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RNA Modifications and the Link to Human Disease

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Abstract

Ribonucleic acid (RNA) is involved in translation and transcription, which are the mechanisms in which cells express genes.[1] The three classes of RNA discussed are transfer RNA (tRNA), messenger RNA (mRNA), and ribosomal RNA (rRNA). mRNA is the transcript encoded from DNA, rRNA is associated with ribosomes, and tRNA is associated with amino acids and is used to read mRNA transcripts to make proteins.[2] Interestingly, the function of tRNA, rRNA, and mRNA can be significantly altered by chemical modifications at the co-transcriptional and post-transcriptional levels, and there are over 171 of these modifications identified thus far.[3,4] Several of these modifications are linked to diseases such as cancer, diabetes, and neurological disorders. In this review, we will introduce a few RNA modifications of biological functions and how dysregulation of these RNA modifications linking to human disease.

Keywords

RNA; Modifications; Disease

N^1-methyladenosine in tRNA

N^1-methyladenosine (m^1A) was first discovered occurring at positions 9, 14, and 58 in tRNA and is important for the tRNA 3-dimensional structure.[5,6] m^1A is also important for structure stabilization of tRNA due to its positive electrostatic charge and also assists in correct folding of tRNA.[6,7] Recently evidence suggests that m^1A in tRNA is under reversible regulation that is installed by a tRNA m^1A methyltransferase (MTase) and is erased by AlkB Homologue 1 (ALKBH1) and AlkB Homologue 3 (ALKBH3).[5] Studies have shown ALKBH1 catalyzed demethylation of tRNA can result in a reduction in translation initiation as well as a decrease in use of the demethylated tRNAs in protein synthesis.[8]

Methylated Guanosines in tRNA

One of the most conserved modifications in tRNA is 7-methylguanosine (m^7G), found at position 46 in the variable loop of tRNA, and is installed by tRNA m^7G46 methyltransferase.[9,10] This modification forms a tertiary base pair with C13 and G22 and stabilizes the structure of the tRNA.[11]

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An additional guanosine modification in tRNA is N2-methylguanosine (m^2G).[12] m^2G forms stable interactions with cytosine as well as uridine.[13,14] The modification is isoenergetic with guanosine in the Watson-Crick G-C base pair at RNA duplexes with cytosine, and does not play a significant role in secondary structure stability.[12] It is located in the dihydrouridine loop (position 10) of tRNA, the CCA stem of $tRNA^{Met}$ (position 6), as well as position 26 in $tRNA^{Lys}$.[13]

N^2, N^2-dimethylguanosine ($m^2{}_2G$) and N^2, N^2, 2'O-trimethylguanosine ($m^2{}_2G_m$) are highly conserved modifications at positions 10 and 26.[15] At position 26, this modification is installed by tRNA($m^2{}_2G$) dimethyltransferase, and at position 10, this modification is installed by the Trm-m22G10 enzyme in archaea.[16,17] $m^2{}_2G$ is located in the bend between the dihydro-uridine stem and the anticodon stem in tRNA, and prevents the folding of tRNA into unusual structures, often adopted by mitochondrial tRNA (mtRNAs).[18] The double methylation of $m^2{}_2G$ allows the guanosine to base pair with adenosine, uridine, or guanosine at position 44, and prevents the base from pairing with cytosine.[18]

Methylation at position N-1 of guanosine occurs at position 37 and position 9 in tRNA.[19] TRMT10A methylates tRNA guanosines at position 9 with S-adenosylmethionine as a methyl donor.[19,20] At position 37, the methyl group is added to guanosine by TrmD from AdoMet.[21] Without this modification at position 37, there can be effects on translation efficiency due to frameshift.[21]

Pseudouridine om tRNA

Pseudouridine (Ψ) is that most abundant modified type of nucleoside across all species of RNA.[22] Ψ is an isomer of uridine, where rather than the usual uracil attached via a nitrogen-carbon glyosidic bond, uracil is attached via a carbon-carbon bond. This modification alters the secondary structure of the RNA by increasing base stacking, making more ridged sugar-phosphate backbones, and improving the base pairing.[23]

5-methylcytosine (m^5C) in tRNA

5-methylcytosine is found at position 34, 38, 40, 48, 49, and 50 in tRNA.[24] m^5C at positions 48 and position 49 are located in the TΨC loop of tRNA, and contributes to the stability of tRNA as well as protein synthesis.[24] This modification is established via Dnmt2 (for cytosine 38) and NSun2 (for cytosine 34, 40, 48, 49, 50).[24] m^5C has been found to cooperate with other modifications on tRNA to protect the tRNA from degradation and is also linked to stress signaling.[24]

Dihydrouridine in tRNA

The dihydrouridine modification is catalyzed by dihydrouridine synthases and is found in the D-loop of tRNA.[25] Dihydrouridine synthases catalyze the reduction of the carbon-carbon double bond at positions 5 and 6 on the uridine base. It is thought that this base modification supports structural flexibility by destabilizing C3'-endoribose conformation and stabilizing the C2'-endoribose form.[26] This conformation allows flexibility and dynamic motion in areas of tertiary interactions and loop formation in tRNA.[26] Dihydrouridine causes the

formation of a stable hairpin structure in the tRNA and is important in the folding and stability of tRNA.[27]

tRNA Modifications Link to Human Disease

It has been found that nonsense mutations in TRMT10A, resulting in loss of function of the methyltransferase, are linked with familial syndromes including intellectual disabilities, microcephaly, and faults in glucose metabolism.[20,28] Mutations in NSun2, a m^5C tRNA methyltransferase, have been found to be associated with intellectual disabilities with a mutation that causes NSun2 to not localize to the nucleus.[29,30] NSun2 has also been linked to Dubowitz syndrome, an autosomal recessive disorder that is characterized by small structure, mild microcephaly, mental retardation, eczema, and distinct facial expression.[31] Patients that have this disorder lack NSun2 specific methylation at nucleotides 47 and 48 on tRNA.[31] The loss of m^5C in tRNA, results in endonucleolytic cleavage by angiogenin and causes the accumulation of 5'tRNA fragments.[28] Additionally, mutations to genes that are associated with the m^7G tRNA methyltransferase complex can lead to microcephalic primordial dwarfism.[10]

Myc, a known proto-oncogene, heterodimerizes with another protein, Max.[32] Together, these Myc/Max heterodimers recruit histone acetyltransferases to activate gene transcription.[33] The overexpression of Myc is linked to cancer, and has been found to activate the RNA methyltransferase NSun2, leading to NSun2 overexpression.[34] An epigenetic cancer therapy, azacytidine, is found to inhibit the m^5C methyltransferase, DNMT2, that catalyzes the m^5C modification on $tRNA^{Asp}$.[35] This has led to the idea that DNMT2 is associated with tumorigenesis.[35] It is suggested that since DNMT2 methylation leads to stabilization of tRNA, the de-methylated tRNAs may be misfolded and impact the rate of protein synthesis in cancer.[35,36]

2'-O-methylation in rRNA

2'-O-methylation (2'-O-me) in rRNA replaces the normal 2'-OH in rRNA with 2'-O-me, and is added postranscriptionally.[37] This modification protects the rRNA from hydrolysis, additionally, 2'-O-me also stabilizes the rRNA into a C3'-endo conformation.[38,37] Additionally, this modification restricts the rRNA strand conformation and flexibility as a result of phosphate restriction.[37] Ribosome structure and function are impacted by 2'-O-me, and this modification is located in more flexible regions of rRNA.[39,40] 2'-O-me plays a role in protein synthesis by regulating translational activity of the ribosome.[37]

Pseudouridine (Ψ) in rRNA

Pseudouridine (Ψ) is found in both the small and large subunit rRNAs, and is the most common single modified nucleoside in rRNA.[22,41] The *N*-1 positions in Ψ are thought to catalyze the transfer of aminoacyl residues from the peptidyl tRNA to aminoacyl tRNA (P site to A site in ribosome).[42] In the large subunit of the ribosome, Ψ is clustered in areas that are connected to the peptidyl transferase center.[41] There is no known clustering of Ψ in the small subunit of the ribosome.[41]

N^1-methyladenosine (m^1A) in rRNA

In addition to tRNA and mRNA, m^1A has been found in rRNA.[5] m^1A is catalyzed by the human nucleolar protein, nucleomethylin (NML) in 28S rRNAs.[43] Rrp8, a NML yeast homolog, methylates m^1A at position 645 of 25S rRNA. This modification impacts the structure of the large subunit as well as translation and ribosome synthesis.[44] In yeast studies, the loss of m^1A modification resulted in anisomycin and peroxide sensitivity as well as defects in ribosomal subunit joining in cells.[45] Additionally, in bacteria, m^1A at position 1408 in 16S rRNA confers aminoglycoside resistance.[46]

N^6-methyladenosine (m^6A) in mRNA

N^6-methyladenosine (m^6A) is the most prevalent modification on messenger RNA (mRNA). This methylation at position $N6$ on mRNA is installed by METTL3 and METTL14, cooperatively.[47] Conversely, this modification is erased by Fat mass and obesity-associated protein (FTO) and/or AlkB Homologue 5 (ALKBH5).[48,49] The m^6A modification is present 3 to 5 times per mRNA and is one of the most common modifications on RNA.[50,51] m^6A can be in *syn* and *anti*-conformations depending on the structure of the RNA.[52] The *syn* conformation is energetically favorable in single stranded RNA and the *anti*-conformation is adopted in the double stranded RNA in order to accommodate Watson-Crick base pairing.[53] The methyl group installed onto the 6^{th} position of RNA is positioned in the major groove of the RNA helix, which leads to the destabilization of the transcript and is a means for the mRNA to switch structurally between double stranded RNA and single stranded RNA.[53] The presence of m^6A changes the way that reader proteins recognize the mRNA transcripts, as well as the transcript's fate.[54]

m^6A is involved in maturation of pre-mRNA into mRNA as well as nuclear export of mRNA.[55] When in the cytosol, this modification can enhance translation efficiency of the mRNA through identification by the reader protein, YTH-domain family member 1 (YTHDF1).[56] Additionally, the IGF2BP class of m^6A reader proteins can promote translation and increase the stability and storage of mRNA targets.[56]

m^6A can also contribute to destabilization and degradation of mRNA through targeting by YTH-domain family member 2 (YTHDF2), another reader of mRNA m^6A.[57] YTHDF2 selectively binds mRNA with m^6A methylation and decays the mRNA, based on this modification. YTHDF2 may also play a role in stability and/or translation since it targets the stop codon region, the 3' untranslated region, and the coding region.[57] Furthermore, the YTHDF2 gene has been thought to be associated with human longevity.[58]

m^6A has been linked to many diseases including cancer, type 2 diabetes, obesity, leukemia, infertility, neuropsychiatric behaviors and depressive disorders.[59,60,61] These diseases have been found to be associated with mutations in FTO, ALKBH2, METTL14, and METTL3.[62] m^6A associated proteins and their link to disease will be discussed in detail.

FTO is known to oxidatively demethylate N^6-methyladenosine residues in mRNA.[48] This demethylase has been found to be associated with obesity and energy homeostasis.[48] Single nucleotide polymorphisms in the FTO gene affects body mass index and predisposes people

to type 2 diabetes.[63] In addition to an association with obesity, FTO is also associated with DNA damage repair and cancer.[64] FTO accumulates at DNA damage sites after ultraviolet irradiation and induces m^{6}A demethylation, regulating mRNA m^{6}A along METTL3.[64] In relation to cancer, FTO is also highly expressed in acute myeloid leukemia and enhances the leukemia oncogenic-mediated cell transformation.[65] This is done through regulating expression of targets by reducing the levels of m^{6}A in mRNA.[65]

METTL3 and METTL14 are associated with promoting tumorigenesis in many cancers, however these methylases have also shown tumor suppressive functions in glioblastoma and hepatocellular carcinoma.[66] The knockdown of METTL3 or METTL14 results in a decrease in m^{6}A modification, and in glioblastoma stem cells, results in the upregulation and expression of oncogenes that downregulate tumor suppressor expression.[67] This results in enhanced glioblastoma stem cell growth, tumorigenesis, and self-renewal.[67] METTL3 upregulation in glioblastoma tumor tissue has also been associated with poor patient survival according to recent studies.[68]

In patients with acute myeloid leukemia (AML), there is an increase in METTL3 as well as an increase in protein expression.[69] METTL3 catalyzes m^{6}A formation, and in AML, this promotes the translation of c-MYC, BCL2, and PTEN, which are proteins that are important for proliferation, survival, and differentiation.[69] When AML cells are depleted of METTL3, the translation of these mRNA is repressed, which results in increase AKT activation and subsequent increase in cell differentiation and apoptosis.[69]

It has been shown that METTL3 is up-regulated in hepatocellular carcinoma tissues and this is associated with poor patient prognosis.[70] Knockdown of METTL3 results in reduced cell proliferation, migration, and colony formation *in vitro*, and restrained hepatocellular carcinoma growth and metastasis *in vitro* and *in vivo*.[70,69] Conversely, overexpression of METTL3 results in growth, metastasis, and tumor growth of hepatocellular carcinoma, *in vivo*.[69] In hepatocellular carcinoma, METTL3 methylates SOCS2, a known tumor suppressor, and decreases SOCS2 stability via a YTHDF2-dependent pathway.[70] It is also shown that there is a decrease in m^{6}A in hepatocellular carcinoma tissue, and METTL14 is associated with this aberrant m^{6}A modification.[71] Consequently, METTL14 knockdown leads to metastasis of hepatocellular carcinoma and overexpression of METTL14 results in less invasiveness and metastasis of hepatocellular carcinoma.[69] The contribution of METTL3 and METTL14 to this cancer is a bizarre methylation tug-of-war.

N^1-methyladenosine (m^{1}A) in mRNA

N^1-methyladenosine (m^{1}A) is influential in tRNA and rRNA both structurally and functionally and it has also been identified as an important modification in mRNA.[72] This modification is installed by S-adenosylmethionine (SAM) and is removed by ALKBH3.[72] m^{1}A gives the adenosine a positive charge, which can affect the structure as well as protein-RNA interactions.[72] m^{1}A is also believed to promote translation initiation.[72]

ALKBH3 is found to be highly expressed in human prostate cancer and non-small cell lung cancer.[73,74] Additionally, this demethylase functions in angiogenesis and apoptotic

resistance, and proliferation in pancreatic cancers.[75] Silencing of ALKBH3 expression induces apoptosis, suppresses cell proliferation, and inhibits angiogenesis in pancreatic cancer.[75] Conversely, overexpression of ALKBH3 increases anchorage-independent growth and invasiveness in pancreatic cancer.[75]

5-methlcytosine (m^5C) in mRNA

The 5-methylcytosine (m^5C) modification is installed by Nsun2 and is thought to be erased by TET enzymes.[76] The m^5C methylation and demethylation pattern suggests that the mRNA could be involved in many biological pathways regulating several functions of an organism.[76] For instance, the m^5C modification is found to play a role in nuclear export of mRNA transcripts.[76] Furthermore, this modification is thought to affect translation efficiency as well as have an effect on codon specificity.[77]

Pseudouridine in mRNA

The Pseudouridine (Ψ) modification is found more frequently in tRNA and rRNA, however it is also found in mRNA. This modification is linked to cell stress, as well as growth under different environmental cues.[78,23] Ψ plays a role in stabilizing RNA structure and could be involved in processes such as enhancing translation initiation efficiency, ribosome pausing, RNA localization, as well as RNA interference.[79]

Diseases associated with mutations in Pseudouridine synthases include mitochondrial myopathy and sideroblastic anemia (MLASA), dyskeratosis congenita, and lung cancer.[79] MLASA is a autosomal recessive oxidative phosphorylation disorder expressed bone marrow and skeletal muscle that is caused when there is a missense mutation in Pseudouridine Synthase 1 (PUS1).[80]

Conclusion

Dynamic regulation on RNA modifications significantly impact the features of the RNA molecules. In mRNA, modifications play a role in stability, translation, decay, location, structure, and export of the transcript. In rRNA, modifications are important for the stability, function, and translation activity of the ribosome. In tRNA, modifications are important for the translation efficiency, structure, flexibility of the tRNA. Additionally, modifications to tRNA can affect protein synthesis in the cell. RNA modifications may seem like a small part of the bigger picture; however, the impact of these modifications is vast. Misregulation of RNA modifications can lead to a plethora of illnesses, from cancer to neurodegenerative disease. However, there remains much to be done, as more quantitative sequencing technologies will be required to precisely map and understand the roles of modifications to different RNA species. Furthermore, the biological functions of site-specific modifications require a deeper understanding. Recent efforts have been made to integrate CRISPR technology to study specific sites of RNA modifications which hold the promise to advance the field.

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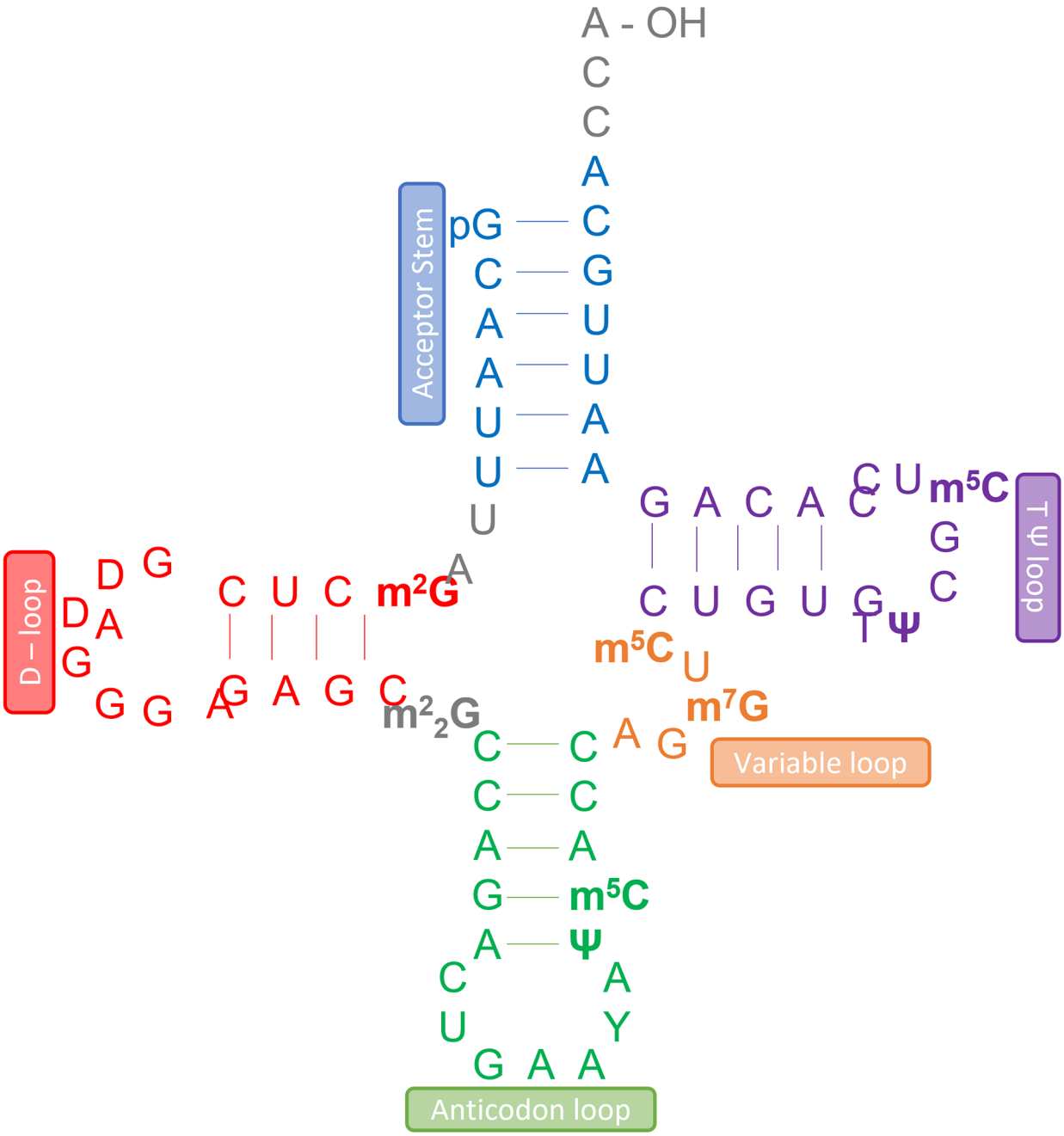

Figure 1:
Modifications made to tRNA.

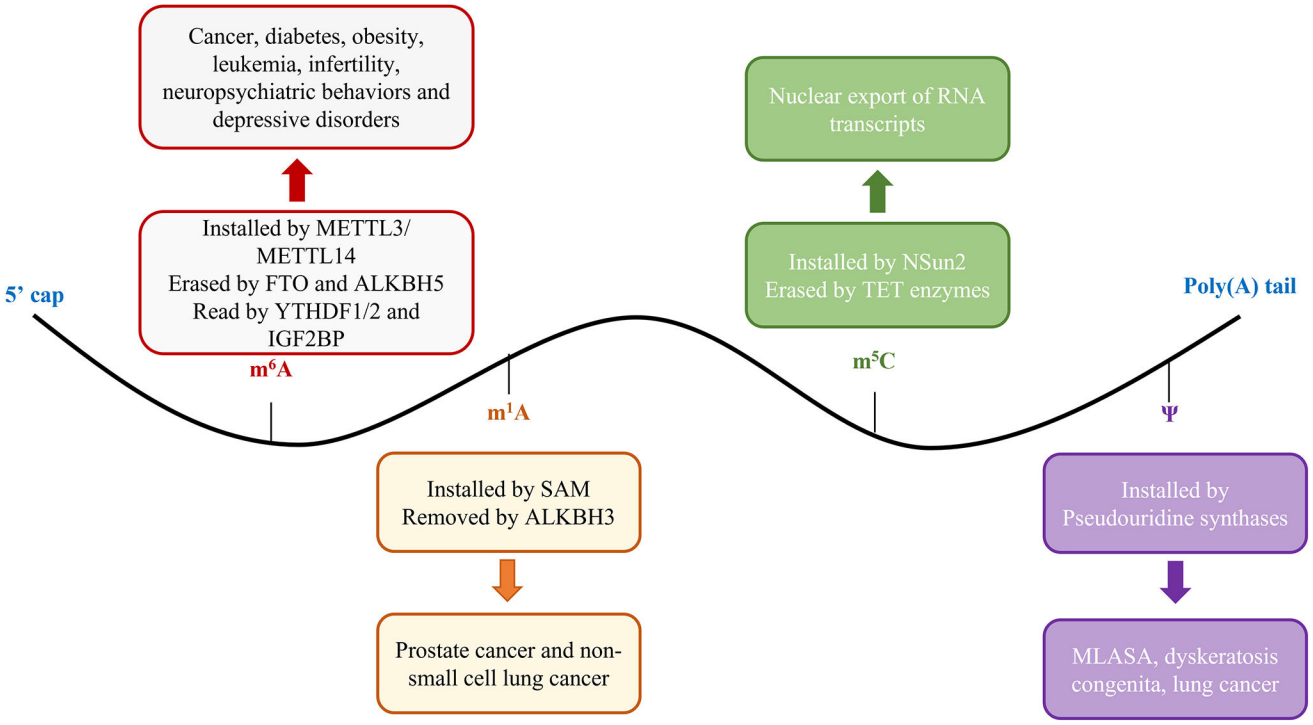

Figure 2:

Modifications made to mRNA. The readers, writers, and erasers associated with these modifications, and their link to different disease.