	Xu et al. (2021b)
YTHDC1/TRIM28	Facilitate H3K9me3 at TEs and silence TEs	Chen et al. (2021)
METTL3 and H3K4me3	METTL3 knockout increases H3K4me3 at TEs	Chelmicki et al. (2021)

(Xu et al., 2021a), which also promotes recovery from DNA damage.

m6A machinery can also repress transcription through interaction with epigenetic factors. Retrotransposons constitute a major part of the mammalian genome, presenting both a threat to genome stability and a driving force for genome evolution. Most retrotransposons have to be silenced in the inner cell mass during embryonic development for genome integrity. In the past, many epigenetic factors were reported to contribute to retrotransposon silencing, among which TRIM28/SETDB1-mediated H3K9me3 plays a key role (Matsui et al., 2010; Rowe et al., 2010). Interestingly, four recent studies found that METTL3/YTHDC1 could promote the silencing of endogenous retrotransposons (Chelmicki et al., 2021; Chen et al., 2021; Liu et al., 2021a; Xu et al., 2021b). Chelmicki et al. (2021) showed that loss of METTL3 increased H3K4me3 at retrotransposons. Besides, they found that METTL3 further promoted silencing of TEs through decreasing TE RNA stability. The other three studies showed that METTL3 and YTHDC1 localized to retrotransposon genomic loci with concordant enrichment of TRIM28/SETDB1 and H3K9me3, which decreased transcription of retrotransposons (Chen et al., 2021; Liu et al., 2021a; Xu et al., 2021b). Mechanistically, physical interaction between METTL3/YTHDC1 and SETDB1/TRIM28 was identified in all three studies (Chen et al., 2021; Liu et al., 2021a; Xu et al., 2021b). Furthermore, METTL3/YTHDC1 is necessary for efficient SETDB1 targeting and H3K9me3 installation at retrovirus elements.

Diverse effects of m6A machinery on transcription

To summarize, the effects of m6A machinery on transcription and chromatin activity are diverse, even opposite for different genomic regions. The underlying mechanisms could be 2-fold. First, the effects of m6A on different regulatory RNAs could be opposite, either promoting their production or decreasing their stability. Second, in different genomic regions, m6A machinery can facilitate the recruitment of either transcription-promoting or transcription-repressing proteins. As discussed in more detail at the end of this review, multivalency likely determines which specific complex is formed. Although it remains largely unknown how m6A machinery itself is differentially targeted to genomic regions, current evidence suggests that DNA sequence-specific regulatory RNA and transcription factors could play a role.

As shown above, interplay with epigenetic machinery or histone modifications plays a pivotal role in transcription regulation by m6A machinery (Table 3). In addition, m6A machinery also possesses biochemical activity on DNA (Woodcock et al., 2019; Yu et al., 2021), and methyl-histone readers may also recognize m6A (Baquero-Perez et al., 2019), although the physiological significance of these biochemical activities remains to be determined.

m6A machinery promotes DNA damage repair

DNA damage repair is critical for genome stability and cell survival, while inducing DNA damage is a mainstream strategy to kill cancer cells for therapeutic purpose. DNA damage repair begins with damage recognition and triggers profound changes in chromatin structure. A multitude of protein factors and protein posttranslational modifications are involved in this process, including histone modifications (Soria et al., 2012; Sulli et al., 2012). Recent studies show that m6A and m6A machinery are also critical for DNA damage repair, which gives novel insights into the DNA damage response.

Xiang et al. (2017) found significant increase of m6A in the nucleus within 2–4 min after ultraviolet A (UVA) or ultraviolet C (UVC) irradiation. This increase of m6A occurs earlier than accumulation of p-H2A.X, a hallmark of DNA damage, indicating that it is an early event during DNA damage response. Upon UVA microirradiation, the increase is shown to localize to the damage foci. Xiang et al. (2017) further showed that METTL3 and METTL14 but not WTAP were recruited to the damage foci. Interestingly, the m6A level decreases to the normal level ∼10 min after damage, consistent with the recruitment of FTO. It is not clear how UV causes recruitment of METTL3 to damage foci but it is shown to depend on PARP, an early-phase regulator of DNA damage response (Xiang et al., 2017). Functionally, loss of METTL3 decreases cell survival and recovery from UV radiation, indicating that transient increase in m6A is critical for UV-induced DNA damage repair. Mechanistically, METTL3 is required for DNA polymerase κ targeting to damaged foci. It remains unknown how m6A carries out such a function as no increased localization of the known m6A reader was identified at the UV-induced damage foci (Xiang et al., 2017).

In a more recent study, Zhang et al. (2020a) found that upon X-ray or zeocin-induced DNA double-strand break, METTL3 is also recruited to damage foci, increasing the local m6A

content. They found that after double-strand break, METTL3 Ser43 was phosphorylated by ATM, a master protein kinase in DNA damage response. Ser43 phosphorylation is necessary for METTL3 recruitment to DNA damage foci. They further showed that YTHDC1, the nuclear m6A reader, is also recruited to the damage foci (Zhang et al., 2020a), which is different from the case in UV radiation (Xiang et al., 2017). The different behavior of YTHDC1 in UV- versus X-ray-induced damage likely reflects different chromatin environments for these two types of DNA damage, including the protein machineries involved in sensing and repairing the damage. Another technical compounding factor is that m6A foci were detected under denaturing condition in Zhang et al. (2020a) in contrast to native condition in Xiang et al. (2017), which means that the two studies might be detecting different groups of m6A. Nevertheless, for double-strand break, increase of localized m6A is critical for the homologous recombination pathway of DNA damage repair, as targeting of RAD51 and BRCA1 to damage foci is lost upon METTL3 knockdown. Consistently, loss of METTL3 or YTHDC1 prolongs repair of DNA damage. Consistent with the role of m6A in DNA damage repair, Zhang et al. (2020a) showed that loss of METTL3 significantly increases the sensitivity of head and neck squamous cell carcinoma to a DNA-damaging agent, implying the therapeutic value of the METTL3 inhibitor.

Class-switch recombination in B cell

VDJ recombination and class-switch recombination underpin antibody diversity, which is key to immune function. Transcription and exosome processing of *cis*-acting non-coding RNA at the IgH regions are critical for class-switch recombination. Recently, Nair et al. (2021) showed that m6A machinery can be recruited to class recombination sites in B cells. METTL3 can install m6A on a non-coding RNA called SμGLT at the IgH region. m6A in turn recruits YTHDC1, which can interact with exosome cofactor MPP6. MPP6 and YTHDC1 in turn promote class-switch recombination by recruiting AID and the RNA exosome to process SμGLT (Nair et al., 2021). Consistently, loss of METTL3 or YTHDC1 inhibits physiological class-switch recombination and instead leads to genomic instability associated with unintended DNA recombination along with alternative DNA end joining (Nair et al., 2021).

Summary

Consistent with m6A modification being co-transcriptional, m6A machinery can be targeted to chromatin by nascent RNA, *trans*-acting regulatory RNA, or chromatin-binding proteins. m6A machinery on chromatin exerts pleiotropic effects on chromatin biology, including transcription, heterochromatin establishment, DNA damage repair, and DNA recombination. While the full mechanisms underlying these functions of m6A machinery merit future investigation, common paradigms are emerging. First, m6A machinery can install m6A on nascent RNA or *trans*-acting regulatory RNA, changing their structure, functionality, or metabolism. Accordingly, in most scenarios, the function of m6A machinery depends on their m6A-catalyzing or m6A-binding activity. Meanwhile, the biological outputs of m6A may depend on the specific RNA substrates. Second, m6A machinery can interact with and facilitate the chromosomal targeting of other proteins, including histone-modifying enzymes. Such a mechanism may be independent of m6A-related activities per se. In reality, these two mechanisms may work in synergy through a self-reinforcing loop as prototypically shown in the case of retrotransposon silencing (Chen et al., 2021; Liu et al., 2021a; Xu et al., 2021b).

m6A machinery on chromatin is instrumental for physiological processes such as ES cell differentiation (Bertero et al., 2018; Liu et al., 2020a, 2021a; Chen et al., 2021; Xu et al., 2021b), B cell development (Nair et al., 2021), DNA damage repair (Xiang et al., 2017; Zhang et al., 2020a), heat-shock response (Knuckles et al., 2017; Ji et al., 2021), and X-chromosome inactivation (Patil et al., 2016). m6A machinery on chromatin is also involved in human diseases, especially in cancer. For example, it is necessary for AML cell proliferation (Barbieri et al., 2017), oncogene-induced senescence (Liu et al., 2021b), liver oncogenesis (Kim et al., 2021), and head and neck squamous cell carcinoma sensitivity to chemotherapy (Zhang et al., 2020a). As illustrated in myeloid leukemia, m6A machinery represents a novel class of cancer therapeutic targets (Yankova et al., 2021).

Chromatin structure impacts every biological process involving DNA. Increasing groups of proteins and RNAs are revealed to localize to chromatin and regulate chromatin biology. Within a given chromatin region, DNA-binding proteins (e.g. transcription factors in transcription), regulatory RNA, RNA-binding proteins, histone modifications, and epigenetic machinery can all function together. In many scenarios, one may run into a chicken–egg problem trying to delineate the recruiting sequential with gene knockdown or knockout. Recent identification of the interplay between m6A machinery and other factors, including regulatory RNA, transcription factors, and chromatin modifications, further complicates the situation. Perhaps the recruitment of these factors does not follow a one-way chain of command. On the contrary, many factors likely simultaneously contribute to the recruitment of others. Within each subgenomic region, different modifications/factors interact with each other extensively. For example, it is well known that many of the epigenetic factors work in multiple-component complexes, which often contain different readers, writers, or erasers (Suganuma and Workman, 2011; Longbotham et al., 2020). Besides, many writers/erasers also contain reader domains and interact with regulatory RNA as well as transcription factors. Consistently, numerous intra-histone or *trans*-histone crosstalks are known (Winter and Fischle, 2010). Coordinations between DNA methylation and histone methylation are also recognized (Hashimoto et al., 2010). The recently identified coordination between m6A/m6A machinery and transcription factors as well as epigenetic modification further increased the complexity. Due to these extensive interplays, the combination of modifications/factors is not random in a genome region. Some modifications/factors are mutually exclusive and other modifications/factors tend to cluster, which

may be compared with an ecosystem. Within each ecosystem, one factor is usually under the influence of many other factors simultaneously. The coordination among all these factors contributes to chromatin biology.

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