Crosstalk between N6-methyladenosine modification and circular RNAs: current understanding and future directions

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Abstract
N6-methyladenosine (m6A) is a prevalent internal modification in eukaryotic RNAs regulated by the so-called “writers”, “erasers”, and “readers”. m6A has been demonstrated to exert critical molecular functions in modulating RNA maturation, localization, translation and metabolism, thus playing an essential role in cellular, developmental, and disease processes. Circular RNAs (circRNAs) are a class of non-coding RNAs with covalently closed single-stranded structures generated by back-splicing. CircRNAs also participate in physiological and pathological processes through unique mechanisms. Despite their discovery several years ago, m6A and circRNAs has drawn increased research interest due to advances in molecular biology techniques these years. Recently, several scholars have investigated the crosstalk between m6A and circRNAs. In this review, we provide an overview of the current knowledge of m6A and circRNAs, as well as summarize the crosstalk between these molecules based on existing research. In addition, we present some suggestions for future research perspectives.

Keywords: N6-methyladenosine, Circular RNA, Crosstalk

Background
RNA modifications (e.g., N6-methyladenosine [m6A], 5-methylcytosine, pseudouridine, N4-acetylcytidine, ribose methylations, and N1-methylguanosine), have recently emerged as vital post-transcriptional epigenetic modulators of gene expression in eukaryotes [1, 2]. Among these RNA modifications, m6A represents the most common and well-studied to date. m6A is a reversible modification that methylated adenosine at the N6 position of almost every type of RNA molecule, including mRNAs, small nuclear RNAs, ribosomal RNAs, and non-coding RNAs [1–3]. m6A was first discovered in the 1970s and developed rapidly during the past few years due to the advances in high-throughput m6A sequencing and methylated RNA m6A immunoprecipitation [4]. Moreover, m6A has been demonstrated to exert critical molecular functions in modulating RNA maturation, localization, translation, and metabolism. m6A dynamically exists and is involved in a variety of physiological and pathological processes, including growth, development, aging and diseases [4–6].

Circular RNAs (circRNAs) are a class of endogenous RNAs with covalently closed single-stranded structures also present in eukaryotes [7, 8]. Most circRNAs are non-coding RNAs while a proportion of cytoplasmic circRNAs have the coding potential to be translated into peptides [9, 10]. These molecules were also discovered several years ago, but has recently attracted the attention of researchers due to the advances in high-throughput RNA sequencing and bioinformatics [11].
Similar to other types of RNAs, circRNAs are involved in the maintenance of the normal physiological function of the human body, as well as the occurrence and development of a variety of human diseases [11–13]. While distinct from other RNA molecules, circRNAs possess unique biogenesis, biology, and characterization. Therefore, they may present peculiarities in response to RNA modifications.

Recently, some scholars have combined these two recent hot topics to investigate the crosstalk between them. In this review, we provide an overview of the current knowledge of m^6^A as well as circRNAs, and summarize the crosstalk between m^6^A modification and circular RNAs based on existing research. In addition, we have found that many questions still remain unanswered in this area and present some suggestions for future research perspectives.

**RNA m^6^A modification**

Similar to DNA methylation, RNA m^6^A methylation is catalyzed and recognized by corresponding enzymes, methyltransferases- “writers”, demethylases- “erasers” and “readers”. Subsequently, these modified RNAs will present with a different fate in maturation, localization, translation and metabolism, thereby influencing various molecular cellular processes. The specific details are described below and a summary is presented in Fig. 1.

**Participants of m^6^A modification: writers, erasers, and readers**

**m^6^A writers**

The m^6^A is installed by the multicomponent m^6^A methyltransferases complex (MTC), known as “writers”. The currently reported writers include methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14), methyltransferase-like 5 (METTL5), methyltransferase-like 16 (METTL16), Cbl proto-oncogene-like 1 (HAKAI), Wilms’ tumor 1-associating protein (WTAP), Vir Like M6A Methyltransferase Associated (VIRMA), RNA Binding Motif Protein 15/15B (RBM15/15B), Zinc Finger CCCH-Type Containing 4 (ZCCHC4), and Zinc Finger CCCH-Type Containing 13 (ZC3H13). These enzymes perform their respective duties and jointly complete the “writing” task. According to current knowledge,
METTL3, METTL5, and METTL16 function as catalytic cores in the complex which catalyze m6A modification via methyltransferase domains [3–5, 14–16]. Other components typically play auxiliary roles, such as structural stabilization, reorganization of special RNA sites, and directing MTC location [3–5, 14–16].

m6A readers
To exert their biological functions, the m6A modifications determined by m6A writers and erasers must be identified by m6A readers. The currently reported readers include the YT521-B homology (YTH) domain family proteins (YTHDF1, YTHDF2, and YTHDF3), YTH domain containing proteins (YTHDC1 and YTHDC2), heterogeneous nuclear ribonucleoprotein (HNRNPC, HNRNPG, and HNRNPA2B1), insulin-like growth factor 2 mRNA-binding proteins (IGF2BP1, IGF2BP2, IGF2BP3), eukaryotic translation initiation factor 3 (EIF3), proline rich coiled-coil 2A (PRRC2A), and staphylococcal nuclease and tudor domain containing 1 (SND1). These RNA binding proteins (RBPs) have conserved m6A-binding domains that can specifically recognize m6A modifications. RBPs bind to m6A methylated RNAs and determine the fate of these RNAs, thus regulating various cellular processes such as transcription, splicing and maturing, exportation, translation, decay and others [3–5, 14–16]. Therefore, the m6A readers represent intermediaries for RNA m6A modification and different RNA fates.

A more comprehensive summary than previous reviews on the classification and functions of m6A writers, erasers, and readers is presented in Table 1. It appears that compared with a simple m6A installation and elimination function of writers and erasers, the roles of readers are more complicated and diverse, which is an area of keen research interest.

Biological functions of m6A modification
The modulation of m6A methylation on RNAs begins during transcription and is largely dependent on the subcellular localization of writers, erasers, and readers. The writers are primarily localized in the nucleus, so the writing processes predominantly occur during the nuclear phase [14–18]. The eraser ALKBH5 mainly exists and functions as a demethylase in the nucleus, and the eraser FTO exerts demethylase activity both in nucleus and cytoplasm [14–18, 54]. Thus, the erasing processes may occur in the nucleus and cytoplasm. Some readers are localized and “read” m6A in the nucleus, which may influence nuclear processes, such as transcription and RNA splicing. In addition, some readers are able to assist with m6A-RNAs export from the nucleus to the cytoplasm. Readers in the cytoplasm may regulate cytosolic processes, such as translation and degradation.

m6A modulates RNA transcription, splicing, and structure
RNA m6A modification is a post-transcriptional regulation which appears not to be related to transcription; however, a recent study demonstrated that m6A modification on chromosome-associated regulatory RNAs (car-RNAs), including promoter-associated RNAs, enhancer RNAs, and repeat RNAs, can induce carRNA decay by YTHDC1 and impact the open chromatin state and downstream transcription [36]. Moreover, RNA m6A modification play a critical role in transcription termination by facilitating the formation of co-transcriptional R-loops to decrease the readthrough activity of Pol II [55]. Reports have confirmed that m6A modification on primary miRNAs (pri-miRNAs) promotes the recognition and processing by the microRNA microprocessor complex protein, DGC8, thereby enhancing miRNA maturation [45, 56]. The regulation of m6A on pre-mRNA splicing has been validated in Drosophila [57], whereas the precise regulation pattern remains largely unknown in mammals. Nevertheless, some efforts have been made to consummate the pathways through which m6A modulates pre-mRNA splicing in mammals. For example, the m6A reader, HNRNPG, may use Arg-Gly-Gly motifs to co-transcriptionally interact with RNA polymerase II and m6A-modified nascent pre-mRNA to modulate alternative splicing [44]. Additionally, YTHDC1 can recruit and promote pre-mRNA splicing factors to enter the binding regions of targeted mRNAs to modulate mRNA splicing [33]. In addition, the m6A modification on U2 and U6 snRNAs may influence the splicing of specific pre-mRNA transcripts [58, 59]. Evidence also shows that m6A can alter RNA structures to affect RNA-protein interactions in cells. For instance, m6A alters the local structure in mRNA and IncRNA and thereby influences the binding of HNRNPA2B1 to mediate pre-mRNA processing [42]. m6A located near splice sites in nascent pre-mRNA modulates HNRNPG binding, which influences RNAII occupancy patterns and promotes HNRNPG-mediated alternative splicing [43, 44].
m^6^A modulates RNA subcellular localization

Mature RNAs modified by m^6^A in the nucleus are recognized by readers, which subsequently mediate subcellular localization. In general, nuclear readers (e.g., YTHDC1 and HNRNPA2B1) can identify m^6^A-RNAs (e.g., mRNAs and circRNAs), and accelerate their exportation from the nucleus to the cytoplasm [34, 45, 60]. However, for some RNAs, m^6^A modification may detain them within the nucleus. For example, m^6^A modification of lncRNA RP11 can increase its accumulation in the nucleus and on chromatin, which may be due to its interaction with HNRNPA2B1 [61]. Interestingly, several RNAs without m^6^A modification can still be exported from the nucleus, indicating that the m^6^A is a facilitator but not an indispensable factor for translocation [14].

m^6^A modulates RNA stability, translation, and binding capacity

RNA exported to the cytoplasm may exert their biological functions or be degraded, and m^6^A modification can impact these processes via multiple cytoplasmic readers. Readers mediate the degradation of m^6^A-mRNAs, including YTHDF1, YTHDF2, YTHDF3, and YTHDC2, and readers enhance m^6^A-mRNA stability, including IGF2BPs, PRRC2A, and SND1 (Table 1) [29–32, 39–41, 47, 52, 53]. Moreover, these readers may regulate RNA stability through diverse mechanisms. For example, the carboxy-terminal domain of YTHDF2 selectively interacts with m^6^A-RNAs, whereas the amino-terminal domain mediates the transposition of the YTHDF2-mRNA complex from the translatable pool to mRNA decay sites (e.g., processing bodies) [62]. IGF2BPs

| Category          | Factors       | Roles                                                                 | Refs                  |
|-------------------|---------------|----------------------------------------------------------------------|-----------------------|
| Writers           | METTL3        | m^6^A catalytic subunit                                              | [14, 15]              |
|                   | METTL14       | Forms heterodimer with METTL3 to stabilize METTL3 and assist recognizing the substrate | [14, 18]              |
|                   | METTL16       | m^6^A catalytic subunit                                              | [19]                  |
|                   | METTL5        | Ribosome 18S m^6^A methyltransferase                                  | [20, 21]              |
|                   | TRMT112       | Forms heterodimeric complex with METTL5 as a methyltransferase activator to stabilize METTL5 | [21]                  |
|                   | ZCCHC4        | Ribosome 28S m^6^A methyltransferase                                  | [22, 23]              |
|                   | HAKAI         | Essential member of the MTC                                          | [24]                  |
|                   | WTAP          | Promote m^6^A methyltransferase activity and localization in nuclear speckles | [25]                  |
|                   | VIRMA         | Binds the MTC and recruit it to specific RNA region                  | [26]                  |
|                   | RBM15/15B     | Binds the MTC and recruit it to specific RNA site                    | [27]                  |
|                   | ZC3H13        | Promotes nuclear localization of MTC to modulate m^6^A in the nucleus | [28]                  |
| Erasers           | FTO           | Eliminates m^6^A by oxidation                                         | [14–18]               |
|                   | ALKBH5        | Eliminates m^6^A by oxidation                                         | [14–18]               |
| Readers           | YTHDF1        | Facilitates the ribosome assembly of m^6^A-mRNAs and interacts with the initiation factor to promote translation; cooperates with YTHDF2 and YTHDF3 to mediate degradation of m^6^A-mRNAs | [29, 30]              |
|                   | YTHDF2        | Reduces m^6^A-mRNAs stability; stabilize m^6^A-mRNAs specifically in cancer stem cells; cooperates with YTHDF1 and YTHDF3 to mediate degradation of m^6^A-mRNAs | [29–31]              |
|                   | YTHDF3        | Cooperates with YTHDF1 and YTHDF2 to mediate degradation of m^6^A-mRNAs | [30, 32]              |
|                   | YTHDC1        | Promotes RNA splicing and translocation; facilitates the decay of m^6^A-modified chromosome-associated regulatory RNAs; together with its target m^6^A-RNAs to regulate chromatin modification and retrotransposon repression; regulate histone methylation | [33–38]              |
|                   | YTHDC2        | Facilitates the translation and decrease the abundance of m^6^A-RNAs; has 3’-5’ RNA helicase activity and decrease the stability of m^6^A-mRNAs | [39–41]              |
|                   | HNRNPC/G      | Responsible for pre-mRNA processing and affect the alternative splicing of target m^6^A-mRNAs | [42–44]              |
|                   | HNRNPA2B1     | Binds to m^6^A-containing pri-miRNAs to promote pri-miRNA processing; may regulate mRNA splicing by binding to m^6^A-containing pre-miRNAs; facilitates m^6^A modification and nucleocytoplasmic trafficking of miRNAs | [45, 46]              |
|                   | IGF2BP1/2/3   | Regulates m^6^A-RNAs stability, subcellular localization and translation | [47–49]              |
|                   | EIF3          | Facilitates translation of m^6^A-mRNAs by recruiting the 43S complex | [50, 51]              |
|                   | PRRC2A        | Enhances m^6^A-mRNAs stability                                       | [52]                  |
|                   | SND1          | Enhances m^6^A-RNAs stability                                        | [53]                  |
probably recruit RNA stabilizers, such as ELAV like RNA binding protein 1 (ELAVL1 or HuR), matrin 3 (MATR3), and poly(A) binding protein cytoplasmic 1 (PABPC1), to maintain the stability of their target m6A-RNAs [47]. Numerous studies have demonstrated that m6A can regulate translation with the assistance of readers, including YTHDF1, YTHDF2, YTHDF3, YTHDC2, IGF2BPs, and EIF3 [29–32, 39–41, 47–49], which involves several distinct mechanisms. For example, YTHDF1 can facilitate the ribosome assembly of m6A-mRNAs and interact with the initiation factor to promote translation [29]. In the absence of the cap-binding factor eIF4E, EIF3 can directly bind to the m6A in the 5’ untranslated region (UTR) and recruit the 43S complex to initiate translation [50]. METTL3 directly binds to the eukaryotic translation initiation factor 3 subunit h (eIF3h) and presumably promotes translation through ribosome recycling [63]. Promoter-bound METTL3 induces m6A in the coding region of mRNA to enhance translation by relieving ribosome stalling [64]. Moreover, m6A on 18S and 28S ribosomal RNA also play critical roles in the maintenance of ribosomal translation dynamics [20, 22]. Apart from the influence of the RNA-RBP interaction described above, m6A may also be indispensable for some RNA-RNA interactions. For example, the sufficient enrichment of the m6A modification on linc1281 is required for the interaction between linc1281 with miRNAs [65], and the m6A modified 353–357 region in the YAP 3’UTR was found to be critical for miR-582-3p targeting [66].

CircRNAs

In contrast to other RNA molecules, circRNAs have a unique circular structure, which requires a unique biogenesis process. Moreover, this stable structure may endow them with distinctive cellular functions, as well as unique approach to degradation. The associated details are described below and a summary is presented in Fig. 2.

Biogenesis of circRNAs

Similar to mRNA maturation, the biogenesis of circRNAs should also undergo processes, including the transcription and splicing of pre-mRNAs [7, 8, 11]. Therefore, the factors that regulate transcription (e.g., epigenetic modifications and transcription factors) may influence the generation of circRNAs [7, 8, 11]. Distinguished from the canonical splicing of mRNAs, the alternative splicing of circRNAs represents a unique mode that competes with mRNA splicing. Instead of the 5’-capping, 3’-polyadenylation and introns removing events of mRNA maturation, a downstream 5’-splice site and a 3’-splice site are connected to form a covalently closed single-stranded structure in circRNA splicing [7, 8, 11–13]. There are several acknowledged mechanisms that can be used to interpret the circularization of circRNAs [7, 13, 67]. The first is intron pairing-driven circularization, in which the flanking of inverted repeat elements form RNA double strands through base-pairing. Another model is RBP-mediated circularization, in which the RBPs bind to the upstream and downstream flanking introns to form dimers. The third is lariat-driven circularization, which is mediated by the lariat structures that form in the exon-skipping events during linear splicing or intronic lariats that escape from the debranching of canonical linear splicing. These regulatory modes serve to bring the downstream splice-donor sites into close proximity with the upstream splice-acceptor site subject to alternative splicing. Based on the involved splicing and the genomic elements, three types of circRNAs are generated: 1) exonic circRNAs (EcRNAs), which are composed by one or more exons; 2) exon-intron circRNAs (EicRNAs) that contain both exon and intron components; and 3) intronic circRNAs (ciRNAs), which consist of only introns [11, 68, 69].

Exportation and distribution of circRNAs

As described above, the biogenesis of circRNAs occurs in the nucleus, and are then exported to the cytoplasm or detained in the nucleus. In general, most circRNAs are exported into the cytoplasm and the vast majority of cytoplasmic circRNAs are EcRNAs without introns [7, 12, 13]. Similar to many linear RNAs, circRNAs involving intron elements (e.g., EicRNAs and ciRNAs) are usually sequestered in the nucleus [7, 12, 13]. There are some underlying mechanisms may interpret the exportation and distribution of circRNAs. The first and the most recently concerned is m6A-mediated circRNA translocation, which will be discussed in detail below. Another mechanism is the length-dependent evolutionarily conserved pathway which involves the association of circRNA lengths with the conserved proteins, UAP56 and URH49 [70]. In addition, since circRNAs are also enriched and stable in exosomes, they also widely exist in extracellular components [71, 72].

Biological functions of circRNAs

Based on the unique characteristics and distribution, circRNAs may exert various biological functions. circRNAs enriched in the nucleus are more likely to modulate transcription and splicing and several underlying mechanisms have been reported. For instance, circSCMH1 may interact with transcription factor methyl CpG binding protein 2 (MeCP2) to restrain its transcriptional activity [73]. circMRPS35 can recruit the histone acetyltransferase, KAT7, to elicit the acetylation of H4K5 in the promoters and directly bind to the
promoters of FOXO1 and FOXO3a genes to activate the transcription [74]. CircRNAs derived from exon 6 of the SEP3 gene in Arabidopsis can bind to its cognate DNA locus to form an RNA:DNA hybrid, pausing transcription and exon 6 skipping in the alternative splicing of SEP3 pre-mRNA [75].

Compared with nuclear circRNAs, cytoplasmic circRNAs are better acquainted. The most frequently reported function of circRNAs is their capacity to act as miRNA sponges. Such sponging refers to the manner by which circRNAs impair miRNA activity through sequestration in a competing endogenous RNA (ceRNA) manner, thereby raising the expression of miRNA target genes [76, 77]. Compared with this explicit inhibitory role on miRNA, circRNAs exhibit diverse binding effects on various proteins [78–81]. For example, circRNAs may not only recruit RBPs to stabilize and translate mRNAs, but also competitively bind to these RBPs to inhibit translation and degradation [62, 82, 83]. In addition, the interaction between RBPs and circRNAs may also influence the functionality and induce degradation through the ubiquitination of RBPs [84, 85].

CircRNAs are once considered as non-coding RNAs, however, recent studies have demonstrated that some cytoplasmic circRNAs carrying an initiation codon and putative open reading frames can be translated into peptides. Although lacking the traditional initiation elements (e.g., 5′ and 3′ untranslated regions), circRNAs carrying an internal ribosome entry site (IRES) may undergo translation in a cap-independent manner [9, 86]. In addition, some circRNAs possess m6A and translation initiation sites may also go through m6A-driven translation with the assistance of the initiation factor, eIF4G2, and m6A reader, YTHDF3 [87]. Due to the same ORF components, several peptides translated by circRNAs are closely related to the proteins translated by their corresponding mRNAs. These peptides may act as substitutes to protect intact proteins from degradation.
or function as competitors to compete for regulators with intact proteins [88–90]. As proteins, while they may also play other functions [91–93], there are few relevant published studies, and further explorations remain to be performed.

Based on abundance and stability, circRNAs located in exosomes can be detected in the circulation and urine. Accumulating studies have confirmed that the circRNA content in the exosomes of some diseases is anomalous, indicating that they are promising diagnostic molecular markers [71, 72]. It is also feasible that cells transfer circRNAs to other cells or even throughout the body via excretion in exosomes. Therefore, they may act as mediators to ensure natural cell-to-cell communications. Besides, exosomes may bring abnormal amount of circRNAs to target cells, which is also an important source of various pathophysiological processes [71, 72].

**Degradation of circRNAs**

Although the structure is highly stable and are resistant to exonucleases [7, 8, 11], they will eventually be degraded through the involvement of several unique and diverse degradation pathways. The binding of miRNAs to circRNAs can initiate the Argonaute 2 (Ago2)-mediated RNA decay, which is executed by the RNA-induced silencing complex (RISC) [94]. However, this phenomenon may not be as common as expected since similar to linear RNAs, the overwhelming majority of circRNAs bear sequences that are only partially complementary to miRNAs [13]. CircRNAs modified by m^6^A may be decayed by the ribonuclease complex RNase P/MRP, which will be discussed in detail below. In addition, there is a structure-mediated RNA decay model (e.g., high overall 3′ UTR structure) formed by base pairing in circRNAs that can be targeted and degraded by UPF1 and G3BP1 [95]. Upon viral infection, circRNAs can also be globally degraded by activated RNase L, which is required for PKR activation [96]. Actually, the above-mentioned degradation pathways are also suitable for some other RNA molecules and not unique to circRNAs.

**Crosstalk between m^6^A and circular RNAs**

Through the above summary, we can find that there are many intersections between the regulatory pathway of m^6^A and the life cycle of circRNAs. Indeed, there have been many studies focusing on the crosstalk between m^6^A and circRNAs. Details are described below and a summary is shown in Table 2 and Fig. 3.

**m^6^A modulates the expression of circRNAs**

Similar to other RNA molecules, m^6^A can also modulate the expression of circRNAs through regulating their generation, stability, or degradation. A recent study

| Table 2 Crosstalk between m^6^A and circRNAs |
|-----------------|-----------------|-----------------|-----------------|
| Crosstalk       | circRNA         | Roles                        | Refs            |
| m^6^A regulates circRNAs expression | circMETTL3      | METTL3 facilitates circMETTL3 expression in an m^6^A-dependent manner | [97] |
|                 | circ1662        | METTL3 induced circ1662 generation by binding its flanking sequences and installing m^6^A modifications | [98] |
|                 | circCUX1        | METTL3 mediates the m^6^A methylation of circCUX1 and stabilizes circCUX1 | [99] |
|                 | circRNA-SORE    | m^6^A modification raises circRNA-SORE level by increasing RNA stability | [100] |
|                 | circRNAs        | m^6^A modification cause circRNAs selectively degraded by RNase P/MPR complex | [101] |
| m^6^A regulates circRNAs distribution | circGFRa1       | METTL14 promotes cytoplasmic export of m^6^A-modified circGFRa1 through the GGACU motif | [102] |
|                 | circNSUN2       | m^6^A modification of circNSUN2 facilitates cytoplasmic export | [60] |
| m^6^A regulates circRNAs function | circRNAs        | Extensive m^6^A modifications in circRNAs drives protein translation in a cap-independent fashion | [87] |
|                 | circRNAs        | m^6^A modification controls circRNA immunity | [103] |
| circRNAs regulate m^6^A | hsa_circ_0072309 | hsa_circ_0072309 upregulates the expression of m^6^A demethylose FTO by targeting miR-607 | [104] |
|                 | circMAP2K4      | circMAP2K4 promote YTHDF1 expression by binding with hsa-miR-139-5p | [105] |
|                 | circRAB11FFP1   | circRAB11FFP1 regulated the m^6^A methylation of ATG5 and ATG7 mRNA via upregulating FTO | [106] |
|                 | circMEG3        | circMEG3 inhibits the expression of METTL3 dependent on HULC | [107] |
|                 | circNOTCH1      | circNOTCH1 regulates the m^6^A modification on Notch1 mRNA by binding to METTL14 | [108] |
|                 | circZbtb20      | circZbtb20 enhances the interaction of ALKBH5 with Nru4a1 mRNA, leading to ablation of the m^6^A on Nru4a1 mRNA | [109] |
|                 | circSTAG1       | circSTAG1 regulates m^6^A modification on FAAH by mediating ALKBH5 translocation | [110] |
confirmed that METTL3 can install m6A in the reverse complementary sequences of flanking introns of circ1662, as well as facilitate the generation of circ1662 based on the intron pairing-driven circularization pattern [98]. Moreover, circRNAs modified by m6A can be recognized by readers and exhibit changes in stability, resulting in altered expression [99, 100]. A subset of m6A-containing circRNAs may be endoribonucleolytically degraded by the RNase P/MRP complex, which depends on the cooperative binding of HRSP12 and YTHDF2 [101]. The dynamic balance or the imbalance of circRNA expression is a consequence of the combined effect of these regulatory factors.

m6A modulates the distribution of circRNAs
Some studies have demonstrated that the m6A modification on circRNAs may modulate their nuclear exportation [60, 102]. This process may depend on the recognition and mediation of m6A readers [60]. Despite these initial findings, the mechanisms underlying the subcellular trafficking of m6A-circRNAs remains largely unknown.

m6A modulates the function of circRNAs
As described above, although considered to be non-coding RNAs, some circRNAs have the potential to encode proteins. Due to their unique structure, the translation of m6A modified circRNAs also differs from that of the linear RNAs. In this process, m6A-circRNAs are identified by the reader, YTHDF3, which recruits the translation initiation factors, eIF4G2 and eIF3A, to initiate translation in a cap-independent manner [87]. The m6A modification also influences the function of circRNAs in the regulation of innate immunity. Unmodified foreign circRNAs can directly trigger RIG-I signaling to promote immune activation; however, m6A-circRNAs may recruit YTHDF2 to form a complex with RIG-I and suppress the RIG-I immune signaling [103].
Conclusions and perspectives

m6A modification and circRNA biology are undoubtedly current research hotspots, and the crosstalk between the two has attracted increasing attention from the researchers. In this review, we describe the complexity of m6A modification and circRNA biology, and present the identified crosstalk between them. Although some efforts have been devoted in this field, the study of correlation between m6A and circRNAs remains in the initial stages. In consideration of the current research realities, lots of questions remain to be addressed. In reference to the crosstalk mentioned in the previous section, we will present some perspectives which may represent potential future hot topics. These perspectives are also displayed in Fig. 3.

First, previous studies have only reported that m6A in the reverse complementary sequences of flanking introns facilitates the generation of circRNA through the intron pairing-driven circularization pattern [98]. However, other studies have verified that the m6A modification in pre-mRNA may regulate RNA-protein interactions and pre-mRNA processing [42–44]. This regulatory mode has been investigated in mRNA maturation but not in circRNAs biogenesis. Here, we propose that the m6A modification in pre-mRNA may affect the binding of some RBPs and regulate the generation of circRNA via RBP-mediated circularization. Whether m6A modification can modulate the lariat-driven circularization of circRNAs is also a topic worth examining.

Second, although it has been confirmed that m6A can regulate the stability of circRNAs [99, 100], in consideration of the specific circular structure and degradation pathway of circRNAs, is there any difference between circRNAs and linear RNAs in the mechanism of m6A regulating stability? Similarly, does the regulation of m6A on circRNAs exportation differ from its regulation on linear RNAs? In addition, studies have revealed that m6A may modulate the degradation of circRNAs [101]; however, whether this mode can participate in the normal life process or disease development has not yet been explored.

Third, while m6A are important initiators in the translation of circRNAs [88], it is unknown whether they are translation sustainers or terminators. Since m6A is able to affect RNA-RBP interactions [47], it may also influence the binding of RBPs to circRNAs. Similarly, the m6A modification on IncRNA is important for the binding of miRNAs [66], and there is an excellent probability that the m6A modification on circRNAs affects miRNA binding. Due to the recent research focus on miRNA sponging, potential m6A-mediated miRNA-circRNA interactions may represent another area of research interest.

Fourth, circRNAs can contend the modification of m6A writers, which may also apply to erasers and readers. In addition, while circRNAs can recruit m6A erasers to ablate m6A modifications, it remains unknown whether they can also recruit m6A writers and readers to install and identify the m6A modifications.
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