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Stefan Jurga
Jan Barciszewski *Editors*

Epitranscriptomics

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Introduction: Understanding Epitranscriptomics

The genetic alphabet consists of the four letters: C, A, G, and T in DNA and C, A, G, and U in RNA. 61 of 64 triplets of these four letters jointly encode 22 different amino acids which construct proteins. This system is universal and functions in all kingdoms of life.

Comparative transcriptomics between mammals has revealed that ∼66% of human genomic DNA is transcribed. RNA plays a critical role in regulating cellular functions. Interestingly, only ∼2% of the transcriptional production is protein-coding messenger RNA (mRNA), while ∼98% encompasses a wide variety of noncoding RNA (ncRNA) molecules. ncRNAs have been classified functionally as either housekeeping or regulatory. The housekeeping ncRNA genes include ribosomal RNA (rRNA), transfer RNA (tRNA), and small nuclear RNA (snRNA), while examples of regulatory ncRNAs are microRNA (miRNA) and long noncoding RNA (lncRNA).

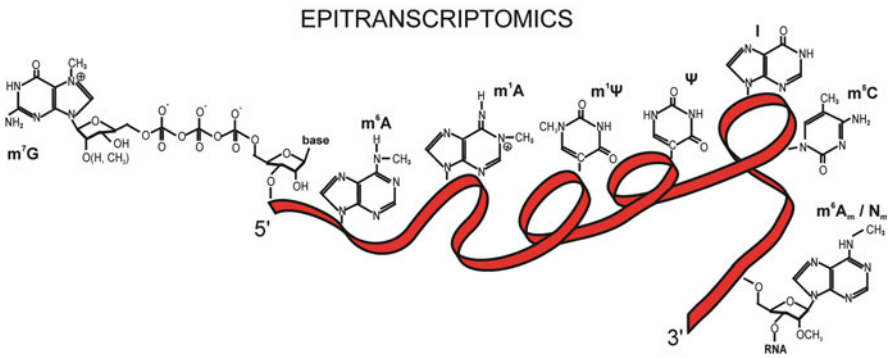
The year 2021 marks the diamond jubilee of the discovery of messenger RNA (mRNA) and the cracking of the genetic code. Sixty years ago, Sydney Brenner, François Jacob, and James Watson isolated mRNA; however, François Jacob and Jacques Monod put mRNA into a theoretical context, arguing for its role in gene expression and regulation. These observations stimulated a new way of thinking about gene function. At the same time, J. Heinrich Matthaei and Marshall Nirenberg showed that poly U encodes the synthesis of polyphenylalanine.

The complexity of RNA is further complicated by numerous post-transcriptional modifications. RNA harbors the potential of being dynamically and reversibly regulated by the addition and removal of distinct chemical moieties. Over 160 post-transcriptional modifications expand the RNA code. These modifications extend the RNA repertoire and alter its chemistry in various ways without changing the nucleotide sequence. The majority of RNA modifications consist of adding a methyl group to certain positions on the nucleobase. RNA methylation can be reversible and convey information *via* recognition of effector proteins. The different positions for methylation carry distinct implications. Although mRNA modification

has been known since the 1970s, its functional importance in mRNA metabolism and its effect on human biology have not been extensively studied in the past.

The process of mRNA maturation involving 5′-capping, splicing, and polyadenylation is well studied. The post-transcriptional modifications found in mRNA mark regions that potentially contribute to the regulation of cellular processes, including gene expression, protein translation, or RNA stability. These modifications can alter the structure and metabolism of mRNA. Only recently methodological and conceptual advances allowed systematic mapping and functional analysis to unfold the role they play in mRNA biology.

Mammalian messenger RNA contains tens of thousands of modified nucleotides, an essential addition to the standard genetic code of four nucleotides in animals, plants, and their viruses. Technological advances that allowed mapping of selected RNA modifications on a transcriptome-wide scale revealed the widespread distribution of N6-methyladenosine (m^6A), pseudouridine (Ψ), ribose 2′-O-methylation (Nm), N1-methyladenine (m^1A), and 5-methylcytidine (m^5C) on mRNA (Figure).



m^6A destabilizes pairing with uracil by altering the energetics of an AU pair through steric hindrance, but the pattern of hydrogen-bonding donors and acceptors remains the same. m^1A methylation endows a positive charge, which may result in strong electrostatic interactions. Also, in m^1A, the methyl group protrudes from the Watson–Crick hydrogen-bonding face of adenine, resulting in the nucleotide remaining unpaired. 2'-O-methylation augments hydrophobicity, protects against nucleolytic attack, and stabilizes RNA helices. Some modifications are incorporated enzymatically by various methyltransferases (writers) and removable by demethylases (erasers).

In comparison to U, Ψ contains an extra imino group (>C═NH), which serves as an additional hydrogen bond donor, while the carbon–carbon (C─C) glycosidic bond linking the sugar to the base is more stable than the carbon-nitrogen (C─N) in U. These two chemical changes confer rigidity to the sugar-phosphate backbone and enhances local base stacking. An additional class of nucleotide modifications, termed RNA editing, creates an irreversible change in the nucleotide sequence. These modifications include insertions, deletions, and base substitutions and occur

in all classes of RNA. When they occur in mRNA, the amino acid sequence of the protein will be altered relative to the sequence encoded by genomic DNA. RNA editing by deamination results in adenosine (A) to inosine (I) and cytosine (C) to uridine (U). A-to-I editing is an abundant class of RNA modifications found throughout metazoans. The conversion of A-to-I residues by base deamination results in the synthesis of distinct proteins, which creates functional diversity and enhances response to rapid environmental changes. RNA editing by deamination is mediated by two major classes of enzymes; the first class is a group of tissue-specific and context-dependent adenosine deaminases called ADARs. The ADAR enzyme class (adenosine deaminases acting on RNA) catalyzes hydrolytic deamination of A-to-I in double-stranded regions of RNA secondary structure. The second class of enzymes, the vertebrate-specific apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) family, promotes C-to-U editing by cytosine deamination. APOBEC1, the first-discovered member of the APOBEC family, was characterized as the zinc-dependent cytidine deaminase which catalyzed a C-to-U modification, resulting in an in-frame stop codon in APOBEC mRNA.

Epitranscriptomics, or RNA epigenetics, is a branch of epigenetics and refers to RNA editing and noncoding RNA regulations. It is a young and fast developing field. mRNA modifications represent another layer of epigenetic regulation to gene expression. Epitranscriptomics is crucial in cell proliferation, migration, invasion, and epithelial–mesenchymal transition. Epitranscriptomics plays essential roles in alternative splicing, nuclear export, transcript stability, and translation of RNA targets to pioneer new ways of cancer treatment.

The rapid advancement of next-generation sequencing and mass spectrometry technologies has recently allowed for the identification and functional characterization of nucleotide modifications in protein-coding and noncoding RNA on a global transcriptome scale. In this book, there will be a summary of transcriptome-wide RNA modification mapping techniques. It will highlight studies exploring the functions of RNA modifications and their association with disease and finally offer insights into the future progression of epitranscriptomics.

Accurate regulation of the transcriptome is critical for gene expression and its subsequent control of cellular functions, including metabolism, proliferation, differentiation, and development. Thus, alterations in transcriptome regulation can disrupt cellular functions and lead to disease. Accumulating evidence has identified and functionally characterized several distinct types of chemical modifications of RNA nucleotides in both protein-coding RNAs and ncRNAs, further advancing the burgeoning field of epitranscriptomics.

The mechanisms and functions of different modifications in mRNA with an emphasis on its effect on human health and disease will be discussed. The low abundance of nucleotide modifications and technical limitations, however, have hampered systematic analysis of their occurrence and functions. Selective chemical and immunological identification of modified nucleotides has revealed global candidate topology maps for many modifications in mRNA.

There are many chapters in this book each dedicated to a particular topic. The role of RNA modifications which represent a novel layer of regulation of gene expression

and offer new possibilities to rapidly alter gene expression upon specific environmental changes will be analyzed. These chapters highlight the importance of epitranscriptomics in different diseases and drug resistance. They also provide new insights on potential therapeutic targets to reverse drug resistance. The field of epitranscriptomics is still in its infancy. We look forward to many exciting discoveries in the coming years.

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Contents

The Emerging Neuroepitranscriptome

Andrew M. Shafik, Emily G. Allen, and Peng Jin

Contents

Abstract Chemical modifications on ribonucleic acid are prominent in the brain, are emerging as regulators of proper brain development, and have been implicated in neurological disorders. Similar to epigenetic modifications that regulate gene expression patterns, epitranscriptomic marks and their associated cellular machinery are involved in the expression of RNA that is required for developmental processes and maintenance of the nervous system. This work provides a detailed view on the current literature surrounding N6-methyladeonosine, N1-methyladeonsine, 5-methylcytidine, pseudouridine, and other RNA modifications in brain development and neurological disorders.

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1 Introduction

Epigenetic modifications such as DNA methylation and histone modifications can regulate gene expression and have been implicated in many biological processes, particularly neurodevelopment. The mechanisms by which these modifications act can be dynamically regulated through "writer" and "eraser" enzymes that install or remove the modifications, respectively. Dysregulation of these mechanisms has been implicated in neurodegenerative, neurodevelopmental, and neurological disorders. For example, DNA methylation and histone modification-mediated gene regulation are crucial for neural cell differentiation (Feng et al. 2007; Hirabayashi and Gotoh 2010; Sun et al. 2011; Yao et al. 2016). However, the effect of RNA modifications in the regulation of biological processes has only just gathered momentum in the past few years. Furthermore, similar to epigenetic modifications, the reversibility and dynamic nature of some post-transcriptional RNA modifications allow them to quickly and tightly regulate various biological processes. Indeed, RNA modifications in the brain are emerging as a critical regulator of many different neuronal pathways, while deregulation of these RNA modifications may be associated with neurological disorders and developmental diseases, including tumorigenesis.

RNA modifications were first identified in abundant RNA species, such as ribosomal RNA and tRNA. However, the occurrence of RNA modifications has recently been documented in less abundant RNAs such as mRNA and long non-coding RNA. These modifications are known to influence many aspects of RNA processing, including splicing (Tang et al. 2018; Zhou et al. 2019), mRNA export (Yang et al. 2017), mRNA stability (Wang et al. 2014; Yang et al. 2019), mRNA translation (Liu et al. 2016a; Mao et al. 2019; Schumann et al. 2020; Wang et al. 2015), and miRNA processing (Alarcon et al. 2015; Song et al. 2020) (Fig. 1). Currently, over 170 chemical modifications have been documented on all four canonical bases and the ribose sugar across all RNA types (Boccaletto et al. 2018). These modifications comprise the "epitranscriptome." Furthermore, the identification of proteins that install ("writers"), remove ("eraser"), and specifically recognize ("readers") RNA modifications has extended our knowledge regarding the role and mechanism by which these modifications affect cellular, developmental, and disease processes (Jonkhout et al. 2017; Kadumuri and Janga 2018). However, research into many RNA modifications is in its infancy, but the advent of high-throughput approaches has paved the way to understand the functions and mechanisms of RNA modifications. In particular, N6-methyladenosine is an abundant modification that has been extensively studied and has been widely implicated as a critical regulator in higher brain processes (see, for example, Shafik et al. 2020). A picture

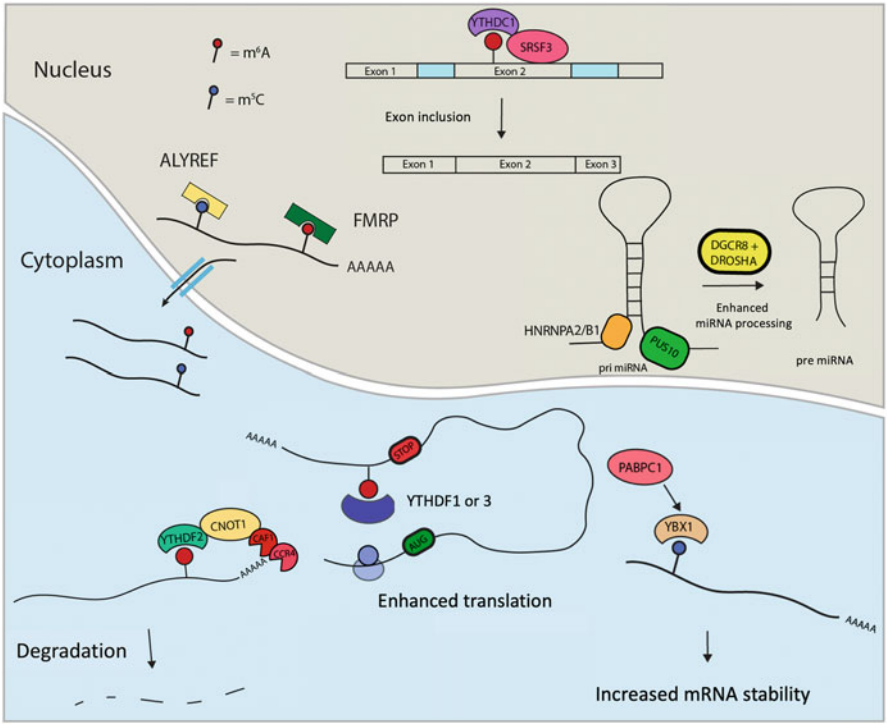

Fig. 1 RNA modifications affect many aspects of RNA metabolism. ALYREF and FMRP facilitate nuclear export of m^5C and m^6A marked transcripts, respectively. YTHDF1 and YTHDF3 specifically recognize m^6A marked transcripts, resulting in enhanced translation through a closed-loop model. YTHDC1 is an m^6A reader which directs the splicing factor SRSF3 to its target, resulting in exon inclusion. HNRNPA2/B1 or PUS10, in complex with DGCR8, recognize primary miRNAs modified by m^6A or pseudouridine respectively, delivering DGCR8 to its target for enhanced miRNA processing. YBX1 recognizes m^5C marked transcripts and functions in stabilizing the mRNA by recruiting PABPC1. m^5C = 5-methylcytidine, m^6A = N6-methyladenosine

of the neuroepitranscriptome is beginning to emerge, which solidifies the importance of RNA modifications in brain development and disease (Fig. 2).

2 N6-methyladenosine

N6-methyladenosine is one of the most abundant internal mRNA modifications and has been extensively studied over the last few years. m^6A has been shown to be a dynamic modification that is involved in the regulation of many biological processes, including mRNA stability, translation, splicing, export, and miRNA processing.

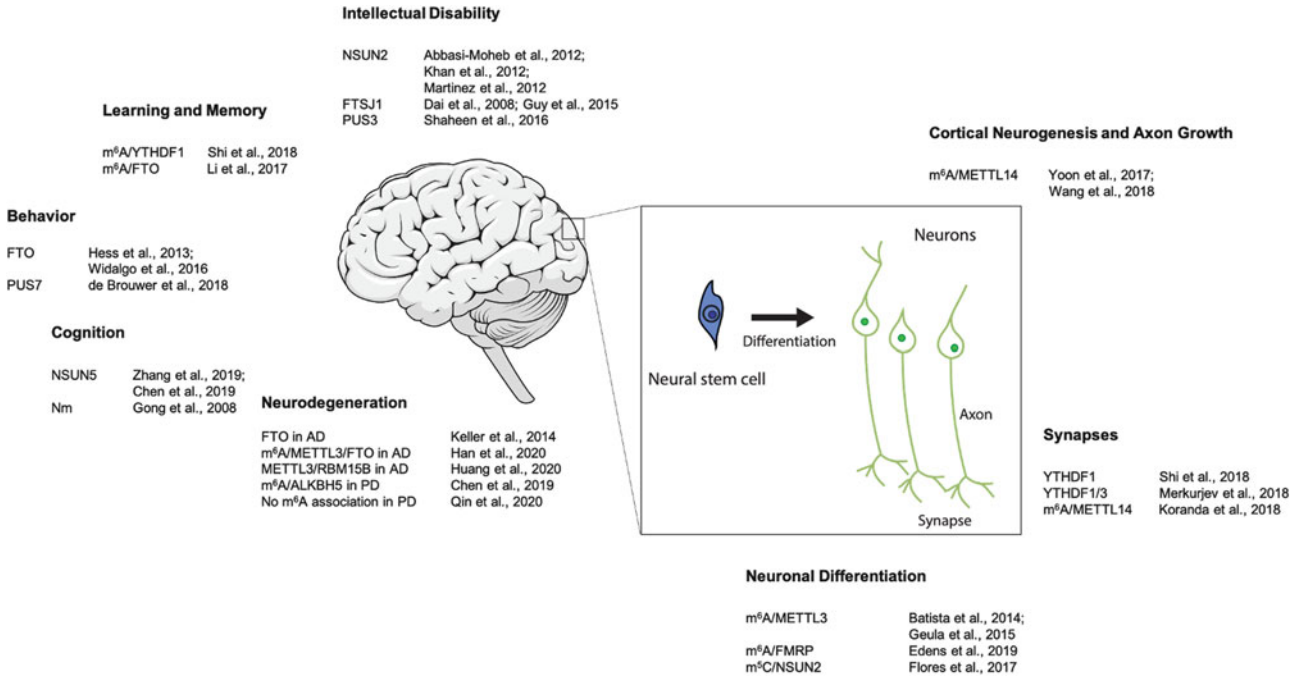

Fig. 2 A summary of the literature implicating RNA modifications and/or the machinery associated with those modifications in many neuronal processes. m^5C, m^6A, pseudouridine, and Nm modifications are broadly required during brain development and are involved in learning and memory, behavior, cognition, intellectual disability, neurodegeneration. Furthermore, m^5C and m^6A have a role in neuronal differentiation, while m^6A is also involved in cortical neurogenesis, axon growth, and synapses. m^5C = 5-methylcytidine, m^6A = N6-methyladenosine, Nm = 2′-*O*-methylation

m^6A is deposited on RNA targets by a complex that consists of the "writer" protein (methyltransferase-like 3) METTL3, which is supported structurally by (methyltransferase-like 14) METTL14 (Bokar et al. 1997; Liu et al. 2014). Further components of the complex include WT1-associated protein (WTAP), which functions in regulating the recruitment of the complex to its mRNA targets (Agarwala et al. 2012), while RNA-binding motif protein 15 directs the complex to appropriate m^6A sites (Patil et al. 2016). Lastly, Vir like m^6A methyltransferase associated (VIRMA) protein guides the catalytic core components (METTL3, METTL14, and WTAP) to selective methylation in the 3′UTR and near the stop codon (Yue et al. 2018). Methyltransferase-like 5 (METTL5) protein has also been shown recently to catalyze m^6A methylation at position 1832 in mouse 18S rRNA (Ignatova et al. 2020). Significantly, the authors demonstrate that *Mettl5* depletion in mouse embryonic stem cells resulted in a global reduction in mRNA translation and loss of pluripotency. Another recently discovered m^6A methyltransferase, methyltransferase-like 16 (METTL16), was shown to methylate a single mRNA (MAT2A) in its 3′ UTR, and this contributes to splicing induction (Pendleton et al. 2017). "Eraser" enzymes (FTO, ALKBH5) reverse the m^6A modification (Jia et al. 2011; Zhao et al. 2014; Zheng et al. 2013), while m^6A function is mediated by proteins that "read" it (e.g., YTHDF1-3, YTHDC1, and YTHDC2) (Mao et al. 2019; Shi et al. 2017; Wang et al. 2014, 2015). Following knockdown of FTO and ALKBH5, m^6A levels increase, validating these proteins as m^6A demethylases (Jia et al. 2011; Zhao et al. 2014; Zheng et al. 2013), while the affinity of "readers" is higher for m^6A methylated mRNA compared to unmethylated mRNA (Theler et al. 2014).

2.1 Detection of m^6A

Initially, m^6A was detected transcriptome-wide using a specific anti-m^6A antibody coupled to next generation sequencing. m^6A peaks are identified compared to a background RNA-seq input of the same sample in an approach termed methylated RNA immunoprecipitation sequencing (MeRIP-seq) (Dominissini et al. 2012; Meyer et al. 2012). Since then, another approach has been developed which allows for the single-nucleotide detection of m^6A (Linder et al. 2015). The authors cross-linked RNA to m^6A antibodies. Reverse transcription of the RNA resulted in a specific pattern of mutations or truncations, which allowed for the identification of m^6A at single-nucleotide resolution. More recently, antibody-free approaches have been developed. DART-seq or deamination adjacent to RNA modification targets coupled with next generation sequencing, employs a fusion protein consisting of cytidine deaminase, APOBEC1, and the m^6A-binding YTH domain (Meyer 2019). APOBEC1-YTH induces C-to-U deamination next to m^6A sites, which can then be identified using RNA-seq. Another antibody-free technique has been developed (Zhang et al. 2019b). They took advantage of the m^6A-sensitive endoribonuclease

(m^6A-sensitve RNA cleavage enzyme) to identify specific m^6A sites at single-nucleotide resolution (Fig. 3).

2.2 *m^6A in Neurodevelopment and Neurogenesis*

Specifically, m^6A is highly enriched in the mammalian brain, and many studies have implicated this RNA modification in neurodevelopment, neurogenesis, and neuropsychiatric disease. A decrease in protein expression of METTL3, METTL14, and WTAP during mouse cerebellar development from postnatal day 7 to postnatal day 60 has been detected (Ma et al. 2018). Furthermore, these proteins were detected in the external granule cell layer, Purkinje cell layer, and inner granule cell layer. With increasing age, the authors noted that expression levels decreased in the internal granular layer but increased in the Purkinje cell layer. Knockdown of *Mettl3* resulted in a dramatic alteration in Purkinje cell numbers, laminal structure, and stunted dendrites, suggesting that m^6A is crucial for proper cerebellar development. Loss of METTL3 has also been shown to result in the impaired embryonic stem cell exit from self-renewal toward differentiation into several lineages (Batista et al. 2014). On this note, Mettl14-induced m^6A depletion resulted in prolonged cell cycle of adult radial glial, while neuron production still occurred into postnatal stages, suggesting m^6A to be a major regulator of cortical neurogenesis (Yoon et al. 2017). Furthermore, Mettl14 conditional knockout results in a loss of late neurons, further suggesting a role for METTL14 in cortical neurogenesis (Wang et al. 2018). It was also demonstrated that METTL14 has a role in the striatum by (Koranda et al. 2018). The authors profiled m^6A in Mettl14-deleted striatum, where they showed a correlation between loss of m^6A and downregulation of mRNAs encoding neuron and synapse-specific proteins. More specifically, they found m^6A methylation in mRNAs known to function in synaptic plasticity, such as Homer1 and Cdk5r1, suggesting a role for m^6A in synaptic signaling. Also implicated WTAP has also been implicated in proper brain development (Ping et al. 2014). In *Drosophila,* they found that embryos injected with a *Wtap* morpholino exhibited smaller head, eyes, brain ventricle, and a curved notochord at 24 h post-fertilization compared to uninjected embryos. Interestingly, they found loss of *Mettl3* to only have a mild effect.

Ythdf1 KO mice have been shown to exhibit difficulties with learning and memory using hidden platform training Morris water maze tests (Shi et al. 2018). Furthermore, loss of Ythdf1 resulted in impaired basal synaptic transmission and long-term potentiation in hippocampal neurons, which further contributes to the defects in learning and memory exhibited by these mice. Re-expressing YTHDF1 in the hippocampus of the KO mice rescued defects in behavior and synapsis. Mechanistically, the authors found that in response to neuronal stimuli, YTHDF1, in the adult mouse hippocampus, recognizes a subset of m^6A marked transcripts, resulting in their enhanced translation and thus facilitating learning and memory. Another m^6A reader, YTHDF2, is highly expressed during the early stages of neural

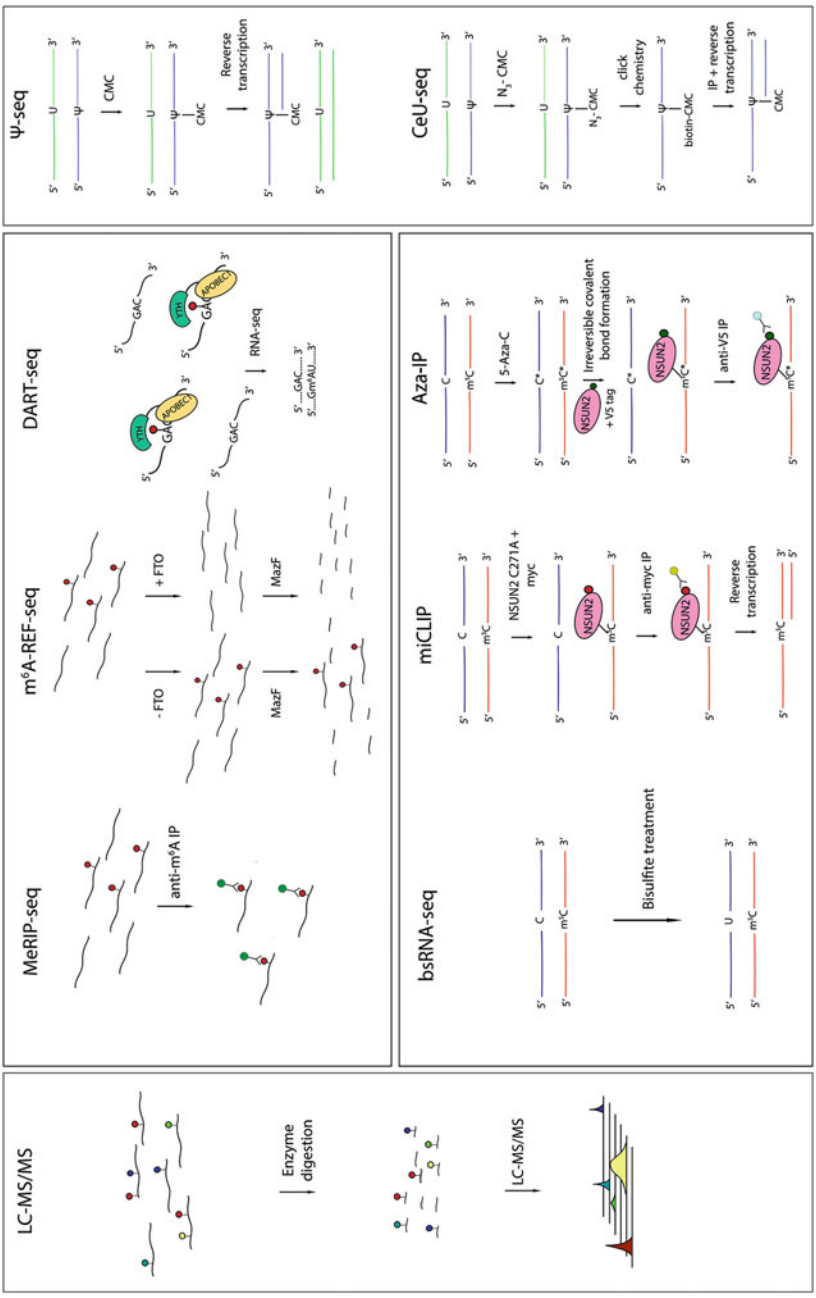

Fig. 3 Approaches to detect RNA modifications. LC-MS/MS allows for the identification of a plethora of RNA modifications in a sample but does not provide the user with information regarding the position of the modifications. The colored circles represent different types of RNA modifications. Each RNA modification produces a different signal. m^6A is detected using m^6A-REF-seq, MeRIP-seq, or DART-seq. m^6A-REF-seq and DART-seq are antibody-free methods. DART-seq uses an APOBEC1-YTH fusion protein to identify m^6A sites, while m^6A-REF-seq employs an m^6A-sensitive RNA cleavage enzyme

development and Ythdf2 −/− mouse embryos at E12.5 and E14.5 were alive but displayed reduced overall cortical thickness as described for Mettl14 cKO mice (Li et al. 2018b). They further demonstrate that loss of *Ythdf2* has a strong negative impact on neural stem/progenitor cell (NSPC) self-renewal and neuron generation in the embryonic neocortex. Also, YTHDF1, YTHDF2, and YTHDF3 are enriched in hippocampal dendrites, and the loss of YTHDF1 or YTHDF3 there resulted in altered spine morphology, dampened excitatory synaptic transmission, and altered cell-surface protein content (Merkurjev et al. 2018). Another protein, fragile X mental retardation protein (FMRP), was shown to bind m^6A marked mRNA (Edens et al. 2019; Zhang et al. 2018). It was demonstrated that upon loss of FMRP, 2035 genes were differentially expressed in the mouse cortex (Zhang et al. 2018). Interestingly, the majority of downregulated FMRP targets harbor an m^6A site. The authors show that FMRP binds the m^6A sites and interacts with YTHDF2, regulating the stability of its m^6A-marked targets. Furthermore, during neural differentiation, FMRP was shown to facilitate the nuclear export of m^6A marked transcripts (Edens et al. 2019). *Fmr1* KO mice were also observed to exhibit an extended neuronal progenitor cell cycle, with neural progenitors still proliferating into postnatal stages, similar to what was described in the Mettl14 cKO mice.

The correct balance of m^6A is essential for the proper development of the brain. Indeed, FTO-catalyzed m^6A demethylation has a significant role in neurogenesis, learning, and memory (Li et al. 2017a). They find FTO in both adult neural stem cells and neurons, observing an increased expression of FTO 1 day to 8 weeks post-birth. Furthermore, they find that many players in the brain-derived neurotrophic factor (BDNF) pathway are regulated by FTO, and this may, in turn, affect postnatal neurogenesis. Furthermore, it has been shown that the levels of FTO and ALKBH5 decreased in the cerebellum from P7 to P60 (Ma et al. 2018). Interestingly, they show that the cerebellum of Alkbh5-knockout mice is not significantly different in weight or morphology to wild-type mice. This may suggest that other demethylases, for example, FTO, may compensate for the lack of ALKBH5. However, since ALKBH5 may have a role in the regulation of cellular processes to hypoxia, the authors postulated that ALKBH5 may protect the brain against hypoxia.

Fig. 3 (continued) (MazF) to identify m^6A sites following treatment with or without the m^6A demethylase FTO. MeRIP-seq uses an antibody to specifically pull out m^6A-marked transcripts. All of these methods are coupled to next generation sequencing. m^5C is detected using either miCLIP, bsRNA-seq, or Aza-IP approaches. Both miCLIP and Aza-IP traps the RNA methyltransferase (NSUN2) to its target. Antibodies against a tagged version of NSUN2 are then used to immunoprecipitate the protein along with the covalently bound RNA, followed by next generation sequencing. bsRNA-seq is an approach where the RNA is treated with bisulfite, which converts unmethylated cytosines to uracil, while methylated cytosines are resistant to the treatment. Pseudouridine is detected through two similar methods. RNA is treated with CMCT, resulting in Ψ-CMC. As the CMC-Ψ is a bulky residue, it can block the reverse transcriptase, resulting in a stop or pause event one nucleotide before the pseudouridine site. The reverse transcription step is followed by next generation sequencing. m^5C = 5-methylcytidine, m^6A = N6-methyladenosine, Ψ = pseudouridine, CMC = carbodiimide

Indeed, they observed that ALKBH5 KO mice have significantly smaller whole brains and cerebellum compared to WT mice after being exposed to hypobaric hypoxia for 48 h. Another recent study found that ALKBH5 is generally expressed in the mouse brain, being more prominent in the cerebellum and olfactory bulb of the adult mouse brain (Du et al. 2020). Also, they detected that ALKBH5 co-localized with the neuronal marker NeuN, suggesting ALKBH5 to be mainly expressed in neurons. Finally, they also observed that ALKBH5 protein expression significantly decreased during brain development. Altogether, these observations suggest further work is required to fully understand the effect of these demethylates in different brain regions where redundancy exists and whether ALKBH5 and FTO are involved in different aspects of brain development (dependent on the region) and/or do they have different targets. Furthermore, m^6A demethylation by FTO plays an important role in dopaminergic transmission, which is involved in the control of complex behaviors (Hess et al. 2013). The authors found that levels of m^6A increased a subset of mRNAs associated with neuronal signaling, including the dopaminergic signaling pathway in the midbrain and striatum of Fto knockout mice. This resulted in several proteins with altered expression and impaired dopaminergic transmission. Another study found knocking down FTO in the mouse medial prefrontal cortex resulted in enhanced cued fear memory, while m^6A levels increased in the same tissue after behavioral training (Widagdo et al. 2016).

2.3 *m^6A and Neurological Disorders*

Currently, there is little known regarding the specific role of m^6A in neurodegenerative and neuropsychiatric diseases. However, there is strong evidence to suggest a fundamental role for m^6A in neurodegenerative and neuropsychiatric disease. For example, genetic variants of FTO have been implicated in Alzheimer's disease (AD), e.g., see Ho et al. (2010), Li et al. (2018a), and Reitz et al. (2012). For example, the interaction between FTO and APOE was found, and individuals carrying both FTO-AA genotype and APOE $\epsilon 4$ were at a higher risk for dementia (Keller et al. 2011). Along those lines, it was shown that variation in introns 1 and 2 of the FTO gene may be associated with AD (Reitz et al. 2012). More recently, a possible association between m^6A and AD was revealed (Han et al. 2020). The authors quantified total RNA m^6A levels in an Alzheimer's mouse model (APP/PS1 transgenic mice), compared to C57BL/6 wild-type mice, revealing increased levels of total RNA m^6A in the cortex and the hippocampus of APP/PS1 transgenic mice. Furthermore, the authors found that the expression of METTL3 and FTO increased and decreased in AD mice, respectively, compared to wild type. This study suggests a possible role for m^6A in regulating Alzheimer's disease, but a more in-depth study is required to actually elucidate the role of m^6A. Another recent study used a public human Alzheimer's brain RNA-seq dataset to determine the expression profiles of m^6A players (Huang et al. 2020). They found that METTL3 and RBM15B were upregulated and downregulated in the hippocampus, respectively. Those observations were independently validated at the protein level in another set of postmortem

human brains. They also suggest that the accumulation of METTL3 positively correlates (albeit weakly) with Tau aggregates. The effect of m^6A has also been looked at in the context of Parkinson's disease (PD) (Chen et al. 2019b). They model Parkinson's disease in rats and PC12 cells by employing 6-OHDA to selectively destroy dopaminergic neurons. Upon this treatment, they found that the m^6A modification was reduced in the PC12 cells in the striatum of the PD rat but not the cortex, hippocampus, or midbrain. This observation seems to correlate with the fact that a significant increase in ALKBH5 is present only in the striatum of PD mice but not in any other region. Interestingly, an extensive study was performed to determine variants of the m^6A players in 1647 sporadic Parkinson patients and 1372 controls of Han Chinese origin (Qin et al. 2020). The authors identified 214 rare variants in METTL3, METTL14, WTAP, FTO, ALKBH5, YTHDF1, YTHDF2, YTHDF3, HNRNPC, and ELAVL1. However, gene-wise association studies did not achieve significance, suggesting that there is no biological association between these m^6A associated proteins and sporadic Parkinson's disease.

m^6A is present in synapses, and aberrant translation at synapses has been associated with autism, fragile X syndrome, and other intellectual disorders, suggesting there may be a role for m^6A in these diseases. For example, mutations in YTHDC2 have been implicated as a risk factor for autism spectrum disorder (Liu et al. 2016b). Also, depressed patients have altered levels of m^6A/m after glucocorticoid stimulation, suggesting that these modifications may also have a role in stress-related psychiatric disorders (Engel et al. 2018). Lastly, FTO polymorphisms have also been found in psychiatric diseases, including major depressive disorder (MDD). Individuals carrying the FTO rs9939609 allele were demonstrated to have an inverse association between obesity risk and depression (Milaneschi et al. 2014). Another study also found an association between MDD and allelic variants of ALKBH5 (Du et al. 2015).

3 N1-methyladenosine

Several enzymes have been reported to install m^1A on rRNA and tRNA, including TRM6, TRM10, and TRM61 (Bujnicki 2001), and the modification can be removed by ALKB, ALBH1, and ALKBH3 (Li et al. 2016; Liu et al. 2016a). No readers have been identified as yet. Furthermore, m^1A, unlike m^6A and m^6Am, perturbs Watson–Crick base pairing and potentially disrupts protein–RNA interactions and RNA secondary structures through electrostatic effects (Zhou et al. 2016).

3.1 Detection of m^1A

Currently, the literature surrounding m^1A is conflicting and not much is known about the modification. Using an antibody approach similar to the m^6A MeRIP-Seq

method, m^1A was recently mapped transcriptome-wide and proposed as a prominent modification in the 5′UTR of mRNAs (Dominissini et al. 2016; Li et al. 2017c; Safra et al. 2017). However, the further analysis presented in a recent study revealed that the m^1A antibody used in these studies had an affinity for the m^7G-cap and resulted in false-positives (Grozhik et al. 2019). Furthermore, the authors used another m^1A antibody that did not cross-react with the m^7G cap, and m^1A was not detected in the 5′ UTR. Indeed, they demonstrate that m^1A sites are rare, if not absent, from mRNAs. Furthermore, m^1A is known to cause truncation during reverse transcription, however, there is much uncertainty associated with this approach. To overcome this, under alkaline conditions, m^1A can be converted to m^6A in a process known as the Dimroth rearrangement. As m^6A does not have a signature during reverse transcription, a disappearance of a reverse transcription signal will be observed after the treatment, signaling the presence of m^1A (Dominissini et al. 2016).

3.2 *m^1A in the Brain*

m^1A abundance has been measured in various tissues using LC-MS/MS, and m^1A was shown to be the highest in the kidney and the brain (Dominissini et al. 2016). Furthermore, they detected four times as much m^1A in the brains of lean (wt/wt) mice compared to obese (ob/ob) mice. That study provides some evidence that m^1A levels vary according to tissue type, is an abundant modification in the brain, and also that the modification can possibly be a dynamic modification in response to physiological signaling. Also, another study determined m^1A levels across 39 different tissues and found that m^1A was highest in whole blood, brain, muscle, and nerve tissues (Ali et al. 2020). Furthermore, m^1A levels increased in the brain of FTO −/− mice compared to wild type, suggesting that in addition to being an m^6A and m^6Am demethylase, FTO mediates demethylation of m^1A (Wei et al. 2018) show that. As loss of m^1A tRNA methylation may regulate translation (Liu et al. 2016a), some of the FTO mediated phenotypes observed in brain tissues (see above) may be in part because of tuning translation through tRNA m^1A demethylation.

4 5-Methylcytidine

RNA m^5C methyltransferases belong to the superfamily of Rossman fold-containing enzymes that use *S*-adenosyl-*L*-methionine (SAM), which functions as a cofactor to donate the methyl group. Currently, known m^5C methyltransferases belong to either the DNMT2 or the NOL1/NOP2/SUN (NSUN) subgroups (Bujnicki et al. 2004; Motorin et al. 2010). In mammals, the NSUN family includes seven enzymes (NSUN1-NSUN7). NSUN2 is the main m^5C methyltransferase and has been shown to target tRNAs, non-coding RNAs, and mRNAs (Amort et al. 2017; Hussain et al. 2013; Khoddami and Cairns 2013; Squires et al. 2012). Substrates for NSUN1

and NSUN5 are 28S rRNA (Heissenberger et al. 2019; Sharma et al. 2013), NSUN4 targets 12S mitochondrial rRNA (Metodiev et al. 2014), NSUN3 and NSUN6 recognize tRNAs (Haag et al. 2015, 2016), and NSUN7 has been shown to methylate enhancer RNAs (Aguilo et al. 2016). Recently, the first m^5C "reader," ALYREF protein was discovered (Yang et al. 2017). The authors showed that ALYREF promotes mRNA export of m^5C-marked mRNAs. m^5C has also been associated with the YBX1 protein that was shown to stabilize a subset of maternal mRNAs by recruiting poly(A)-binding protein cytoplasmic 1 during the maternal to zygotic transition in zebrafish (Yang et al. 2019). The modification has also been implicated as potentially having a role in regulating mRNA translation. For example, specific m^5C methylation in the 3′UTR of the cell cycle regulators CDK1 and p21 resulted in enhanced translation in vitro and using a gene reporter in vivo (Li et al. 2017b; Xing et al. 2015). Also, m^5C may reduce translation when introduced at any position within a codon and altered codon specificity when in the second position of the codon (Hoernes et al. 2016). However, this observation was achieved using a bacterial system and still remains to be validated for eukaryotic translation.

4.1 Detection of m^5C

Currently, several high-throughput m^5C detection methods exist. m^5C was first mapped transcriptome-wide using bisulfite RNA sequencing (Squires et al. 2012). In the presence of sodium bisulfite, cytosine is converted into uracil by deamination while methylated cytosines are resistant to the treatment. A standard RNA-seq then allows for the identification of m^5C. Other currently used detection techniques are m^5C RNA immunoprecipitation (m^5C-RIP), 5-azacytidine-mediated RNA immunoprecipitation (Aza-IP) (Khoddami and Cairns 2013), or methylation-individual nucleotide resolution cross-linking immunoprecipitation (miCLIP) (Hussain et al. 2013). m^5C-RIP uses m^5C specific antibodies to selectively enrich for m^5C-containing RNA. Aza-IP randomly incorporates the cytidine analog, azacytidine, into nascent RNA transcripts in place of cytosine. This results in an RNA-methylase covalent adduct that can be immunoprecipitated with enzyme-specific or tag antibodies. Lastly, release of RNA from the complex results in hydrolytic opening of the azacytidine ring, which is read as a guanine following reverse transcription. The miCLIP approach employs a mutant form of the m^5C writer NSUN2, which allows for capturing NSUN2 bound RNA complexes. This is coupled with an individual-nucleotide resolution cross-linking immunoprecipitation (iCLIP) approach and next generation sequencing (Fig. 3).

4.2 m^5C in Neurodevelopment

There is some evidence to suggest that m^5C may be a significant regulator of brain development processes. For example, recently, an ultrahigh performance liquid

chromatography–multiple reaction monitoring tandem mass spectrometry (UHPLC–MRM-MS/MS) analysis identified m^5C in mRNAs from six mouse tissues (small intestine, heart, muscle, brain, kidney, and liver) (Yang et al. 2017). This revealed the highest concentration of m^5C in the brain compared to the other tested tissues. A total of 4371 m^5C sites in 1655 mRNAs were detected. Of those 4371 sites, 1918 are specific to the brain and correlate with the highest mRNA expression in the brain compared to the other tissues. These transcripts are involved in trans-synaptic signaling, nervous system development, cell-cell signaling, and neurogenesis processes, pointing to a role for m^5C in neurodevelopment. m^5C in the mouse brain was also detected by Amort et al. (2017). They found ~7500 m^5C sites (>20% methylation) mapping to 1650 mRNAs were detected in embryonic stem cells (ESCs) and 2075 m^5C sites mapping to 486 mRNAs in the brain, with m^5C being its highest in the 3′ UTR of transcripts methylated in the brain. Interestingly, mRNAs that are specifically methylated in ESCs were expressed but not methylated in the brain. The uniquely methylated transcripts in the brain are not expressed in ESCs and are involved in ion transport or synapse function biological processes. The authors also found an overlap between the 3′ UTR m^5C sites and RNA-binding protein sites, including UPF1 and splicing factors SRSF3 and SRSF4. Despite this association being statistically significant, very low numbers of overlapping sites were actually observed, suggesting that any gene regulation through such a mechanism may be specific to a particular transcript.

hm^5C is generated through the active demethylation of m^5C by the Tet enzyme. hm^5C in RNA was shown to be at the highest levels in the brainstem, hippocampus, and cerebellum using a dot blot approach (Miao et al. 2016). Interestingly, they detected less hm^5C in an MPTP-induced Parkinson's disease mouse model compared to wild type. Another study found that hydroxymethylated RNA and the Tet enzyme are prominent in the Drosophila brain (Delatte et al. 2016). Depletion of Tet results in decreased RNA hydroxymethylation and consequently impaired Drosophila brain development. Clearly, both m^5C and hm^5C have significant roles in the brain, and further work is required to fully elucidate the impact and mechanisms of m^5C on neurodevelopment.

Expression of NSUN2, the m^5C writer, is highest in the cortex, hippocampus, and striatum of the developing mouse brain (Blanco et al. 2014). Furthermore, loss of NSUN2 resulted in the increased cleavage of NSUN2 tRNA targets by the endonuclease angiogenin, which in turn resulted in the accumulation of 5′ tRNA fragments (Blanco et al. 2014) and consequently a decrease in global protein translation (Gebetsberger et al. 2012; Ivanov et al. 2011). This manifested itself as increased cellular stress and reduction in size in those tissues where NSUN2 levels are highest. Importantly, both cellular stress and microcephaly can be rescued through the inhibition of angiogenin (Blanco et al. 2014). In humans, NSUN2 is expressed in early neuroepithelial progenitors, and levels progressively decrease during differentiation of neuroepithelial stem (NES) cells in vitro, and the process is impaired by both absence of NSUN2 and the presence of angiogenin (Flores et al. 2017). The authors also observed that loss of *Nsun2* caused an accumulation of intermediate progenitors and a decrease in differentiated upper-layer neurons in the cortex. Also,

NSUN5 expression was found to be highest in oligodendrocyte precursor cells compared to neurons or astrocytes (Zhang et al. 2019a). On this note, the authors found that the loss of *Nsun5* inhibits NMDA receptor activity in neuronal cells, possibly as a result of impaired development and function of oligodendrocyte precursor cells. Another study focused on the role of NSUN5 in the mouse cerebral cortex (Chen et al. 2019a). At P10, the authors found a significant reduction in cortical thickness and abnormal laminar organization in Nsun5-KO mice compared to wild-type littermates. At embryonic day (E) 12.5 to E16.5, the authors find that NSUN5 is expressed in radial glial cells of the cerebral cortex, where it is required to properly maintain the radial glial scaffolds in order to regulate neocortical neuron migration. Clearly, proper connections within the brain and cortex organization are required to ensure the correct development of complex social-cognitive functions, and these findings suggest that NSUN5 plays a particularly important role in cortex development.

4.3 m^5C in Neurological Disorders

Loss-of-function mutations in the m^5C writer, NSUN2, in both mouse and human have been linked to intellectual disability phenotypes, including growth retardation, microcephaly, impaired cognition, and motor function (Abbasi-Moheb et al. 2012; Martinez et al. 2012). A missense mutation at a conserved residue in NSUN2 causes a failure to localize to the nucleolus of Purkinje cells in the cerebellum, contributing to the intellectual disability phenotype (Khan et al. 2012).

Williams–Beuren syndrome is a neurodevelopmental disorder characterized by social-cognitive disorder. The NSUN5 gene, which encodes an m^5C methyltransferase, has been shown to be deleted in the syndrome. Adult *Nsun5*-KO mice that have been generated show spatial cognitive defects (Chen et al. 2019a; Zhang et al. 2019a).

5 Pseudouridine

Pseudouridylation can occur through two different mechanisms. In the first, enzymes belonging to the pseudouridine synthase (PUS) family recognize sequence and/or secondary structural elements of the RNA target and catalyze the isomerization of uridine to pseudouridine. There are ten pseudouridine synthases that are compartmentalized into six families (TruA, TruB, TruD, RsuA, RluA, and Pus10) (Spenkuch et al. 2014); however, all PUS enzymes share a conserved catalytic domain and it is likely that they all function through a conserved catalytic mechanism. In the second mechanism, the pseudouridylation event is guided by snoRNPs (H/ACA snoRNA assembled with core proteins). The core proteins include Cbf5/NAP57/Dyskerin, Nhp2/L7Ae, Nop10, and Gar1 (Massenet et al. 2017). The

snoRNA functions as a guide that base pairs with the substrate RNA, directing the core protein to the site for pseudouridylation. Interestingly, all recent transcriptome-wide studies mapping pseudouridine showed that the majority of mRNA pseudouridylation is catalyzed by the independent PUS enzymes. Lastly, pseudouridylation has been shown to result in stop codon read through or nonsense suppression in mRNAs (Karijolich and Yu 2011), while another study found pseudouridylated mRNAs to be highly inducible in response to serum starvation in humans (Carlile et al. 2014). Recently, Pus10 has been implicated in regulating miRNA processing by directly binding to primary miRNAs interacting with the microprocessor to promote miRNA biogenesis (Song et al. 2020).

5.1 Detection of Pseudouridine

There are two main methods to detect pseudouridine in RNA. The first method is based on *N*-Cyclohexyl-*N*′-(2-morpholinoethyl) carbodiimide methyl-*p*-toluenesulfonate (CMCT) modification of pseudouridine (Li et al. 2015; Lovejoy et al. 2014). Uridine and guanine can also be modified with CMCT resulting in Ψ-CMC, U-CMC, and G-CMC adducts. Fortunately, mild alkaline conditions can reverse the CMCT modification of uridine and guanine but not pseudouridine. Finally, as the CMC-Ψ is a bulky residue, it can block the reverse transcriptase, resulting in a stop or pause event one nucleotide before the pseudouridine site. There are presently two very similar approaches that use this idea termed Ψ-seq and CeU-seq (Fig. 3). The second method exploits site-specific cleavage and labeling of RNA (Zhao and Yu 2004). In this approach, RNA is cleaved at the target uridine/pseudouridine sites, the cleaved site is then radiolabeled, followed by nuclease digestion into mononucleotides, and, finally, analysis by thin-layer chromatography.

Pseudouridylation was shown to be as prevalent as m^6A in mRNAs using quantitative mass spectrometry (Li et al. 2015). Furthermore, using the chemical pulldown-based approach, the authors found high levels of pseudouridine in mRNAs from various mouse tissues, with particular enrichment in the brain and lung. They detected 1741 sites in mouse brain, and the pseudouridine-marked genes were enriched in nervous system development and signal transduction. These findings, as well as the fact that pseudouridylation can be dynamically regulated (Schwartz et al. 2014), raise the possibility that pseudouridine modification is involved in the regulation of neurodevelopmental processes.

5.2 Pseudouridine in Neurological Disorders

Novel homozygous protein-truncating variants in PUS3 and PUS7, respectively, which encode RNA-independent pseudouridylate synthases (de Brouwer et al. 2018; Shaheen et al. 2016). Patients with these mutations exhibited an intellectual

disability phenotype, including speech delay, a smaller physique, microcephaly, and aggressiveness. Furthermore, a significant decrease in pseudouridine modification at positions 38 and 39 in tRNA of patient cells was observed, consistent with a loss of PUS3 in these patients (Shaheen et al. 2016), while these protein-truncating mutations resulted in the loss of PUS7, and consequently loss of pseudouridine on tRNA and mRNA targets (de Brouwer et al. 2018). They validated their findings by studying the effects of PUS7 knockout in Drosophila. Deletion of *Pus7* resulted in a number of behavioral defects, including increased activity, disorientation, and aggressiveness. These findings suggest that PUS7-mediated RNA pseudouridylation is required to maintain proper neurodevelopment and function. These observations were also independently validated by a related study (Shaheen et al. 2019). Those authors also found one missense and one frameshift mutation in PUS7 that resulted in the abolishment of the enzyme, with consequent loss of pseudouridine at position 13 in PUS7 tRNA targets. This manifested itself in a strong intellectual disability phenotype and progressive microcephaly.

6 Other RNA Modifications

Since RNA modifications on small non-coding RNAs (sncRNAs) have been implicated as important regulators of physiological and pathological processes (e.g., Abe et al. 2014), it was investigated whether RNA modifications on small RNAs have an impact on Alzheimer's disease (AD) (Zhang et al. 2020). To this end, they analyzed modification profiles on both small RNAs (15–25 nt) and tRNA fractions extracted from the human cortex using LC-MS/MS (Fig. 3). In the 15–25-nt RNA fraction, the authors found increases in 2′-*O*-methylcytidine (Cm), 7-methylguanosine (m^7G), 2′-*O*-methylguanosine (Gm) and decreased levels of *N2*,*N2*,*N7*-trimethylguanosine ($m^{2,2,7}G$) and *N2*,*N2*-dimethylguanosine ($m^{2,2}G$) modifications in AD brains compared with controls. Interestingly, in the 30–40-nt fraction, a significant reduction in rRNA-derived small RNA (rsRNA)-5S, tRNA-derived small RNA (tsRNA)-Tyr, and tsRNA-Arg fragments were observed in AD patients compared with controls; however, the authors do not show any association between these changing RNA levels and RNA modifications. Ultimately, more work is required to understand whether these RNA modifications play a part in AD etiology and how this is achieved mechanistically.

Also, various tRNA modifications have been indirectly linked with neurological disorders. For example, mutations in the FtsJ methyltransferase homolog 1 (FTSJ1) gene have been linked with non-syndromic X-linked mental retardation and intellectual disability (Dai et al. 2008), and genetic variants of FTSJ1 have been associated with general cognitive ability, verbal comprehension, and perceptual organization (Gong et al. 2008). This gene encodes for a methyltransferase that is responsible for 2′-*O*-methylation modifications in $tRNA^{Leu}$, $tRNA^{Trp}$, and $tRNA^{Phe}$ at positions 32 and 34 (Gong et al. 2008; Guy et al. 2015). Furthermore, two genetically independent lymphoblastoid cell lines from non-syndromic X-linked

mental retardation and intellectual disability patients had loss-of-function *FTSJ1* mutations and a near complete loss of 2′-*O*-methylation at position 32 and 32 of $tRNA^{Phe}$ (Guy et al. 2015). This observation suggests 2′-*O*-methylation is linked to neurodevelopmental disorder. However, whether loss of the modification is a driving force for onset or is just a by-product of *FTSJ1* mutation remains to be determined. In any case, aberrant 2′-*O*-methylation is clearly associated with neurological disorder.

Furthermore, a homozygous frameshift mutation in human tRNA methyltransferase 1 has been associated with recessive cognitive disorders (Najmabadi et al. 2011). tRNA methyltransferase 1 catalyzes the dimethylation of guanosines ($m^{22}G$) at position 26 of tRNAs. More recently, studies have associated ELP2, ELP3, and ELP4, three subunits of the multisubunit elongator protein complex, with neurodevelopmental disability, amyotrophic lateral sclerosis, and atypical Rolandic epilepsy, respectively (Kojic and Wainwright 2016). Moreover, the elongator complex has an essential role in tRNA uridine modifications. Altogether these observations suggest that these tRNA modifications may be linked to neurological disorders. However, whether the modification has a direct or indirect role in the onset of these disorders and the possible mechanisms remains to be fully elucidated.

7 Future Directions and Perspectives

RNA modifications clearly afford another layer of regulation in the maintenance of the mammalian brain. However, further work is required to understand the role of the mechanisms by which the RNA modifications act in brain development and disease. Mapping RNA modifications, especially at the single-nucleotide level, will go a long way to further understanding the role of these modifications. For example, what cues (cellular or environmental) control whether a transcript is modified or unmodified, and how do the different modifications work in concert? Single-cell sequencing studies have started to emerge outlining the view of the transcriptome in each cell type in the brain, and this is being extending to look at the epitranscriptome. Currently, the advent of single-molecule direct RNA sequencing may shed light on the different RNA modifications present on a given transcript. This will allow us to begin to fully determine the impact of modifications on gene expression. Such technologies, as those that are being developed by Nanopore Sequencing, will be important to determine, using a small input of RNA, the combinations of modifications that are present on individual transcripts, in a specific cell type, at a specific time. This is critical to gain an understanding of how different modifications work together or against each other to contribute to development and disease. On this note, it is important to know whether it is the modification itself or the cellular machinery that "writes," "reads," or "erases" the modification that actually contributes to disease etiology. For example, it is the loss of methylation or the loss of the writer that plays a role in the onset of disease? Clearly, more work is required to fully

understand the mechanism by which these RNA modifications exert their function in neurodevelopment and disease.

References

Abbasi-Moheb L, Mertel S, Gonsior M et al (2012) Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 90(5):847–855

Abe M, Naqvi A, Hendriks GJ et al (2014) Impact of age-associated increase in 2′-O-methylation of miRNAs on aging and neurodegeneration in Drosophila. Genes Dev 28(1):44–57

Agarwala SD, Blitzblau HG, Hochwagen A et al (2012) RNA methylation by the MIS complex regulates a cell fate decision in yeast. PLoS Genet 8(6):e1002732

Aguilo F, Li S, Balasubramaniyan N et al (2016) Deposition of 5-methylcytosine on enhancer RNAs enables the coactivator function of PGC-1alpha. Cell Rep 14(3):479–492

Alarcon CR, Lee H, Goodarzi H et al (2015) N6-methyladenosine marks primary microRNAs for processing. Nature 519(7544):482–485

Ali AT, Idaghdour Y, Hodgkinson A (2020) Analysis of mitochondrial m1A/G RNA modification reveals links to nuclear genetic variants and associated disease processes. Commun Biol 3 (1):147

Amort T, Rieder D, Wille A et al (2017) Distinct 5-methylcytosine profiles in poly(A) RNA from mouse embryonic stem cells and brain. Genome Biol 18(1):1

Batista PJ, Molinie B, Wang J et al (2014) m(6)A RNA modification controls cell fate transition in mammalian embryonic stem cells. Cell Stem Cell 15(6):707–719

Blanco S, Dietmann S, Flores JV et al (2014) Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. EMBO J 33(18):2020–2039

Boccaletto P, Machnicka MA, Purta E et al (2018) MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res 46(D1):D303–D307

Bokar JA, Shambaugh ME, Polayes D et al (1997) Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA 3 (11):1233–1247

Bujnicki JM (2001) In silico analysis of the tRNA:m1A58 methyltransferase family: homology-based fold prediction and identification of new members from eubacteria and archaea. FEBS Lett 507(2):123–127

Bujnicki JM, Feder M, Ayres CL et al (2004) Sequence-structure-function studies of tRNA:m5C methyltransferase Trm4p and its relationship to DNA:m5C and RNA:m5U methyltransferases. Nucleic Acids Res 32(8):2453–2463

Carlile TM, Rojas-Duran MF, Zinshteyn B et al (2014) Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. Nature 515(7525):143–146

Chen P, Zhang T, Yuan Z et al (2019a) Expression of the RNA methyltransferase Nsun5 is essential for developing cerebral cortex. Mol Brain 12(1):74

Chen X, Yu C, Guo M et al (2019b) Down-regulation of m6A mRNA methylation is involved in dopaminergic neuronal death. ACS Chem Neurosci 10(5):2355–2363

Dai L, Xing L, Gong P et al (2008) Positive association of the FTSJ1 gene polymorphisms with nonsyndromic X-linked mental retardation in young Chinese male subjects. J Hum Genet 53 (7):592–597

de Brouwer APM, Abou Jamra R, Kortel N et al (2018) Variants in PUS7 cause intellectual disability with speech delay, microcephaly, short stature, and aggressive behavior. Am J Hum Genet 103(6):1045–1052

Delatte B, Wang F, Ngoc LV et al (2016) RNA biochemistry. Transcriptome-wide distribution and function of RNA hydroxymethylcytosine. Science 351(6270):282–285

Dominissini D, Moshitch-Moshkovitz S, Schwartz S et al (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485(7397):201–206
Dominissini D, Nachtergaele S, Moshitch-Moshkovitz S et al (2016) The dynamic N(1)-methyladenosine methylome in eukaryotic messenger RNA. Nature 530(7591):441–446
Du T, Rao S, Wu L et al (2015) An association study of the m6A genes with major depressive disorder in Chinese Han population. J Affect Disord 183:279–286
Du T, Li G, Yang J et al (2020) RNA demethylase Alkbh5 is widely expressed in neurons and decreased during brain development. Brain Res Bull 163:150–159
Edens BM, Vissers C, Su J et al (2019) FMRP modulates neural differentiation through m(6)A-dependent mRNA nuclear export. Cell Rep 28(4):845–854. e845
Engel M, Eggert C, Kaplick PM et al (2018) The role of m(6)A/m-RNA methylation in stress response regulation. Neuron 99(2):389–403. e389
Feng J, Fouse S, Fan G (2007) Epigenetic regulation of neural gene expression and neuronal function. Pediatr Res 61(5 Pt 2):58R–63R
Flores JV, Cordero-Espinoza L, Oeztuerk-Winder F et al (2017) Cytosine-5 RNA methylation regulates neural stem cell differentiation and motility. Stem Cell Rep 8(1):112–124
Gebetsberger J, Zywicki M, Kunzi A et al (2012) tRNA-derived fragments target the ribosome and function as regulatory non-coding RNA in Haloferax volcanii. Archaea 2012:260909
Gong P, Li J, Dai L et al (2008) Genetic variations in FTSJ1 influence cognitive ability in young males in the Chinese Han population. J Neurogenet 22(4):277–287
Grozhik AV, Olarerin-George AO, Sindelar M et al (2019) Antibody cross-reactivity accounts for widespread appearance of m(1)A in 5′UTRs. Nat Commun 10(1):5126
Guy MP, Shaw M, Weiner CL et al (2015) Defects in tRNA anticodon Loop 2′-O-methylation are implicated in nonsyndromic X-Linked intellectual disability due to mutations in FTSJ1. Hum Mutat 36(12):1176–1187
Haag S, Warda AS, Kretschmer J et al (2015) NSUN6 is a human RNA methyltransferase that catalyzes formation of m5C72 in specific tRNAs. RNA 21(9):1532–1543
Haag S, Sloan KE, Ranjan N et al (2016) NSUN3 and ABH1 modify the wobble position of mt-tRNAMet to expand codon recognition in mitochondrial translation. EMBO J 35 (19):2104–2119
Han M, Liu Z, Xu Y et al (2020) Abnormality of m6A mRNA methylation is involved in Alzheimer's disease. Front Neurosci 14(98)
Heissenberger C, Liendl L, Nagelreiter F et al (2019) Loss of the ribosomal RNA methyltransferase NSUN5 impairs global protein synthesis and normal growth. Nucleic Acids Res 47 (22):11807–11825
Hess ME, Hess S, Meyer KD et al (2013) The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry. Nat Neurosci 16(8):1042–1048
Hirabayashi Y, Gotoh Y (2010) Epigenetic control of neural precursor cell fate during development. Nat Rev Neurosci 11(6):377–388
Ho AJ, Stein JL, Hua X et al (2010) A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proc Natl Acad Sci USA 107 (18):8404–8409
Hoernes TP, Clementi N, Faserl K et al (2016) Nucleotide modifications within bacterial messenger RNAs regulate their translation and are able to rewire the genetic code. Nucleic Acids Res 44 (2):852–862
Huang H, Camats-Perna J, Medeiros R et al (2020) Altered expression of the m6A methyltransferase METTL3 in Alzheimer's disease. eNeuro 7(5)
Hussain S, Sajini AA, Blanco S et al (2013) NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. Cell Rep 4(2):255–261
Ignatova VV, Stolz P, Kaiser S et al (2020) The rRNA m(6)A methyltransferase METTL5 is involved in pluripotency and developmental programs. Genes Dev 34(9-10):715–729
Ivanov P, Emara MM, Villen J et al (2011) Angiogenin-induced tRNA fragments inhibit translation initiation. Mol Cell 43(4):613–623