m^{6}A-mediated alternative splicing coupled with nonsense-mediated mRNA decay regulates SAM synthetase homeostasis

Watabe E, Togo-Ohno M, Ishigami Y et al. EMBO J. 2021 Jul 15;40(14): e106434.

Presenter: Chih-Yu Lin **Date/Time:** 2022/9/22, 16:00-17:00
Commentator: Shih-Chieh Lin, PhD **Location:** Medical Building Classroom 601

Background:

Alternative splicing (AS) produces variably spliced mRNAs, which is a mechanism for increasing the proteome diversity, it can also control gene expression by generating mRNA isoforms with a premature termination codon (PTC). Such unproductively spliced mRNAs tend to be degraded through a mRNA surveillance system termed nonsense-mediated mRNA decay (NMD), and the intact process was named alternative splicing coupled with nonsense-mediated mRNA decay (AS-NMD). To study the mechanism of AS-NMD, organism model, *Caenorhabditis elegans*, possess advantages included editable NMD factors that do not affect development or fertility, genome rich in introns like those of higher organisms, and majority of its protein-coding genes undergo alternative splicing. In their previous study, the author demonstrated the mechanism of RNA translation and processing genes autoregulate their own expression through AS-NMD.

Objective:

To study the regulatory mechanism of AS-NMD other than the authors' previous research of autoregulation by RNA-binding proteins (RBPs).

Results:

In this study, the author performed high-throughput long-read sequencing of mRNAs to reveal their full-length sequences in a NMD-deficient mutant strain with a mutation in the smg-2 gene, encoding the crucial NMD factor UPF1. The author identified 259 AS-NMD regulated genes that produce 289 PTC-containing and smg-2-enriched mRNA isoforms in C. elegans. Other than genes involved in RNA translation and processing, metabolism related genes are also significantly enriched. The author focused on S adenosyl-L-methionine (SAM) synthetase genes which are involved in metabolic pathways. Semi-quantitative RT-PCR of translation inhibitor, emetine, treated *C. elegans* indicated that splicing regulation of the *sams* genes do not depend on synthesis of new proteins. Nevertheless, they demonstrated that homeostasis of SAM synthetase activity is maintained by negative feedback through AS-NMD in *C. elegans*. *In vitro* LC-MS/MS and *in vivo* m^{6}A-RNA immunoprecipitation studies indicated that m^{6}A modification at the invariant 3' splice site (SS) AG dinucleotide of SAM synthetase genes by a methyltransferase METT-10 switches the choice of competing 3' SSs, leading to AS-NMD for homeostasis of the enzyme. The author further utilized direct RNA sequencing data coupled with machine learning and found that 73–100% of the endogenous sams-3 or sams-4 mRNA isoforms carrying the putative m^{6}A modification.

Conclusion:

Homeostasis of SAM synthetase in C. elegans is maintained by alternative splicing regulation through m6A modification at the 3'SS of the *sams* genes.