RNA DECAY

Stabilizing stemness through m^{6}A

> m^{6}A modification seems to increase mRNA decay

N^6-methyladenosine (m^{6}A), the most frequent internal modification of mRNA, is required to maintain the properties of mouse embryonic stem (ES) cells, according to new research published in *Nature Cell Biology*. This is one of the first demonstrations of a mammalian function for m^{6}A modification, through effects on mRNA stability.

Two mammalian m^{6}A methyltransferases, METTL3 and METTL14, have been predicted on the basis of sequence analysis. Using short hairpin RNAs to target *Mettl3* or *Mettl14*, the authors generated knockdown ES cell lines and showed that m^{6}A levels were decreased, as compared with controls, in cells depleted of either methyltransferase. A direct RNA methylation assay confirmed that METTL3 and METTL14 mediate m^{6}A formation individually and can function synergistically to increase m^{6}A modification.

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Genome-wide analysis identified 4,766 genes in *Mettl3* knockdown ES cells and 4,749 genes in *Mettl14* knockdown ES cells that had significantly decreased m^{6}A mRNA levels compared with controls. There was substantial overlap between these target genes, and METTL3 and METTL14 were shown to interact *in vivo*, which supports the idea of functional synergy between these m^{6}A methyltransferases. Furthermore, METTL3 was almost absent in *Mettl14* knockdown ES cells, despite only a small decrease in *Mettl3* mRNA (and vice versa), which indicates that METTL3 and METTL14 might stabilize each other at the protein level.

Microarray analysis and genome-wide gene-set enrichment analysis of the *Mettl3* and *Mettl14* knockdown ES cell lines showed that the expression of most pluripotency factors was downregulated compared with control cells, whereas the expression of some developmental regulators was significantly upregulated. Thus, m^{6}A modification seems to be required to maintain the 'ground' state of ES cells; indeed, m^{6}A levels in the RNA encoding five out of eight developmental regulators were significantly decreased by day 12 of ES cell differentiation.

So, how might m^{6}A maintain pluripotency? Loss of m^{6}A modification in knockdown cells was more significantly associated with gene upregulation than downregulation, and the stability of methyltransferase RNA targets was increased in knockdown cells compared with controls. Hence, m^{6}A modification seems to increase mRNA decay; this destabilizing effect was shown to target developmental regulators in particular, which are more significantly enriched for METTL3 and METTL14 target sequences than are pluripotency genes.

The RNA stabilizer protein Hu-antigen R (HUR; also known as ELAVL1) showed increased binding to demethylated RNA from knockdown cells compared with m^{6}A-modified RNA from control cells, which could explain how loss of m^{6}A modification increases mRNA stability and gene expression. For example, the expression of the METTL3 and METTL14 target gene *Igfbp3* (insulin-like growth factor-binding protein 3) is increased in knockdown ES cells; m^{6}A demethylation of *Igfbp3* in these cells is associated with increased HUR binding and increased stability of *Igfbp3* mRNA, whereas *Igfbp3* stability is decreased to control levels in methyltransferase-knockdown ES cells that also lack HUR. Finally, the authors showed that HUR increases the stability of *Igfbp3* in methyltransferase-knockdown cells by blocking binding of the RNA-induced silencing complex (RISC).

Together, the data indicate that the pluripotency of ES cells is maintained, in part, through an RNA regulatory mechanism involving the m^{6}A modification of developmental regulators, which blocks HUR binding, increases RISC binding and decreases mRNA stability to decrease gene expression. As thousands of mammalian mRNAs and long non-coding RNAs show m^{6}A modification, this mechanism might have more widespread implications in various cell types.

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