Nucleoside analogs for messenger RNA metabolic labeling and sequencing

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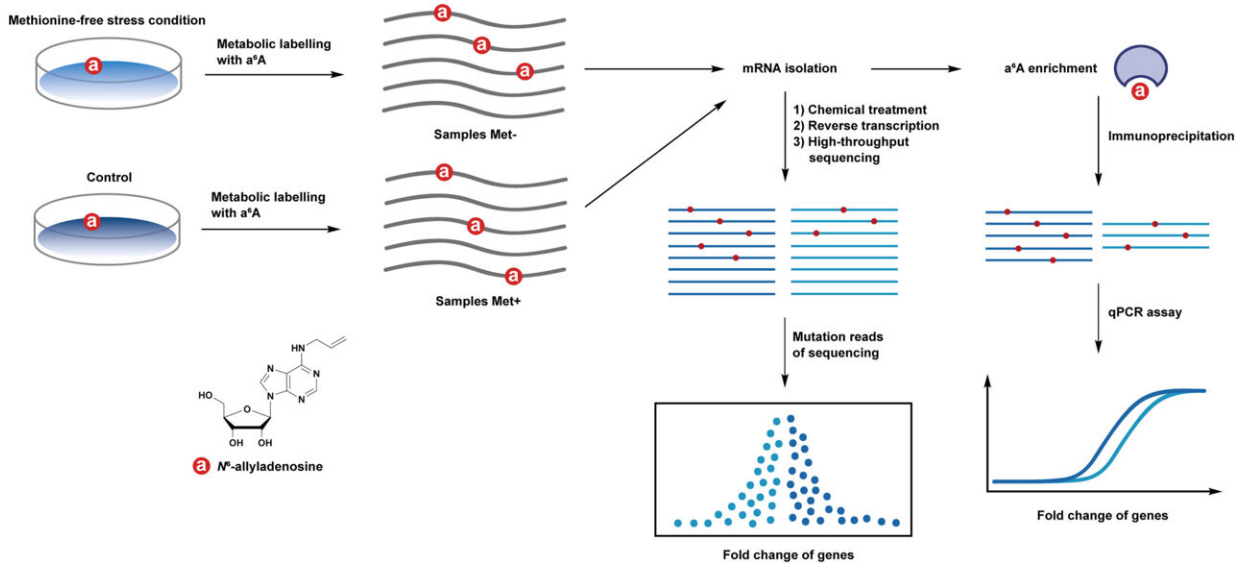

A schematic illustration to study the changes of gene expression profiles under methionine-free condition by a^{6}A-seq. The mutation reads can be used to identify the produced a^{6}A-containing transcripts over a time scale. The combination of a^{6}A-specific antibody immunoprecipitation and qPCR assay can be applied to validate the high-throughput a^{6}A-seq data. Credit: *Fundamental Research* (2023). DOI: 10.1016/j.fmre.2023.04.010

High-throughput RNA-sequencing (RNA-seq) is widely used to profile global cellular gene expression and gain insights into cellular biological processes; however, effectively tracking dynamic changes in cellular mRNA expression remains a challenge. Being able to do so can help

researchers to investigate transcripts over a time frame.

In a study published in the journal *Fundamental Research*, a team of researchers has pioneered a novel method. It incorporated an adenosine analog, N^6-allyladenosine (a^6A) into newly transcribed RNAs by nucleoside salvage pathways.

Following the metabolic labeling, the researchers employed a chemical iodination treatment to convert a^6A within the transcripts into a distinct variant structure. This modified structure can then be accurately identified as base mutation sites using high-throughput sequencing technology.

"We believe that an increasing number of nucleoside analogs will be explored for uses as chemical sequencing tags with the hope of achieving specific labeling on different kinds of RNA, especially mRNA," says Jianzhao Liu, corresponding author of the study, and a professor at the Institute of Biological Macromolecules at Department of Polymer Science and Engineering of Zhejiang University.

The team found that a^6A can be recognized by RNA polymerase and incorporated into the newly synthesized cellular mRNA. Meanwhile, the allyl group on a^6A is bio-orthogonally treated with iodine and a^6A is converted into a structure of $1,N^6$-cyclized adenosine. Consequently, this modification induces base mismatch during RNA reverse transcription, leading to the generation of A- to C/T mutation reads in subsequent RNA-seq analysis.

At present, an antibody suitable for the immunoprecipitation of a^6A-containing RNA is commercially available. Leveraging these resources, the researchers used a^6A in their investigation of alterations in cellular gene expression profiles under methionine-free conditions. Furthermore, they utilized a^6A-IP as a validation tool to confirm the

findings obtained through sequencing. This integrated approach allowed for comprehensive and reliable analysis of gene expression dynamics.

"Our technique will enable researchers to track the newly formed a^6A-containing transcripts and investigate the dynamics of these transcripts, including RNA synthesis and decay," says Liu. "a^6A provides not only a chemical tag for sequencing, but also a tag for antibody enrichment."

More information: Xiao Shu et al, a6A-seq: N6-allyladenosine-based cellular messenger RNA metabolic labelling and sequencing, *Fundamental Research* (2023). DOI: 10.1016/j.fmre.2023.04.010

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