N6-methyladenosine modification of mRNA and its role in gene expression

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Objectives

Overview of adenosine N6 methylation (m^6A) in mammalian mRNA by describing the m^6A modifying enzymes, interactors, and its role in gene expression. Also, explaining human cancers caused by incorrect modification control, focusing on acute myeloid leukemia (AML).

Introduction

N6-methyladenosine (m^6A) is the first example of reversible RNA methylation and the most common internal (non-cap) epigenetic modification of mRNA in all higher eukaryotes. It regulates gene expression at post-transcriptional stage in different ways; modifying mRNA secondary structure to enable interaction with regulatory proteins, modifying splicing, intracellular distribution, cytoplasmic degradation, and determining its translation potential.
As a result, m^6A RNA methylome regulates cell development and signaling, and improper regulation may result in various illnesses such as cancer.

Protein factors involved in the m^6A modification system

Mammals have 3-5 m^6A sites per mRNA and the consensus motif DRACH is the most prevalent (D = A/G/U; R = G/A; H = A/C/U, where **A** is converted to m^6A).

m^6A methylation is abundant around the stop codon, CDS, and mRNAs' 3' UTR, and it is more prevalent in introns of pre-mRNA than mature mRNA.

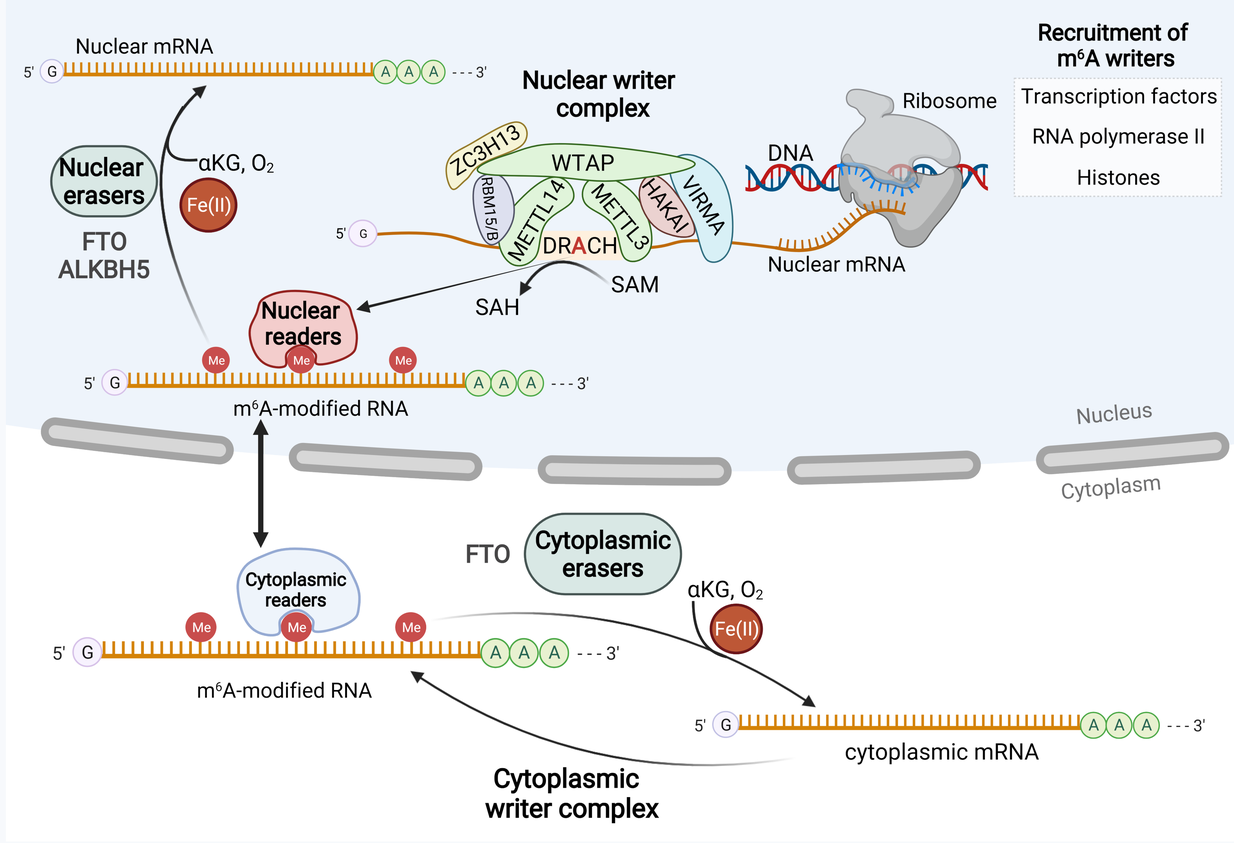

Figure 1. Cellular pathways for m^6A-based RNA modification.

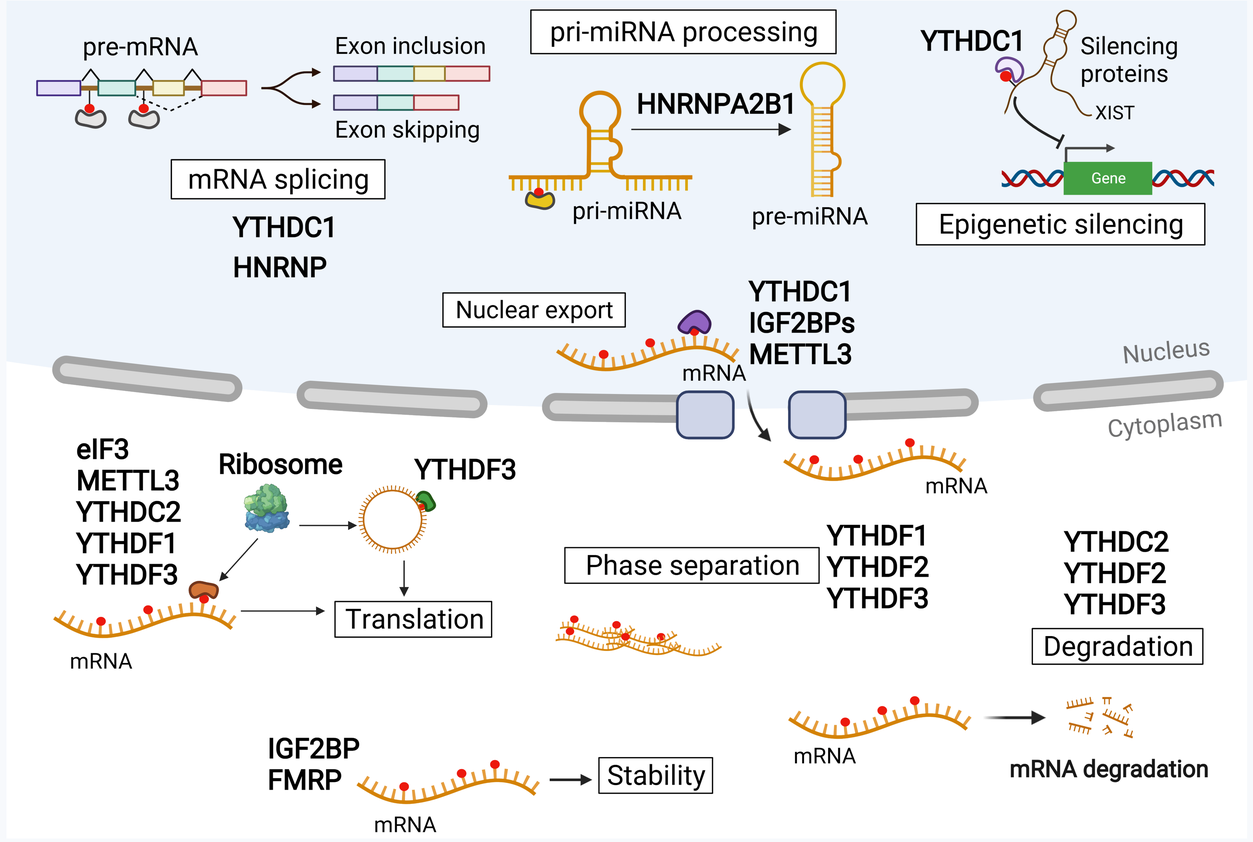

Figure 2. Representation of the different m^6A-binding proteins and their biological functions.

Writers	**METTL3**	Methylates mRNA
	METTL14	Gives conformational stability to METTL3 and binds RNA
	WTAP	Recruits METTL3-14 to nuclear speckles
	VIRMA	Recruits WMM complex in the 3'UTR near the stop codon
	RBM15 RBM15B	Binds to mRNA uridylate-rich sequences and interact with WTAP
	ZC3H13	Binds RBM15/15B and connects them to WTAP
	HAKAI	E3 ubiquitin ligase that interacts with WTAP

Table 1. Summary of m^6A writers functions.

Erasers	**FTO**	Oxidatively demethylate m^6A in mRNA
	ALKBH5	Oxidatively demethylate m^6A in mRNA

Table 2. Summary of m^6A erasers functions.

Other potential m^6A readers	**Ribosomes**	Cytoplasm	Influence stability, and translation
	eIF3	Cytoplasm	Promotes translation
	IGF2BPs	Nucleus, Cytoplasm	Maintain stability, and nuclear-cytosol transport
	FMRP	Nucleus, Cytoplasm	Maintain stability
	METTL3	Nucleus, Cytoplasm	Promotes translation, and nuclear-cytosol transport

Table 3. Summary of m^6A potential readers functions.

YTH domain readers	**YTHDC1**	Nucleus	Transport, splicing, and epigenetic silencing
	YTHDC2	Nucleus, Cytoplasm	Enhances mRNA degradation, and promotes translation
	YTHDF1	Cytoplasm	Promotes translation
	YTHDF2	Cytoplasm	Enhances mRNA degradation
	YTHDF3	Cytoplasm	Promotes translation, and enhances mRNA degradation
Non-YTH domain readers	**HNRNP**	Nucleus	Splicing

Table 4. Summary of m^6A readers functions.

Cancer and m^6A

Cancer type	Enzyme	Target RNA	Effect of enzyme on target RNA	Role of m^6A in cancer
Breast cancer	**ALKBH5**	NANOG	Stabilization	↑ Tumor initiation capacity and metastasis
Glioma	**ALKBH5**	FOXM1	Expression	↑ Tumorigenesis
Lung cancer	**METTL3**	Cancer-related genes	Translation	↑ Cell survival, proliferation, and invasion
AML	**METTL3**	c-MYC, BCL2, PTEN	Translation	↑ Cell proliferation
		SP1, SP2	Translation	↓ Cell differentiation and apoptosis

Table 5. Roles of m^6A enzymes in cancer.

METTL3 and Acute Myeloid Leukemia

STM2457 is the first RNA methyltransferase inhibitor proven to have *in vivo* efficacy and therapeutic effectiveness against cancer. It is a SAM competitive inhibitor that targets key AML stem cell populations and reverses the AML phenotype, preventing or slowing cancer progression.

Figure 3. The METTL3 function in AML is shown graphically. A. METTL3 is recruited by CEBPZ on specific promoter regions, resulting in co-transcriptional m6A methylation of different mRNAs. B. Enhanced m6A methylation enhances mRNA translation and increases protein levels. C. Representation of MEETL3-METTL14 interaction and the inhibitor STM2457, obtained from PDB (ID 7O21).

Conclusions

m^6A regulation is tissue and development-specific, and it modifies the final protein expression level, determining the phenotype of eukaryotic cells.

Understanding the effects on cell metabolism of pathological unregulated m^6A mechanism would allow the development of novel molecular and cellular drugs to treat them.

Although m^6A biology has advanced significantly in recent years, numerous challenges require additional research, such as: which mechanism controls methylation, determining the similarity between the cytosol and the nucleus writer complex and understanding HNRNP's, YTHDC2, and eIF3 mechanism of action. Finally, it is also essential to validate the other identified m^6A demethylases inhibitors *in vivo*.

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