Trends in Genetics

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REVIEWS

Review

Molecular Mechanisms Driving mRNA Degradation by m^{6}A Modification

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N^6-Methyladenosine (m^{6}A), the most prevalent internal modification associated with eukaryotic mRNAs, influences many steps of mRNA metabolism, including splicing, export, and translation, as well as stability. Recent studies have revealed that m^{6}A-containing mRNAs undergo one of two distinct pathways of rapid degradation: deadenylation via the YT521-B homology (YTH) domain-containing family protein 2 (YTHDF2; an m^{6}A reader protein)–CCR4/NOT (deadenylase) complex or endoribonucleolytic cleavage by the YTHDF2–HRSP12–ribonuclease (RNase) P/mitochondrial RNA-processing (MRP) (endoribonuclease) complex. Some m^{6}A-containing circular RNAs (circRNAs) are also subject to endoribonucleolytic cleavage by YTHDF2–HRSP12–RNase P/MRP. Here, we highlight recent progress on the molecular mechanisms underlying rapid mRNA degradation via m^{6}A and describe our current understanding of the dynamic regulation of m^{6}A-mediated mRNA decay through the crosstalk between m^{6}A (or YTHDF2) and other cellular factors.

Highlights

N^6-Methyladenosine (m^{6}A) as an mRNA modification plays multiple roles in various steps/characteristics of mRNA processing and metabolism, such as splicing, export, translation, and stability.

YTHDF2 preferentially recognizes m^{6}A and recruits RNA-degrading enzymes or adaptor proteins to trigger rapid degradation of the m^{6}A-containing mRNA.

Depending on the presence of HRSP12-binding sites in m^{6}A-containing mRNAs, YTHDF2 elicits one of two RNA decay pathways: deadenylation by the YTHDF2–CCR4/NOT deadenylase complex or endoribonucleolytic cleavage via the YTHDF2–HRSP12–RNase P/MRP complex.

The stability of m^{6}A-containing mRNAs is regulated by the dynamic crosstalk between m^{6}A and other cellular factors, such as RNA-binding proteins, RNA structures, and/or other types of modification.

RNA Modification: An Emerging Layer of Post-Transcriptional Gene Regulation

Many recent studies point to the role of RNA modification as a mode of post-transcriptional gene regulation and this field has been termed 'epitranscriptomics' [1–4]. To date, approximately 150 post-transcriptional modifications have been associated with various RNA species, including mRNAs, tRNAs, rRNAs, noncoding RNAs (ncRNAs), and viral RNA genomes [5–7]. In this review, we summarize recent reports on m^{6}A deposition and function. In particular, we discuss recent findings regarding how m^{6}A contributes to mRNA stability at the molecular level.

Features and Dynamics of the m^{6}A Modification

Although first discovered in the 1970s, m^{6}A modification recently returned to the spotlight with the development of RNA-seq techniques and the characterization of the proteins involved in the m^{6}A modification [4,8]. This modification is found in mRNA expressed in various mammalian cell types including blood, muscle, liver, intestinal, and neuronal cells. At the molecular level, the m^{6}A modification functions at almost all stages of the mRNA life cycle, including splicing, export, and translation, and regulates mRNA stability (Figure 1). The m^{6}A modification has also been implicated in a variety of cellular and physiological events including spermatogenesis [9], embryogenesis [10], cortical neurogenesis [11], and carcinogenesis [12–14]. As the most prevalent internal mRNA modification, approximately 25% of cellular mRNAs harbor one or more m^{6}A bases [4,8]. In general, the m^{6}A modification is enriched around translation stop codons and in the 3′ untranslated region (UTR) [4,8,15], although this varies among different mRNAs.

Accumulating evidence indicates that the m^{6}A RNA modification is a dynamic and reversible event (Figure 1). The coordinated action of methyltransferases (m^{6}A writers) and demethylases (m^{6}A erasers) contributes to the deposition and depletion of this modification. **Methyltransferase-like protein 3 (METTL3)** (see Glossary), also known as MT-A70, and **METTL14** function as a catalytic core complex known as the **m^{6}A–METTL complex (MAC)**. This complex recognizes the DRACH motif (where D = A, G, or U; R = purine; and H = A, C, or U)

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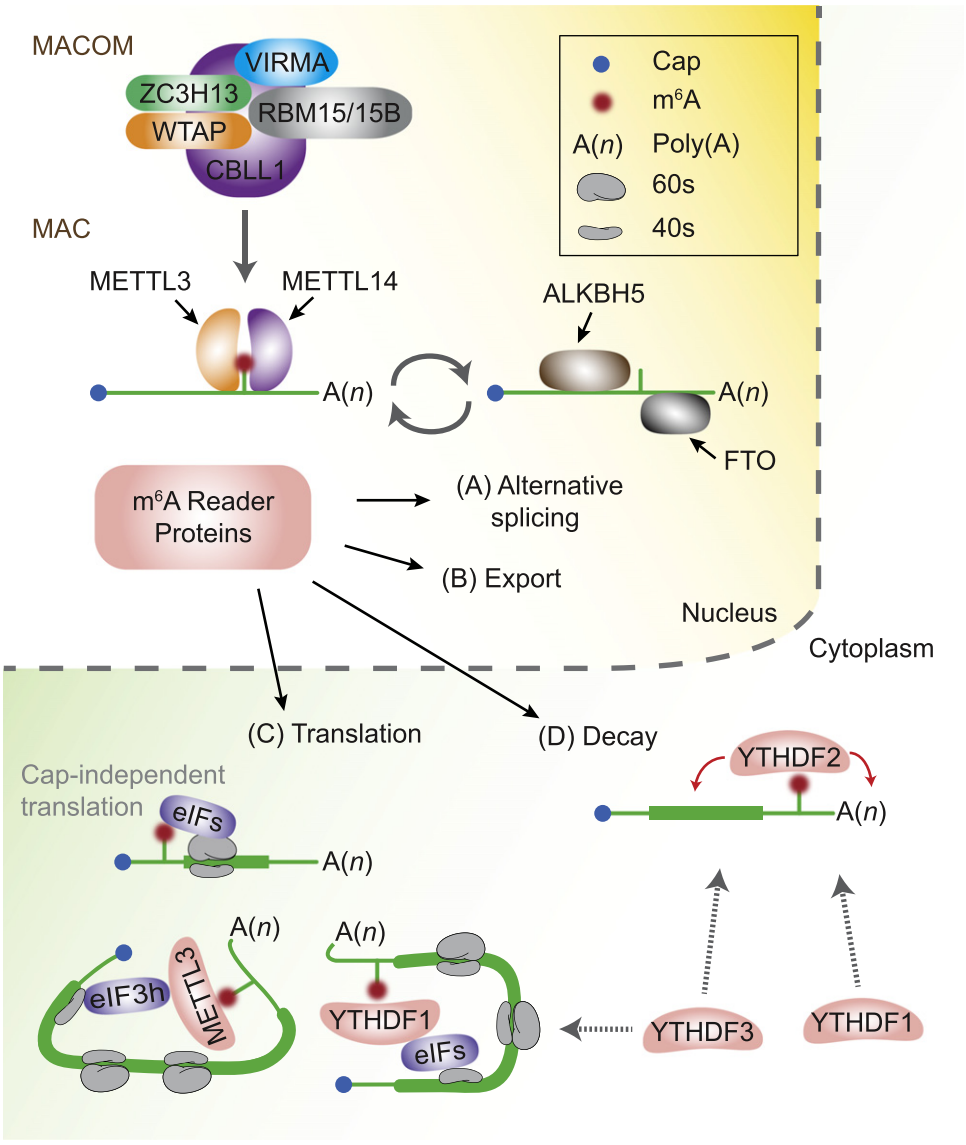

Figure 1. An Overview of N^6-Methyladenosine (m^6A) Functions. m^6A modification is installed in the nucleus by the methyltransferase-like protein (METTL)3–METTL14 core complex, known as the MAC. A regulatory complex known as MACOM (comprising WTAP, ZC3H13, VIRMA, and WTAP) assists the MAC, localizing it to specific regions of nascent transcripts. Various m^6A-specific reader proteins determine the fate of m^6A-containing mRNAs; for example, alternative splicing (A), export (B), translation (C), or decay (D). Abbreviation: FTO, fat mass and obesity-associated protein.

and introduces m^6A into nascent transcripts [4,8,15]. Notably, METTL3 has catalytic activity, whereas METTL14 forms a heterodimer with METTL3 and contributes to the binding of the complex to target RNA [16–18].

The methylation activity of MAC functions in conjunction with a regulatory protein complex – the **m^6A–METTL-associated complex (MACOM)** – comprising Wilms tumor 1-associated protein (WTAP) (also known as female-lethal[2]d), RNA-binding motif 15 (RBM15), Vir-like m^6A methyltransferase-associated (VIRMA) (also known as Virilizer or KIAA1429), Cbl proto-

Glossary

α-Ketoglutarate-dependent dioxygenase alk B homolog 5 (ALKBH5): mRNA demethylase that removes the methyl group from m^6A.
Adenylate- and uridylate-rich element (ARE)-mediated mRNA decay: molecular mechanism eliciting rapid degradation of mRNAs containing AREs in their 3′UTR. The degradation efficiency is regulated by stabilizing factors (e.g., HuR) and destabilizing factors (e.g., TTP, BRF1/2).
Endoribonuclease: enzyme that cleaves the phosphodiester bond in a polynucleotide chain of either single-stranded or double-stranded RNA.
Exoribonuclease: enzyme that cleaves nucleotides at either the 5′ or the 3′ end of a polynucleotide chain.
Exosome: multiprotein complex with a 3′-to-5′ exoribonuclease activity that catalyzes the degradation of various types of RNA.
Fat mass and obesity-associated protein (FTO): mRNA demethylase that removes the methyl group from m^6A. Additionally, FTO demethylates m^6A_m at the first position after the 5′ cap.
Fragile X mental retardation protein (FMRP): protein that is essential for cognitive development and related to fragile X syndrome and Parkinson's disease. FMRP is also a recently identified context-dependent m^6A reader that stabilizes m^6A-containing mRNAs.
Human antigen R (HuR) or ELAV-like protein 1: RBP that binds to U-rich regions and is known to stabilize its target mRNAs.
m^6A–METTL-associated complex (MACOM): complex comprising WTAP, RBM15, VIRMA, CBLL1, and ZC3H13. One or more of these factors interacts with the MAC to direct methylation at specific sites.
m^6A–METTL complex (MAC): m^6A mRNA bound by the METTL3–14 heterodimer.
Methyltransferase-like 3 (METTL3): catalytically active part of the methyltransferase complex. METTL3 is also known as MTA-70, MTA, or IME4.
Methyltransferase-like 14 (METTL14): component of the m^6A methyltransferase complex; appears to be catalytically inactive, but supports the methyltransferase activity of METTL3.
miRNA: small ncRNA ~22 nucleotides in length that functions in post-transcriptional gene silencing. A miRNA

oncogene-like protein 1 (CBLL1) (also known as Hakai), and zinc-finger CCCH-type-containing 13 (ZC3H13) [19]. Although the MACOM itself lacks methyltransferase activity, the coordinated interaction of its components with the MAC promotes the localization of the MAC to specific RNA sites for m^{6}A modification. RBM15 and its paralog RBM15B interact with METTL3 in a WTAP-dependent manner and preferentially bind to U-rich sequences near m^{6}A sites [20,21]. As a result, RBM15 and/or RBM15B recruit the MAC–WTAP complex to sites proximal to m^{6}A consensus motifs. It has been suggested that VIRMA preferentially mediates the m^{6}A modification near stop codons and participates in alternative polyadenylation through its association with the polyadenylation cleavage factor CFIm (a tetramer complex of CPSF5 and CPSF6) in an RNA-dependent manner [22]. Depletion of VIRMA or METTL3 induces 3′UTR lengthening, with a reduced amount of m^{6}A modification. By contrast, depletion of CPSF5 leads to shortening of the 3′UTR, with an increased abundance of the m^{6}A modification in the 3′UTR, near stop codons. Considering that stop codons are defined in the cytoplasm, whereas 3′UTR lengthening occurs in the nucleus, the molecular details underlying the VIRMA-mediated m^{6}A modification near stop codons should be investigated in future studies. ZC3H13 is required for the nuclear localization of the ZC3H13–WTAP–VIRMA–CBLL1 complex in mouse embryonic stem cells [23]. ZC3H13 also serves as an adapter protein between WTAP and RBM15, to enable efficient methylation [21].

It is now well established that m^{6}A is installed cotranscriptionally on nascent transcripts [12,24–26]. CCAAT/enhancer-binding protein zeta (CEBPZ) binds to a transcription start site and recruits METTL3 to the promoter region independent of METTL14, thereby inducing the m^{6}A modification in the protein-coding region of the associated transcripts [12]. It is also known that METTL3 is recruited to chromatin in a transcription-dependent manner and cotranscriptionally methylates nascent transcripts [24]. In particular, a recent report showed that the cotranscriptional conversion of A bases into m^{6}As depends on the activity of RNA polymerase II. A low rate of transcriptional elongation leads to a greater number of m^{6}A bases throughout the nascent transcript [26]. Furthermore, it is known that the majority of m^{6}As are formed in exon sequences in chromatin-associated nascent transcripts during transcription [25].

The possible reversibility of the m^{6}A mRNA modification was demonstrated by the identification of two mammalian m^{6}A demethylases: the **α-ketoglutarate-dependent dioxygenase alk B homolog 5 (ALKBH5)** protein and **fat mass and obesity-associated protein (FTO)** [27,28]. ALKBH5 preferentially demethylates m^{6}A in a consensus DRACH motif-dependent manner, whereas FTO demethylates a broad spectrum of substrates including m^{6}A [28]. Therefore, it is plausible that ALKBH5 is more involved than FTO in global m^{6}A demethylation. FTO was originally implicated in overweight and obesity in humans [29,30]. Later, FTO was shown to demethylate m^{6}A in polyadenylated RNAs [27,31]. Although several studies have provided evidence that FTO depletion results in the upregulation of total m^{6}A [27,32], several recent reports suggest that FTO participates more in the demethylation of ***N*^{6},2′-*O*-dimethyladenosine (m^{6}A$_m$)**, which is found adjacent to the 7-methylguanosine cap on mRNA and affects mRNA stability [33]. More recently, FTO was also found to demethylate ***N*^{1}-methyladenosine (m^{1}A)** in tRNAs [34].

Degradation of m^{6}A-Containing mRNAs

A variety of gene-regulatory pathways and biological effects mediated by the m^{6}A modification have been summarized in several recent review papers [35,36]. It should be noted that these molecular and biological functions involving m^{6}A are mostly mediated by m^{6}A-recognizing RNA-binding proteins (RBPs) (m^{6}A reader proteins), such as **YTH domain-containing proteins** (YTHDF1, YTHDF2, YTHDF3, YTHDC1, and YTHDC2), eukaryotic translation initiation factor 3, heterogeneous nuclear ribonucleoprotein (hnRNP) C, hnRNP G, and hnRNPA2B1. Here, we highlight recent progress in our understanding of the molecular details of m^{6}A-mediated mRNA decay.

base pairs with complementary sequences in its target mRNA, leading to rapid degradation of the mRNA, its inefficient translation, or both.

***N*^{1}-Methyladenosine (m^{1}A):** reversible modification found in tRNAs and mRNAs. The m^{1}A modification of adenine at position 58 in tRNA is known to be crucial for tRNA stability.

***N*^{6},2′-*O*-Dimethyladenosine (m^{6}A$_m$):** reversible modification found at the +1 position from the 5′ cap in mRNA that affects mRNA fate.

Neuronal RNA granules: motile granules delivering translationally arrested mRNPs from the cell body to axons and dendrites.

Nonsense-mediated mRNA decay: the best-characterized mRNA surveillance mechanism, by which faulty mRNAs containing premature termination codons are selectively recognized and rapidly degraded. In addition, it functions as a post-transcriptional regulatory pathway by targeting normal physiological mRNAs, ensuring the proper cellular response to a variety of intrinsic and extrinsic cues.

NSUN2: RNA methyltransferase that introduces m^{5}C into tRNAs, mRNAs, miRNA, and ncRNAs.

Processing bodies (P bodies): nonmembranous cytoplasmic foci where some transcripts are considered to be degraded and/or translationally repressed. Some transcripts can exit P bodies and resume translation.

Stress granules: dense cytosolic aggregates (comprising translationally arrested mRNPs) that appear when the cell is exposed to various stresses.

YT521-B homology (YTH) domain-containing proteins: family of reader proteins that specifically recognize m^{6}A.

The destabilization of m^{6}A-containing mRNAs was first identified in studies that uncovered an increase in the half-life of mRNAs after m^{6}A writer protein (METTL3 or WTAP) downregulation in both human and mouse cells [37–39]. Then, after the discovery of m^{6}A-specific YTH reader proteins and structural studies that showed they are conserved across various species [40,41], it became possible to characterize the details of RNA destabilization by m^{6}A (Figure 2, Key Figure). Thus far, it seems that all three YTHDF proteins (YTHDF1, 2, and 3) can work together to destabilize the same subset of transcripts [42–44]. Nonetheless, recent reports outlining the mechanism behind the decay of m^{6}A-containing mRNAs seem to consistently indicate that YTHDF2 is the major decay-inducing reader protein [45,46].

Key Figure

Molecular Details of the YTH Domain-Containing Family Proteins (YTHDF)2-Mediated Decay of *N*^{6}-Methyladenosine (m^{6}A)-Containing mRNAs

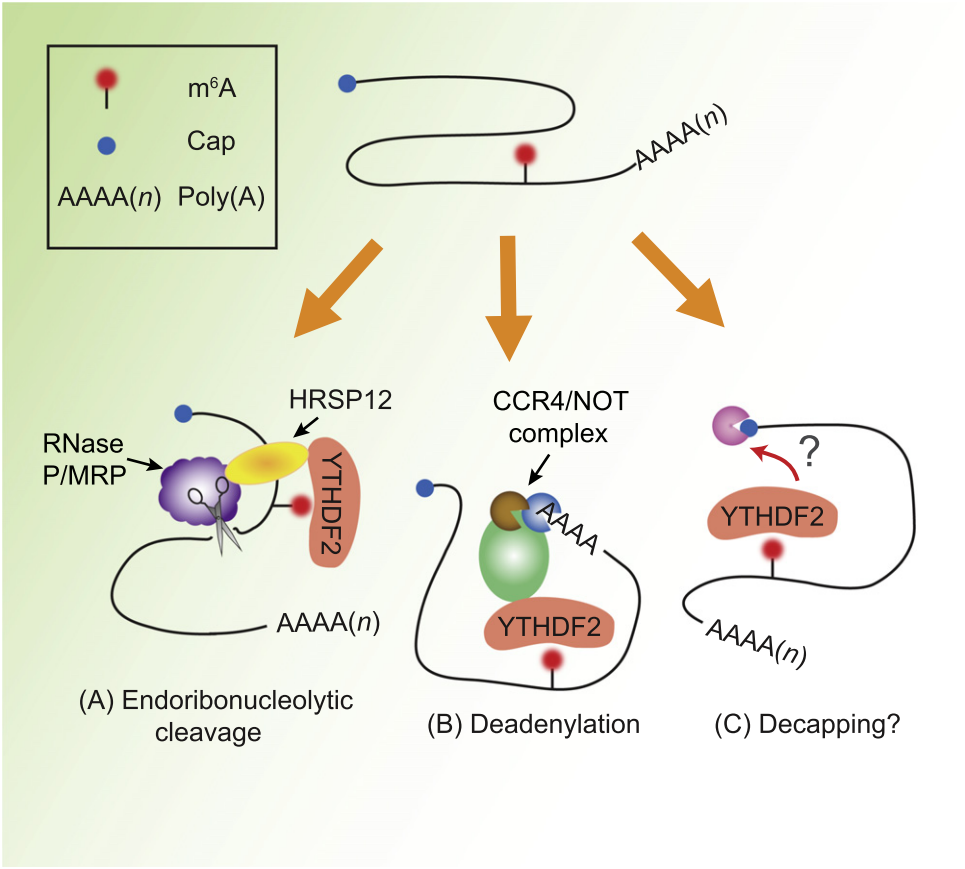

Figure 2. An m^{6}A-containing mRNA bound by YTHDF2 can be degraded via several pathways, including endoribonucleolytic cleavage in the presence of an heat-responsive protein (HRSP)12-binding site (A) or deadenylation in the absence of such a site (B). Considering that YTHDF2 associates with processing bodies (P bodies) [47,48], it is possible that an m^{6}A-containing mRNA localizes to P bodies for its complete degradation. In particular, it is known that the exosome (3′-to-5′ exoribonuclease) is not localized to P bodies and that deadenylation is a prerequisite for the formation of P bodies and for mRNA degradation. Therefore, CCR4/NOT-mediated deadenylation and subsequent 3′-to-5′ decay of an m^{6}A-containing mRNA may precede the targeting of the mRNA to P bodies. Besides these pathways, a decapping complex may be recruited with the help of unidentified adaptor proteins (C).

Growing evidence shows that YTHDF2 is responsible for localizing transcripts from translating pools to **processing bodies (P bodies)** [47,48], where cellular proteins participating in mRNA degradation are enriched [49,50]. A recent study revealed that, under stress conditions, the complex of an m^{6}A-containing mRNA and YTHDF proteins partitions into intracellular phase-separated compartments, such as P bodies, **stress granules**, or **neuronal RNA granules** [48]. However, another research group reported that YTHDF2 directly recruits the CCR4/NOT deadenylase complex to m^{6}A-containing mRNA independently of the association between YTHDF2 and P body components, thereby triggering deadenylation of m^{6}A-containing RNAs [45]. It is known that deadenylation of an mRNA precedes the formation of P bodies [51] and that the **exosome** (3′-to-5′ **exoribonuclease** complex), which is engaged in rapid mRNA degradation after deadenylation, is not enriched in P bodies [49–51]. Therefore, it is plausible that CCR4/NOT-mediated deadenylation and subsequent exosome-mediated 3′-to-5′ exoribonucleolytic decay may initiate the degradation of an m^{6}A-containing mRNA outside of P bodies. The remaining mRNA intermediate may then be subject to decapping, followed by 5′-to-3′ exoribonucleolytic cleavage in P bodies, where the decapping complex and 5′-to-3′ exoribonuclease (XRN1) are enriched [49,50].

An additional route for YTHDF2-mediated mRNA decay was reported recently [46]. m^{6}A-containing mRNAs bound by YTHDF2 associate with RNase P/MRP, an **endoribonuclease** (Box 1). The association between YTHDF2 and RNase P/MRP is bridged by an adaptor protein: heat-responsive protein 12 (HRSP12) (also known as reactive intermediate imine deaminase A homolog, UK114 antigen homolog, or 14.5 kDa translational inhibitor protein). Experiments based on crosslinking immunoprecipitation followed by next-generation sequencing have characterized HRSP12 as a new RBP with a binding preference for the sequence GGUUC. Of note, this sequence is located in the 5′ half of a potential palindromic sequence, suggesting that HRSP12 may recognize an RNA stem–loop structure as well as primary sequences. Besides serving as an adaptor, HRSP12 facilitates the binding of YTHDF2 to mRNA. Moreover, YTHDF2 promotes the binding of HRSP12 to target mRNAs. With the help of this cooperative binding, RNase P/MRP is eventually recruited to an m^{6}A-containing mRNA and performs endoribonucleolytic cleavage [46]. Currently, it remains unknown whether this endoribonucleolytic-cleavage event is associated with P bodies.

Considering that the N-terminal half of YTHDF2 contains an unstructured low-complexity region rich in proline, glutamine, and asparagine without a clear domain and that the C-terminal half forms a rigid YTH domain for m^{6}A recognition [40,41], the N-terminal half may provide a binding

Box 1. Molecular Properties of RNase P/MRP

RNase P and RNase MRP are both RNP complexes that are conserved across a variety of species including humans, yeast, mice, and flies [86]. RNase P was first identified as an endonuclease that cleaves the 5′ leader sequence of a precursor form of tRNAs. The protein subunits of RNase P can interact with various cellular proteins and this combinatorial assembly can give rise to myriad RNP complexes. RNase MRP was first found to cleave mitochondrial RNA in mouse cells (hence the name), but subsequent research has revealed that RNase MRP is not associated with mitochondria and is now widely known as a nuclease for 5.8S rRNA processing. Notably, in humans, RNase P and RNase MRP share at least seven protein components (POP1, POP5, RPP20, RPP25, RPP30, RPP38, and RPP40) and have similar secondary and tertiary structures. Other than several protein components, RNase P and RNase MRP are distinguished by their unique ncRNA components: *RPPH1* and *RMRP* RNA, respectively.

Targets of RNase P/MRP are not limited to tRNA, but also include long ncRNAs and mRNAs. As for mRNAs, RNase MRP has been reported to accumulate at a particular cytoplasmic location and destabilize *CLB2* mRNA to promote cell cycle progression. In addition, viperin mRNA has been shown to be directly cleaved by RNase P/MRP in human cells. Furthermore, a recent study by Park *et al.* indicates that RNase P/MRP directly binds to m^{6}A-containing mRNAs in the cytoplasm and internally cleaves them [46].

Box 2. Biogenesis of circRNAs

At approximately the same time that pre-mRNA splicing was discovered, *in vitro* research showed that a form of alternative splicing yields products where the 3′ end is spliced head to tail to the 5′ end of the RNA, thereby uncovering the following paradox: exons located genetically downstream can end up in front of the exons that are genetically upstream [87]. The resulting covalently closed circular products are known as circRNAs and for decades these molecules have been dismissed as byproducts of alternative splicing and have been assumed to have no protein-coding ability or function in the cell. However, with advances in next-generation sequencing, recent data indicate that circRNA formation is a widespread phenomenon among cell types across a variety of species. Although not definitively, circRNAs have been reported to enhance transcription, compete with linear splicing, or act as a translation decoy or an miRNA sponge [87].

circRNAs are mostly produced by a cotranscriptional backsplicing reaction (Figure I), where the spliceosome catalyzes the joining of the 5′ donor site of a downstream exon to a 3′ acceptor site of an upstream exon. Although the majority of the backspliced products are circRNAs that comprise only exons (A), other circularized RNA products can be generated, such as circular intronic RNAs (ciRNAs), which are intron lariats (B), and exon–intron circRNAs (ElciRNAs), which contain both exons and introns (C). Several regulatory elements can aid this process. Base pairing of inverted repeats, such as Alu elements, in the introns flanking circularized exons and several RBPs, such as quacking (QKI), muscleblind (MBL), and fused-in-sarcoma (FUS), have been reported to facilitate the circularization process. Nonetheless, the exact molecular mechanism and the RNP complex responsible for efficient backsplicing remain unknown.

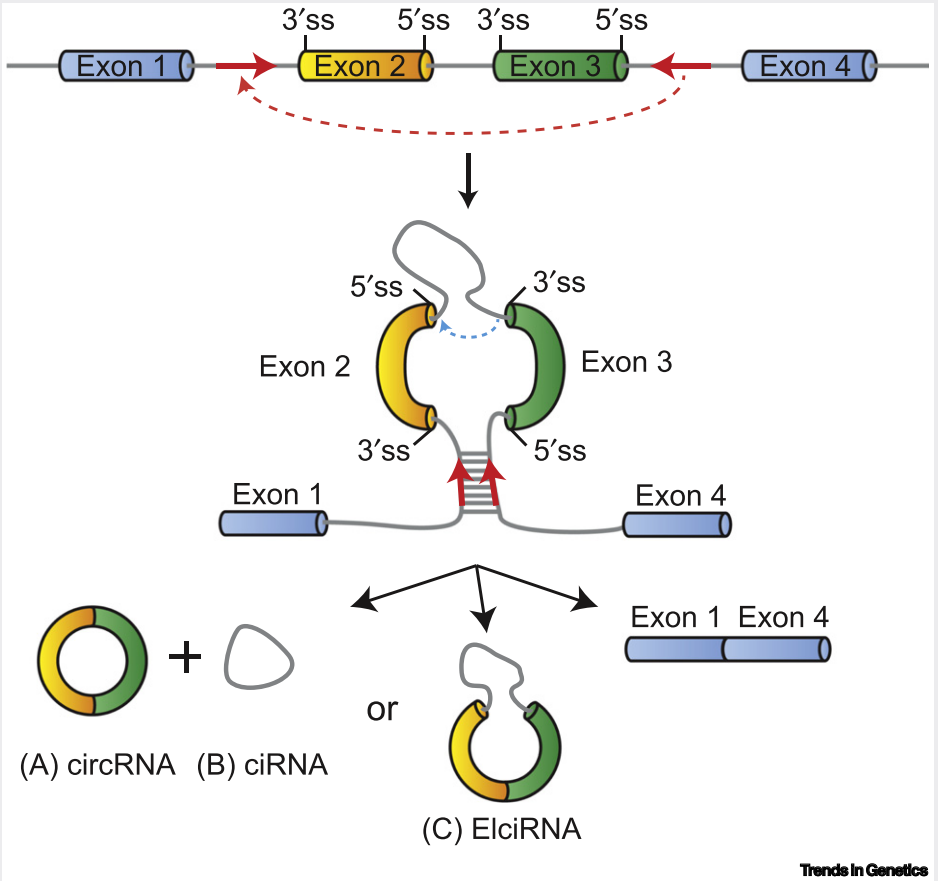

Figure I. An Overview of Biogenesis of Circularized RNAs by Backsplicing Reaction. Abbreviations: circRNA, circular RNA; ciRNA, circular intronic RNA; ElciRNA, exon-intron circular RNA.

platform for the recruitment of regulatory proteins. The minimal site for binding to the CCR4/NOT deadenylase complex is found in amino acids 101–200 of the N-terminal region of YTHDF2 [45]. In addition, the interaction site for HRSP12 is mapped to positions 1–100 of YTHDF2 [46]. In line

with this notion, it is likely that a putative decapping adaptor or decapping complex may also be recruited through the N-terminal half of YTHDF2. In summary, YTHDF2-mediated mRNA decay may proceed via two distinct pathways, depending on the presence of an HRSP12-binding site in a messenger ribonucleoprotein (mRNP): either the endoribonucleolytic-cleavage pathway, via RNase P/MRP, when the HRSP12-binding site is present or the CCR4/NOT-mediated deadenylation pathway in the absence of this site.

A Subset of m^{6}A-Containing circRNAs Also Undergoes YTHDF2-Mediated Decay

It was recently reported that some circRNAs (Box 2) are also m^{6}A modified [52,53]. circRNAs have received much attention due to their unique features [54,55]. Due to the lack of a 5′ cap and a 3′ poly(A) tail, circRNAs are resistant to degradation by exoribonucleases and this property gives them a greater chance of evading degradation than linear RNAs [56]. Accordingly, the only way to initiate the degradation of circRNAs appears to be endoribonucleolytic cleavage. It was recently demonstrated that YTHDF2–HRSP12–RNase P/MRP-mediated endoribonucleolytic cleavage is also applicable to m^{6}A-containing circRNA decay [46]. Endogenous circRNAs that have been shown to associate with YTHDF2 in an HRSP12-dependent manner become more abundant when a component of RNase P/MRP is downregulated [46]. This finding suggests that RNase P/MRP is an endoribonuclease that can initiate the decay of a subset of circRNAs.

Cellular Factors Affecting the Stability of an m^{6}A-Containing mRNA

As mentioned above, YTHDF2 is the main regulator triggering the rapid decay of m^{6}A-containing mRNAs. In the case of YTHDF2–HRSP12–RNase P/MRP-mediated m^{6}A mRNA decay, HRSP12 tends to bind approximately 800 nucleotides upstream of a YTHDF2-binding site and RNase P/MRP preferentially cleaves the mRNA approximately 400 nucleotides downstream of the YTHDF2-binding site [46]. Such a long-range interaction is achieved by the cooperative interaction between HRSP12 and YTHDF2 [46], eventually influencing the selection of the target site for RNase P/MRP-mediated endoribonucleolytic cleavage. Additionally, it is possible that all three YTHDF proteins can act cooperatively to destabilize m^{6}A-containing transcripts. To a lesser extent, YTHDF1 and 3 have been reported to promote deadenylation [45]. In addition, YTHDF1, 2, and 3 can interact with HRSP12, although YTHDF2 has the strongest binding affinity [46]. Furthermore, a recent transcriptome-wide analysis revealed that YTHDF1–3 share binding sites in ~470 transcripts [43], suggesting that possible competition among YTHDF1, 2, and 3 for binding to the common sites may contribute to the differential regulation of transcript stability. Alternatively, given that all three proteins are conserved in their C-terminal half and vary in their N-terminal half, different decay factors or adaptors may bind to each N-terminal half, thereby activating different decay pathways.

There is increasing evidence that YTHDF2-mediated mRNA decay is affected by additional RBPs (Figure 3). For instance, recent studies indicate that **fragile X mental retardation protein (FMRP)** is likely to compete with YTHDF2 for binding to m^{6}A-containing mRNAs, thus preventing the mRNAs from being degraded by YTHDF2 [57,58]. FMRP can bind to m^{6}A in a sequence context-dependent rather than an m^{6}A methylation status-dependent manner [57,58]. Its preferred binding motifs are YGGA (Y = C or U) and GAC, which largely overlap with the DRACH motif for m^{6}A modification [57]. In another study, systematic mass spectrometry-based screening of m^{6}A interactors revealed that a stress granule protein, Ras-GAP SH3 domain-binding protein 1 (G3BP1), is repelled by m^{6}A in an RNA sequence context-dependent manner, thereby affecting the stability of the m^{6}A-containing mRNA [58]. Transcriptome-wide analysis of G3BP1-binding sites showed ~88% overlap between m^{6}A sites and G3BP1-binding sites. When m^{6}A levels are reduced by METL3 downregulation in HeLa cells, there is a significant increase in the half-

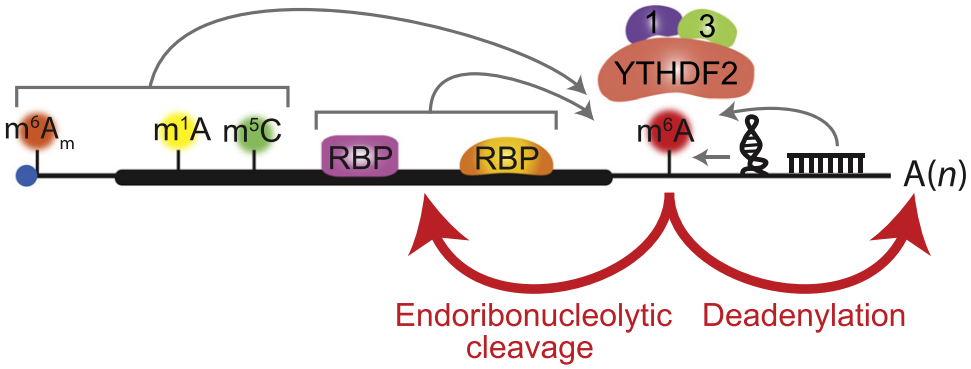

Figure 3. Cellular Factors Contributing to the Regulation of the Stability of N^6-Methyladenosine (m^6A)-Containing mRNAs. Thus far, ~150 different RNA modifications have been characterized [5–7]. In addition, recent approaches to the proteome-wide identification of RNA-binding proteins (RBPs) have identified >1000 RBPs in mammalian cells [83–85]. Therefore, the stability of an m^6A-containing mRNA may be regulated through dynamic crosstalk between m^6A and various cellular factors, including RBPs, other RNA modifications, RNA structures around the m^6A, miRNAs, and/or a change in the levels of a writer, reader, or eraser.

lives of G3BP1-bound mRNAs (which applies to 143 mRNAs) and this is negated by the double downregulation of METTL3 and G3BP1. This suggests that the increase in mRNA stability after METTL3 downregulation is in part due to G3BP1 binding to these mRNAs. In addition, insulin-like growth factor 2 mRNA-binding protein (IGF2BP) 1, 2, and 3 have been reported to recognize m^6A and stabilize m^6A-containing mRNAs [59]. Considering that only ~1% of IGF2BP-binding sites overlap with YTHDF2-binding sites, the opposing functions of IGF2BPs and YTHDF2 imply distinct binding preferences for m^6A target selection. A recent report showed that proline-rich coiled-coil 2 A (PRRC2A) binds to a consensus GGACU motif in the *Olig2* mRNA in an m^6A-dependent manner and stabilizes *Olig2* mRNA, as indicated by the increase in mRNA half-life after PRRC2A overexpression [60]. It is also known that **human antigen R (HuR) [also known as ELAV-like protein 1 (ELAVL1)]**, which stabilizes mRNAs targeted for **adenylate- and uridylate-rich element (ARE)-mediated mRNA decay**, binds to mRNA, depending on the distance between the HuR-binding site and m^6A site [61]. In METTL3/14 target mRNAs with HuR-binding sites (which applies to 571 mRNAs), when an m^6A base is positioned in close proximity to the HuR-binding site, m^6A promotes the binding of HuR to the mRNA [8,61]. By contrast, when the m^6A site is positioned sufficiently far from the HuR-binding site, m^6A blocks HuR binding to the mRNA, thereby leading to rapid decay of the m^6A-containing mRNA [61]. Because m^6A and HuR-binding sites do not usually colocalize in mRNAs, the authors proposed that, in general, m^6A inhibits HuR binding to mRNA, thus triggering rapid mRNA decay [61]. Of note, recent studies indicate that HuR binding to m^6A plays an essential role in the stabilization of m^6A-containing *SOX2* mRNA and *FOXM1* mRNA during the proliferation and maintenance of glioblastoma stem-like cells [62,63].

A growing number of studies suggest that, in addition to RBPs, m^6A can either stabilize or destabilize the neighboring RNA structure depending on the sequence context around the m^6A. In general, steric hindrance due to the methyl group in m^6A destabilizes the adjacent structure [64–66]. By contrast, when m^6A is located at dangling ends or in apical loops, m^6A stabilizes the local duplex [66]. In addition, when m^6A is near a 5′ bulge, the base pairing between m^6A and U is stabilized in a Mg^{2+}-dependent manner [67]. The change in local RNA structure due to the m^6A modification may affect the access of YTHDF2 to m^6A and, consequently, the stability of the m^6A-containing mRNA [64–67].

In addition, the stability of an m^{6}A-containing mRNA can be affected by changes in the amount or activity of writers, readers, or erasers of m^{6}A via various factors, such as cellular stresses, post-translational modification, **miRNAs**, and the type of cell or tissue. For instance, the exposure of human breast cancer cells to hypoxia affects the expression of ALKBH5, METTL14, and YTHDF3 (and YTHDF2, albeit less than YTHDF3), consequently leading to a change in the amount of m^{6}A-containing target mRNAs [68]. As another example, a recent study showed that *miR-145* downregulates *YTHDF2* mRNA and, as a result, increases the cellular level of m^{6}A [69].

Finally, it should be noted that a transcriptome-wide analysis revealed that m^{6}A peaks are enriched at miRNA target sites [70]. miRNA increases m^{6}A abundance at its target sites in an mRNA via a sequence pairing between the miRNA and the target mRNA. In support of this notion, artificial changes in either the amount or the seed sequence of miRNAs affect m^{6}A abundance at miRNA target sites, possibly through modulating the association between METTL3 and an miRNA target site in an mRNA [70]. Of note, in the case of miRNA-mediated gene silencing, the efficient loading of an miRNA onto a target site in an mRNA is known to require Argonaute (Ago) proteins. Nevertheless, miRNA-mediated m^{6}A regulation is independent of Ago proteins [70], implying that the loading of a miRNA onto its target mRNA for m^{6}A modification may be guided by other, uncharacterized RBPs. Future studies should address the molecular details of the regulation of miRNA-mediated stability of m^{6}A-containing mRNA.

Possible Crosstalk Between m^{6}A-Mediated mRNA Decay and Other mRNA Modifications

RNA modifications are abundant in a variety of RNA species and specific RNA modifications can work synergistically or antagonistically with each other. For example, the combinatorial effect of two or more tRNA modifications is important for stress sensitivity, growth inhibition, and ribosomal pausing (and, therefore, the translation of mRNAs) [71,72]. Although many post-transcriptional modifications in eukaryotic mRNAs [e.g., m^{1}A, 5-methylcytosine (m^{5}C), pseudouridine, 2′-*O*-methylnucleosides] have received increasing attention recently [73], information about the crosstalk between m^{6}A and other modifications in mRNA is limited, because research on modifications other than m^{6}A is still in its infancy. Nonetheless, one recent report uncovered a direct case of crosstalk between two different mRNA modifications [74]. Both m^{5}C (generated by **NSUN2**) and m^{6}A (generated by the METTL3–METTL14 complex) occur in the 3′UTR of the mRNA encoding cyclin-dependent kinase inhibitor 1A (CDKN1A) and they synergistically enhance *CDKN1A* mRNA translation. In addition, another recent report identified YTHDF1–3 and YTHDC1 as m^{1}A reader proteins [75], thus pointing to possible crosstalk between m^{6}A and m^{1}A. As observed in these cases, the dynamic crosstalk among different mRNA modifications may be important for the regulation of the stability of a particular transcript (Figure 3). Consequently, future research on the decay mechanism of m^{6}A-containing mRNAs should also consider the effects of the crosstalk between m^{6}A and other mRNA modifications.

Possible Interplay Between m^{6}A-Mediated mRNA Decay and Other mRNA Decay Pathways

Dynamic crosstalk between different mRNA decay pathways has been reported. For example, **nonsense-mediated mRNA decay** competes with staufen1-mediated mRNA decay by sharing a common factor, upstream frameshift 1 (UPF1) [76,77]. In addition, the ARE-mediated mRNA decay of tumor necrosis factor α mRNA requires cellular factors (Ago2 and its associated *miRNA-16*) involved in miRNA-mediated gene silencing [78]. Furthermore, a recent report suggests that the miRNA-mediated decay of mRNAs harboring a long 3′UTR requires the cooperative action of Ago2 and the nonsense-mediated mRNA decay factors UPF1 and SMG7 [79].

Box 3. GMD

GCs, which belong to the family of steroid hormones, play diverse roles in many biological and physiological processes, including glucose metabolism and inflammation [88]. GCs freely traverse the plasma membrane and directly bind to the cytosolic GR, a nuclear receptor for GCs. Free GR (not bound to a GC) is mostly located in the cytosol and remains inactive. By contrast, after binding a GC, the GR is activated and relocates to the nucleus. In the nucleus, the GC–GR complex binds to specific DNA sequences, thus causing transcriptional activation or repression. In this way, the GR has long been thought to act on DNA as a transcription factor.

Recent accumulating evidence suggests that the GR also binds to specific RNA sequences [80,81]. Although the GR binds to DNA only in the presence of a GC, it can associate with mRNAs independent of GCs. By contrast, in the presence of a GC, the mRNA-bound GR more strongly interacts with proline-rich nuclear receptor coregulatory protein 2 (PNRC2), which functions as an adaptor linking UPF1 and decapping enzyme 1A (DCP1A). Because of these protein–protein interactions, a GC–GR-bound mRNA becomes vulnerable to rapid degradation, a process known as GMD. Besides PNRC2, UPF1, and DCP1A, additional cellular RBPs, such as HRSP12 and Y-box-binding protein 1, have been identified as GMD factors. In particular, HRSP12 is required for the maintenance of a functionally active GMD complex.

In genome-wide analyses, ~100 potential GMD target substrates have been identified [80,81]. Among them, *CCL2* mRNA has been confirmed as a *de novo* substrate for GMD. It directly binds to the GR and is rapidly degraded in the presence of a GC. Downregulation of the CCL2 protein by GMD inhibits the chemotaxis of monocytes, indicating that GMD is engaged in the regulation of inflammation by acting on mRNAs.

Outstanding Questions

There are two distinct modes of m^{6}A-containing mRNA degradation: deadenylation mediated by the YTHDF2–CCR4/NOT deadenylase complex and endoribonucleolytic cleavage via the YTHDF2–HRSP12–RNase P/MRP complex. What is their biological significance? Which m^{6}A-mediated biological and cellular events depend on HRSP12?

What are the mechanisms that underlie the preferential binding of HRSP12 upstream of a YTHDF2-binding site and selective endoribonucleolytic cleavage by RNase P/MRP downstream of the YTHDF2-binding site?

Are the deadenylation and endoribonucleolytic-cleavage pathways of m^{6}A mRNA decay active in the same intracellular compartment (P bodies vs cytosol)?

Are there any RBPs other than HRSP12 (functional homologs of HRSP12) that interact with YTHDF2 and elicit rapid mRNA degradation by recruiting a specific RNA-degrading enzyme?

Does an RNA modification occur in a cell type-specific or organism-specific manner? What factors, if any, determine the specificity? Many of the studies involving different cells or organisms reveal opposing cellular functions of the same RNA modification. How do these opposite events induced by the same modification occur?

As illustrated above, m^{6}A-mediated mRNA decay can also engage in crosstalk with other mRNA decay pathways. HuR, a well-characterized RBP involved in the stabilization of mRNAs targeted for ARE-mediated mRNA decay, binds to m^{6}A and stabilizes m^{6}A-containing mRNAs [8,61–63]. As another example, HRSP12, a recently identified RBP involved in YTHDF2-mediated mRNA decay [46], was first reported to participate in glucocorticoid (GC) receptor (GR)-mediated mRNA decay (GMD) (Box 3) [77,80,81]. Although these two decay pathways are mechanistically different [46], it is possible that m^{6}A (or YTHDF2)-mediated mRNA decay communicates with GMD through the common protein HRSP12. Further research is needed to determine the exact role and the molecular function of HRSP12 in each mRNA decay pathway.

Concluding Remarks and Future Directions

Recent progress in the m^{6}A field has uncovered various molecular pathways regulating the stability of m^{6}A-containing mRNA. Considering the importance of m^{6}A in a variety of biological and physiological events, diverse intrinsic and/or extrinsic factors may affect the decision to follow a certain degradation pathway for m^{6}A-containing mRNAs (see Outstanding Questions). Currently, the molecular details of how the crosstalk between RNA modification and various cellular factors can affect m^{6}A (or YTHDF2)-mediated mRNA decay are still being investigated. Additionally, a variety of RNA modifications and RBP-binding sites in mRNA are being identified with recent advances in biochemical assays and high-throughput sequencing. However, among the various RNA modifications identified so far, the effects of only a few mRNA modifications have been studied [82]. This state of affairs suggests that the profiles of RNA modifications and RBP-binding sites may be much more diverse than currently thought, making it difficult to attribute a particular phenotype to a single modification. Therefore, the crosstalk between m^{6}A and other modifications and the communication between m^{6}A and other cellular factors, including RBPs, should be taken into consideration to fully elucidate the mechanisms whereby mRNA fate is controlled by post-transcriptional modification.

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