



RESEARCH HIGHLIGHT

On demand CRISPR-mediated RNA N6-methyladenosine editing

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There are over 170 types of chemical modifications discovered across all the RNA categories in living organisms,¹ which constitute the epitranscriptome.² Emerging as an additional mechanism of post-transcriptional regulation of genetic information, these RNA modifications and the epitranscriptome have been tightly associated with the RNA fate and various human diseases.³ Among these distinct RNA modifications, m⁶A is the most prevalent and involved in coordinating many aspects of biological activities. It has been known that the deposition and removal of the m⁶A modification are dynamic and reversible processes in cells and regulated by different groups of enzymes and proteins, including the m⁶A writers, erasers and readers.^{2,3} The m⁶A writers include the methyltransferase-like 3 (METTL3), methyltransferase-like 14 (METTL14) and Wilms' tumor 1-associating protein (WTAP), which are responsible for adding the methyl group to N6 position on adenosine to generate the m⁶A modification, and the fat-mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5) have been identified as the enzymes to remove it.³ The YTH family and heterogeneous nuclear ribonucleoproteins (HNRNPs) as well as the eukaryotic initiation factor (eIF) and insulin like growth factor 2 (IGF2) are discovered to be the endogenous proteins to recognize the m⁶A modification and mediate its biological functions. Dissecting the precise roles of m⁶A in biological or disease

processes has been complicated by the fact that the m⁶A's function is position, transcript, cell and tissue context-dependent. Conventional genetic methods manipulating m⁶A writers and erasers not only lead to the global change of m⁶A levels across the whole transcriptome but also lack temporal controls. Without proper research tools, the relationship between m⁶A and the phenotypic outcomes within particular RNA and cellular contexts remains elusive.

To address these roadblocks, the clustered regularly interspaced short palindromic repeats (CRISPR) technology has recently been applied for targeted m⁶A editing by fusing the de-activated CRISPR associated protein (dCas protein) with the m⁶A modification related enzymes.⁴ These strategies allow the m⁶A writing and erasing to take place at a specific site on a particular RNA transcript instead of causing transcriptome-wide changes. However, these tools lack temporal control for m⁶A editing, which can be critical for deciphering cellular context dependent functions of m⁶A. Furthermore, the constitutive binding of m⁶A editing enzymes on the targeted RNA site raises the concern for the potential disruption on interactions between other cellular components with the target RNA or m⁶A. To address these issues, Liang and co-workers reported a platform by integrating CRISPR with the abscisic acid (ABA)-based chemically induced proximity (CIP) strategy to achieve the temporal induction and reversal of m⁶A editing under the control of a chemical ligand, ABA (Fig. 1).⁵ In this platform, ABA controlled the binding between ABI and PYL proteins, which are individually fused to the RNA-targeting dCas13b protein and an m⁶A editing enzyme. Combined with the customizable single guide RNA

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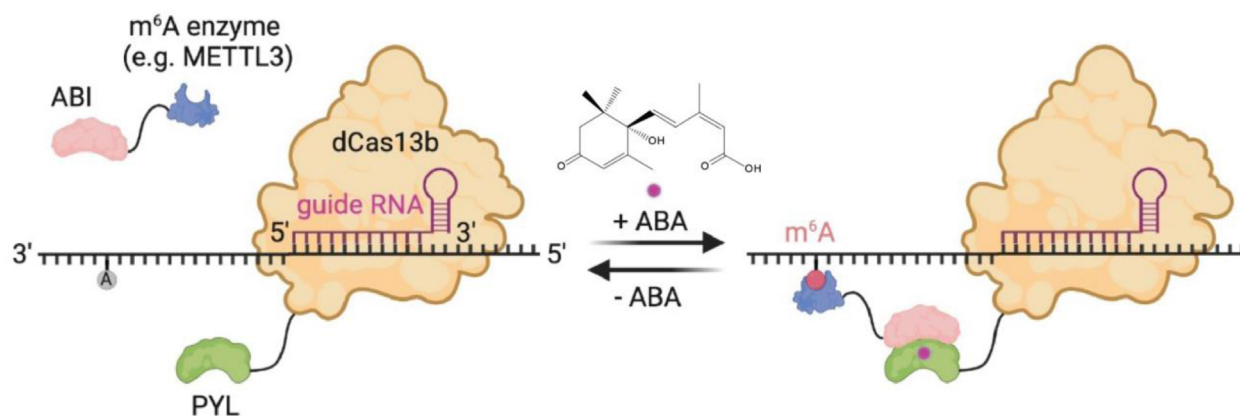


Figure 1 Scheme for the inducible and reversible m⁶A editing platform. The addition of ABA triggers the recruitment of m⁶A writer or eraser to the dCas13b/sgRNA targeting site on the RNA. This process can be reversed after ABA removal.

(sgRNA), the editing of m⁶A can be achieved in both RNA site/transcript and temporal specific manners.

In this work, the authors first developed and tested the ABA-controlled platform for m⁶A writing. By coupling dCas13b and the m⁶A writing enzyme, METTL3, separately to PYL and ABI, they showed that the addition of ABA led to increased levels of m⁶A and METTL3 at the targeted RNA site, but not on other non-target RNAs. More importantly, the ectopically installed m⁶A resulted in the destabilization of the target RNA as expected. Moreover, this system can be easily reprogrammed for site-specific m⁶A deposition on different RNAs by switching the spacer sequence on sgRNA for tailored pairing with other target RNAs.

Another interesting aspect of this work was from the observation that the ABA-induced m⁶A editing as well as the associated biological effect can be reversed after ABA removal.⁵ Liang and co-workers showed that after the recruitment of METTL3 to the target mRNA for m⁶A writing, the enzyme could be released from the RNA after ABA removal, accompanied by the decrease of m⁶A to the background level and restored mRNA stability. These results indicated that this ABA controlled m⁶A writing platform was not only inducible but also reversible.

The authors also demonstrated that this modular m⁶A writing platform could be easily re-engineered for inducible m⁶A erasing by replacing the m⁶A writer, METTL3, with the m⁶A eraser, ALKBH5, to achieve the site-specific m⁶A removal on the target RNA. Moreover, to offer additional way to control the m⁶A editing process, ABA was replaced by a synthetic photo-caged ABA compound, which remained inactive until been activated by the UV light. As a result, they observed that the targeted m⁶A writing can be controlled by light stimulation, which potentially provided another layer of spatiotemporal control in m⁶A editing.

Taken together, Liang and co-worker developed a programmable and robust platform to achieve temporal control of site-specific m⁶A writing and erasing on RNAs of interest through either chemical or light control, which could be reversed after ABA withdrawal. This new

technology is a welcome addition to the growing toolkit for RNA epigenetic research and is expected to facilitate studies in revealing m⁶A functions in various cellular and pathological processes.

Author contributions

Ying Xu and Fu-Sen Liang wrote the manuscript.

Conflict of interests

The authors declare no conflicts of interest.

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Figure was created using the BioRender.com.

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