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Stem Cell RNA Epigenetics: M^{6}Arking Your Territory

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Modifications of mRNAs can have a profound effect on cellular function and differentiation. In this issue of *Cell Stem Cell*, Batista et al. (2014) describe fundamental parameters of N^6-methyl-adenosine modification of mRNAs in embryonic stem cells and provide strong evidence that modification plays a role in exit from pluripotency toward differentiation.

Epigenetic modifications associated with the DNA genome that regulate gene expression are well described. Interestingly, an analogous phenomenon in RNA—N^6-methyl-adenosine (m^{6}A) modifications—has recently begun to enter the limelight. While the presence of m^{6}A in mRNA was first described 40 years ago, advances in m^{6}A antibodies and sequencing technology have revealed important parameters of this modification (Dominissini et al., 2012). m^{6}A modifications have been found in multiple eukaryotes and viruses and can occur on both mRNAs and noncoding RNAs. The methyltransferases METTL3 and METTL14, in conjunction with the mammalian splicing regulator WTAP, form a complex to add m^{6}A to target transcripts (Liu et al., 2014). The m^{6}A modification can also be reversed by demethylases such as FTO and ALKBH5, whose altered expression has detrimental effects on cells and is associated with several pathological conditions including obesity, cancer, and developmental defects (Jia et al., 2011; Zheng et al., 2013). Control of multiple aspects of the mRNA life cycle has been attributed to m^{6}A modification, but its best characterized role to date is in mRNA turnover. YTH domain family proteins can recognize m^{6}A-modified mRNAs and relocalize them to cellular sites of decay (Wang et al., 2014a). Because of its dynamic nature, m^{6}A-mediated transcript regulation could prove to be indispensable for stem cells, which must have quick and coordinated responses to environmental cues.

In this issue of *Cell Stem Cell*, Batista et al. (2014) utilized global sequence analyses of mRNAs immunoprecipitated with an m^{6}A RNA-specific antibody to define the mRNA methylome in mouse and human embryonic stem cells (ESCs). Interesting factors encoded by m^{6}A -modified mRNAs include multiple core pluripotency factors and transcripts involved in development and the cell cycle. In addition, m^{6}A modifications in mouse and human ESCs were frequently found near stop codons, at the beginning of 3′ untranslated regions (UTRs), and in long internal exons, indicating that m^{6}A site preference may be tied to functional roles in regulating the RNA life cycle, as described previously in somatic cells (Meyer et al., 2012). Unmodified and m^{6}A transcripts had comparable rates of transcription, but methylated RNAs had shorter half-lives and reduced translation efficiencies. This phenotype signifies a prominent role for m^{6}A in ESC RNA turnover in accordance with recent findings in stem and somatic cells (Liu et al., 2014; Wang et al., 2014a, 2014b).

The authors used gene editing to completely knock out *Mettl3* in mESCs to determine the effects of reducing m^{6}A modifications of mRNA in stem cells. Strikingly, the *Mettl3* KO cells showed improved self-renewal and proliferation, but their ability to differentiate was significantly reduced compared to wild-type mESCs. Knockdown of *METTL3* in hESCs produced similar results. These findings contrast with a recent paper in which *Mettl3* and *Mettl14* knockdowns in mESCs led to decreased self-renewal (Wang et al., 2014b), indicating a need for further investigation to discern the full effects of m^{6}A dynamics in stem cells. Comparison of the *Mettl3* KO and wild-type mESCs revealed a global loss of m^{6}A sites, including those found in pluripotency genes such as *Nanog*. While METTL3 is not the only methyltransferase, it may specifically affect a subset of transcripts associated with pluripotency. In addition, teratomas derived from the KO cells were poorly differentiated and retained high expression of pluripotency markers. These findings suggest that RNA methylation may be the key that allows stem cells to exit a proliferative pluripotent state and enter a path of differentiation (Figure 1).

In addition to m^{6}A, recent evidence suggests widespread and dynamic modification of mRNAs with pseudouridine (Schwartz et al., 2014). Interestingly, mutations in dyskerin, the enzyme responsible for pseudouridine formation, have been reported to be associated with defects in hematopoietic stem cell differentiation (Bellodi et al., 2013). Messenger RNAs modified with pseudouridine and 5-methyl C, another mRNA modification, efficiently reprogram somatic cells to form induced pluripotent stem cells. Thus mRNA modifications in general appear to be tightly associated with cell fate transitions associated with stem cells.

The burning question of the underlying molecular mechanism or mechanisms responsible for the biological impact of m^{6}A mRNA modifications in stem cells remains to be answered. At least three major areas of posttranscriptional gene expression appear to be in play. First, the modification may influence the association of factors such as RNA binding proteins and miRNAs with mRNA targets. Since m^{6}A modification occurs at a well-defined consensus sequence (RRACU) in all cell types analyzed to date, this should assist in identifying nearby RNA elements whose *trans*-acting factor interactions may be influenced by the modification. Second, the m^{6}A modification could influence local mRNA structure because the N6 position is involved in base triples and Hoogsteen base pairs and has been previously shown to

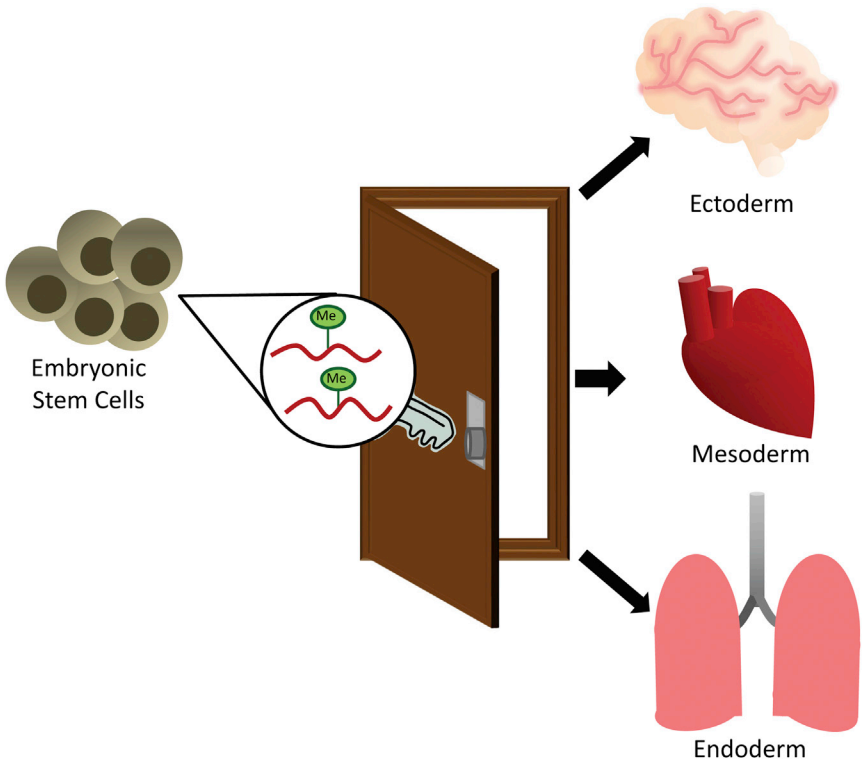

Figure 1. m^{6}A Modification of Transcripts Allows Stem Cells to Effectively "Open the Door" to Various Paths of Differentiation

influence the thermodynamic stability of RNA duplexes. Changes in mRNA folding can reveal or mask regulatory elements that influence the fate of a transcript. Finally, m^{6}A modifications could mark mRNAs as having passed quality control tests in the cell. Stem cells may be reluctant to differentiate without this modification on key transcripts. 3′ UTR length and termination codons are major parts of mRNA surveillance pathways. Thus, the strong bias for m^{6}A modifications in the 3′ UTR near stop codons of mature mRNAs is suggestive of a role in mRNA quality control.

In summary, the extensive work in furthering the characterization of the ESC mRNA methylome by Batista et al. provides a strong foundation for experiments to determine in-depth answers to precisely how m^{6}A modification influences stem cell fate. There are undoubtedly exciting times ahead in this area of stem cell biology.

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