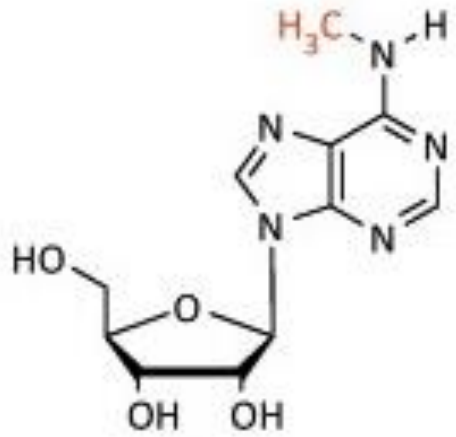
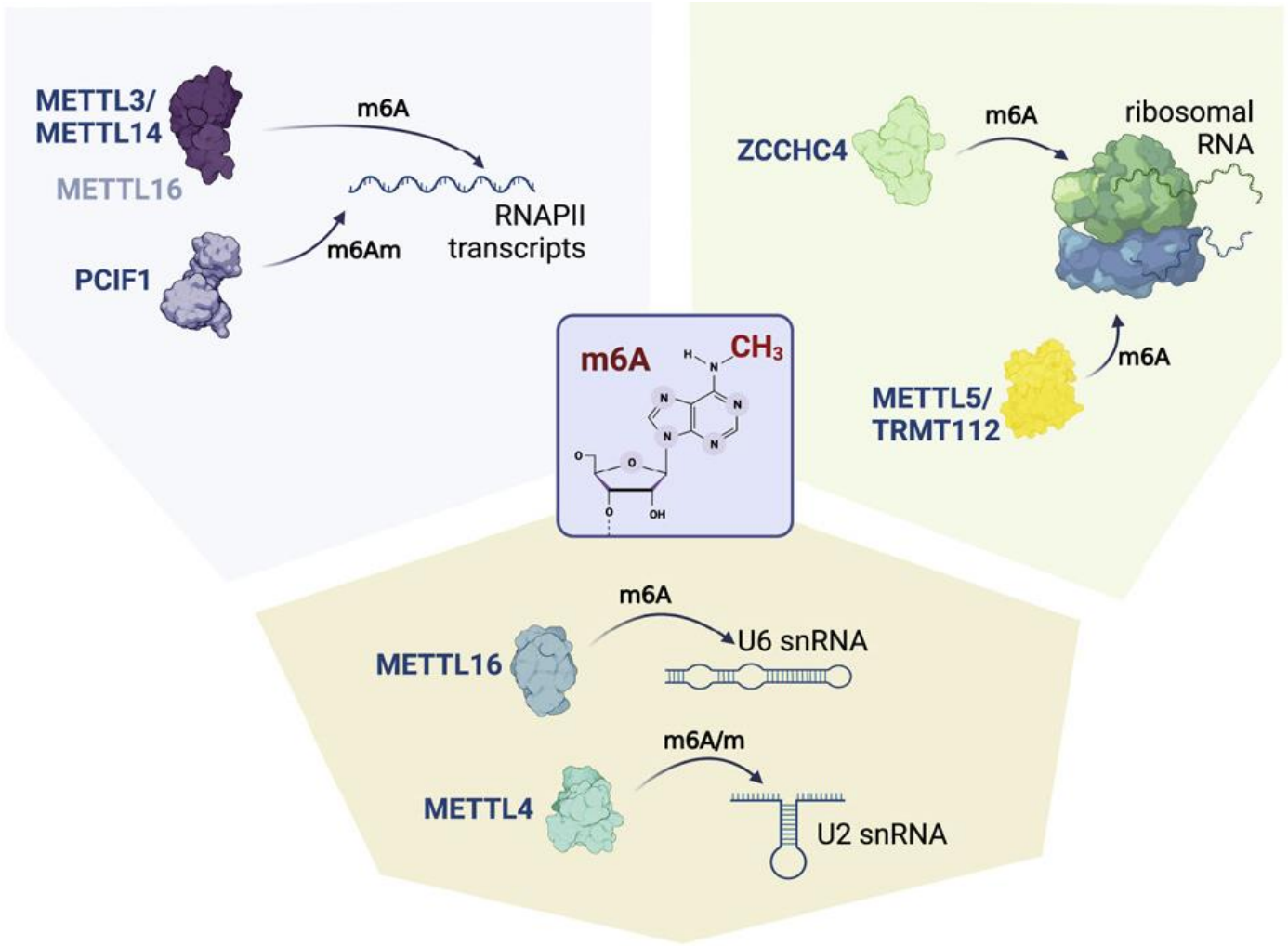
N^6-Methyladenosine (m^6A)

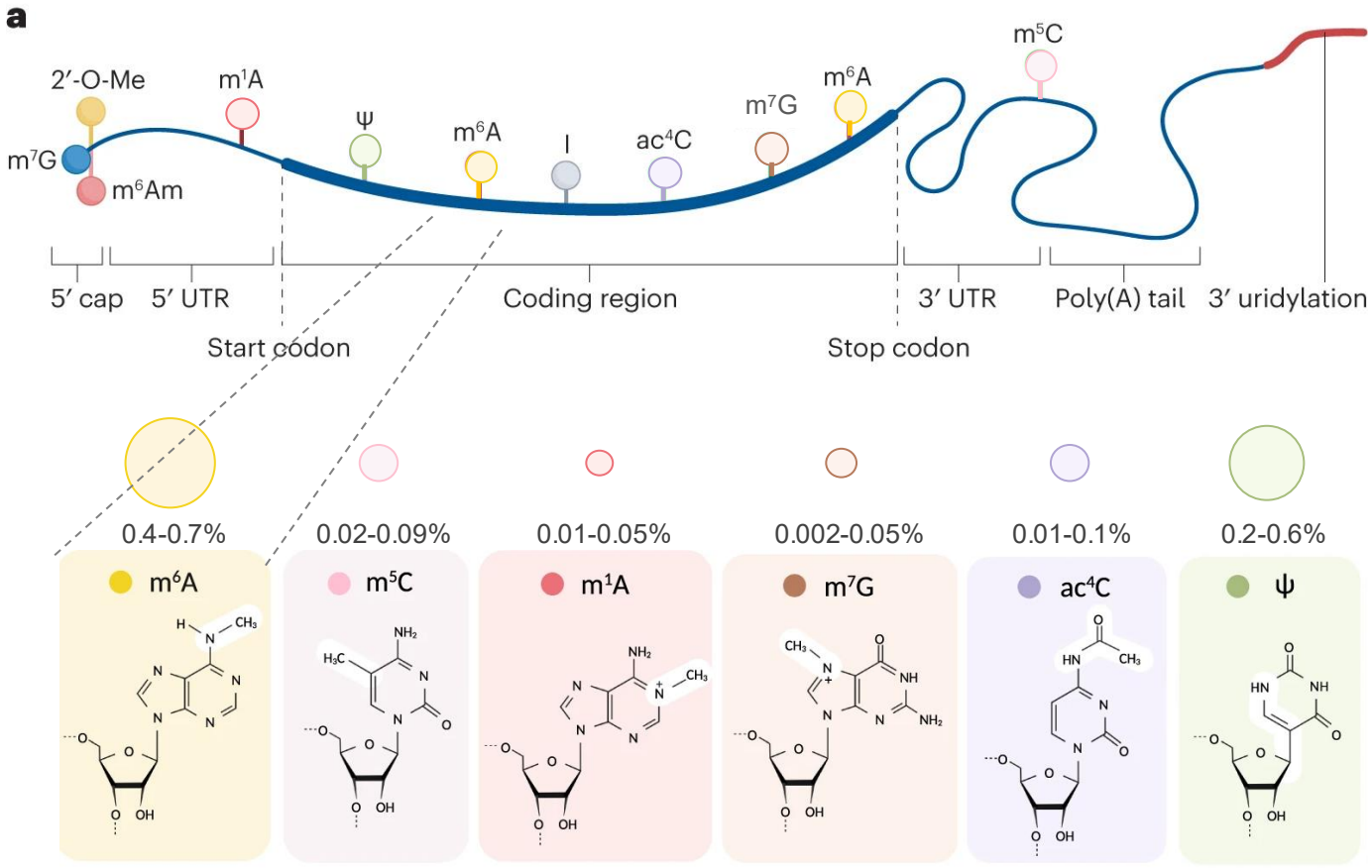


N^6-methyladenosine
(m^6A)

m^{6}A is deposited across a wide range of RNA species

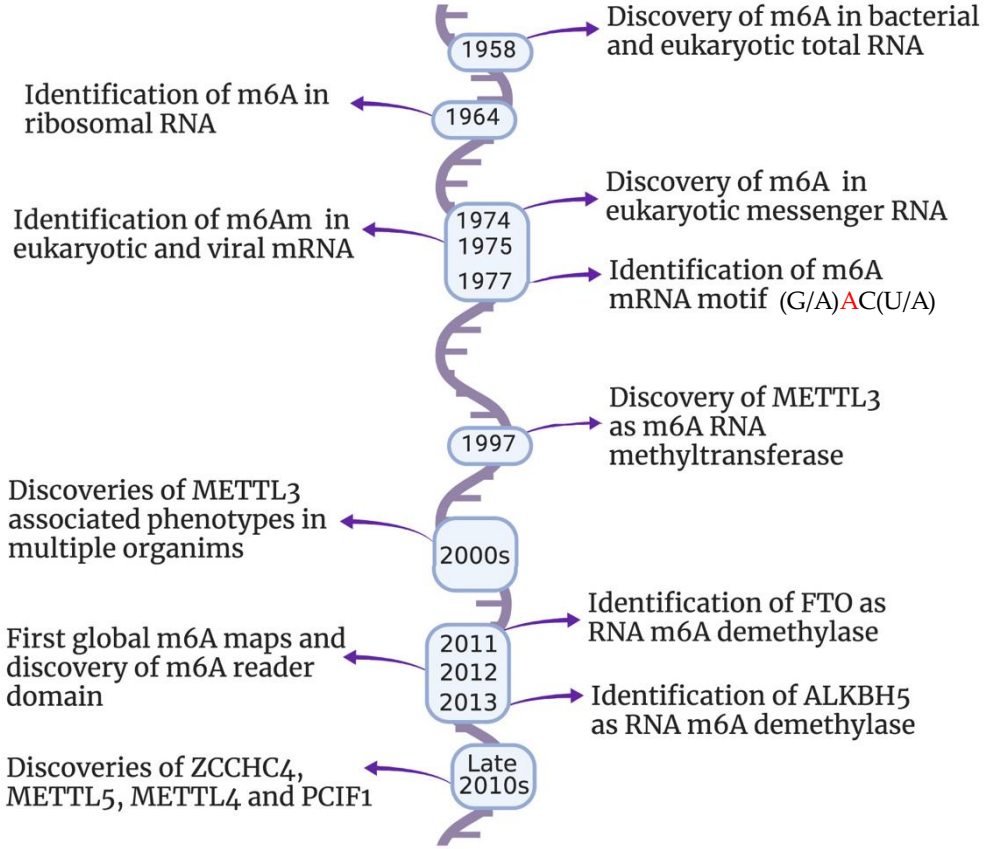


Nucleotide modifications in mRNA



Delaunay et al. 2024 Nature Rev Gen

A new….. old phenomenon



A new….. old phenomenon

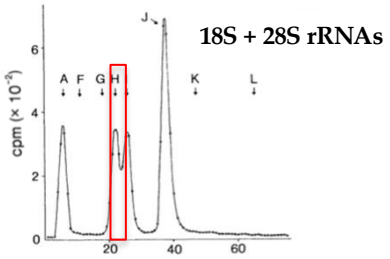
Proc. Nat. Acad. Sci. USA
Vol. 71. No. 10. pp. 3971–3975. October 1974

Identification of Methylated Nucleosides in Messenger RNA from Novikoff Hepatoma Cells

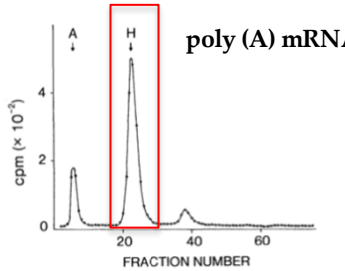
(RNA methylation/RNA processing/methylnucleoside composition)

RONALD DESROSIERS, KAREN FRIDERICI, AND FRITZ ROTTMAN*

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



18S + 28S rRNAs

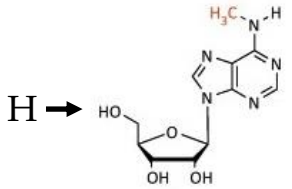


poly (A) mRNA

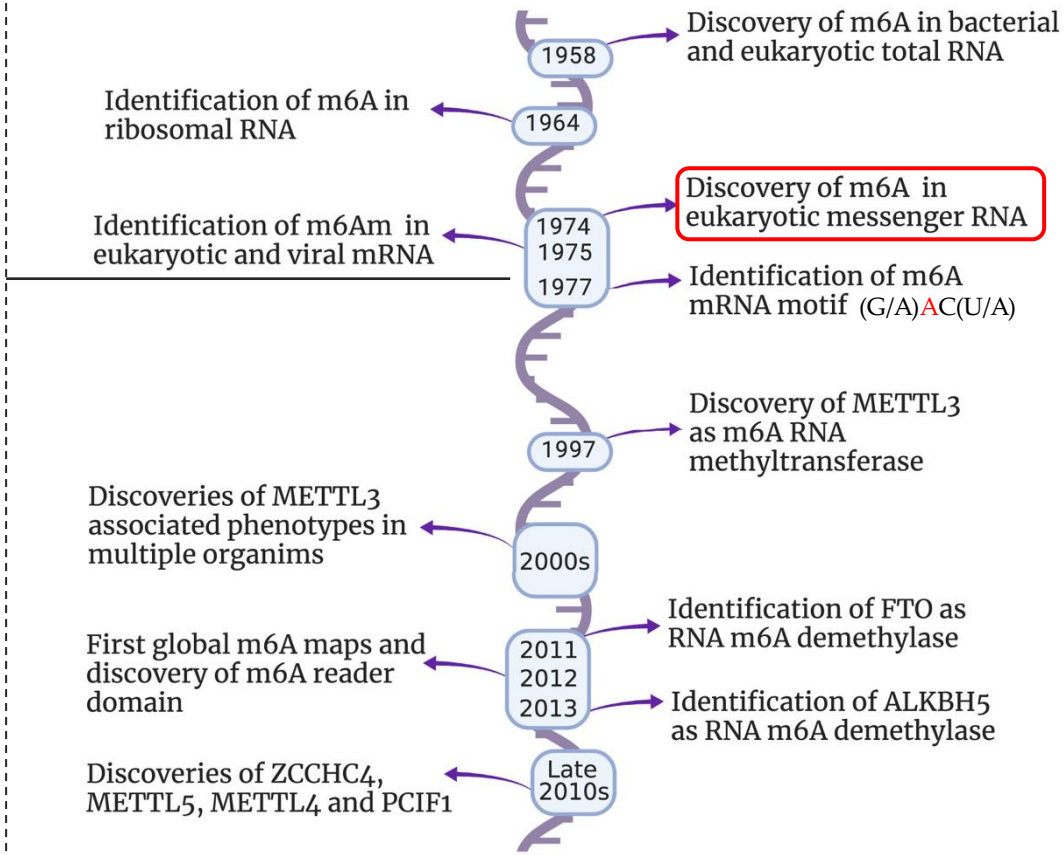
Cell, Vol. I, No. 1, January 1974, Copyright © 1974 by MIT

Existence of Methylated Messenger RNA in Mouse L Cells

R. P. Perry and D. E. Kelley
The Institute for Cancer Research,
Fox Chase Center for Cancer
and Medical Sciences
Philadelphia, Pennsylvania 19111



N⁶-methyladenosine (m⁶A)



A new….. old phenomenon

Nucleic Acids Research, Vol. 18, No. 19 5735

Sequence specificity of the human mRNA N6-adenosine methylase *in vitro*

Joan E.Harper*, Sheila M.Miceli[1], Richard J.Roberts[1] and James L.Manley
Department of Biological Sciences, Columbia University, New York, NY 10027 and [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

Biochem. J. (1992) **288**, 233–240 (Printed in Great Britain)

Partial purification of a 6-methyladenine mRNA methyltransferase which modifies internal adenine residues

Martin T. TUCK
Department of Chemistry and the Programme in Molecular and Cellular Biology, Ohio University, Athens, OH 45701, U.S.A.

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 269, No. 26, Issue of July 1, pp. 17697–17704, 1994

Printed in U.S.A.

Characterization and Partial Purification of mRNA N^6-Adenosine Methyltransferase from HeLa Cell Nuclei

INTERNAL mRNA METHYLATION REQUIRES A MULTISUBUNIT COMPLEX*

(Received for publication, February 3, 1994, and in revised form, April 25, 1994)

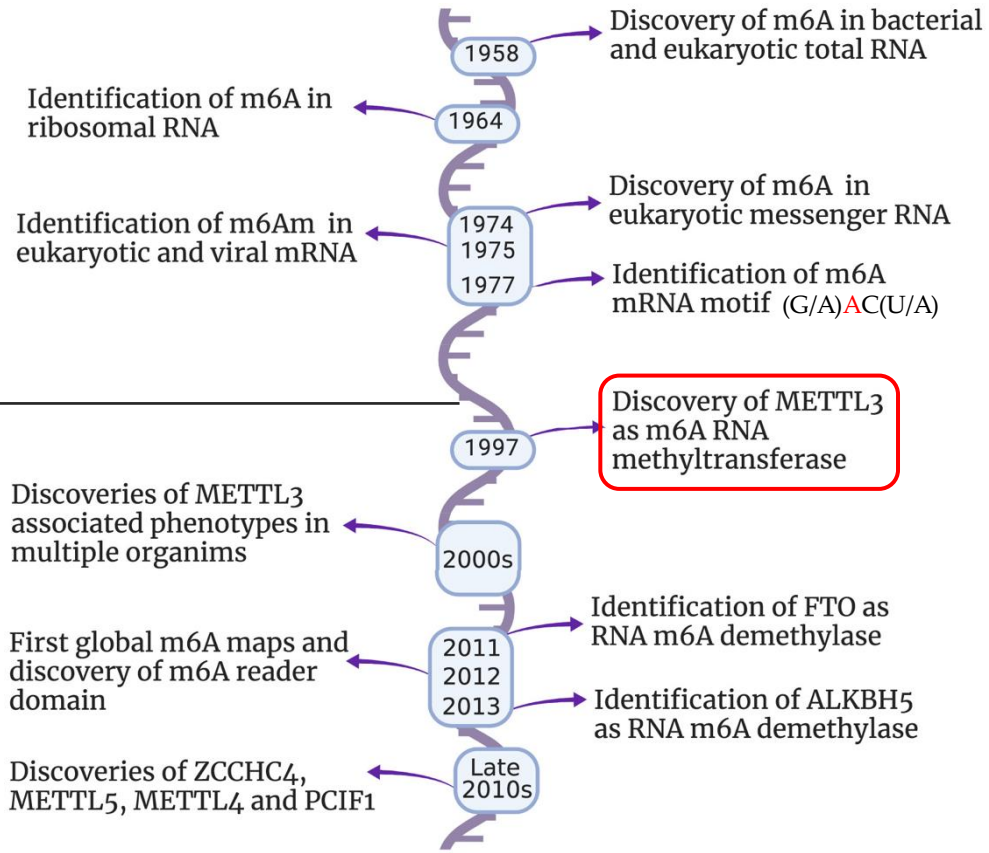
Joseph A. Bokar‡§, Mary Eileen Rath-Shambaugh, Rachael Ludwiczak, Prema Narayan¶, and Fritz Rottman‖

From the Department of Molecular Biology and Microbiology and the ‡Department of Internal Medicine, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase

J. A. Bokar, M. E. Shambaugh, D. Polayes, A. G. Matera and F. M. Rottman

RNA 1997 3: 1233-1247

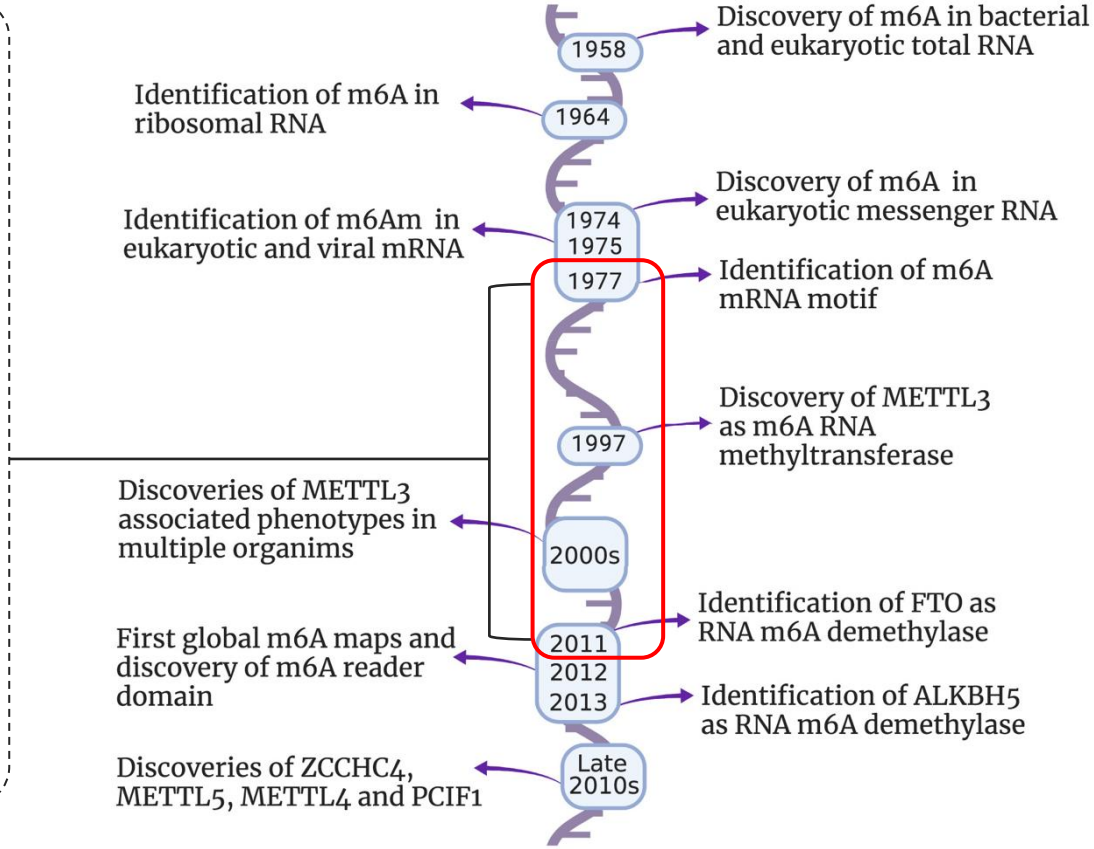


A new….. old phenomenon

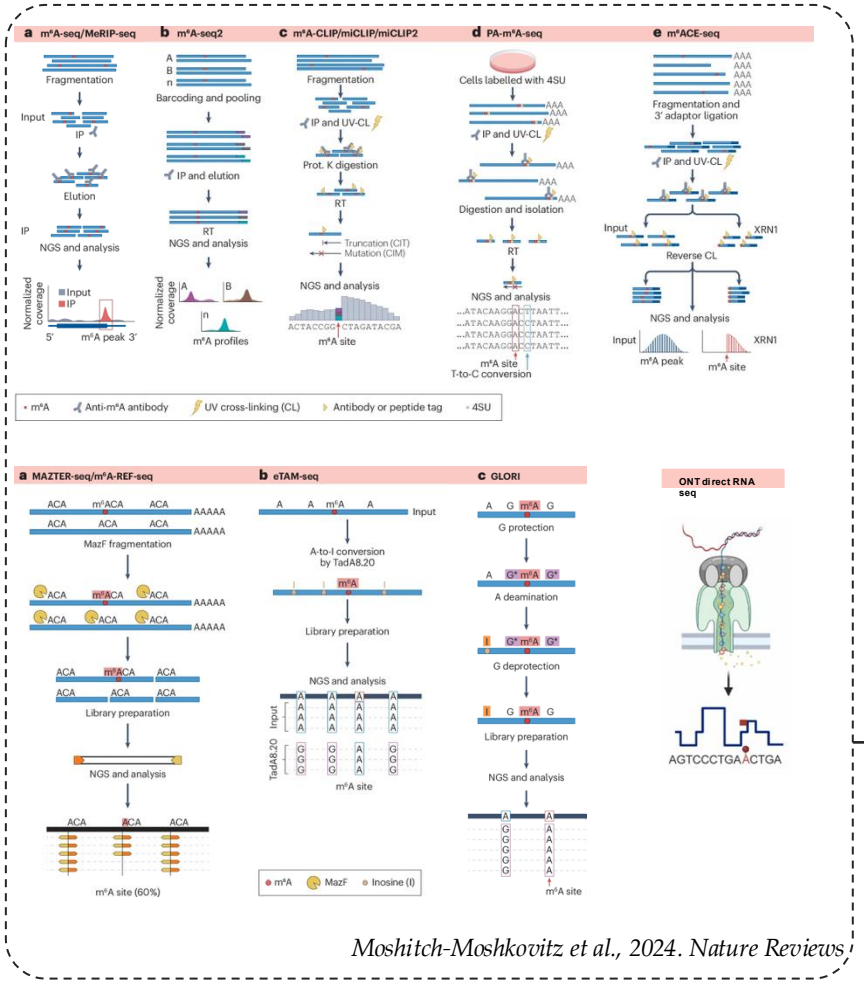
1974-2012: m^6A modification in mRNA was believed not biological relevant

Reasons:

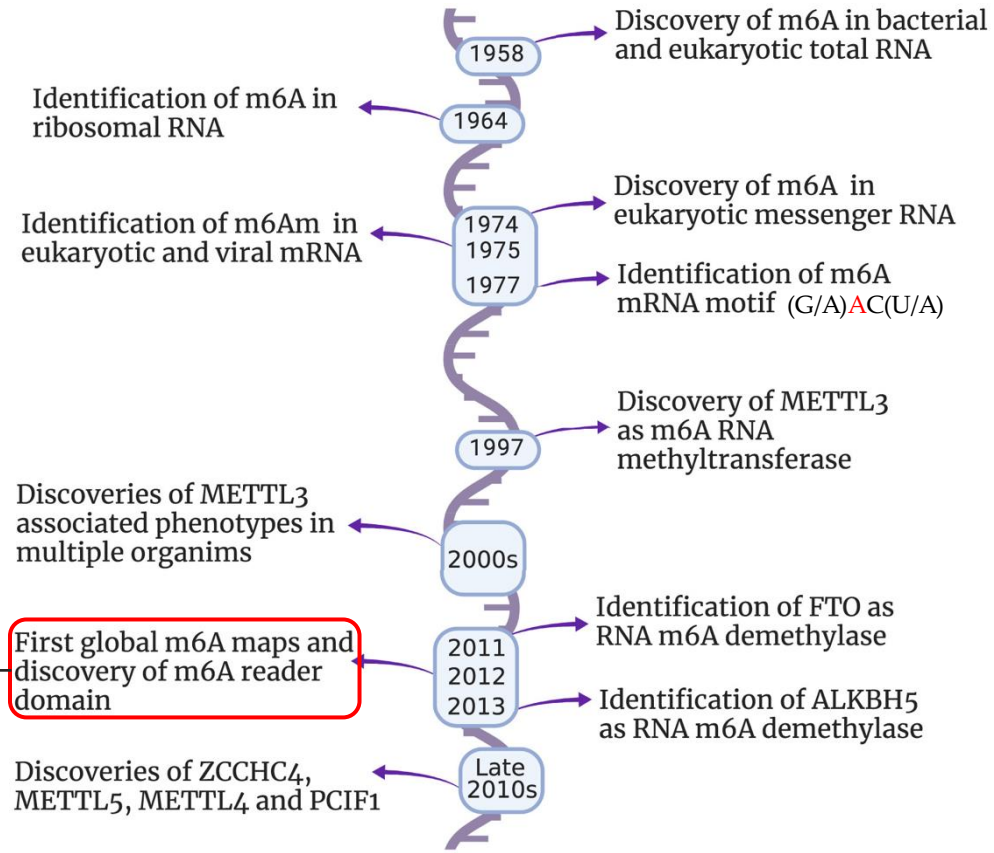
- contamination artefact,
- mutation of specific m^6A sites in viral RNA did not affect processing or the viral lifecycke (Kane and Beemon, 1987),
- no methods for global detection of m^6A sites in the transcriptome.



A new….. old phenomenon



Moshitch-Moshkovitz et al., 2024. Nature Reviews

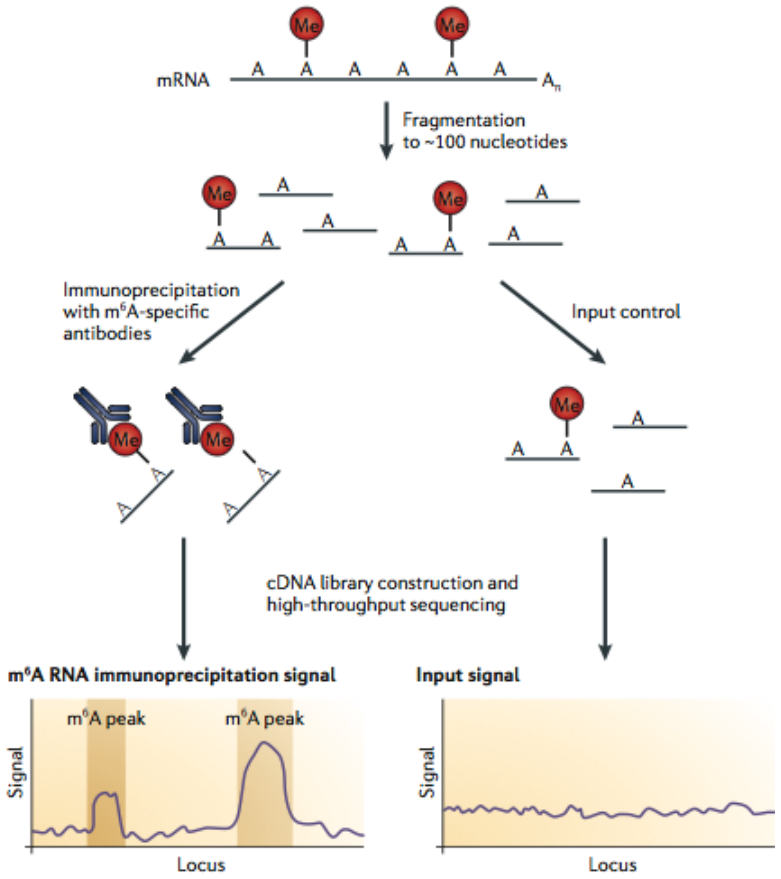


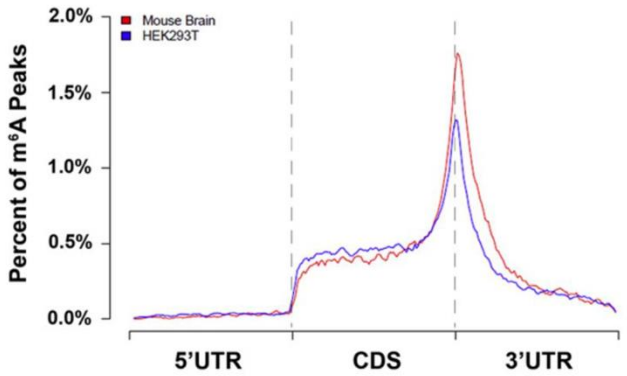
Before 2012, the genome-wide distribution of m^{6}A was unknown, until two independent studies developed an m^{6}A RNA immunoprecipitation approach followed by high-throughput sequencing (MeRIP–seq) to map the m^{6}A RNA methylomes with a ~100-nucleotide resolution. Numerous other methodologies have since followed over the years.

Methods for detecting m^{6}A

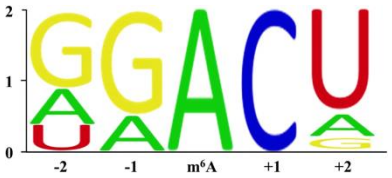
2012

MeRIP-Seq / m6A-Seq





m^{6}A motif: DRACH



- ~10,000 sites identified, average ~3 m^{6}A sites/transcript.
- Enrichment around the STOP codon (mRNA) or last exon (mRNA and lncRNA).
- 20% DRACH sequences modified.

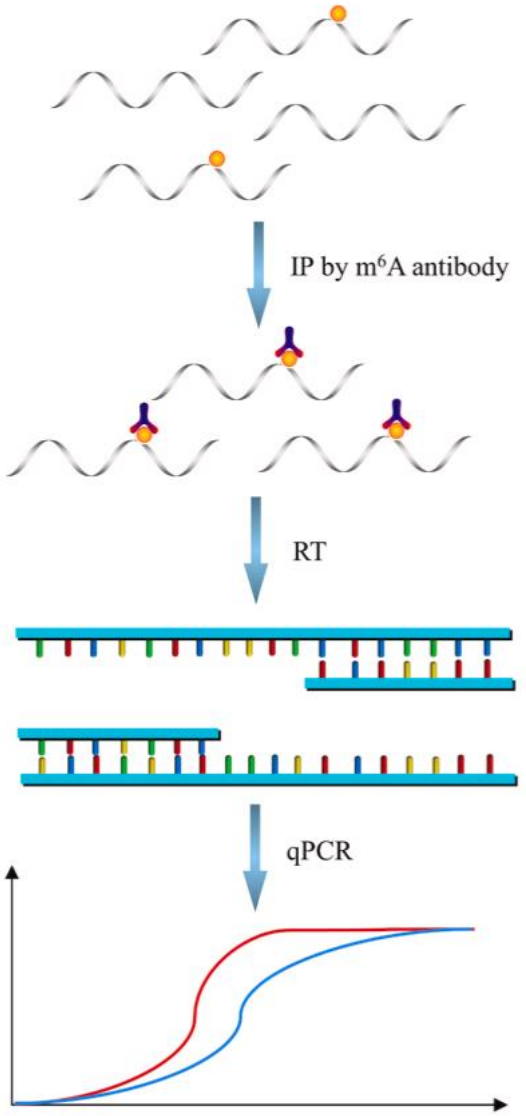
Resolution: ~100 nucleotides

Dominisini et al. 2012 Nature; Meyer et al., 2012 Cell

Methods for detecting m^{6}A

MeRIP-qPCR is suitable for m^{6}A identification at single mRNA target. MeRIP-qPCR uses premixed m^{6}A antibody immunobeads to obtain full-length m6A modified mRNA from total RNA directly. The enriched RNA-antibody complex is first digested by protease to remove antibodies, and then the mRNA is subjected to qPCR. The MeRIP-Seq/m6A-seq method can identify m^{6}A mRNA modifications, and then MeRIP- qPCR can verify the accuracy of results while studying the function of m^{6}A modifications on specific transcripts. The combination of MeRIP-Seq/m6A-seq and meRIP-qPCR is suitable for studying the majority of m^{6}A mRNA modifications.

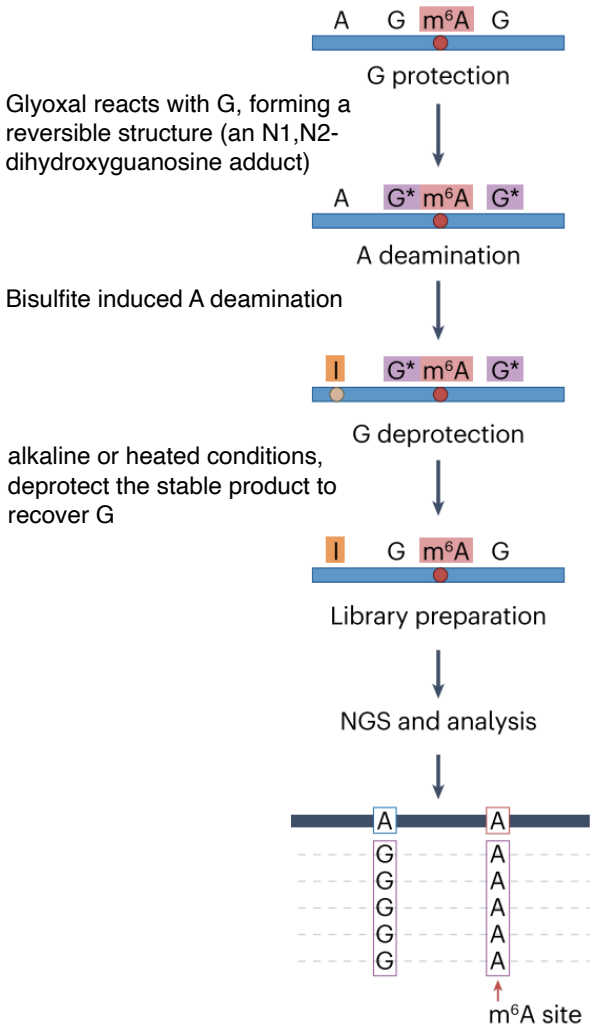
MeRIP–qPCR



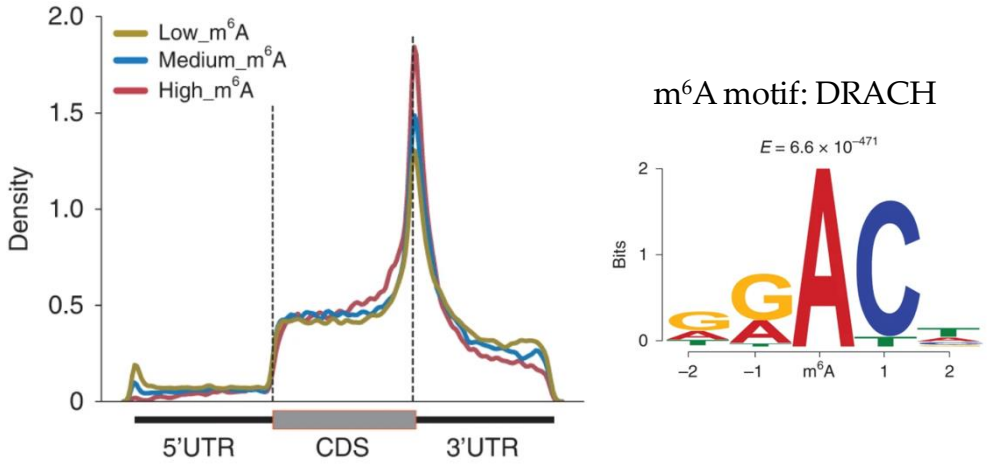
Methods for detecting m^{6}A

2023

m^{6}A mapping by glyoxal and nitrite-mediated adenosine deamination (GLORI)



Resolution: single nucleotide



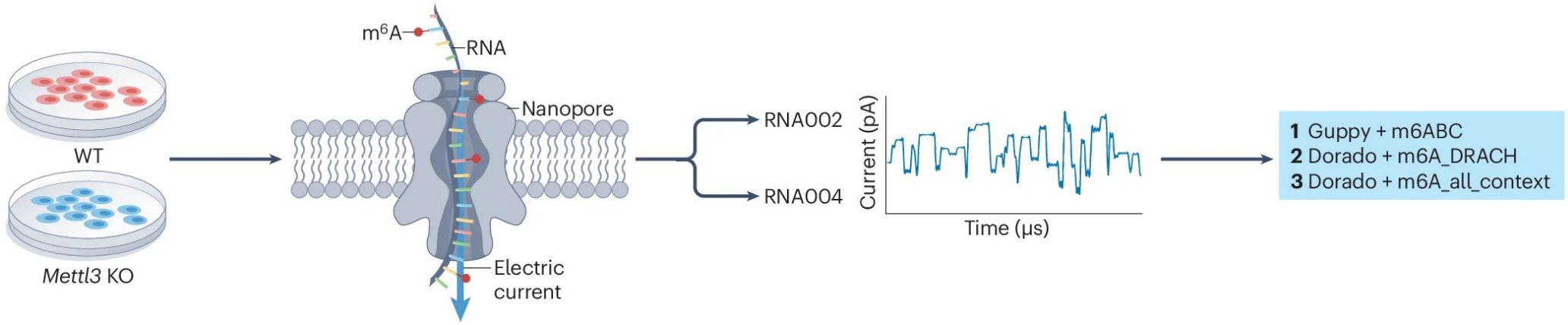
m^{6}A motif: DRACH

- >200,000 identified, average ~10 m^{6}A sites/transcript with several genes possessing even more than 100 modification sites.
- Enrichment around the STOP codon (mRNA) or last exon (mRNA and lncRNA).
- 40% DRACH sequences modified.

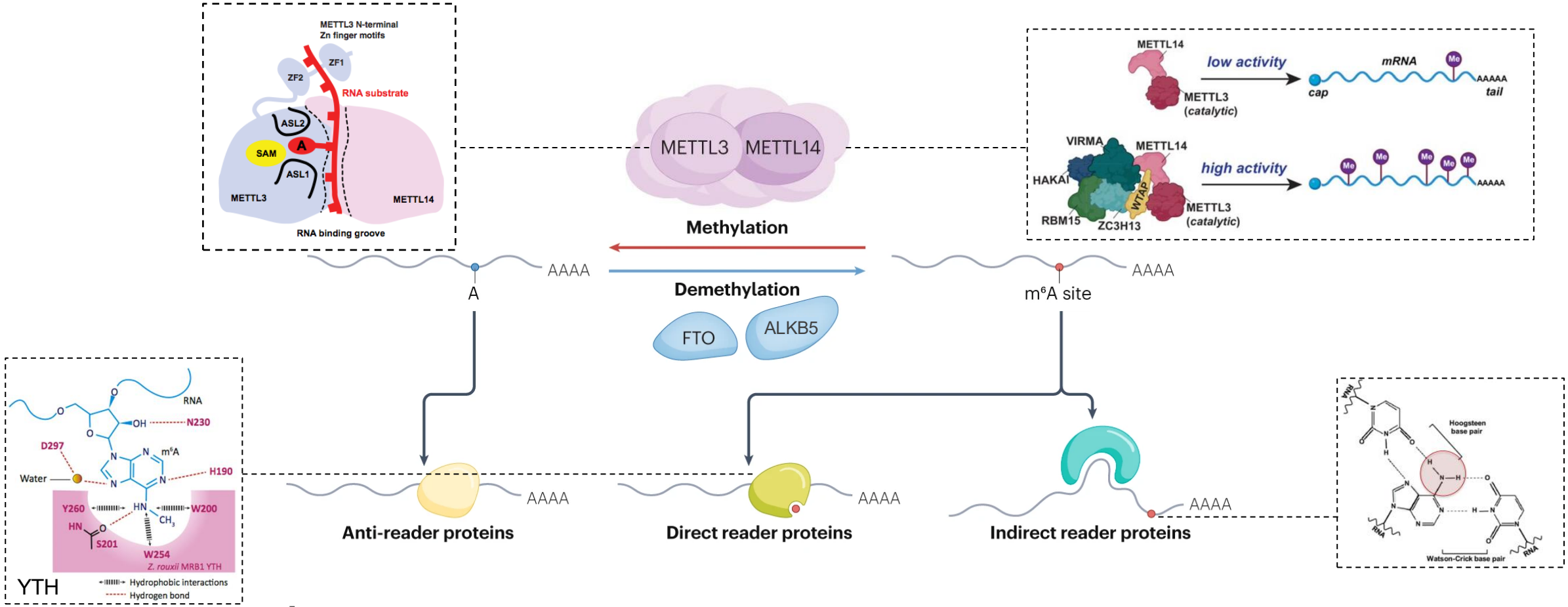
Liu et al. 2023 Nature Biotech.

Methods for detecting m^{6}A

Direct detection of RNA modification using nanopore sequencing has many advantages over NGS. No PCR amplification is required during sequencing, so there is no PCR bias. It can also identify multiple m^{6}A modification sites on a single RNA and multiple RNA modifications simultaneously in one experiment. However, some RNA modifications cause only small current changes, making it difficult to distinguish accurately between certain modifications, and potentially leads to high false positive rates. Computational methods have been developed based on the property that RNA modification causes an increase of "error" in the output of the base-calling algorithm.



m^{6}A writers, erasers and reaaders



b

Transcription silencing
- YTHDC1

Transcription activation
- YTHDC1

Splicing
- YTHDC1
- HNRNPC
- HNRNPG

Nuclear export
- YTHDC1

RNA stability and decay
- YTHDF2
- YTHDF1
- YTHDC1
- IGF2BP1
- IGF2BP2
- IGF2BP3

Translation
- YTHDF1
- YTHDF3
- eIF3

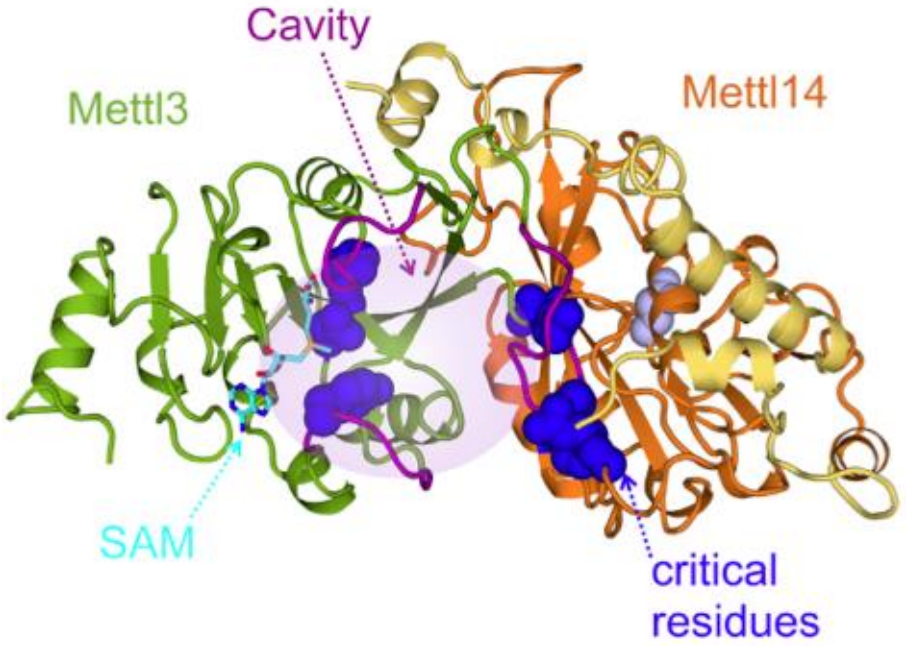
miRNA biogenesis
- HNRNPA2B1

Alternative polyA
- YTHDC1

- Indirect reader proteins
- Direct reader proteins

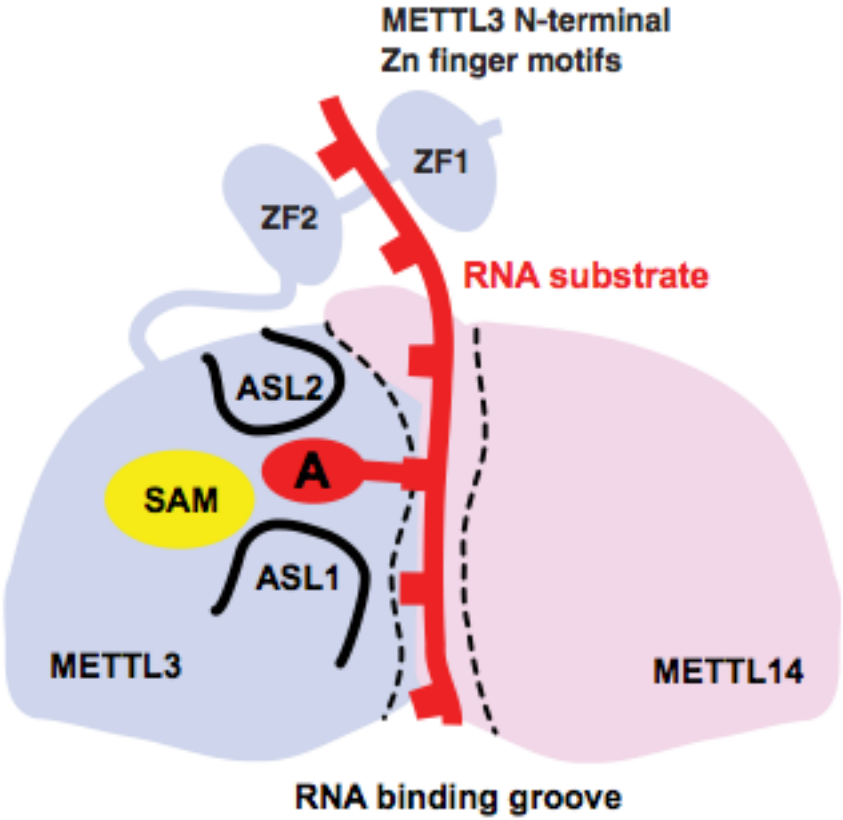
Moshitch-Moshkovitz et al., 2024. Nature Reviews

METTL3 is the catalytic subunit of the complex



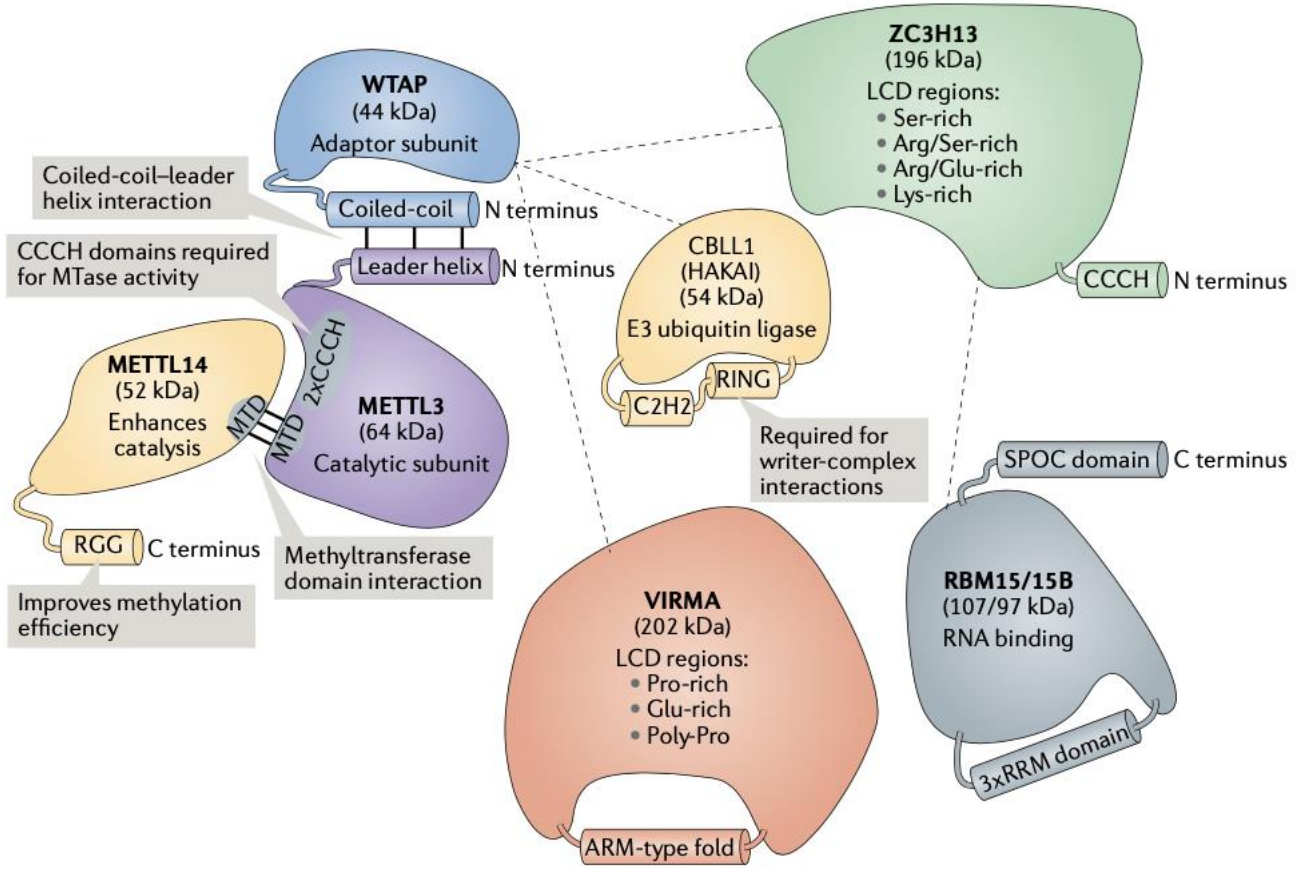
METTL14 has a degenerate active site and plays non-catalytic roles in maintaining complex integrity and substrate RNA binding.

The RNA binding activity of METTL3 depends on the presence of a conserved cluster of positively charged residues across the METTL3/METTL14 heterodimer interface and a N-terminal Zinc finger domain in the METTL3 protein



The m^{6}A writer-complex components

The m^{6}A writer complex is regulated by the MACOM complex, which contains diverse proteins. Depletion of MACOM components inhibits m^{6}A methylation. Why m^{6}A formation is mediated by such a large complex is not known, but the individual proteins probably have specific functions or may integrate different cellular signals in order to regulate methylation.



Zaccara et al. 2019. Nat. Rev. Mol. Cell. Biol.

m^6A writers show evolutionary conservation and important biological roles

Species	Methyltransferases	Auxiliary factors	Biological roles
Saccharomyces cerevisiae	IME4	MUM2, SLZ1	Required for meiosis and sporulation (Clancy et al. 2002). SLZ1 localizes the complex to the nucleolus for m^6A methylation (Schwartz et al. 2013).
Drosophila melanogaster	IME4	FL(2)D	IME4 is essential for viability (Hongay and Orr-Weaver 2011). IME4 is required for Notch signaling during oogenesis (Hongay and Orr-Weaver 2011). FL(2)D is required for splicing of *Sxl* and *tra* pre-mRNAs that are responsible for sexual determination (Penalva et al. 2000).
Arabidopsis thaliana	MTA	FIP37	Required for embryonic development (Zhong et al. 2008). Required for normal growth patterns, apical dominance, and plant development (Bodi et al. 2012).
Danio rerio	METTL3, METTL14	WTAP	METTL3 and WTAP are required for normal embryogenesis (Ping et al. 2014).
Mammals	METTL3, METTL14	WTAP	METTL3 and METTL14 regulate stem cell differentiation and reprogramming (Batista et al. 2014; Wang et al. 2014b; Geula et al. 2015). METTL3 regulates circadian periods (Fustin et al. 2013). Depletion of METTL3 and METTL14 leads to apoptosis in cancer cells (Bokar 2005). WTAP localizes METTL3–METTL14 to nucleus speckles (Ping et al. 2014). WTAP regulates cell cycle, splicing, and embryonic development (Horiuchi et al. 2006, 2013; Ping et al. 2014).

METTL3 knock-out mice exhibit embryonic lethality between E3.5 and E8.5.

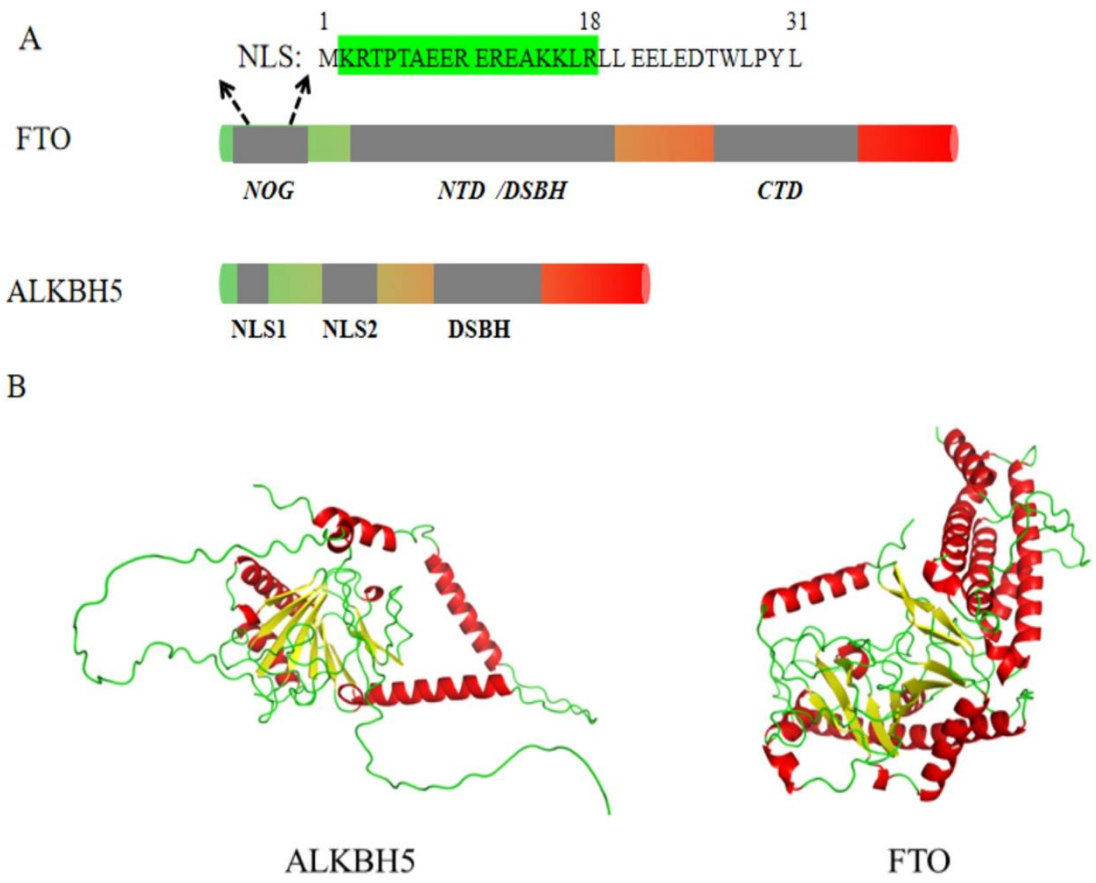
m^{6}A writers show evolutionary conservation and important biological roles

Protein	Genetic alteration (method)	System	Phenotypes
METTL3	RNA interference (Morpholino)	Zebrafish embryo	• Endothelial-to-hematopoietic transition ↓ • HSPC emergence ↓
	Endothelial KO (*Vec-Cre*)	Murine embryo (E10.5 AGM)	• HSPC and HEC frequency ↓ • In vitro CFU-forming ability and in vivo repopulating ability ↓
	Hematopoietic KO (*Vav-Cre*)	Murine embryo (E14.5 fetal liver)	• BM failure and perinatal lethality in homozygous knockout embryos • Cellularity, erythroid maturation, terminal myeloid differentiation ↓ • LSK and LT-HSC absolute number ↑ • In vitro CFU-forming and serial replating ability ↓ • In vivo repopulating ability ↓
	pIpC-inducible KO (*Mx1-Cre*)	Adult mouse	• Pancytopenia, splenomegaly, extramedullary hematopoiesis • BM cellularity, terminal differentiation of megakaryocyte and erythroid ↓ • BM phenotypic HSC frequency and absolute number ↑ • In vitro CFU-forming ability of HSCs ↓ • In vivo repopulating ability of purified HSCs ↓
	Myeloid KO (*Lysm-Cre*)	Adult mouse	• BM and SP cellularity, complete blood count, no$^{\Delta}$ • Homeostatic and lipopolysaccharide-induced myelopoiesis, no$^{\Delta}$
	RNA interference (shRNA)	Human CD34$^+$ HSPC (cultured ex vivo)	• CFU-forming ability and cell growth ↓ • Apoptosis, no$^{\Delta}$ • Myeloid differentiation ↑
METTL14	Tamoxifen-inducible KO (Cre^{ERT})	Adult mouse	• Complete blood count, no$^{\Delta}$ • In vivo repopulating ability of BM cells ↓
	pIpC-inducible KO (*Mx1-Cre*)	Adult mouse	• BM cellularity and BM LSK frequency, no$^{\Delta}$ • In vivo long-term repopulating ability of BM cells ↓
	RNA interference (shRNA)	Human CD34$^+$ HSPC (induced toward myelopoiesis ex vivo)	• CFU-forming ability ↓ • Cell growth, apoptosis, no$^{\Delta}$ • Monocytic differentiation ↑

m^6A erasers

FTO and ALKBH5 belong to the AlkB family of Fe(II) and α-ketoglutarate-dependent dioxygenases, capable of removing alkyl adducts from bases through oxidative demethylation.

FTO, and ALKBH5 exhibit differences in intracellular functions and biological roles, likely stemming from variances in structure, substrate selection, cellular localization, and protein interactions.

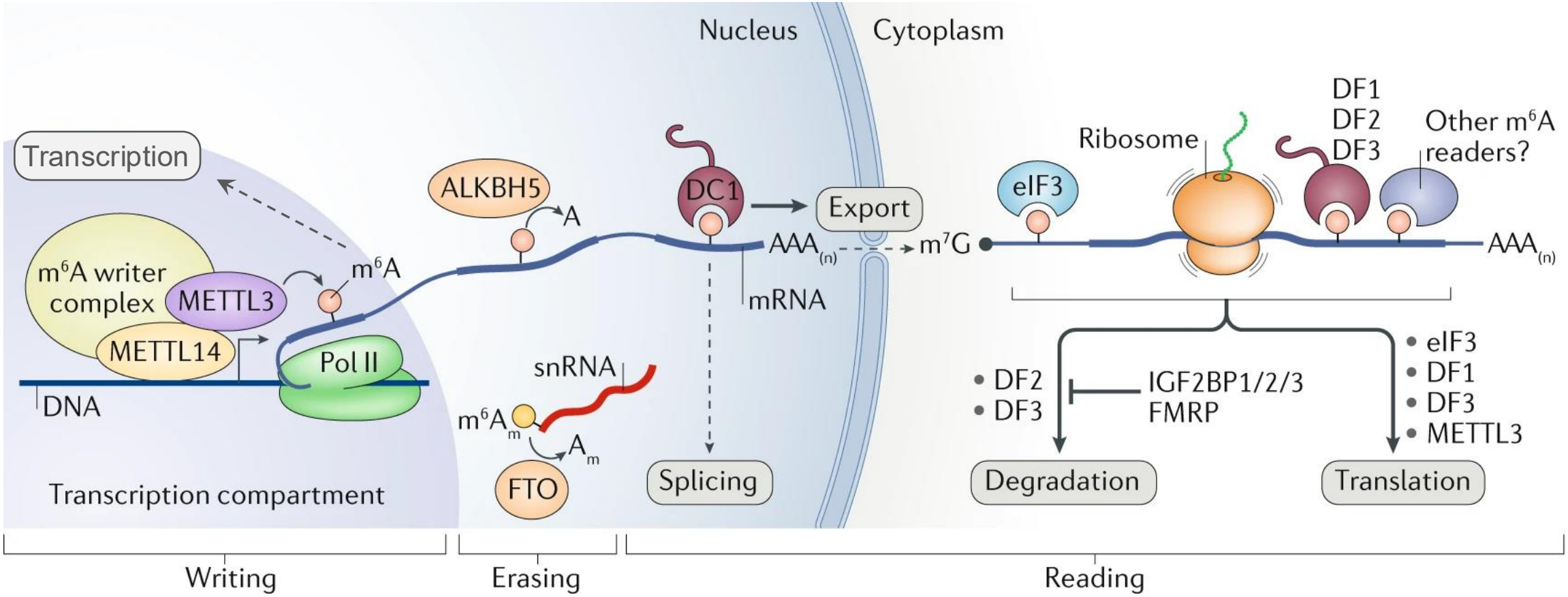


DSBH represents the double-stranded β-helix domain, NOG stands for the N-oxoglutarate binding domain, and CTD represents the carboxyl-terminal domain. The nuclear localization signal is the specific NLS sequence in the NOG structure of FTO

m^6A erasers

Protein	FTO	ALKBH5
Target	ssDNA, ssRNA m^6A , m^6Am, m^1A	ssDNA, ssRNA m^6A
Localization	Nucleus, cytoplasm	Nucleus
Function in tissues	Alternative splicing, regulates cap-independent translation upon heat shock, affects neuronal development	mRNA export, splicing in mouse male germ cells
KO phenotype in mouse	Impaired adipocytes differentiation, lean phenotype, growth retardation	Infertility and impaired spermatogenesis
Highest expression	Brain	Testis
Connection with disease	Neuronal and metabolic defects, AML, breast cancer, glioblastoma	Breast cancer, glioblastoma, infertility

The m^{6}A mRNA life cycle

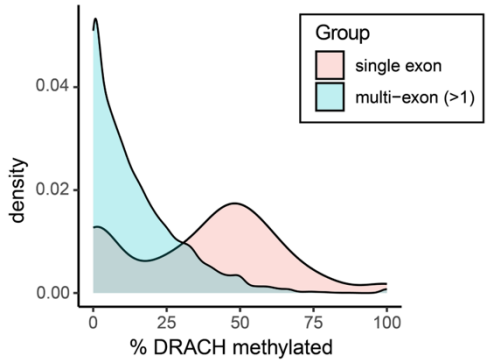


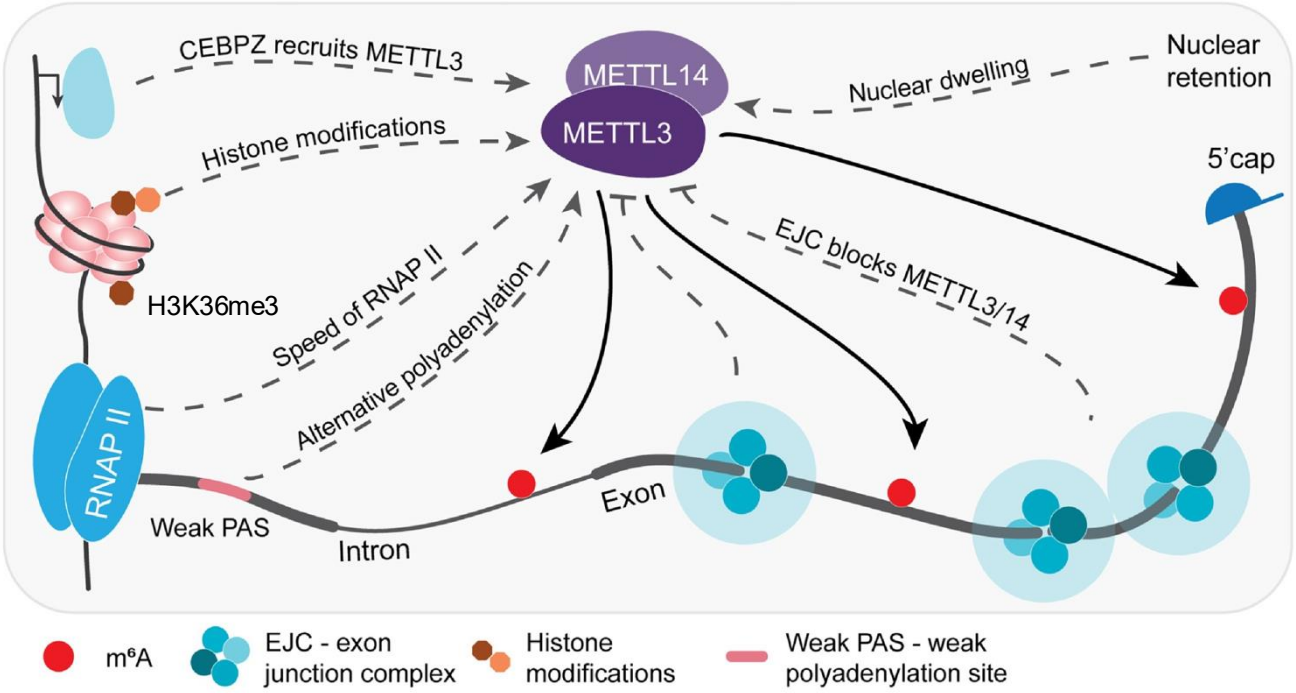
METTL3 is the m^{6}A methylases specific for mRNA (99%), **METTL16** for U6 snRNA and few mRNAs (1%), while ZCCHC4 and METTL5 are specific for rRNAs.

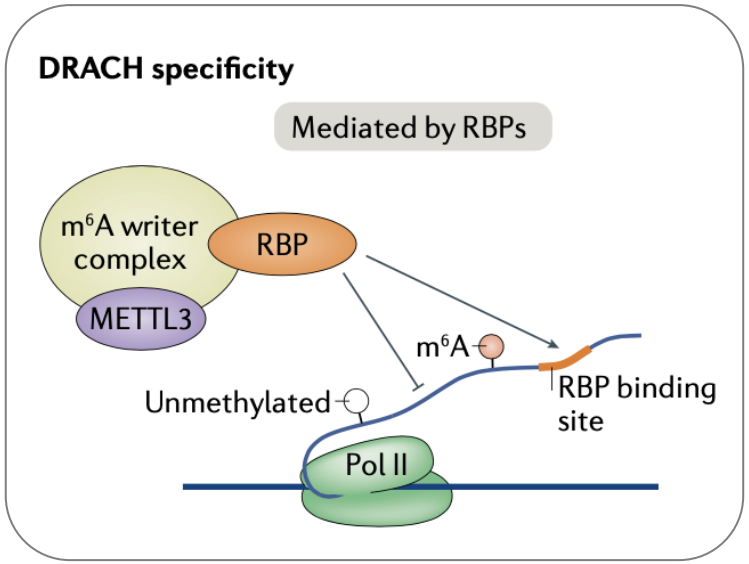
adapted from Zaccara et al., 2019. Cell

Factors influencing m^{6}A deposition

- METTL3/14 recruitment by TFs
- Histone modifications
- RNA Pol II elongation rate
- Nuclear retention
- EJC and SR protein deposition
- METTL3/14 recruitment by RBPs

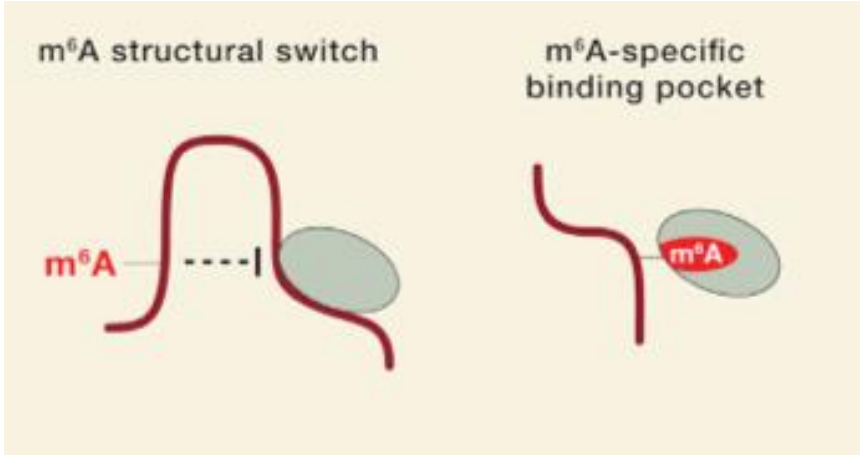




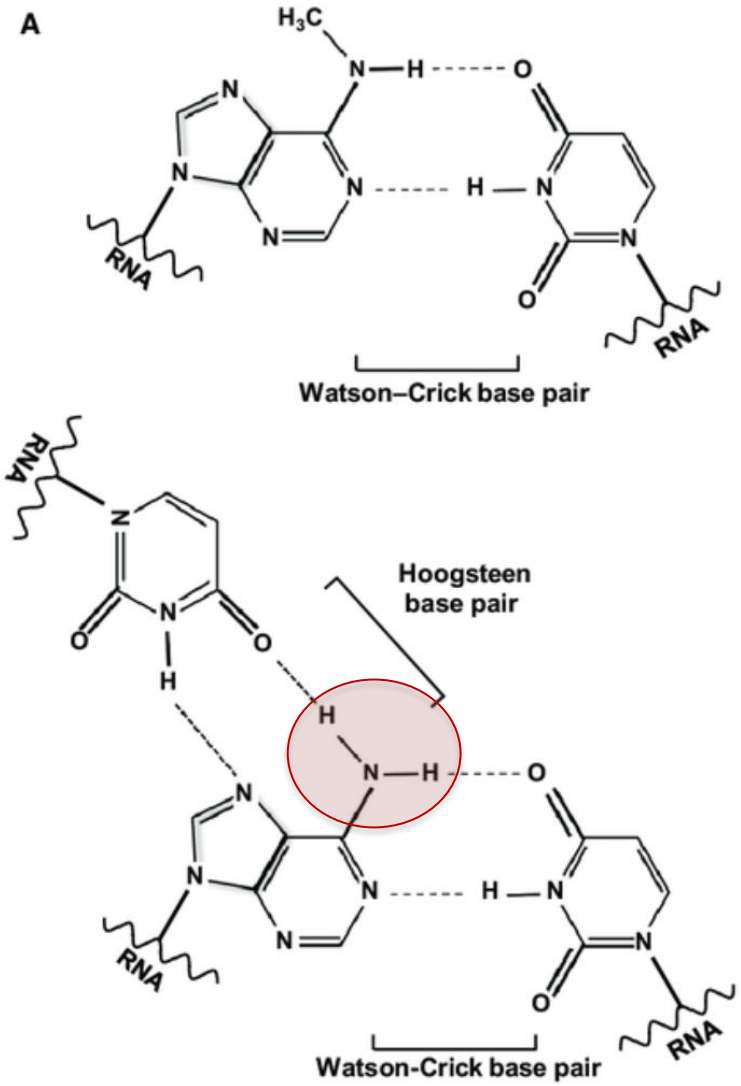


m^6A methylation affects protein-RNA interactions through multiple mechanisms

- Methylation can perturb the secondary structure of mRNA, exposing or masking potential RNA-binding motifs.
- Selective m^6A-binding proteins exhibit increased affinity for methylated mRNAs and in turn incorporate these transcripts into various steps of mRNA metabolism.



m^6A can disrupt RNA structures that are dependent on Hogsteen base pair



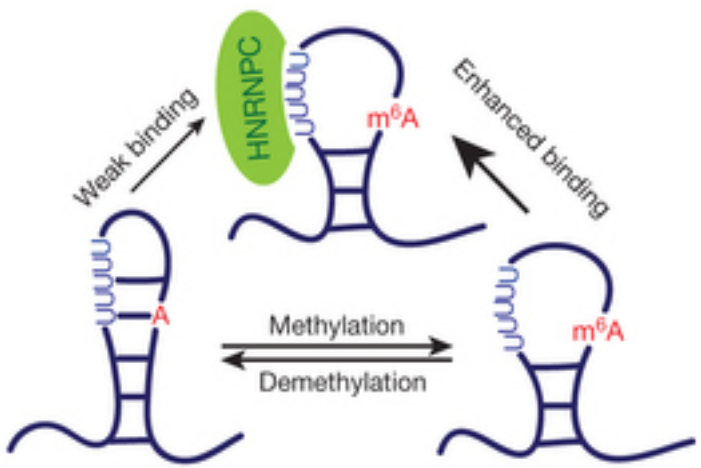
nature

N^6-methyladenosine-dependent RNA structural switches regulate RNA–protein interactions

Nian Liu[1], Qing Dai[1], Guanqun Zheng[2], Chuan He[1,2,3,4], Marc Parisien[2]† & Tao Pan[2,3]

Nature **518**, 560–564. 2015

m^{6}A alters the local structure in mRNA and lncRNA to facilitate binding of heterogeneous nuclear ribonucleoprotein C (HNRNPC), an abundant nuclear RNA-binding protein responsible for pre-mRNA processing. Thus, affecting the abundance as well as alternative splicing of target mRNAs.



"m^{6}A switch"

m^{6}A readers

Direct reader proteins

Indirect reader proteins

"m^{6}A switch"

Transcription silencing
- YTHDC1

Transcription activation
- YTHDC1

Splicing
- YTHDC1
- HNRNPC
- HNRNPG

Nuclear export
- YTHDC1

RNA stability and decay
- YTHDF2
- YTHDF1
- YTHDC1
- IGF2BP1
- IGF2BP2
- IGF2BP3

Translation
- YTHDF1
- YTHDF3
- eIF3

miRNA biogenesis
- HNRNPA2B1

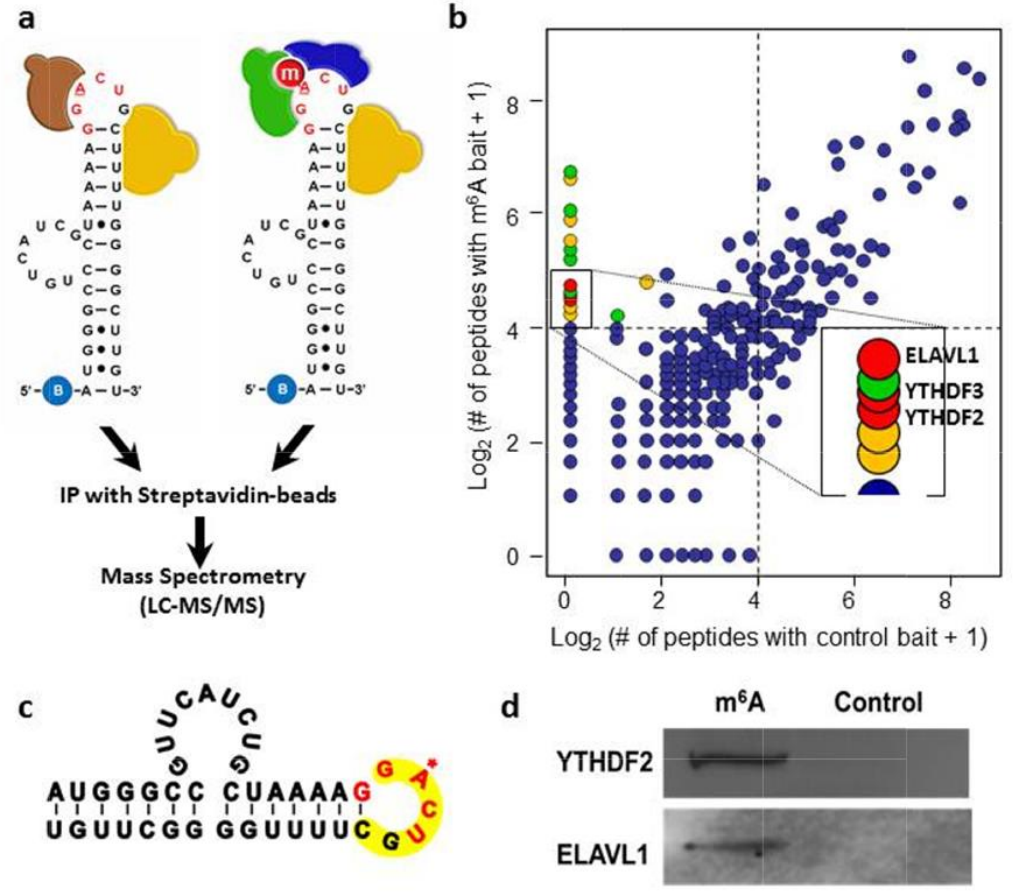
Alternative polyA
- YTHDC1

- Indirect reader proteins
- Direct reader proteins

m^{6}A direct readers

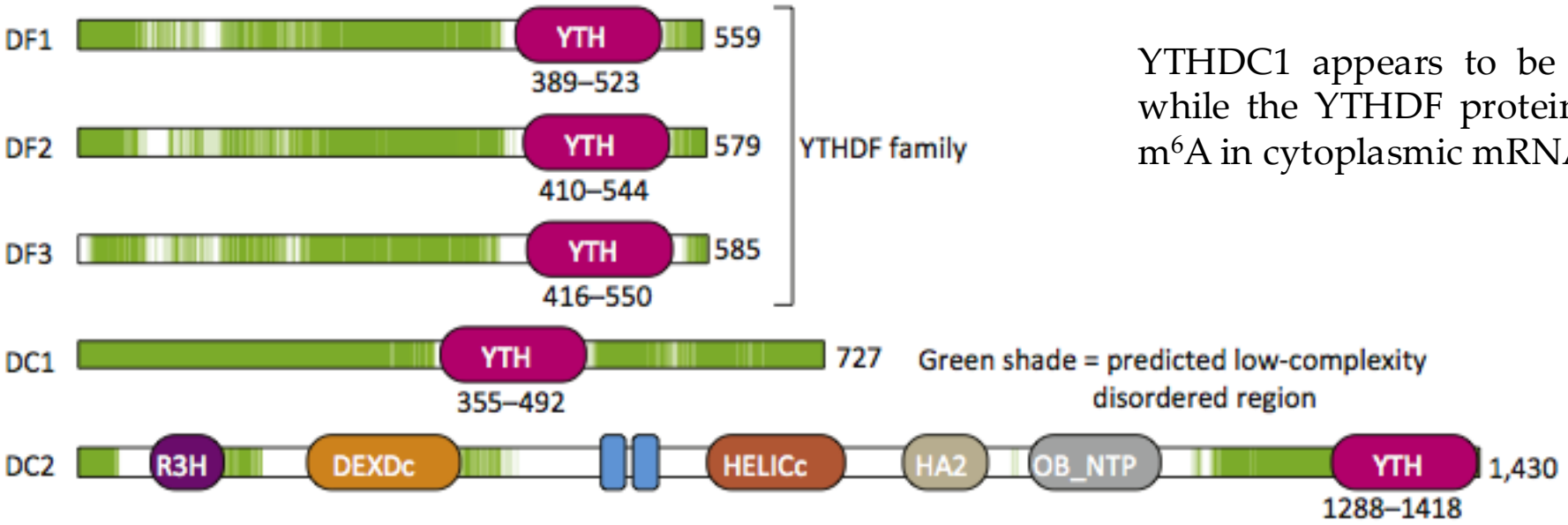
RNA pull-down experiments, using methylated and control versions of an RNA bait followed by mass spectrometry, was used to identify novel m^{6}A-binding proteins.

Three cytoplasmic proteins of the YTH domain family, **YTHDF1–3**, have been identified as selective m^{6}A-binding proteins in mammalian cell extracts.

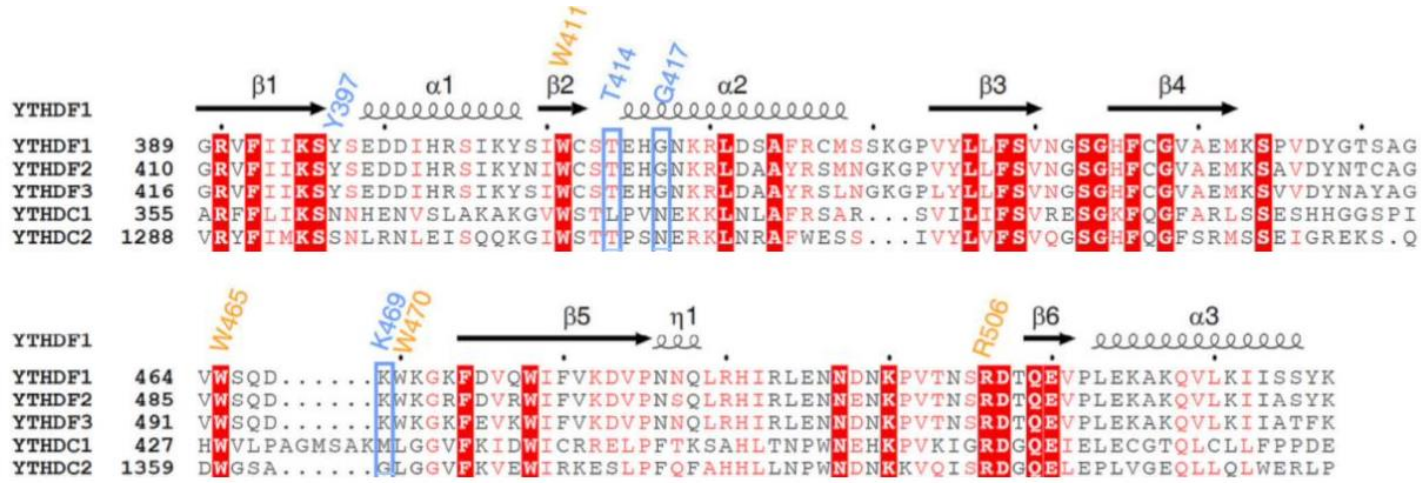


Overlap between YTH binding consensus (yellow) and methylation consensus (red)

m^{6}A direct readers



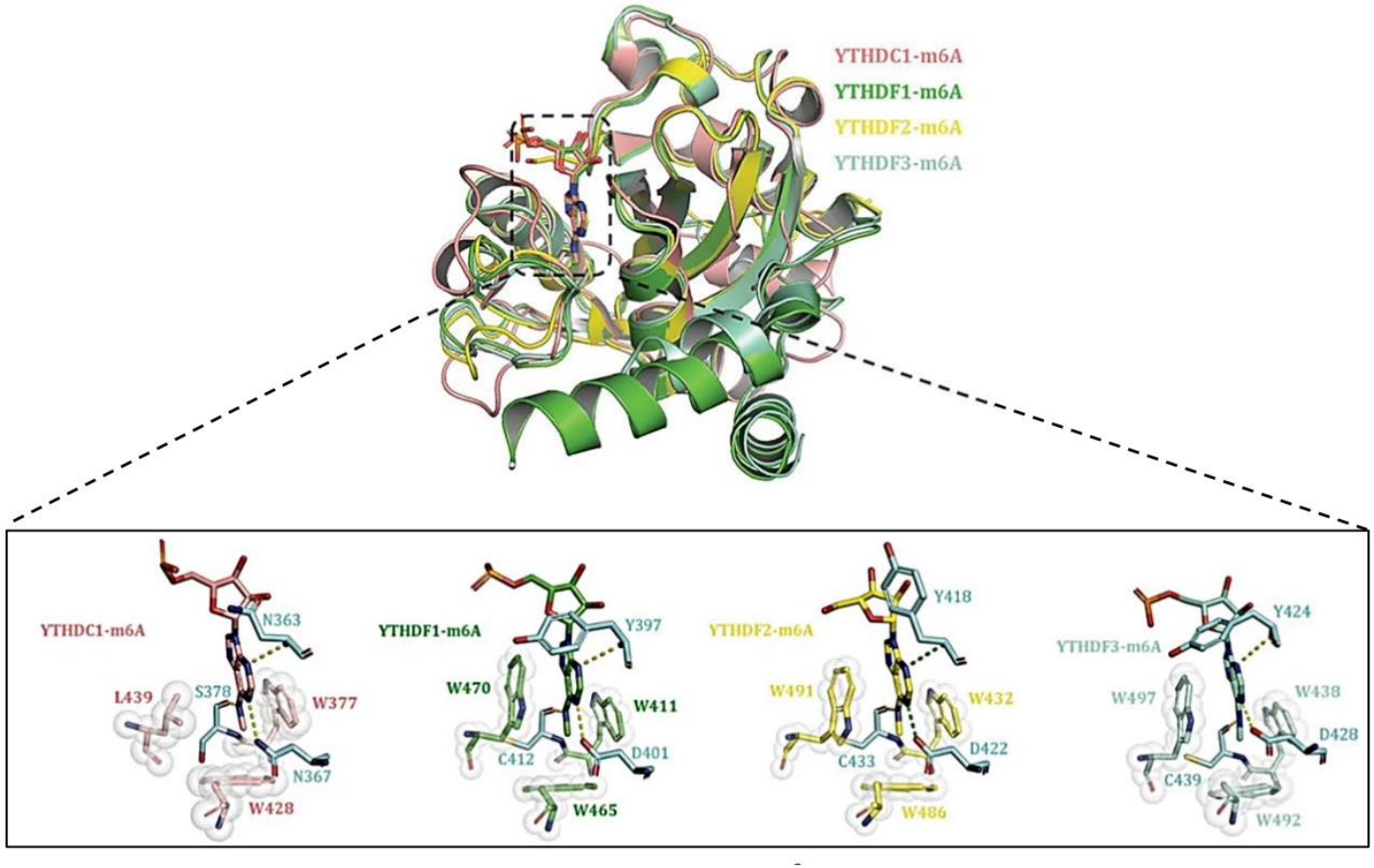
YTHDC1 appears to be the only nuclear reader, while the YTHDF proteins bind predominantly to m^{6}A in cytoplasmic mRNAs.



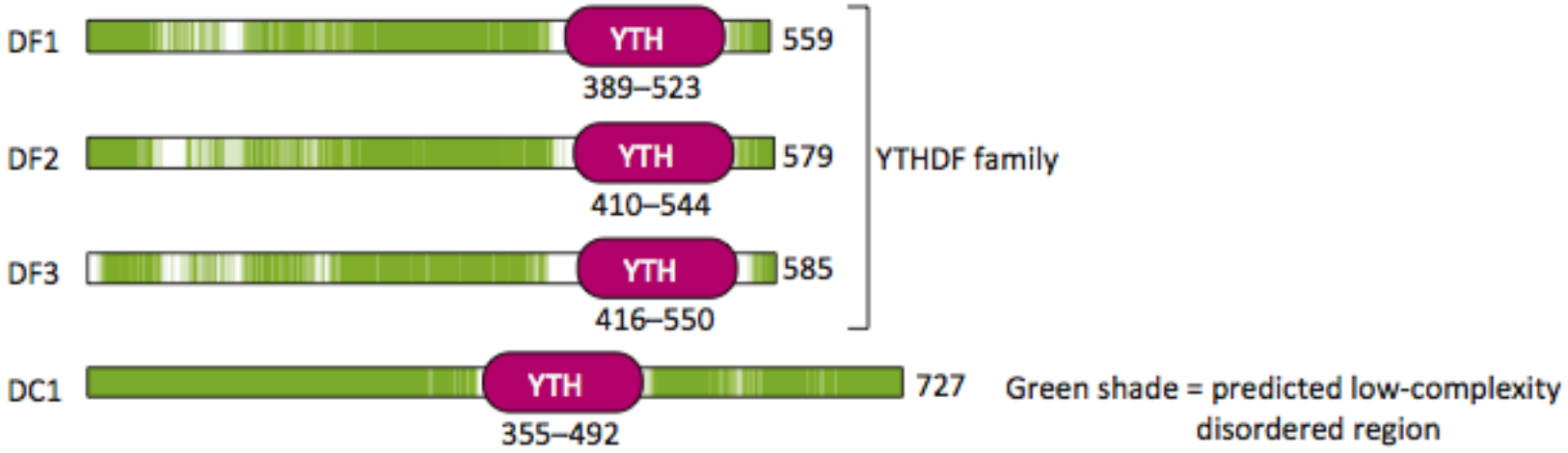
The YTH domain is highly conserved with more than 86% identity

m^{6}A direct readers

The YTH domain hosts the m^{6}A recognition site within the aromatic cage. The m^{6}A moiety fits into a small pocket lined with hydrophobic residues (0.1-3 μM), including one tyrosine and two or three tryptophan residues (i.e., Trp411 and Trp470 in YTHDF1, Trp432 and Trp491 in YTHDF2, Trp438 and Trp497 in YTHDF3). YTH specificity is not restricted to the m^{6}A moiety; it can also recognise with lower affinity m^{1}A and m^{5}C (KD m1A = 5.8 ± 1.7 μM; KDm5C = 17.3 ± 4 μM). YTH domain does not require a specific *consensus* sequence for binding.



m^{6}A direct readers



The N- and C-terminal regions, termed Low Complexity Domains (LCDs), exhibit disordered structural organisation and limited sequence similarity, particularly among YTHDFs (49-52%). These regions determine paralog identity and are thought to mediate interactions with specific effector proteins within dynamic, subcellular, membraneless condensates by liquid-liquid phase separation LLPS, such as P-bodies and SG

Consequences of m6A methylation on gene expression

m^{6}A modification on pre-mRNA and mRNA may produce different outputs, including:

- **regulation of transcription and chromatin modifications**
- **regulation of alternative splicing and polyadenylation**
- **regulation of mRNA stability**
- **regulation of mRNA translation**
- **enhanced mRNA export**
- **increased nuclear microRNA processing**

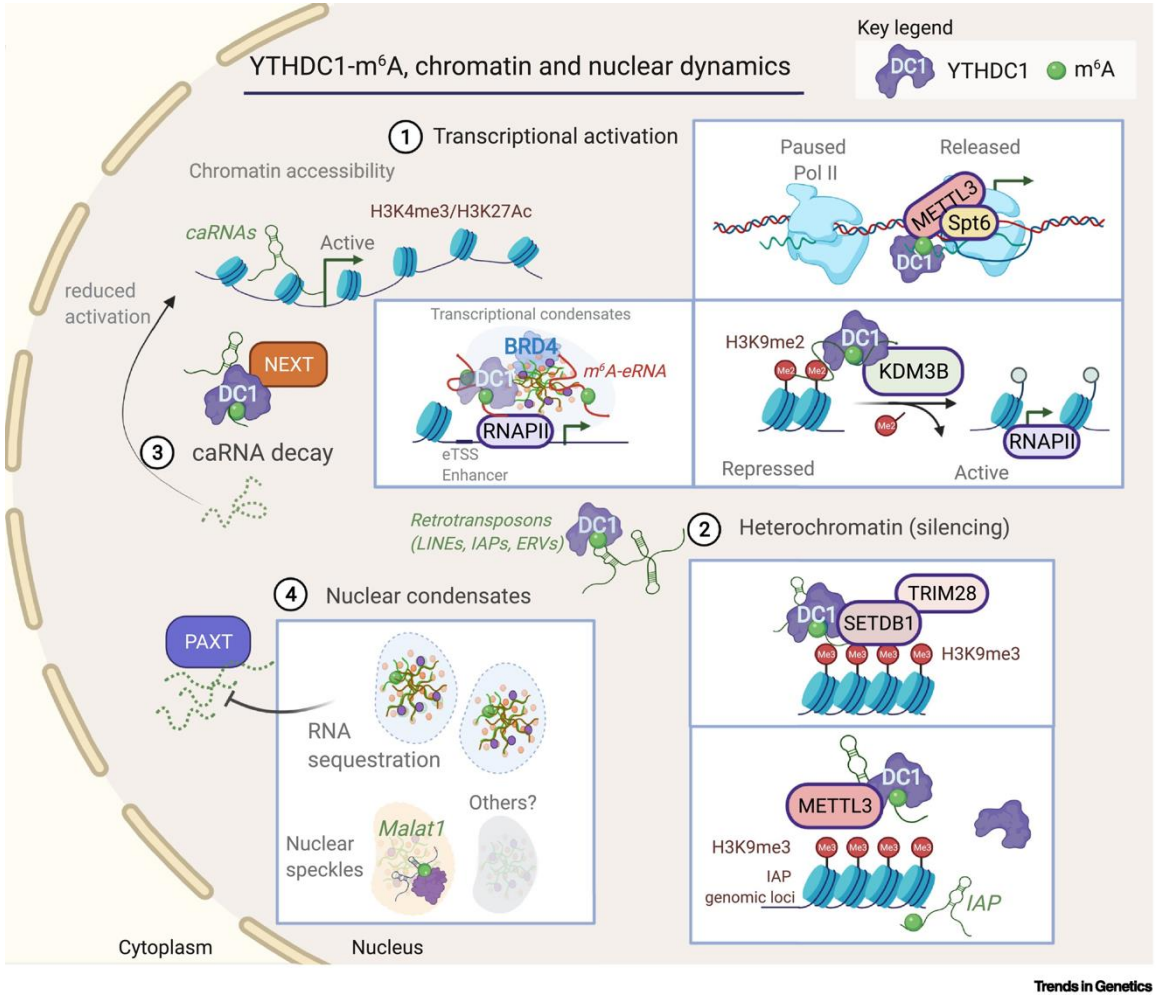
Regulation of transcription and chromatin modifications

Nuclear m^{6}A reader YTHDC1 regulates, transcription, alternative splincing and polyadenilation

- YTHDC1 is essential for embryo viability and is required for oocyte growth and maturation.
- YTHDC1 interacts with m^{6}A-modified RNAs to regulate multiple steps of RNA metabolism in the nucleus.
- YTHDC1 is widely associated with transcriptional activation (via enhancer RNA-mediated crossregulation with active epigenetic marks).
- YTHDC1 transcriptional repressive action is largely associated with transposable elements and long ncRNAs.
- The diversity in YTHDC1–m^{6}A functions is linked to their ability to promote membraneless nuclear subcompartments, such as the nuclear speckles.
- YTHDC1 also regulates alternative splicing and polyadenylation

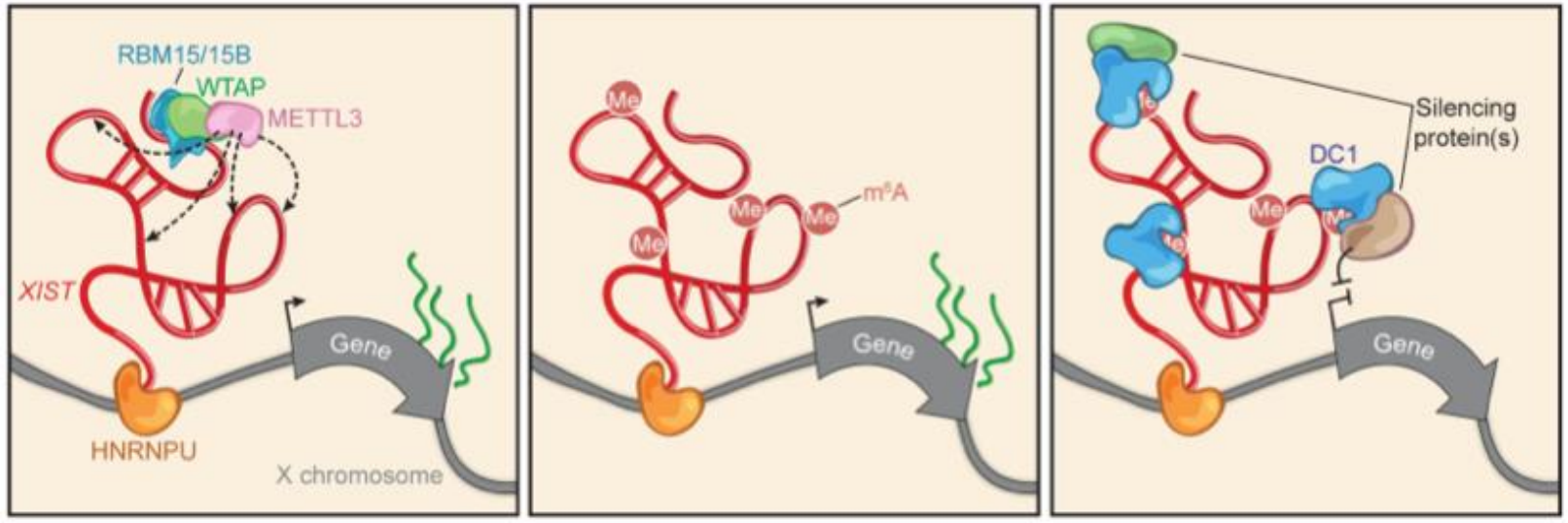
Distinct molecular mechanisms associated with YTHDC1 nuclear and chromatin regulation

(1) Transcriptional activation can be driven by transcriptional condensates formed by the multivalent interaction of YTHDC1–m6A–enhancer RNAs and the BRD4 transcriptional activator, regulation of promoter-proximal pausing of RNAPII (Drosophila S2R cells), or de-repression via KDM3B interaction which promotes the removal of H3K9me2 repressive marks (mESCs). **(2) Transcriptional silencing** by YTHDC1 predominantly targets ncRNAs and transposable elements as m^{6}A targets. Recruitment of YTHDC1 to m6A-modified retrotransposon transcripts triggers heterochromatin formation in mESCs, acting in cis in regulating the intracisternal A-type particle (IAP, bottom), a type of endogenous retroviral sequences, and in trans (top) via direct interaction with SETDB1 and TRIM28, which leads to the deposition of H3K9me3. YTHDC1 and METTL3 interaction provides positive feedback to enhance m^{6}A-associated local heterochromatin formation (mESCs). **(3)** Further gene repression in mESCs can be achieved by **degradation of regulatory RNAs** termed chromatin-associated RNAs (caRNAs), which have chromatin relaxation activity, including LINE-1 (mESCs). **(4) Nuclear condensates** induced by YTHDC1 phase separation with m^{6}A-RNAs and other IDR-containing nuclear proteins can modulate nuclear processing via spatial control of gene expression.



YTHDC1 promotes XIST-mediated transcriptional repression

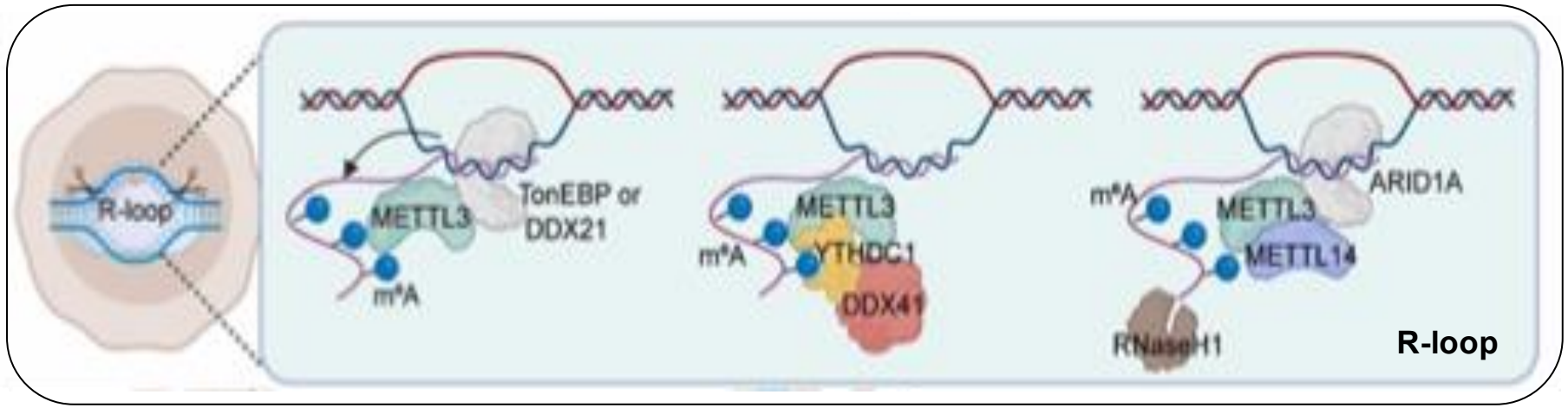
XIST is highly methylated with at least 78 m6A. RBM15 binding enables methylation of adjacent adenosine residues in DRACH consensus sites. Knockdown of RBM15 and RBM15B, or knockdown of METTL3 impairs XIST-mediated gene silencing. The m^6A residues act as recruitment sites for YTHDC1, which may facilitate and stabilize the assembly of silencing proteins on *XIST*.



Patil et al., 2017. Nature

m^{6}A and R-loop regulation

m^{6}A modification has also been found enriched in R-loop and plays a role in maintaining genome stability and facilitating transcription termination. YTHDC1 recognizes m^{6}A-modified R-loops and participates in DNA damage responses induced by R-loops. Although the consequences of m^{6}A-deficiency in DNA damage are consistent in many studies (e.g. increased sensitivity to DNA damaging agents), the role of RNA m^{6}A modification in R-loops formation remains to be further clarified.

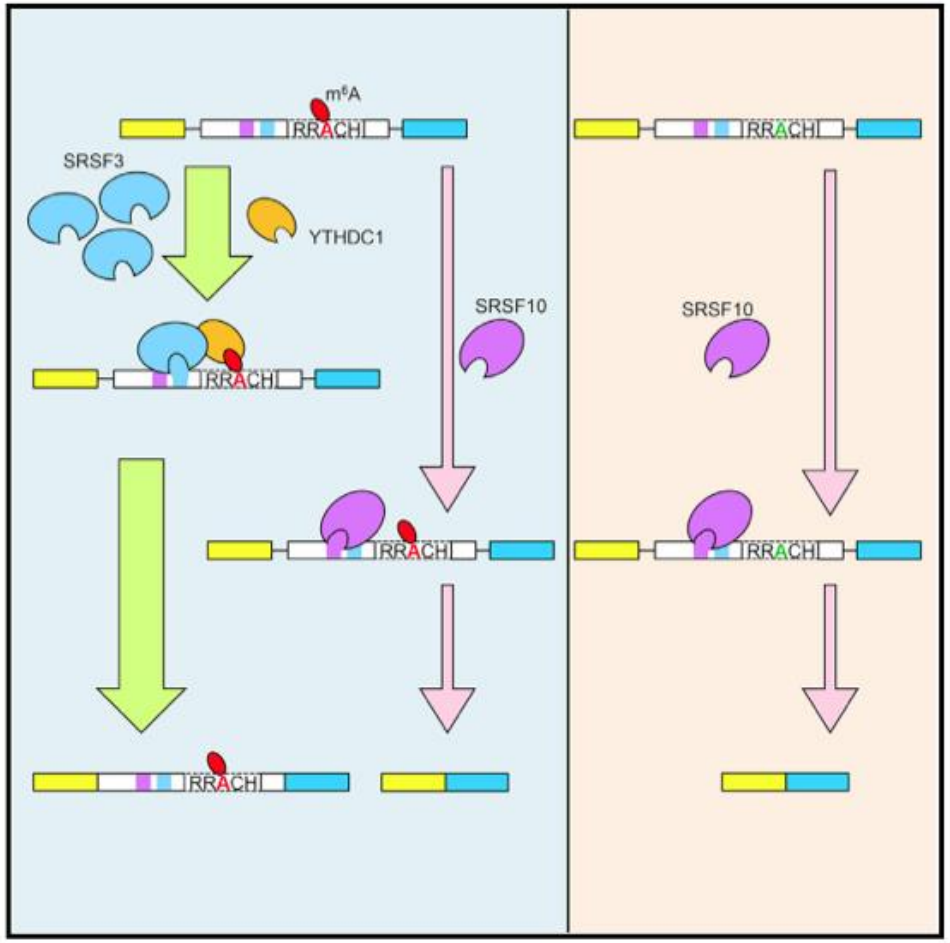


adapted from Zhang et al., 2025. Cell Invest.

Regulation of alternative splicing and polyadenylation

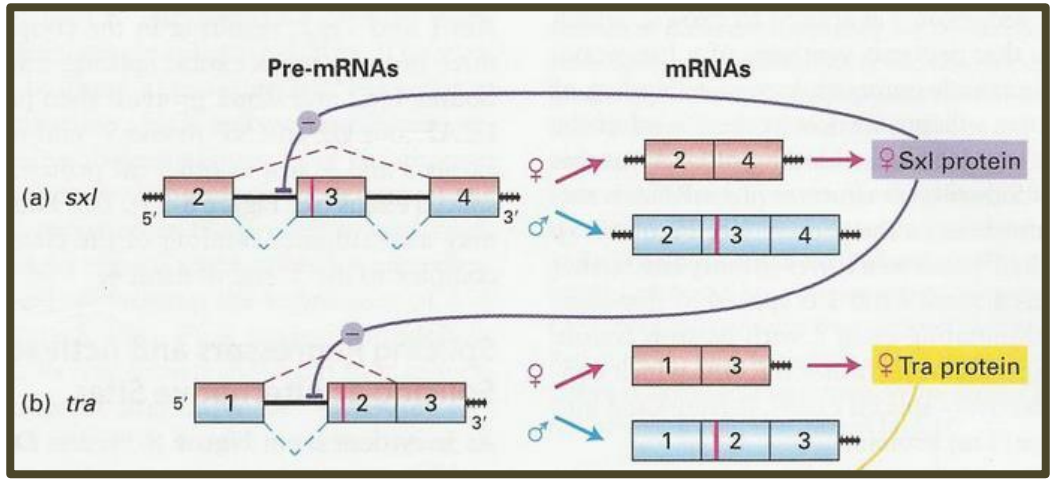
YTHDC1 regulates alternative pre-mRNA splicing

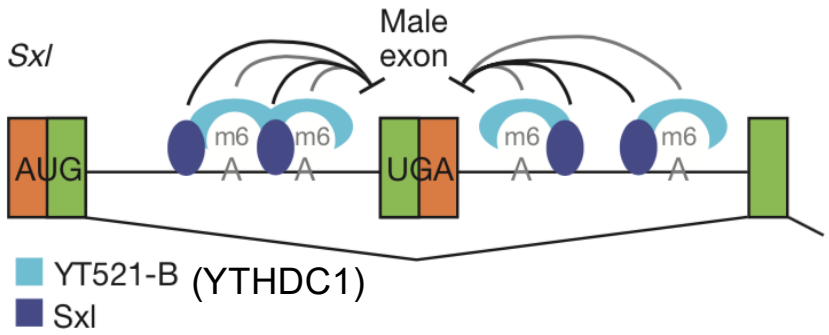
YTHDC1 promotes exon inclusion of targeted mRNAs through facilitating SRSF3 while blocking SRSF10 mRNA binding. YTHDC1 directly regulates mRNA splicing by bridging interactions of *trans*- and *cis*- regulatory elements.



Xiao et al. 2016. Mol Cell

YTHDC1 is required for female-specific alternative splicing of *Sxl*



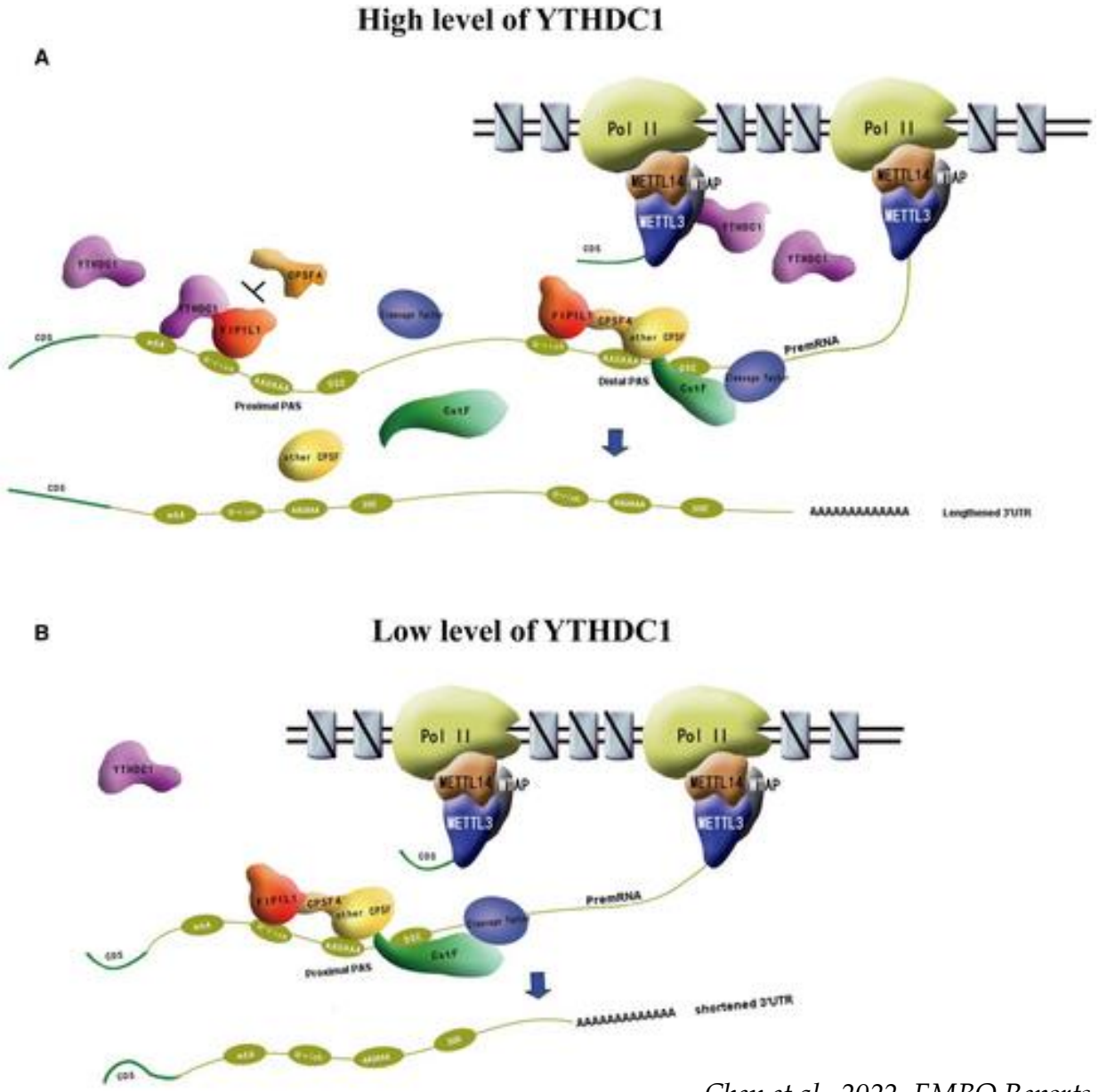


Female splicing

YTHDC1 regulates alternative polyadenylation

A. YTHDC1 is associated with Pol II during transcription by interacting with MTC and binds to the modification sites upstream of proximal APA sites when m^{6}A modification is finished. Then, YTHDC1 interacts with FIP1L1 and disrupts the recruitment of CPSF4 and other CPSF factors, resulting in the suppression of proximal APA sites and longer 3′ UTRs.

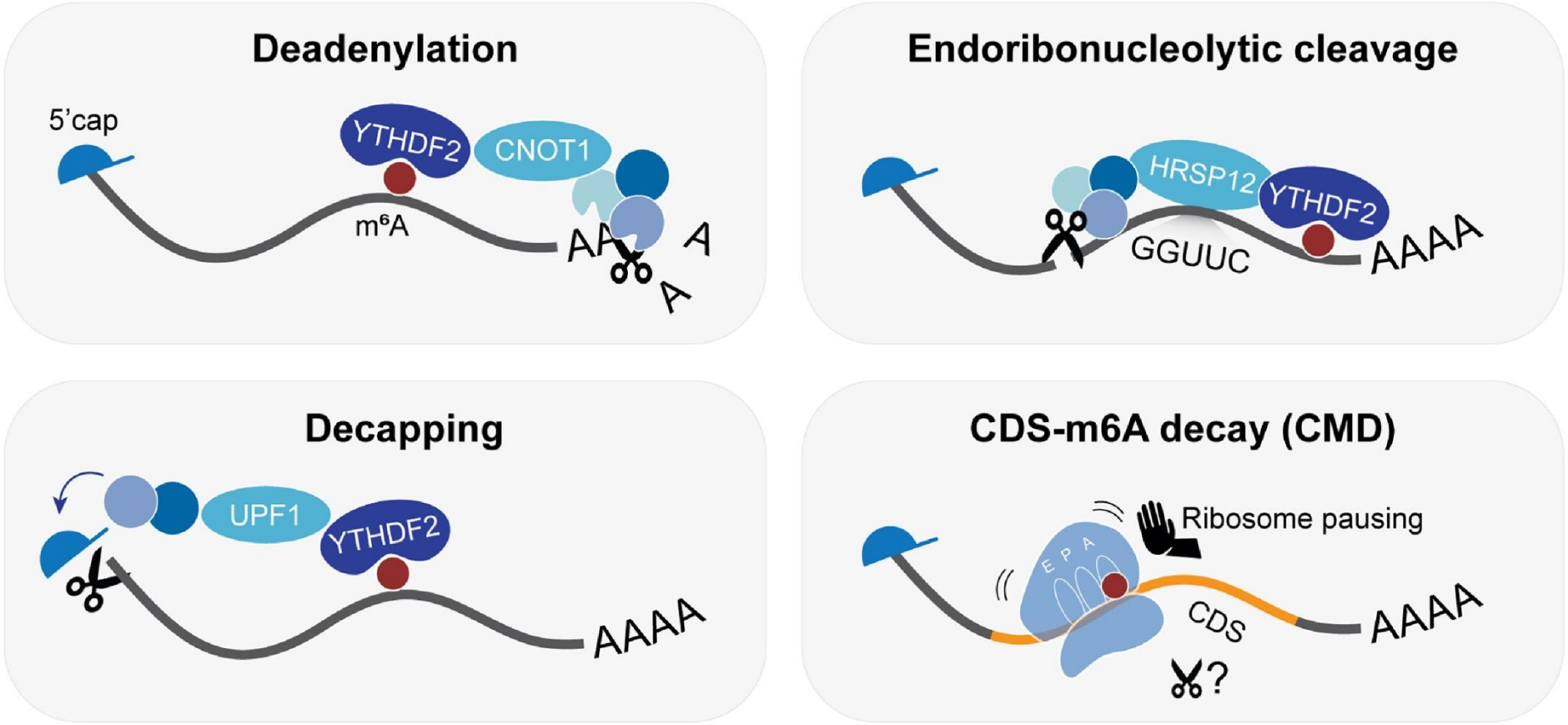
B. This inhibition is abrogated at low levels of YTHDC1 or in the absence of m^{6}A modification. FIP1L1 interacts with CPSF4, which recruits other CPSF factors and promotes the usage of proximal APA sites, resulting in shortened 3′ UTRs.



Chen et al., 2022. EMBO Reports

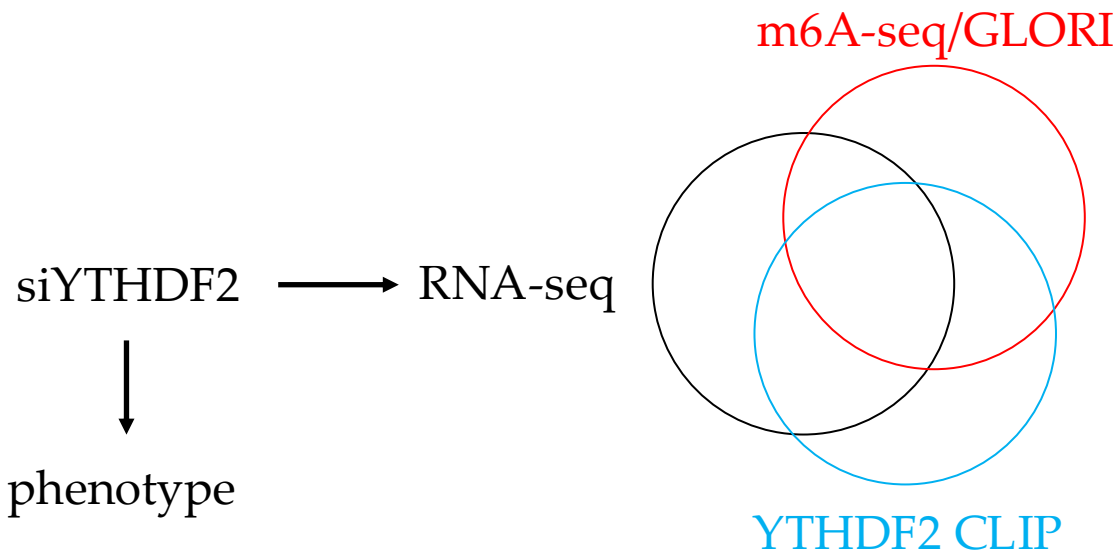
Regulation of mRNA stability

m^6A-mediated RNA degradation



Trends in Genetics

Methodologies for identifying YTHDF2-regulated RNA targets

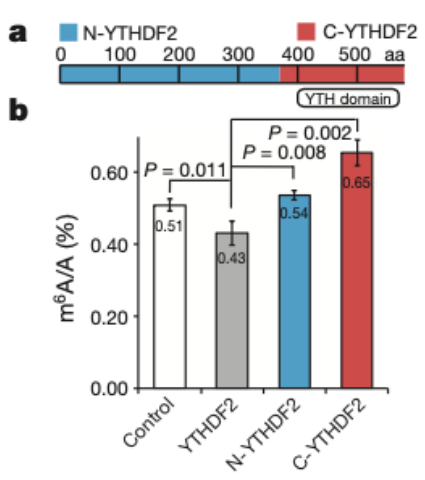


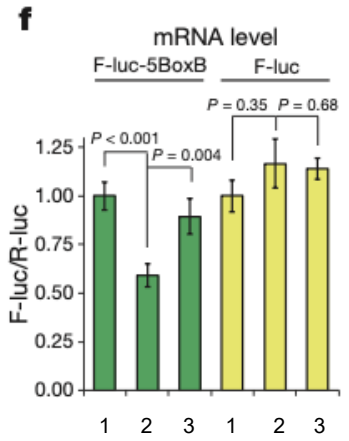
YTHDF2 stimulates mRNA degradation

- YTHDF2 knockdown led to prolonged (~30% in average) lifetimes of its mRNA targets in comparison to non target mRNAs (RNA-seq data on YTHDF2 knockdown and control samples obtained at different time points after transcription inhibition with actinomycin D).

- Overexpression of full-length YTHDF2 in HeLa cells led to reduced levels of m^6A after 24 h (measured by LC-MS), whereas overexpression of N-YTHDF2 or C-YTHDF2 increased the m^6A/A ratio of the total mRNA (a, b).

- Tethering YTHDF2–λ to F-luc-5BoxB (five Box B sequence was inserted into the 39 UTR of the mRNA reporter) led to a significantly reduced mRNA level (f).



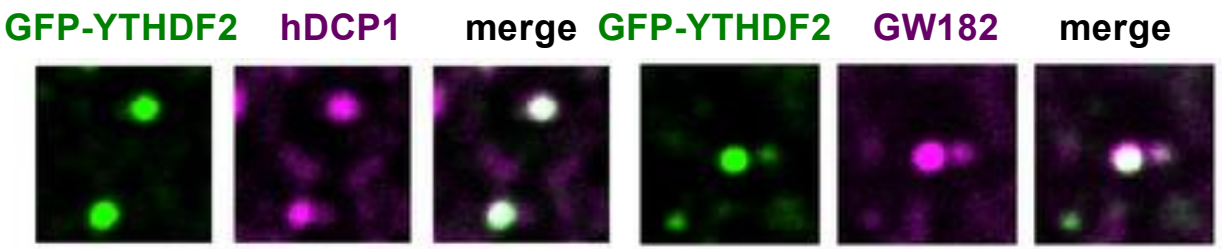


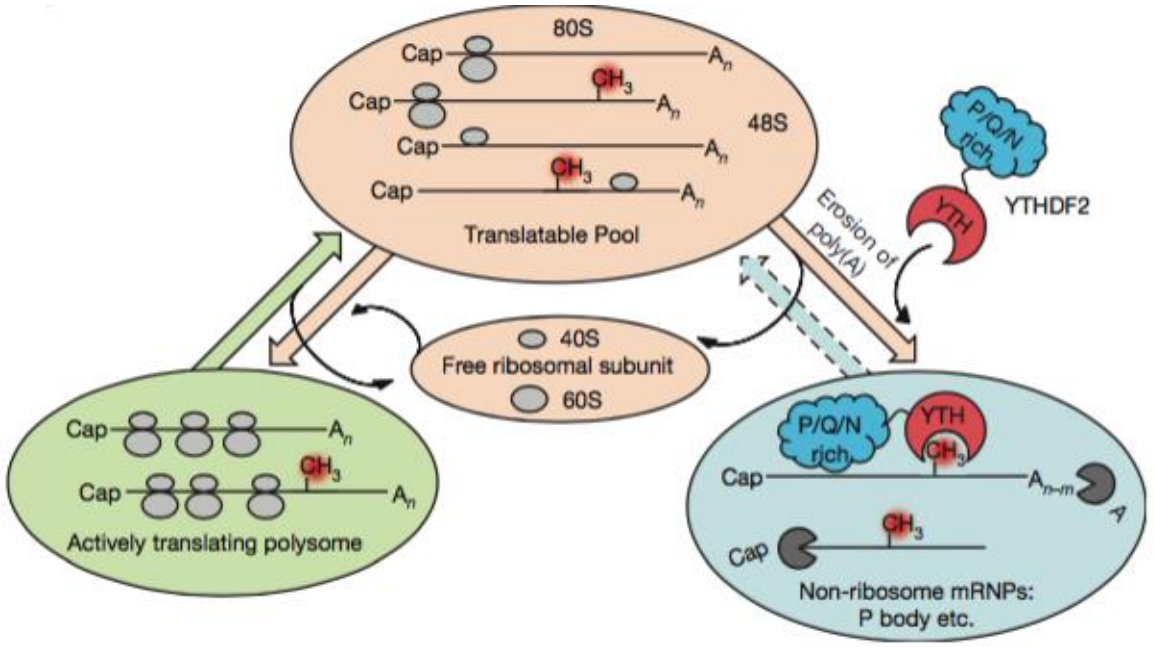
1: λ
2: YTHDF2–λ
3: YTHDF2

Whang et al., 2017 Nature

YTHDF2 induces mRNA localization to P bodies

Via its P/Q/N-rich N-terminal domain, YTHDF2 localizes to processing bodies (P bodies) in the cytoplasm where mRNA turnover factors are concentrated.



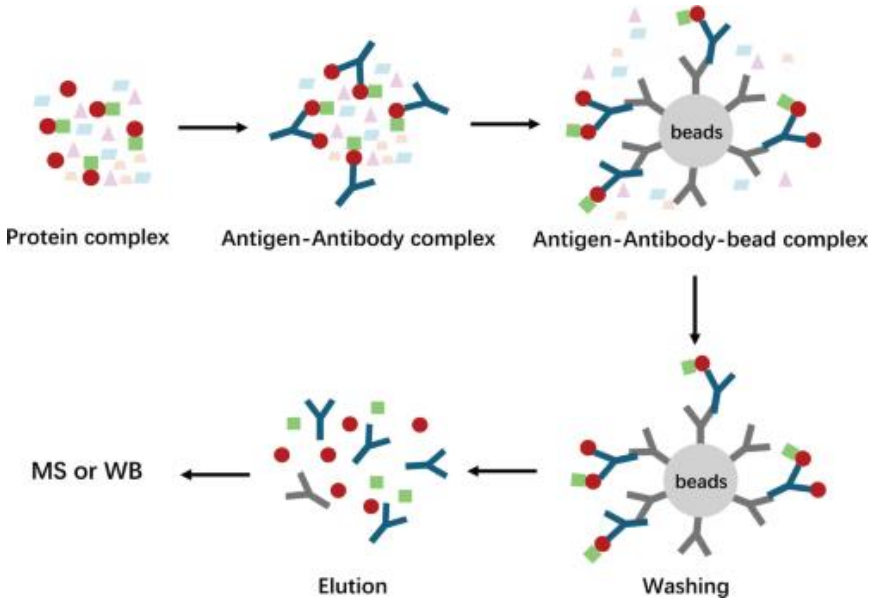


Whang et al., 2017 Nature

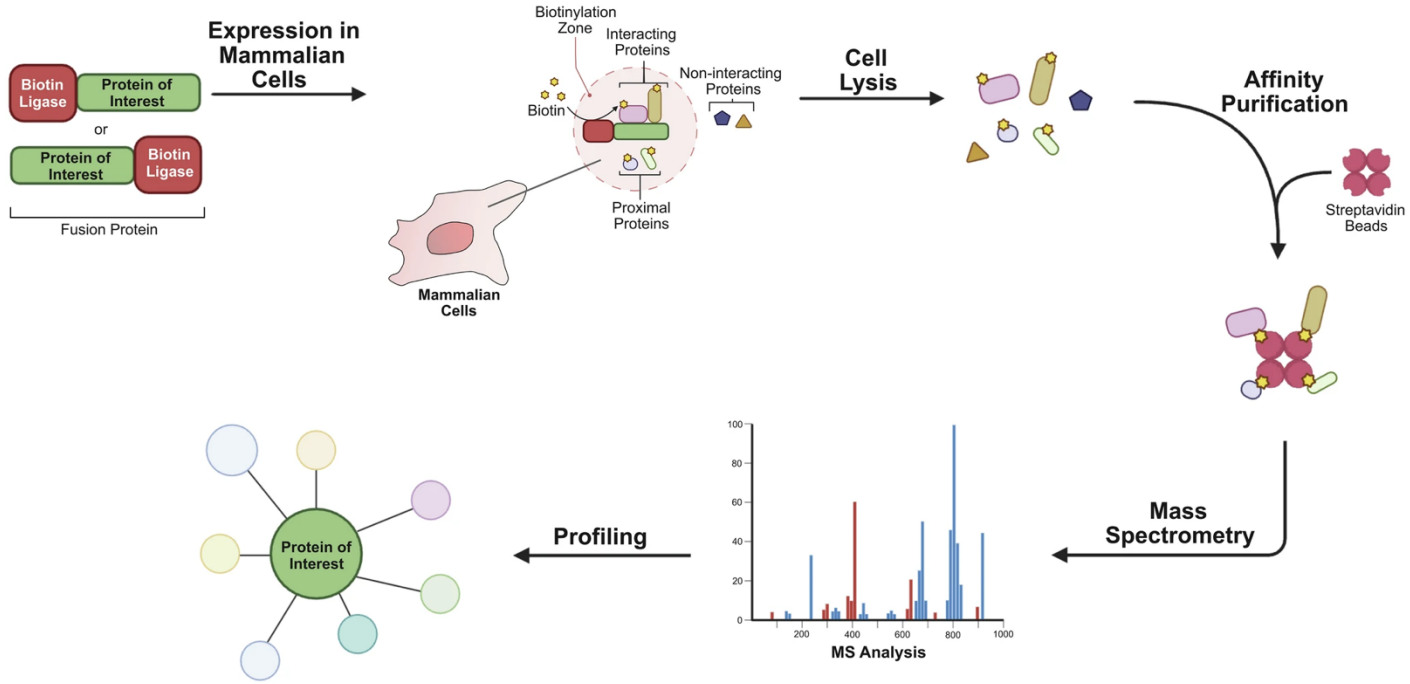
YTHDF2 stimulates mRNA degradation

YTHDF2 interactors were identified by Co-IP or Proximity-dependent Bioin identification (BioID)

Co-IP



BioID



YTHDF2 stimulates mRNA degradation

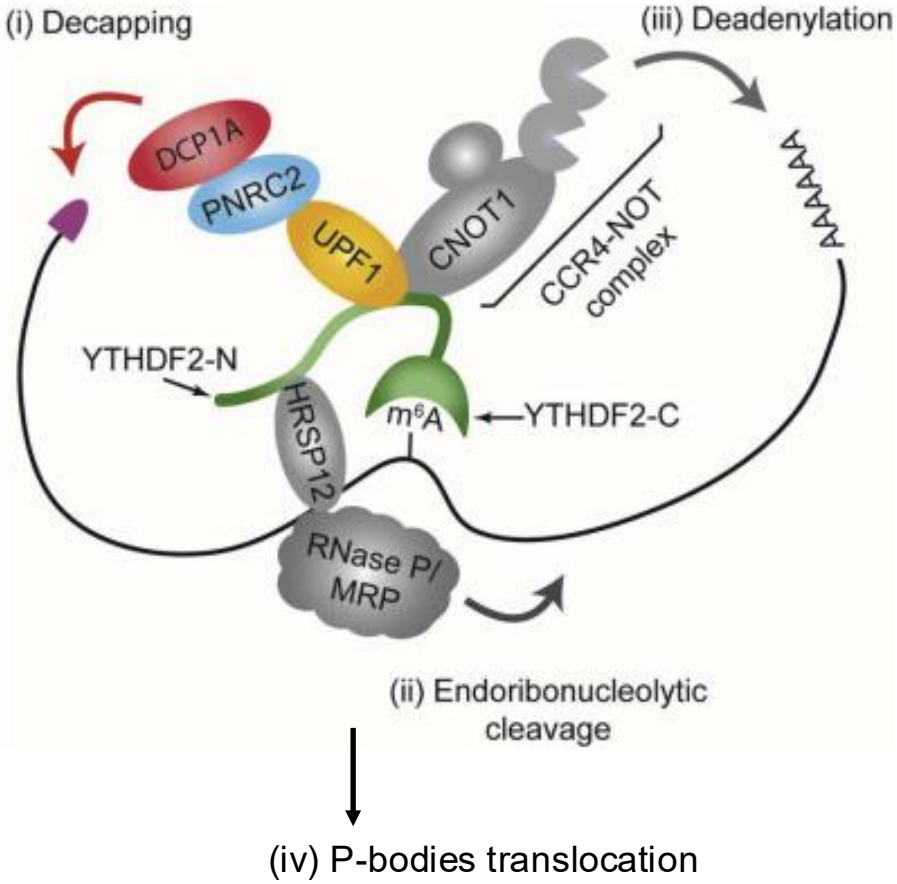
YTHDF2 recognizes m^{6}A in mRNAs by its C-terminal YTH domain. Then, the N-terminal half of YTHDF2 recruits various effectors, such as UPF1, HRSP12, and/or CNOT1, to trigger rapid degradation of m^{6}A mRNAs through one of the three following pathways:

i) decapping followed by 5′-to-3′ exoribonucleolytic cleavage via the YTHDF2-UPF1-PNRC2-DCP1A axis,

ii) endoribonucleolytic cleavage via the YTHDF2-HRSP12-RNase P/MRP axis, and

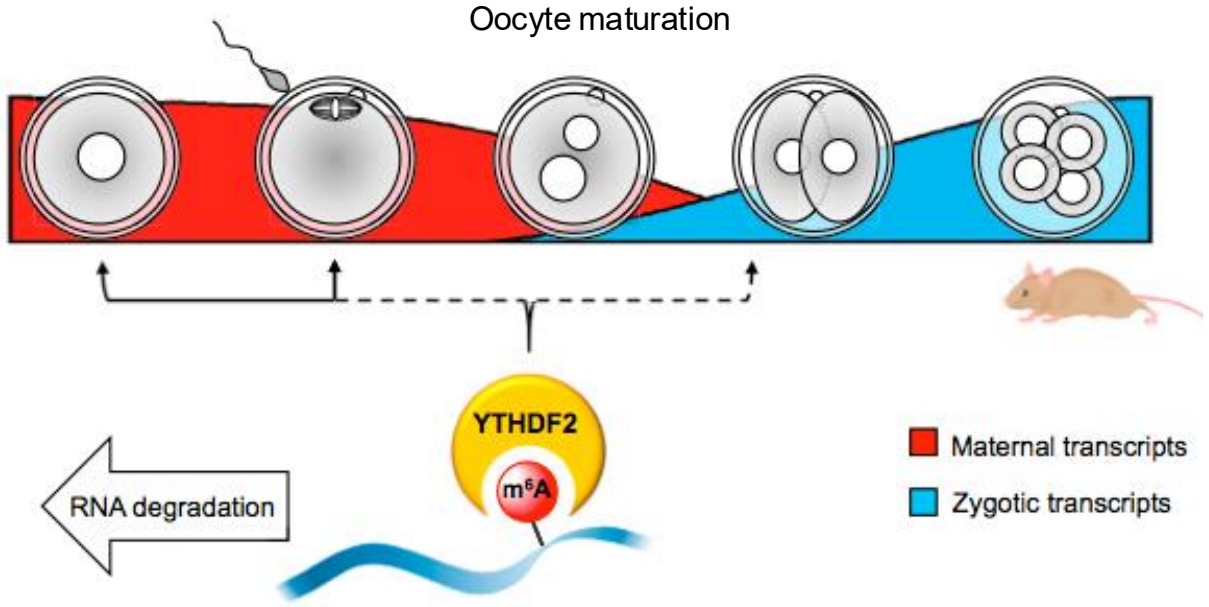
iii) deadenylation followed by 3′-to-5′ exoribonucleolytic cleavage via the YTHDF2-CCR4-NOT axis.

iv) P-body translocation



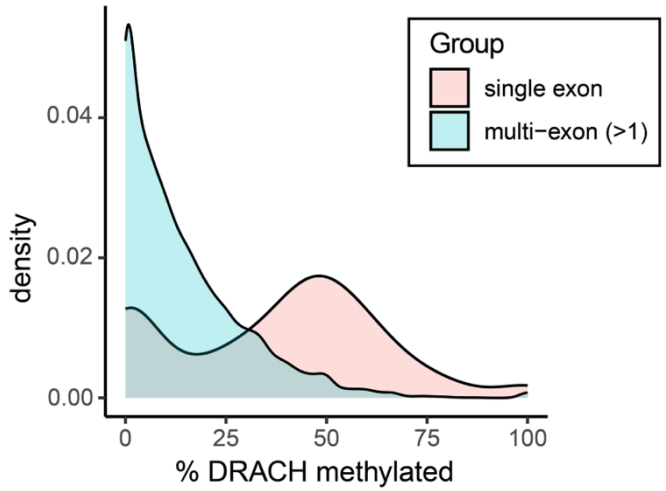
YTHDF2 Exerts Essential Functions in mouse Female Fertility

Knockout of *Ythdf2* revealed that depletion of YTHDF2 causes female-specific infertility despite successful ovulation. YTHDF2 regulates transcript degradation during oocyte maturation, which is essential for the competence of oocytes to sustain early zygotic development. Ythdf2-KO male mice showed mild degenerative changes in the seminiferous tubules. Accordingly, these males were hypofertile. Ythdf2 heterozygote mice need at least one functional copy of another Ythdf reader to escape embryonic mortality.

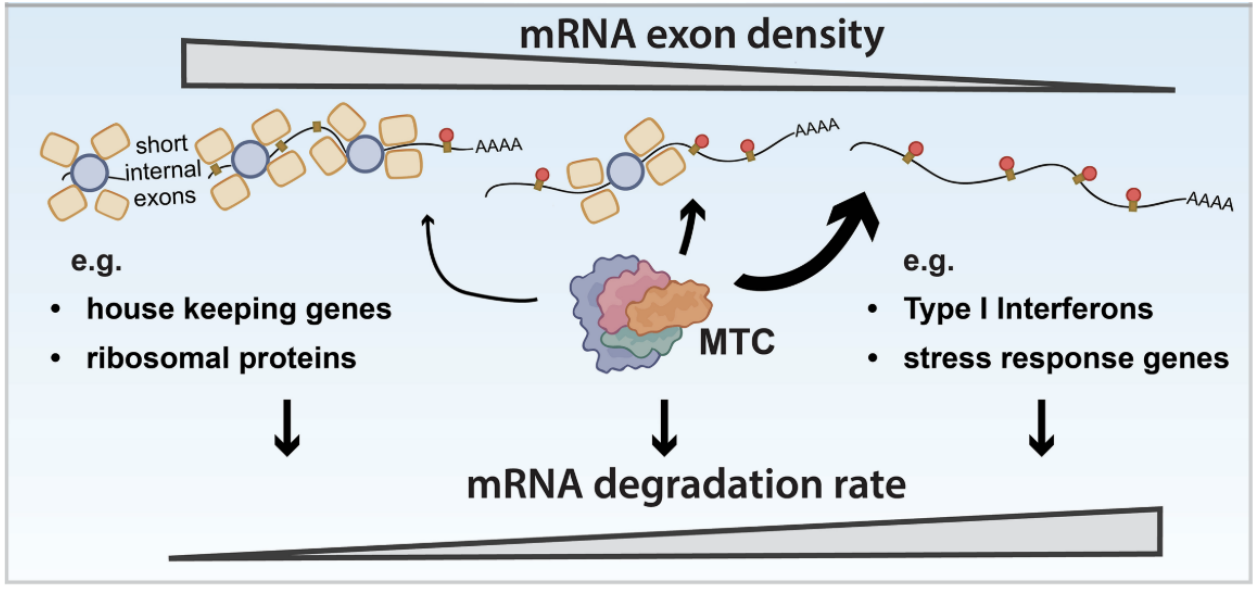


Ivanova et al., 2017 Mol Cell; Lasman et al., 2020 Genes & Dev.

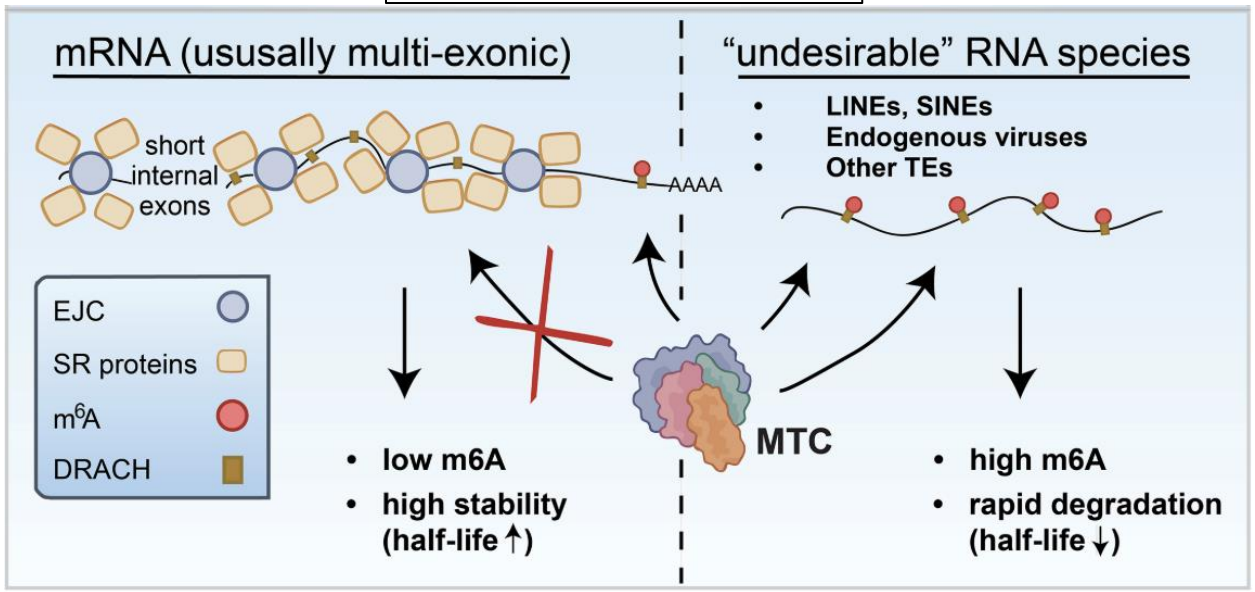
mRNA half-life is primarily dictated by m^{6}A



mRNA dosage regulation

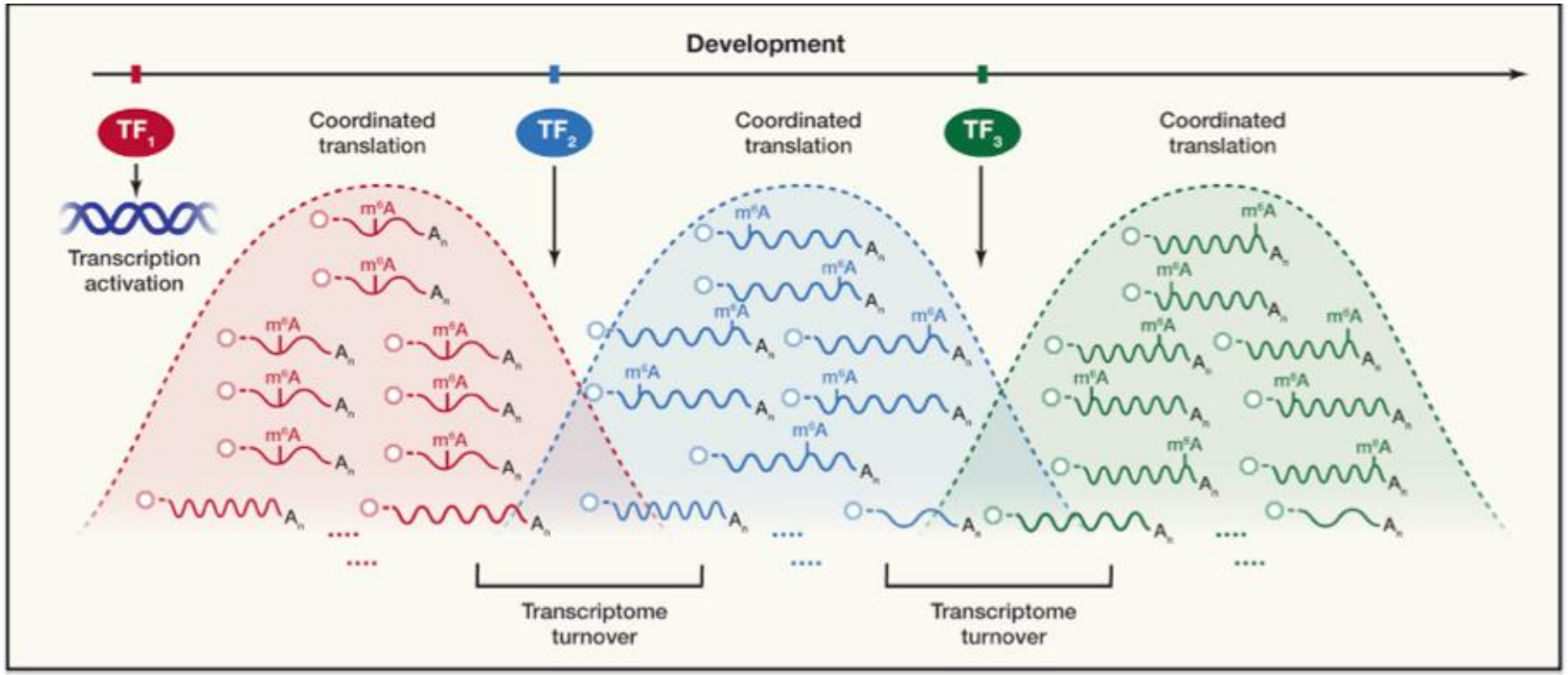


RNA surveillance



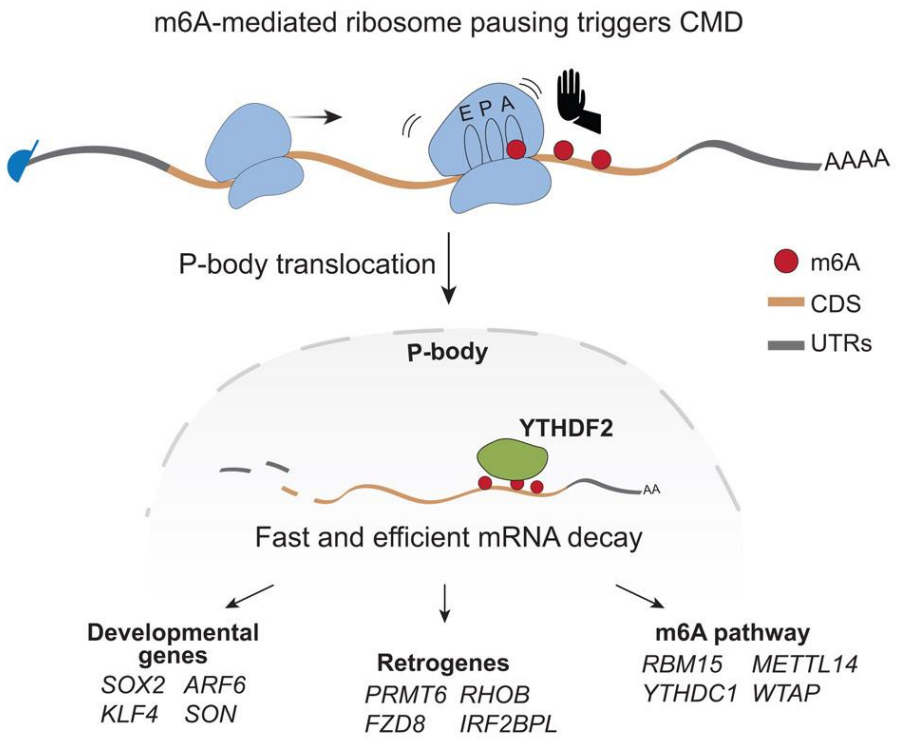
Dierks and Schwartz, 2026 Mol Cell

RNA Modification Groups Transcripts for Cellular Processes



CDS-m^{6}A decay (CMD)

m^{6}A sites in the coding sequence trigger **CDS-m6A decay (CMD),** a pathway that is distinct from previously reported m^{6}A-dependent degradation mechanisms. Mechanistically, CMD depends on translation, whereby m^{6}A deposition in the CDS triggers ribosome pausing and transcript destabilization. The subsequent decay involves the translocation of the CMD target transcripts to P-bodies and recruitment of the m^{6}A reader YTHDF2.



Zhou et al., 2024 Mol. Cell

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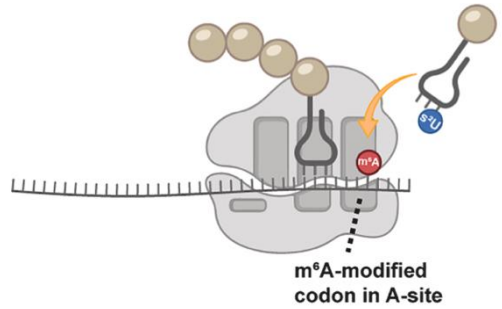
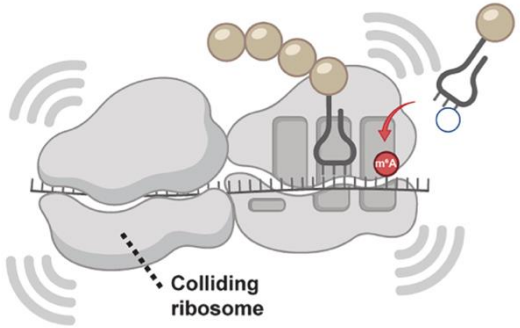
Article

tRNA modifications tune m^{6}A-dependent mRNA decay

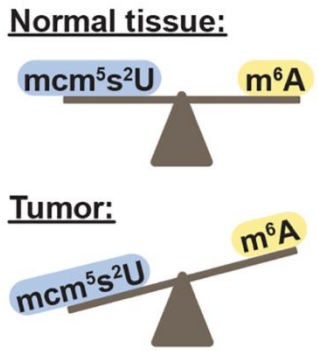
Bastian Linder,[1,11,10] Puneet Sharma,[2,3,10] Jie Wu,[2,3,4,9,10] Tosca Birbaumer,[3,4] Cristian Eggers,[3] Shino Murakami,[5] Roman E. Ott,[3] Kai Fenzl,[1] Hannah Vorgerd,[2] Florian Erhard,[6,7] Samie R. Jaffrey,[5] Sebastian A. Leidel,[2,3,12,*] and Lars M. Steinmetz[1,8,9,*]

Codons that are modified with m^{6}A are decoded inefficiently by the ribosome, rendering them "non-optimal" and inducing ribosome collisions on cellular transcripts. This couples mRNA translation to decay. 5-Methoxycarbonylmethyl-2-thiouridine (mcm^{5}s^{2}U) in the tRNA anticodon loop counteracts this effect. This unanticipated link between the mRNA and tRNA epitranscriptomes enables the coordinated decay of mRNA regulons, including those encoding oncogenic signaling pathways. In cancer, dysregulation of the m^{6}A and mcm^{5}s^{2}U biogenesis pathways—marked by a shift toward more mcm^{5}s^{2}U—is associated with more aggressive tumors and poor prognosis.



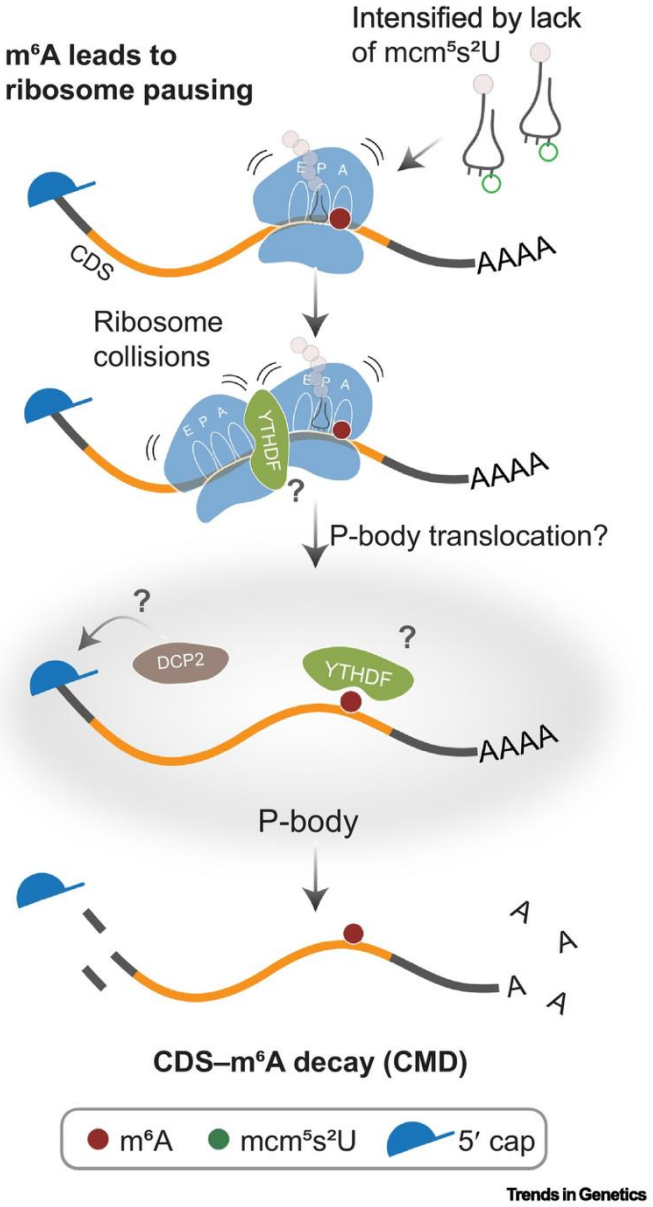


mRNA decay
(CCR4-NOT4)



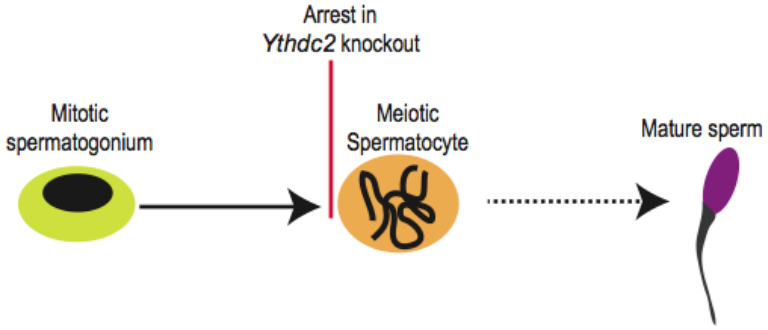
Coding sequence–m^{6}A-dependent mRNA decay (CMD)

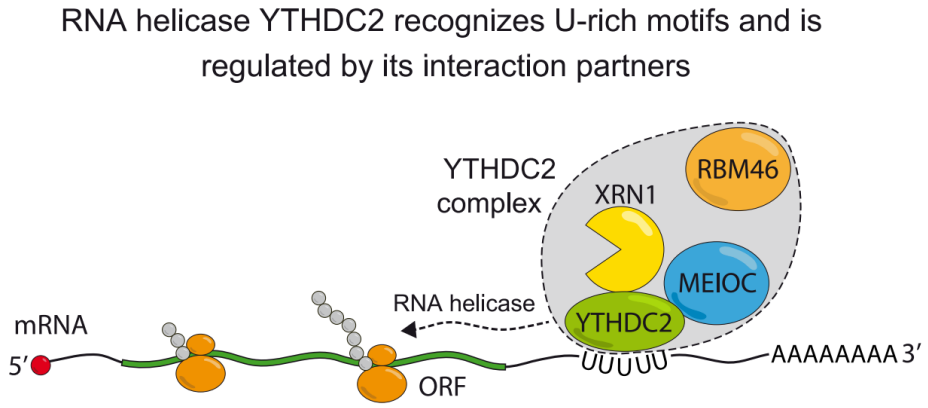
m^{6}A modification within ribosomal aminoacyl (A)-site codons slows down ribosome decoding, which may result in ribosome pausing or stalling. This impaired translation can trigger downstream decay via multiple mechanisms that remain unresolved. YTHDF proteins may be recruited to stalled ribosomes or bind downstream, and either promote decay directly or via translocation to processing bodies (P-bodies). Ribosome collisions – potentially intensified by loss of tRNA modifications such as 5-methoxycarbonylmethyl-2-thiouridine (mcm^{5}s^{2}U) – amplify decay signals. It remains unclear whether degradation occurs at the site of pausing, within P-bodies, or both, and whether decapping enzymes such as DCP2 play a role in this process



YTHDC2 is essential for fertility in mice

- Homozygous *Ythdc2*$^{-/-}$ mutant females and males are infertile.
- YTHDC2 regulates levels of m6A-modified transcripts to ensure meiosis
- YTHDC2 interacts with the 5'/3' exoribonuclease XRN1
- The function of DC2 in fertility has been recently shown to be independent from the YTH domain.





Wojtas et al., 2017. Mol Cell; Li et al., 2022 Mol Cell

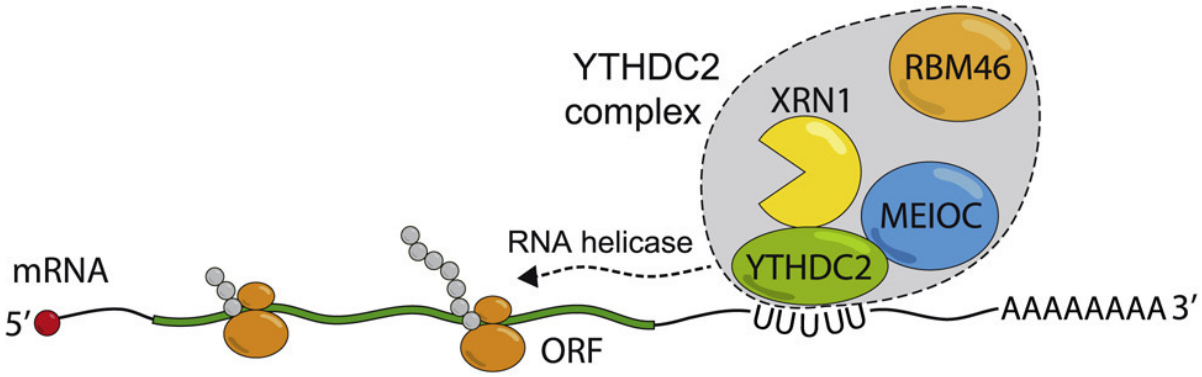
YTHDC2 is the largest member of the YTH family, is cytosolic, and expressed exclusively in the animal germline.

While its m^{6}A-binding property is dispensable *in vivo* in mice. However, its RNA helicase activity is essential for mammalian fertility.

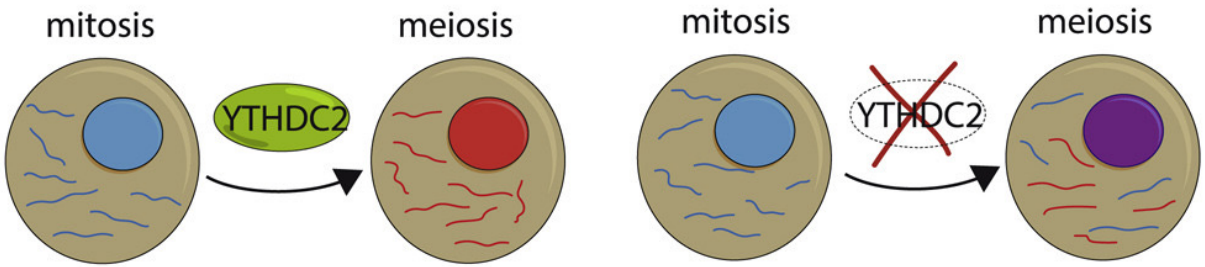
Biochemical studies reveal that the weak helicase activity of YTHDC2 is enhanced by its interaction with the 5′→3′ exoribonuclease XRN1

Single-cell transcriptomics allowed the identification of a role for YTHDC2 in ensuring proper separation of transcriptomes when the germ cells transition from mitosis to meiosis, because mutant cells display a mixed transcriptome identity that is not conducive for proper meiotic progression

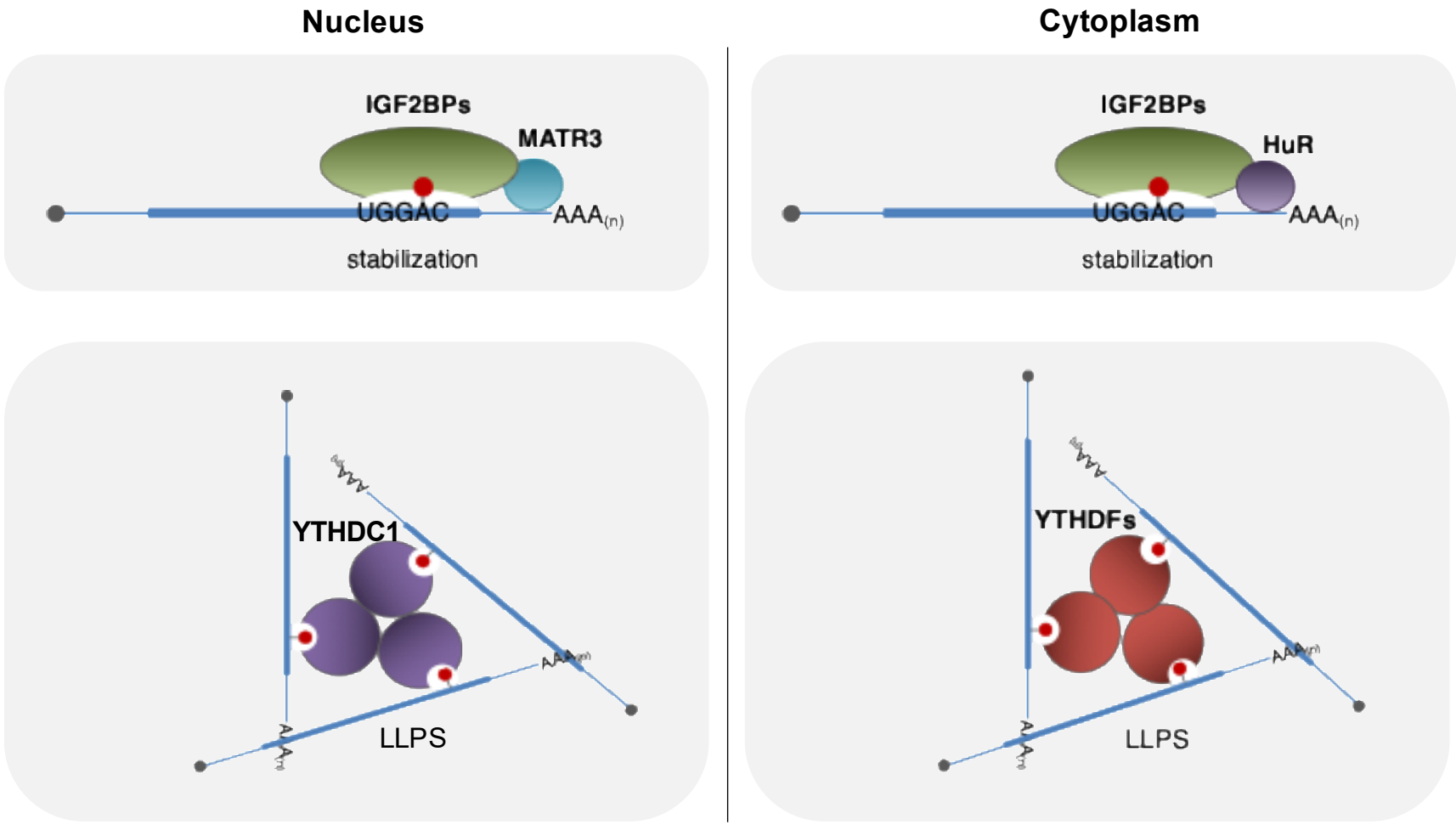
RNA helicase YTHDC2 recognizes U-rich motifs and is regulated by its interaction partners



YTHDC2 separates the mitotic and meiotic transcriptomes

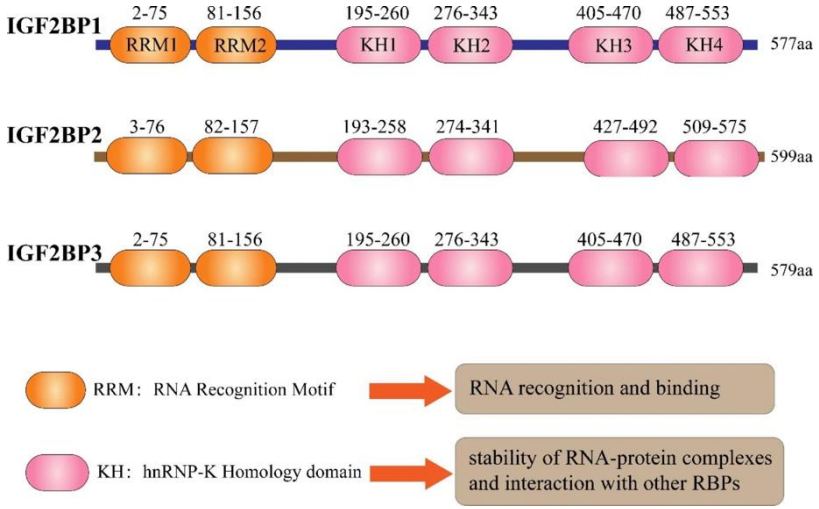


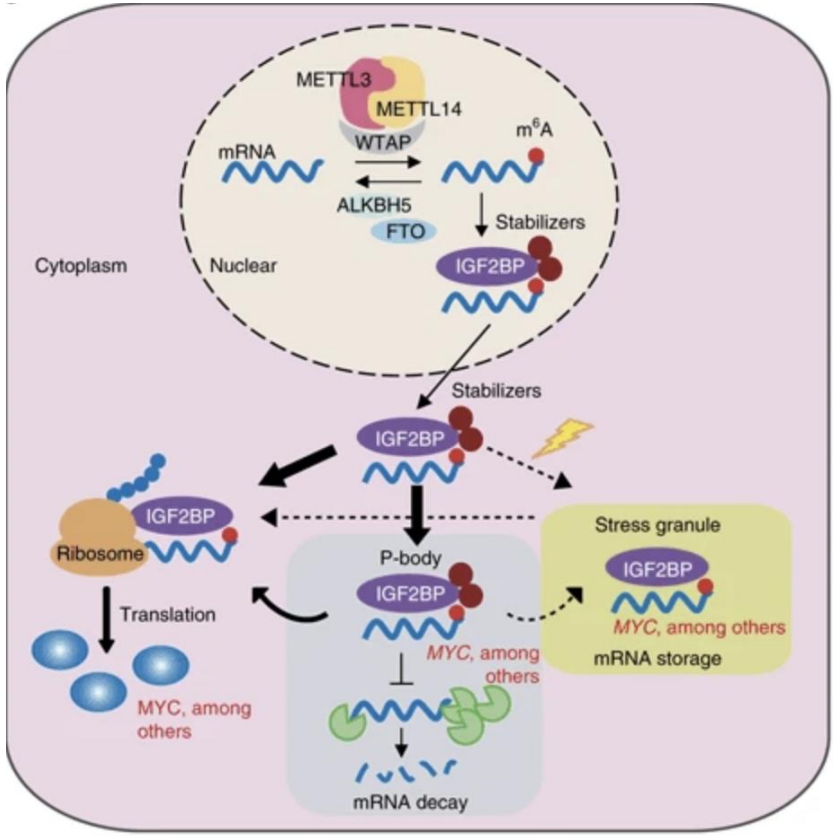
m^{6}A-mediated RNA stabilization



IGF2BPs are oncogenic m^{6}A readers

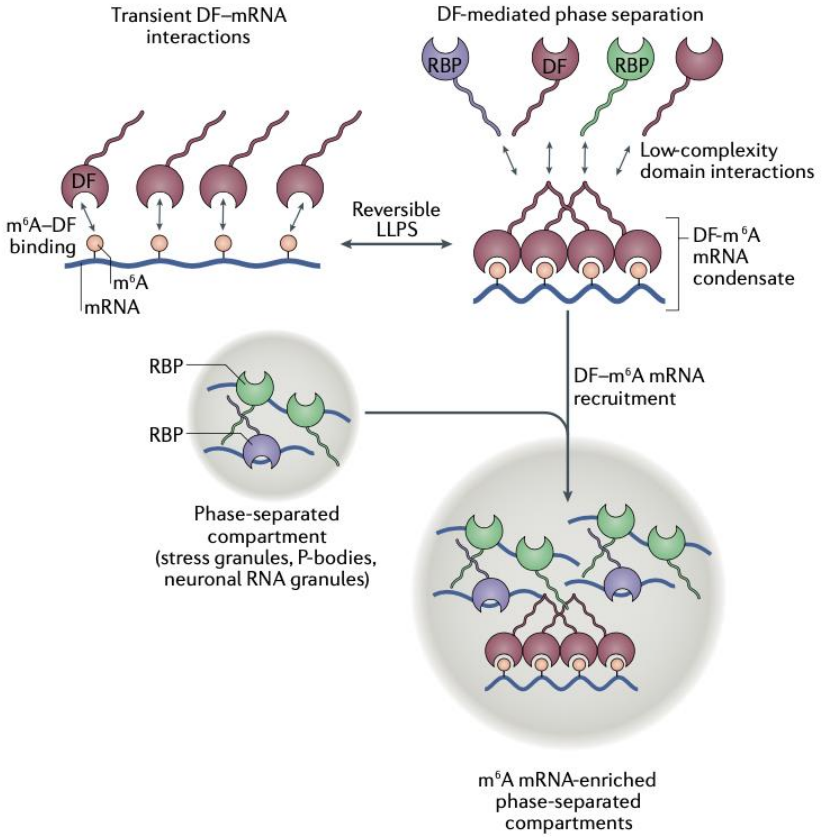
By recruiting mRNA stabilizers, such as HuR and MATR3, IGF2BPs protect target mRNAs from degradation in the P-body while facilitating translation after being exported to the cytoplasm. In particular, IGF2BPs regulate *MYC* expression in an m^{6}A-dependent manner.





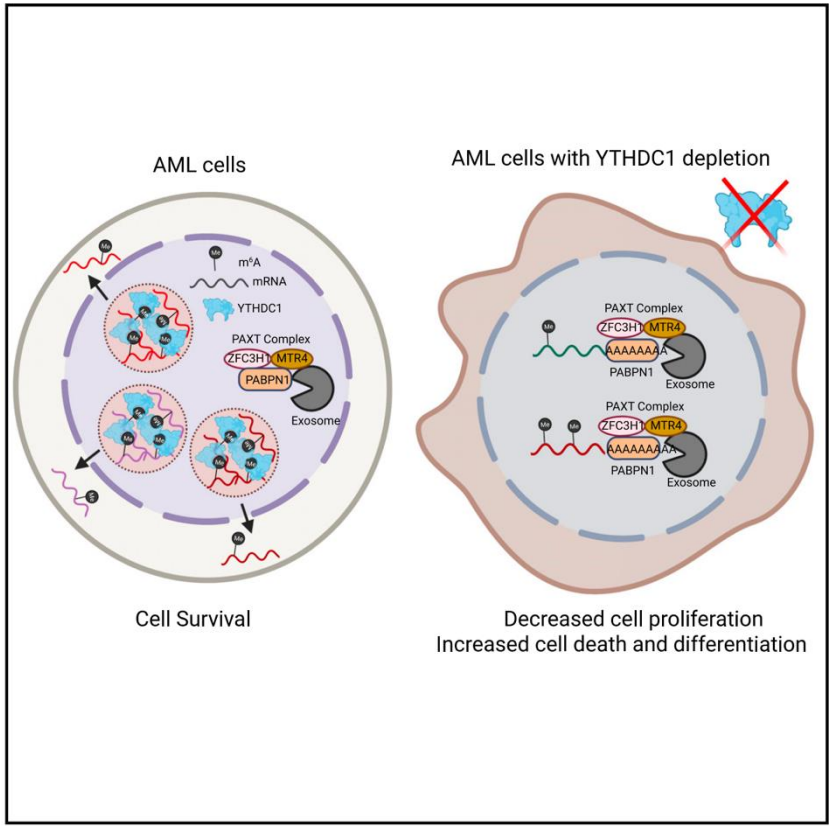
m^{6}A recruits YTHDF proteins, leading to their phase separation

YTHDF (DF) proteins and YTHDC! were shown to undergo liquid–liquid phase separation (LLPS) in the presence of mRNAs containing multiple m^{6}A residues. The low-complexity domain of each reader has the ability to mediate protein–protein interactions that induce the formation of phase-separated 'condensates'. These DF–m^{6}A mRNA condensates are then recruited to pre-existing membraneless compartments formed by phase separation, such as stress granules, P-bodies and neuronal RNA granules.



YTHDC1 is required for AML cell survival and leukemogenesis

YTHDC1 undergoes liquid-liquid phase separation by binding to m6A to form dynamic nuclear condensates. These nuclear bodies are increased in acute myeloid leukemia (AML) cells and protect mRNAs (i.e., MYC and others) from the PAXT-exosome complex.

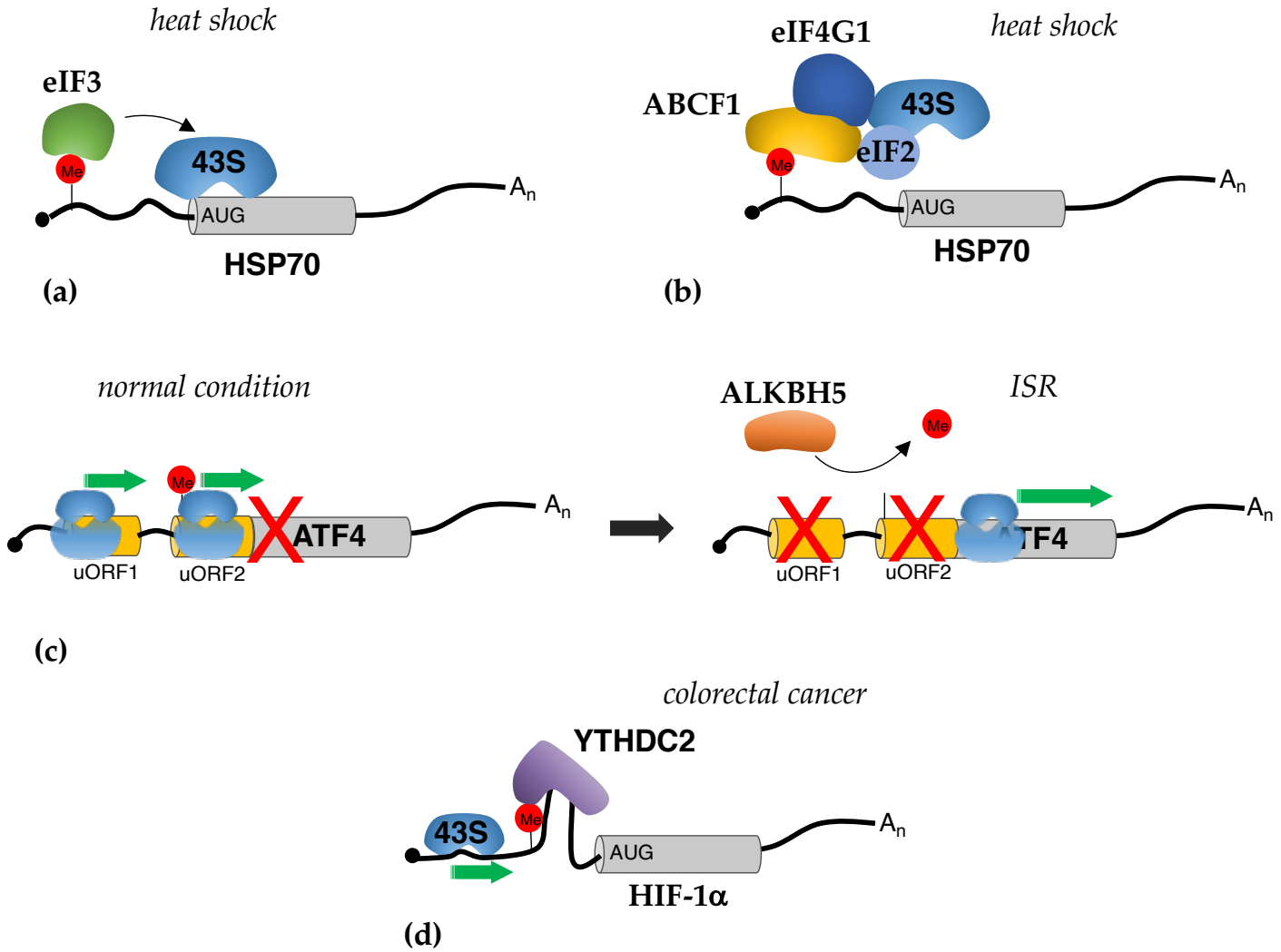


Cheng et al., 2021, Cancer Cell 39, 1–15

Regulation of mRNA translation

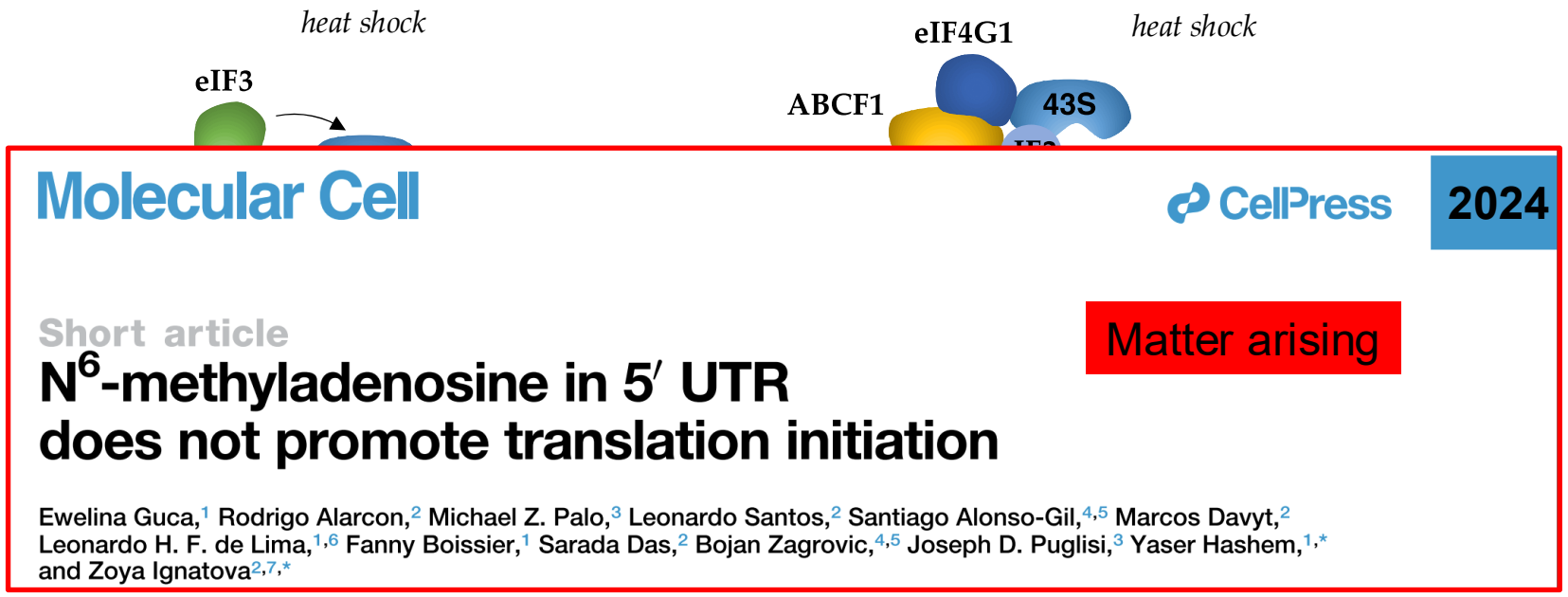
m^{6}A regulates mRNA translation

- Cap -independent translation initiation (m^{6}A in 5′-UTR)

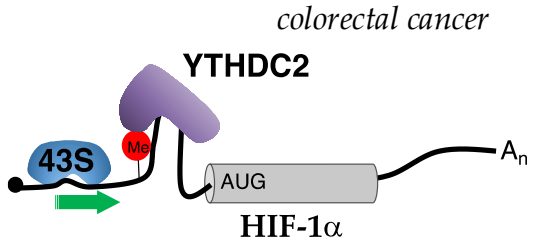


m^{6}A regulates mRNA translation

- Cap -independent translation initiation (m^{6}A in 5′-UTR)



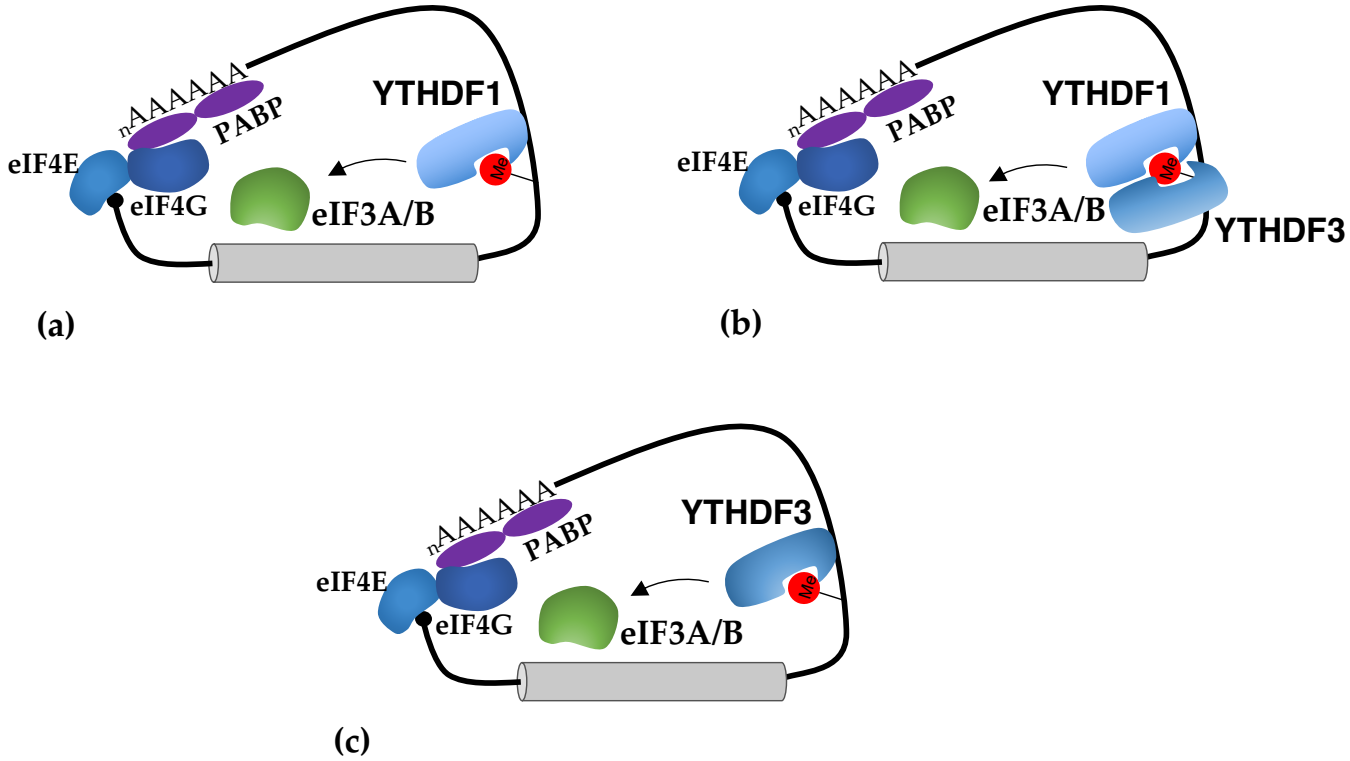
(c)



(d)

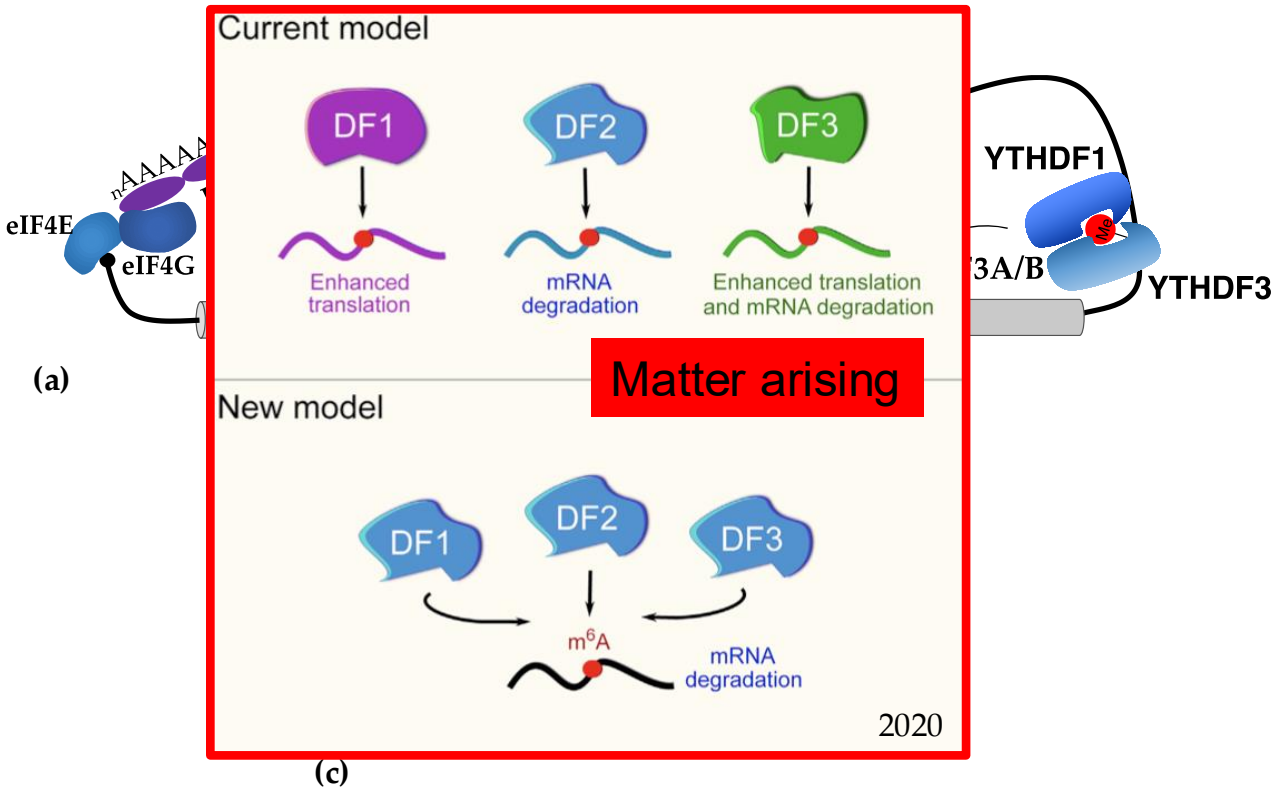
m^{6}A regulates mRNA translation

- Cap-dependent translation initiation (m^{6}A in 3′-UTR)



m^{6}A regulates mRNA translation

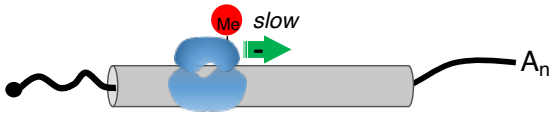
- Cap-dependent translation initiation (m^{6}A in 3′-UTR)



m^{6}A regulates mRNA translation

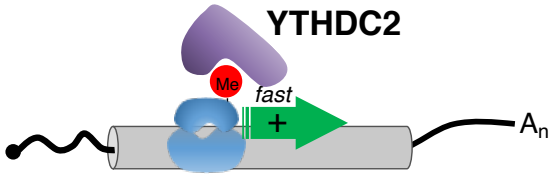
- Translation elongation (m^{6}A in coding regions)

Bacteria, HEK293T, breast cancer cells, X. laevis



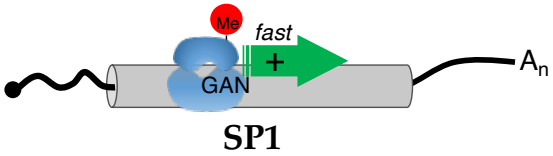
(a)

MEFs



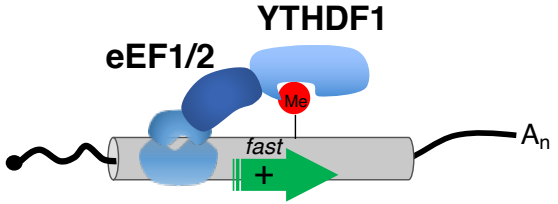
(b)

AML



(c)

HeLa, breast cancer, gastrointestinal tumors

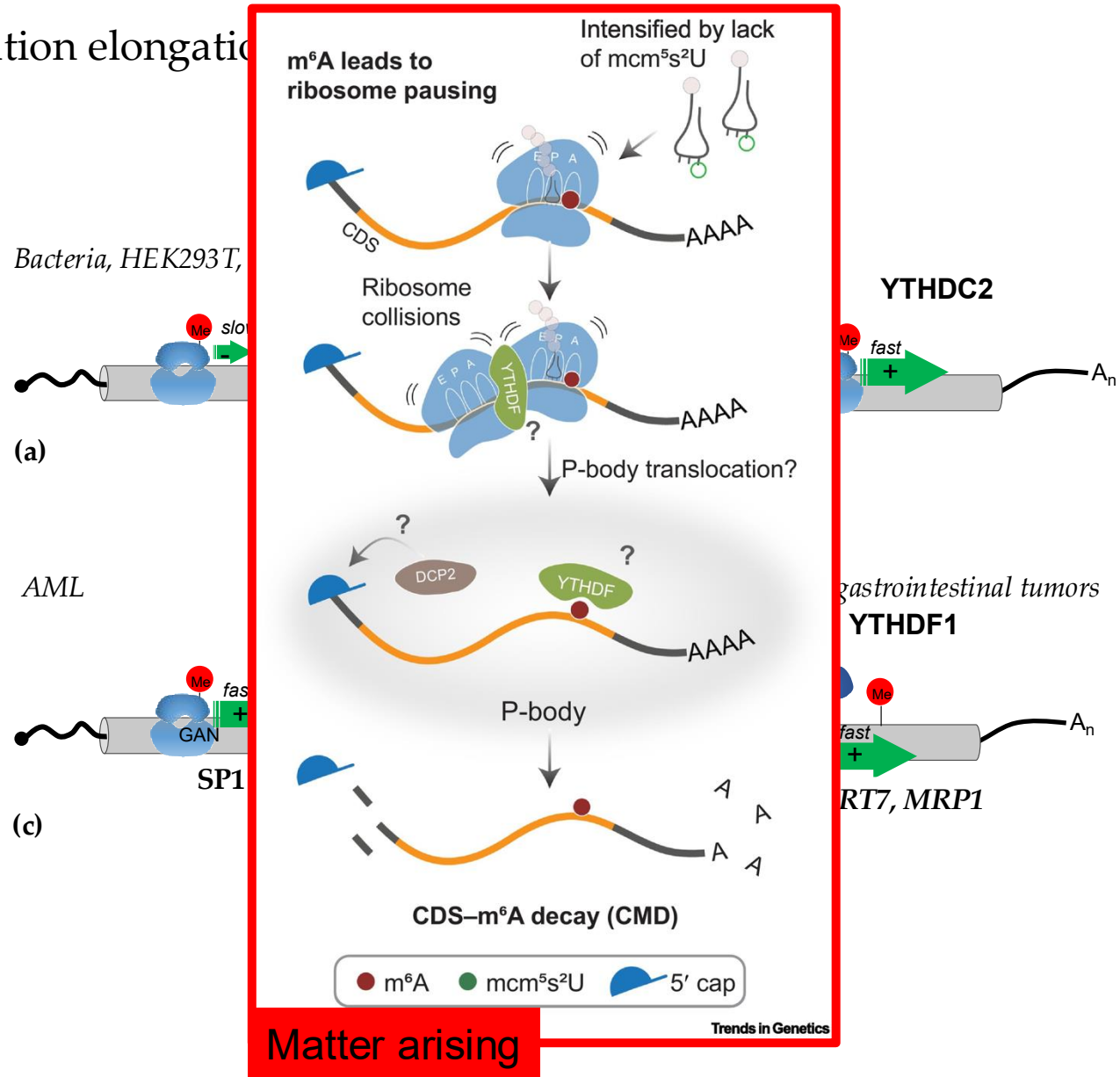


Snail, KRT7, MRP1

(d)

m⁶A regulates mRNA translation

- Translation elongatio

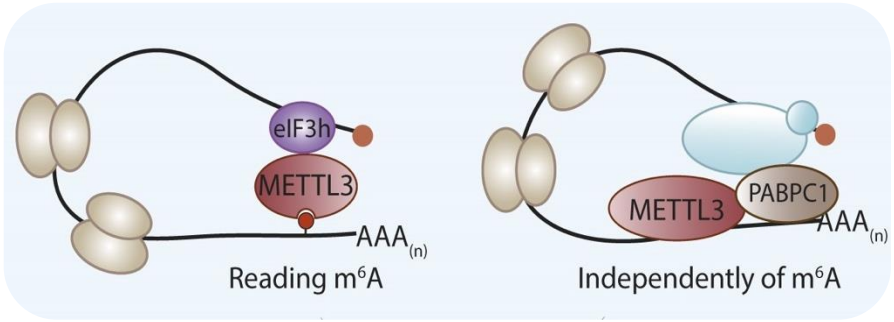


Matter arising

m^{6}A regulates mRNA translation

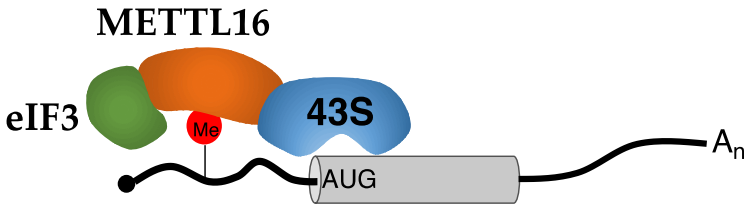
- Direct translational regulation by m^{6}A methyltransferases

Lung cancer, CML



(a)

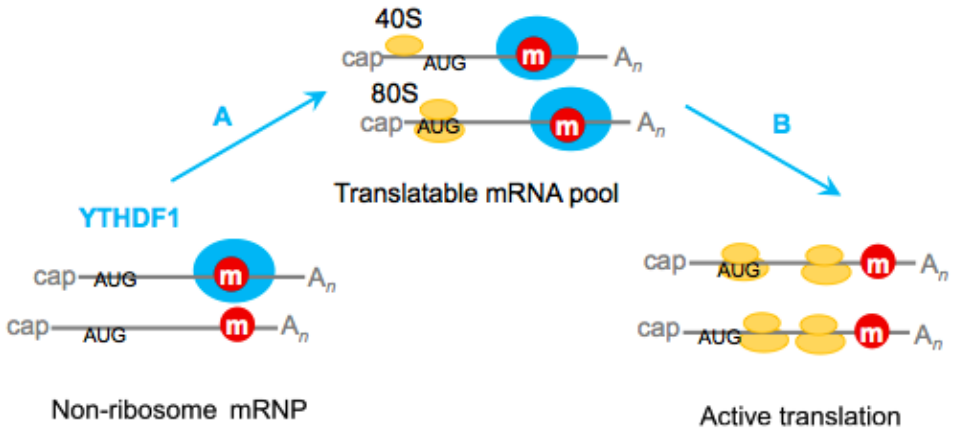
Hepatocellular carcinoma

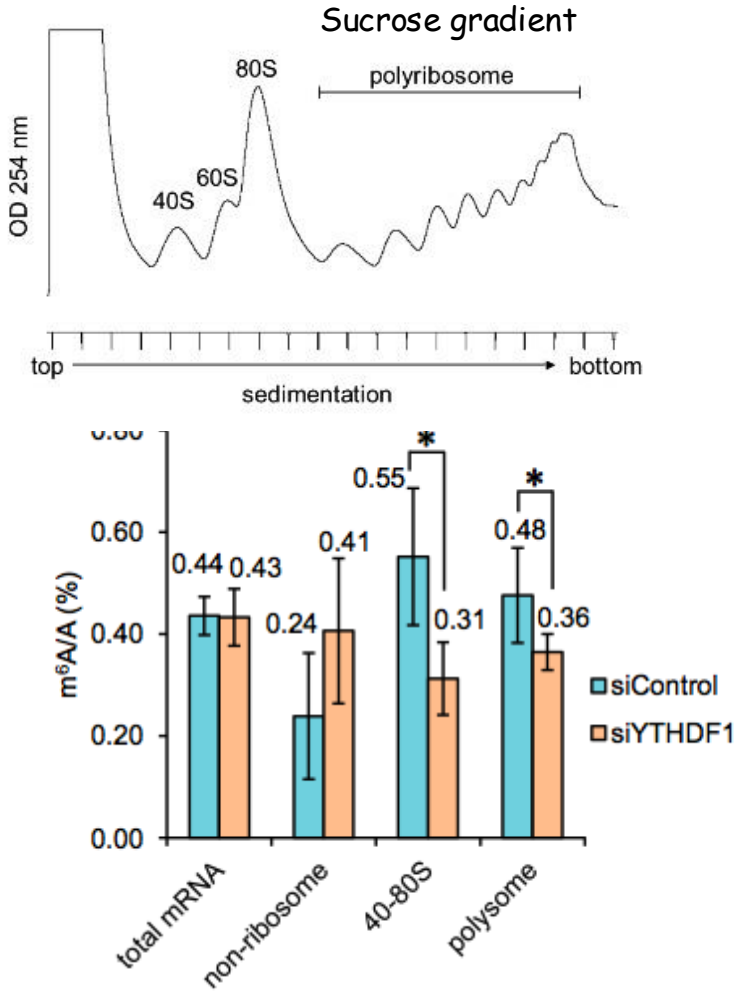


(b)

YTHDF1 stimulates mRNA translation

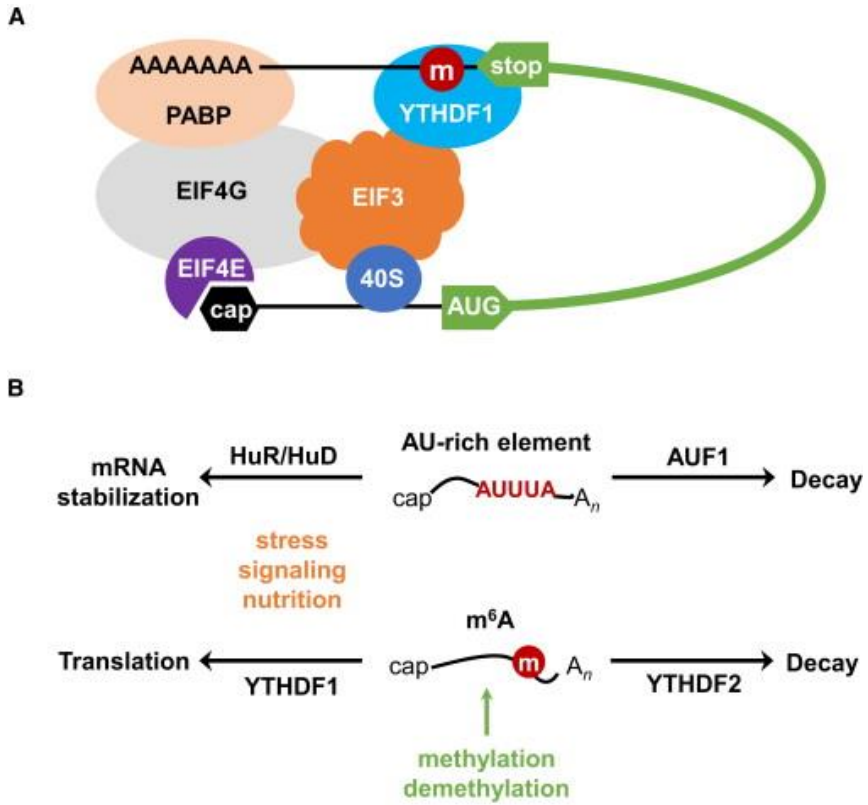
The knockdown of **YTHDF1** does not change the m^{6}A/A ratio of total mRNA. Upon the knockdown of YTHDF1, the amount of methylated mRNA increases in mRNPs and decrease within the translating and translatable pools. These results do indicate that YTHDF1 is functionally distinct from YTHDF2 and may not be directly involved in mRNA decay.



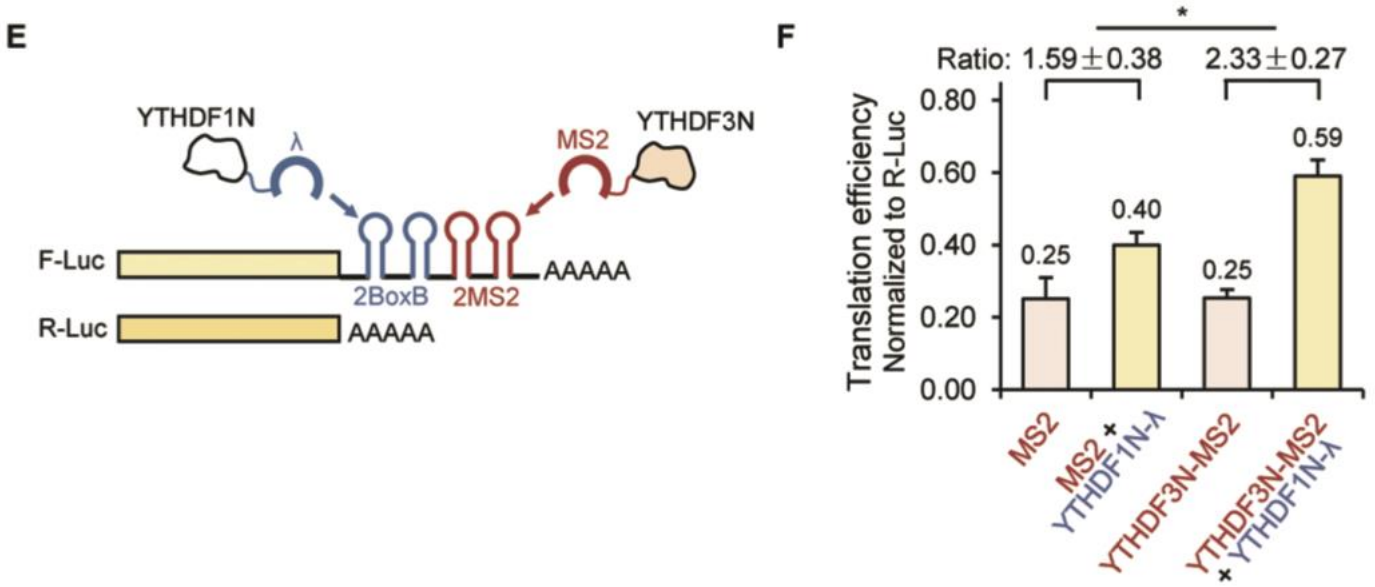


Proposed Model of Translation Promotion by YTHDF1

(A) YTHDF1 recruits m^{6}A-modified transcripts to facilitate translation initiation. The association of YTHDF1 with translation initiation machinery may be dependent on the loop structure mediated by eIF4G and the interaction of YTHDF1 with eIF3.
(B) The m^{6}A-based regulation through binding of YTHDF1 and YTHDF2 shares similarities with that of the AU-rich element which is regulated by HuR/HuD and AUF1.



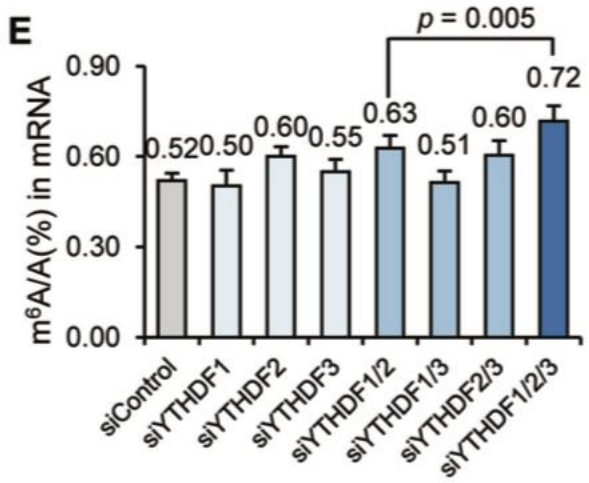
YTHDF3 facilitates translation through interacting with YTHDF1



In the reporter assay, YTHD- F3N-MS2 itself did not promote translation

Shi et al., 2017. Cell Research

All three YTHDFs contribute collectively to accelerating the metabolism of m^{6}A-modified mRNAs in the cytosol

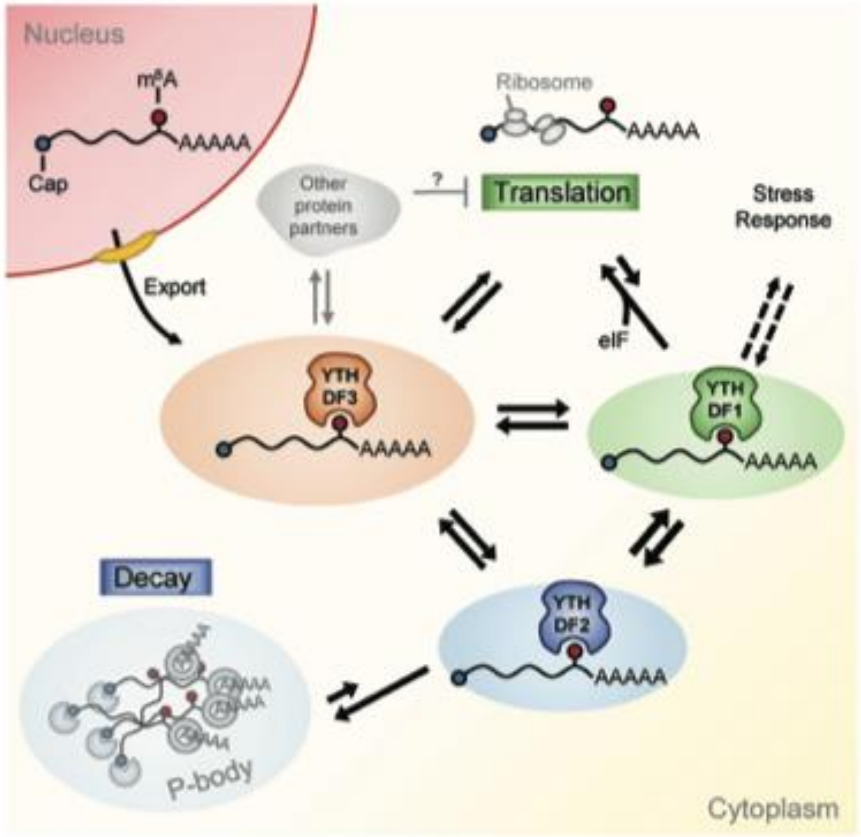


LC-MS/MS quantification of m6A levels of HeLa cells treated with siControl, siYTHDF1, siYTHDF2, siYTHDF3, and combinations of those oligoes.

Shi et al., 2017. Cell Research

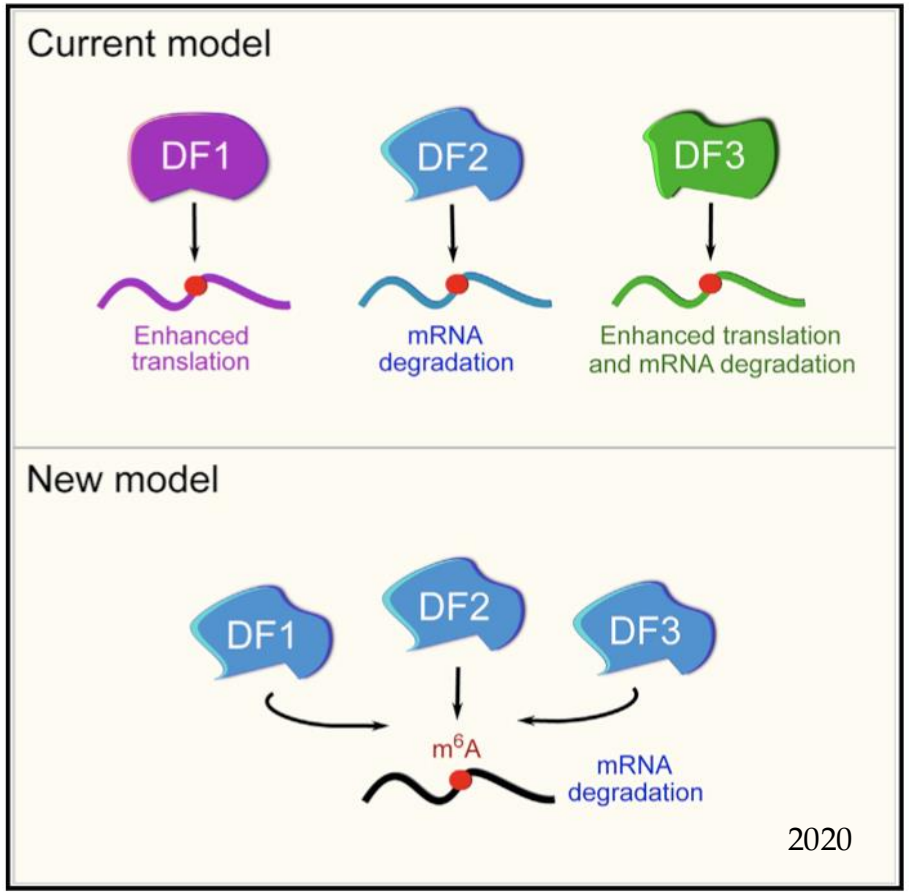
YTHDF3 facilitates translation and decay of m^{6}A-modified RNA

YTHDF3 could serve as a hub for fine-tuning the RNA accessibility of YTHDF1-2. These three mRNA pools controlled by YTHDF1-3 could be interchangeable and highly dynamic, resulting in an interconnected and dynamic mRNA modulation through m6A.



Shi et al., 2017. Cell Research

A Unified Model for the Function of YTHDF Proteins in Regulating m^{6}A-Modified mRNA

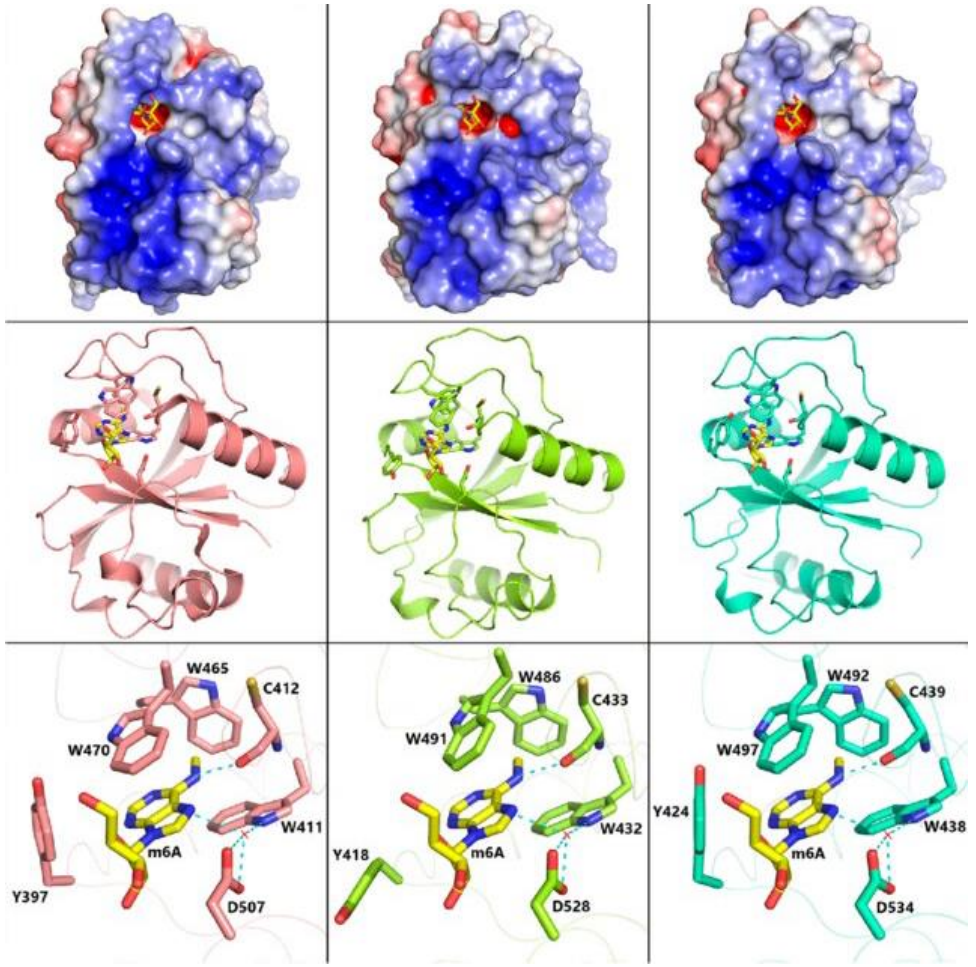


Zaccara et al. 2020. Cell

DF Proteins Bind the Same m^{6}A Sites throughout the Transcriptome

YTH domains are essentially identical in structure and are expected to have the same binding specificity. The crystal structures of the YTH domain show a single RNA-binding domain, which is the m6A-binding domain.

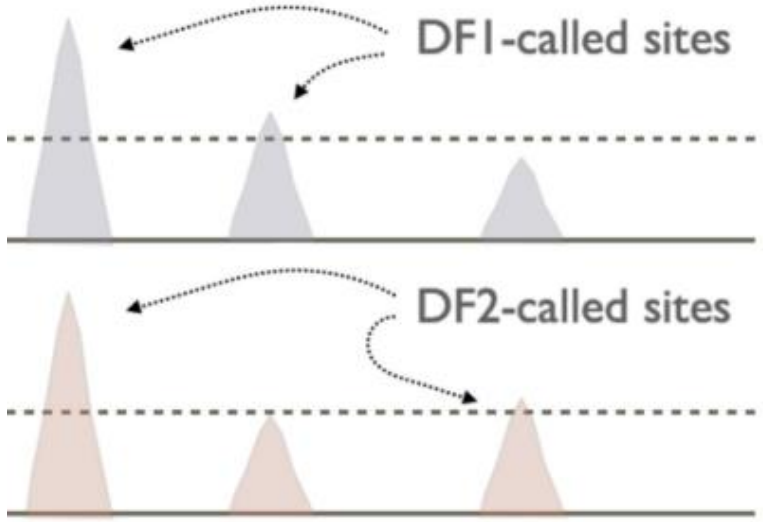
The amino acids that contact m^{6}A and the m^{6}A-proximal nucleotides are conserved in the DF1, DF2, and DF3 YTH domain.



Zaccara et al. 2020. Cell

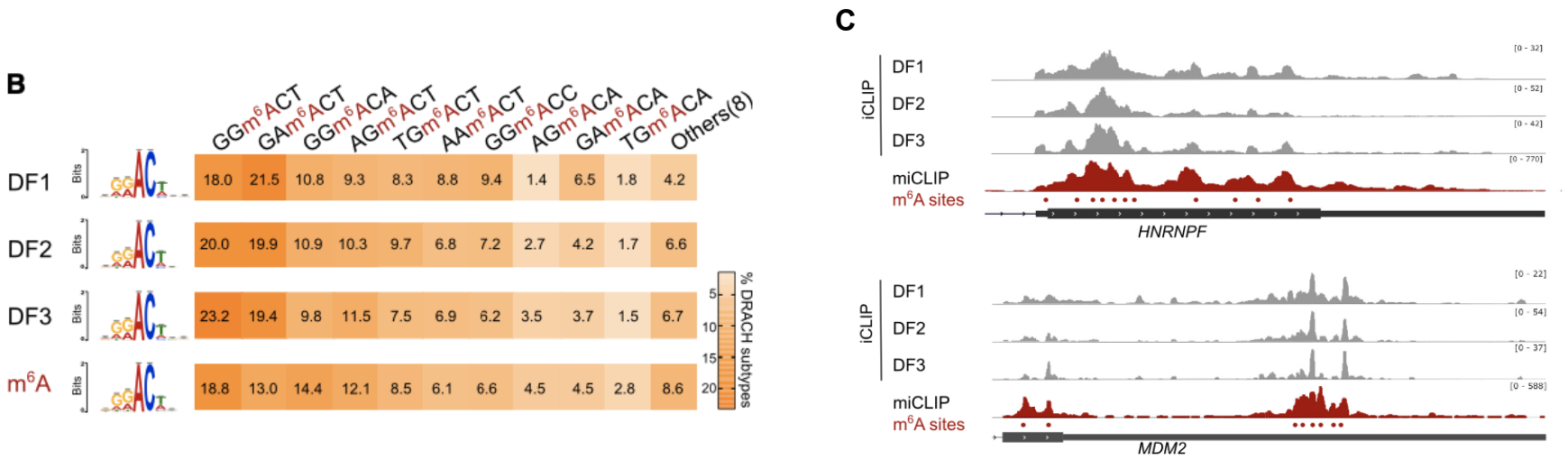
Problem with using cutoff thresholds to call peaks

In CLIP experiments, multiple reads that overlap a specific region of a gene comprise a peak. Despite the low background in CLIP experiments, nonspecific reads are present, which therefore requires "peak calling" algorithms. These algorithms call a peak based on peak height relative to other regions in the transcript. However, some peaks are true positives (i.e., actual binding sites) and some are false positives. Therefore, a statistical cutoff threshold is used to decide which peaks will be used for further analysis. Since the cutoff threshold is arbitrary, a slightly lower threshold would have called both peaks. Thus, using cutoffs creates an incorrect impression that an mRNA binds one YTHDF protein and not the other.



DF proteins bind the same m^{6}A sites throughout the transcriptome

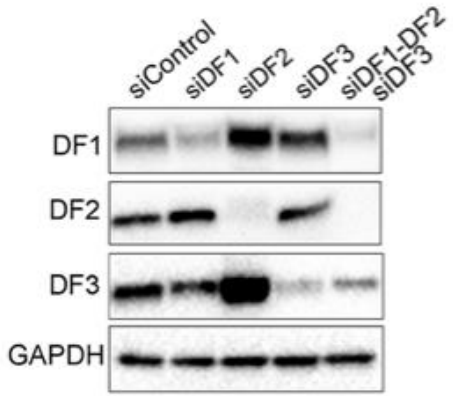
By using a correlation of read numbers at predicted binding sites, DF1, DF2, and DF3 have similar binding preferences for different m^{6}A submotifs. Shown is the prevalence of different binding sites recognized by DF1, DF2, and DF3 based on iCLIP binding data (**B**). DF1, DF2, and DF3 iCLIP reads show a similar distribution in mRNAs that resembles the miCLIP read distribution. Shown are representative examples of DF1, DF2, and DF3 iCLIP read distribution and miCLIP read distribution on HNRNPF and MDM2. The iCLIP and miCLIP data shown here were obtained in HEK293T cells and were similar to data obtained in HeLa cells (**C**).

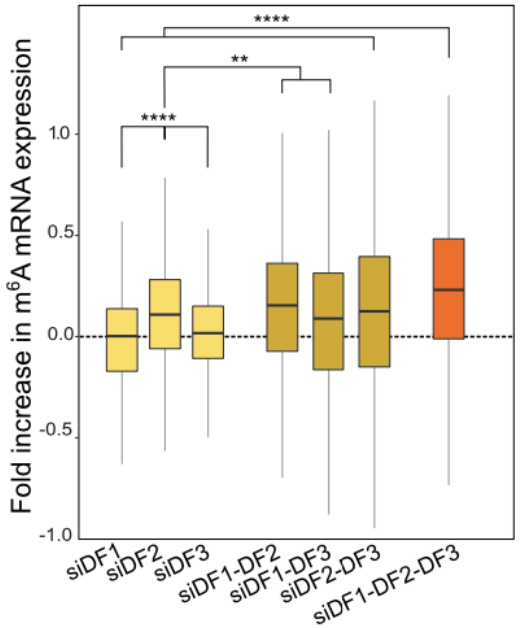


Zaccara et al. 2020. Cell

The combined activity of DF proteins leads to degradation of m^{6}A-modified mRNA

As in a previous study, they found no effect of DF1 depletion on m^{6}A-mRNA abundance compared with non-methylated mRNAs. In contrast, as reported previously, depletion of DF2, the most highly expressed DF paralog in HeLa cells was associated with a small but statistically significant increase in the abundance of m^{6}A-mRNAs. No increase in m^{6}A-mRNA abundance was observed following DF3 depletion which, like DF1, is more lowly expressed in HeLa cells. However, knockdown of any of the DF paralogs was associated with a compensatory increase in the expression of the other paralogs. Thus, compensatory upregulation of the other DF paralogs could further mask an effect of knockdown. Knocking down any two DF paralogs increased the overall abundance of m^{6}A-mRNA. Importantly, the selective increase in m^{6}A-mRNA expression was largest upon triple knock- down, and, in each case, it was directly correlated with the number of m^{6}A sites per mRNA

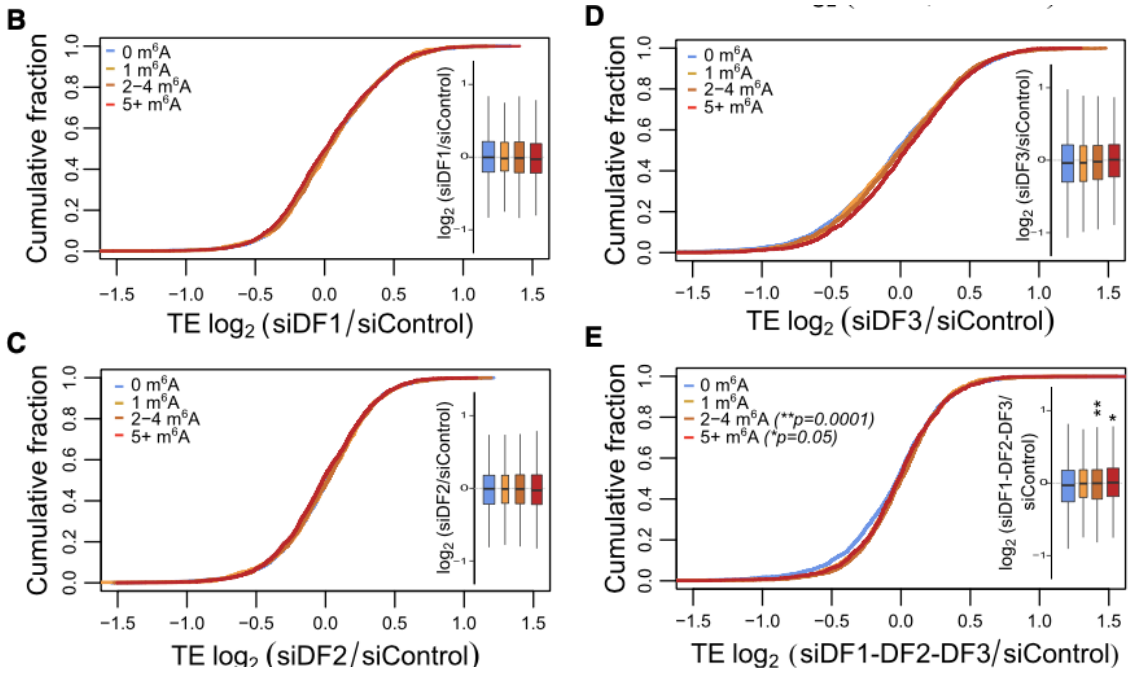


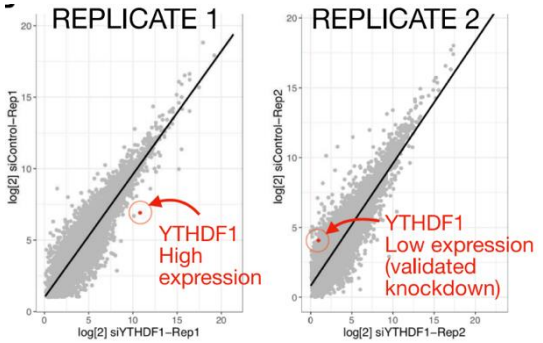


Zaccara et al. 2020. Cell

DF paralogs do not affect the translation of m^{6}A-modified mRNAs

They reanalyzed published dataset of ribosome footprinting. The number of ribosome-protected fragments bound to each mRNA was normalized to the abundance of the respective mRNA to calculate translation efficiency (TE). The TE of m^{6}A-mRNAs is not reduced upon silencing of *DF1* or any DF paralog.



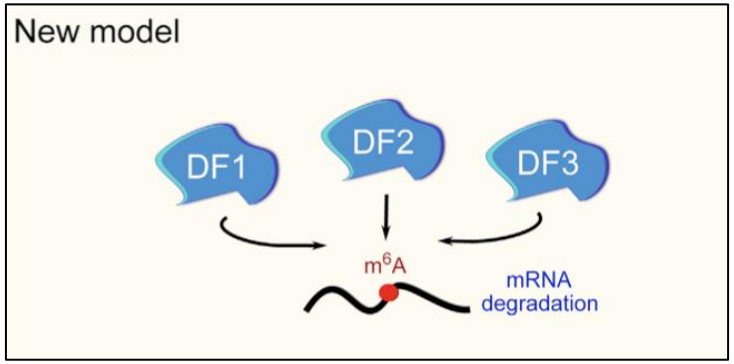


mRNAs were binned based on the number of m^{6}A sites. The distribution of each of m^{6}A mRNA subgroups is quantified in the boxplots.

Zaccara et al. 2020. Cell

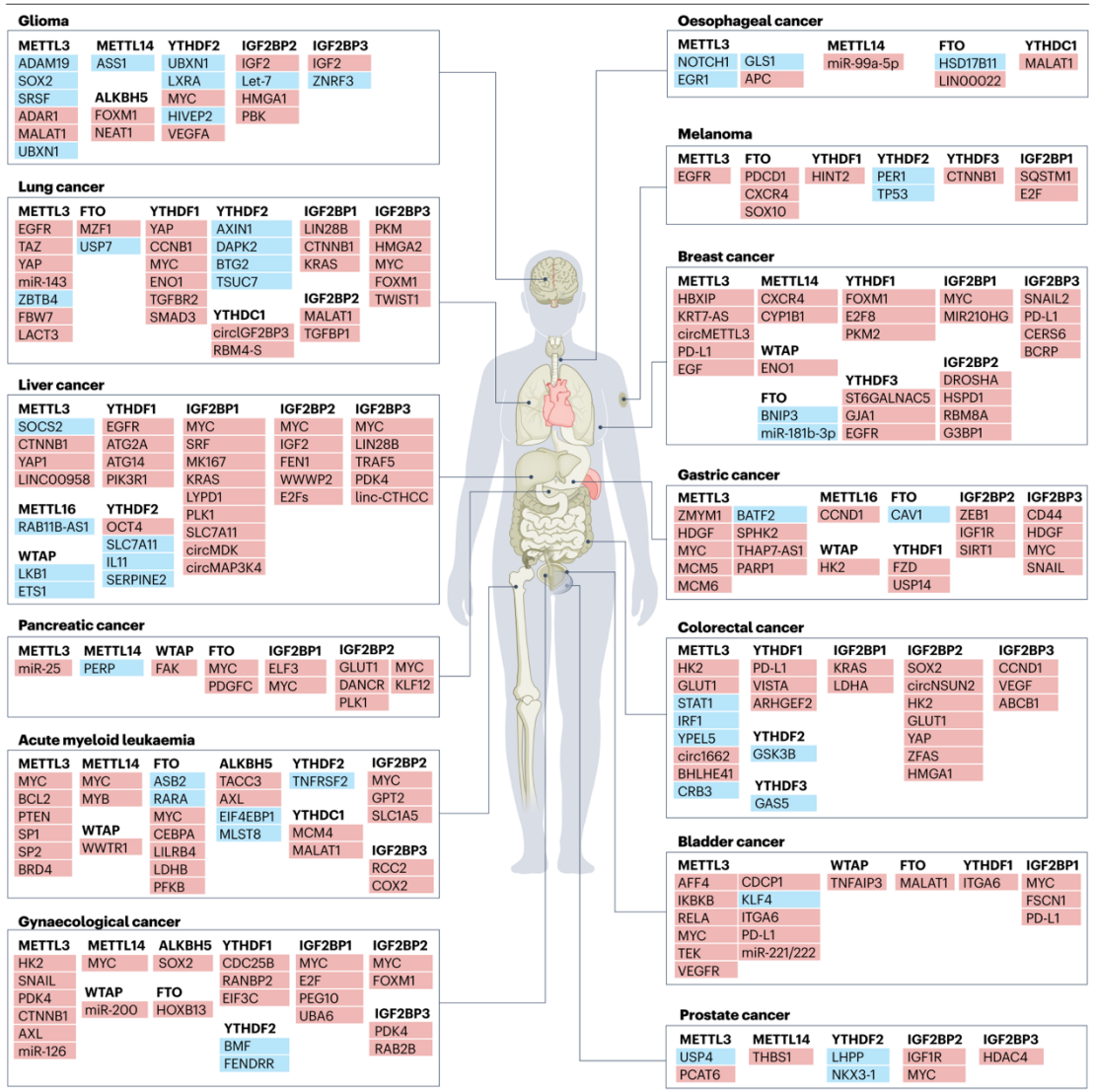
A Unified Model for the Function of YTHDF Proteins in Regulating m^{6}A-Modified mRNA

The three DF proteins function together to mediate degradation of m6A-containing mRNAs. However, depletion of different DF paralogs can have different effects. This is because DF proteins exhibit markedly different expression levels. Therefore, depletion of a low-abundance DF paralog, such as DF3, is likely to only affect a small number of highly sensitive mRNAs, whereas depletion of a higher-abundance DF paralog would affect a larger sub- set of mRNAs, causing a different phenotype. Additionally, because DF paralogs may be expressed at different levels in different tissues, single DF paralog depletion can result in tissue-specific phenotypes



Zaccara et al. 2020. Cell

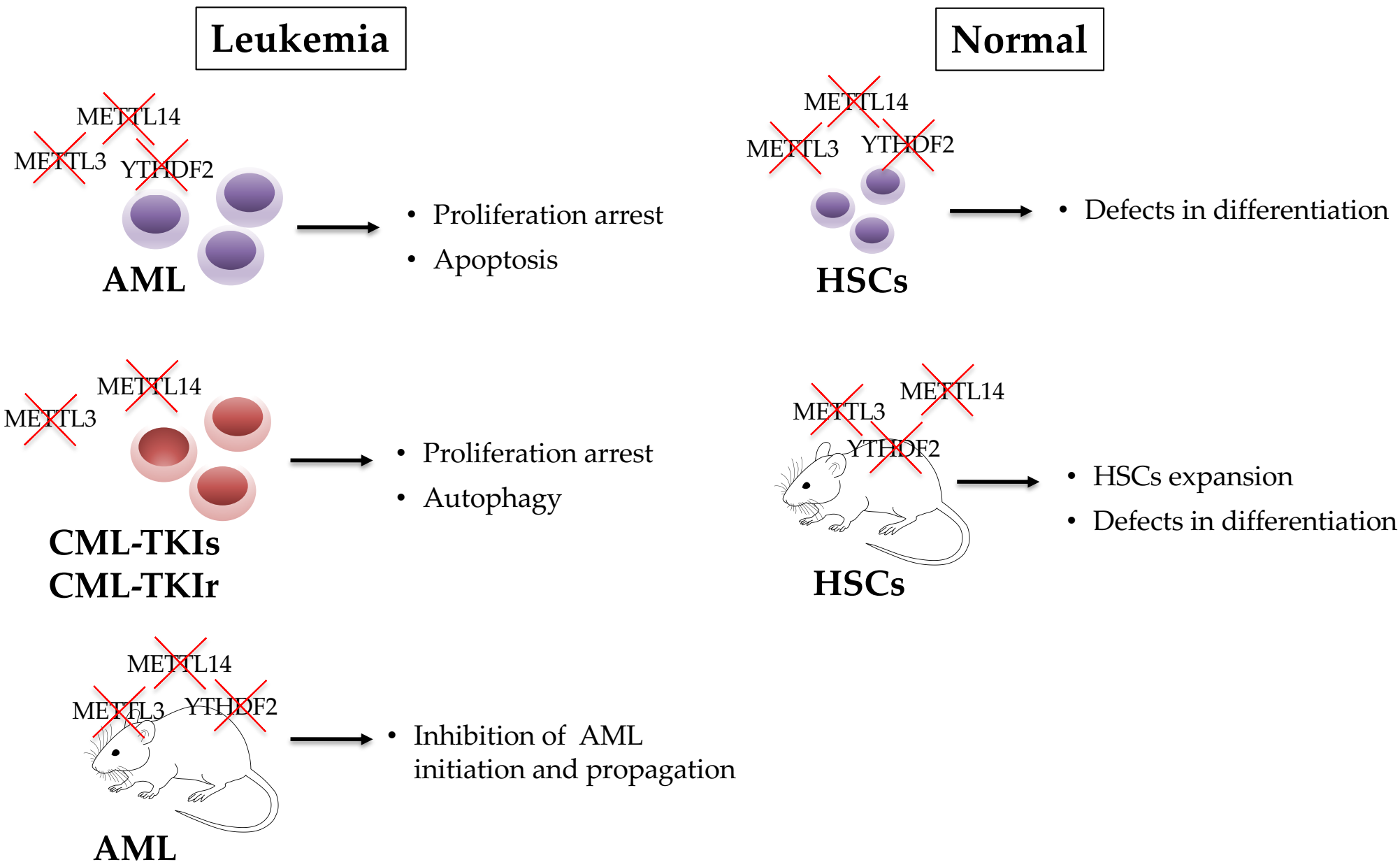
Oncogenic roles of m^{6}A modifiers



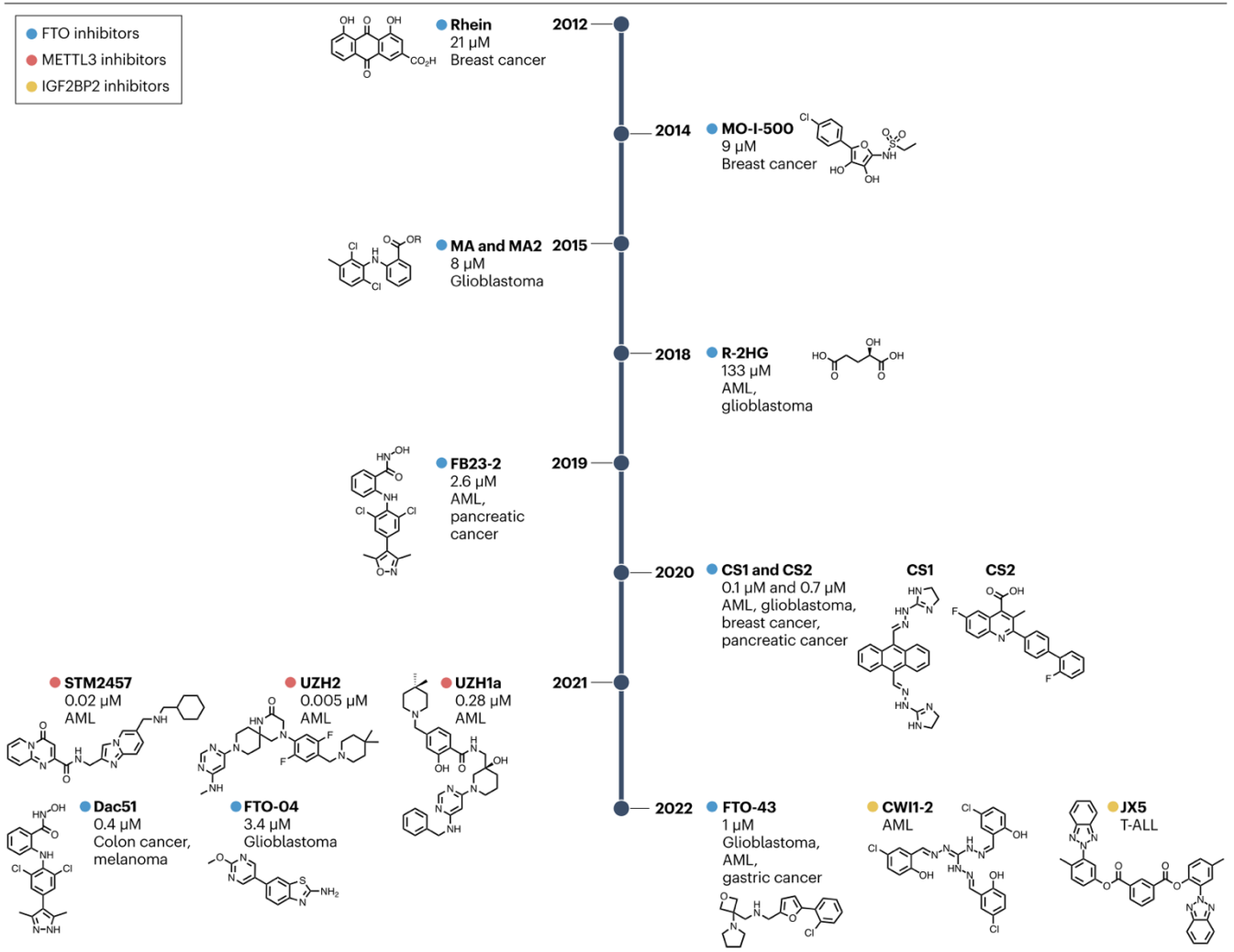
Targets positively regulated (upregulated) by m6A modifiers are in red, while negatively regulated (downregulated) targets are in blue.

Deng et al., 2023. Nature Reviews Clinical Oncology

m^{6}A is a good therapeutic target in leukemias



Small-molecule inhibitors targeting m6A modifiers



COMPANIES TARGETING RNA EPIGENETICS

Company	Named targets	Likely lead indication	Estimated phase I trial start date
STORM Therapeutics	METTL3, other methyl transferases	AML[a]	2021[a]
Accent Therapeutics	METTL3, ADAR1	AML[a], NSCLC[a]	2021[a], 2022[b]
Gotham Therapeutics	METTL3, undisclosed 'reader', undisclosed 'eraser'	AML[a]	2021[a]
EPICS Therapeutics	Undisclosed RNA modifying enzymes	Cancer	ND
Twentyeight-Seven Therapeutics	Undisclosed RNA modifying enzymes	Cancer	ND
Korro Bio	ADAR1	ND	ND

AML, acute myeloid leukaemia. [a]For METTL3 inhibitor. [b]For ADAR1 inhibitor. ND, no data available.

9th October 2024
STORM Therapeutics to Present Phase 1 Data on its First-in-Class Lead Product STC-15 at EORTC-NCI-AACR Symposium

11th November 2024
STORM Therapeutics Presents New Clinical Data on its First-in-Class METTL3 Inhibitor STC-15 at SITC 2024

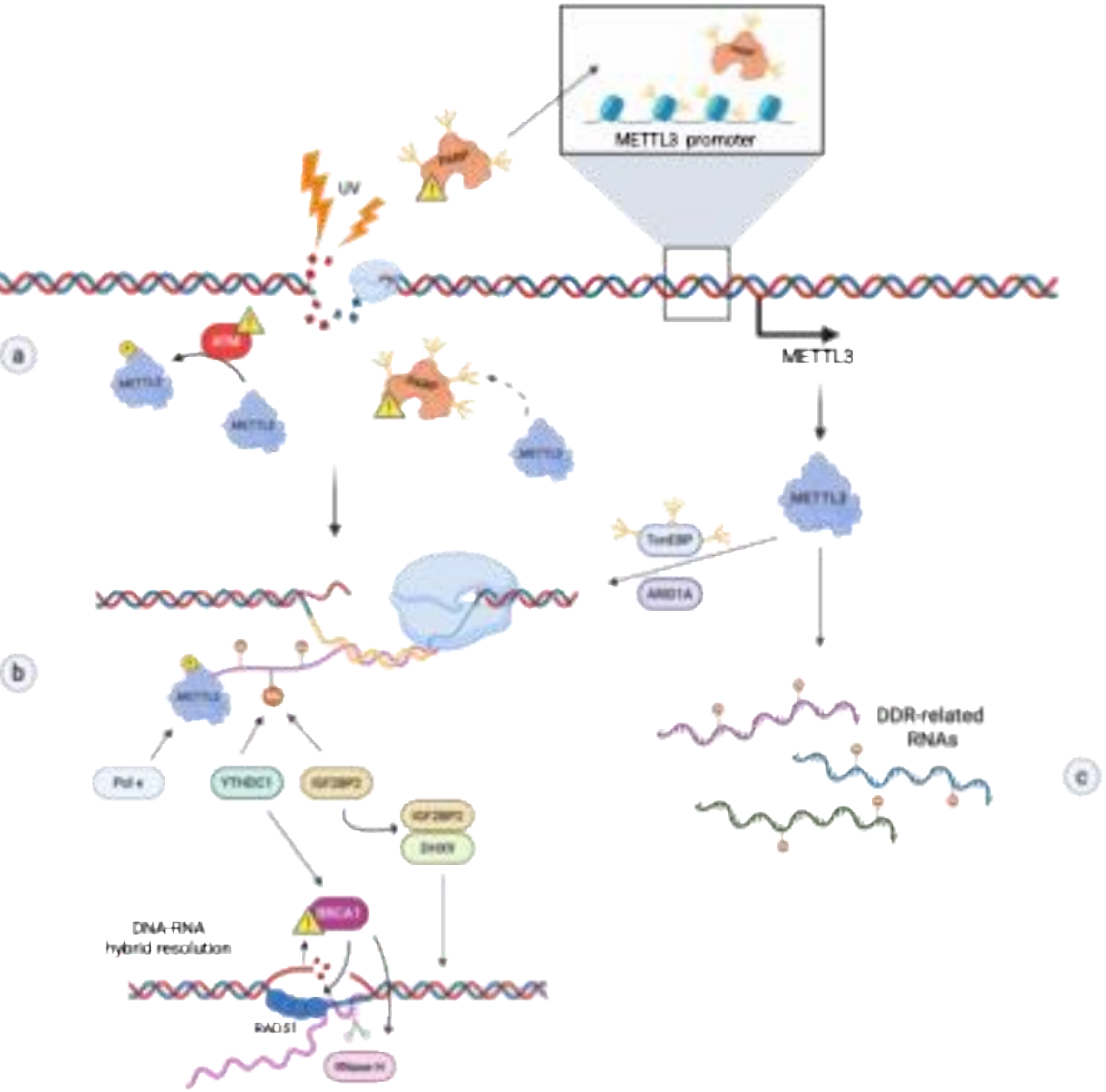
STC-15 is well tolerated and demonstrates promising signs of clinical activity observed in multiple tumor types

27th May 2025
STORM Therapeutics Announces First Patient Dosed in Clinical Collaboration to Evaluate STC-15 in Combination with LOQTORZI® and Appointment of Atif Abbas, M.D., as Chief Medical Officer

STC-15 has demonstrated durable clinical activity and tolerability to progress into a combination study with a checkpoint inhibitor

16th April 2026
STORM Therapeutics Secures $56 Million Series C Financing and Doses First Patient in Phase 2 Sarcoma Trial of STC-15

m^{6}A can regulate DNA damage repair through several pathways

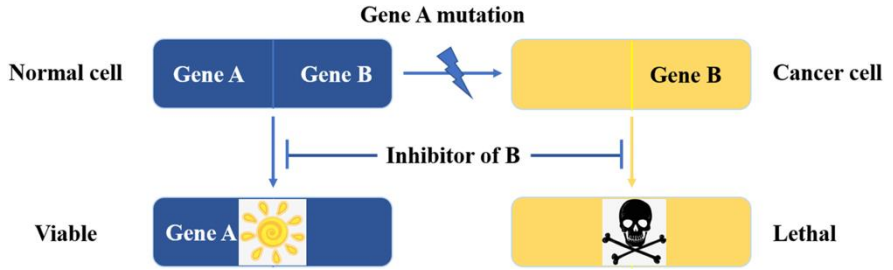


a) METTL3 recruitment on chromatin is coordinated by distinct mechanisms. First, DSBs (DNA Double-Strand Breaks) are recognized by ATM, which subsequently phosphorylates and activates METTL3. Additionally, PARP1 auto-PARylation leads to its activation and subsequent binding to the METTL3 promoter, making it transcriptionally competent and upregulating METTL3 mRNA. PARP1 also mediates PARylation at damaged sites, thereby rendering them permissive for METTL3 loading. Alternatively, METTL3 recruitment can also be mediated by TonEBP (which recognizes R-loops) and ARID1A (a component of the SWI/SNF chromatin-remodeling complex).

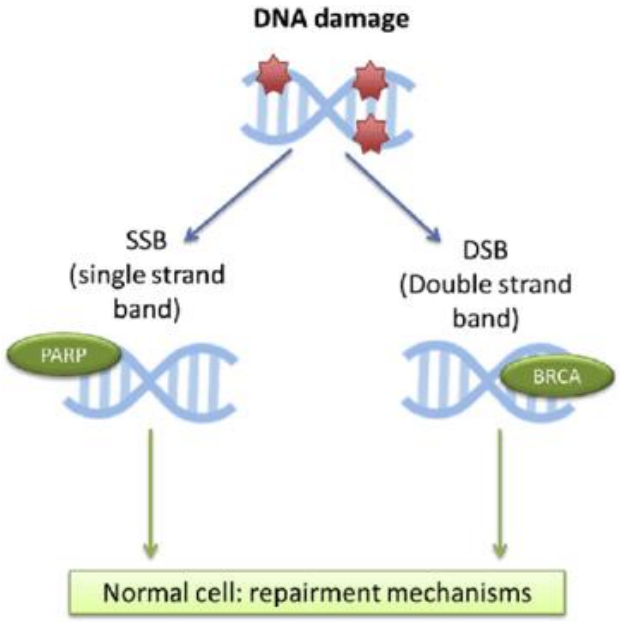
b) While on chromatin, METTL3 engages Pol κ (Polymerase Kappa) and methylates RNAs within DNA: RNA hybrids, subsequently recruiting the DNA repair machinery through the m^{6}A readers YTHDC1 and IGF2BP2.

c) METTL3 also acts directly on mRNAs of genes involved in the DNA Damage Response (DDR-related RNAs), modulating their expression.

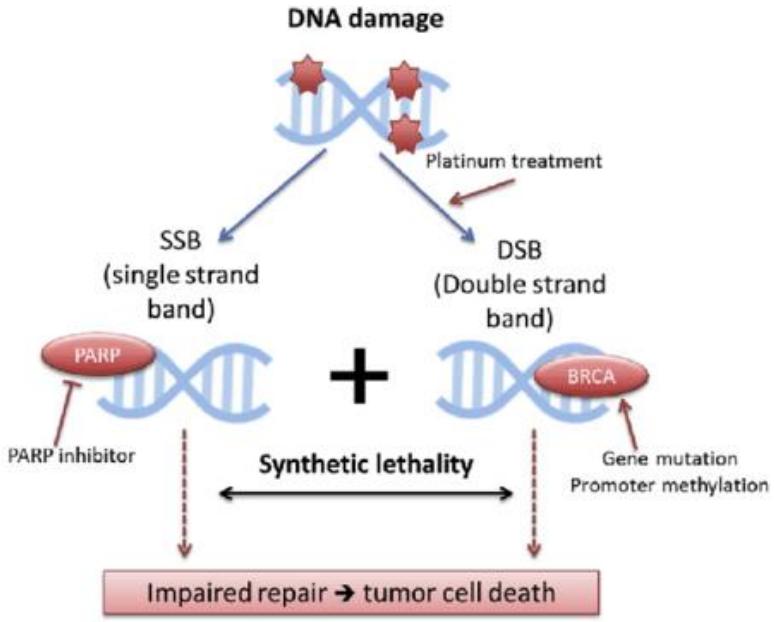
DNA damaging therapies in cancer patients



Homologous recombination proficiency (HRP) status

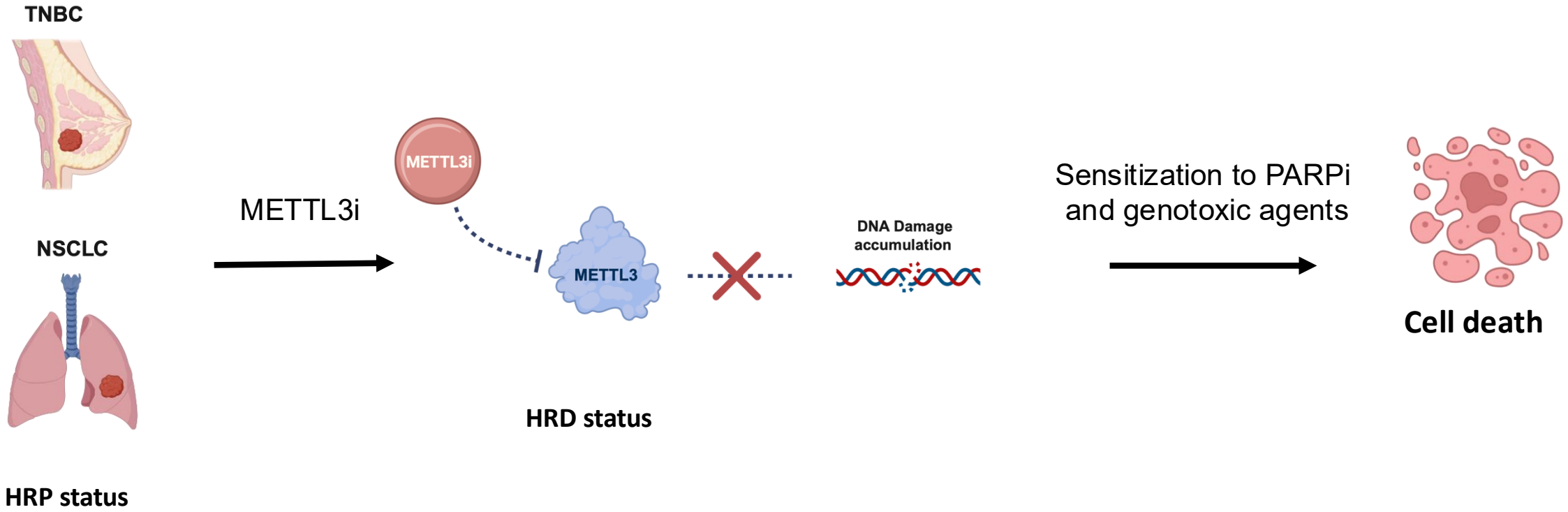


Homologous recombination deficiency (HRD) status



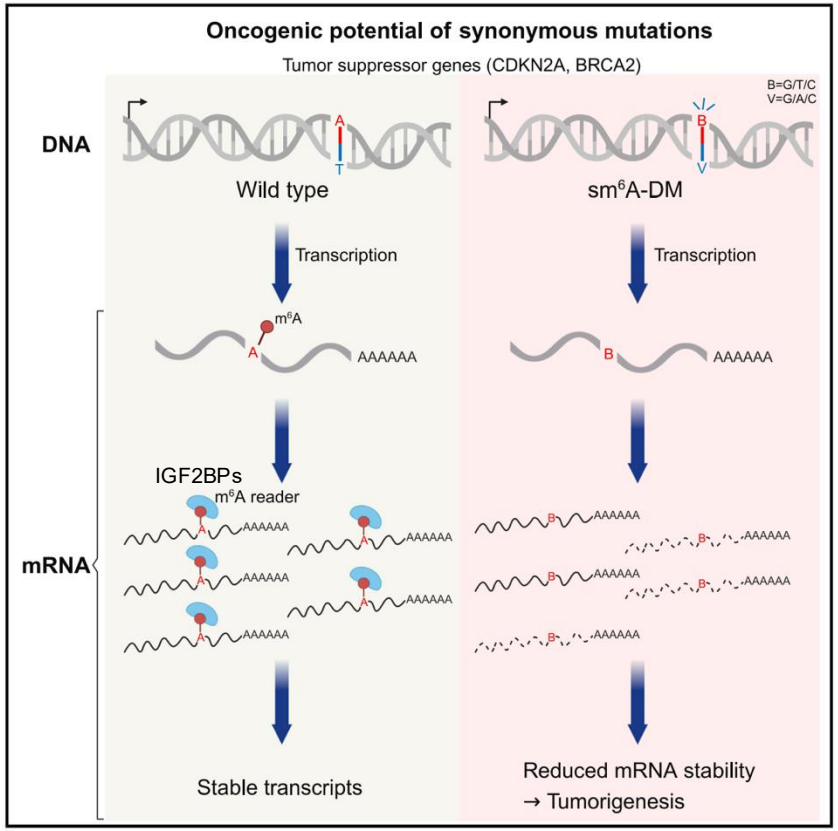
Mota et al. 2019. ScienceDirect

METTL3 inhibition increases the response to DNA damage-based treatment

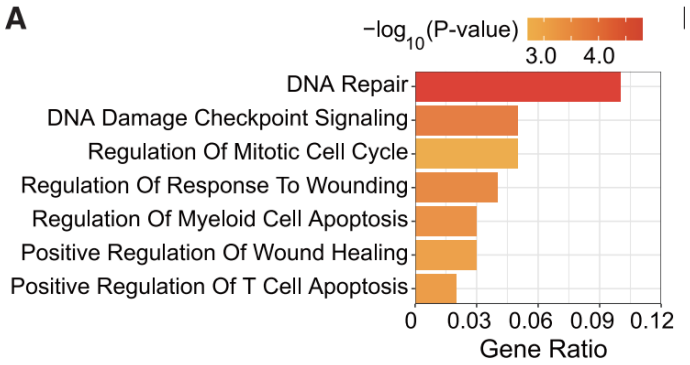


Synonymous mutations promote tumorigenesis by disrupting m^{6}A-dependent mRNA metabolism

Cancer cells acquire numerous mutations during tumorigenesis, including synonymous mutations that do not change the amino acid sequence of a protein. These mutations, called ‘‘m^{6}A disruption mutations (m^{6}A-DMs), can potentially perturb m^{6}A modification patterns, thus altering mRNA expression levels.



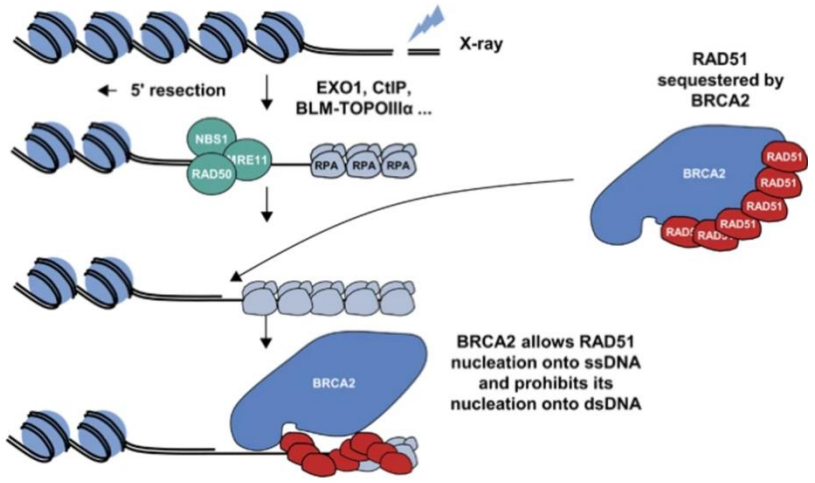
GO enrichment analysis for the top 100 genes with the highest observed m^{6}A-DM rates.

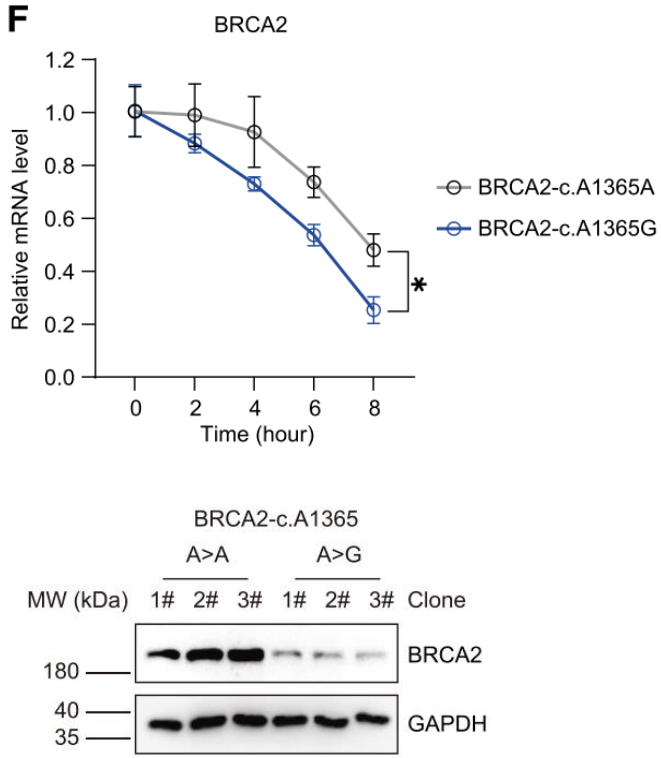


Lan et al., 2025. Cell

BRCA2 is a relevant sm^{6}A-DM-containing genes

The BRCA2-c.A1365 mutation (1365A>G) was identified in prostate cancer. They utilized a knockin approach to generate the BRCA2-c.A1365G mutation in the prostate cancer cell line PC-3. BRCA2 mRNA stability and BRCA2 protein levels were downregulated in cells with the BRCA2- c.A1365G mutation.

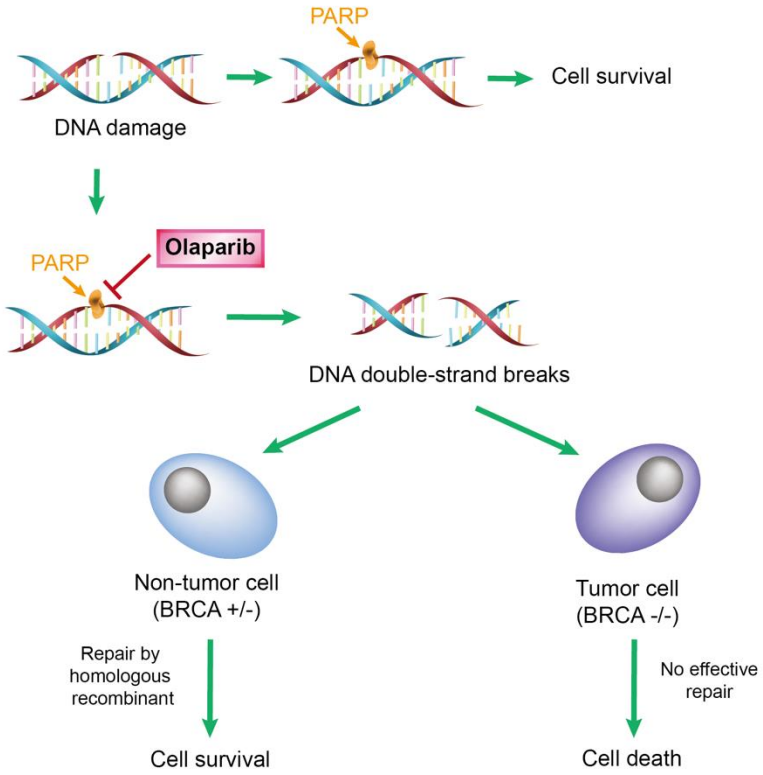


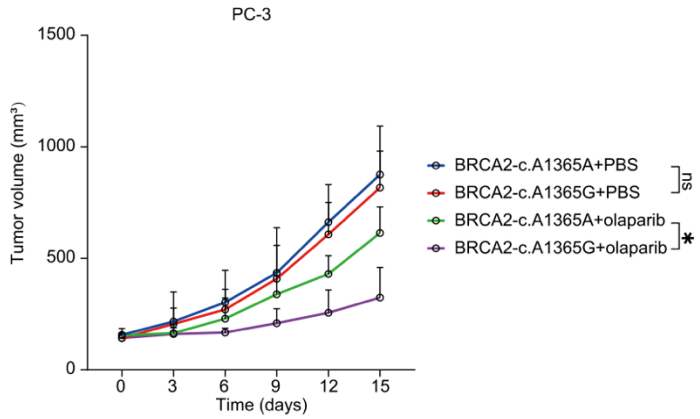


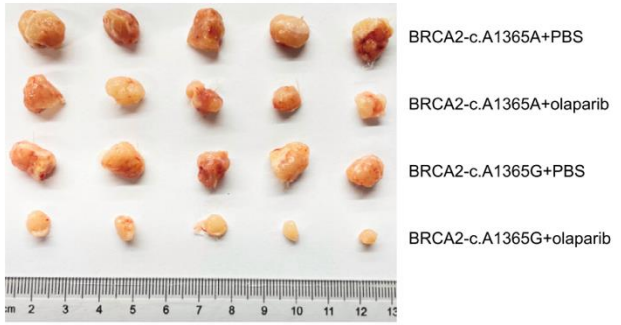
Lan et al., 2025. Cell

BRCA2 is a relevant sm^{6}A-DM-containing genes

Given that deficiencies in BRCA1 and BRCA2 sensitize cancer cells to poly (ADP-ribose) polymerase inhibitors (PARPis), loss of m^{6}A at this site increased cancer cell sensitivity to the PARPi Olaparib.





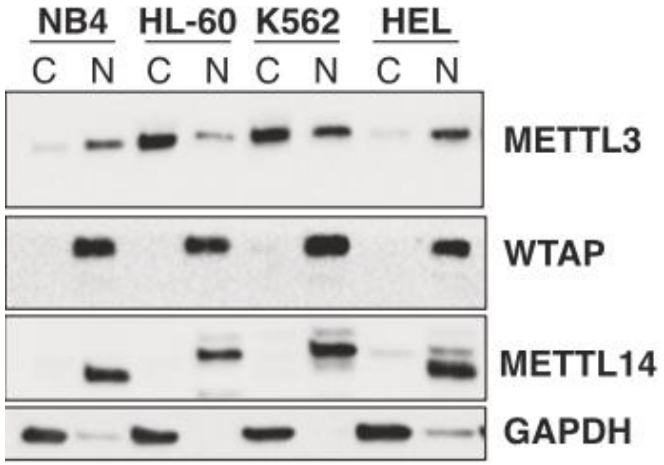


Lan et al., 2025. Cell

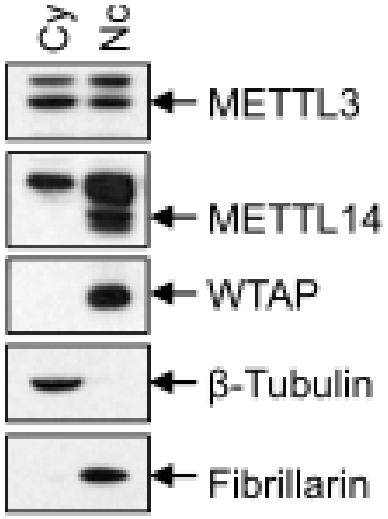
Catalytic independent role of METTL3 in cancer

The m^{6}A Methyltransferase METTL3 Promotes Translation in Human Cancer Cells

In human cancer cells (lung cancer and myeloid leukemia) METTL3 is mislocalised in the cytoplasm and interacts with translation initiation machinery to promote translation of a subset of m^{6}A containing mRNAs independently of its methyltransferase activity and m^{6}A readers.



Myeloid leukemia cell line

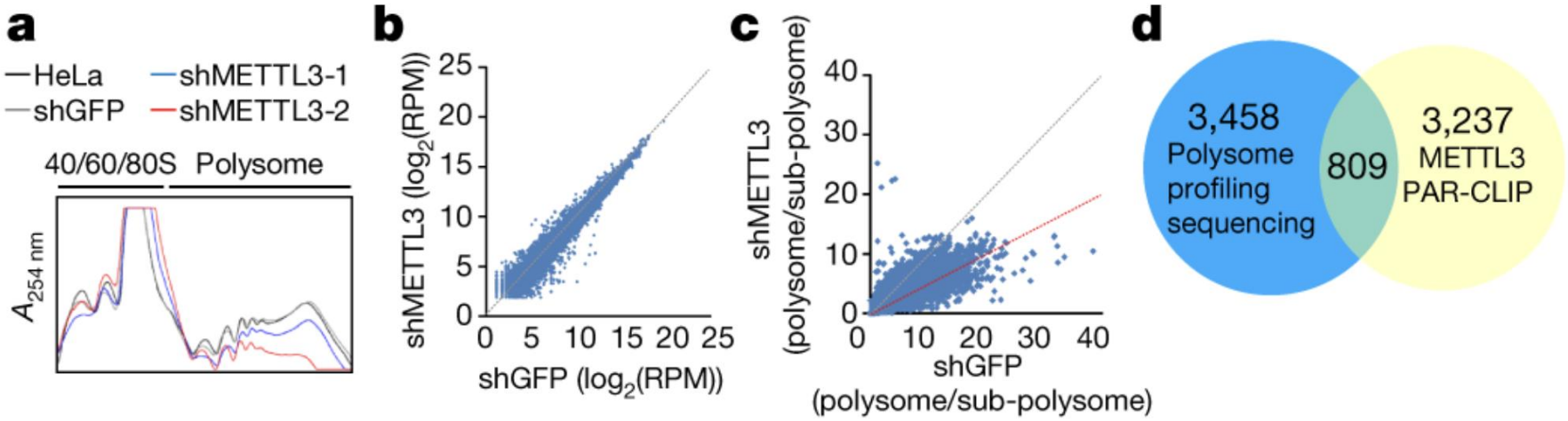


Lung cancer cell line

Sorci et al., 2018. Cell Death & Dis.

Lin et al., 2016. Mol Cell

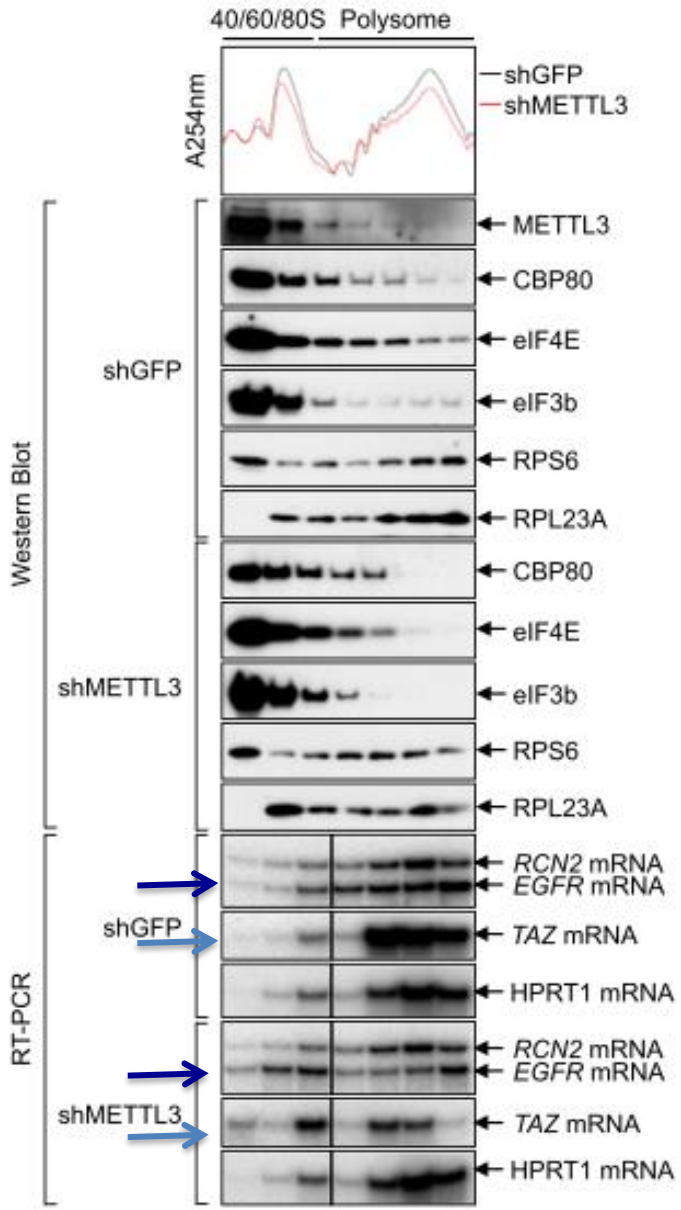
METTL3 promotes translation of a large subset of mRNAs



METTL3 depletion caused an increase in the 80S ribosome peak and a corresponding reduction of polyribosome peak (**A**). METTL3 depletion had a negligible effect on steady-state mRNA abundance whereas translation efficiency of a large subset (4,267) of mRNAs was reduced by at least twofold in METTL3-depleted cells (**B** and **C**). Comparison of these genes with previously reported METTL3 photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) data identified 809 mRNAs that are both bound and translationally regulated by METTL3 (**D**).

Choe et al., 2018. Nature

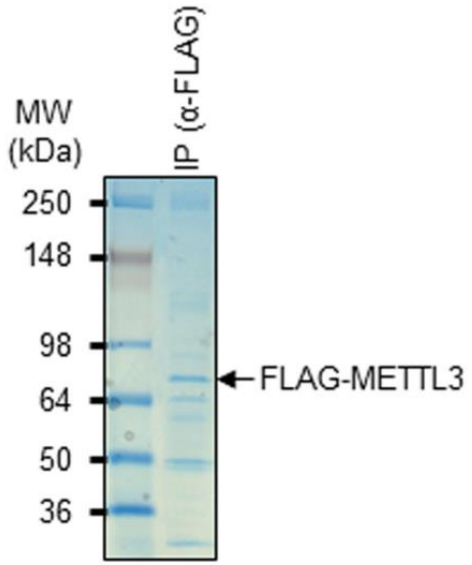
METTL3 promotes translation of a large subset of mRNAs



Lin et al., 2016. Mol Cell

METTL3 enhances translation of target mRNAs by interacting with eIF3h

A large-scale purification and mass spectroscopy characterization of complexes containing Flag–METTL3 identified numerous translation factors. Gene Ontology analysis of the METTL3-interacting proteins identified ‘mRNA metabolic processes’, ‘RNA processing’ and ‘Translation’ as the most significantly enriched categories. Between the, they identifyed the translation factor eIF3.

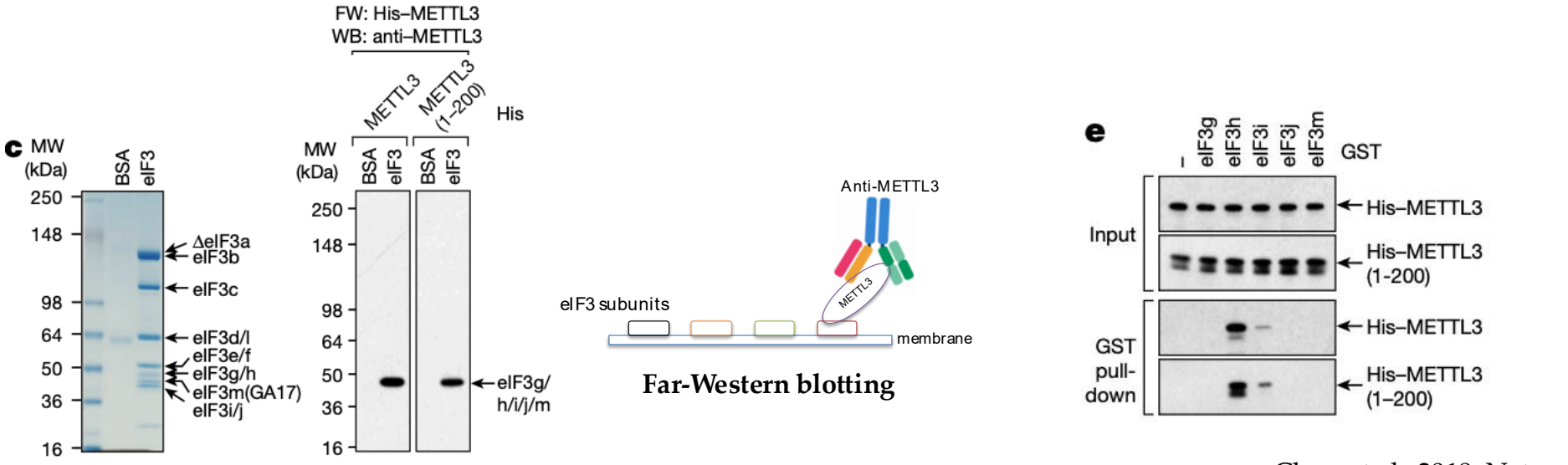


Translation involving factors	
Name	No. of Proteins
Ribosomal proteins	87
Eukaryotic translation initiation factors	21
Eukaryotic translation elongation factors	5
Nuclear cap-binding protein, CBP80	1

Choe et al., 2018. Nature

METTL3 enhances translation of target mRNAs by interacting with eIF3h

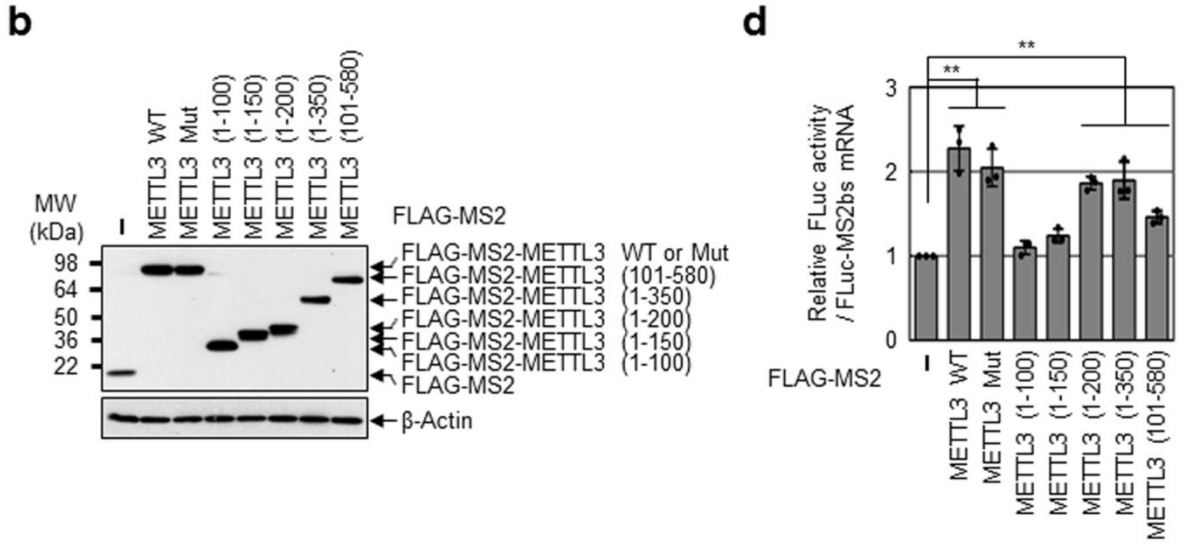
To test whether METTL3 interacts with any of the 13 subunit(s) of eIF3, recombinant METTL3 and 1–200 a.a. (a fragment of METTL3 that promote translation) were used for far-western blotting with a purified human eIF3 complex (**c**). METTL3 and 1–200 a.a. both specifically bound to a single band that probably corresponds to eIF3g, eIF3h, eIF3i, eIF3j or eIF3m. To further confirm this interaction and to define the particular subunit(s) that interacts with METTL3, they individually expressed and purified the GST-tagged eIF3 subunits from bacteria and tested them for binding to His–METTL3 by GST-pull-down (**e**). METTL3 (and 1–200 a.a.) were found to specifically interact with eIF3h.



Choe et al., 2018. Nature

METTL3 promotes translation when tethered to a reporter mRNAs

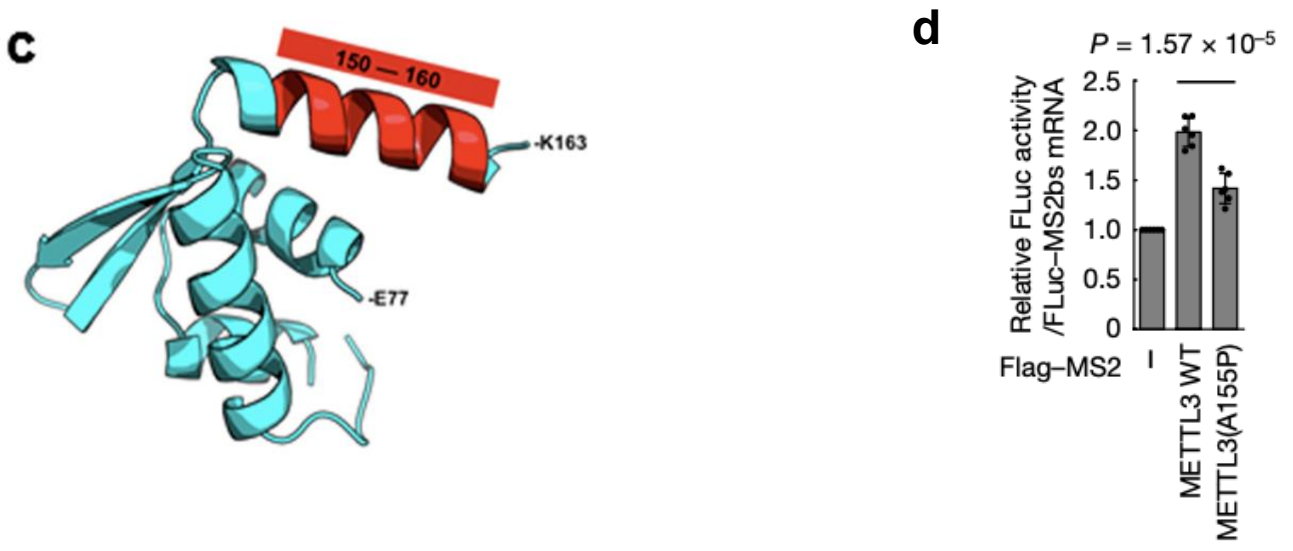
Tethering assay to measure translation efficiency of reporter mRNAs (b, d). 1–200 a.a. fragment can promote translation in tethering experiments whereas 1–150 a.a. does not. They reasoned that a region between 150–200 a.a. must be important for the physical and functional METTL3–eIF3h.



Choe et al., 2018. Nature

Identification of a conserved alanine residue in the N-terminal region of METTL3 required for its interaction with eIF3h

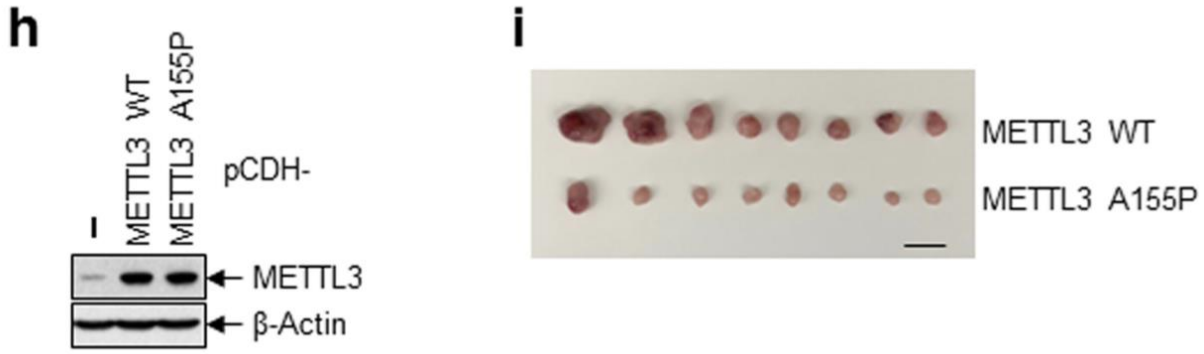
Secondary structure predictions identified a putative alpha helix (150–161 a.a.) that is highly conserved in mammals. Moreover, 3D modelling identified a putative structured module (**c**). They therefore generated a mutant version of METTL3 with a single amino acid substitution of a highly conserved alanine (A155P) to disrupt this putative helical structure. Co-immunoprecipitation confirmed substantially impaired interaction of METTL3(A155P) with translation initiation factors (not shown). Tethering experiments demonstrated that the METTL3(A155P) mutant is strongly impaired in promoting mRNA translation (**d**).



Choe et al., 2018. Nature

METTL3 promotes tumorigenesis

The oncogenic function of METTL3 was studied in the mouse xenografts. NIH-3T3 cells with ectopic expression of wild-type METTL3, METTL3(A155P) or the empty-vector control were injected into nude mice to determine their in vivo tumorigenic capacities. Overexpression of wild-type METTL3 promoted in vivo tumour growth, whereas METTL3(A155P) showed an impaired ability to promote tumour growth.

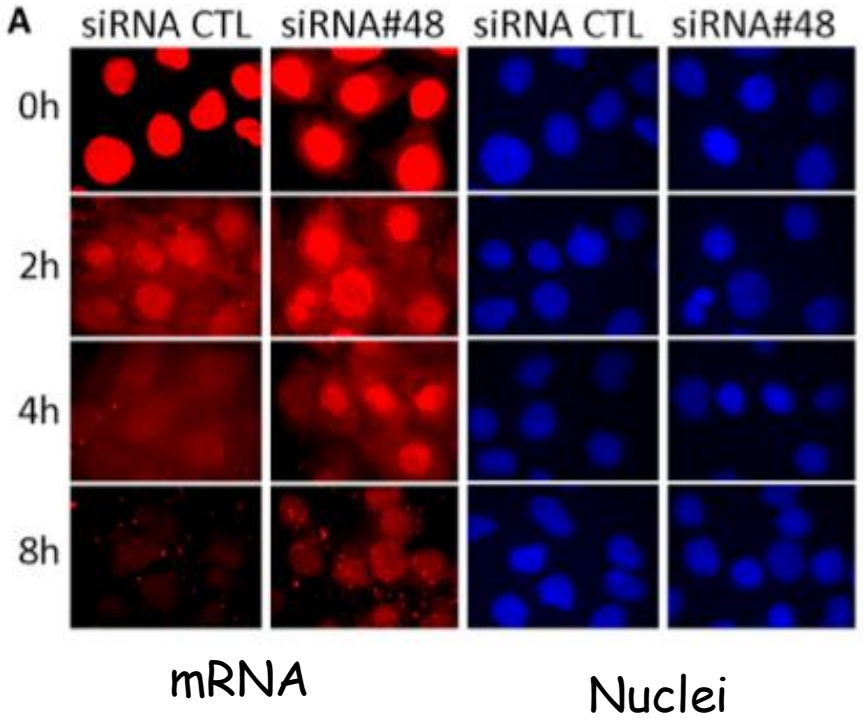


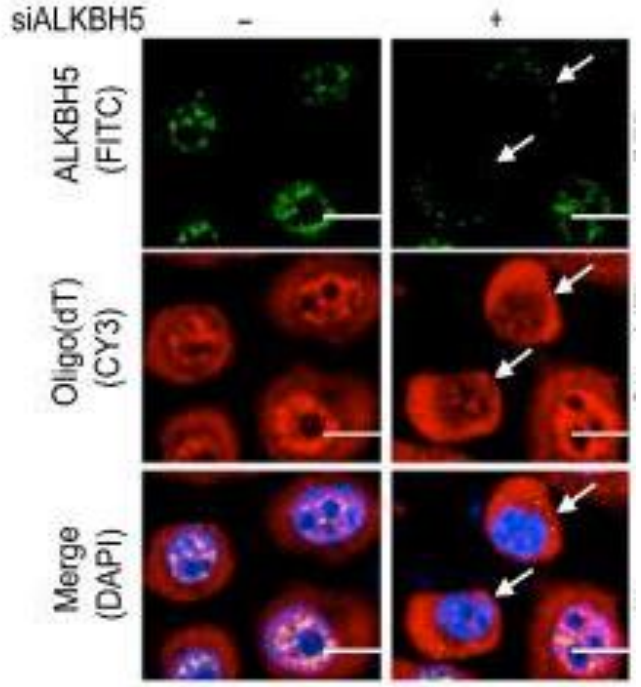
Choe et al., 2018. Nature

m6A promotes mRNA export

Inhibition of m^{6}A-RNA Methylation Delays mRNA Export

Mettl3 knockdown by siRNA(#48) leads to overall RNA nuclear retention, whereas depletion of ALKBH5 enhanced mRNA export to the cytoplasm. Mechanistic details are yet to be reported, but it is conceivable that nuclear readers have an active role in this process.

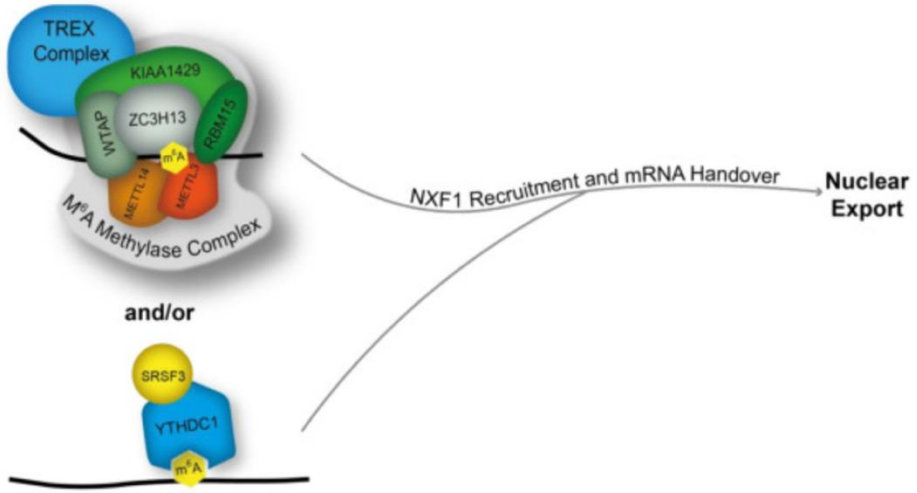




Zheng et al., 2013. Mol Cell; Fustin et al., 2015. Cell

The m^{6}A machinery and mRNA export

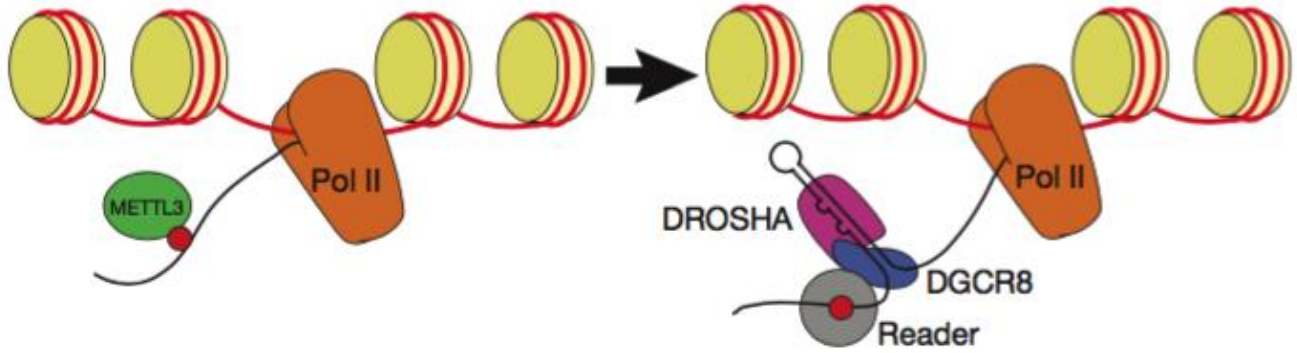
The MACOM complex interacts with the TREX complex aiding in the export of specific mRNAs. The reader YTHDC1 associates with SRSF3. Both pathways may work in unison to recruit the export receptor NXF1(TAP)/P15 to initiate nuclear export.



m^6A promotes pri-miRNA processing

METTL3 regulates miRNA biogenesis

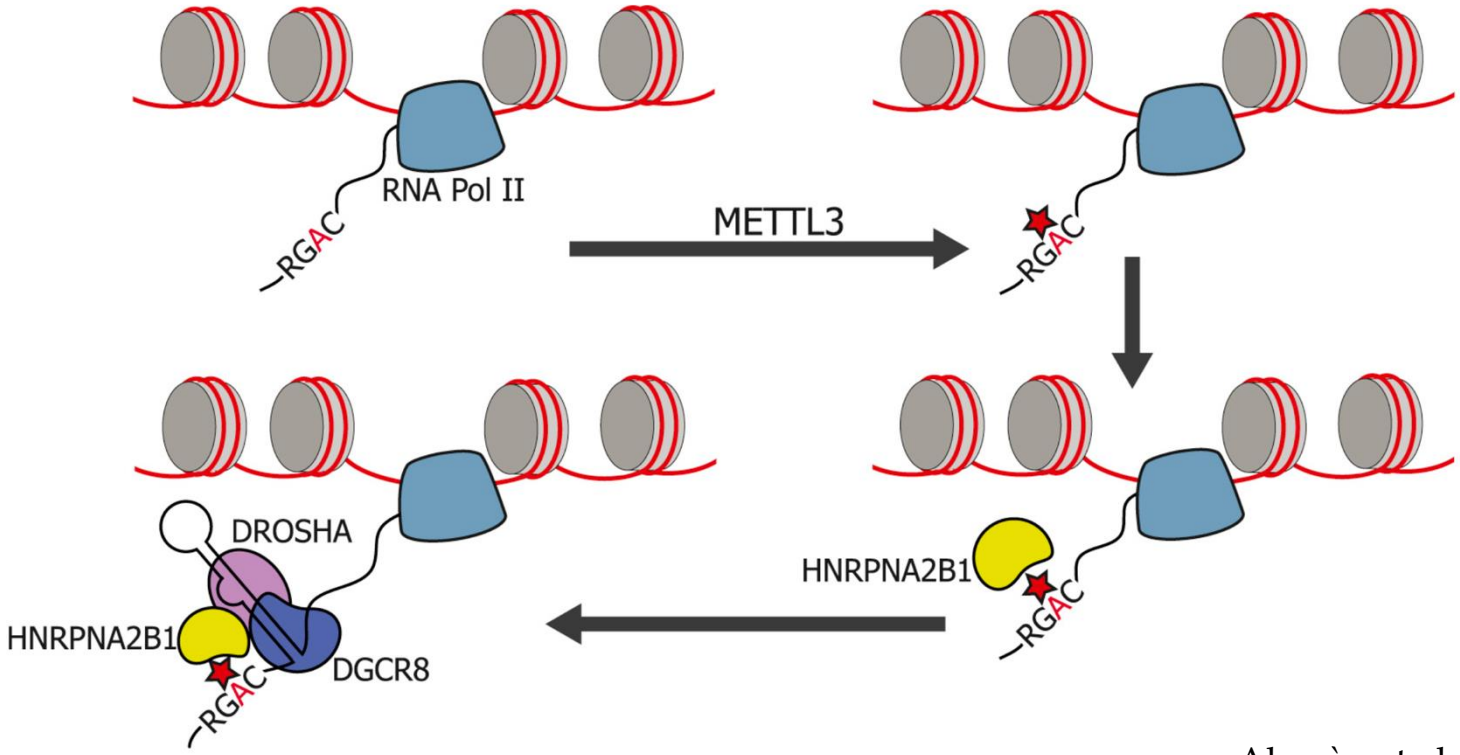
The m^6A modification facilitates the recognition of pri-miRNA sequences and marks an initiation event in miRNA biogenesis. The m^6A mark thus has an important role in the nucleus, allowing the microprocessor complex to recognize its specific substrates. Additionally, altered METTL3 expression in various human malignancies may contribute to the aberrant expression of miRNAs seen in cancer.



Alarcòn et al. 2015. Nature

HNRNPA2B1 is a nuclear reader of the m^{6}A methylation mark

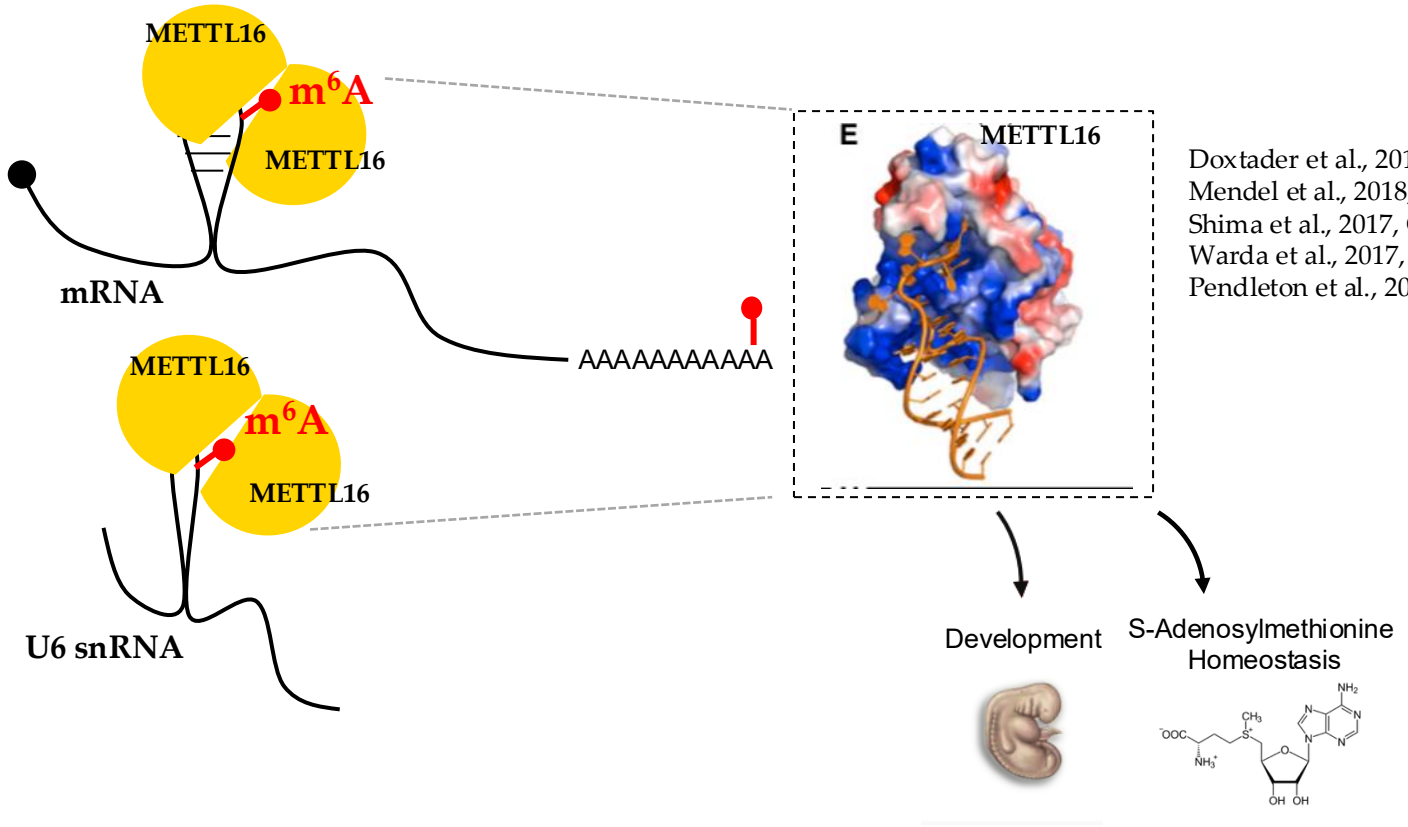
HNRNPA2B1 acts as a reader of the m^{6}A mark in the nucleus and mediates, in part, the effects of m^{6}A/METTL3 on microRNA processing. It is still unclear whether HNRNPA2B1 recognizes m^{6}A residues directly, like the YTH-domain proteins, or indirectly, like the HNRNPC protein.



Alarcòn et al. 2015. Cell

The m^{6}A Writer METTL16

Methyltransferase-like protein 16 (METTL16) is responsible for the modification of the U6 snRNA and specific mRNAs and lncRNAs containing the UACAGAGAA sequence within a specific stem-loop structure. Reader proteins cannot distinguish between METTL3- and METTL16- dependent m^{6}A modification.

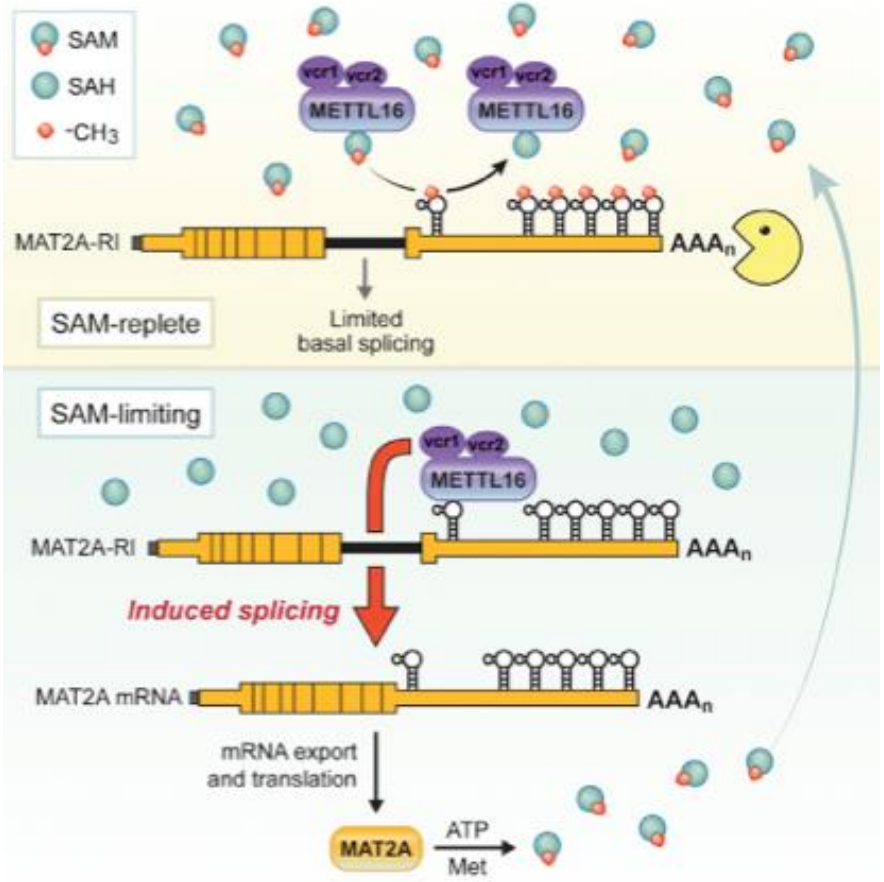


Doxtader et al., 2018, Molecular Cell
Mendel et al., 2018, Molecular Cell
Shima et al., 2017, Cell Reports
Warda et al., 2017, EMBO Reports
Pendleton et al., 2017, Cell

METTL16 Regulates S-adenosylmethionine (SAM) Synthetase Intron Retention

Maintenance of proper levels of SAM is critical for a wide variety of biological processes. METTL16 regulates expression of human MAT2A, which encodes the SAM synthetase. Upon SAM depletion by methionine starvation, cells induce MAT2A expression by enhanced splicing of a retained intron.

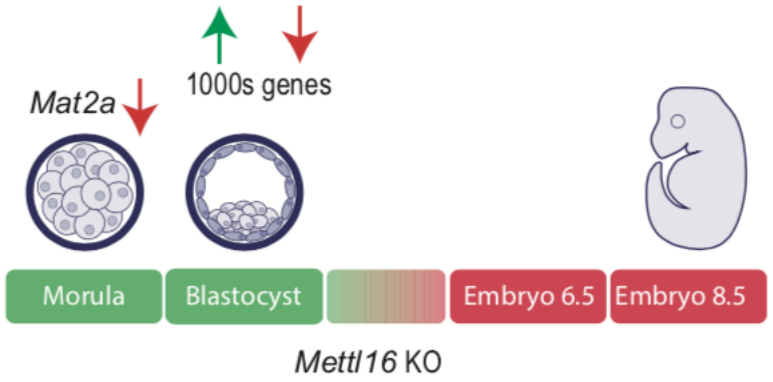
Induction requires METTL16 and its methylation substrate, a vertebrate conserved hairpin in the MAT2A 3'-UTR. Under SAM-limiting conditions, METTL16 occupancy increases due to inefficient enzymatic turn-over, which promotes MAT2A splicing.



Pendleton et al. 2017. Cell

METTL16 is Essential for Mouse Embryonic Development

The downregulation of the SAM synthetase Mat2a mRNA in Mettl16 KO E2.5 morula is potentially a trigger for subsequent massive alteration in gene expression in the E3.5 blastocysts. Such mutant embryos fail to proceed further in development



Mendel et al., 2018. Mol Cell