Settling the m^{6}A debate: methylation of mature mRNA is not dynamic but accelerates turnover

Nicolle A. Rosa-Mercado,[1] Johanna B. Withers,[1] and Joan A. Steitz[1,2]

[1]Department of Molecular Biophysics and Biochemistry, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06536, USA; [2]Howard Hughes Medical Institute, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06536, USA

Post-transcriptional modification of RNA nucleosides has been implicated as a pivotal regulator of mRNA biology. In this issue of *Genes & Development*, Ke and colleagues (pp. 990–1006) provide insights into the temporal and spatial distribution of *N*^{6}-methyladenosine (m^{6}A) in RNA transcripts by analyzing different subcellular fractions. Using a recently developed biochemical approach for detecting m^{6}A, the researchers show that m^{6}A methylations are enriched in exons and are added to transcripts prior to splicing. Although m^{6}A addition is widely thought to be readily reversible, they demonstrate in HeLa cells that once RNA is released from chromatin, the modifications are surprisingly static. This study integrates data from previous publications to clarify conflicting conclusions regarding the role of m^{6}A in mRNA biogenesis and function. Ke and colleagues found that m^{6}A methylation levels negatively correlate with transcript half-life but are not required for most pre-mRNA splicing events.

Although *N*^{6}-methyladenosine (m^{6}A) modification of RNA was discovered in the 1970s, the prevalence of this modification in mammalian mRNAs has become evident only recently, with the advent of transcriptome-wide m^{6}A detection. Advances in the field have shown that m^{6}A methylations are introduced into mRNA by METTL3 in complex with METTL14 and WTAP (Liu et al. 2014). Experiments manipulating the levels of these proteins and other proteins that interact with m^{6}A have suggested that this modification negatively affects mRNA stability and alters splicing patterns (Dominissini et al. 2012; Wang et al. 2014). These findings motivated researchers to investigate further the importance of this modification to mRNA biology. However, the mechanisms modulating m^{6}A modification and its precise role in mRNA biogenesis and regulation have remained debatable. In this issue of *Genes & Development*, Ke et al. (2017) clarify many of these questions by analyzing the presence of m^{6}A in RNAs from different HeLa subcellular fractions and investigating how the distribution and extent of m^{6}A modification relate to splicing and turnover of transcripts.

The precise mapping of m^{6}A in cellular transcripts was facilitated by the development of m^{6}A cross-linking immunoprecipitation (m^{6}A-CLIP) (Ke et al. 2015). Single-nucleotide resolution of the positions of m^{6}A modification is achieved by UV cross-linking immunoprecipitated RNA bound to anti-m^{6}A antibody prior to high-throughput sequencing. The reduced background of m^{6}A-CLIP enables researchers to identify the location of m^{6}A modifications at unprecedented resolution. By analyzing the distribution of m^{6}A in total RNA using this technique, Ke et al. (2015) confirmed that most m^{6}A peaks are located in the last exon of a transcript such that nearly half of all m^{6}As in mRNAs occur in 3′ untranslated regions (UTRs) (Meyer et al. 2012). Ke et al. (2017) furthered their investigation of RNA methylation by comparing the distribution of m^{6}A in chromatin-associated, nucleoplasmic, and cytoplasmic RNA fractions obtained from HeLa cells. They found that even for incompletely spliced chromatin-associated pre-mRNA, the majority of m^{6}A modifications is in exonic sequences. Additionally, the number and location of m^{6}As do not change between nucleoplasmic and cytoplasmic RNAs (Fig. 1). Prior studies suggested that this modification is dynamic based on the presence of m^{6}A demethylases in the nucleus (Jia et al. 2011); however, the findings of Ke et al. (2017) show that changes in the methylation state are minimal and occur at the pre-mRNA level prior to release from chromatin.

After demonstrating that m^{6}A methylation occurs before splicing is complete, the researchers assessed the number of m^{6}A modifications in relation to splice sites. Although chromatin-associated RNA contains slightly more m^{6}A modifications near 5′ and 3′ splice sites than

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Corresponding author: joan.steitz@yale.edu
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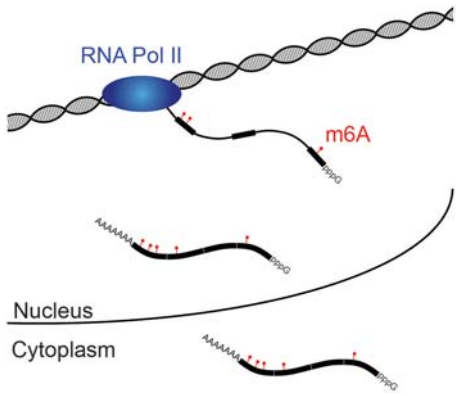

Figure 1. m^{6}A modifications are added to exonic sequences before splicing and shorten mRNA half-life. RNA polymerase II (blue) synthesizes the pre-mRNA transcript, and, prior to the completion of splicing, a methylation complex containing METTL3 selectively adds m^{6}A modifications to exonic sequences (thicker black bars). After release of spliced mRNA first into the nucleoplasm and ultimately into the cytoplasm, no further additions or subtractions of m^{6}A are detected. The presence of m^{6}A in an mRNA accelerates turnover.

the same regions in nucleoplasmic or cytoplasmic transcripts, a reduction in the density of methylation marks was observed within 50 nucleotides of splice sites in all fractions. Furthermore, no enrichment of this modification was found at either end of exons that can be alternatively spliced. Because this observation contradicts findings by other groups, Ke et al. (2017) reanalyzed previously published data using their own bioinformatic analysis pipeline. This reaffirmed their conclusion that m^{6}A is not enriched at the ends of alternatively spliced exons. Finally, the researchers knocked out METTL3 in mouse embryonic stem cells and observed no significant changes in splicing patterns compared with those of wild-type cells. Once again, they were able to corroborate this observation by reanalyzing previously published data.

Several laboratories have concluded that the presence of m^{6}A results in faster mRNA turnover (Sommer et al. 1978; Wang et al. 2014; Geula et al. 2015). Ke et al. (2017) confirmed this observation by studying the half-life of m^{6}A-containing mRNAs after treating cells with actinomycin D. In general, mRNA transcripts containing multiple m^{6}As have shorter half-lives than those containing a single m^{6}A methylation. mRNAs that have m^{6}As in both the coding sequence and the 3′ UTR have the shortest half-lives. Knocking out METTL3 resulted in longer half-lives of m^{6}A-containing mRNAs, confirming that the differences in RNA life spans can be attributed to the presence of this RNA modification.

Taken together, the findings presented by Ke et al. (2017) provide a better understanding of the distribution of m^{6}A at different stages of mRNA processing and demonstrate that the presence of m^{6}A modification influences the half-life of an mRNA. However, these results raise several interesting new questions and highlight the importance of standardizing bioinformatic analyses to ensure that researchers can achieve reproducible results across the RNA modification field. The observation that the m^{6}A distribution is constant in the nucleoplasmic and cytoplasmic fractions highlights the need to revisit the idea that m^{6}A modification is dynamically regulated. The data presented do not preclude the possibility that m^{6}A modification may be dynamic in certain stress conditions or developmental processes. The findings also call for the elucidation of the spatiotemporal relationship between the activities of RNA polymerase II, m^{6}A methylases and demethylases, and the spliceosome with respect to the nascent RNA. The conclusion that m^{6}A is added selectively to exons before the mRNA is spliced raises the questions of how exons are defined and whether m^{6}A plays a role in establishing exon identity. This comprehensive analysis by Ke et al. (2017) will hopefully begin to unify the field so that these newly arising questions can be addressed.

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