A Methyltransferase-Assisted Chemical Labeling Method to Differentiate N^6-Methyladenosine from Adenosine in RNA

3130103752. Xiao Shu and Jianzhao Liu

Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

Introduction:

N^6-methyladenosine (m^6A) is the most prevalent modification in messenger RNA (mRNA) of higher eukaryotes, and it plays an important role in regulation of biological processes. To understand the function of m^6A, it is of great significance to locate it in RNA. Therefore, development of a method that can differentiate m^6A from adenosine at single-base resolution is highly desirable. Here we report an enzyme-assisted chemical labeling method to solve the problem. Through combination of Human's mRNA m^6A methyltransferases METTL3/METTL14 and synthetic cofactor derivative allyl-*S*-adenosyl methionine (allyl-SAM), we can label an allyl group on the adenosine of RNA to obtain N^6-allyladenosine(allyl-6A). Then N^1,N^6 cyclization of allyl-6A occurs upon iodination, which leads to mutations when reversely transcribed to complementary DNA. In contrast, m^6A in RNA is not labeled with allyl group and no mutation occurs. We prove the mechanism of this method.

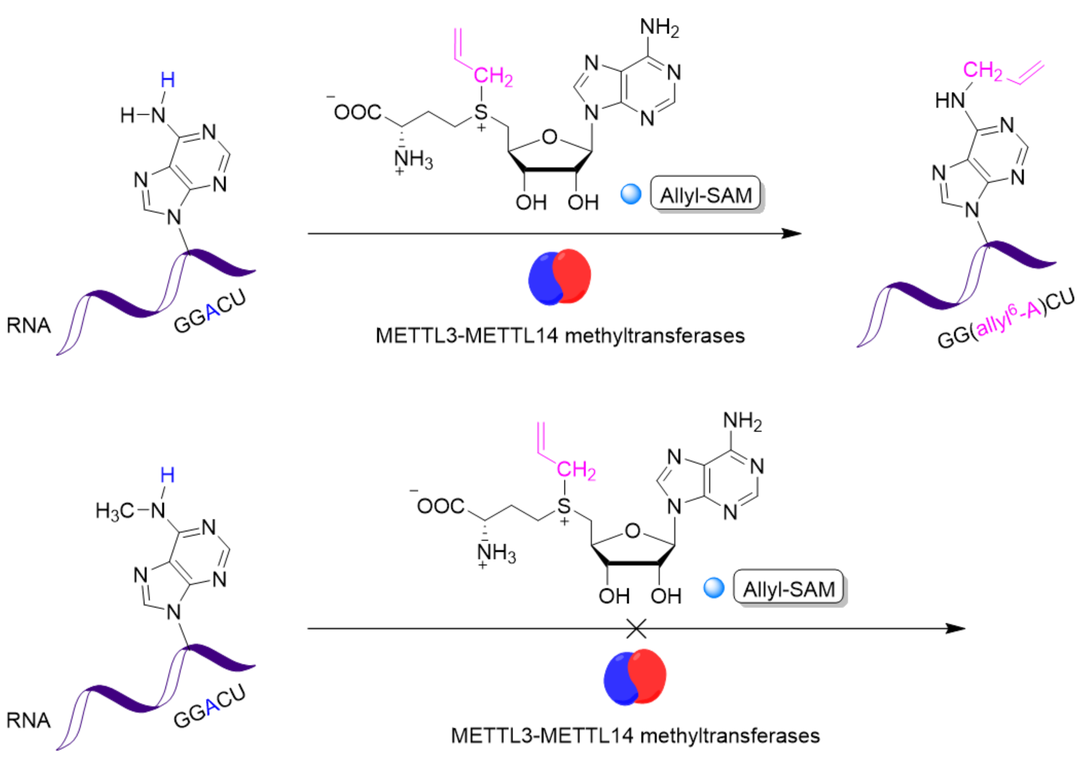

Fig. 1 Methyltransferase-assisted chemical labeling method through combination of methyltransferase and synthetic cofactor allyl-SAM

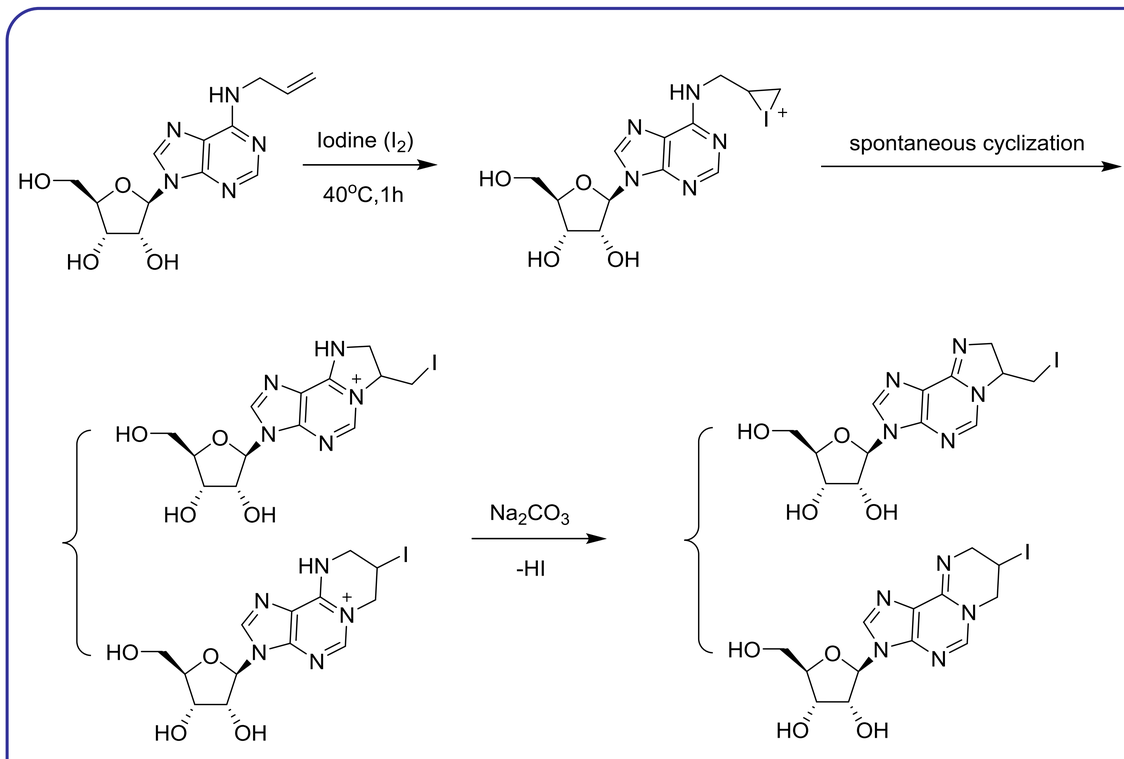

Fig. 2 Iodination of N^6-allyladenosine leads to formation of N^1, N^6-cyclized adenosine which induces mutation during reverse transcription

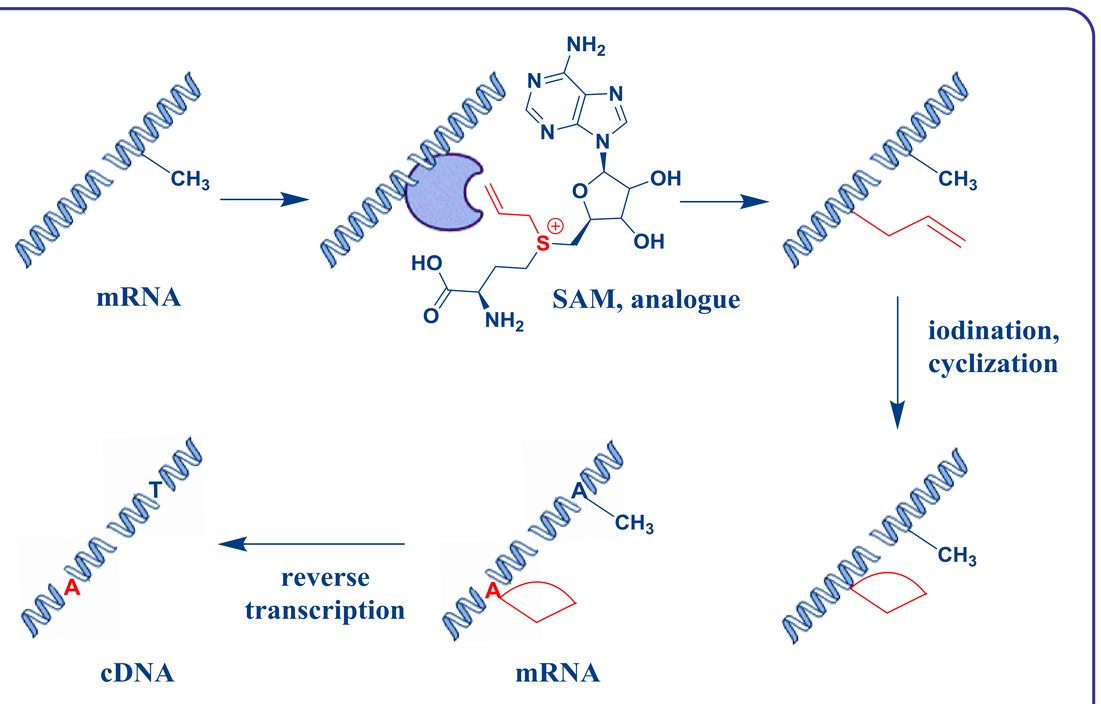

Fig.3 Specific labeling of adenosine to differentiate from m^6A within mRNA

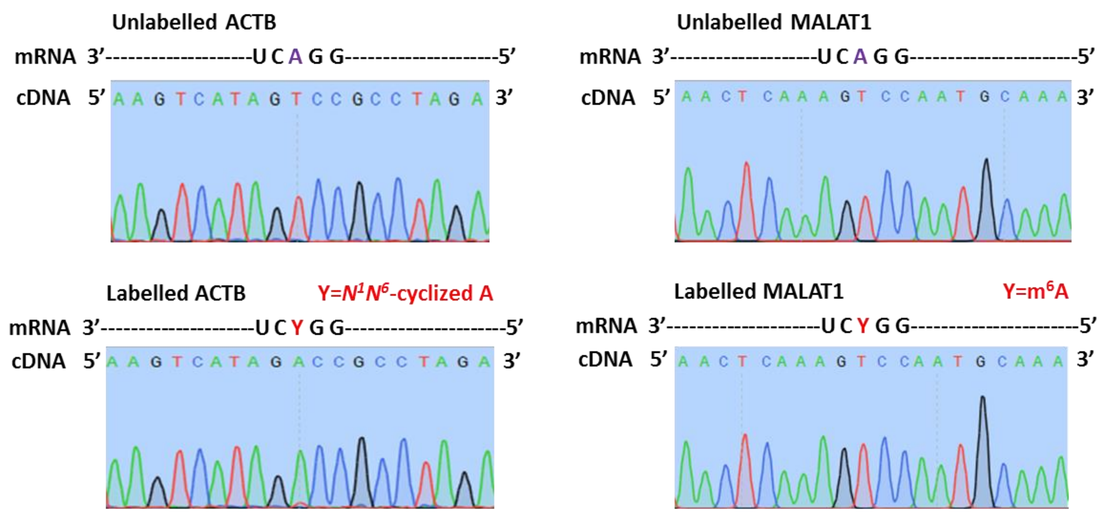

Fig. 4 Purified mRNAs from human HeLa cells were treated according to the above method. Known m^6A sites of ACTB and MALAT1 mRNAs were validated by cDNA sequencing.

Conclusions:

We combined the use of human native mRNA m^6A methyltransferases and synthetic allyl-SAM cofactor to develop a labeling method for differentiation of A from m^6A at base resolution. This methyltransferase-assisted chemical labeling approach offers a chance to specifically label unmodified A within the m^6A consensus motif and then to calculate mRNA m^6A stoichiomery transcriptome-wide.

Acknowledgements:

We thank the National Natural Science Foundation of China (21642015) and National Key Research and Development Program of China (2017YFA0506800). J. L. thanks the Thousand Young Talents Plan of China and Hundred Talents Program of Zhejiang University.

References:

[1] Liu, J. Z.; Yue, Y. et al. *Nat. Chem. Biol*. **2014**, *10*, 93.
[2] Liu, J. Z.; Zhu, Y..; Luo, G. Z.; Wang, X. X. et al. *Nat. Commun*. **2016**, *7*, 13052.