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A new methyl mark on messengers

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Abstract

The presence of an N^1 methyl group on adenine bases in DNA and RNA was thought to be a form of damage. Results now show that it also occurs at specific sites in messenger RNAs, where it affects protein expression.

The fact that chemical modifications to DNA bases can alter gene expression without changing the nucleic-acid sequence has been known for more than a decade. But the key regulatory function of many such epigenetic modifications to messenger RNA molecules has been recognized only recently^{1–3}. A simple mark — the methyl group — is widely observed in DNA and its associated histone proteins, and has been studied in mRNA in the forms of 5-methyl-cytosine and N^6 -methyladenosine. On page 441 of this issue, Dominissini *et al.*⁴ present a new member of the mRNA methyl-marked family, N^1 -methyladenosine, and propose that its presence in mRNAs has an influence on biological processes. What is surprising about this modification is that it has previously been described as a form of cellular damage⁵.

N^1 -methyladenosine (m^1A) is unusual in having a positive charge at physiological pH (other bases are uncharged) and a methyl group that blocks the Watson–Crick base-pairing edge of adenine. The modification was previously documented in transfer RNA molecules, where it plays a crucial part in the formation of tertiary RNA structure. The methyl group forces the m^1A base to pair with a non-Watson–Crick configuration⁶, and the positive charge has also been hypothesized to exert an electrostatic influence on protein interactions⁷. Dominissini *et al.* propose that this base modification may have similar biophysical effects in mRNAs: it could affect base-pairing interactions close to the site at which protein translation starts, and might alter RNA folding and electrostatic interactions.

m^1A was previously known to be formed by the exposure of RNA molecules to alkylating agents, and under alkaline conditions it is converted to N^6 -methyladenosine (m^6A) through the Dimroth rearrangement⁸ (Fig. 1). This is potentially a big problem in analysis, because m^6A is a considerably more abundant modification in mRNA, and thus obscures the rarer m^1A . Dominissini *et al.* found methods to suppress this confounding chemical instability of m^1A . Through carefully controlled sample preparation, they reduced the incidence of the m^1A -to- m^6A rearrangement to less than 10%, and the use of an isotopically labelled internal control during mass-spectrometry analysis allowed them to accurately quantify m^1A . These techniques enabled the researchers to identify the overall amounts of the modification in cells, and to map its locations in mRNAs.

The authors find that, although the N^1 methylation of adenine occurs several times less often than N^6 methylation, it is still found in thousands of mRNAs. They note an enrichment of m^1A signals near the translational start site of these mRNAs, and most of the molecules analysed were methylated only once, suggesting that the group has a single role in a given RNA. In the same region, they found sequences rich in the bases guanine and cytosine; such GC-rich motifs correlate with thermodynamically stable secondary RNA structure, which could be involved in directing the placement of m^1A . Moreover, the authors' mapping of m^1A across all RNA transcripts in the cell (the transcriptome) revealed that the modification in mRNAs is associated with higher levels of proteins.

What role does m^1A have in the cell? Dominissini *et al.* found that the fraction of mRNAs containing m^1A correlates positively with the level of gene expression, and may affect changes in cellular metabolism. The data also suggest the presence of a greater number of alternative translation initiation sites in the methylated RNAs than in RNAs lacking this modification. Most notable was the finding that m^1A -modified RNAs produced 1.7-fold higher protein levels than did non-methylated ones. Deeper analysis of the translation process led the authors to suggest that m^1A is involved in the processes by which pre-mRNA molecules are 'spliced' to form mature mRNAs. Thus, influencing protein expression seems to be one of the strongest effects of this methyl mark.

Importantly, the m^1A pattern in yeast RNAs differed from that in human and mouse RNAs: in yeast, the mark was distributed across the coding sequence without preferred locations, suggesting that more-sophisticated organisms have evolved m^1A marking as a distinct pathway. Comparing mouse and human mRNAs, the authors observed that the positions of m^1A modification showed 33% conservation, and that N^1 -methylation in 5'-untranslated regions and close to the start site exhibited even higher conservation. The researchers also demonstrated that the m^1A level varies significantly between different mouse tissues. Furthermore, exposing cultured cells to different stress conditions resulted in changes in the m^1A level, which suggests that m^1A is a dynamic modification with a role in cellular stress responses and signalling processes.

A key question raised by these findings is how the cell differentiates between damage and intentional, stable cellular markers. When is m^1A a 'bug', and when is it a useful feature? Methylation of adenine at position 1 is well documented to arise from exposure to chemical methylating agents⁹ (Fig. 1), and thus 1-methyladenine has been widely studied as a form of damage in DNA and RNA. At least one enzyme (ALKBH3) has been documented to remove this lesion and reduce its cytotoxicity¹⁰. A recent study that also documents m^1A in non-damaged mRNAs shows explicitly that this 'repair' enzyme can remove much of this methyl mark¹¹. It will be important to determine how the stable m^1A mark is recognized as being distinct from that resulting from alkylation damage, and thus protected from immediate repair. And if m^1A is truly dynamic, in the sense of being specifically placed and removed from existing RNA as a cellular switch, then what methylase enzyme puts the mark in place, and what enzymes — ALKBH3 or other — remove it?

Dominissini and colleagues' findings represent an intriguing step for epitranscriptomics. More work is needed to understand the mechanism by which m^1A influences translation

initiation and regulation, as well as the changes in its levels in stress responses. It will be exciting to see how this modification affects, and is affected by, RNA structure. Moreover, we need to know about the hypothesized proteins that are ‘writers’, ‘readers’ and ‘erasers’ of m¹A and thus take part in the dynamics of its regulation. Finally, as yet another modified base has been identified in mRNAs, one wonders how many more exist: is this the final one, or are we just seeing the tip of the epitranscriptome iceberg?

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Figure 1. Dynamic methylation of adenine

Methylation at the N^1 position of the base adenine generates the N^1 -methyladenosine (m^1A) modification in RNA molecules, and occurs as a result of damage by chemical methylating agents⁹. Dominissini *et al.*⁴ report the presence of m^1A in undamaged messenger RNA molecules, and propose that it represents a cellular epigenetic mark. In response to damage, the repair enzyme ALKBH3 can remove this methyl group to repair the base, but it is not clear if this or other enzymes function to remove the mark in an epigenetic context, nor how this mark is added¹¹. Another form of methylated adenine, m^6A , is also recognized as an epigenetic mark in mRNA, added by the enzyme METTL3/14 and removed by FTO or ALKBH5. Under alkaline conditions, m^1A is converted to m^6A through the Dimroth rearrangement.