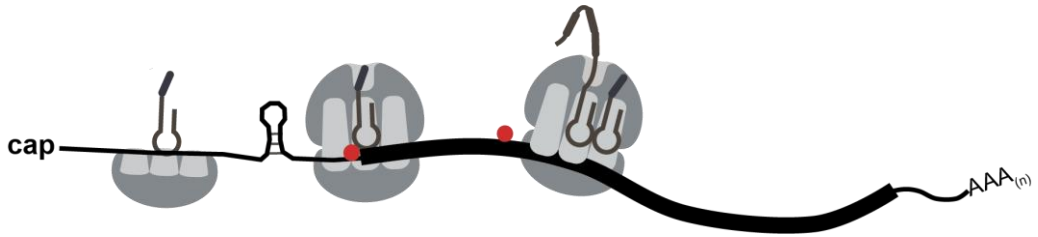
Regulation of Protein Synthesis by the Epitranscriptome



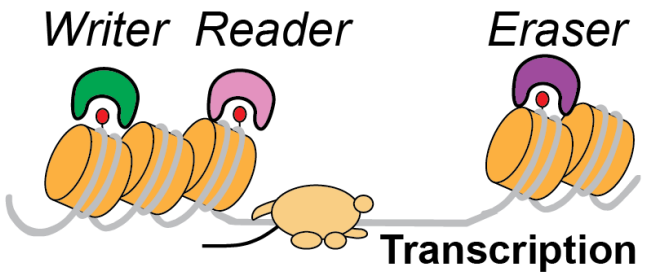
Daniel Arango, Ph.D.
Assistant Professor
Department of Pharmacology
https://sites.northwestern.edu/arangolab/

Northwestern Medicine®
Feinberg School of Medicine

Nucleotide modifications:
a regulatory layer of gene expression

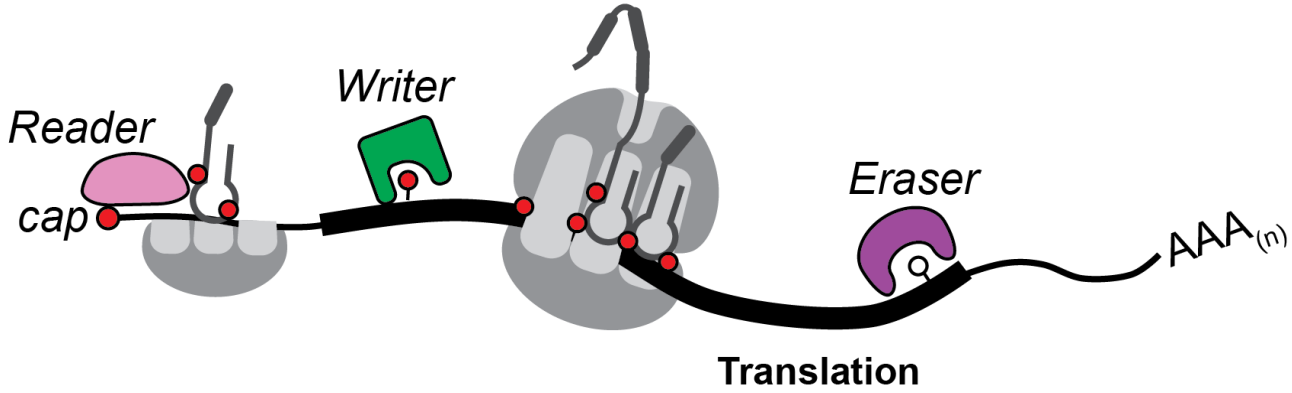
- Modified Nucleotide : chemical modifications of canonical A, C, T/U, G

Epigenome



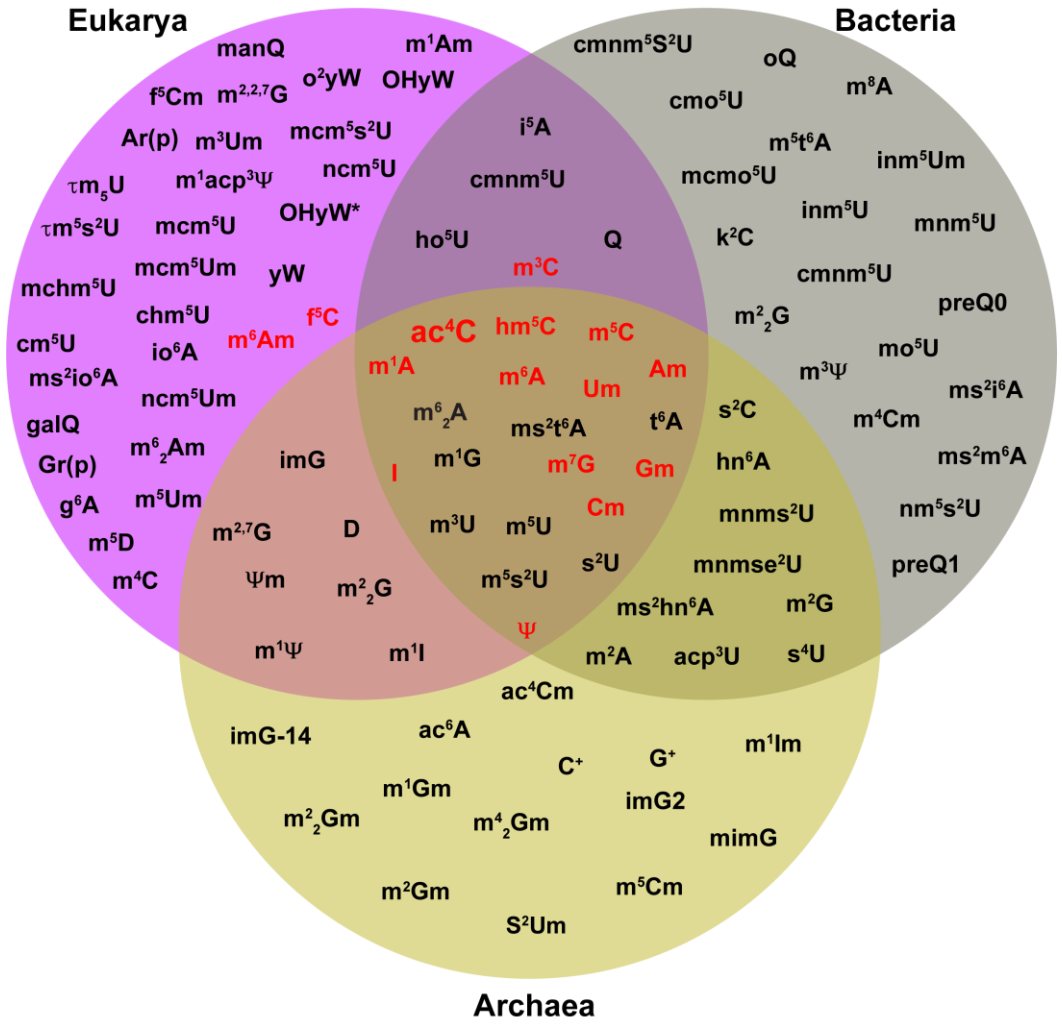
Chromatin structure
Transcription
Co-transcriptional RNA processing

Epitranscriptome

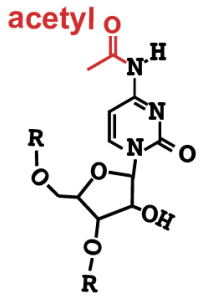


RNA Structure
RNA Processing
RNA Stability
Translation

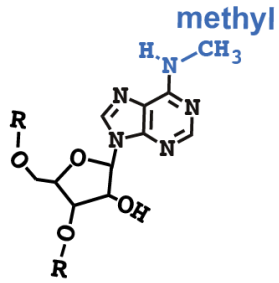
RNA Modifications: sheer numbers and diversity



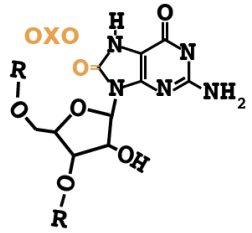
ac⁴C



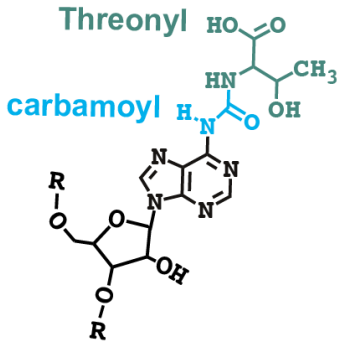
m⁶A



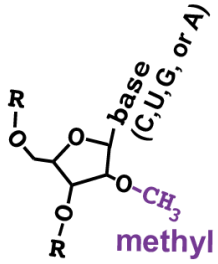
oxo⁸G



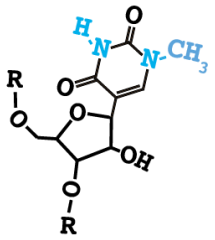
t⁶A



2'O-me



m¹Ψ



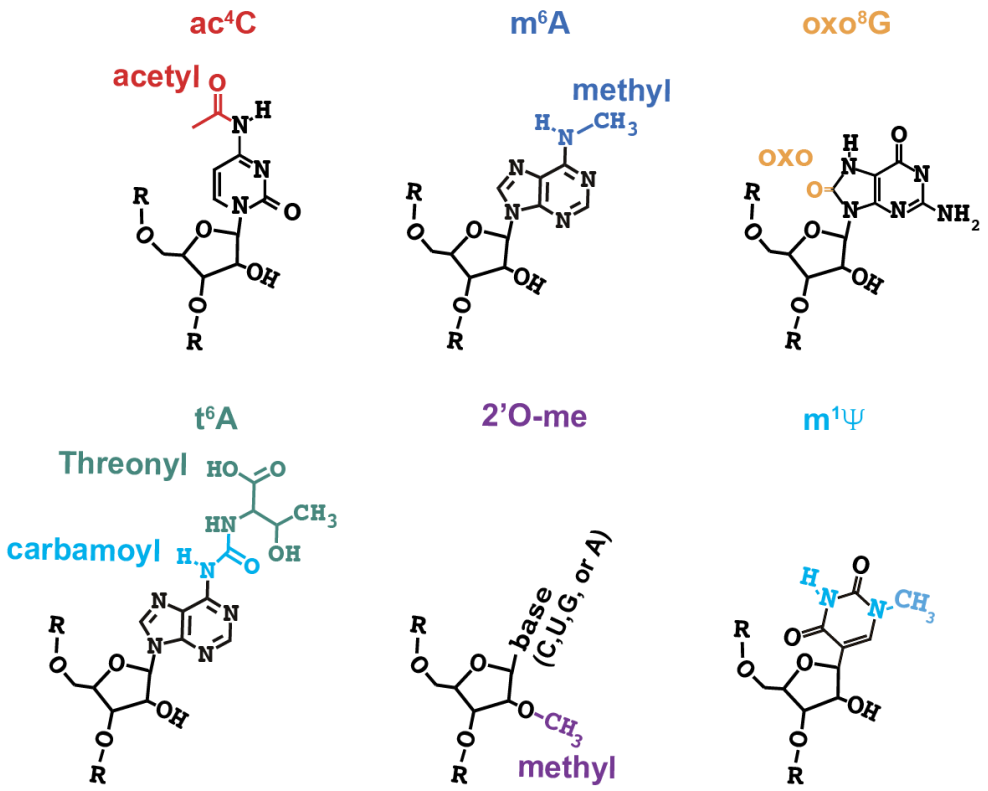
> 150 ribonucleoside modifications

For most modifications, function is unknown

RNA Modifications: sheer numbers and diversity

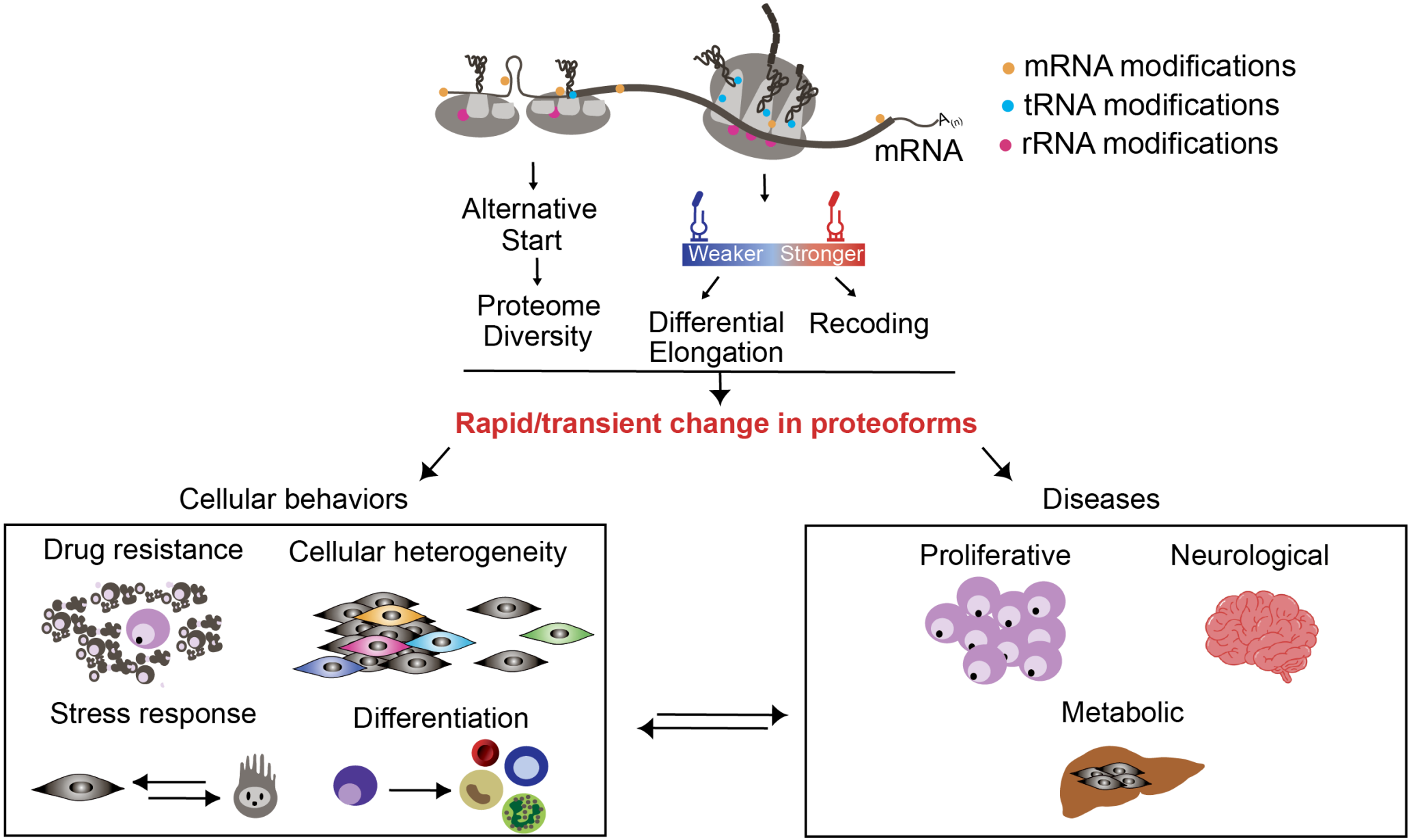
papa

papá

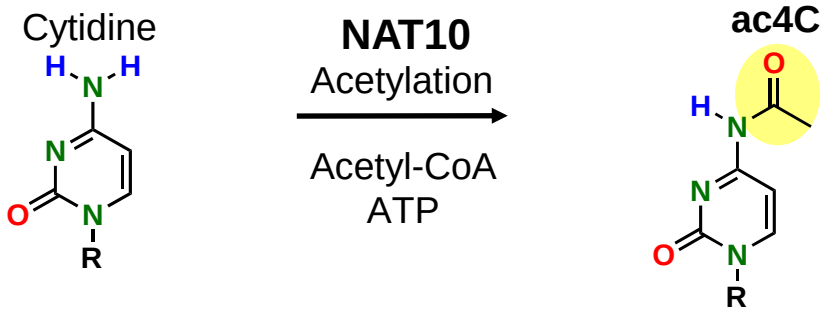


key in the development of mRNA vaccines

Decrypting the epitranscriptome



N4-acetylcytidine:
catalyzed by the disease-associated enzyme NAT10



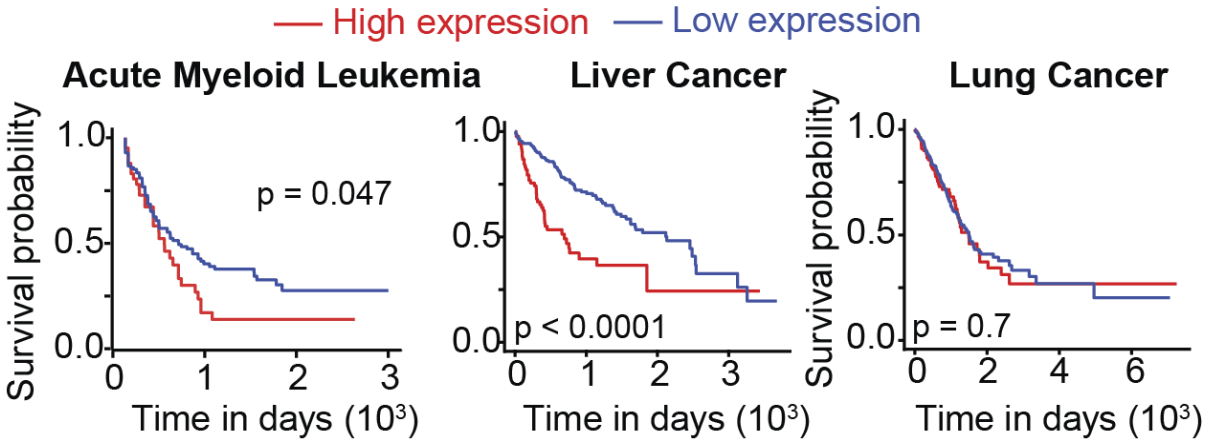
Evolutionary Conserved: Eukarya, Archaea, Bacteria

Sole eukaryotic RNA acetyltransferase

Hutchinson-Gilford progeria syndrome (HGPS)

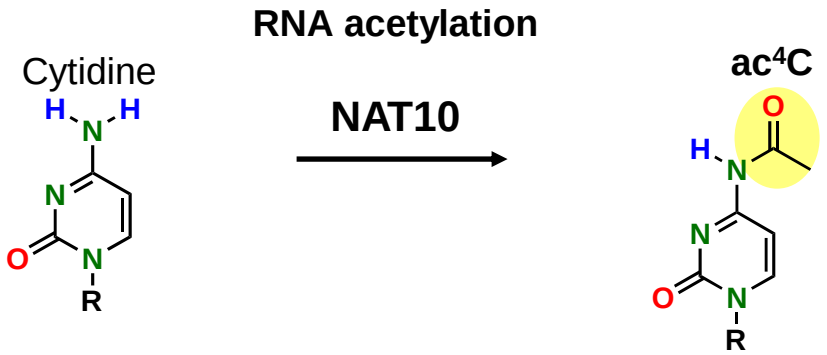
Scaffidi *et al.*, Plos Biol, 2005
Larrieu et al., Science 2014

Effect of NAT10 expression in patient survival

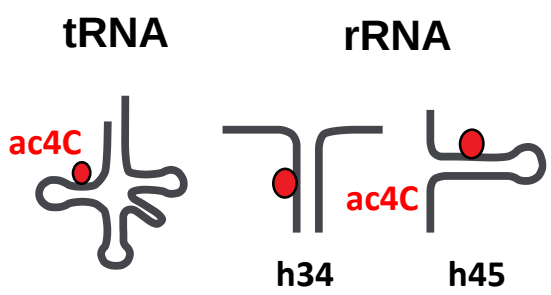


from cBioportal

Uncovering the distribution of RNA acetylation

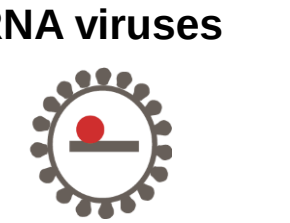


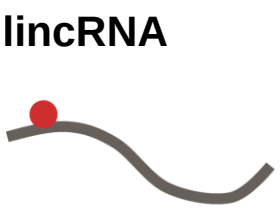
Evolutionary Conserved: Eukarya, Archaea, Bacteria



Zachau et al. 1966 Angew Chem Int Ed Engl
Thomas et al. 1978 JBC
Sharma et al. 2015 NAR

Arango et al., 2018. Cell

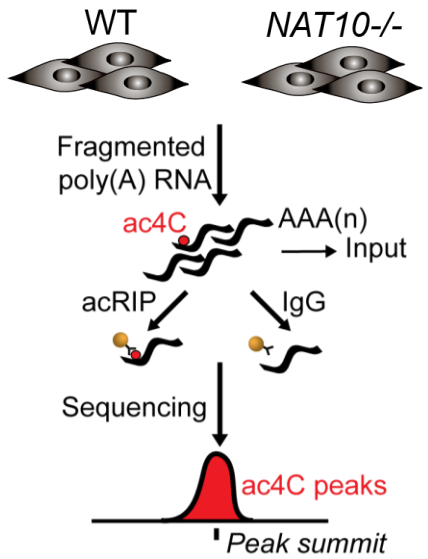


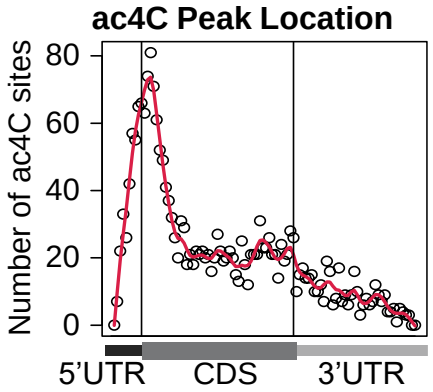


Tsai *et al. 2020 Cell Host Microbe*
Feng *et al. 2022 J Hematol Oncol*

Developing methods to decipher the epitranscriptome: the case of ac^{4}C

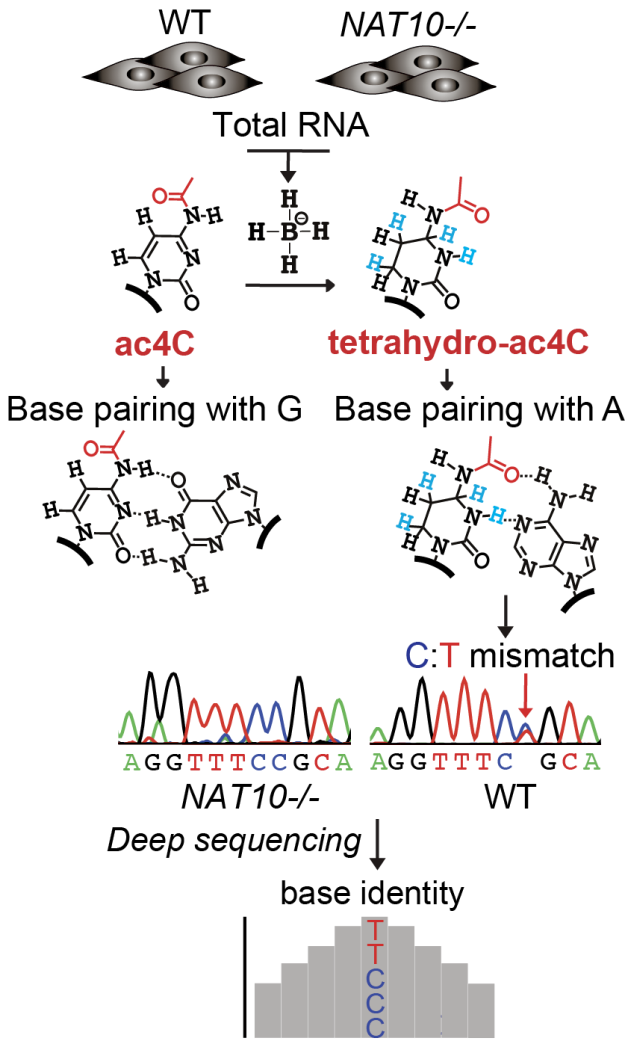
acRIP-seq





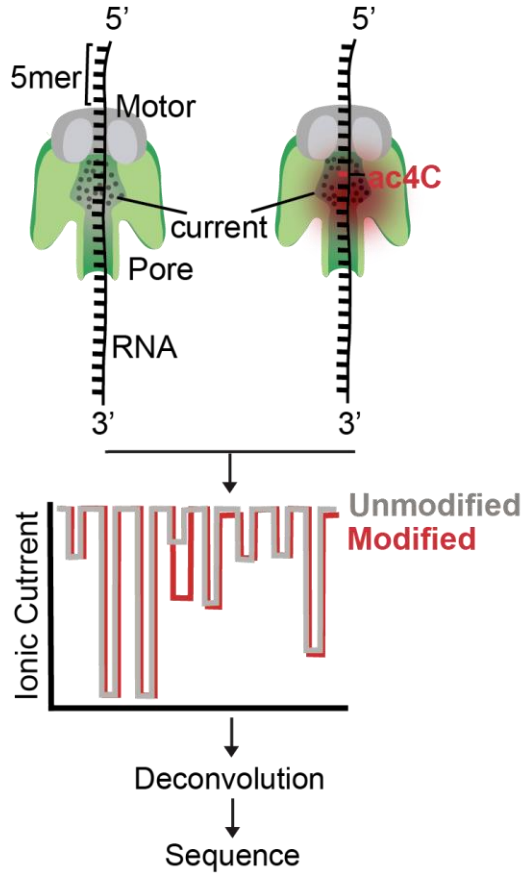
Arango et al., Cell, 2018
Arango et al., Bio-Protocols, 2019

RedaC:T-seq



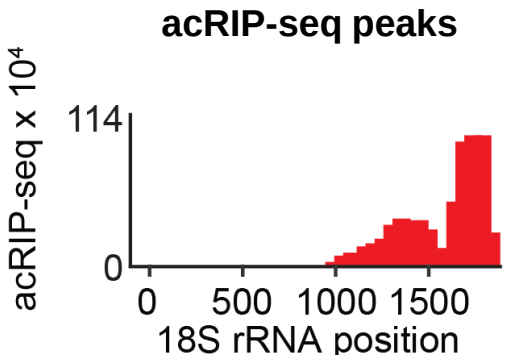
Arango et al., Mol Cell, 2022
Sturgill et al., STAR Protocols, 2022

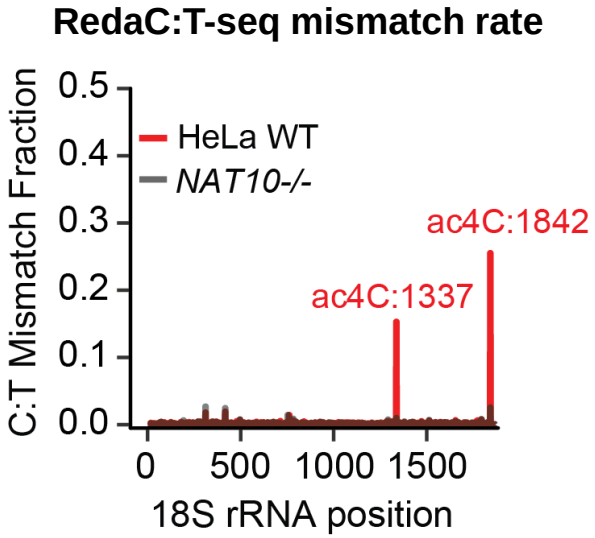
Nanopore Direct RNA-seq

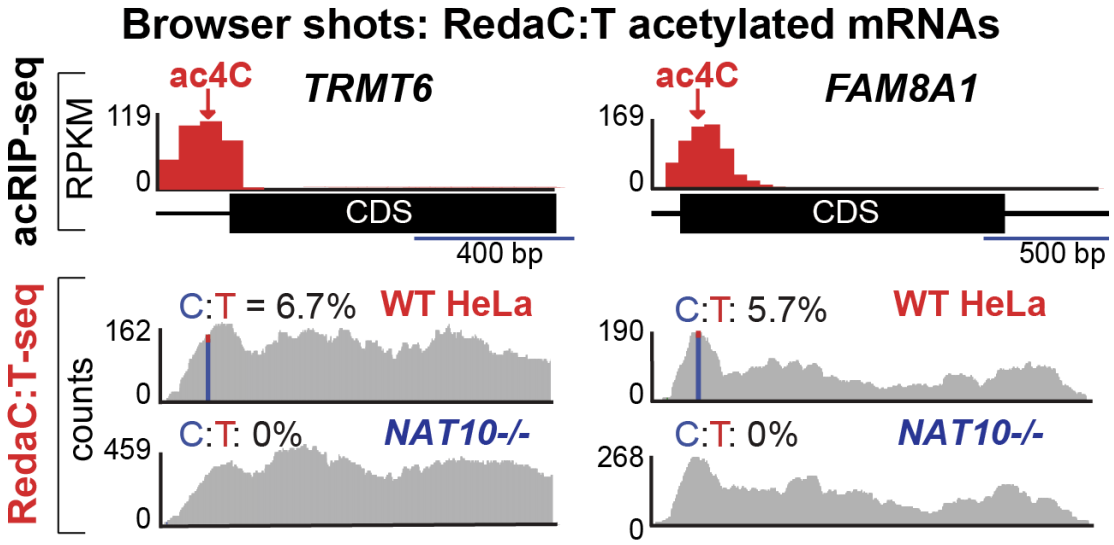


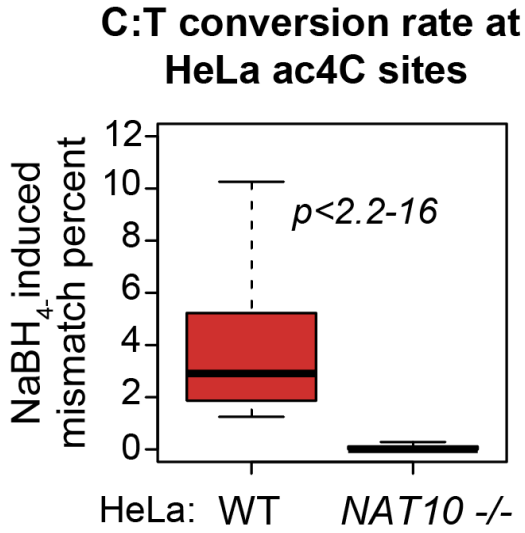
Ongoing

Developing methods to decipher the epitranscriptome: the case of ac^{4}C

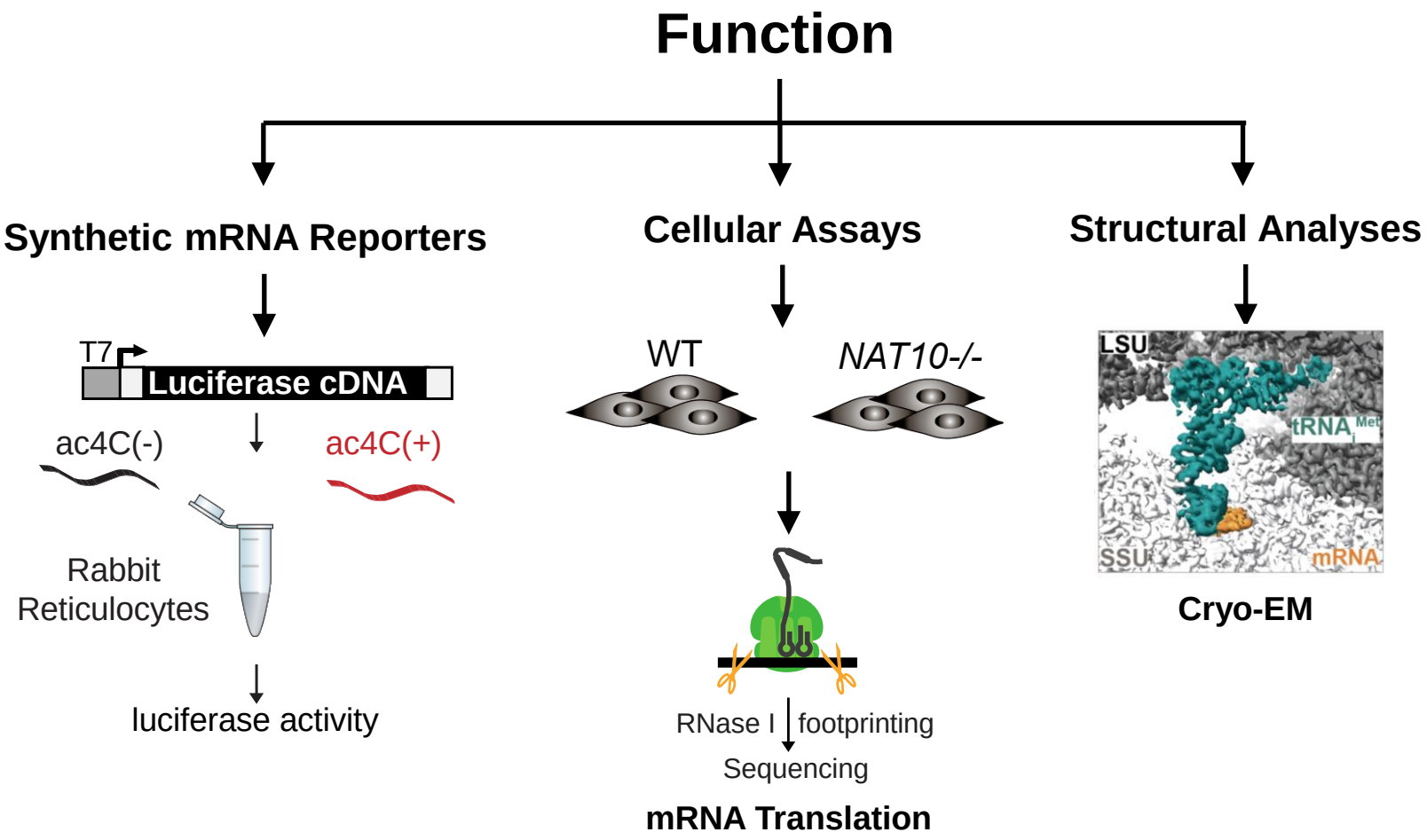




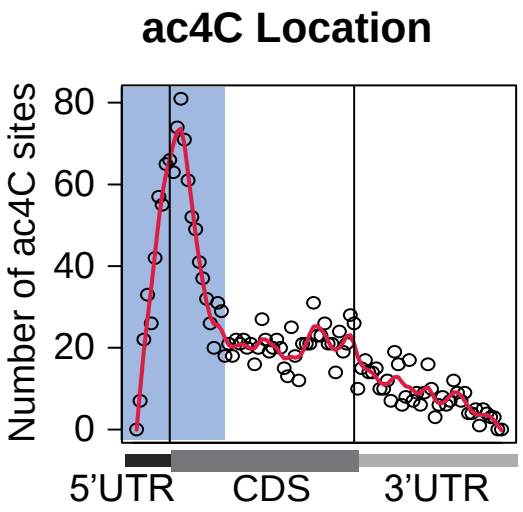


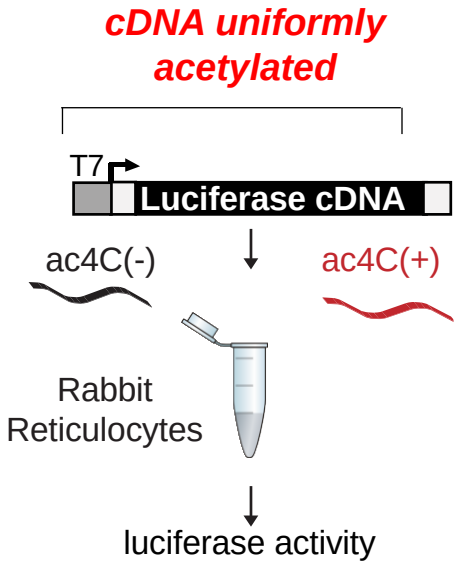


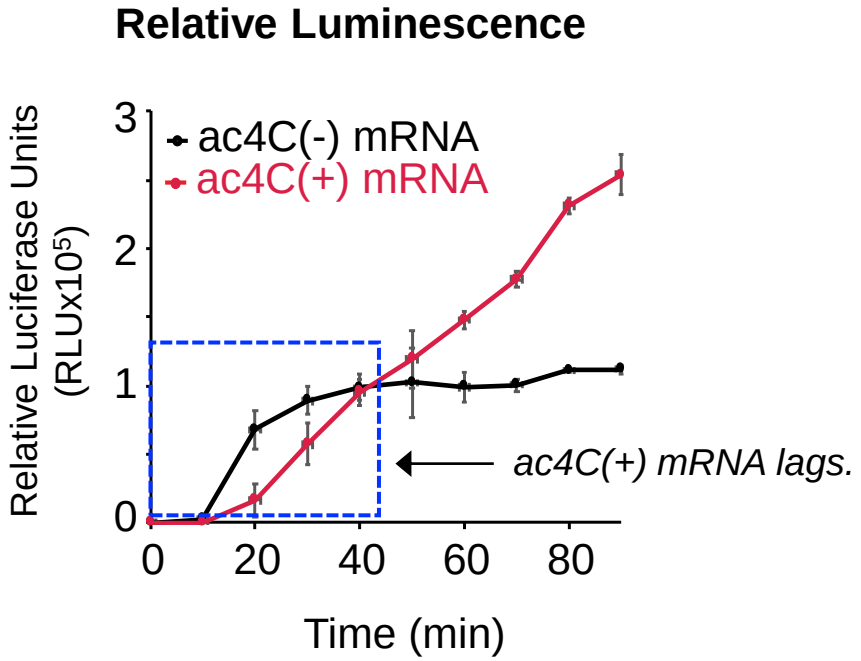
Uncovering the function of RNA modifications: the case of ac^{4}C



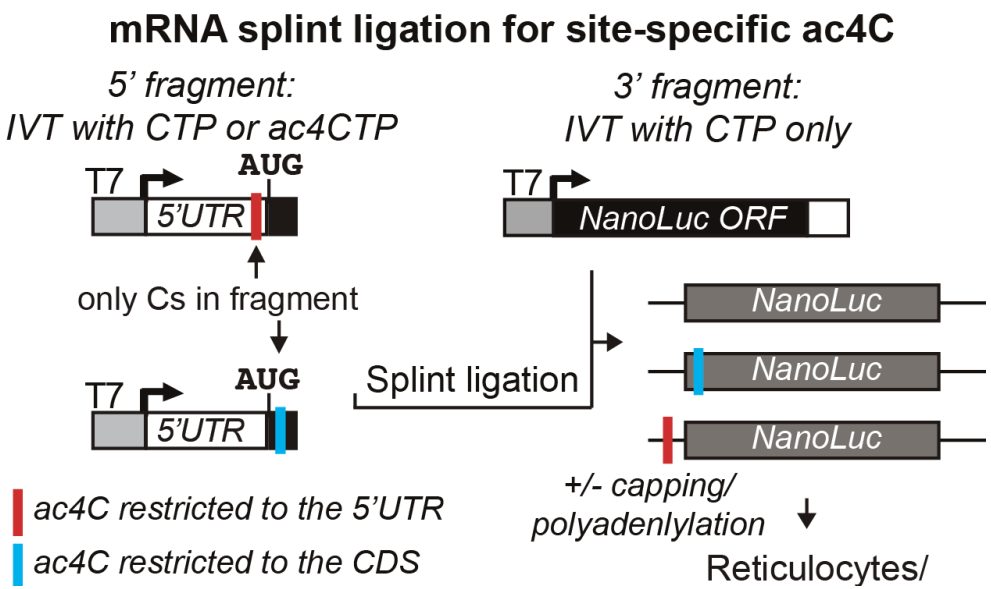
Is the position within transcripts a determinant of function?

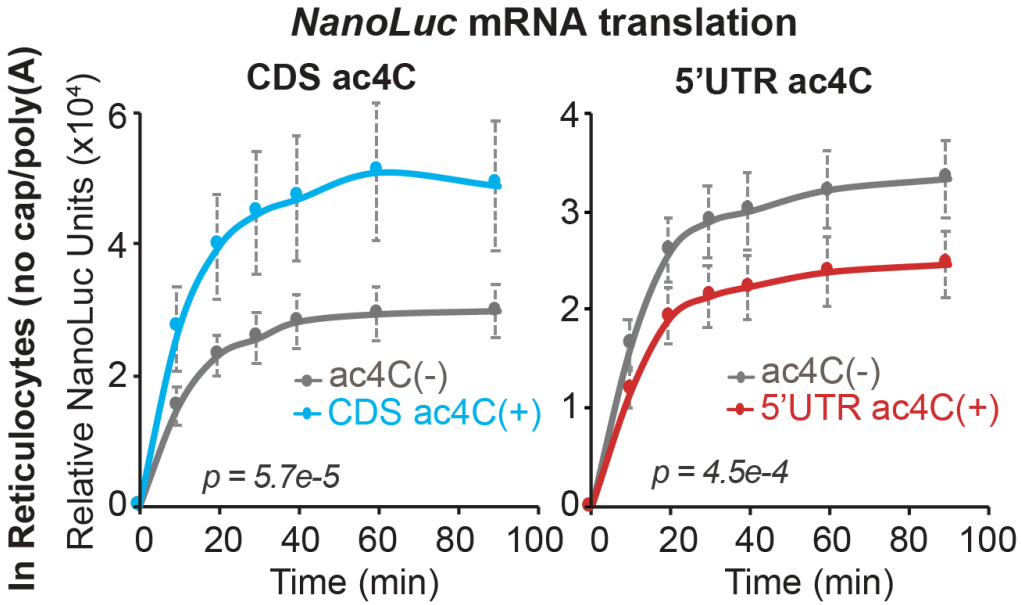






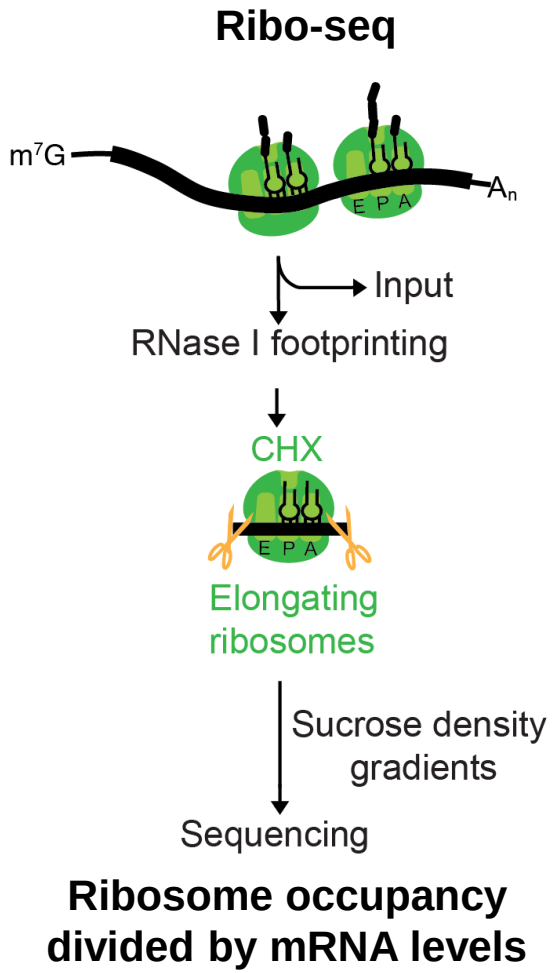
5’UTR and CDS acetylation mediate distinct roles in translation

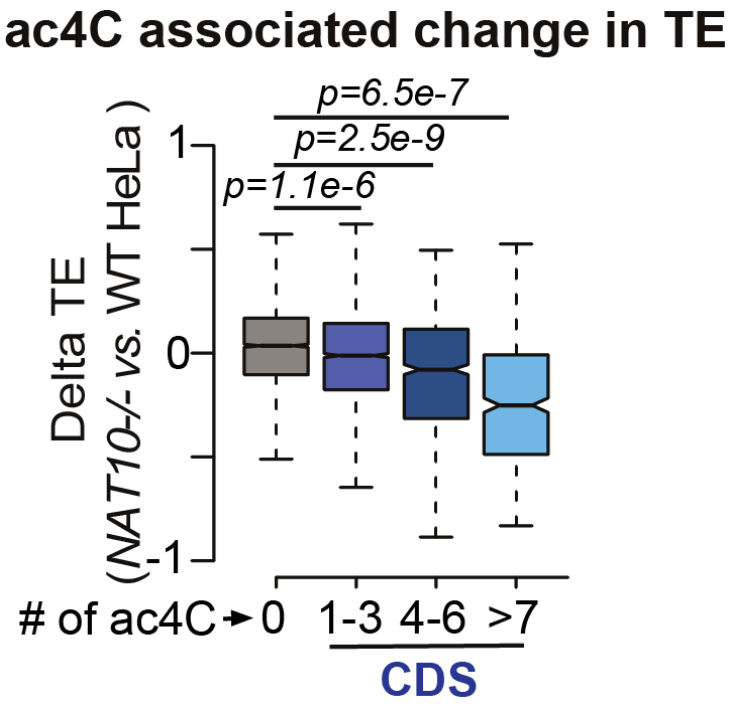




Arango et al., Mol Cell, 2022

CDS acetylation promotes translation efficiency





n=5,646 ac4C sites in CDS

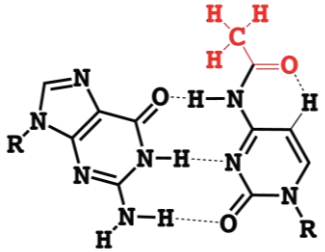
Arango et al., Mol Cell, 2022

ac4C promotes codon recognition in bacteria

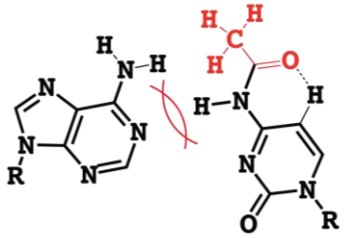
Possible wobble pairing

With acetylated cytidine

G • ac4C



A ac4C

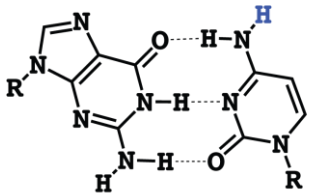


Strengthen Interaction

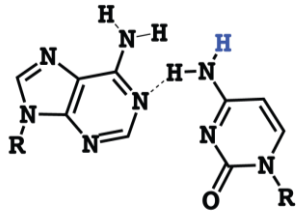
Increased G:C base pairing

Non-acetylated cytidine

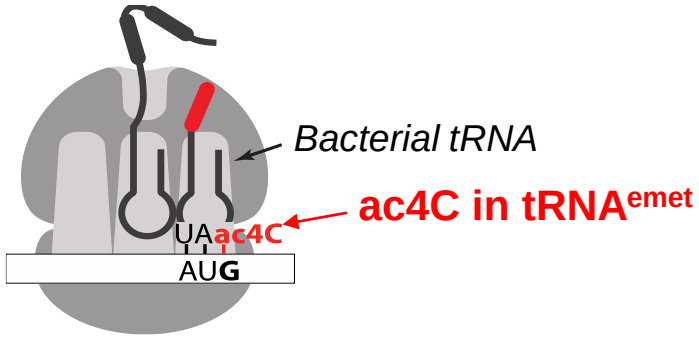
G • C

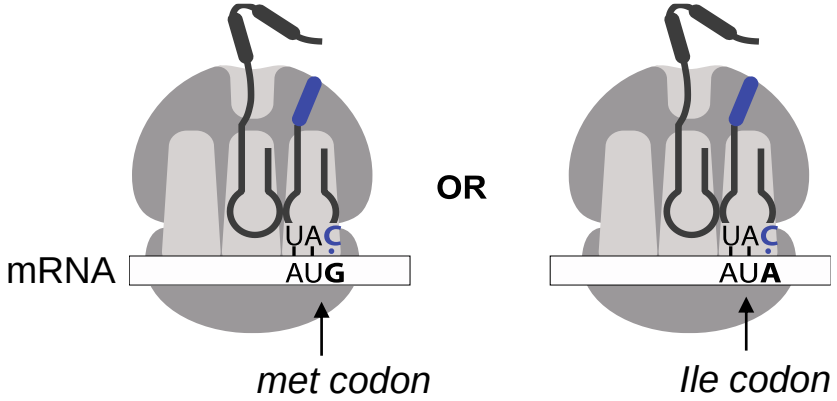


A • C



outcome

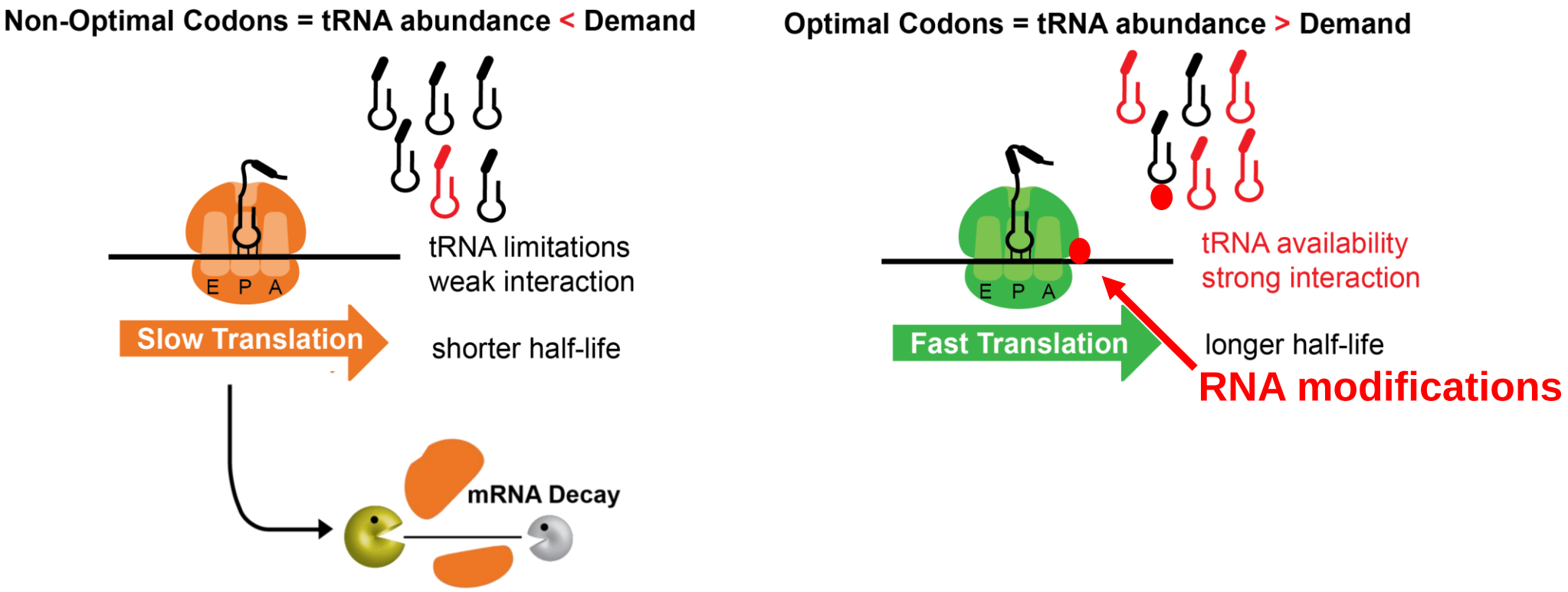




**Methionine insertion at Isoleucine-encoding site.*

Stern and Schulman, JBC, 1978

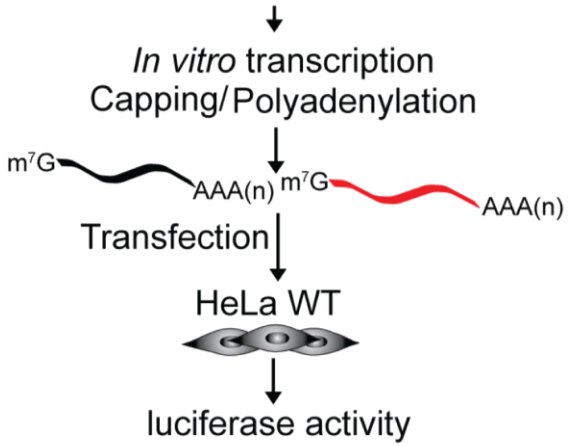
Efficiency of codon recognition promotes mRNA stability and translation

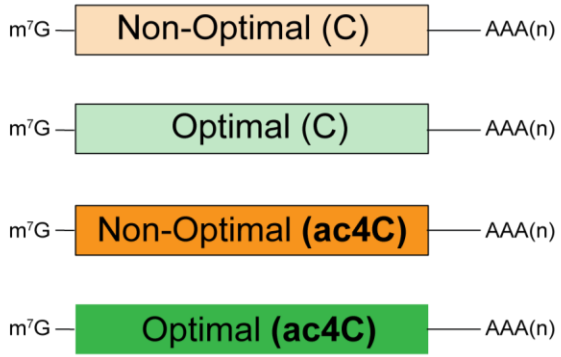


RNA modifications increase or decrease the strength of the codon:anticodon recognition

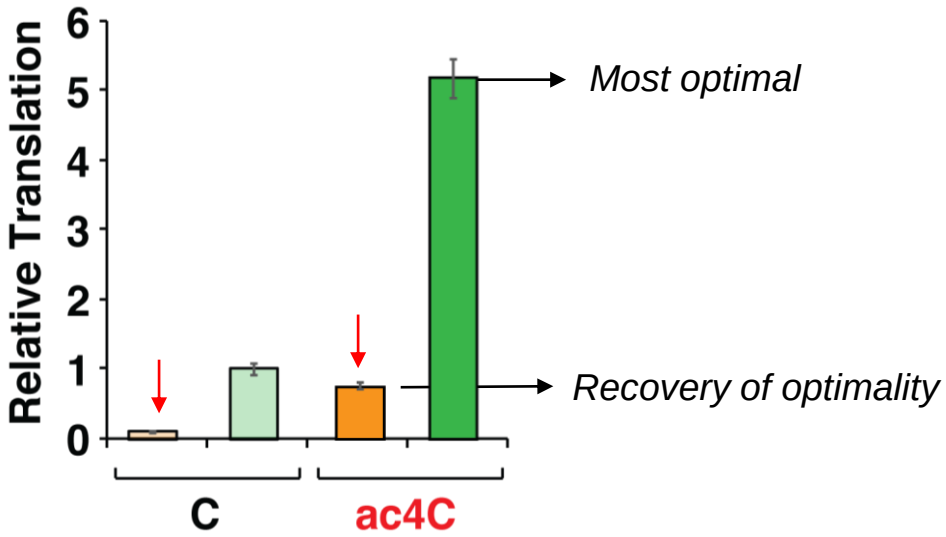
ac4C Promotes Translation of Transcripts with Low Codon Optimality

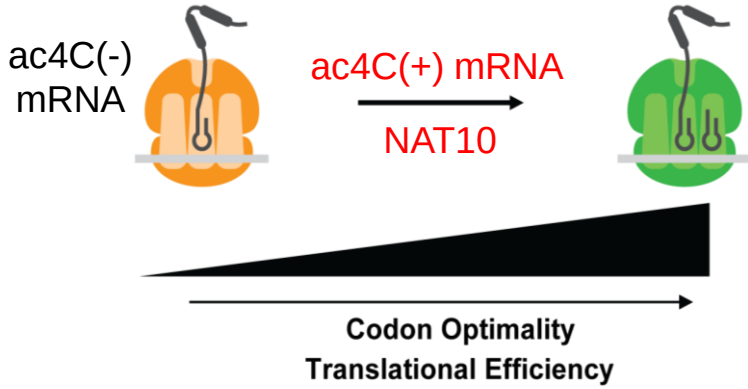
Synonymous Mutations C > A, G, U (change codon optimality)





Relative Luminescence

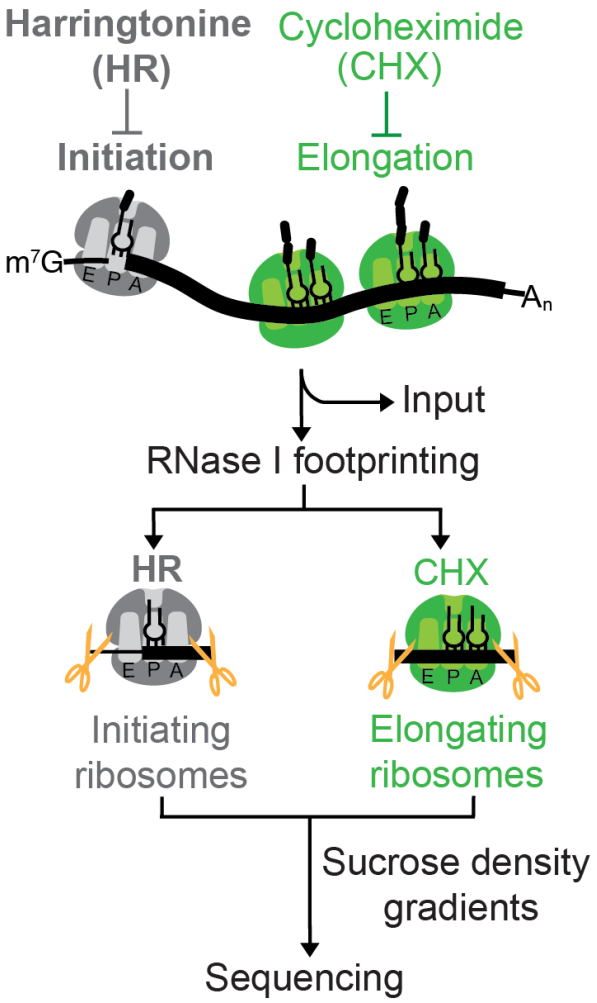


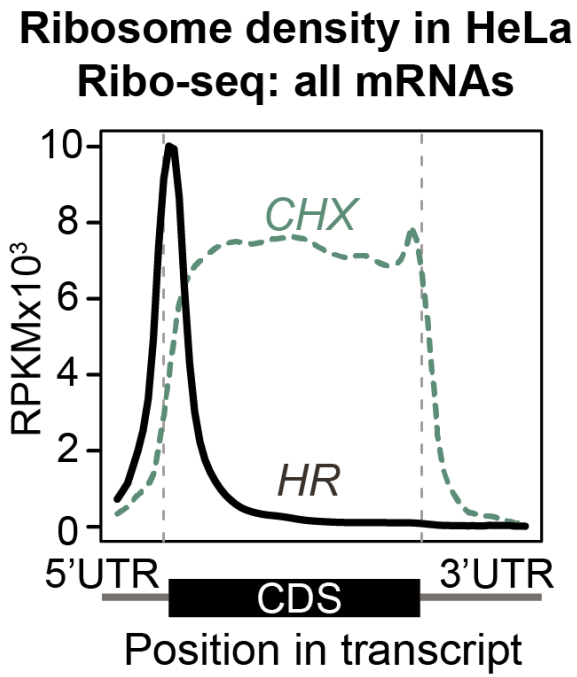


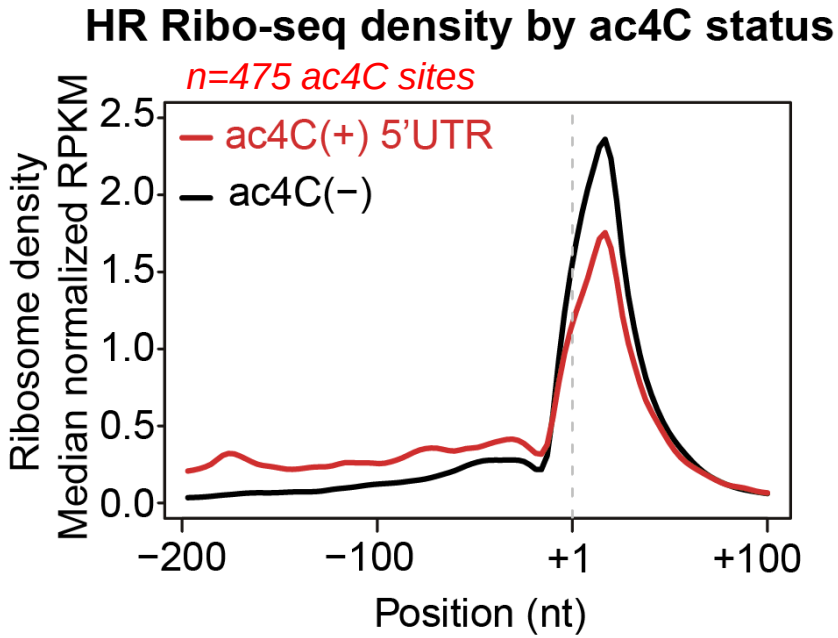
Arango et al., Cell, 2018

In Collaboration with Jeff Coller. Johns Hopkins

Examining the role of 5'UTR ac4C role in translation initiation

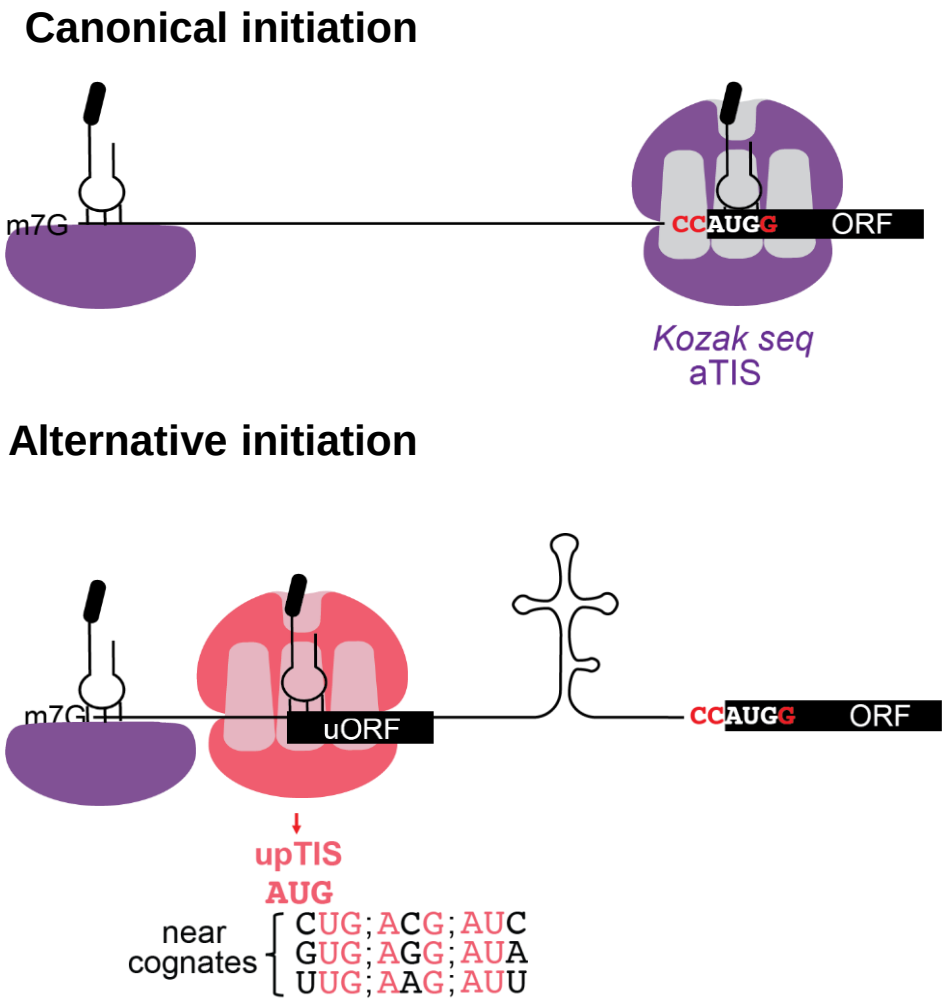


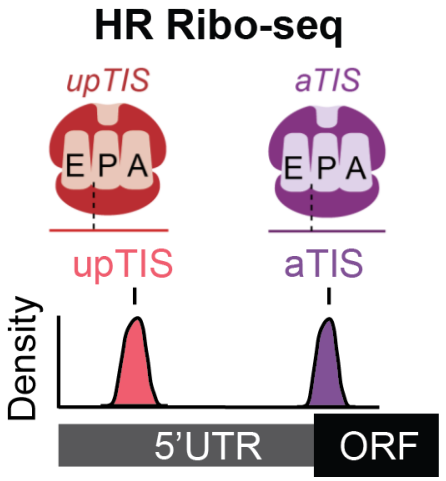


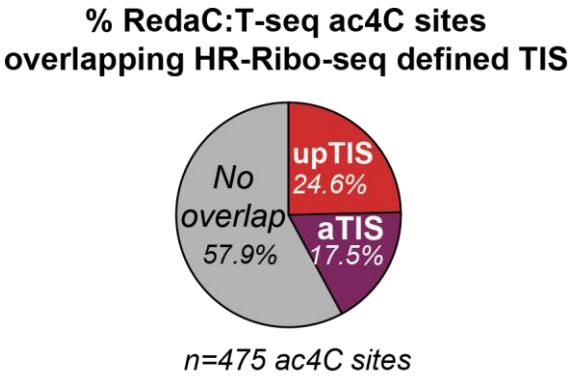


5'UTR ac4C is associated with upstream translation initiation

Regulation of translation initiation







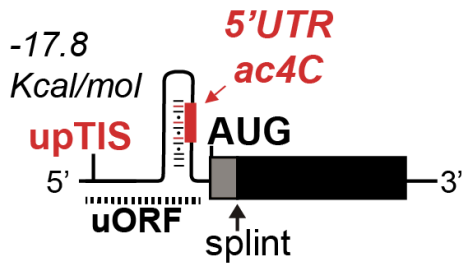
aTIS: annotated translation initiation site

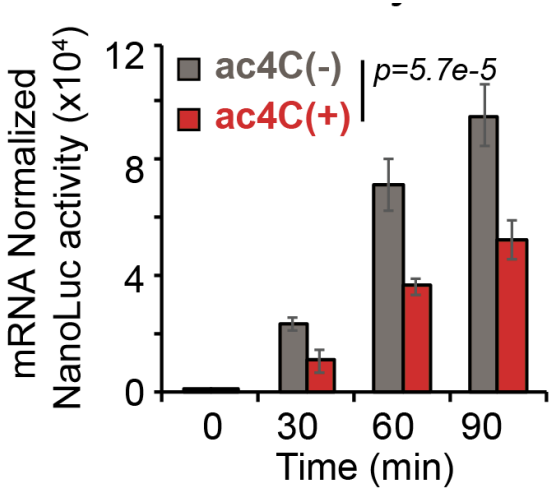
upTIS: upstream translation initiation site

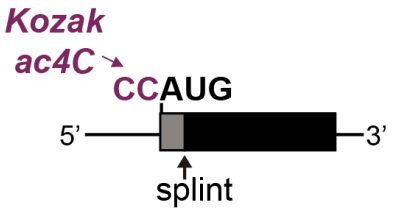
ORF: Open reading frame = CDS

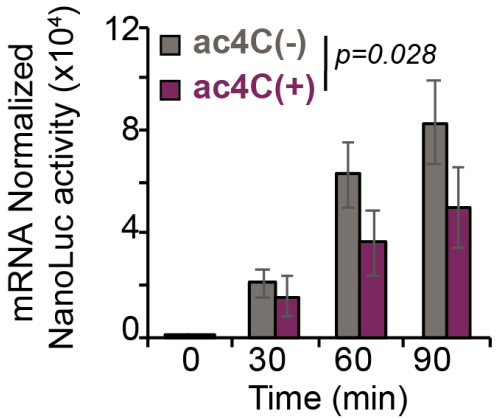
uORF: upstream open reading reading

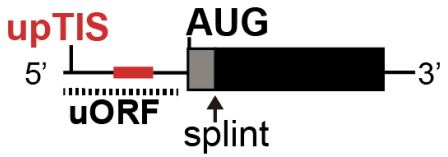
ac4C influences translation initiation in a position specific-manner

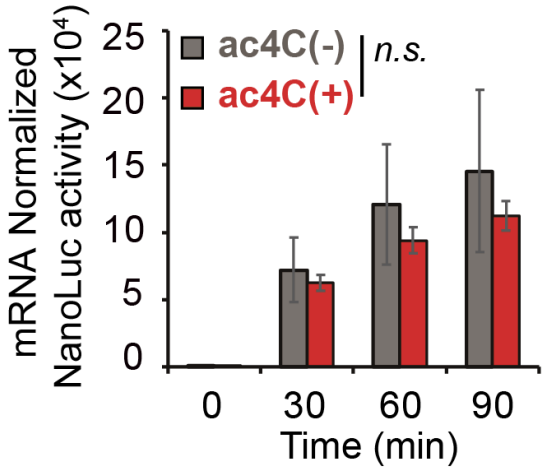


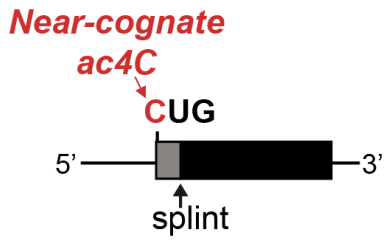


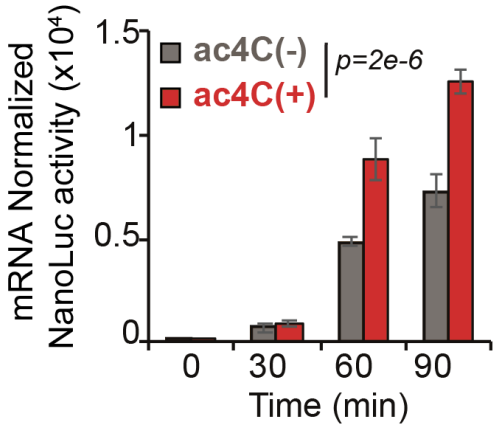






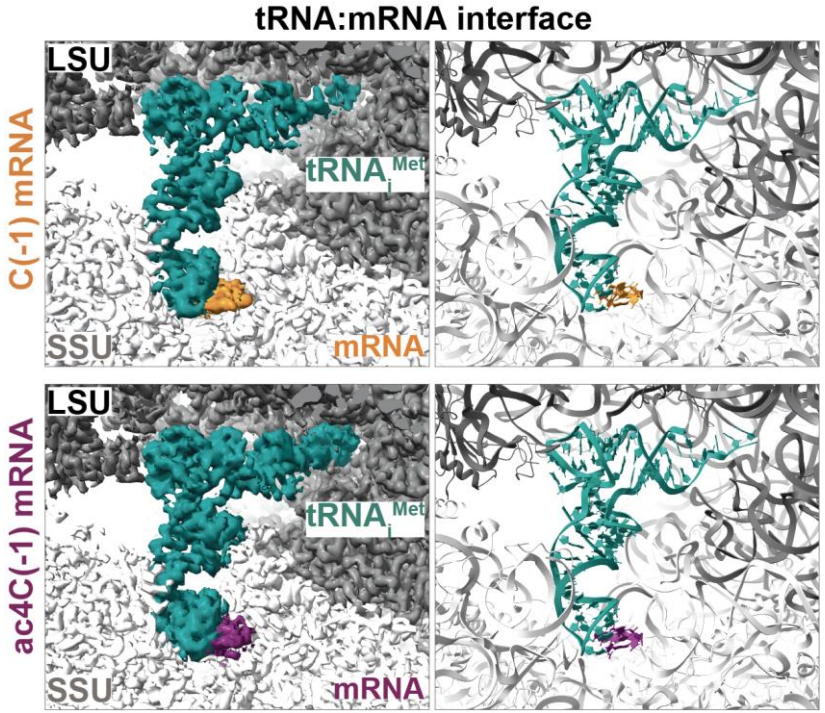


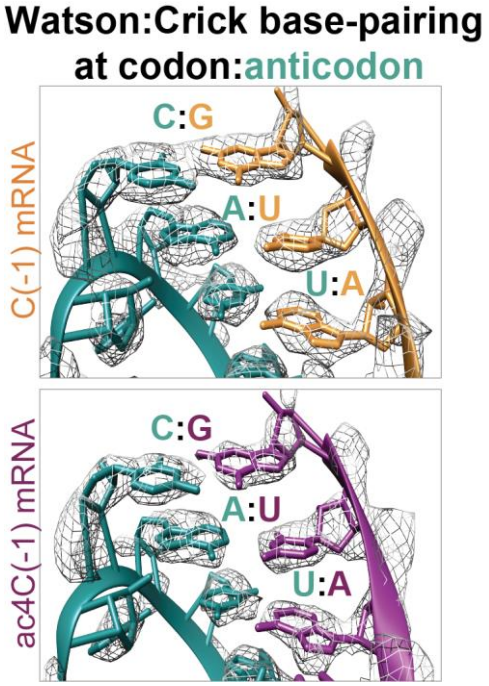




Arango et al., Mol Cell, 2022

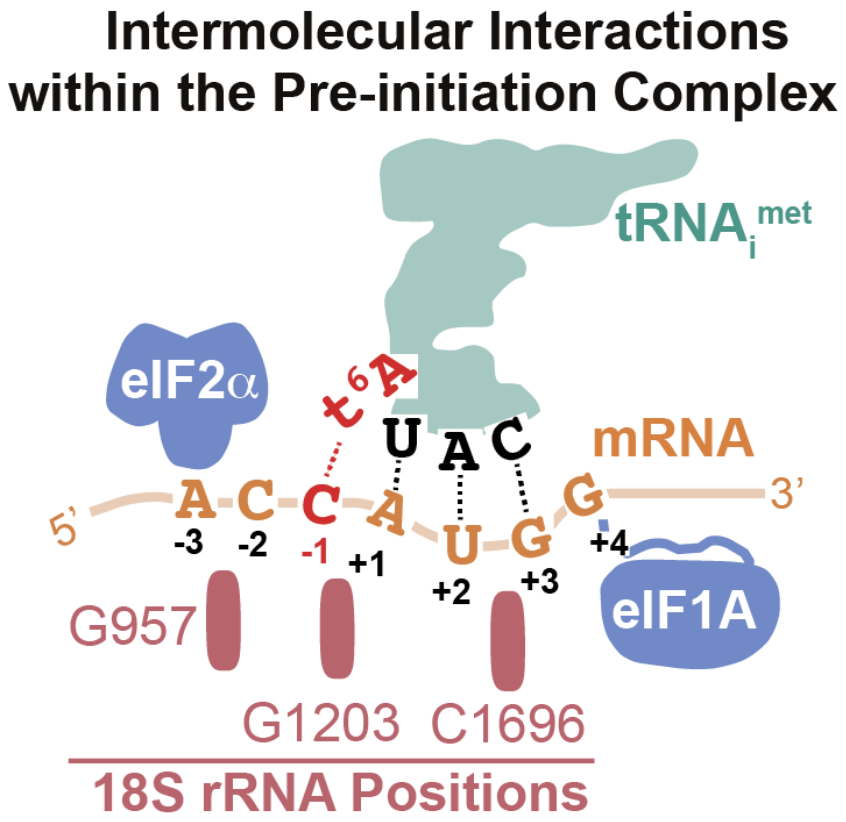
Direct influence of ac4C on translation initiation?



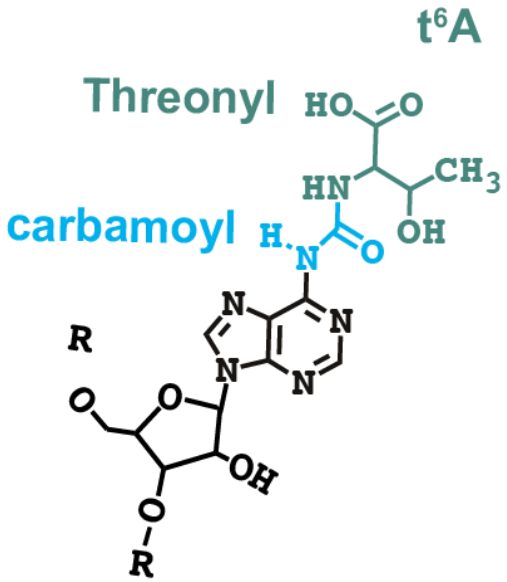


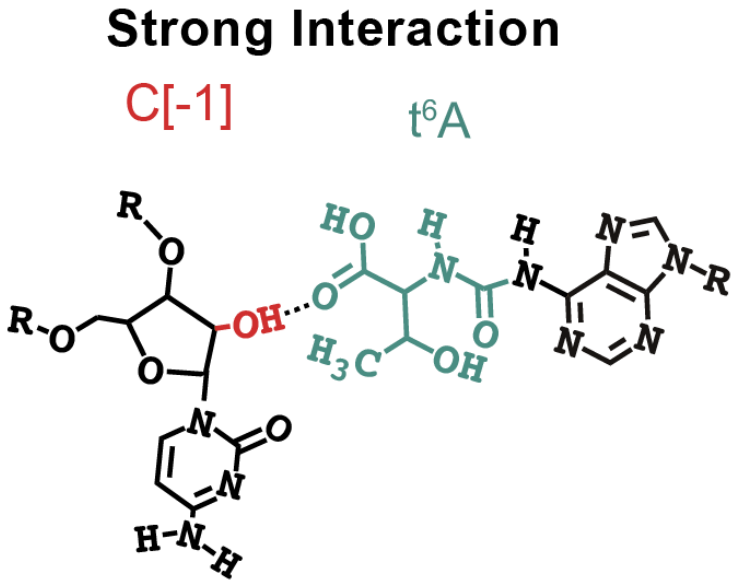
In Collaboration with Renbin Yang. National Cancer Institute

Kozak optimality is defined by the Interactions within the pre-initiation complex

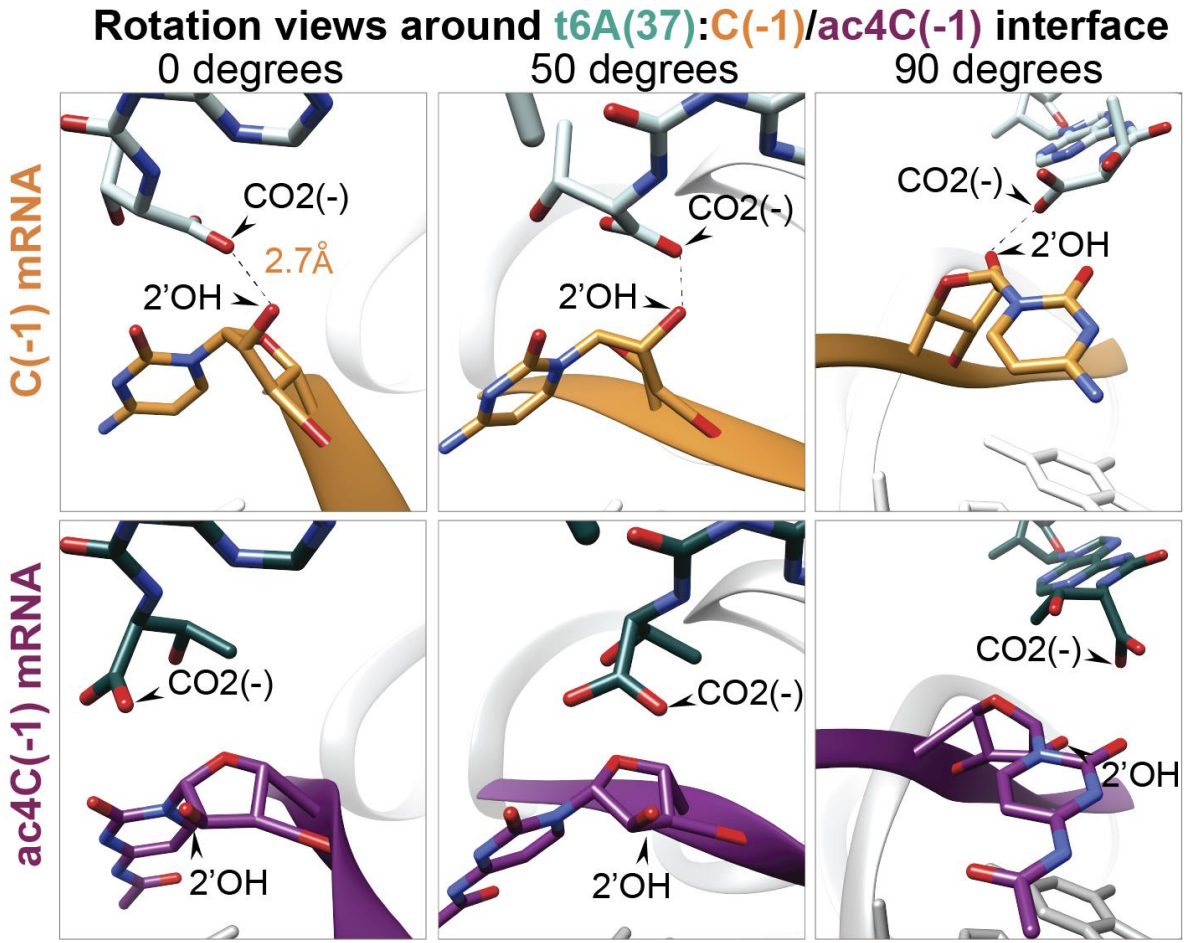


Adapted from Simonetti et al. 2020 Cell Reports



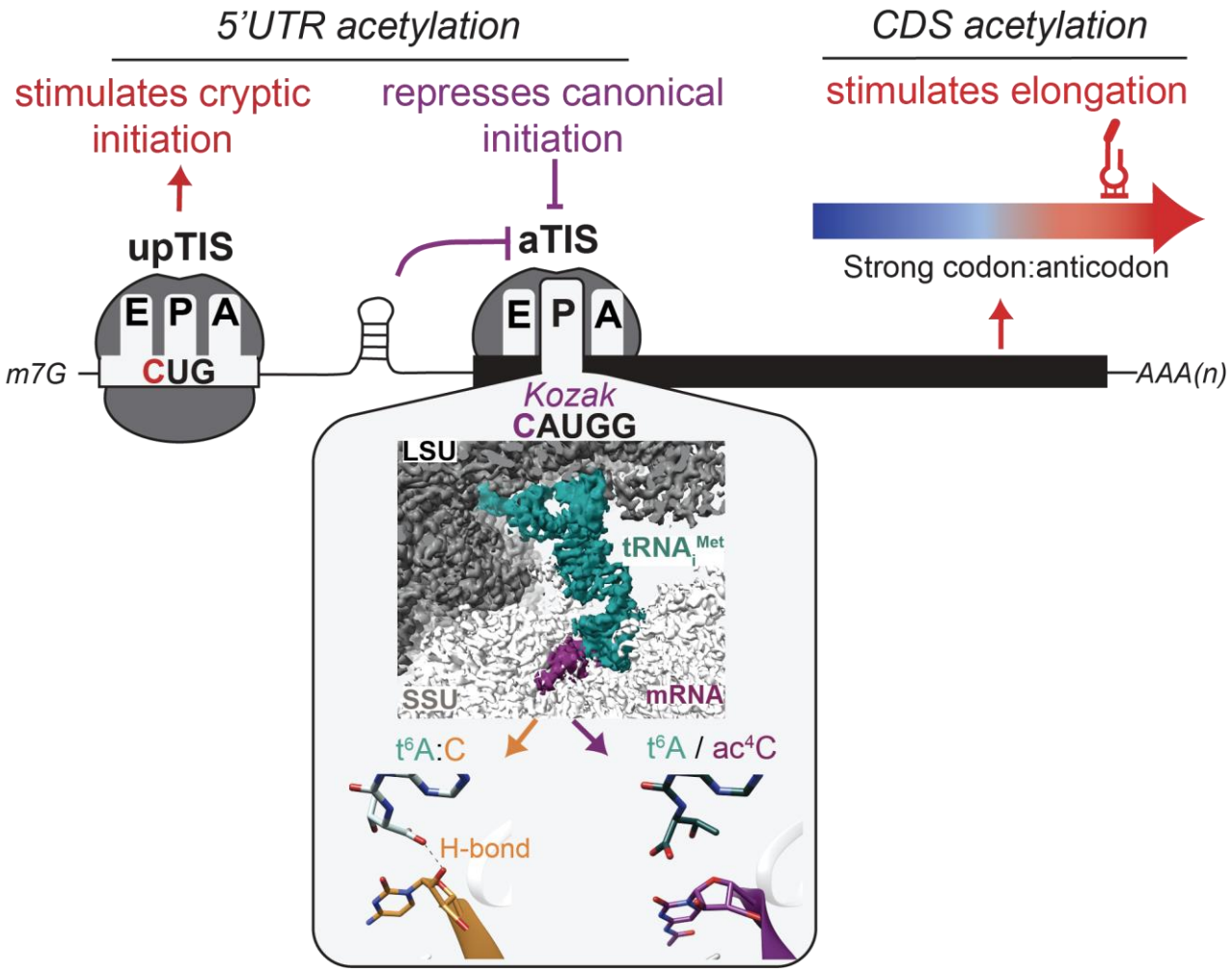


ac4C impairs the intermolecular interactions of the initiation complex

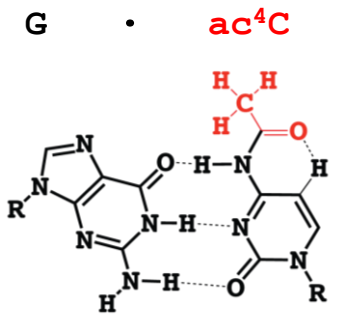


Arango et al., Mol Cell, 2022

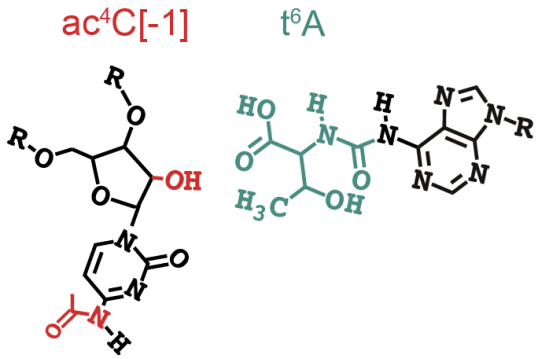
RNA acetylation modulates protein synthesis in a position-specific manner



Strengthens canonical interactions

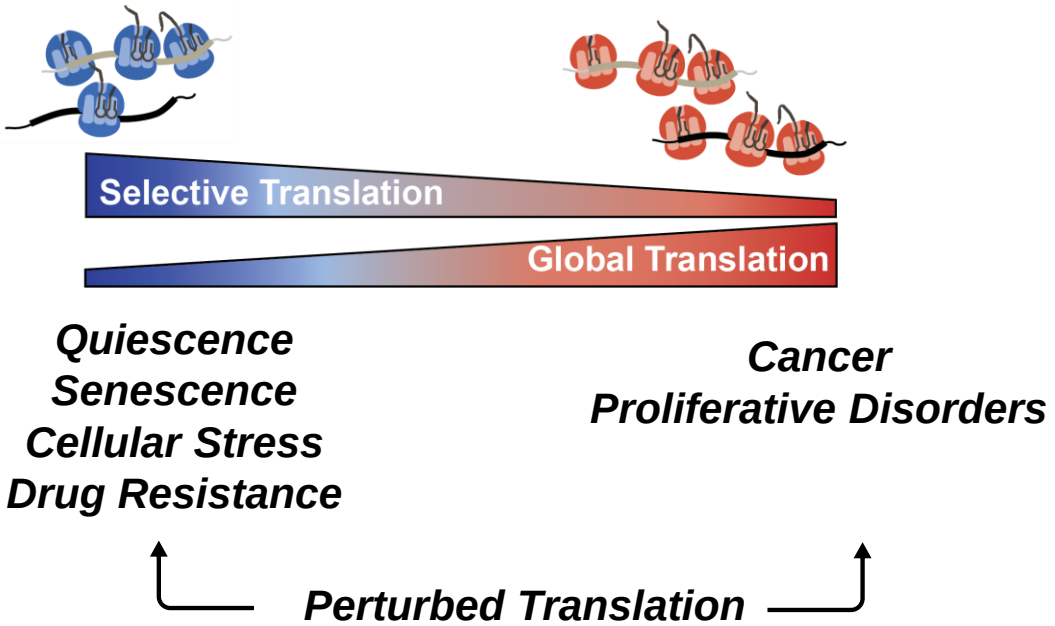


Weakens non-canonical interactions



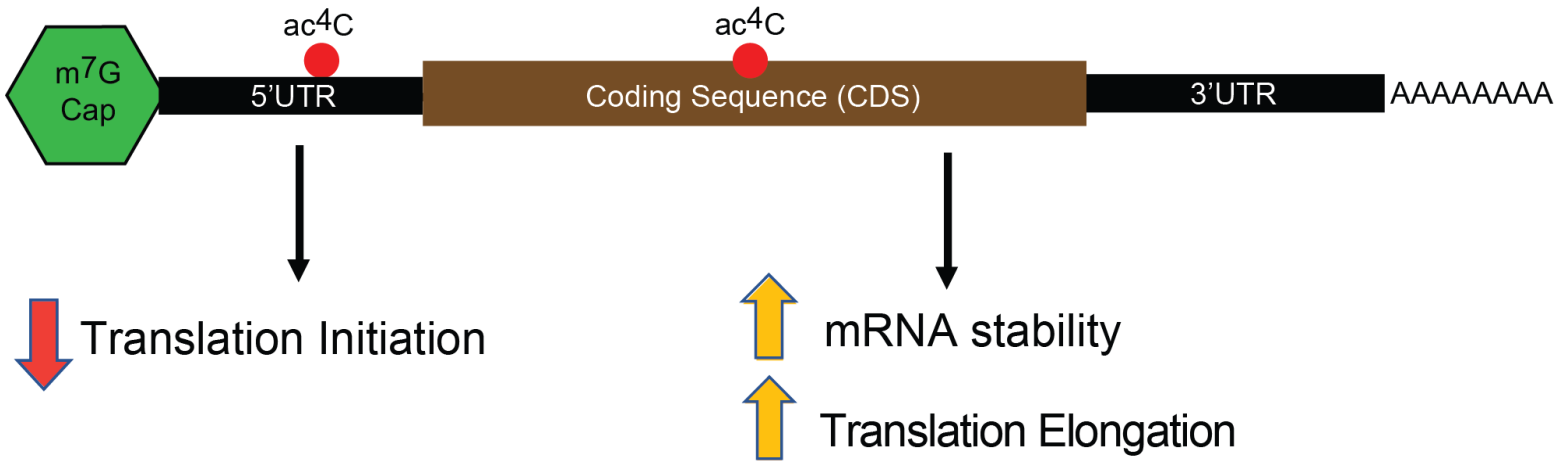
Why Does Modulating Protein Synthesis Matter?

Translation regulation is a determinant of cancer plasticity



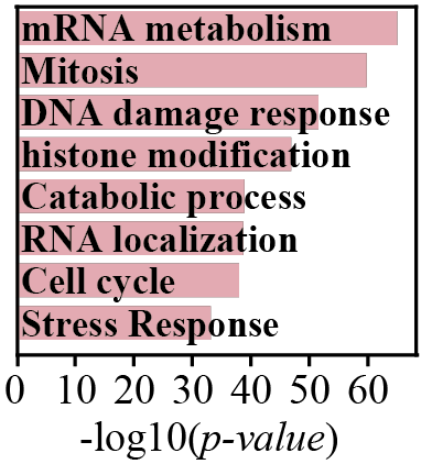
- **When is mRNA acetylation happening?**
- **How is NAT10 promoting cell proliferation and cancer growth?**

RNA acetylation is associated with cell proliferation and stress response

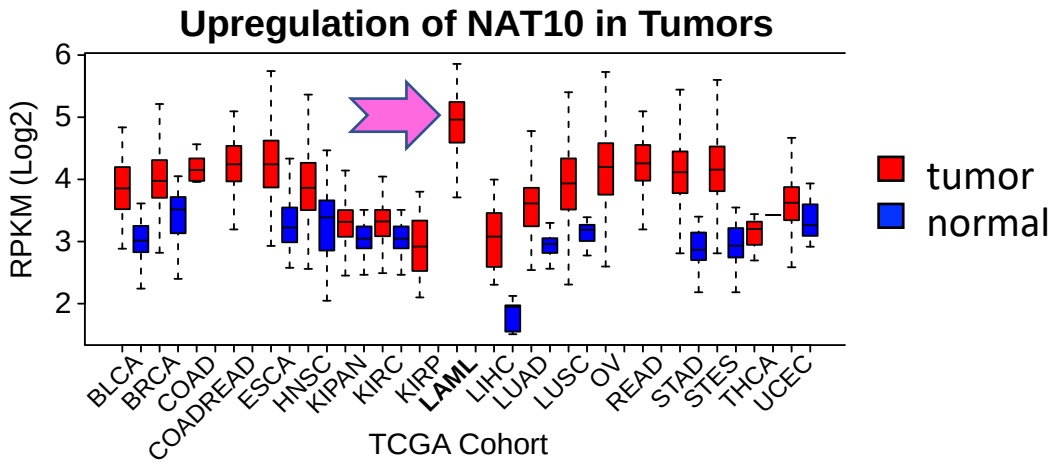


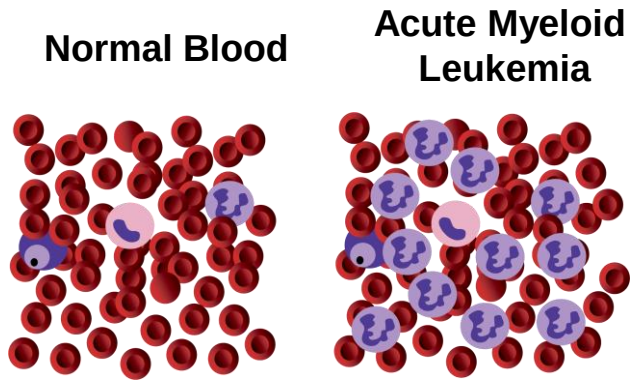
Arango et al., 2018. Cell
Arango et al., 2022. Mol Cell

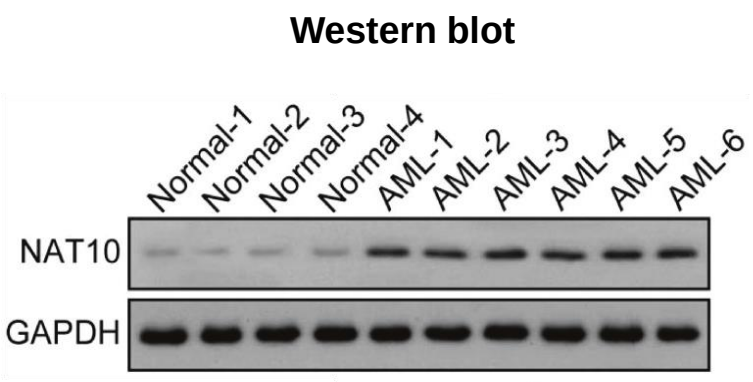
Gene enrichment ontology of acetylated RNAs



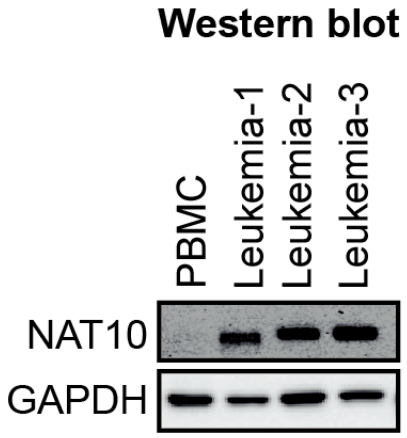
NAT10 is overexpressed in cancers







Zhi et al 2020.

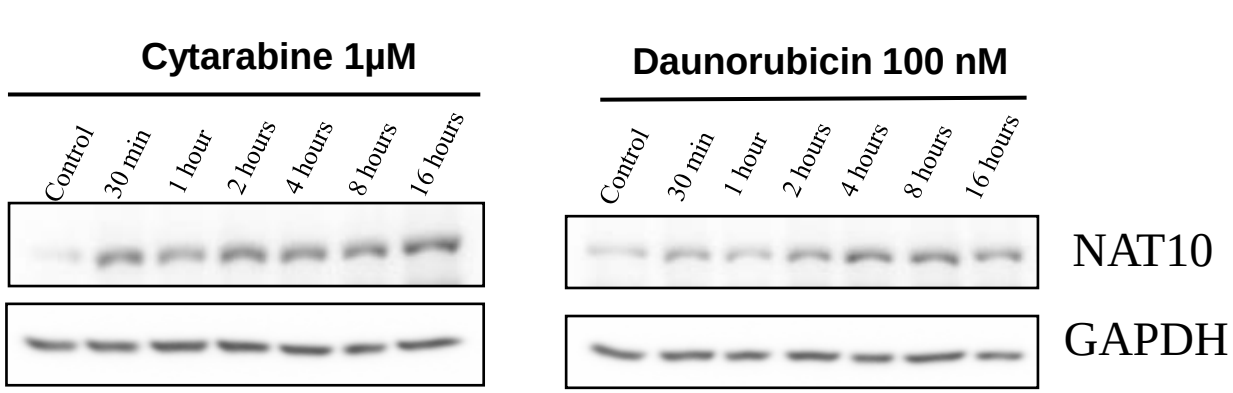


NAT10 expression is induced in response to chemotherapy drugs

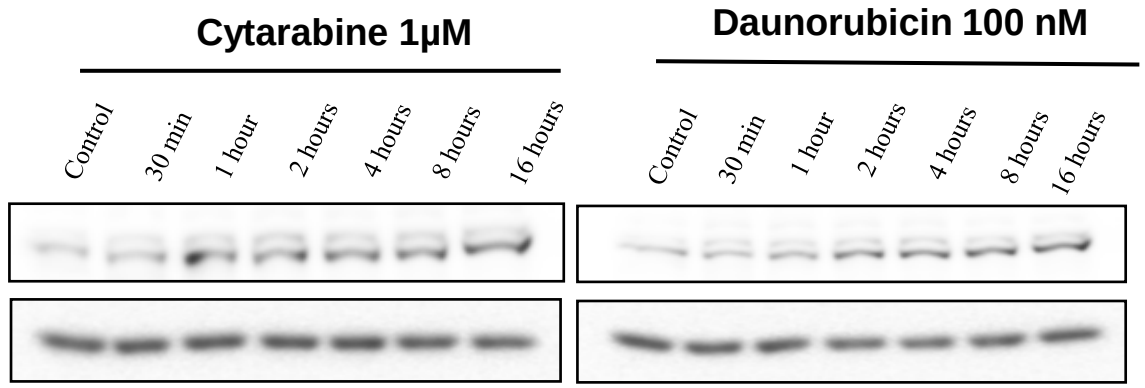
Cytarabine + Daunorubicin

Most common AML treatment

Resistance arises very often



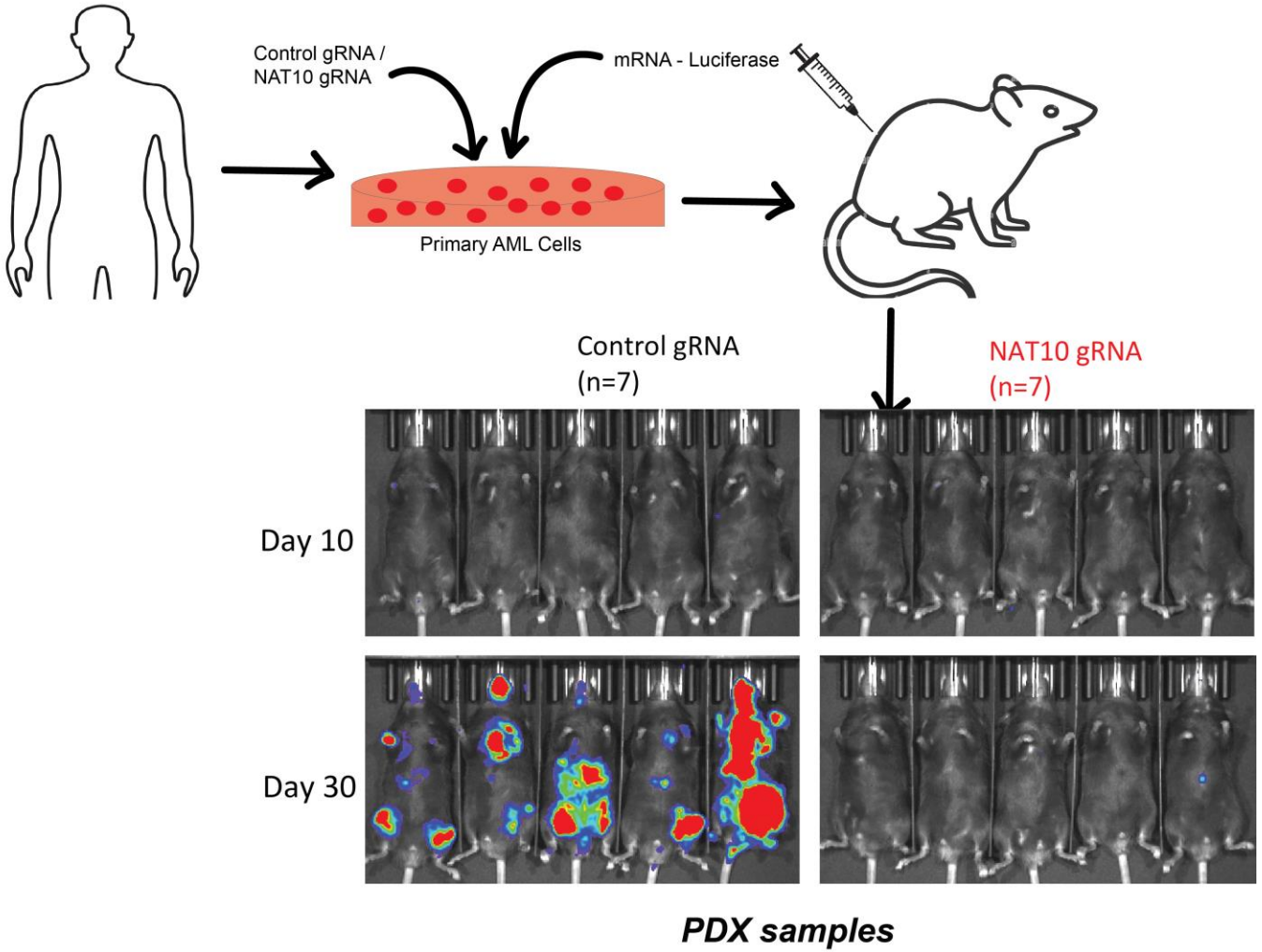
MOLM13 Cells



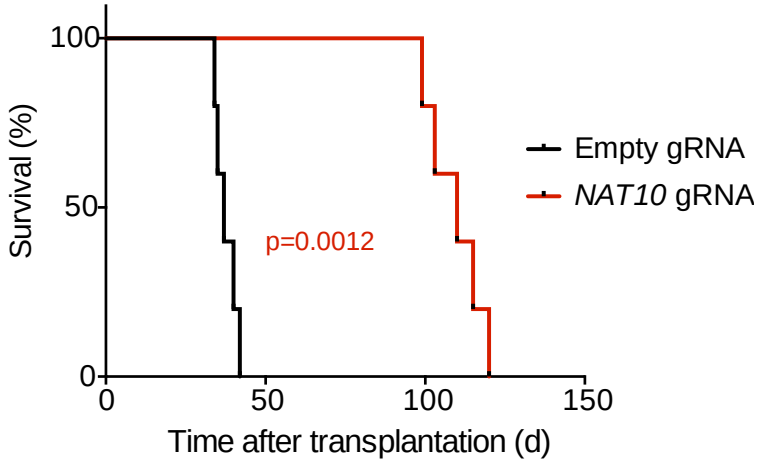
K562 Cells

Unpublished. Please do not post

NAT10 is a vulnerability in AML



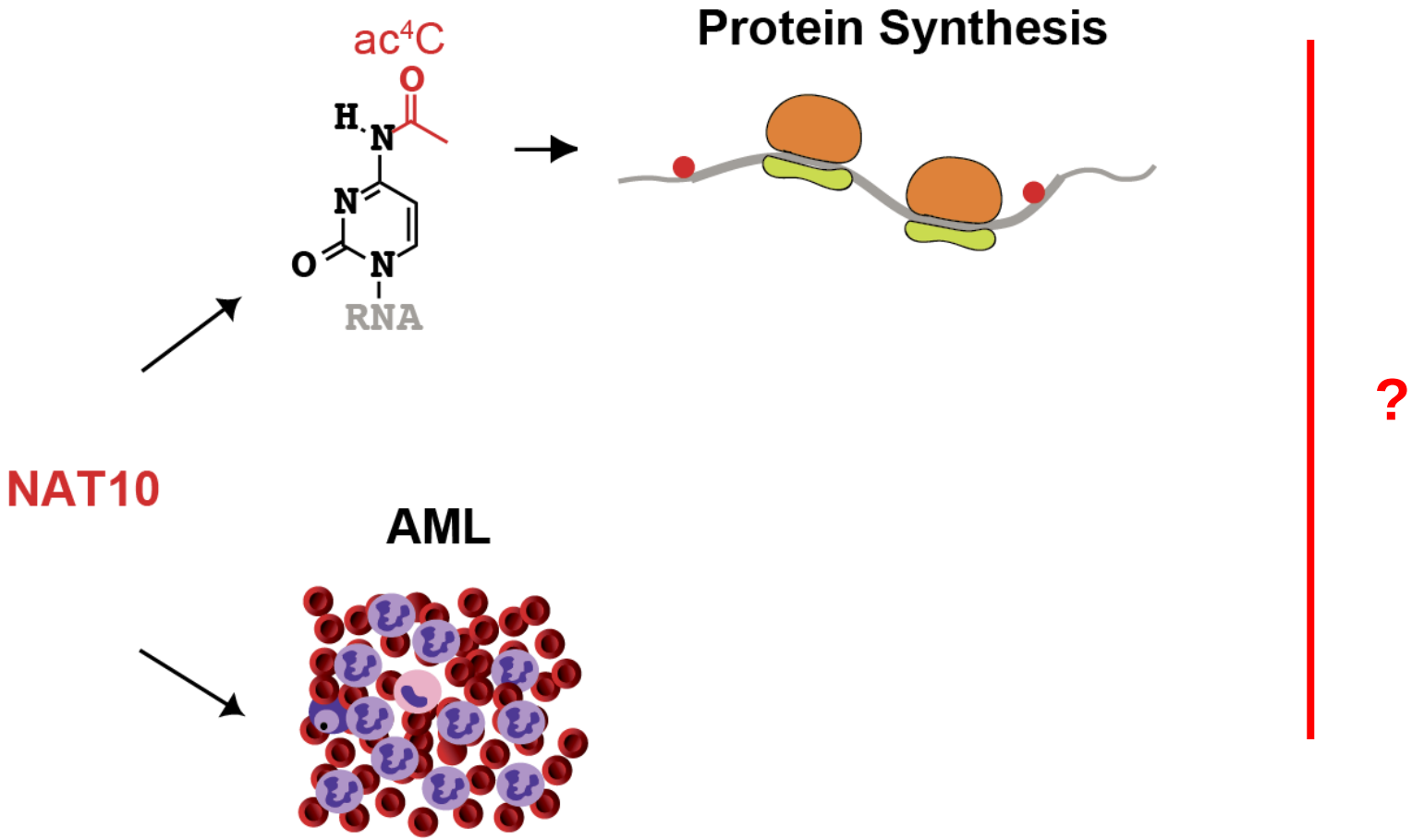
Survival curves of NPM1c PDX mice



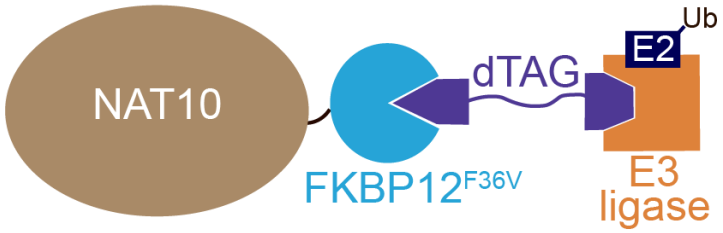
In collaboration with Kostas Tzelepis. University of Cambridge

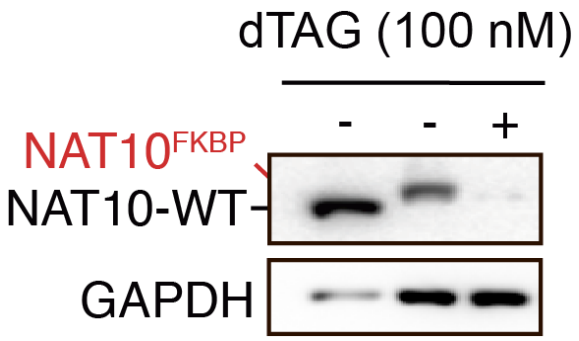
Unpublished. Please do not post

Investigating the oncogenic mechanisms of NAT10

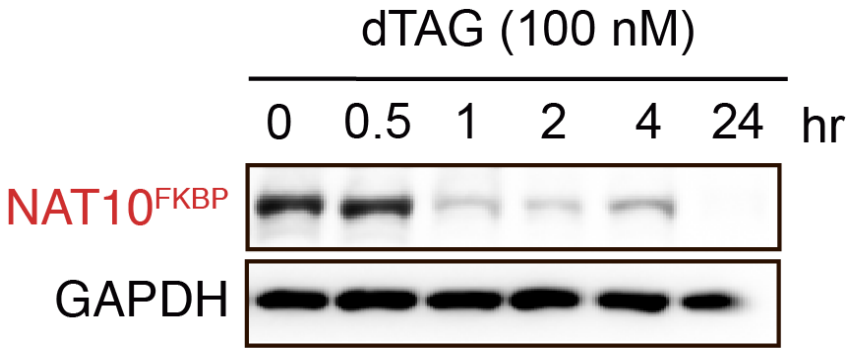


Generating PROTAC degraders for controlled depletion of NAT10





Sweta Raikundalia, Ph.D.



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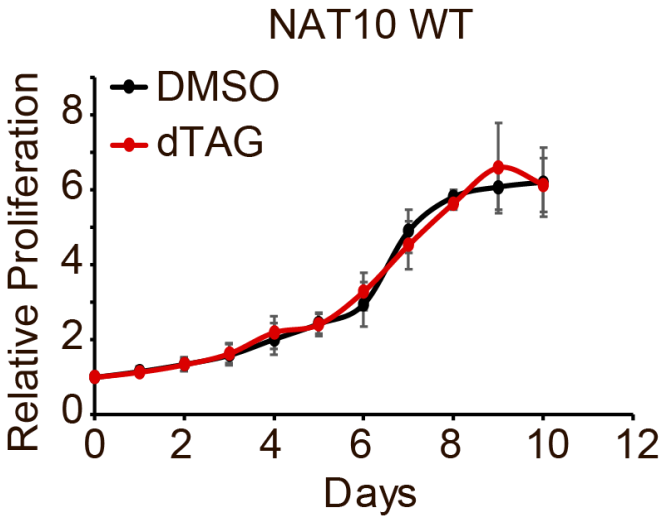
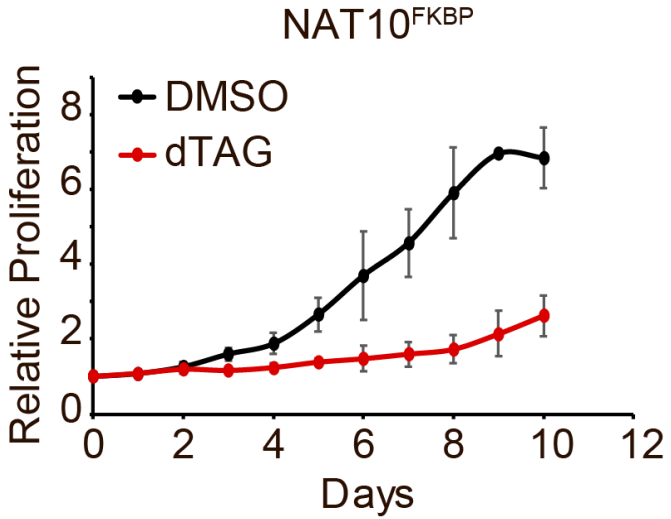
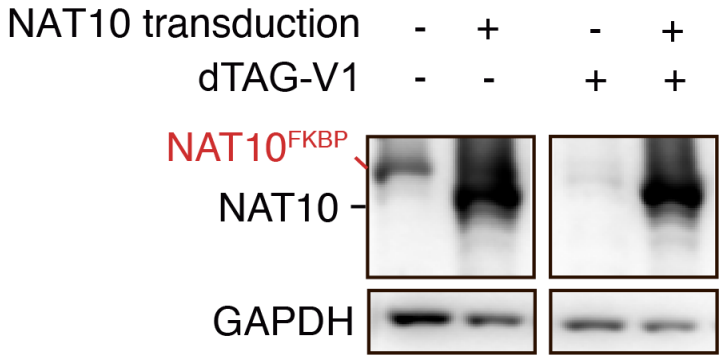
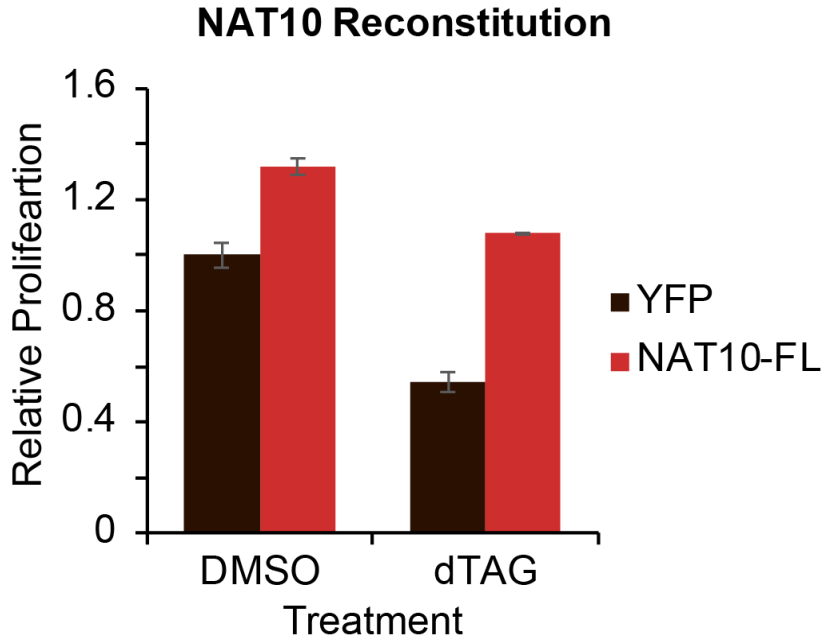
NAT10 promotes leukemia cells proliferation





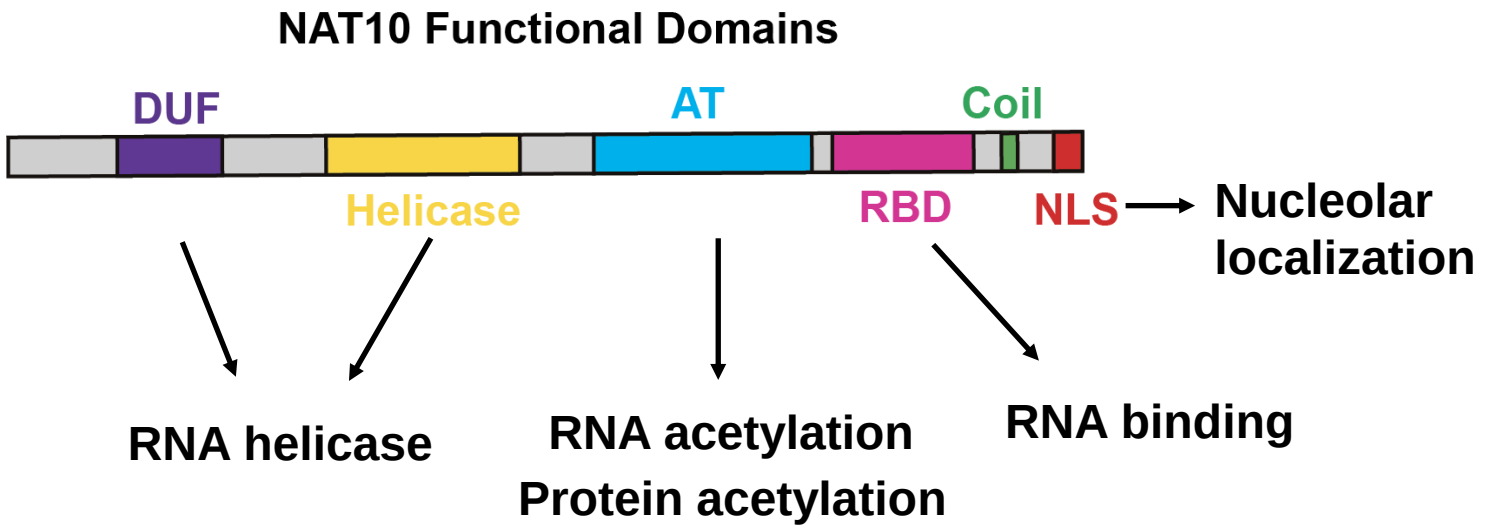




No induction of cell death

Unpublished. Please do not post

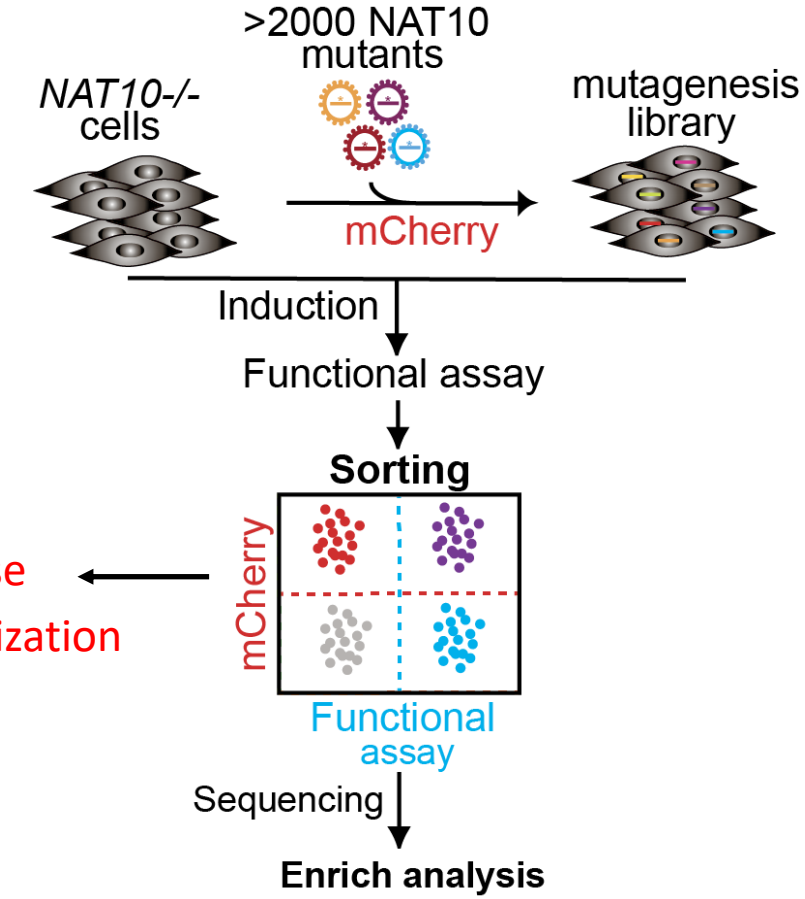
Investigating the oncogenic mechanisms of NAT10



Investigating the oncogenic mechanisms of NAT10

High-throughput approaches

Deep Mutagenesis Scanning



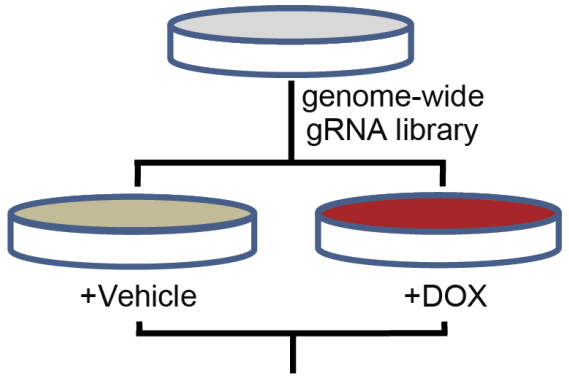
RNA helicase

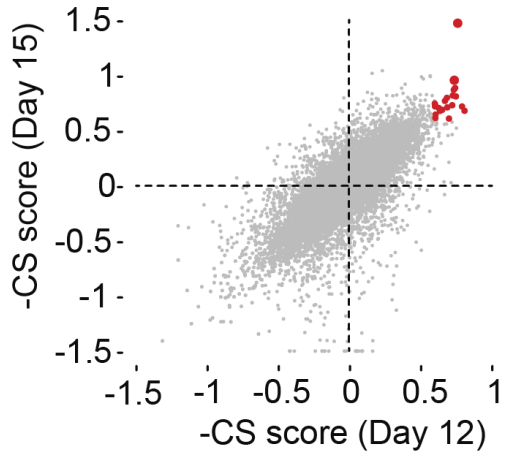
Subcellular localization

CRISPR screens

Counter Screen

Cas9-expressing cancer cell lines +shNAT10

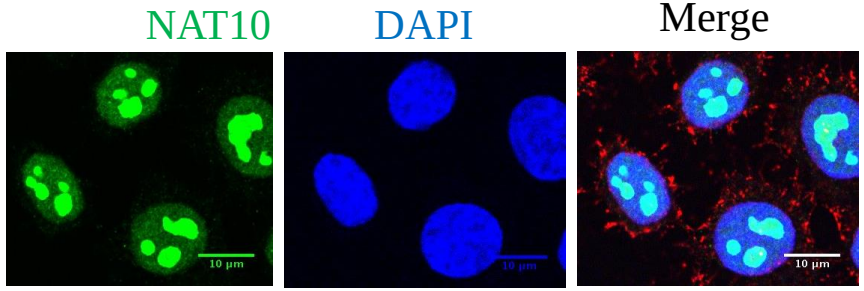


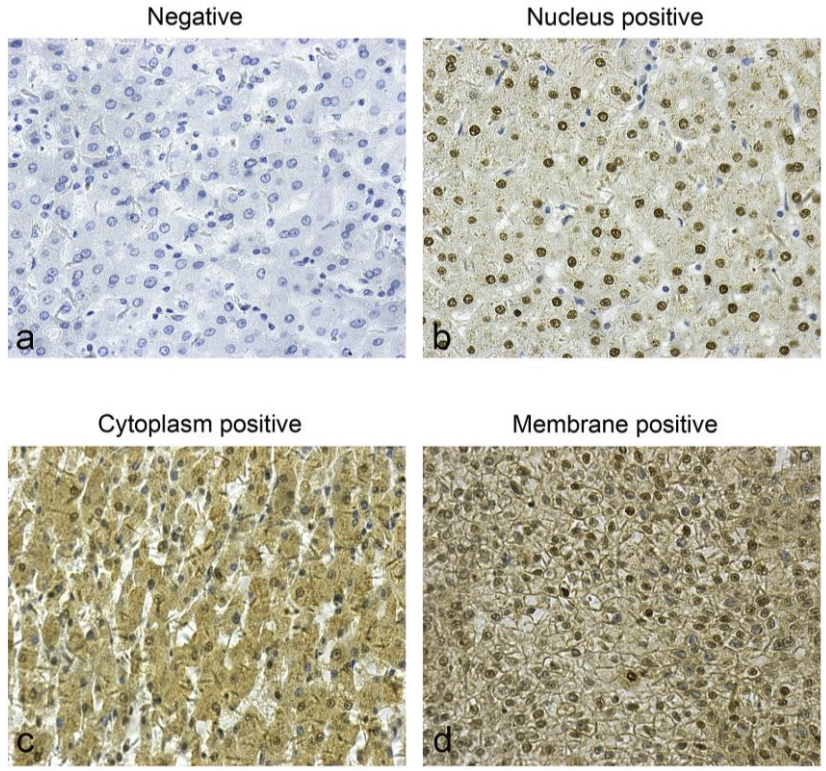


Translation regulators

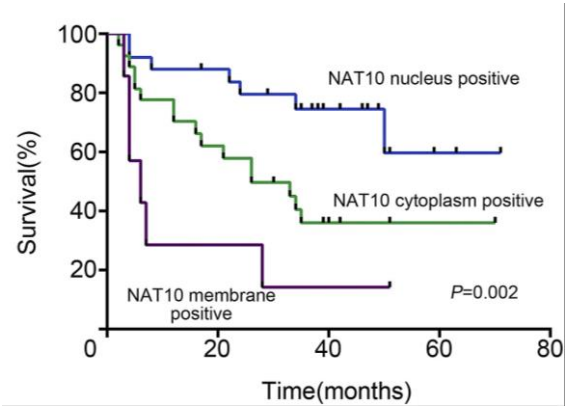
Cytoplasmic NAT10 is associated with poor prognosis in cancer

NAT10 is predominantly nucleolar

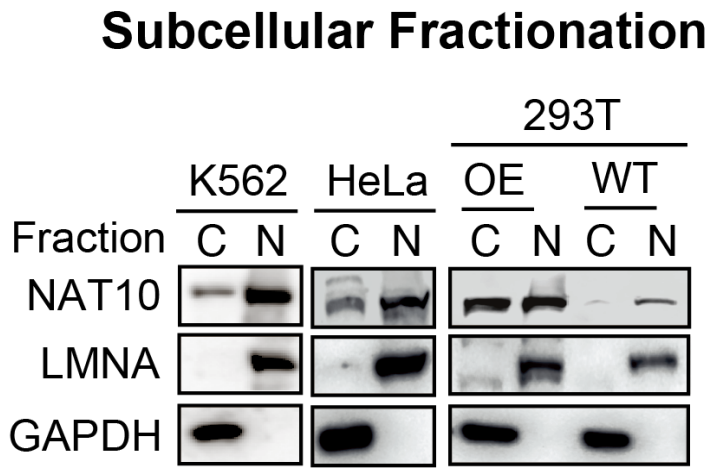




Tan et al., 2018 Biochem Biophys Res Comm

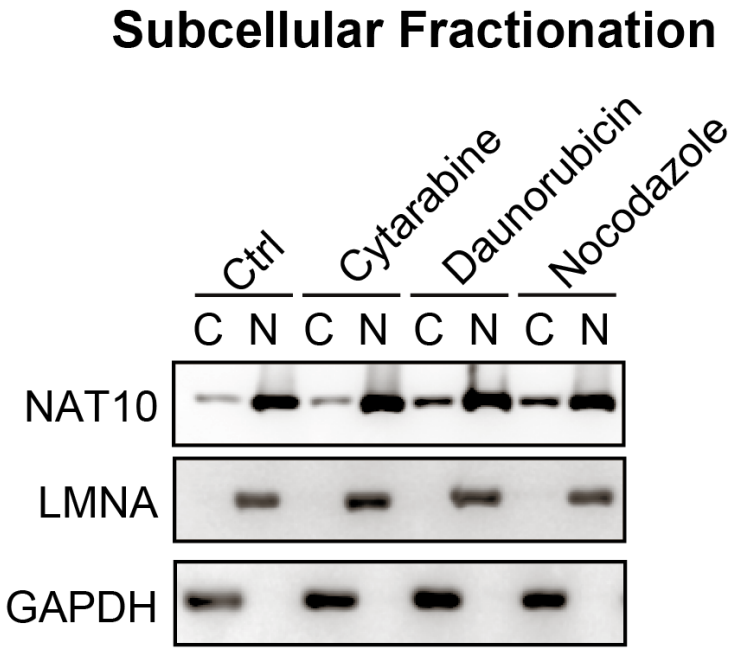


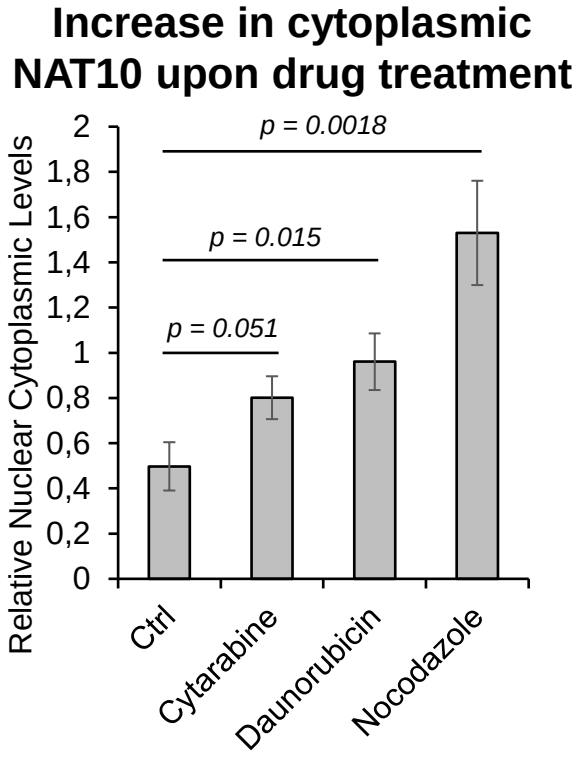
Cytoplasmic NAT10 correlates with mRNA acetylation



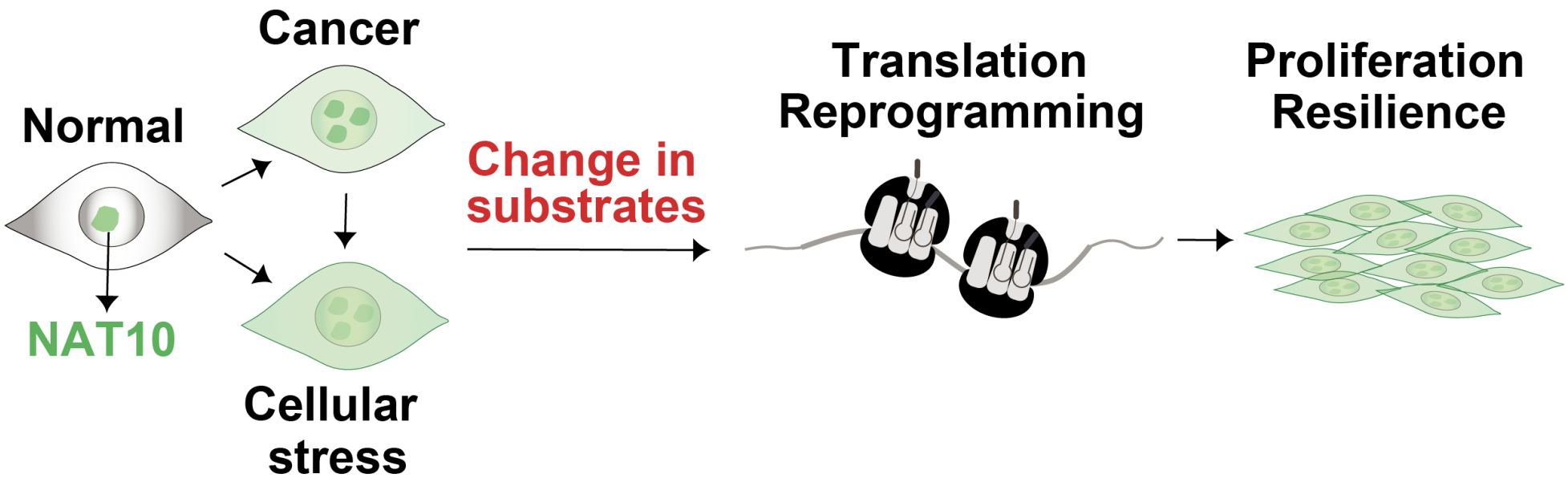
mRNA acetylation + ++ + -

Cytoplasmic NAT10 is observed in cancer cells treated with chemotherapeutic drugs





Working Model



Acknowledgements

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Lab members

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Mahmood Dalhat Postdoc
Kevin Vasquez, Graduate Student
Sharath Narayan, Graduate Student
Emmely Patrasso, Visiting Scholar
Adam Suh, Undergrad Student

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Masaki Hosogane, Tohoku University
Pablo Penaloza-MacMaster, Northwestern University
Grant Barish, Northwestern University

Former Mentors

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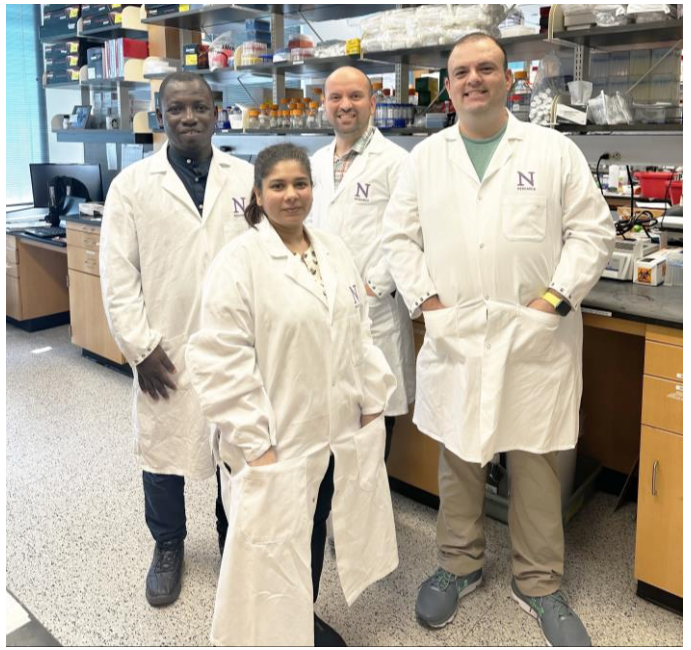
Mauricio Camargo, Universidad de Antioquia



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AACR American Association for Cancer Research

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