

Medicinal Chemistry and Chemical Biology Highlights

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Epitranscriptomics: RNA Biology Moves into the Focus of Medicinal Chemistry

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Keywords: Alkbh5 eraser enzyme · Epitranscriptomics · m⁶A · Mettl3/Mettl14 writer enzyme · RNA-modifying enzymes · YTH reader domain

Recent advances in the rapidly expanding field of RNA biology have resulted in several novel findings that will unlock new therapeutic opportunities for a broad spectrum of different diseases. Many new RNA classes with previously unknown roles have been identified, and RNA has matured from a formally elusive target class for small molecule-based drug discovery to a structurally well-defined entity that warrants structure-guided medicinal chemistry approaches. A steadily increasing number of high-resolution complex structures of low-molecular weight compounds bound to RNA molecules with defined tertiary structures highlight the druggability of those novel targets,^[1,2] and a number of biotech companies have been founded that aim to develop small molecules against RNA targets.^[3] Most prominent RNA targets for medicinal chemistry-centric approaches are riboswitches, micro RNA, RNA repeat elements, G-quadruplex structures, or the splicing machinery of pre-mRNA.^[1–3] Small molecule splicing modifiers that have advanced into clinical development bind to distinct transient pre-mRNA structures, thereby stabilizing the interaction with a small nuclear ribonucleic protein complex. This small molecule–RNA interaction reverts splicing deficits as found in spinal muscular atrophy by structural pre-organization of all the molecular components required for correct and efficient pre-mRNA splicing.^[4]

In addition to targeting RNA directly, there is a long list of newly discovered protein targets that operate on RNA. As of today, more than 100 distinct chemical modifications on coding and non-coding RNAs have been documented, offering medicinal chemistry a huge playground since those modifications are installed and removed post-transcriptionally by distinct enzymes and are recognized by specific receptor proteins.^[5] The enzymes involved utilize a broad range of different catalytic reactions, including methylations, deaminations, thiolations, glycosylations, and isomerizations.

In this context, the field of epitranscriptomics^[6] has gained enormous scientific interest over the last few years, focusing on the modulation of chemical modifications carried by mRNA (Fig. 1).

While chemical modifications on the DNA and histone proteins have established the conceptual basis for the field of epigenetics with several new reader, writer, and eraser proteins as putative drug targets that provide an additional layer of gene expression control (epi-genome: on top of the genome), mRNA molecules were long considered static entities that simply shuttle DNA-encoded information from the cell nucleus to the cytoplasmic ribosomes for translation. Today it is well-established that distinct chemical modifications of mRNA also provide an additional layer of gene-expression control, and it is known that

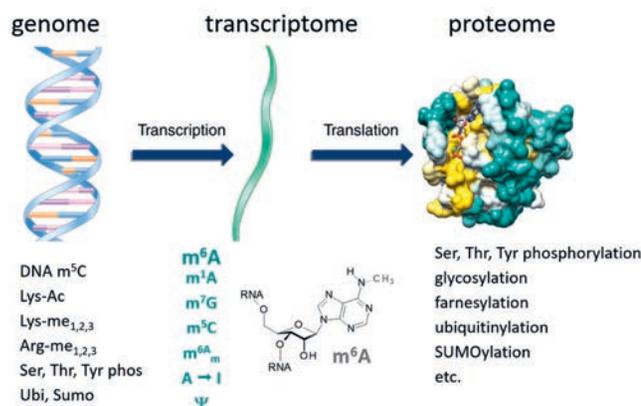


Fig. 1. Top: Genetic information stored within the genome is shuttled from nucleus to the cytosol *via* the transcriptome (*i.e.* the sum of all mRNAs) to produce the proteome by ribosomal protein synthesis, according to the central dogma of molecular biology. All three molecular entities, *i.e.* DNA:histone complexes, mRNA, and proteins carry an additional level of modifications that establish the epi-genome, epi-transcriptome, and epi-proteome. Bottom: A selection of distinct modifications – epigenetic on the left, epitranscriptomic in the middle with m⁶A as the most prominent modification, and posttranslational modifications on proteins on the right – are highlighted explicitly.

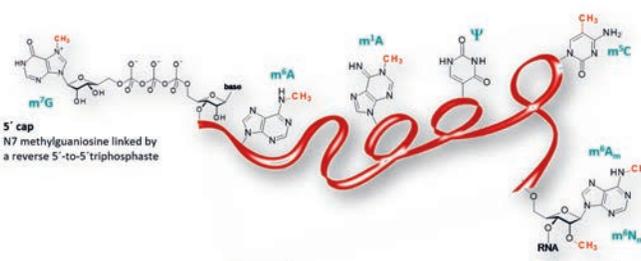


Fig. 2. Schematic presentation of a generic mRNA structure with most prominent chemical modifications shown. The mRNA oligonucleotide backbone is indicated with a red ribbon, modified nucleobases, nucleosides and nucleotides are exemplified.

the intricate network of readers, writers, and erasers responsible for recognizing, installing, and removing mRNA modifications confer a dynamic layer of complexity to what was once thought of as only a gene expression intermediate.^[7] The most prominent chemical modifications identified on mRNA molecules are shown in Fig. 2.

The most prevalent internal modification in eukaryotic mRNA is the addition of one methyl group to the exocyclic amino group of the adenosine nucleobase, called m⁶A (N⁶-methyladenosine). As of today, around 10.000 m⁶A sites have been identified in around 25% of human transcripts. m⁶A sites are enriched at the terminal 3'UTR region, near stop codons, and in long introns, and they frequently occur in clusters. The total count of m⁶A sites in mRNA can range from single one to several 100 modifications per transcript, and the quantitative ratios of distinct m⁶A sites vary from 5% to 80%. The dynamic equilibrium between N⁶-methylated and unmodified adenosine governs a variety of physiologically relevant processes such as nuclear splicing of pre-

mRNA, mRNA stability, nuclear export of mRNA, and mRNA translation. Pathophysiological changes have mechanistic impact in a variety of disease states, including hematological and other types of cancer and neuronal and immunological disorders (see for example refs [8–11] and references therein). The **m⁶A pathway** has been further elucidated over the last few years and several key enzymes and receptor proteins have been identified and validated as viable drug targets (Fig. 3).

Several drug discovery projects are currently pursued in biotech companies, targeting one or more of the epitranscriptomic target proteins presented in Fig. 3,^[12] and it can be assumed that pharmaceutical companies might prosecute similar projects. The heterodimeric methyltransferase **Mettl3/Mettl14** seems to gain most interest, at least three companies have active projects aiming at S-adenosyl-methionine (SAM) competitive compounds, among them are Accent Therapeutics near Boston, US,^[13] Storm Therapeutics in Cambridge UK,^[14] and Gotham Therapeutics in New York, US and in Munich, Germany.^[15] The therapeutic potential of Mettl3/Mettl14 inhibitors is aimed at different types of blood cancer, as well as treating a variety of solid tumors.^[11] The enzyme has a well-defined co-substrate binding pocket within the catalytically competent Mettl3 subunit, while Mettl14 seems to function as a scaffolding domain for Mettl3 and constitutes the mRNA binding site at the interface between both monomers (Fig. 4). Similar therapeutic effects to Mettl3/Mettl14 inhibition can be achieved by preventing the downstream recognition event of m⁶A-modified transcripts by blocking reader domains. Five different reader domains from the YTH family have been identified, one subgroup comprising YTHDC1 and YTHDC2, and three isoforms of the **YTHDF** family, notably the DF1, DF2, and DF3 proteins. The reader domains of the DF family use a highly conserved tryptophan cage comprised of three indole sidechains for selective recognition of m⁶A-containing mRNA (Fig. 4), while in the two DC proteins one tryptophan is exchanged against a leucine residue. To our knowledge, only Gotham Therapeutics reports on an active antagonist program targeting a YTHDF protein with small-molecule approaches. In certain disease states such as glioblastoma m⁶A levels are found to be too low, thereby

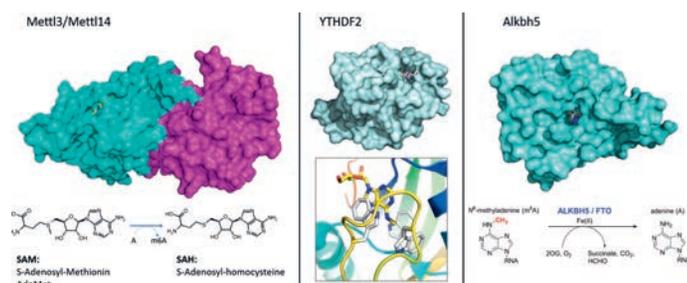


Fig. 4. Schematic presentation of three major constituents of the m⁶A mRNA pathway that qualify as drug targets for small-molecule intervention. Left: heterodimeric structure of the Mettl3/Mettl14 complex (5tey.pdb) and depiction of the chemical methyltransfer reaction catalyzed by the enzyme. Middle: reader protein YTHDF2 (4rdn.pdb) with m⁶A in stick mode bound to the tryptophan cage (bottom). Right: Alkbh5 demethylase (4nro.pdb) and depiction of chemical demethylation reaction catalyzed by the enzyme.

qualifying the demethylase **Alkbh5** as a potential target protein for small-molecule intervention. Alkbh5 belongs to the *jumonji* domain-containing demethylases that catalyzes an oxidative, metal ion-dependent removal reaction converting m⁶A to adenosine (Fig. 4).

In summary, the rapidly emerging field of epitranscriptomics provides for an increasing number of attractive enzymes and receptor proteins that unlock new therapeutic approaches for medicinal chemistry-centric drug discovery campaigns. Mettl3/Mettl14, YTHDF2, and Alkbh5 have been introduced to exemplify just three well-druggable targets proven for disease states in which m⁶A levels need to be either attenuated or elevated. New findings from the broad area of modern RNA biology will without any doubt further expand the target portfolio for which today's and tomorrow's medicinal chemistry is expected to find useful chemical matter.

Received: January 29, 2020

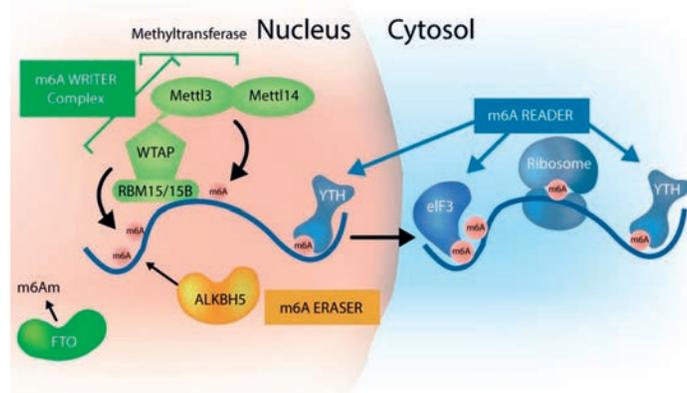


Fig. 3. Schematic presentation of the m⁶A mRNA pathway. Enzymatic 'writer' activity is located in the nucleus and carried out by the Mettl3/Mettl14 heterodimeric SAM-dependent methyltransferase complex. The reverse 'eraser' activity is accomplished by the demethylase Alkbh5, while a related enzyme FTO operates on the di-methylated version m⁶Am. m⁶A-containing mRNA is specifically recognized by 'reader' domains of the YTH type within the nucleus and within the cytoplasm.^[8,11]

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