RNA modification in cancer

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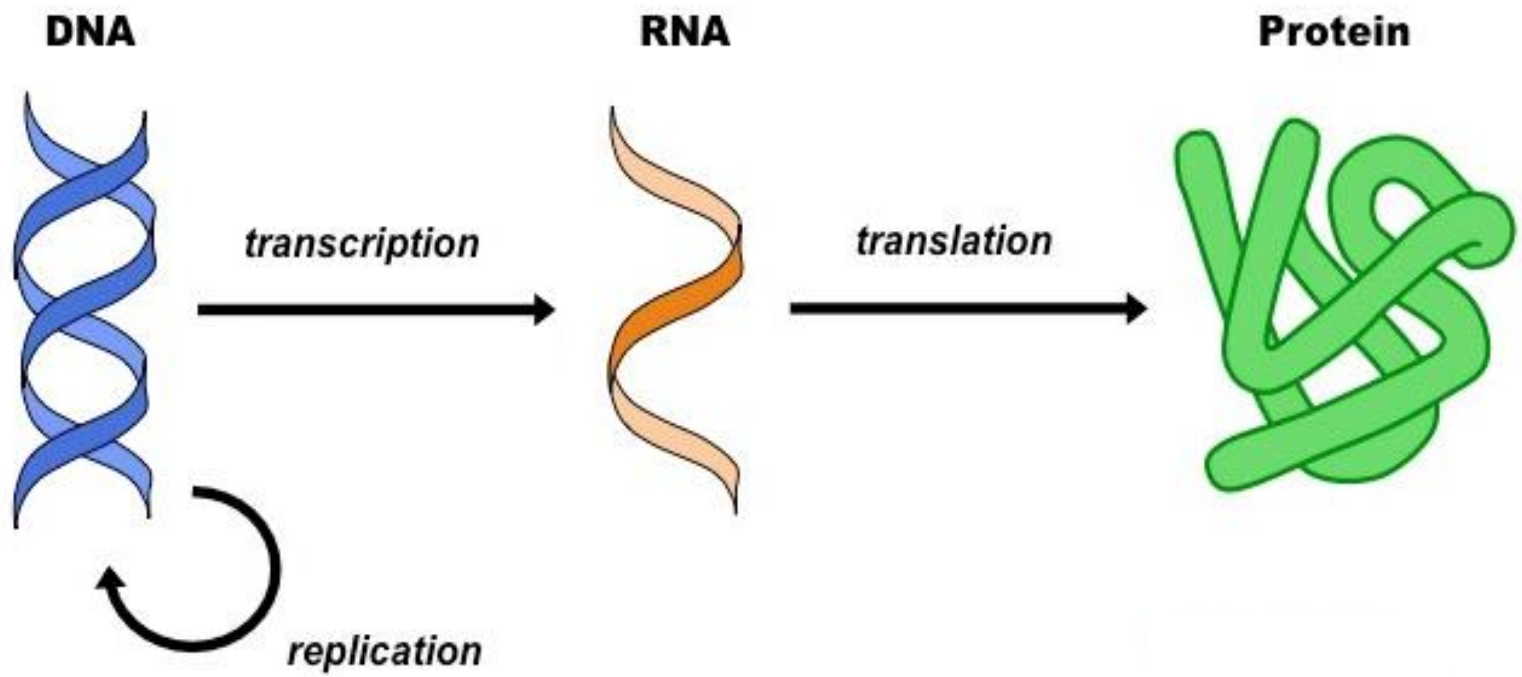
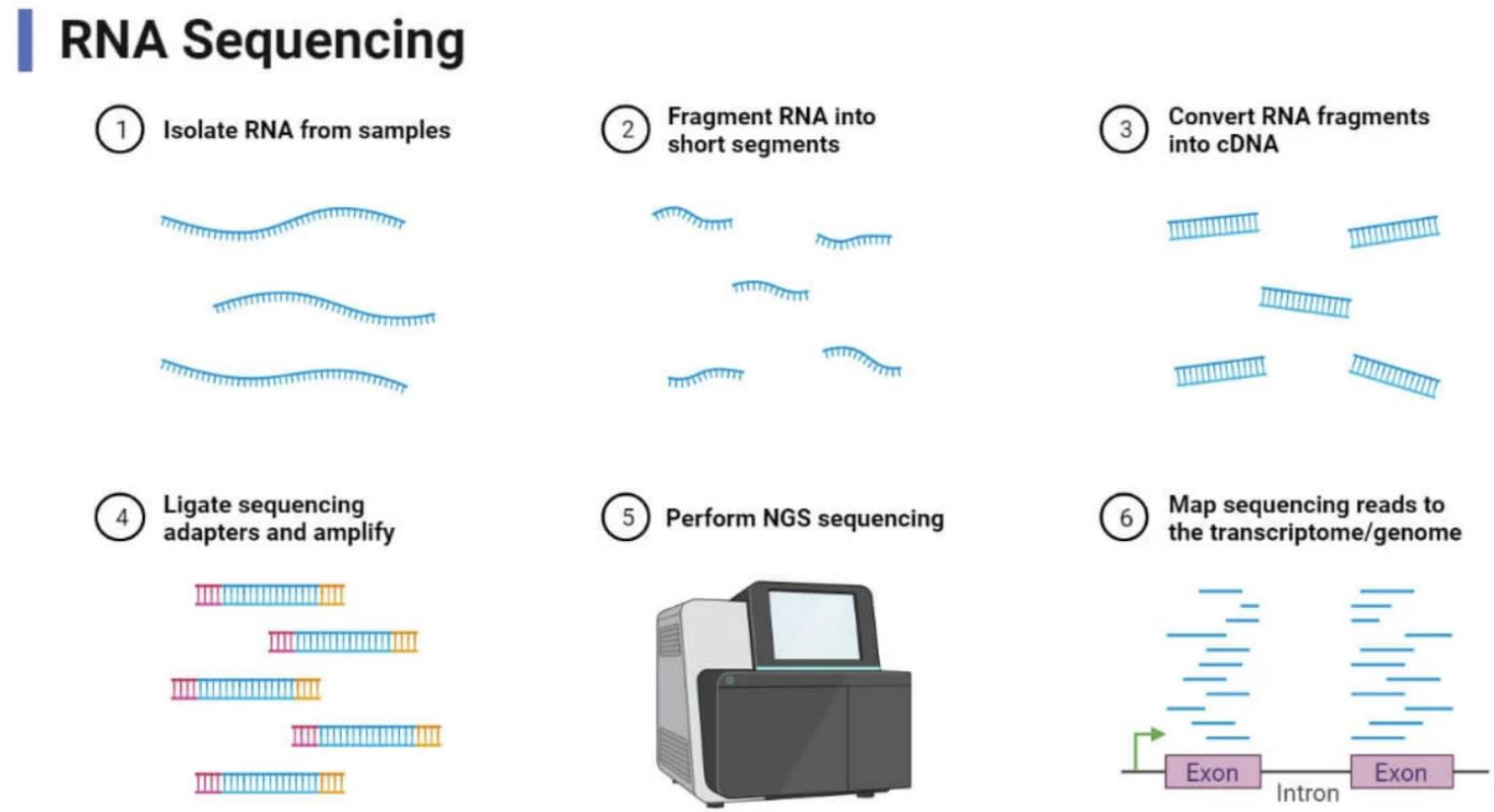
What we will discuss:

- Discovery of mRNA modification
- Technologies to detect mRNA modifications
- Regulation of mRNA modifications in cancer (example of AML)
- Application of modification in future therapeutics

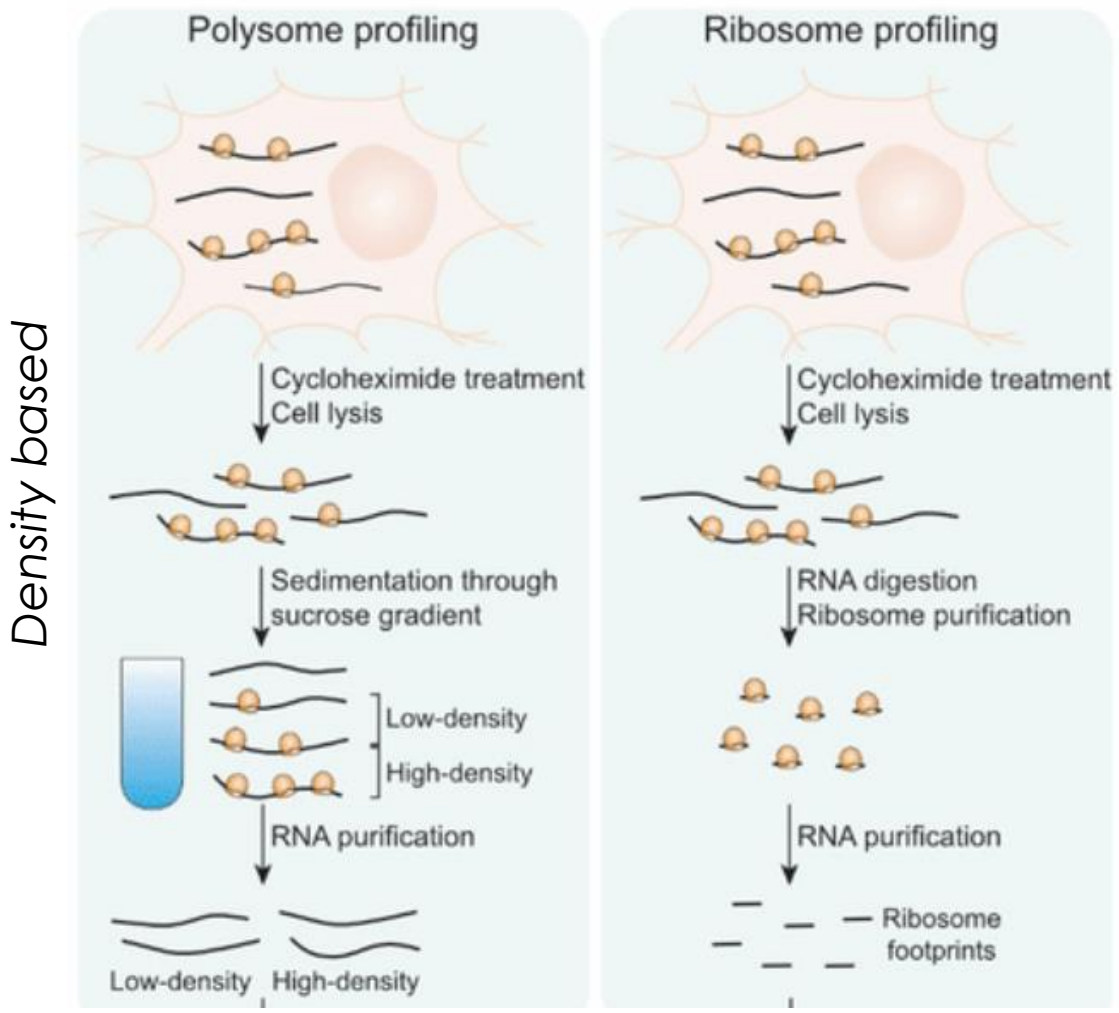
Central dogma (1957)



How do we detect which RNAs are transcribed?

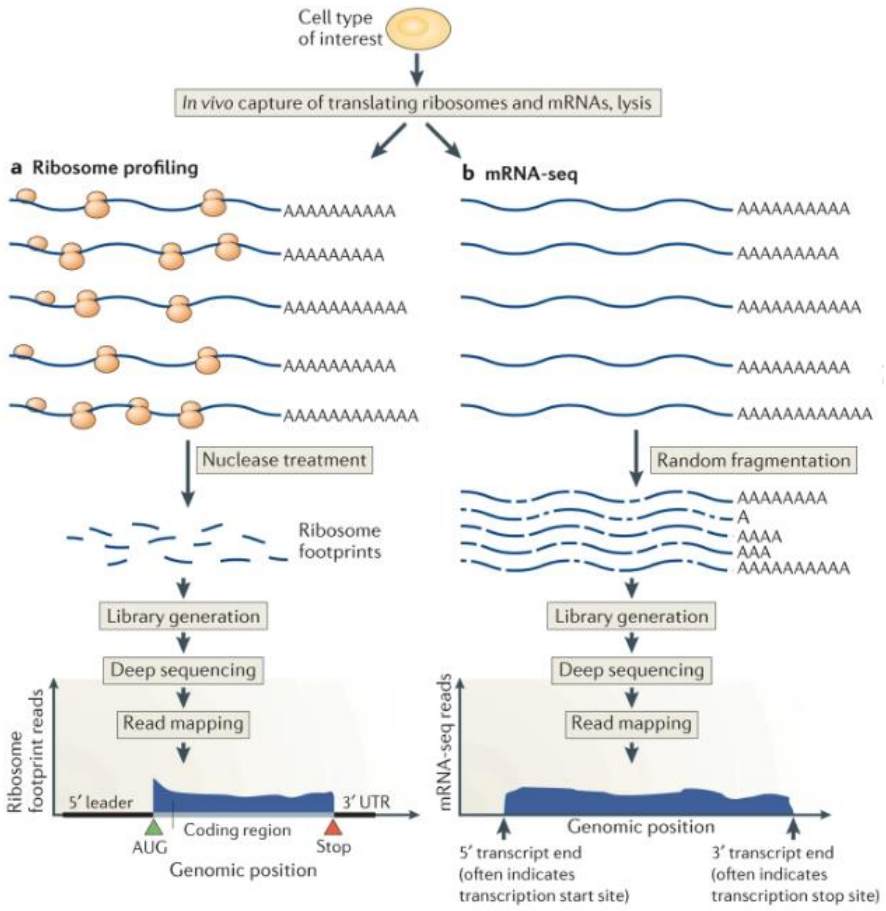


How do we detect which RNAs are translated?



Arava Y., et al: Genome-wide analysis of mRNA translation profiles in . Proc. Natl. Acad. Sci. U. S. A. 2003
Morales, Analysis of translation using polysome profiling, NAR, 2016

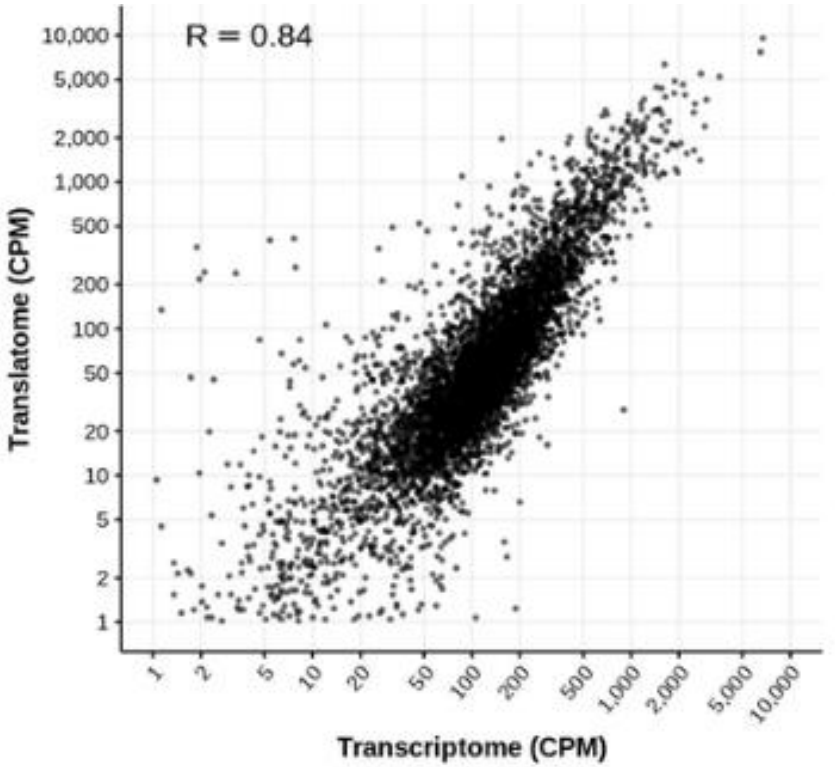
How do we detect which RNAs are transcribed and translated?



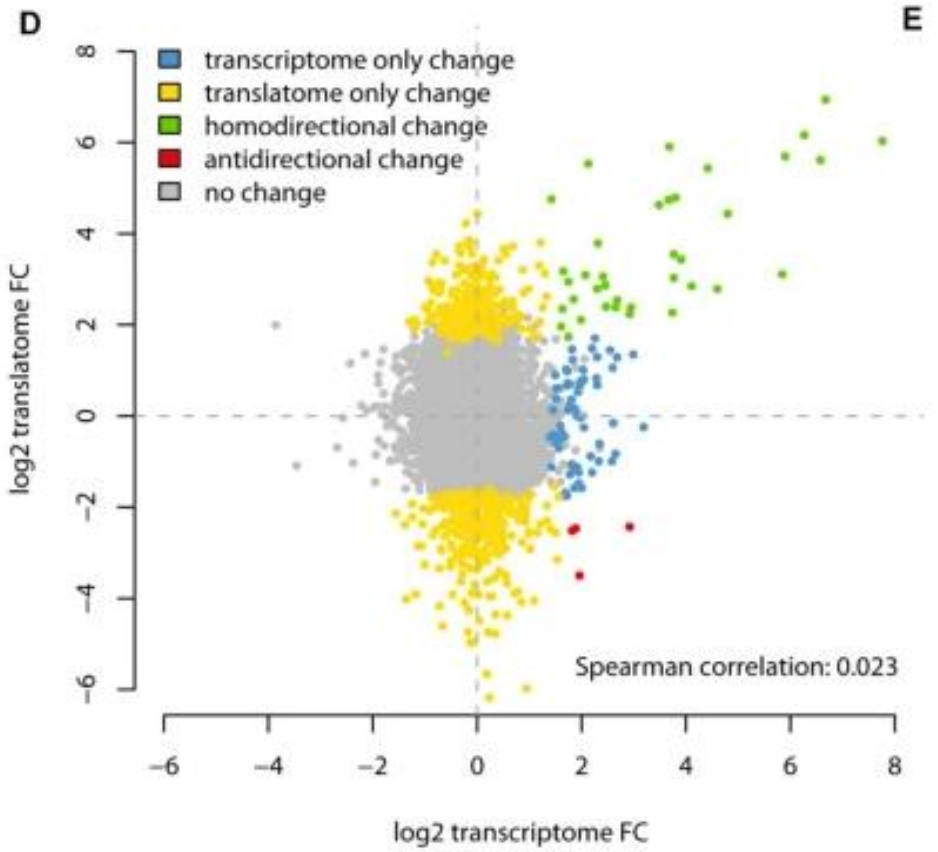
Bar, Weissman, Nature Reviews Molecular Cell Biology (2015)

Are transcriptome and translatome correlated?

Normal condition



EGF treatment



Why the transcriptome does not correlate with the translatome?

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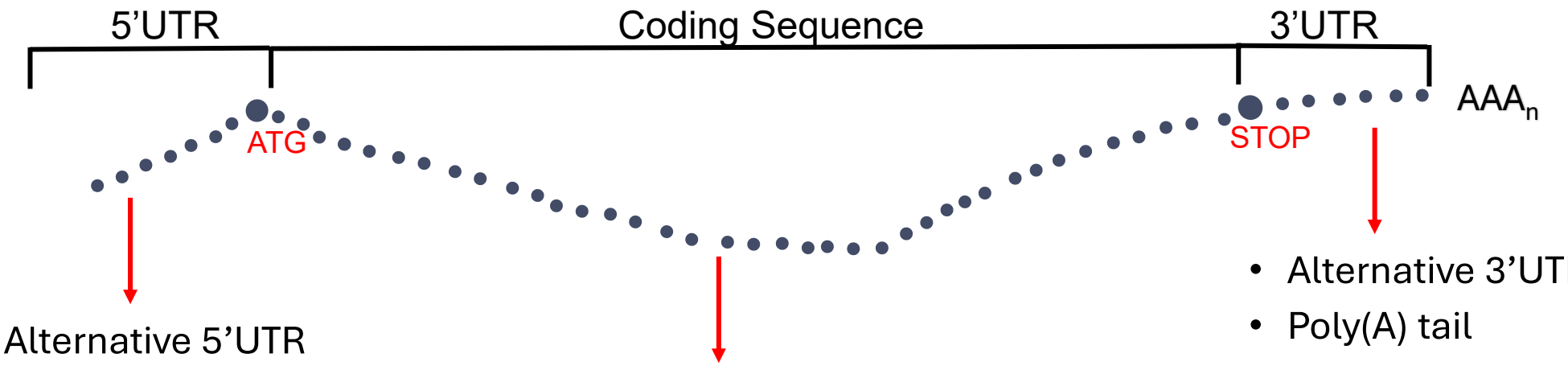


- Alternative 5'UTR
- uORF
- Epitranscriptome
- RBPs

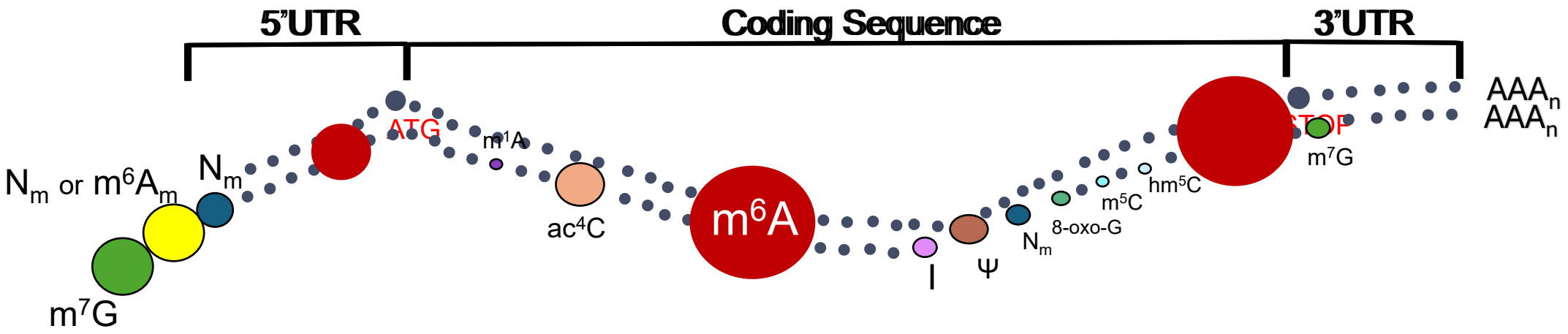
- Splicing
- RNA turnover
- RNA compartmentalization/localization
- RBPs

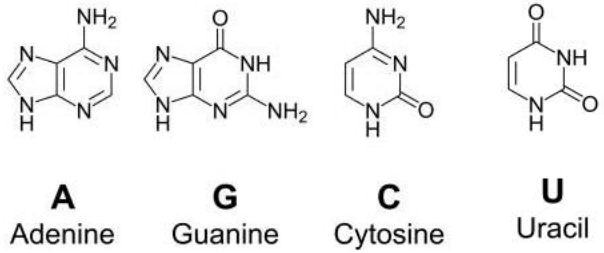
- Alternative 3'UTR
- Poly(A) tail
- Epitranscriptome
- microRNA
- RBPs

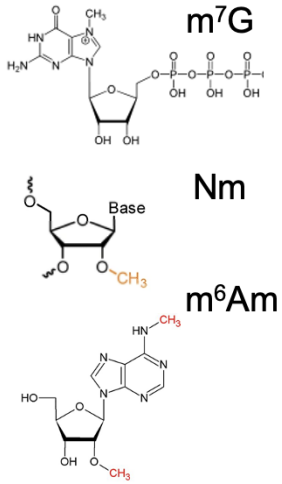
Why the transcriptome does not correlate with the translatome?

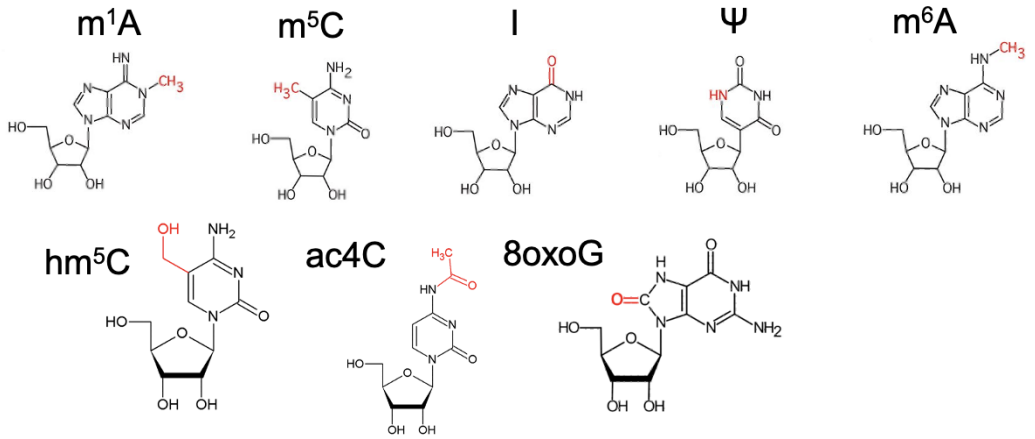
mRNA modifications

RNA modifications on mRNAs

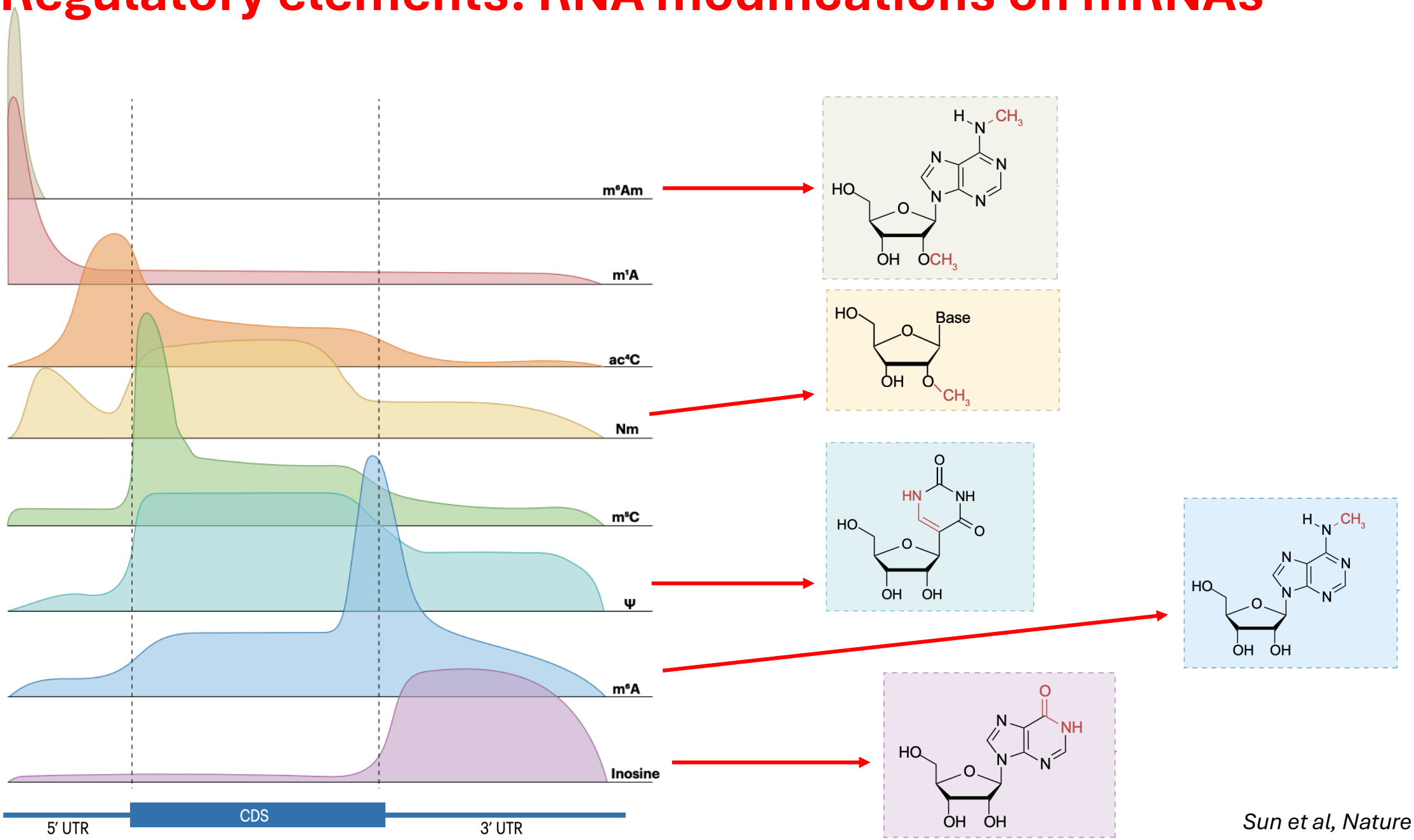








Regulatory elements: RNA modifications on mRNAs



Sun et al, Nature Reviews (2022)

RNA modifications on mRNAs: m^{6}A

When it was discovered…

Proc. Nat. Acad. Sci. USA
Vol. 71, No. 10, pp. 3971–3975, October 1974

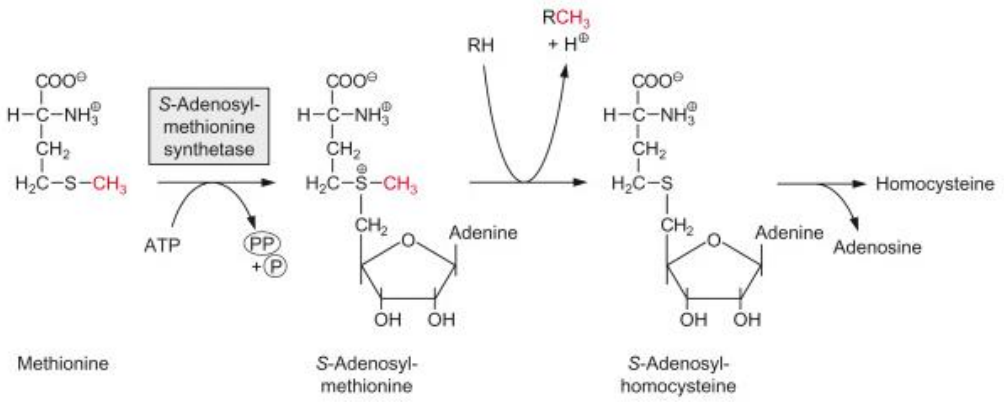
Identification of Methylated Nucleosides in Messenger RNA from Novikoff Hepatoma Cells

(RNA methylation/RNA processing/methylnucleoside composition)

RONALD DESROSIERS, KAREN FRIDERICI, AND FRITZ ROTTMAN*

The Department of Biochemistry, Michigan State University, East Lansing, Mich. 48824

Communicated by Anton Lang, July 8, 1974



ABSTRACT The poly(A) tract found in eukaryotic mRNA was used to study methylation in mRNA obtained from Novikoff hepatoma cells. Methyl labeling of RNA was achieved with L-[*methyl*-^{3}H]methionine under conditions that suppress radioactive incorporation into the purine ring. RNA that contains a poly(A) segment was obtained from polysomal RNA by chromatography on oligo(dT)–cellulose. Sucrose density gradient centrifugation of this RNA revealed a pattern expected for mRNA. The composition of the methyl-labeled nucleosides in the RNA was analyzed after complete enzymatic degradation to nucleosides. By use of DEAE–cellulose (borate) chromatography, which separates 2′-*O*-methylnucleosides from normal and base-methylated nucleosides, about 50% of the radioactivity was recovered in the 2′-*O*-methylnucleoside fraction and 50% in the base-methylnucleoside fraction. High-speed liquid chromatography (Aminex A-5) of the 2′-*O*-methylnucleoside fraction produced four peaks coincident with the four 2′-*O*-methylnucleoside standards. Analysis of the base-methylnucleoside fraction revealed a unique pattern. While ribosomal RNA and tRNA possessed complex base-methylnucleoside patterns, the distribution in mRNA was quite simple, consisting predominantly of N^6-methyladenosine. These results demonstrate a unique distribution of methylated nucleosides in mRNA. By analogy to ribosomal RNA synthesis, the presence of methylnucleosides in mRNA may reflect a cellular mechanism for the selective processing of certain mRNA sequences.

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RNA modifications on mRNAs: m^{6}A

When it was discovered…

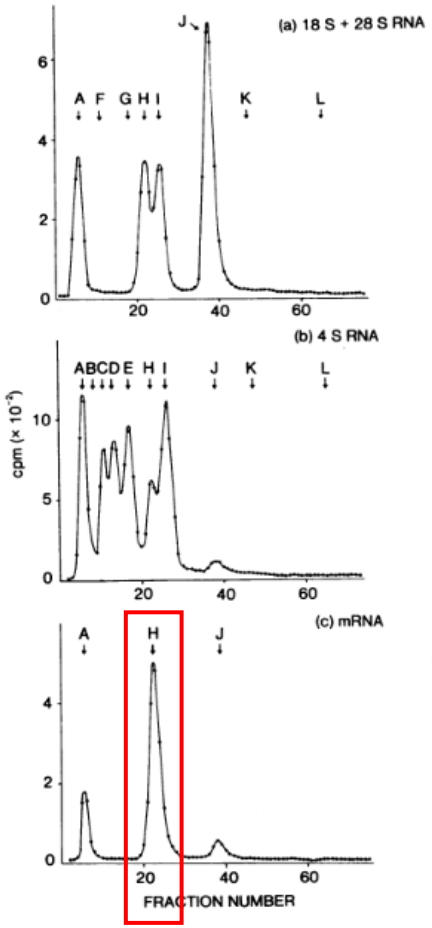

FIG. 3. High-speed liquid chromatography of base-methylnucleoside fraction. Two hundred fifty microliters of each base-methylnucleoside fraction, dissolved in H_2O, were injected onto a high-speed liquid chromatography column. The column was developed at 2500 lbs./inch² at 31.5°. The flow rate was 0.8 ml/min, and 0.67-ml fractions were collected. At fraction 35, the volume collected per fraction was changed to 1.35 ml. The letters correspond to the location of the following standards: A, 3-methyluridine, thymine riboside, and uridine; B, 1-methylinosine; C, 1-methylguanosine; D, N^2-dimethylguanosine; E, N^2-methylguanosine; F, guanosine; G, adenosine and N^4-methylcytidine; H, N^6-methyladenosine; I, 5-methylcytidine; J, N^6-dimethyladenosine; K, 1-methyladenosine; L, 7-methylguanosine. (a) 28S + 18S RNA; (b) 4S RNA; (c) mRNA.

ABSTRACT The poly(A) tract found in eukaryotic mRNA was used to study methylation in mRNA obtained from Novikoff hepatoma cells. Methyl labeling of RNA was achieved with L-[*methyl*-^{3}H]methionine under conditions that suppress radioactive incorporation into the purine ring. RNA that contains a poly(A) segment was obtained from polysomal RNA by chromatography on oligo(dT)–cellulose. Sucrose density gradient centrifugation of this RNA revealed a pattern expected for mRNA. The composition of the methyl-labeled nucleosides in the RNA was analyzed after complete enzymatic degradation to nucleosides. By use of DEAE–cellulose (borate) chromatography, which separates 2′-*O*-methylnucleosides from normal and base-methylated nucleosides, about 50% of the radioactivity was recovered in the 2′-*O*-methylnucleoside fraction and 50% in the base-methylnucleoside fraction. High-speed liquid chromatography (Aminex A-5) of the 2′-*O*-methylnucleoside fraction produced four peaks coincident with the four 2′-*O*-methylnucleoside standards. Analysis of the base-methylnucleoside fraction revealed a unique pattern. While ribosomal RNA and tRNA possessed complex base-methylnucleoside patterns, the distribution in mRNA was quite simple, consisting predominantly of N^6-methyladenosine. These results demonstrate a unique distribution of methylated nucleosides in mRNA. By analogy to ribosomal RNA synthesis, the presence of methylnucleosides in mRNA may reflect a cellular mechanism for the selective processing of certain mRNA sequences.

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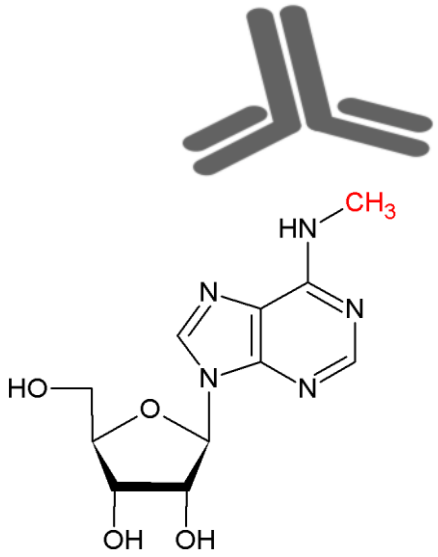
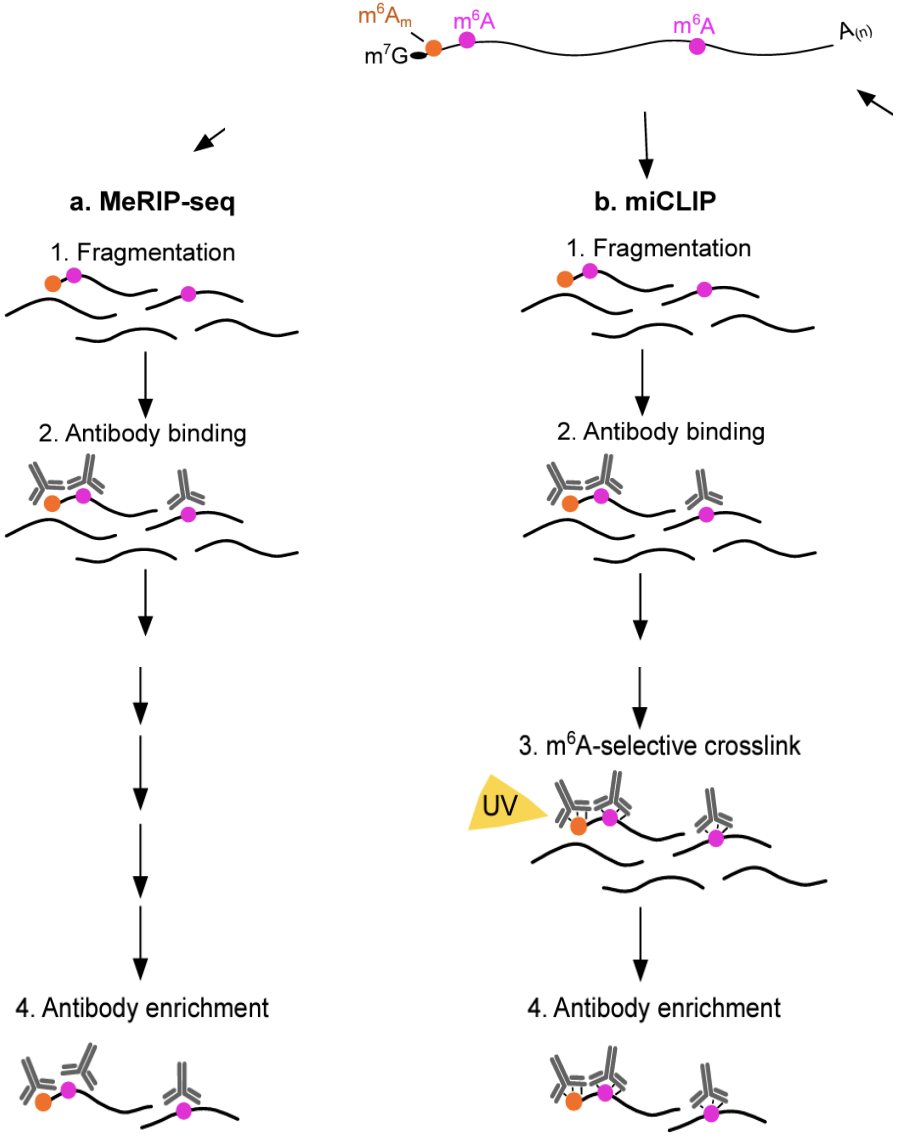
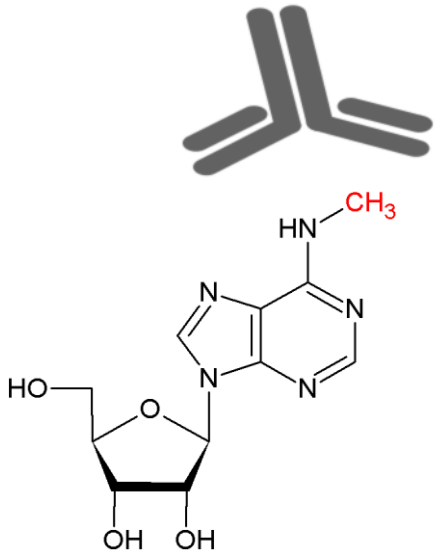
RNA modifications on mRNAs: m^{6}A

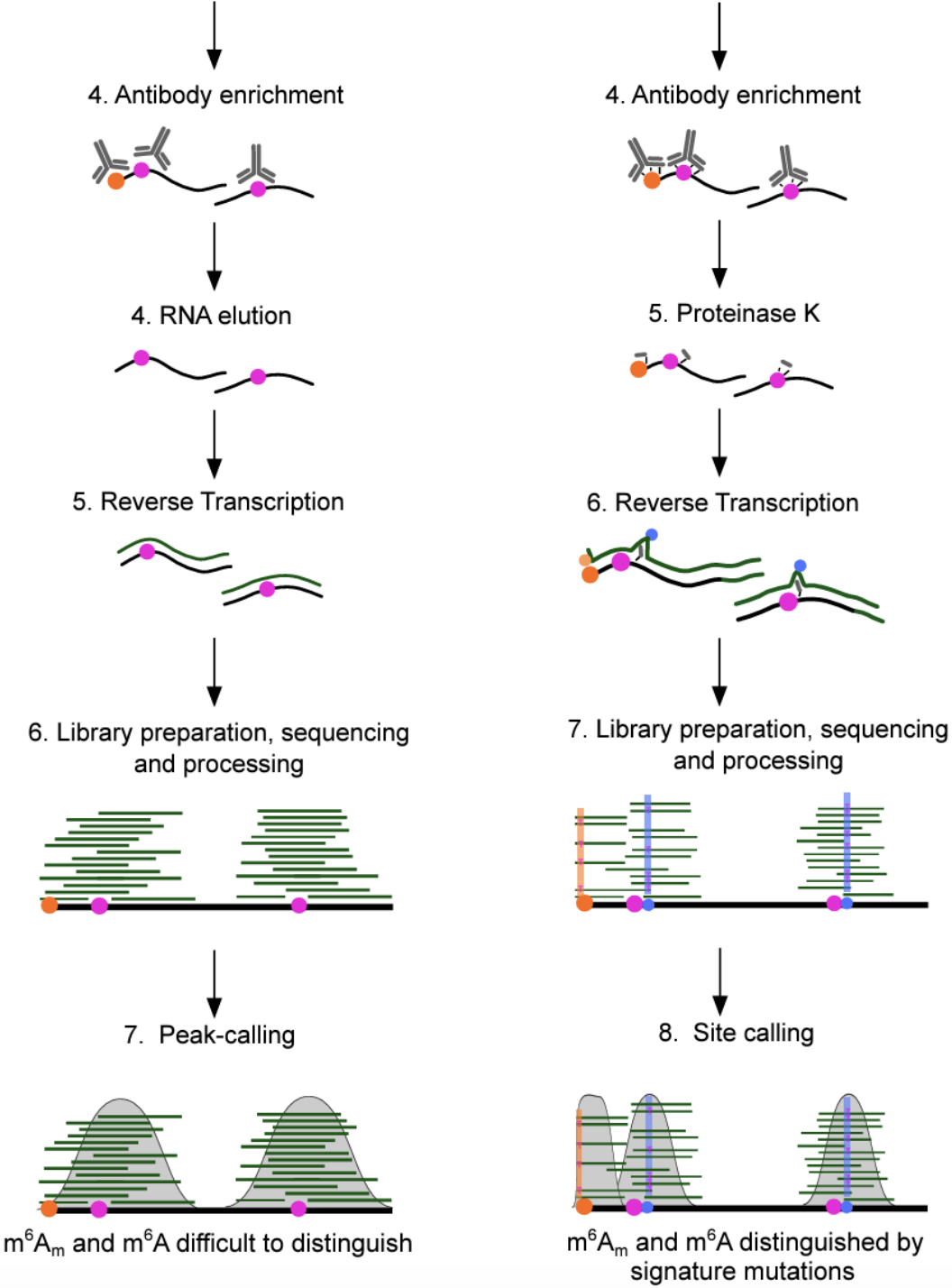
When it was mapped using m^{6}A-seq (antibody-based method)





When it was mapped using m⁶A-seq (antibody-based method)

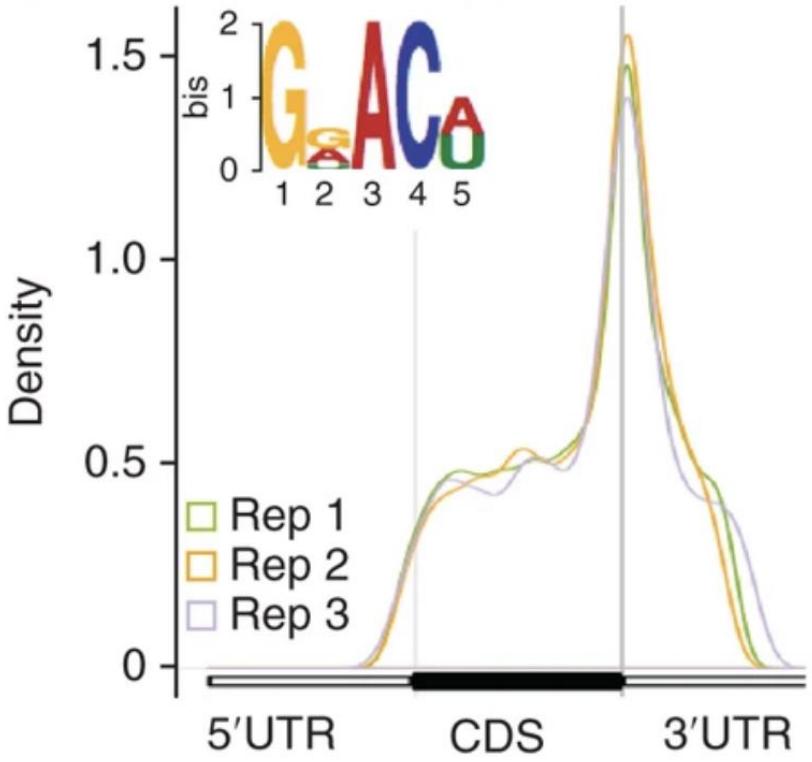




RNA modifications on mRNAs: m^{6}A

Major findings:

- ≈ 35% of the transcriptome is modified by m^{6}A
- Present in “DRACH” sequences in coding sequence (CDS) and 3’ UTR



RNA modifications on mRNAs: m^6A

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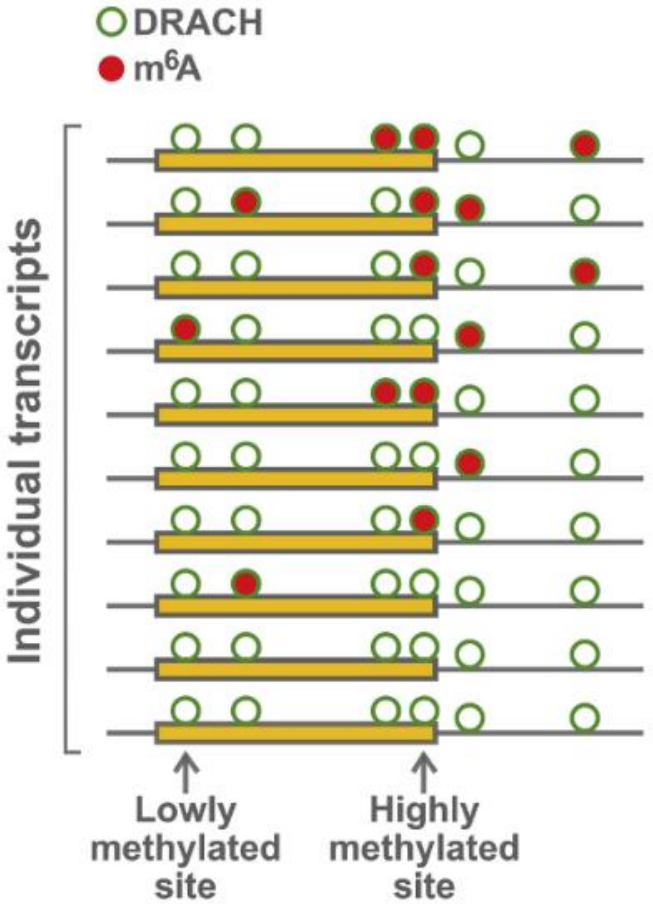
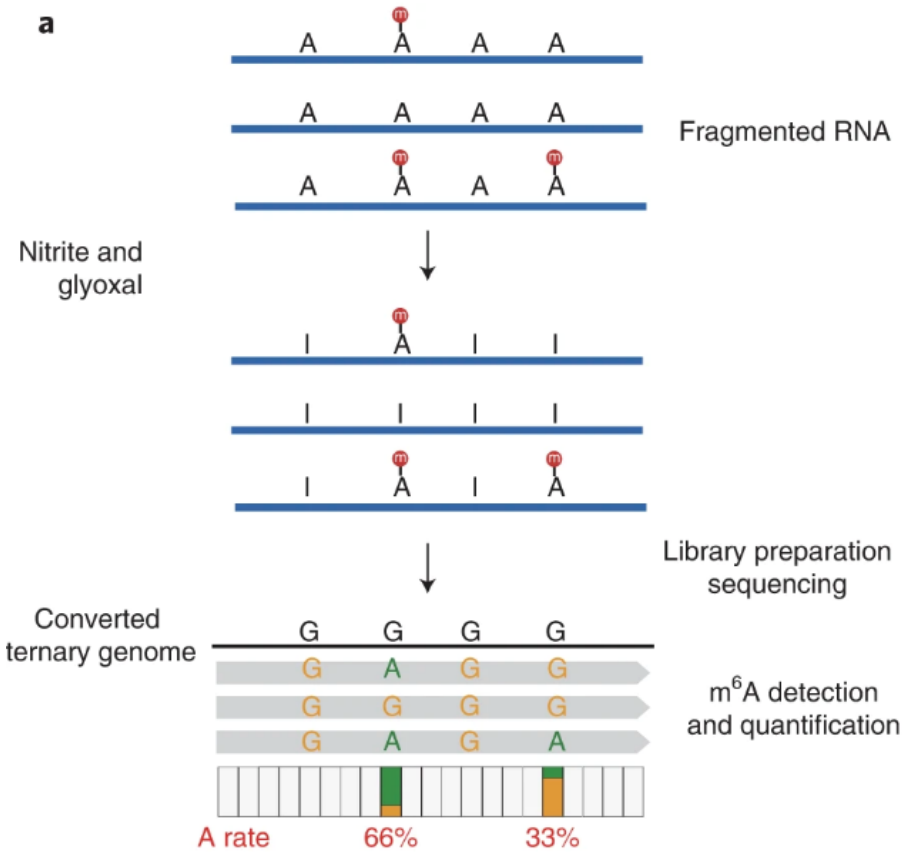
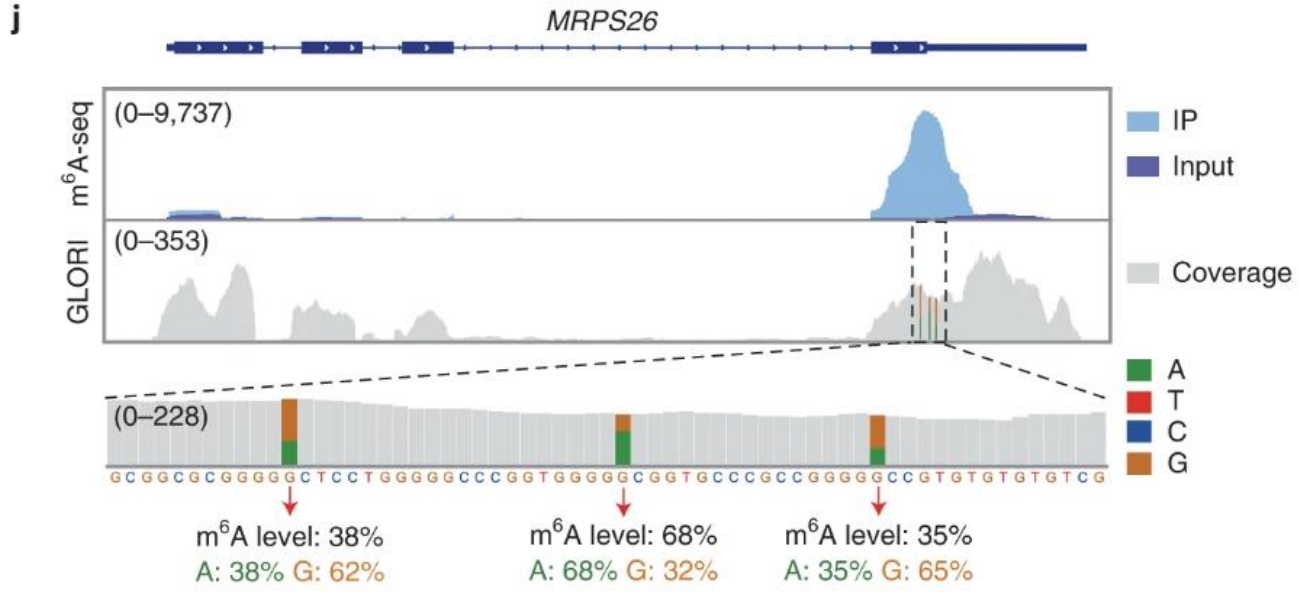
- Present in “DRACH” sequences in coding sequence (CDS) and 3’ UTR
- multiple m^6A sites per transcript



RNA modifications on mRNAs: m^{6}A

Now mapped using GLORI (chemical-based method)





RNA modifications on mRNAs: m^6A

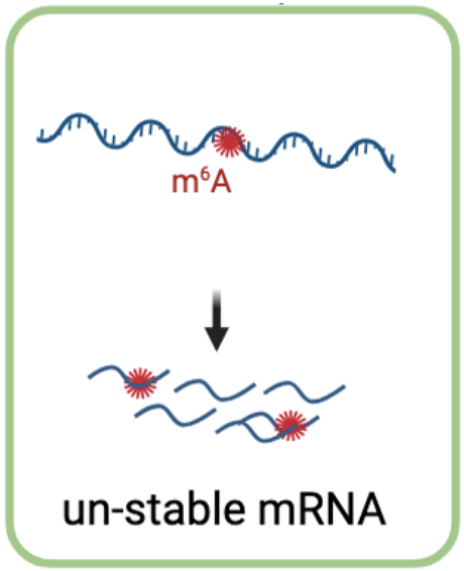
Now mapped using Nanopore (Direct RNA seq)

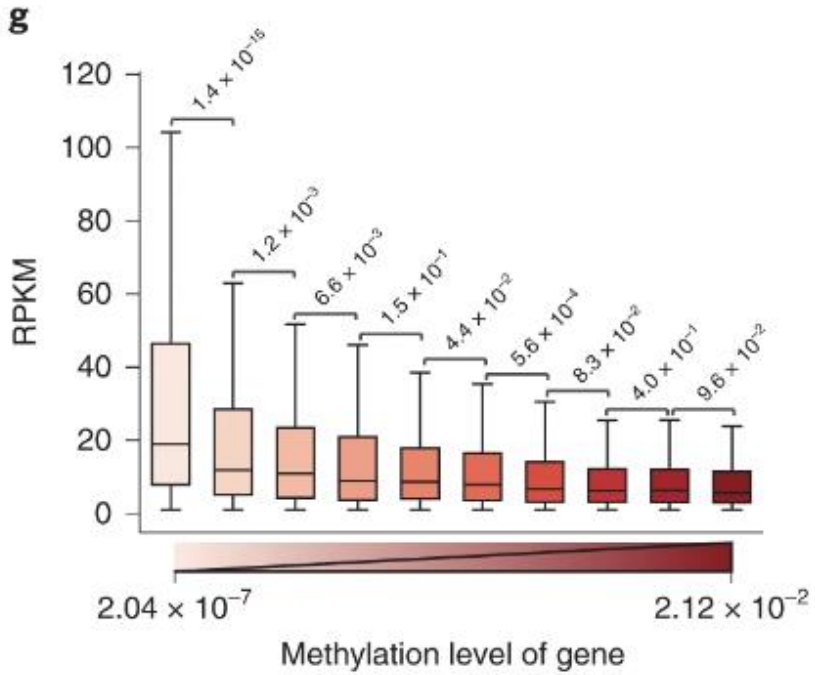
Pro:
Direct RNA seq
Long reads
Sequence anywhere
Fully scalable
Real-time sequencing

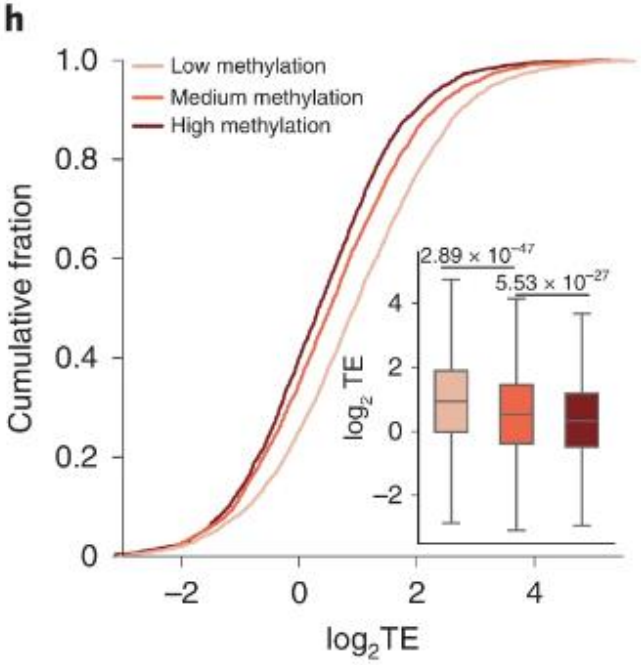
Cons:
Generation of less data
Less accuracy
Specific analysis pipeline

RNA modifications on mRNAs: m^{6}A

m^{6}A is a mark of mRNA degradation



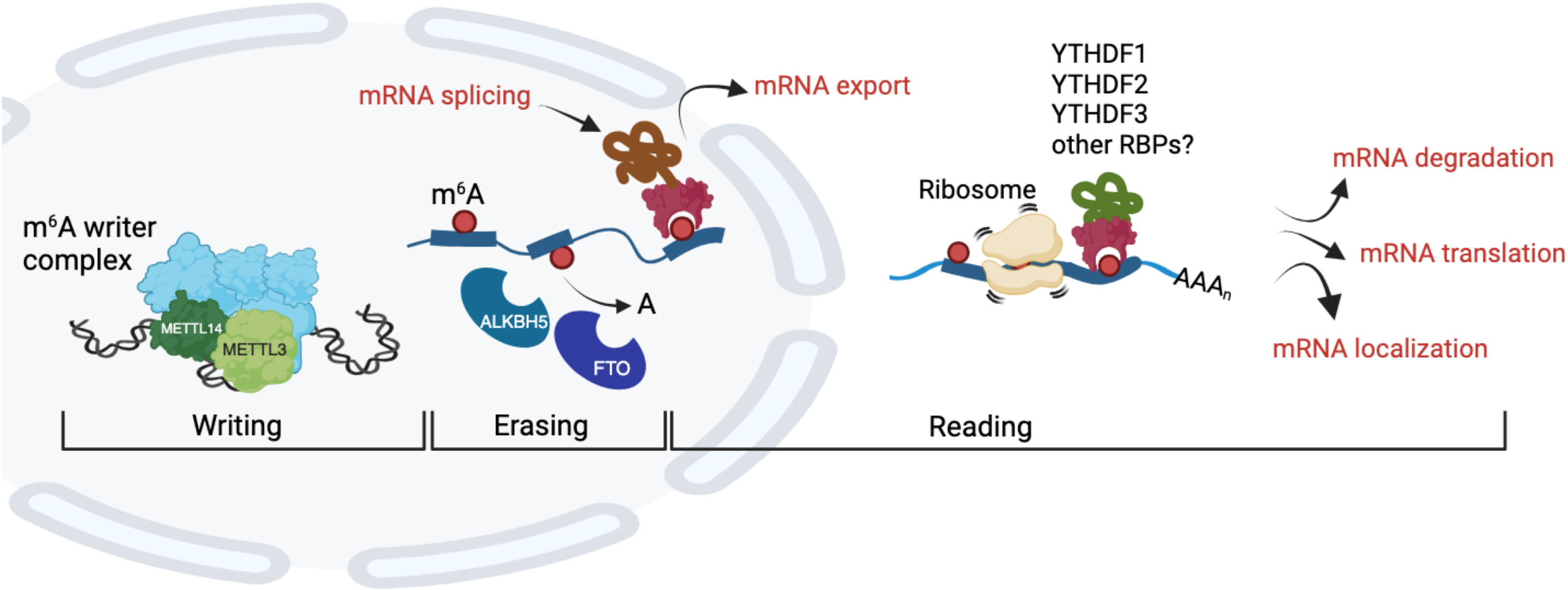




Q: Do you think that a housekeeping gene would be methylated?

The m^{6}A mRNA life cycle

What controls m^{6}A?

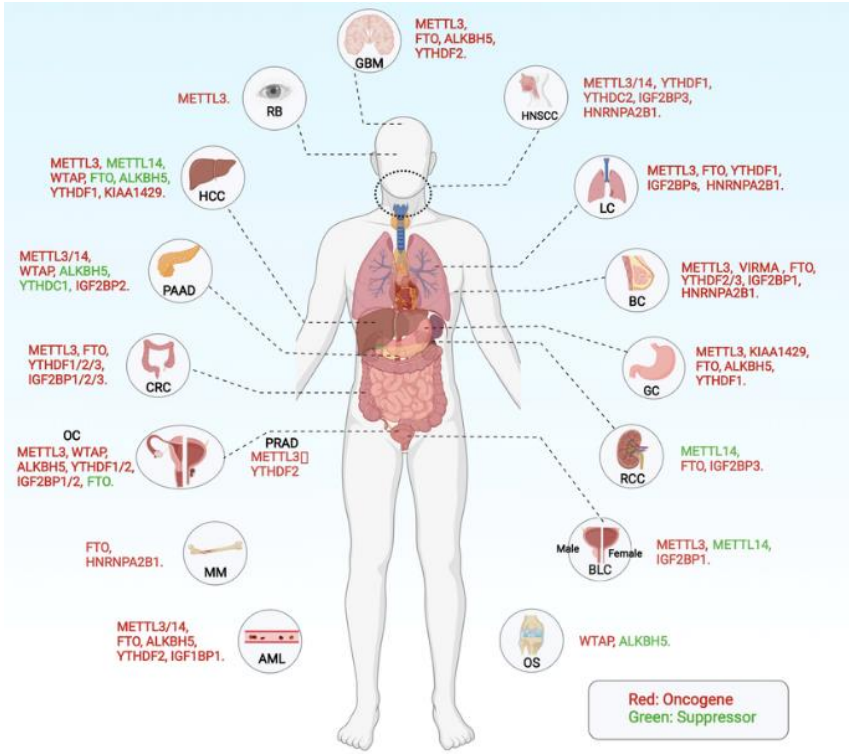


Adapted from Zaccara et al., Nat Rev Mol Cell Biol 2019

m^{6}A regulators are deregulated in cancer

Major findings:

- m^{6}A regulators are rarely mutated in cancer
- Common is the de-regulation of m^{6}A regulators (upregulation/downregulation)



m^{6}A regulators are deregulated in cancer

Major findings:

- m6A regulators are rarely mutated in cancer
- Common is the de-regulation of m^{6}A regulators (upregulation/downregulation)
- Impact on <u>specific m^{6}A mRNAs</u>

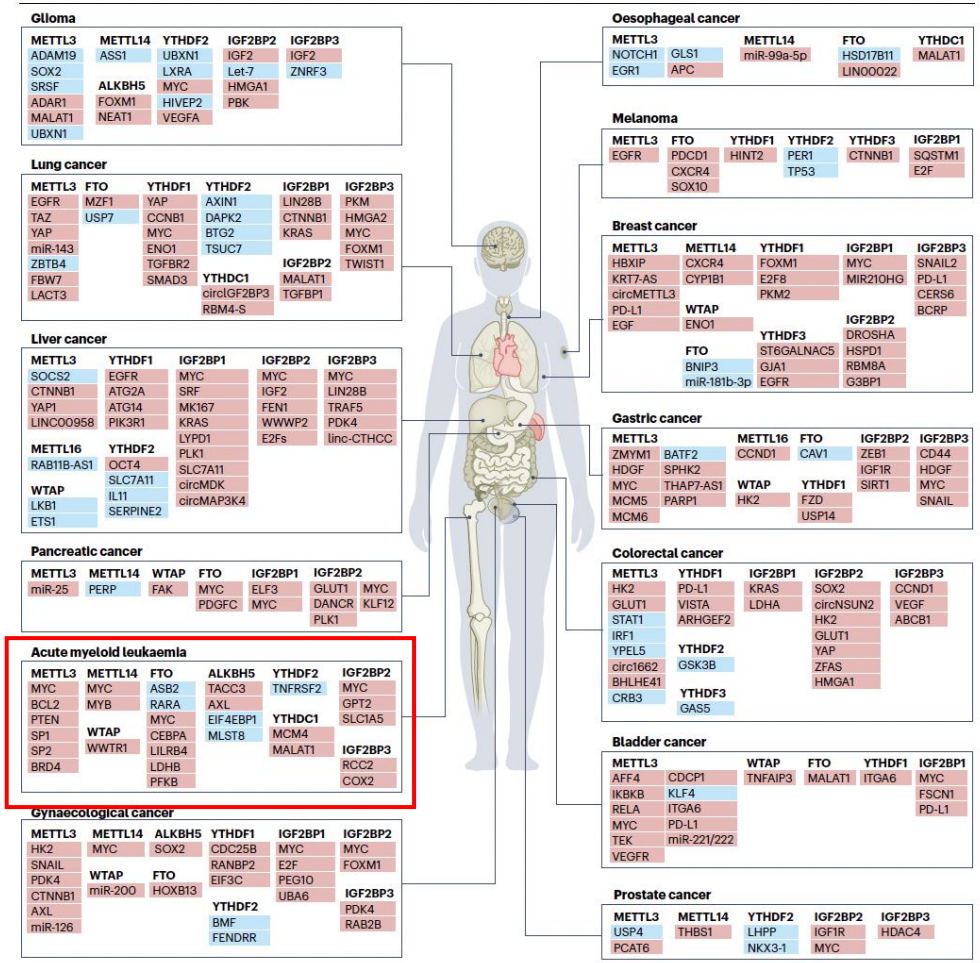
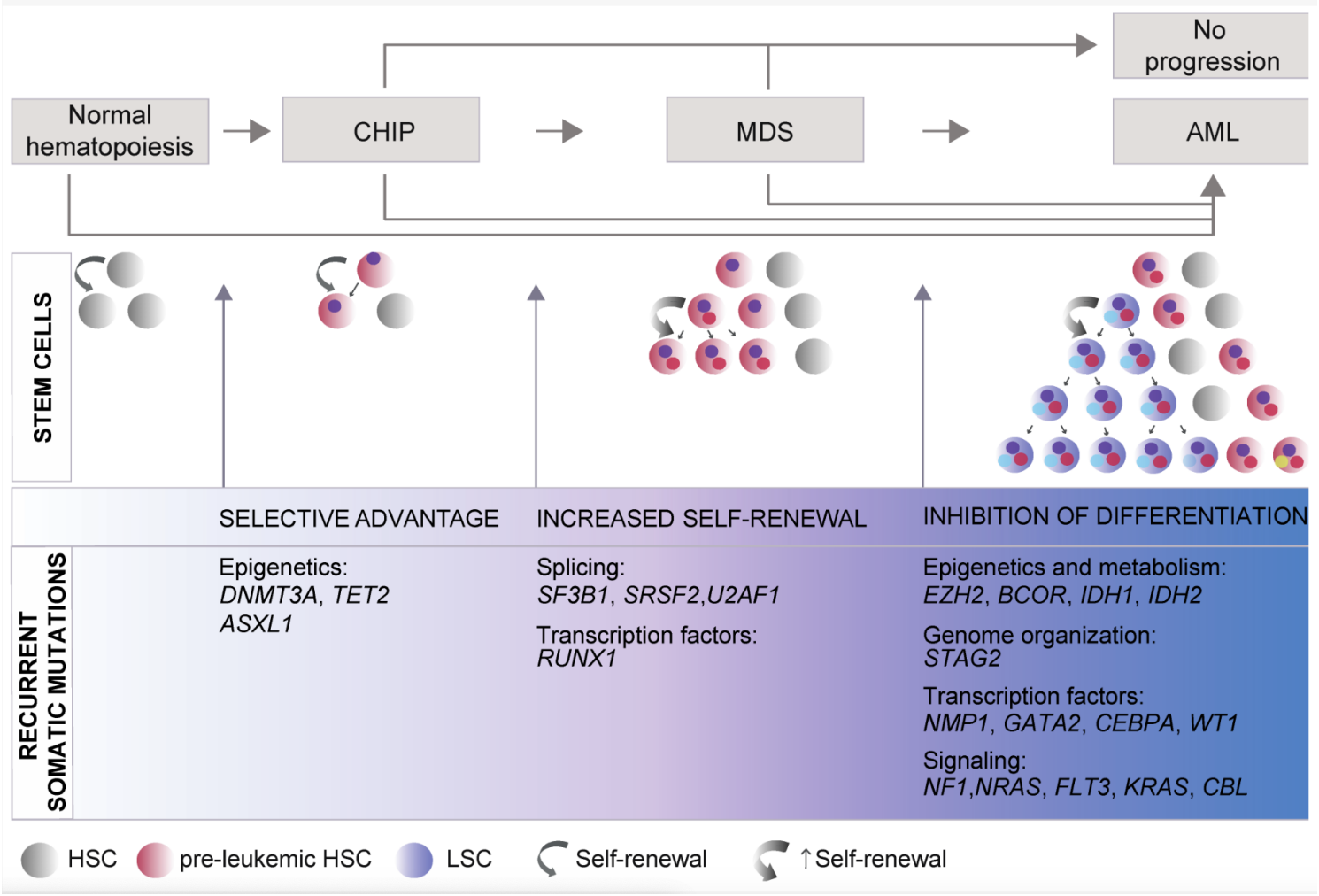
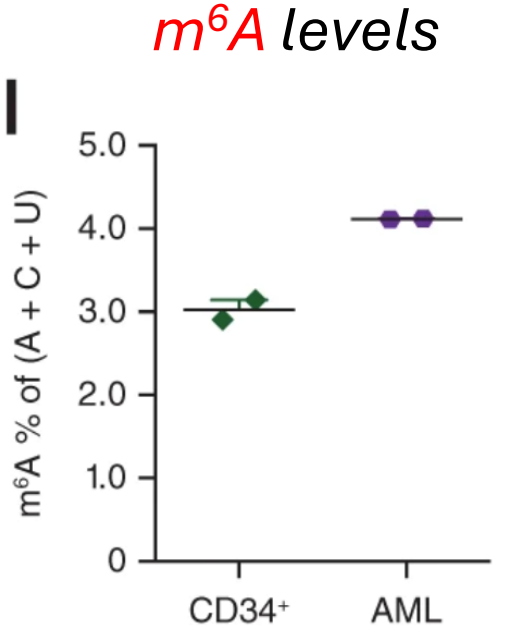


Fig. 2 | Oncogenic roles of m^{6}A modifiers. Tumour-promoting roles of N^6-methyladenosine (m^{6}A) modifiers, including m^{6}A writers (METTL3, METTL14, METTL16 and WTAP), m^{6}A erasers (FTO and alkB homologue 5 (ALKBH5)) and m^{6}A readers (insulin-like growth factor 2 mRNA-binding proteins 1–3 (IGF2BP1–3), YTH domain-containing family proteins 1–3 (YTHDF1–3) and YTH domain-containing protein 1 (YTHDC1)), and their respective downstream targets, including coding and non-coding RNAs, are listed for the relevant cancer types. Targets positively regulated (upregulated) by m^{6}A modifiers are in red, while negatively regulated (downregulated) targets are in blue.

Effect of m^{6}A on Acute Myeloid Leukemia:



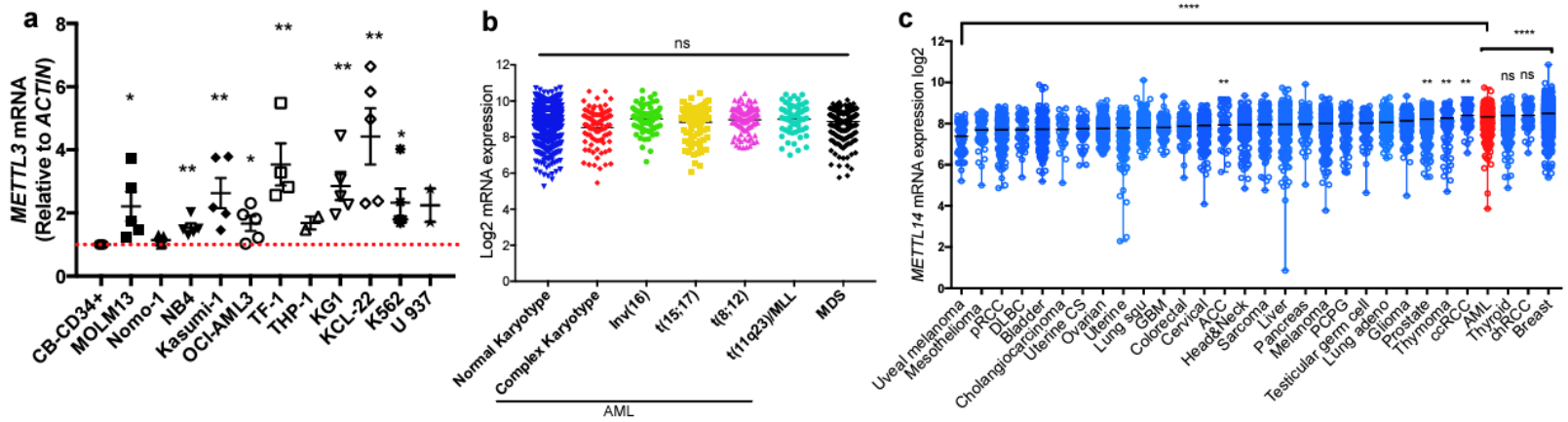


Andreas Trumpp and Simon Haas, Cell, 2022
Vu et al, Nature Medicine 2017

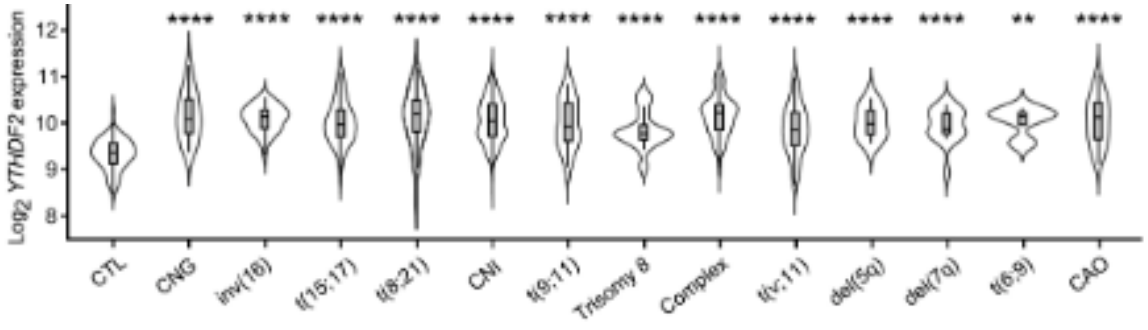
m^{6}A and AML

m^{6}A regulators are upregulated in AML

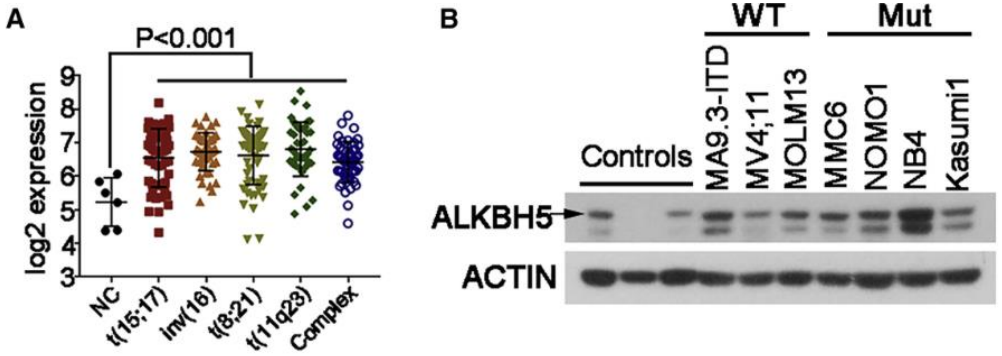
METTL3



YTHDF

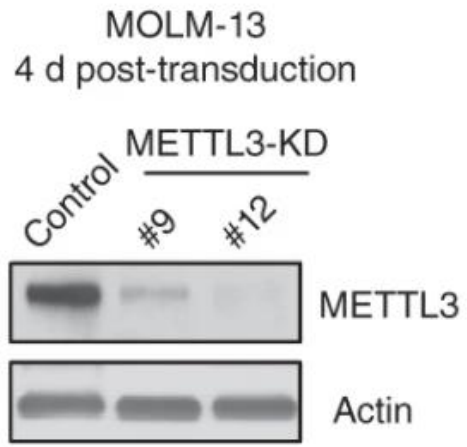


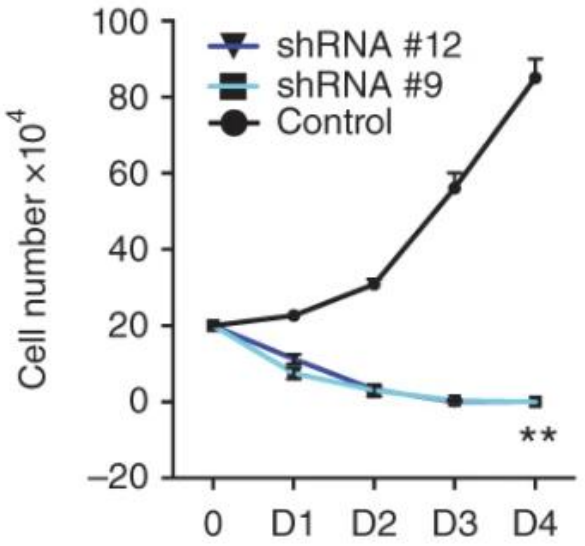
ALKBH5

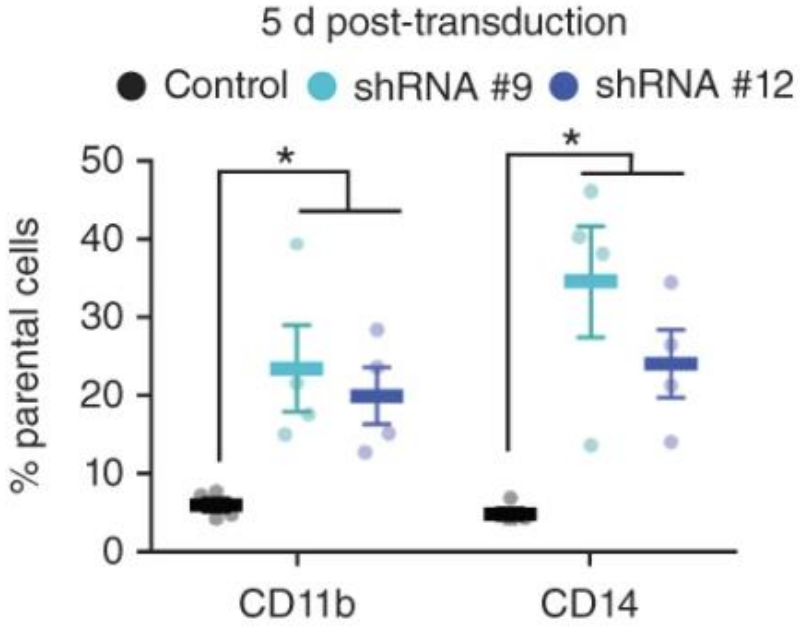


m^6A controls the differentiation of AML cells

The role of METTL3 in AML





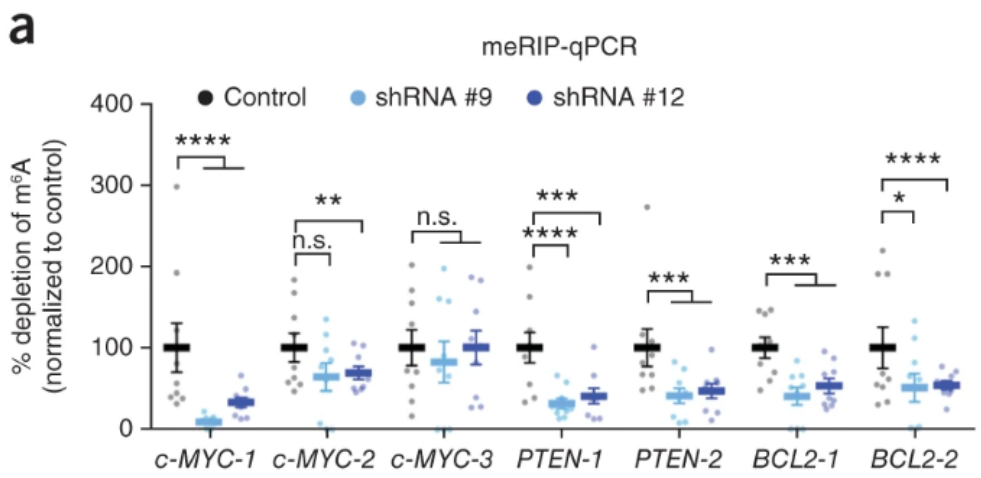


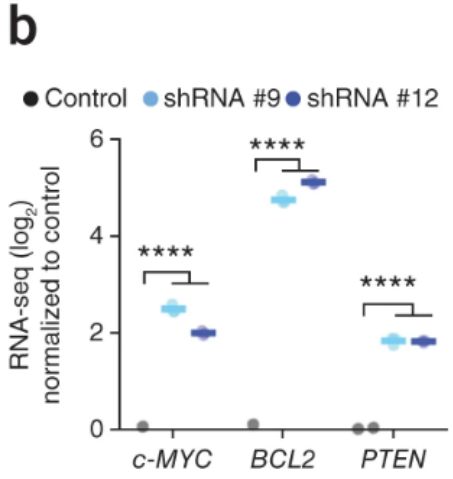
Vu et al., Nature Medicine 2017

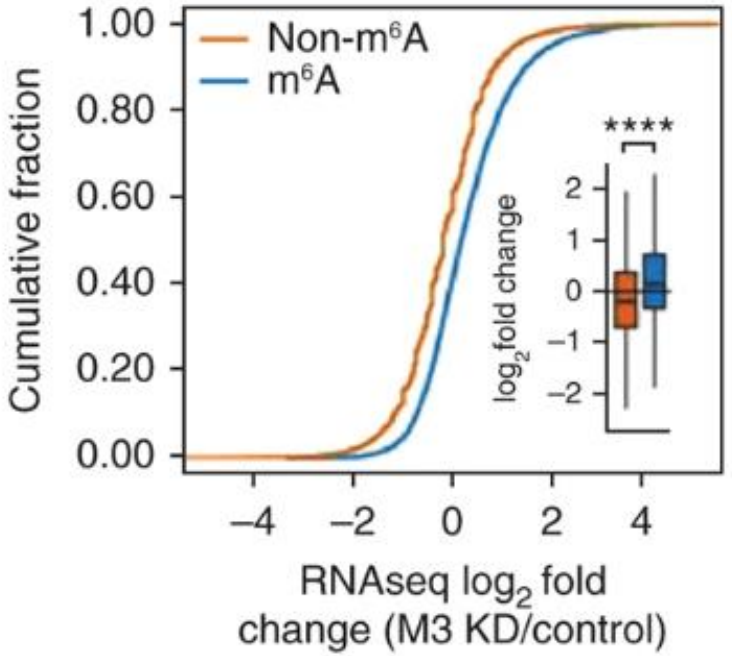
m^{6}A controls the differentiation of AML cells

The role of METTL3 in AML

Upon METTL3 depletion, m^{6}A mRNAs are more stable





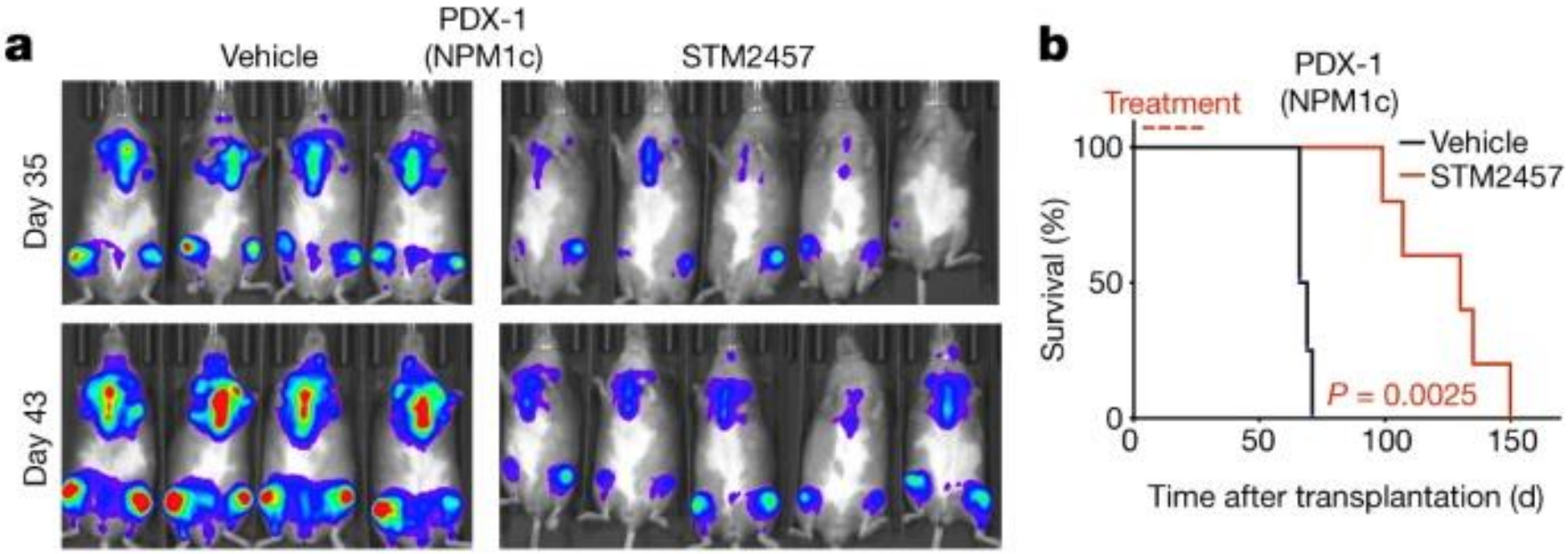


m^{6}A controls the differentiation of AML cells

The role of METTL3 in AML

This inhibitor is now in clinical trial for AML patients

Fig. 4: STM2457 prevents AML expansion and reduces the number of key leukaemia stem cells in vivo.

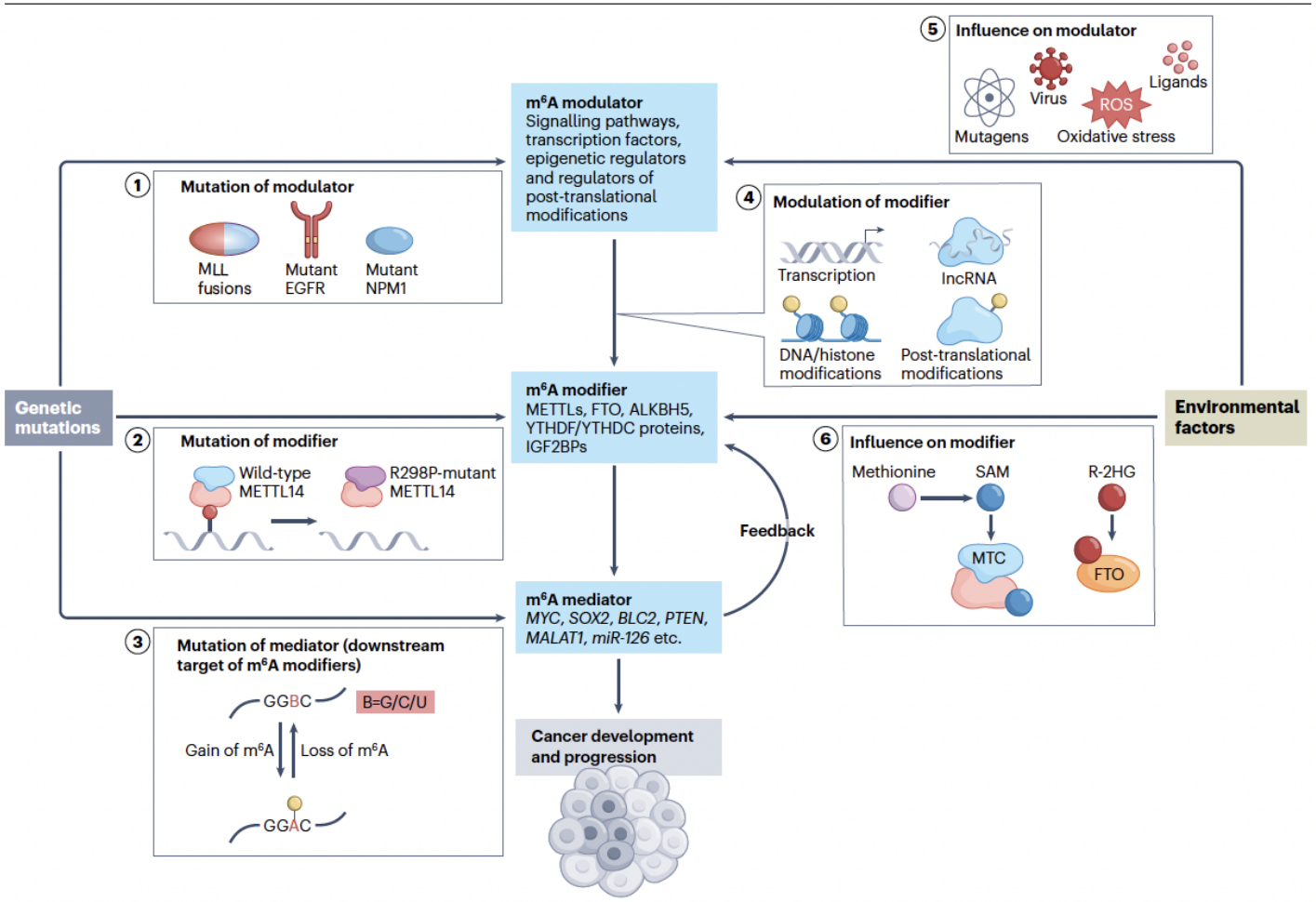


m^6A regulators are deregulated in cancer

- m^6A is a marker of instability
- It is controlled by m^6A regulators
- Changes in these regulators have been associated with cancer

m^{6}A regulators are deregulated in cancer

- Mutations of modulators or modulation of transcription factors
- Changes in metabolites
- Changes in PTMs or localization of m^{6}A regulators
- Mutation in GAC sequences



The future of the RNA modification field is to understand how they are controlled

RNA modifications on mRNAs: m^{6}A

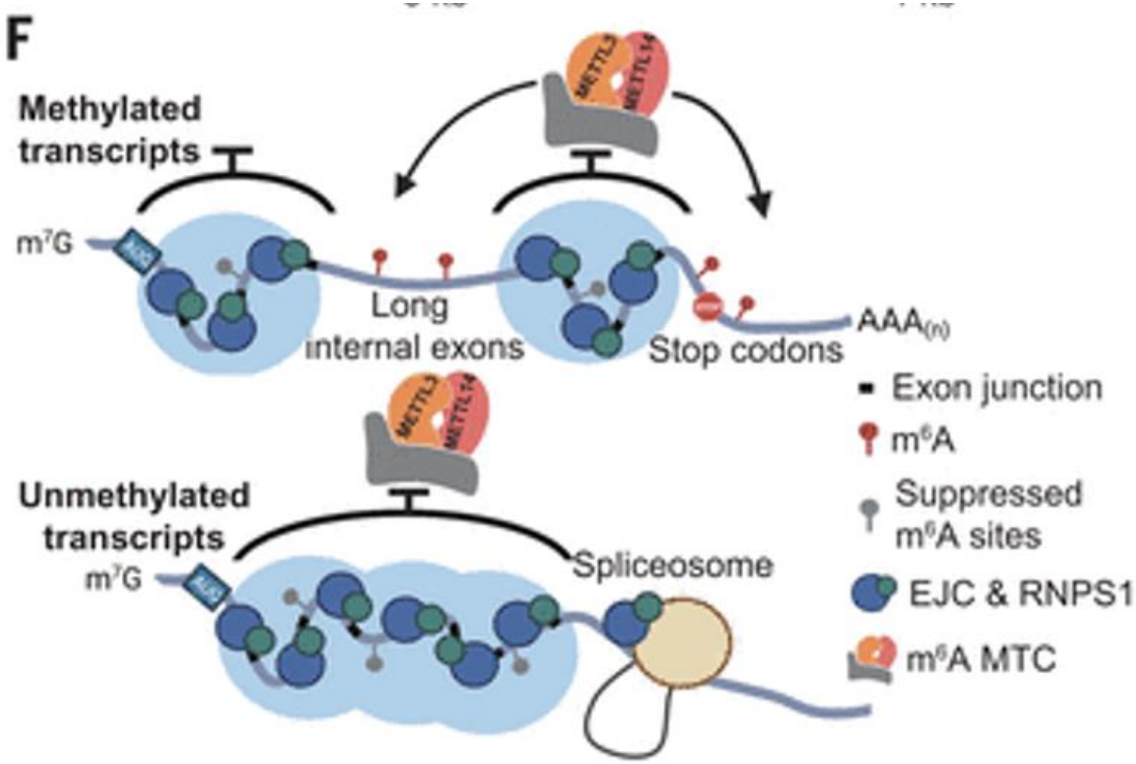
Gene architecture defines where m^{6}A gets added

Major findings:

- m^{6}A is hard-coded

- Long exons are methylated,

shorter exons (<100nt) are not methylated because the EJC (exon junction complex) does not leave space for METTL3 to bind



The future of the RNA modification field is to understand how they are controlled

Regulatory elements: RNA modifications on mRNAs

Why do we need RNA modifications?

- Change mRNA degradation rate
- Change mRNA localization
- Control cellular differentiation and multiple other cellular events
- **Differentiate self vs non-self RNA**

THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE 2023

Illustrations: Niklas Elmehed

Katalin Karikó Drew Weissman

"for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19"

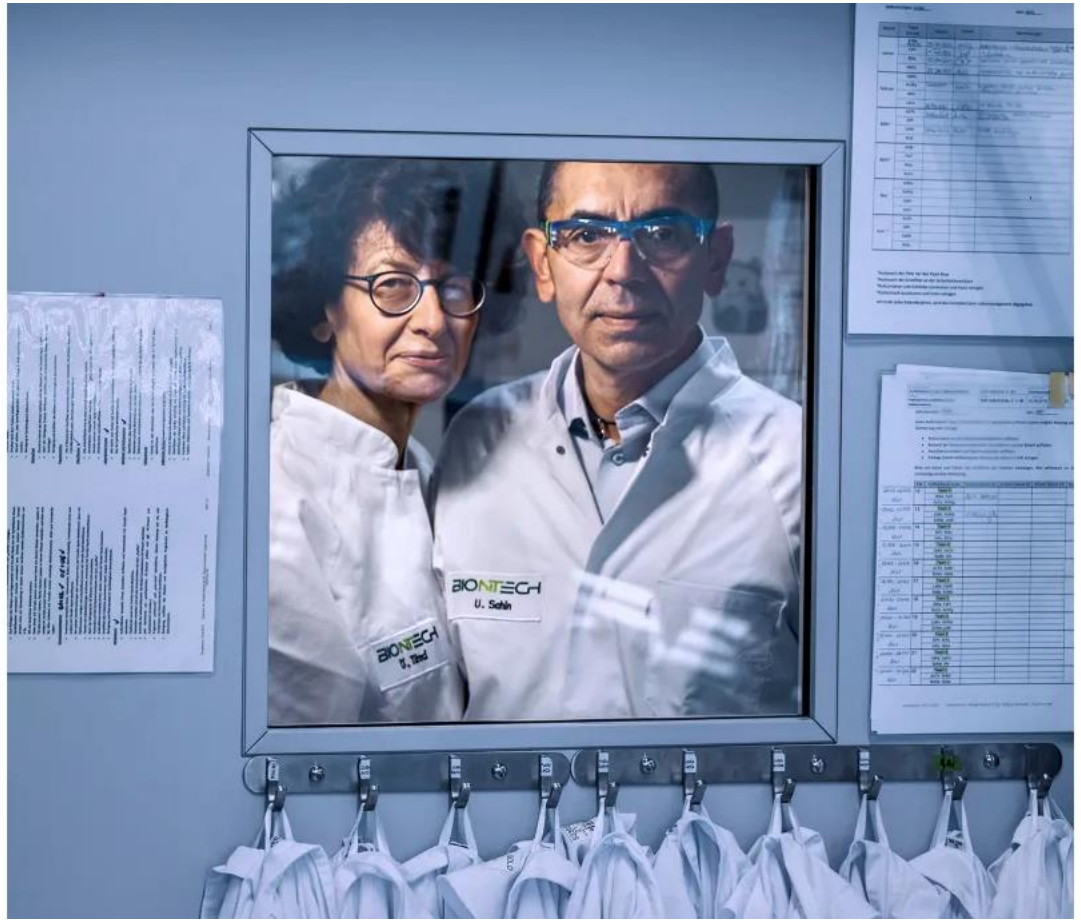
THE NOBEL ASSEMBLY AT KAROLINSKA INSTITUTET

Regulatory elements: RNA modifications on mRNAs

TIME

← THE 100 MOST INFLUENTIAL PEOPLE OF 2023

Ozlem Tureci and Ugur Sahin



Regulatory elements: RNA modifications on mRNAs

Immunity, Vol. 23, 165–175, August, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.immuni.2005.06.008

Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA

Katalin Karikó,[1,*] Michael Buckstein,[2] Houping Ni,[2] and Drew Weissman[2]
[1]Department of Neurosurgery
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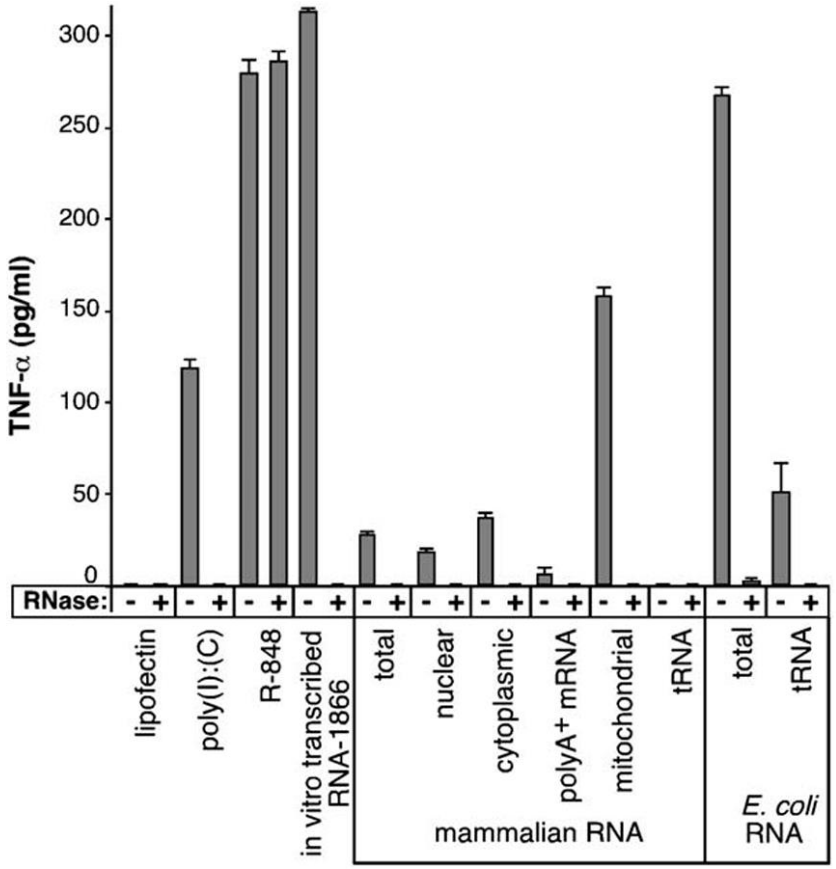
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

"It has been known for decades that selected DNA and RNA molecules have the unique property to activate the immune system. The sequence and structural microheterogeneity of DNA was starting to be appreciated. However, a question emerges whether the immunogenicity of RNA is under the control of similar types of modifications."



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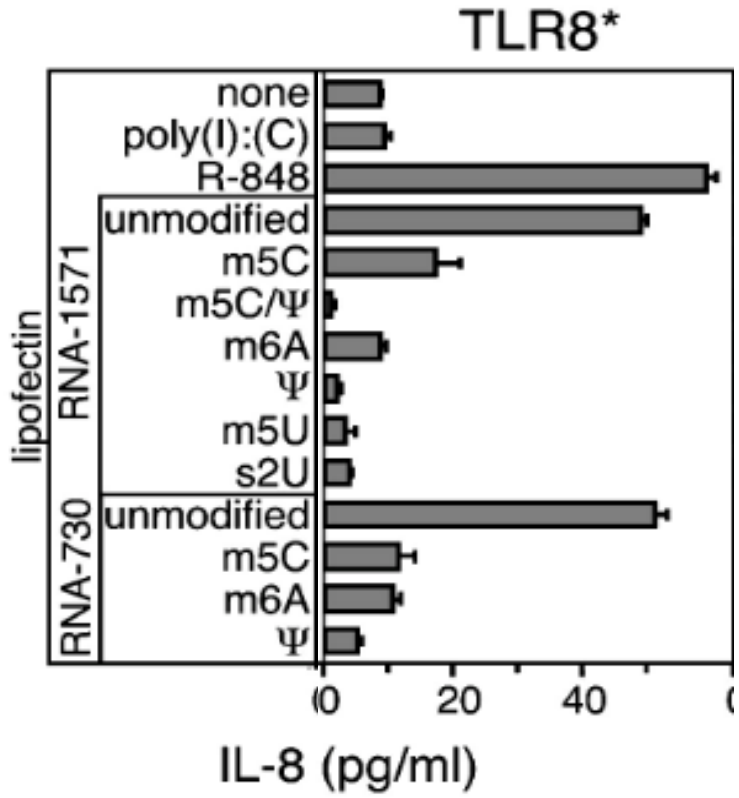
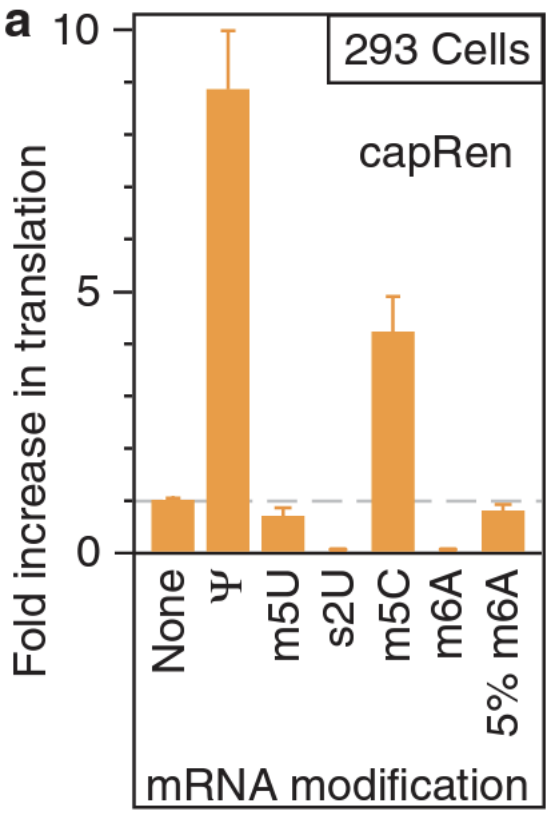
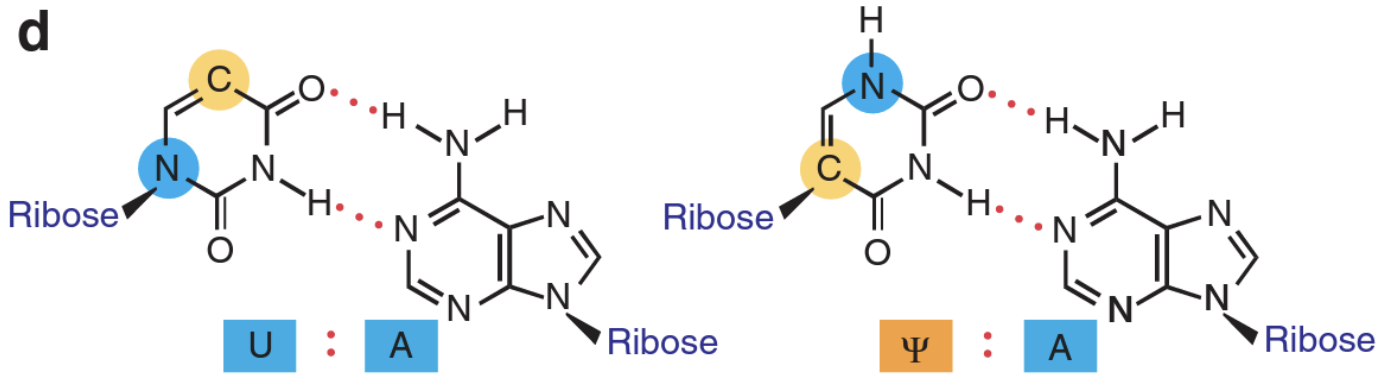


Regulatory elements: RNA modifications on mRNAs

Ψ-modified mRNA is also more translated

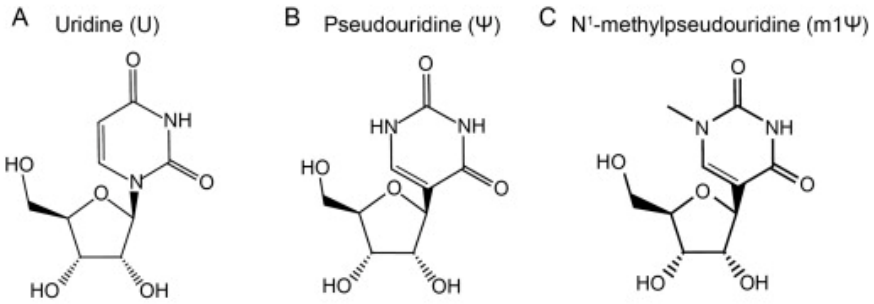


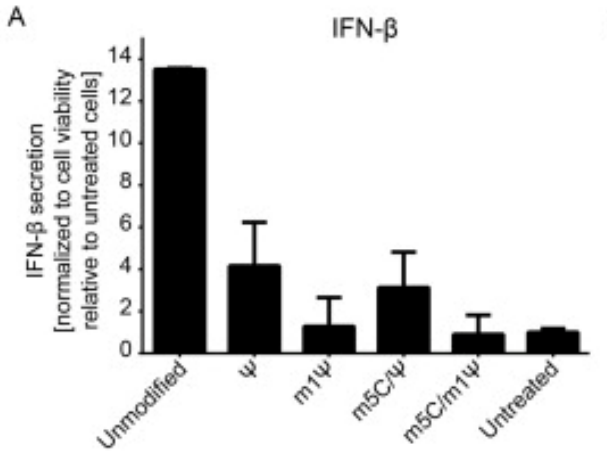


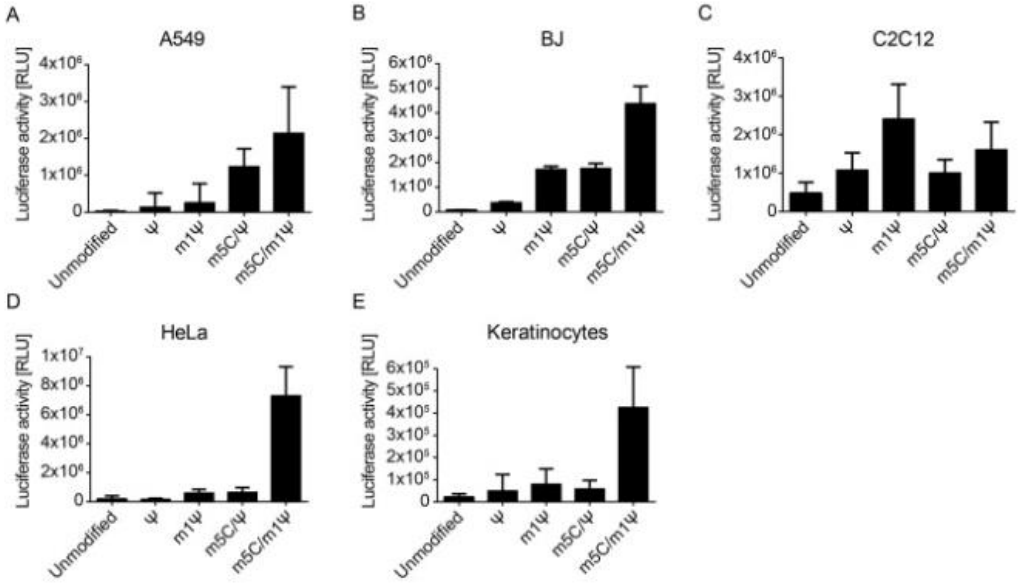
Kariko' et al, Molecular Therapy (2008)

Regulatory elements: RNA modifications on mRNAs

- *m1Ψ-modified mRNA is even more translated and less capable* of stimulating the innate immune signaling pathways than *Ψ-modified mRNA*
- *Our Covid-vaccine has m1Ψ-modified mRNA*







Andries et al, J Control Release (2015)

What we discussed:

- **Discovery of mRNA modification**
- **Technologies to detect mRNA modifications**:

--antibody, chemical, direct RNA seq methods

--they revealed what we currently know about m^6A

--for some modifications, we do not have good sequencing technologies

- **Regulation of mRNA modifications in cancer**

■ m^6A regulators are upregulated/downregulated in cancer

■ specific mRNAs are regulated, but we do not know how they are regulated

■ the major effect of m6A is on mRNA stability. However, it can impact splicing, localization, etc…

- **Application of modifications in future therapeutics**

--modifications can allow to distinguish self from non-self RNA

--their use in vaccine

m^{6}A in cancer:

- https://www.nature.com/articles/s41571-023-00774-x

METTL3 inhibitor:
https://www.nature.com/articles/s41594-021-00606-5

m^{6}A regulation:
https://www.sciencedirect.com/science/article/pii/S1097276522004968?via%3Dihub

Beyond m^{6}A:

- https://pubmed.ncbi.nlm.nih.gov/33188361/