

Supplementary information S2: Roles of m⁶A in RNA species other than mRNAs

In addition to the functions of *N*⁶-methyladenosine (m⁶A) in mRNA, m⁶A exists and may have regulatory roles in other RNA species.

- MicroRNA (miRNA)

A crosstalk between m⁶A and miRNA function has been hypothesized, as both miRNA-binding sites and a large fraction of m⁶A sites are found in mRNA 3' untranslated regions. m⁶A was first shown to impact miRNA biogenesis by regulating the processing of primary miRNAs (pri-miRNAs)¹: heterogeneous nuclear ribonucleoproteins A2/B1 was proposed to be the m⁶A reader for pri-miRNAs processing that recruits the Microprocessor complex to facilitate miRNA maturation². A later study also suggested the existence of m⁶A on mature miRNAs, and that the m⁶A eraser fat mass and obesity-associated protein may affect the steady-state level of several miRNAs³. Interestingly, a most recent study reported that miRNAs promote site-specific m⁶A deposition by mediating the binding of the m⁶A writer methyltransferase-like protein 3, thereby affecting cell reprogramming⁴.

- Long non-coding RNA (lncRNA)

lncRNAs have several sites of m⁶A⁵⁻⁷. The modified adenosine fraction can range from less than 10% to greater than 90% between different lncRNAs⁷. This heterogeneity complicates the elucidation of the role of m⁶A in lncRNA function. The lncRNA X-inactive specific transcript (XIST) was reported to contain 78 m⁶A sites which are required for XIST function of transcriptional silencing of genes on the X chromosome⁸.

- Transfer RNA (tRNA)

tRNA is heavily modified in a wide range of organisms^{9,10}. Of these modifications, many feature large chemical moieties with roles in tRNA processing, localization, quality control and stabilization¹¹. Yet only one m⁶A site has been found on tRNA, located in tRNA^{Val} of *Escherichia coli*¹², and the positions and role of m⁶A in eukaryotic tRNA are largely unknown.

- Ribosomal RNA (rRNA)

rRNA is decorated with several distinct modifications, most of which are involved in mRNA decoding or biochemical processing and stability of the ribosome. m⁶A has been found on rRNA in both bacteria and human, but not in yeast¹³. Human rRNA contains only two known sites of m⁶A modification, at position A1832 in 18S rRNA and position A4190 in 28S rRNA¹⁴. Currently the enzymes responsible for these modifications are not known, nor are the modifications' roles in ribosomal biogenesis or function¹⁴⁻¹⁶.

- Other small RNAs

m⁶A has been found in many small nuclear RNA species including in the splicing factors U2, U4, and U6¹⁷⁻¹⁹. Additionally, small nucleolar RNAs, which commonly guide the formation of rRNA modifications through sequence-specific base-pairing, contain several m⁶A sites with unknown function²⁰.

References:

- 1 Alarcon, C. R., Lee, H., Goodarzi, H., Halberg, N. & Tavazoie, S. F. N⁶-methyladenosine marks primary microRNAs for processing. *Nature* **519**, 482-485 (2015).
- 2 Alarcon, C. R. *et al.* HNRNPA2B1 Is a Mediator of m⁶A-Dependent Nuclear RNA Processing Events. *Cell* **162**, 1299-1308 (2015).
- 3 Berulava, T., Rahmann, S., Rademacher, K., Klein-Hitpass, L. & Horsthemke, B. N⁶-adenosine methylation in MiRNAs. *PLoS ONE* **10**, e0118438 (2015).
- 4 Chen, T. *et al.* m⁶A RNA methylation is regulated by microRNAs and promotes reprogramming to pluripotency. *Cell Stem Cell* **16**, 289-301 (2015).
- 5 Dominissini, D. *et al.* Topology of the human and mouse m⁶A RNA methylomes revealed by m⁶A-seq. *Nature* **485**, 201-206 (2012).
- 6 Meyer, K. D. *et al.* Comprehensive Analysis of mRNA Methylation Reveals Enrichment in 3' UTRs and near Stop Codons. *Cell* **149**, 1635-1646 (2012).
- 7 Liu, N. *et al.* Probing N-6-methyladenosine RNA modification status at single nucleotide resolution in mRNA and long noncoding RNA. *RNA* **19**, 1848-1856 (2013).
- 8 Patil, D. P. *et al.* m⁶A RNA methylation promotes XIST-mediated transcriptional repression. *Nature* **537**, 369-373 (2016).
- 9 G R Bjork *et al.* Transfer RNA Modification. *Annu Rev Biochem* **56**, 263-285 (1987).
- 10 Agris, P. F., Vendeix, F. A. P. & Graham, W. D. tRNA's Wobble Decoding of the Genome: 40 Years of Modification. *J Mol Biol* **366**, 1-13 (2007).
- 11 El Yacoubi, B., Bailly, M. & de Crecy-Lagard, V. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. *Annu Rev Genet* **46**, 69-95 (2012).

- 12 Saneyoshi, M., Harada, F. & Nishimura, S. Isolation and characterization of N6-methyladenosine from Escherichia coli valine transfer RNA. *Biochim Biophys Acta* **190**, 264-273 (1969).
- 13 Sergiev, P. V. *et al.* N6-Methylated Adenosine in RNA: From Bacteria to Humans. *J Mol Biol* **428**, 2134-2145 (2016).
- 14 Piekna-Przybylska, D., Decatur, W. A. & Fournier, M. J. The 3D rRNA modification maps database: with interactive tools for ribosome analysis. *Nucleic Acids Res* **36**, D178-D183 (2008).
- 15 Maden, B. E. H. in *Progress in Nucleic Acid Research and Molecular Biology* 241-303 (Academic Press, 1990).
- 16 Choi, Y. C. & Busch, H. Modified nucleotides in T1 RNase oligonucleotides of 18S ribosomal RNA of the Novikoff hepatoma. *Biochemistry (Mosc)* **17**, 2551-2560 (1978).
- 17 Shimba, S., Bokar, J. A., Rottman, F. & Reddy, R. Accurate and efficient N-6-adenosine methylation in spliceosomal U6 small Nuclear RNA by HeLa cell extract in vitro. *Nucleic Acids Res* **23**, 2421-2426 (1995).
- 18 Gu, J., Patton, J. R., Shimba, S. & Reddy, R. Localization of modified nucleotides in Schizosaccharomyces pombe spliceosomal small nuclear RNAs: modified nucleotides are clustered in functionally important regions. *RNA* **2**, 909-918 (1996).
- 19 Bringmann, P. & Lührmann, R. Antibodies specific for N6-methyladenosine react with intact snRNPs U2 and U4/U6. *FEBS Lett* **213**, 309-315 (1987).
- 20 Linder, B. *et al.* Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. *Nat Meth* **12**, 767-772 (2015).