Implications of Widespread Covalent Modification of mRNA

Thomas A. Cooper

Vertebrate ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNAs (mRNAs) as well as viral RNAs can be methylated at the N6 position of adenosines to produce N6-methyladenosine (m6A). Although the presence of m6A modifications in mRNA has been known for decades and a handful of mRNAs methylated at the N6 position of adenosines to produce N6-methyladenosine (m6A). Although the presence of m6A modifications in mRNA has been known for decades and a handful of mRNAs has been identified, the extent of m6A modification in mRNAs was unknown. Two recent articles using massively parallel sequencing found that mRNAs methylated at the N6 position of adenosines to produce N6-methyladenosine (m6A). Although the presence of m6A modifications in mRNA has been known for decades and a handful of mRNAs have either positive or negative effects on interactions between RNA and protein. How does this potential modulate the functions of individual pre-mRNAs and mRNAs? The second level of inquiry is what is the larger physiological relevance of the m6A modifications are conserved between mouse and humans. Particularly intriguing is the recent discovery that FTO gene, a risk gene for obesity and metabolic abnormalities, demethylates the modified residues, suggesting a link between RNA methylation and disease.

Whole transcriptome analysis reveals that one third of human and mouse genes express mRNAs containing methylated adenosines. Fat mass and obesity-associated (FTO) gene, a risk gene for obesity and metabolic abnormalities, demethylates the modified residues, suggesting a link between RNA methylation and disease. Vertebrate ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNAs (mRNAs) as well as viral RNAs can be methylated at the N6 position of adenosines to produce N6-methyladenosine (m6A). Although the presence of m6A modifications in mRNA has been known for decades and a handful of mRNAs methylated at the N6 position of adenosines to produce N6-methyladenosine (m6A). Although the presence of m6A modifications in mRNA has been known for decades and a handful of mRNAs have either positive or negative effects on interactions between RNA and protein. How does this potential modulate the functions of individual pre-mRNAs and mRNAs? The second level of inquiry is what is the larger physiological relevance of the m6A modifications are conserved between mouse and humans. Particularly intriguing is the recent discovery that FTO gene, a risk gene for obesity and metabolic abnormalities, demethylates the modified residues, suggesting a link between RNA methylation and disease.
the HepG2 cell line. Interestingly, METTL3 knockdown was associated with a change in the ratios of mRNA splice variants that correlated with exons and introns that contain m6A modifications. Furthermore, m6A modifications were enriched among exons and introns that are alternatively spliced. A second link between m6A and splicing is the finding that modified residues are highly enriched within unusually long internal exons (>400 nt). The average size of an internal exon is ≈140 nt, and internal exons larger than ≈300 nt are spliced less efficiently as a result of constraints within the splicing machinery, which remain to be defined. In addition to the splice sites at the exon–intron junctions that are essential for splicing, vertebrate pre-mRNAs contain a splicing code of short (5–8 nt) motifs within introns and exons that prevent recognition of cryptic splice sites and enhance recognition of bona fide splice sites by the splicing machinery. Large exons are thought to require additional information for their efficient splicing. The results of Dominissini et al. suggest that m6A could contribute to the splicing code to ensure efficient splicing of large exons.

The preferential modification of mRNAs near the translation termination signal suggests a role for methylation either in translation regulation or in an aspect of RNA metabolism associated with the 3′ untranslated region. For example, Meyer et al. found that m6A modifications are enriched within miRNAs that contain microRNA (miRNA)-binding sites which are typically found within the 3′ untranslated region. Although modified miRNAs are more likely to contain miRNA-binding sites, there was an inverse correlation between the locations of miRNA-binding sites and m6A modifications such that the modification was located 5′ of the miRNA-binding sites. It is possible that miRNAs influence methylation sites or that methylation affects miRNA binding or function. It is also possible that this correlation reflects an indirect relationship reflective of a yet-to-be discovered phenomena.

Beyond defining the specific molecular functions of m6A modification of individual mRNAs, results from both articles address the broader biological significance of m6A modifications. One question addressed is the dynamic nature of the modification: are changes in methylated adenosines used to regulate gene expression programs? The extent of m6A modification increases dramatically during postnatal brain development, suggesting functionality during this critical transition. Comparison between adult mouse brain and the human HEK293 cell line showed extensive similarities by MeRIP-Seq illustrating striking conservation, but also suggesting the lack of cell-specific regulation on a global scale. Similarly, MeRIP-Seq on HepG2 cells subjected to a variety of stresses showed only minimal differences in m6A sites. Although the results do not explain why a large fraction of mRNAs are modified, they provide a limited number of genes subject to altered methylation, with potential relevance to a physiological consequence; something that is often not available after a genome-wide assay.

A particularly intriguing recent finding is that the FTO gene encodes an m6A demethylase, suggesting an important link between m6A modifications and metabolism, obesity, and heart disease. Several genome-wide association studies have linked FTO with obesity and metabolic abnormalities. The next task is to connect the molecular function of FTO in m6A demethylation to a physiological role in metabolism. One can imagine a role for FTO in setting a critical baseline modification of the transcriptome or in the dynamic modification of a few specific mRNAs. The FTO demethylase is localized in the same nuclear structures as the methyltransferase (using the METTL3 subunit as a proxy). One can also imagine that, in the end, the m6A signature results from coordinated methylation and trimming by the FTO demethylase.

Although m6A is thought of as a posttranscriptional modification, the actual timing of modification with regard to transcription is not clear. mRNA processing (capping, splicing, cleavage/polyadenylation) is primarily cotranscriptional and because m6A modifications were found within introns as well as exons, it is likely that a large fraction of the target pre-mRNAs is cotranscriptionally methylated. Consistent with this is the finding that METTL3 and FTO are located in nuclear speckles that also contain components of the splicing machinery. Although speckles are thought to be storage areas rather than sites of active splicing, colocalization suggests a potential splicing-associated function. It is also likely that the fraction of modified sites within introns is an underestimate, because the procedures used for MeRIP-Seq were biased toward the more abundant spliced mRNAs. An analysis of nuclear RNA for m6A modifications would produce an interesting map of m6A enriched within pre-mRNA. It would also be interesting to use MeRIP-Seq in a time course after acute gene activation and in RNA from different cellular components as recently described to define the dynamics of modifications within different RNA populations.

The results from these studies overwhelmingly support an important role for the m6A modification in one or more aspects of gene regulation. The results also illustrate the bursts of insight...
that result from systematic analysis using genome-wide approaches. Ultimately however, it will be an analysis of individual genes that will connect the effects of m6A modifications within specific mRNAs to a broader understanding of physiological significance. These results have turned our collective heads to look in a new direction. Now, it will require the tools and creativity of molecular, cellular, and computational biologists to zero in on the specific targets of m6A modification that are relevant to each of the areas of potential physiological impact.

Disclosures

None.

References


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