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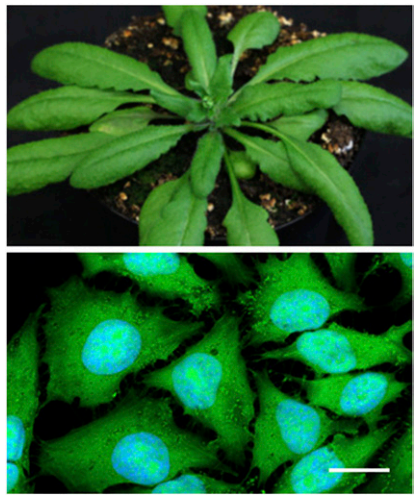
IN BRIEF

The Story Continues: Following the Fate of m^{6}A Marks in the Eukaryotic Transcriptome[OPEN]

Methylation of the N^6 position of adenine in RNA (N^6-methylated adenine [m^{6}A]) is the most common internal mRNA modification in eukaryotes. This modification regulates gene expression during a myriad of processes ranging from leaf and trichome development in *Arabidopsis thaliana* (Arribas-Hernández et al., 2018; Scutenaire et al., 2018; Wei et al., 2018) to cell fate determination in humans. These marks, which are deposited at specific mRNA sequences by m^{6}A writers and removed by m^{6}A erasers, recruit and anchor m^{6}A binding proteins (readers) that play vital roles in pre-mRNA splicing, mRNA degradation, and translation (reviewed in Meyer and Jaffrey, 2017). While many studies have honed in on the roles of m^{6}A marks in specific processes, as well as the underlying mechanisms, **Chen et al. (2018)** took a step back and asked this fundamental question: What happens to m^{6}A after mRNA turnover? When m^{6}A-containing mRNA is degraded in the cytoplasm, N^6-methylated AMP (N^6-mAMP) is released. What prevents this N^6-mAMP from being converted to N^6-mATP, which could be randomly incorporated into newly forming mRNA by RNA polymerase II, thus bypassing the well-regulated activity of m^{6}A writers? The authors reasoned that a degradation pathway specific for N^6-mAMP must exist and that something must prevent the conversion of any excess N^6-mAMP to N^6-mATP.

As suspected, N^6-mAMP does not accumulate in aging Arabidopsis leaf tissue, implying that it is indeed catabolized in plants. The hydrolysis of N^6- and O^6-alkylated purine mononucleotides is catalyzed by human ADAL (adenosine/AMP deaminase-like) in vitro. A similar enzyme is encoded by the Arabidopsis genome, prompting the authors to speculate that



Arabidopsis plant and HeLa cells. The HeLa cells (lower panel) were stained with HCS Cell Mask (green) and Draq5 (nucleus; blue). Bar = 20 µm. (*Figure courtesy of M.A. Olayioye.*)

Arabidopsis and human ADAL catalyze the breakdown of N^6-mAMP in vivo, converting it to inosine monophosphate and methylamine. Arabidopsis ADAL (renamed N^6-mAMP deaminase [MAPDA]) heterologously expressed in wild tobacco (*Nicotiana benthamiana*) had the expected cytosolic localization, as well as the expected activity. The Arabidopsis T-DNA insertional mutants *mapda-1* and *mapda-2* exhibited higher N^6-mAMP/AMP ratios and contained more N^6-mATP than the wild type, confirming the notion that MAPDA hydrolyzes N^6-mAMP. These mutants also specifically overaccumulated free N^6-methyladenosine, as did HeLa (human ovarian tumor) cells (see figure) with knocked-down expression of the human homolog of *MAPDA*. However, the authors did not detect increased misincorporation of m^{6}A marks into mRNA in *mapda-1* or *mapda-2*, perhaps because the frequency was too low for reliable detection. The *mapda-1* and *mapda-2* mutants exhibited slightly reduced root growth but no other obvious phenotypes; the reason for this is unclear. Phylogenetic analysis suggested that MAPDA is structurally and functionally conserved in eukaryotic organisms ranging from algae and plants to fish and mammals, although many fungi partially or fully lack this protein, reflecting the minor role of m^{6}A marks in fungi.

N^6-mAMP is several times more abundant than N^6-mATP in *mapda-1* and *mapda-2*, indicating that N^6-mAMP is not efficiently converted into the trinucleotide in these mutants. Therefore, additional molecular filters likely suppress the phosphorylation of N^6-mAMP as opposed to standard AMP. Kinetic analysis of enzyme activity suggested that adenylate kinases represent such filters, as they selectively phosphorylate AMP versus N^6-mAMP. Thus, by stepping back and asking a fundamental question about a well-described process, the authors uncovered a multilayered molecular protection system that prevents N^6-mATP accumulation and thereby likely averts random incorporation of m^{6}A-marks into mRNA in Arabidopsis, humans, and likely many other organisms. What happens to other modified nucleotides after mRNA is degraded? Read on.

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