RNA modification
RNA Modifications

• $N^6$-methyladenosine ($m^6A$) was first discovered in the 1970s and is the most prevalent post-transcriptional modification in mammalian mRNA.

• $m^6A$ is conserved across plants and vertebrates.
  • Also found in viruses, bacteria, archaea, and yeast.

• Today, 170 modified bases in RNA have been reported (database: http://modomics.genesilico.pl/modifications/).

• 5-methylcytidine ($m^5C$) is also a widespread epigenetic mark found in abundance in noncoding RNAs.


Li et al. 2017 Nature Methods
m$^6$A Key Points

- m$^6$A is enriched in consensus sequences within long exons, near stop codons, at 3’ untranslated regions (3’ UTRs), and in pre-mRNAs.
- m$^6$A influences the export of mRNA, mRNA translation, and mRNA decay.
- m$^6$A has regulatory roles in circadian rhythm maintenance, stem cell differentiation, and stress responses.
- m$^6$A may facilitate cell-state transitions by regulating the metabolism of transcripts of key transcription factors.
- METTL3 KO blocks ESC’s ability to differentiate.

MeRIP-seq:
- m6A-specific methylated immunoprecipitation
- Resolution: 100~200bp

miCLIP:
- crosslinking immunoprecipitation
- Resolution: single base

Li et al. 2017 Nature Methods
Detecting m5C

m5C-RIP:
- m5C RNA immunoprecipitation
- Resolution: 100~200bp

Bisulfite-seq:
- Bisulfite treatment
- Resolution: single base

Li et al. 2017 Nature Methods
mRNA m$^6$A is critical in embryo development and cell differentiation

- m$^6$A methylomes in ESCs mark the pluripotency network. (MeRIP-seq)
- Highly conserved in human and mouse ESCs.
- m$^6$A is associated with mRNA turnover.

Batista et al. 2014 Cell Stem Cell
Geula et al. 2015 Science
mRNA $m^6A$ is critical in embryo development and cell differentiation

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- Highly conserved in human and mouse ESCs.
- $m^6A$ is associated with mRNA turnover.
- $m^6A$ loss promotes ESC self-renewal and hinders differentiation. ($Mettl3$ KO)

Batista et al. 2014 Cell Stem Cell
Geula et al. 2015 Science
mRNA m⁶A offers rapid transcriptome switching mechanism in cell differentiation

• Zebrafish MZT (maternal-to-zygotic transition)
• m⁶A reader Ythdf2 loss leads to developmental delay in zebrafish.

Zhao et al. 2017 Nature
Roundtree et al. 2017 Cell
mRNA $m^6A$ offers rapid transcriptome switching mechanism in cell differentiation

- Zebrafish MZT (maternal-to-zygotic transition)
- $m^6A$ reader $Ythdf2$ loss leads to developmental delay in zebrafish.
- Maternal mRNAs are marked with $m^6A$.
- $m^6A$ facilitates maternal RNA decay during MZT.

Zhao et al. 2017 Nature
Roundtree et al. 2017 Cell
mRNA m$^6$A marks the tempo of cortical neurogenesis

- m$^6$A depletion leads to prolonged cell-cycle progression of cortical neural progenitors (Mettl14 cKO).
- m$^6$A depletion promotes progenitor proliferation and delays differentiation.
mRNA m⁶A marks the tempo of cortical neurogenesis

• m⁶A depletion leads to prolonged cell-cycle progression of cortical neural progenitors \((\text{Mettl14 cKO})\).

• m⁶A depletion promotes progenitor proliferation and delays differentiation.

• m⁶A promotes decay of tagged neurogenesis-related transcripts.

Yoon et al. 2017 Cell
Boles et al. 2017 Neuron
HIV-1 Triggers RNA Methylation in CD4+ T-Cells

- 56 genes gain m⁶A only when infected with HIV-1.
  - GO analysis on these genes enriches for viral gene expression.
  - 19 with known interactions with HIV.
  - As compared to the total cellular transcriptome there is more m⁶A in 5'UTR and CDS than 3'UTR.
- Silencing of METTL3/METTL14 (writers) lowers viral replication.
- Silencing of ALKBH15 (eraser) increases viral replication.

Lichinchi et al. *Nature Microbiology* volume1, Article number: 16011 (2016)
Topology of m$^6$A in HIV Genome

Lichinchi et al. *Nature Microbiology* volume 1, Article number: 16011 (2016)
HIV Structure

- The Reverse Response Element (RRE) is present within the viral envelope and in the presence of Rev, viral RNAs with the RRE can be exported from the nucleus to the cytoplasm for packaging.
- Peak 11 (previous slide) is in the stem loop IIB structure of the RRE which is critical for binding with Rev.
- A7877 and A7883 are methylated in vivo.
- Rev-RRE binding is reduced by METTL3/METTL14 silencing, but increased by ALKBH15 knockdown. Also in the same direction for nuclear export.
- Mutation of A7883 effectively blocks nuclear export.

*Image from Wikipedia*

Lichinchi et al. *Nature Microbiology* volume 1, Article number: 16011 (2016)
HIV-1 Mutational Frequency

- Analyzed ~2,500 HIV-1 sequences.
- ~11.16% A mutation rate in the entire sequence.
- ~9% A mutation rate in the RRE Stem Loop IIB Structure.
- 5.44% at the A7877 site.
- .28% at the A7833 site.

Lichinchi et al. Nature Microbiology volume1, Article number: 16011 (2016)
Confirming Importance

• Mutant 2 (A7883G) severely attenuates viral fitness.
• Mutant 1 (A7887G) really does not have an effect.
• Effect of mutant 2 is not modulated by METTL3/METTL14 knockdowns.

Lichinchi et al. *Nature Microbiology* volume1, Article number: 16011 (2016)