RESEARCH HIGHLIGHT

m⁶A deposition: a boost from TGFβLior Lasman¹ and Jacob H. Hanna¹ Cell Research (2018) 28:505–506; <https://doi.org/10.1038/s41422-018-0037-3>

The regulation of m⁶A deposition is yet to be fully understood. Recently, Bertero et al. describe direct regulation of m⁶A methyltransferase complex by TGFβ signaling in human stem cells.

The mechanisms of regulating gene expression have been studied for many years in an attempt to understand cellular function and behavior. In recent years, another layer of gene expression regulation has started to be unveiled—RNA modifications. There are over 100 different chemical modifications on different types of RNA,¹ and N⁶-Methyladenosine (m⁶A) is the most abundant one on mRNAs.² The m⁶A modification is established as an important determinant of mRNA homeostasis,³ however, the signals regulating its deposition are yet to be fully understood. In a recent paper published in *Nature*, Bertero et al. report that extracellular signaling, such as TGFβ, can directly modulate the m⁶A dynamics.⁴

N⁶-Methyladenosine, also known as m⁶A, is a methylation of the adenosine base at the nitrogen-6 position. m⁶A is widely conserved among eukaryotes, from yeasts, flies to mammals, indicating its importance. In 2012, a leap forward was made in the research of m⁶A, as for the first time m⁶A modification sites were mapped in mammals, using a novel method of RNA immunoprecipitation followed by high-throughput sequencing.⁵ Since then, many groups all over the world started to study this modification, revealing its importance in regulating mRNA metabolism by affecting RNA stability and translation.³

The m⁶A methyltransferase complex, which is responsible for the deposition of m⁶A, was isolated biochemically for the first time in 1994.⁶ Since then, the proteins comprising this “writer” complex were identified as two catalytic components, Methyl transferase-like 3 (METTL3) and Methyl transferase-like 14 (METTL14),⁷ and one regulatory unit, Wilms tumor 1 associated protein (WTAP).⁸ The subunits of the complex act in synergy to add the m⁶A modification to RNA molecules, but the signals regulating these dynamics remain elusive. However, by using human embryonic stem cells as a model, Bertero et al. uncovered a novel mechanism of m⁶A deposition regulation from an unexpected direction—investigating the well-studied TGFβ signaling pathway.

The TGFβ pathway was previously shown to play a significant part in cell growth and differentiation, embryonic development, tissue repair, and disease. Those processes are mediated through the SMAD2 and SMAD3 effectors, which are known to control the activity of target genes by interacting with transcriptional regulators. Bertero et al. decided to investigate the molecular interactions (interactome) of SMAD2/3 in a dynamic cellular process, differentiation of human primed pluripotent stem cells (PSCs) into endoderm. Applying systematic proteomic experiments, they were able to show that SMAD2/3

interacts with the METTL3-METTL14-WTAP complex, linking for the first time between m⁶A modification and the ACTIVIN/TGFβ signaling.

The m⁶A modification has been shown to have a critical regulatory role by facilitating a timely down-regulation of naïve pluripotency.⁹ However, its role in human pluripotent stem cells (hPSCs) and during differentiation to a specific germ layer is yet to be fully understood. To further investigate the interaction described between SMAD2/3 and the METTL3-METTL14-WTAP complex, Bertero et al. blocked the TGFβ pathway by inhibiting the Activin signaling, which is known to be important for maintaining pluripotency by upregulating NANOG expression.¹⁰ Using nuclear-enriched m⁶A-methylated-RNA immunoprecipitation followed by deep sequencing, they show that inhibition of Activin signaling leads to decreased m⁶A levels in specific transcripts, which significantly overlap with genes bound to SMAD2/3, including the pluripotency factor gene NANOG (Fig. 1).

In attempt to discover the mechanism behind these interactions, Bertero et al. performed nuclear RNA immunoprecipitation, and showed that inhibition of ACTIVIN signaling impairs the binding of the m⁶A “writer” protein WTAP to m⁶A-labeled transcripts, which interact with SMAD2/3 when the Activin signaling is not repressed. These results indicate that while Activin signaling is on, SMAD2/3 has an important role in the recruitment of the m⁶A methyltransferase complex toward nuclear RNAs (Fig. 1). These findings not only reveal a novel regulation of m⁶A deposition, but also show that this process can be dynamically changed due to extracellular signaling.

However, how this mechanism of regulation affects hPSC differentiation tendency? To answer this question, Bertero et al. carried out inducible knockdown of each component of the m⁶A “writer” complex, WTAP, METTL14, and METTL3. Despite the residual expression levels of methyltransferase complex proteins following the knockdown strategy used, the authors were able to show that the m⁶A deposition decreases NANOG mRNA stability. Following inhibition of ACTIVIN signaling, this decrease in NANOG stability facilitates the transition from pluripotency toward neuroectoderm differentiation.

To conclude, starting from analysis of SMAD2/3 interactome in human primed/conventional PSCs, Bertero et al. were able to demonstrate a regulatory axis of m⁶A modification that is executed by the TGFβ signaling pathway and mediated through the interaction between SMAD2/3 and the m⁶A methyltransferase complex. This interaction upregulates m⁶A deposition on several transcripts, and among them are transcripts that are Activin signaling targets and function as pluripotency regulators. This mechanism leads to a break in the pluripotent circuit and

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Fig. 1 The model suggested by Bertero et al., demonstrating that ACTIVIN signaling, mediated by SMAD2/3, promotes m^6A deposition on nuclear RNAs (M3, Mettl3; M14, Mettl14)

induction of neuroectoderm differentiation following inhibition of Activin signaling.

Looking to the future, the novel understanding that extracellular cues, such as the $TGF\beta$ signaling pathway, can directly regulate the dynamics of the m^6A modification on RNA for a rapid cellular response, opens a new direction for research. As $TGF\beta$ is known to affect diseases such as cancer,¹¹ it might reveal a new connection between mRNA modifications and pathological conditions. In addition, it will be important to define response of naïve vs. primed human PSCs to depletion of each component of the m^6A “writer” complex, and via knockout approaches when no residual enzyme expression is maintained.

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