NEURAL REGENERATION RESEARCH
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Dysregulation of RNA modification systems in clinical populations with neurocognitive disorders

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https://doi.org/10.4103/1673-5374.385858

Date of submission: April 27, 2023

Date of decision: July 5, 2023

Date of acceptance: August 10, 2023

Date of web publication: September 22, 2023

From the Contents

Abstract

The study of modified RNA known as epitranscriptomics has become increasingly relevant in our understanding of disease-modifying mechanisms. Methylation of N6 adenosine (m^6A) and C5 cytosine (m^5C) bases occur on mRNAs, tRNA, mt-tRNA, and rRNA species as well as non-coding RNAs. With emerging knowledge of RNA binding proteins that act as writer, reader, and eraser effector proteins, comes a new understanding of physiological processes controlled by these systems. Such processes when spatiotemporally disrupted within cellular nanodomains in highly specialized tissues such as the brain, give rise to different forms of disease. In this review, we discuss accumulating evidence that changes in the m^6A and m^5C methylation systems contribute to neurocognitive disorders. Early studies first identified mutations within *FMR1* to cause intellectual disability Fragile X syndromes several years before FMR1 was identified as an m^6A RNA reader protein. Subsequently, familial mutations within the m^6A writer gene *METTL5*, m^5C writer genes *NSUN2*, *NSUN3*, *NSUN5*, and *NSUN6*, as well as *THOC2* and *THOC6* that form a protein complex with the m^5C reader protein ALYREF, were recognized to cause intellectual development disorders. Similarly, differences in expression of the m^5C writer and reader effector proteins, *NSUN6*, *NSUN7*, and *ALYREF* in brain tissue are indicated in individuals with Alzheimer's disease, individuals with a high neuropathological load or have suffered traumatic brain injury. Likewise, an abundance of m^6A reader and anti-reader proteins are reported to change across brain regions in Lewy bodies diseases, Alzheimer's disease, and individuals with high cognitive reserve. m^6A-modified RNAs are also reported significantly more abundant in dementia with Lewy bodies brain tissue but significantly reduced in Parkinson's disease tissue, whilst modified RNAs are misplaced within diseased cells, particularly where synapses are located. In parahippocampal brain tissue, m^6A modification is enriched in transcripts associated with psychiatric disorders including conditions with clear cognitive deficits. These findings indicate a diverse set of molecular mechanisms are influenced by RNA methylation systems that can cause neuronal and synaptic dysfunction underlying neurocognitive disorders. Targeting these RNA modification systems brings new prospects for neural regenerative therapies.

Key Words: 5-methylcytosine methylation; Alzheimer's disease; cognitive diseases; epitranscriptomics; intellectual disability; Lewy body diseases; N6 adenosine; RNA modification

Introduction

The study of modified RNA species, known as epitranscriptomics, has led to unprecedented discoveries in RNA regulatory systems which are increasingly relevant in our understanding of disease-modifying mechanisms. Over a hundred and fifty different types of post-transcriptional RNA modifications are presently recognized to occur on rRNA, tRNA, and mRNA species and include N6-methyladenosine modification (m^6A), 5-methylcytosine (m^5C), N^6,2′-O-dimethyladenosine (m^6Am) and other mRNA capping modifications, N1-methyladenosine (m^1A), pseudo-uridine (Ψ) and dihydrouridine (Boccaletto et al., 2018; Eyler et al., 2019; Finet et al., 2022). Novel forms of RNA modification in mammals and their functional consequences are still being discovered. For example, 3-methylcytidine (m^3C) methylation has recently been described to modify eukaryotic tRNAs, and while controversial, potentially mRNAs (Ma et al., 2019; Lentini et al., 2022). Although suggested to be involved in the regulation of translation in the cytoplasm as well as in mitochondria, m^3C functional specificity and range of molecular functions in these compartments are still relatively unknown. Similarly, modification by acetylation of N4-cytidine in mammalian rRNAs (ac^4C) has only recently been characterized and is suggested to moderate rRNA processing and ribosome biogenesis but its biological significance is yet to be established (Arango et al., 2022).

The most prevalent commonly stable RNA modifications in mammals are methylation of N6 adenosine (m^6A) and C5 cytosine (m^5C) bases. m^6A and m^5C modification occurs at internal sites within mRNA transcripts. However, both forms of methylation also exist on tRNA, mt-tRNA, and rRNA species as well as non-coding RNAs. In tRNA, rRNA, and mt-tRNA, m^5C modification provides structural stability and improves the accuracy of translation (Van Haute et al., 2019; Schumann et al., 2020). In comparison, m^5C methylation of mRNA is indicated to increase RNA stability and abundance, regulate nuclear exportation as well as negatively correlating with translation efficiency (Yang et al., 2017; Liu et al., 2021). The biological consequences of m^5C RNA methylation on cellular function are numerous and differ depending on the developmental stage but include regulating fundamental processes such as cell proliferation and migration, DNA repair, and cell senescence (Zhang et al., 2012; Xue et al., 2019; Yang et al., 2019; Chen et al., 2020). m^6A methylation is the most abundant internal modification in mRNA and long non-coding RNAs and has been shown to regulate mRNA nuclear splicing, translation efficiency, and RNA degradation. It has also been implicated in regulating numerous physiological processes such as stem cell differentiation, embryonic development, as well as neuronal and glial cell function.

Methylation status is moderated by groups of proteins referred to as effector proteins. These proteins differ between the m^5C and m^6A systems. Methyltransferases, known as writer proteins, add methyl groups to nucleotides. m^5C methyltransferases include NOL1/NOP2/SUN (NSUN) domain family proteins and a tRNA (cytosine(38)-C(5))-methyltransferase also known as DNMT2. NSUN1, NSUN2, NSUN5, NSUN6, NSUN7, and DNMT2 target rRNA, mRNA and tRNA, and enhancer RNAs (Brzezicha et al., 2006; Bourgeois et al., 2015; Aguilo et al., 2016) whilst NSUN3 and NSUN4 are predominately located within mitochondria where they modify mitochondrial tRNA and rRNAs, respectively (Metodiev et al., 2014; Nakano et al., 2016). In contrast, in mammals the writer proteins involved in m^6A mRNA methyltransferase activity consist of METTL3 and METTL14, WTAP, ribosomal binding motif 15 (RBM15), and its paralogue RBM15B. An additional, newly discovered m^6A writer protein, METTL16, has recently been shown to be involved in the methylation of coding RNAs (Brown et al., 2016; Pendleton et al., 2017) as well as non-coding U6 spliceosomal small nuclear RNAs (Warda et al., 2017). Furthermore, ribosomal RNA can be m^6A modified at two sites namely, 18S rRNA at position A1832 and 28S at position A4220a (Maden, 1986, 1988) and that is catalyzed by the writer protein METTL5 (van Tran et al., 2019).

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Funding: *This work was funded by Nottingham University and the Neuroscience Support Group Charity, UK (to HMK). Adriana PerezGrovas-Saltijeral was supported by a CONACYT PhD scholarship. The author MDÖ was supported by the Postdoctoral Research Fellowship Program of TUBITAK.*

How to cite this article: *Knight HM, Demirbugen Öz M, PerezGrovas-Saltijeral A (2024) Dysregulation of RNA modification systems in clinical populations with neurocognitive disorders. Neural Regen Res 19(0):000-000.*

The erasers effector proteins, the demethylases, are proteins that remove the methyl group. Removal of m^5C modification is mediated by the ALKBH1, a pleitropic dioxygenase that equally demethylates other forms of modification such as m^1A and m^3C (Falnes et al., 2004; Ougland et al., 2004; Kawarada et al., 2017). ALKBH1 has also been shown to mediate modifications of cytosine-34 (m^5C34) in mitochondrial tRNAs (mt-tRNAmet) (Haag et al., 2016). Three additional eraser proteins TET1, TET2, and TET3 enzymes also demethylate m^5C by oxidation and are involved in the demethylation of DNA as well as RNA. The m^6A demethylase eraser proteins, FTO and ALKBH5, belong to the AlkB subfamily of the Fe(II)/2-oxoglutarate dioxygenase superfamily. However, they differ in their mode of demethylation with FTO demonstrating oxidative methylase activity, while ALKBH5 directly removes methyl groups from single-stranded RNA non-oxidatively (Han et al., 2010; Feng et al., 2014). Importantly, ALKBH5 which is highly abundant in brain tissue, is reported to be specific to m^6A whereas, FTO also mediates tRNA m^1A demethylation.

RNA binding proteins belonging to the reader effector protein category, recognize specific chemical modifications and direct binding events. Several RNA binding proteins have been suggested to be m^5C reader proteins. Nonetheless, the most well-studied m^5C readers include the THO complex subunit 4 (ALYREF) that interacts with the methyltransferase NSUN2 and mediates mRNA transport in and out of the nucleus (Yang et al., 2017); and YBX1 that is involved in mRNA stabilization (Chen et al., 2000) and splicing (Raffetseder et al., 2003). The best-characterized m^6A reader effector proteins belong to the YTH domain family that consists of YTHDF1, YTHDF2, YTHDF3, YTHDC, and YTHDC2. The YTHDF1-3 proteins have similar binding affinities toward preferred RNA motifs and recent studies support dosage-dependent redundancy in their function to regulate m^6A-dependent mRNA stability and translation (Li et al., 2020). However, context-specific factors may be determining spatial-temporal changes in their expression and hence individual roles unique in moderating RNA metabolism. In addition to YTH domain reader proteins that interact with m^6A RNA-modified sites, several proteins have been identified as auxiliary readers such as FMR1, FXR1, and FXR2. Indeed a subset of proteins have recently been characterized and are referred to as m^6A anti-readers (Arguello et al., 2017) as they preferentially interact with unmodified m^6A RNA binding sequences, i.e., they are repelled by "m^6A" RNA modifications. The expression and cellular localization of these m^5C and m^6A writers, eraser and reader/anti-reader effector proteins are likely to have a direct effect on RNA methylation regulatory systems and consequential cellular function that could influence the expression of disease. To date, clinical investigations of RNA modification deficits in human cohorts and using human tissue have been focused more on cancer, and only very recently has the field of epitranscriptomics started to explore neurodegenerative and neuropsychiatric conditions. The purpose of this review is to sumarize the current evidence, ranging from genetic, RNA, and protein expression studies, and quantitative modification analysis, which supports that alterations in m^5C and m^6A RNA modification influence brain disease processes underlying cognitive disorders.

Search Strategy

The citation database used was PubMed and literature was searched between January and June 2023. All years were chosen in the search.

Evidence of Disrupted m^5C Effector Protein Expression in Neurodevelopmental and Neurodegenerative Disease

m^5C RNA methylation, although less prevalent than m^6A RNA modification, has been characterized across multiple tissues and is found highest in abundance within mRNAs in the brain (Huang et al., 2019). Evidence initially from pedigree studies in which causative mutations were identified, led to the discovery that several NSUN writers cause neurodevelopmental conditions (**Figure 1**). For example, *NSUN2* is known to cause forms of autosomal recessive intellectual disability (Abbasi-Moheb et al., 2012; Khan et al., 2012; Martinez et al., 2012; Sun et al., 2020), as well as Dubowitz syndrome, a rare multiple congenital syndrome characterized by growth retardation, microcephaly, distinctive facial dysmorphism, and intellectual deficits (Martinez et al., 2012). *NSUN3*, a mitochondrial tRNA writer, causes autosomal recessive mitochondrial encephalomyopathy characterized by global developmental delay (Van Haute et al., 2016; Paramasivam et al., 2020). Likewise, the rRNA writer, *NSUN5*, is located within the critical region for Williams Beuren syndrome, a rare neurodevelopmental condition; and haploinsufficiency of *NSUN5* in fibroblasts from Williams Beuren syndrome patients causes a partial loss of 28S rRNA m^5C methylation (Heissenberger et al., 2019). Furthermore, in a very recently published report, biallelic mutations within a fourth NSUN writer, *NSUN6*, were described to cause autosomal recessive neurodevelopmental disorders (Mattioli et al., 2023). The different mutations identified, i.e., two homozygous frame shift mutations and a homozygous missense mutation, were suggested, by the authors, to lead to a loss of functional NSUN6 protein abundance and hence to a loss of m^5C modification of tRNAs. Finally, the m^5C reader, ALYREF, is part of the TRanscription-EXport (TREX) protein complex that regulates mRNA transcription and processing and shuttles mRNA out of the nucleus (Dufu et al., 2010). Mutations within *THOC2* and *THOC6* that are protein binding partners of the TREX mRNA-export complex are reported to cause X-Linked intellectual disability and rare autosomal recessive syndromic intellectual disability (Najmabadi et al., 2011; Kumar et al., 2015).

Regulatory processes important for neurodevelopment and which are disrupted in such neurodevelopmental diseases are commonly found dysregulated in the aging brain. Indeed, evidence that changes in m^5C machinery abundance contribute to neurodegeneration is beginning to emerge. Dementia, a progressive decline in neurocognitive ability, is the principal cause of disability in the elderly population. Alzheimer's disease (AD) is the most common form of dementia and is characterized by the neuropathological accumulation of intraneuronal aggregation of tau protein known as neurofibrillary tangles and the accumulation of insoluble beta amyloid peptide termed senile and neurite plaques. These pathological features progress through stages of severity that correlate to the burden of pathology and anatomical localization (Braak et al., 1991), and are assessed as neuropathological Braak and CERAD scores. Neuropathological lesions are also commonly found in individuals exposed to head trauma that leads to either focal or diffuse traumatic brain injury (TBI) (Hyman et al., 2012). TBI can have both acute or chronic effects with the latter having a greater link to a decline in cognition.

One approach commonly undertaken to understand how pathology develops through changes at the cellular level, is to examine changes in RNA expression profiles using RNA sequencing data for large AD cohorts (Gardner et al., 2019; Marques-Coelho et al., 2021). In a recent study by PerezGrovas-Saltijeral et al. (2023), gene expression profiles of m^5C RNA effector transcripts were examined in AD and TBI clinical populations as well as age-matched healthy control individuals (PerezGrovas-Saltijeral et al., 2023). RNA-sequencing for 107 individuals, of which 51 had a clinical diagnosis of AD and 56 were healthy controls, was obtained from the aging, dementia, and traumatic brain injury study generated initially for the Adult Changes in Thought study (Miller et al., 2017). Information was available for four brain regions, the hippocampus, the superior temporal gyrus of the temporal cortex, the inferior parietal cortex, and the white matter from the parietal cortex. Twenty-six individuals had a history of TBI and 30 had no history of TBI. The m^5C effector protein investigated by PerezGrovas-Saltijeral et al. (2023) were the writers, *NSUN1*, *NSUN2*, *NSUN3*, *NSUN4*, *NSUN*5, *NSUN6*, *NSUN7*, and *DNMT2*; the readers, *ALYREF* and *YBX1*; and erasers, *ALKBH1*, *TET1*, *TET2*, and *TET3*. Expression profiles were compared between AD and controls, Braak and CERAD scores of neuropathology, and individuals with a history of TBI. The study found that in healthy brain tissue, RNA effector protein transcripts exhibited the overall highest relative expression in the white matter tissue from the parietal cortex. However, differences in abundance for sets of effector transcripts were observed across the different brain regions. For example, in the hippocampus, a region associated with contextual learning and memory, gene expression of the m^5C NSUN writer effector proteins was found to be moderately high. In contrast, the reader *YBX1* and eraser effectors *TET1* and *TET2* were the most abundant transcripts in the hippocampus and white matter tissue, whereas in the superior temporal gyrus of the temporal cortex and the inferior parietal cortex, the reader *ALYREF*, and erasers *ALKBH1* and *TET3*, were more highly expressed. Such observations suggested that in the healthy aged brain, there are tissue-specific m^5C RNA methylation effector protein mechanisms.

In the same study, RNA effector proteins expression in the AD population showed a similar overall expression profile to healthy controls across the brain regions. However, in AD individuals, significantly lower expression of *NSUN6* was found in the superior temporal gyrus and the white matter tissue; while a second writer, *NSUN7*, showed significantly higher abundance in the hippocampus in AD individuals. These changes were consistently observed for both NSUN writers across different stages of AD neuropathology for neurofibrillary tangles, Braak stages, within the hippocampus. Interestingly, *NSUN7* was the only m^5C effector protein that showed changes in abundance for the neuropathological score assessing amyloid load, CERAD. In addition to the changes in *NSUN6* and *NSUN7* gene expression, the m^5C reader *ALYREF* showed lower expression in the most severe Braak stages in the hippocampus and inferior parietal cortex. Finally, all individuals with a history of TBI showed significantly lower expression of m^5C writer *NSUN6* across the temporal gyrus when compared to healthy aged controls, which was consistent across samples regardless of whether there was a diagnosis of AD or not.

Although this study did not directly assess changes in m^5C-modified RNAs in the human brain, the findings do describe consistent alterations in the methylation machinery associated with neurodegenerative disease or neuropathology status. As RNA methylation effector proteins are known to target various types of RNA species, e.g. mRNA, tRNA, rRNA and mt-RNA and alternative non-coding RNA species such as enhancer RNA, vault RNA and circular RNA, understanding the functional role of these differentially expressed effector proteins in the cellular context, is important for understanding of how the m^5C system may be contributing to cognitive disease and TBI. The function of NSUN7 is still relatively uncharacterized although it has been suggested to regulate the stability of enhancer RNAs of genes targeted by the transcriptional co-activator PGC-1α (Aguilo et al., 2016). Nevertheless, PGC-1α is a known mitochondria and energy metabolism regulator associated with neuronal survival and synaptic maintenance, and its dysregulation is suggested to be involved in the pathogenesis of neurodegenerative diseases.

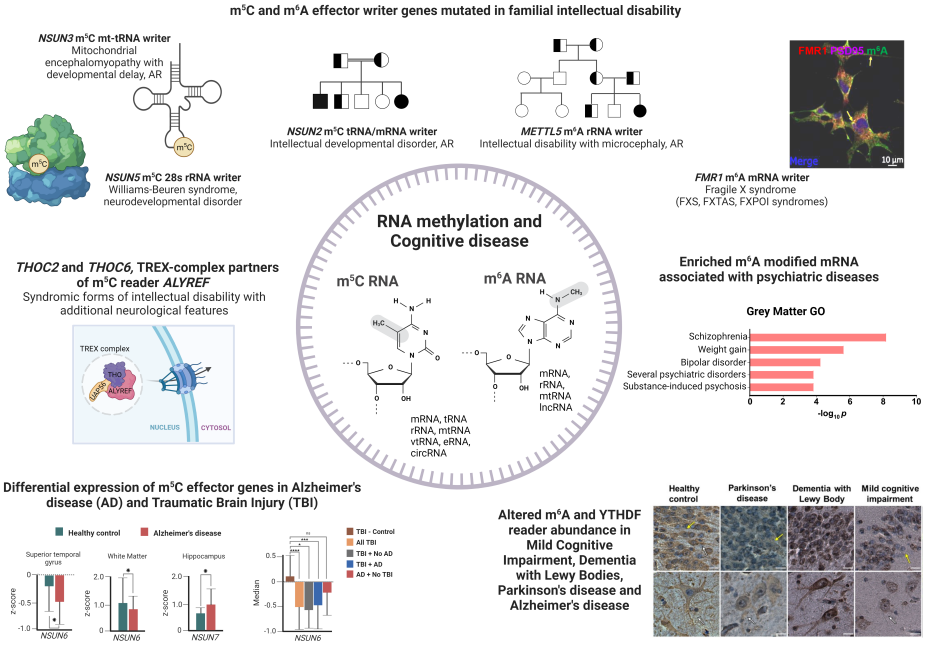

Figure 1 | **Alterations in m^{5}C and m^{6}A RNA modification systems contribute to neurocognitive disorders.**
Mutations within the m^{5}C writer effector protein genes *NSUN2* (Abbasi-Moheb et al., 2012; Khan et al., 2012; Martinez et al., 2012; Sun et al., 2020), *NSUN3* (Paramasivam et al., 2020), and *NSUN5* (Heissenberger et al., 2019) cause intellectual disability and neurodevelopmental disorders. Loss-of-function mutations within the genes *THOC2* and *THOC6* which are TREX-complex protein partners of the m^{5}C reader, ALYREF, cause X-linked and syndromic forms of intellectual disabilities (Kumar et al., 2015; Mattioli et al., 2019). Similarly, familial mutations within the m^{6}A rRNA writer gene *METTL5* cause autosomal recessive intellectual disability with microcephaly (Richard et al., 2019), whereas damaging variants within the m^{6}A mRNA reader gene, *FMR1*, cause fragile X associated disorders (Devys et al., 1993; Zhang et al., 2018). Changes in expression of the m^{5}C writers, *NSUN6* and *NSUN7*, and the reader *ALYREF*, are associated with Alzheimer's disease and Traumatic Brain Injury (PerezGrovas-Saltijeral et al., 2023). Likewise, changes in abundance and the cellular location of m^{6}A-modified RNAs, are significantly altered in Lewy body diseases and mild cognitive impairment brain tissue (Martinez De La Cruz et al., 2023). m^{6}A-modified RNAs are also found enriched in mRNA transcripts which are associated with psychiatric disorders (Martinez De La Cruz et al., 2021). All artwork is original, created with BioRender.com and using information cited in the referenced papers. ALYREF: Aly/REF export factor; FMR1: fragile X messenger ribonucleoprotein 1; m^{5}C: 5-methylcytosine; m^{6}A: N6-methyladenosine; *METTL5*: methyltransferase 5; *NSUN2*: NOP2/Sun domain family, member 2; *NSUN3*: NOP2/Sun RNA methyltransferase 3; *NSUN5*: NOP2/Sun RNA methyltransferase 5; *NSUN7*: NOP2/Sun RNA methyltransferase family member 7; *THOC2*: THO complex subunit 2; *THOC6*: THO complex subunit 6.

Conversely, NSUN6 methylates tRNA^{Thr} and tRNA^{Cys} molecules in human cells, and alongside NSUN2, was recently discovered to determine most mRNA transcription-wide m^{5}C sites. Moreover, NSUN6 is associated with "type II" m^{5}C mRNA sites, which are predicted to be located in the loops of putative hairpin structures. Like NSUN6, type II m^{5}C mRNAs are commonly found in the cytoplasm of cells and in NSUN6 knock-out cells, type II modification correlated with a modest overall increase in translation efficiency (Liu et al., 2021). Furthermore, within *NSUN6* transcript coding regions, a circular RNA transcript is predicted to be encoded, which is evident across several regions of the human brain. As circular RNAs can regulate mRNA through binding to RNA-binding proteins and have been shown to be abnormally expressed in brain diseases (Dube et al., 2019), these observations highlight the potential for a more complex transcriptional regulatory system involving *NSUN6* underlying AD and TBI pathology. Such complex transcriptional regulatory mechanisms may also be involved in disease-driving processes giving rise to neurodevelopmental disorders associated with *NSUN6* (Mattioli et al., 2023). The PerezGrovas-Saltijeral et al. (2023)'s study also highlighted that the expression of the m^{5}C reader ALYREF increased with increasing Braak pathology. This reader is a core regulator of m^{5}C-modified mRNA export out of the nucleus and is a component of the TREX protein complex. Interestingly, mutations in the TREX complex genes, THOC2 and THOC6, are known to cause forms of intellectual disabilities (Kumar et al., 2015), which include neurodevelopmental delay and growth disorders. Furthermore, the recognition and hence nuclear-cytoplasmic shuttling of specific transcripts by ALYREF is reduced with the knockdown of NSUN2 suggesting that NSUN2 is required for both NSUN6 and ALYREF's role in the distribution of m^{5}C mRNA sites as well as for m^{5}C mRNAs nuclear export.

One limitation of the PerezGrovas-Saltijeral et al., 2023's study is that heterogeneous cellular tissue sections were used to examine changes in expression and were therefore not cell-type population or subcellular region specific. Alterations in cell type composition in brain tissue with increased pathological staging is well documented and could be a significant factor influencing effector protein transcript expression. Another drawback of the study is that it is unknown whether changes in effector transcript expression lead to changes in protein abundance and hence whether post-transcription regulatory mechanisms, proteomic changes, or a combination of both mechanisms, are influencing pathological processes. Indeed, the knockdown of mouse *Nsun2*, which like NSUN6, is part of the tRNA regulome, results in decreased tRNA m^{5}C levels, deficits in tRNA glycine codon-specific defects, and a loss of Gly-rich synaptic proteins (Blaze et al., 2021). Whether such consequential molecular changes are co-moderated by *Nsun2* effector transcript mechanisms, remains to be determined. Nevertheless, cell-population-specific high throughput transcriptomic and proteomic studies of dementia and TBI pathological brain tissue will be needed to elucidate methylome-specific mechanisms and consequences.

Evidence of m^{6}A Abundance Changes in Neurocognitive Disease

Like m^{5}C writer effector proteins that can cause intellectual disability, heritable mutations within m^{6}A writer effector proteins are reported to cause neurodevelopmental phenotypes. For example, the m^{6}A rRNA writer, *METTL5*, is reported to cause autosomal recessive intellectual disability syndrome with microcephaly (Richard et al., 2019), and functional studies of truncated *METLL5* suggest that *METTL5* expression rather than cellular localization is changed in the mutated form. In addition, the auxiliary m^{6}A readers, the fragile X gene family, Fragile X messenger ribonucleoprotein 1 (*FMR1*), FMR1 Autosomal Homolog 1 (*FRX1*), and Fragile X Mental Retardation Syndrome-Related Protein 2 (*FXR2*), are associated with causing fragile X syndrome, an X-linked dominant neurodevelopmental disease characterized by moderate to severe intellectual disability (Devys et al., 1993). These RNA binding proteins that interact with m^{6}A sites along mRNAs, are associated with translation at ribosomes, and, form ribonucleoprotein complexes that are involved in trafficking RNAs between the nucleus and cytoplasm. FMR1 also binds to mRNAs at synaptic sites and represses translation by stalling mRNA-linked ribosomes (Darnell et al., 2011).

In 2018, using tissue from mouse brains, Merkurjev et al., first provided evidence that modified RNA existed at synapses. Furthermore, through reducing YTHDF1 reader expression and observing deficits in excitatory synaptic transmission, the same authors indicated that the m^{6}A modification system was involved in synaptic function (Merkurjev et al., 2018). To further elucidate how m^{6}A-modified RNAs might be involved in synaptic function and thereby contribute to brain disease driving mechanisms, Martinez De La Cruz et al. (2021) used advanced microscopy techniques in human differentiated neuronal cell lines and visualized m^{6}A-modified RNAs using an antibody that specifically binds to m^{6}A. They first showed in differentiated cell cultures that modified m^{6}A RNAs are located at both pre- and post- synaptic regions which were confirmed in situ by scanning transmission electron microscopy imaging of single mature synapses. To assess changes in m^{6}A abundance with the YTHDF1, YTHDF3, FMR1 reader, and ALKBH5 eraser proteins after synaptic activation during an early plasticity stage (15 minutes) and late plasticity stage (24 hours), neuronal cells were activated using the glutamate neurotransmitter receptor agonist, NMDA. They found that at activated

glutamatergic post-synaptic sites, both the YTHDF1, YTHDF3, and FMR1 reader and the ALKBH5 eraser proteins increased in co-localization to m^{6}A-modified RNAs during early plasticity. To determine the role of modified RNA and effector proteins in local protein synthesis at synapses undergoing plasticity, Martinez De La Cruz et al. (2021) used biochemical inhibitor assays to stall translation at active ribosomes. They found that after synaptic activation there was a significant increase in modified m^{6}A RNAs at active ribosomes, as well as increases in YTHDF1, YTHDF3, and ALKBH5 proteins with m^{6}A-modified RNAs. Furthermore, they observed that before synaptic activation the eraser protein ALKBH5 was predominantly localized to the nucleus but upon synaptic activation, ALKBH5 is transported from the nucleus to cytoplasmic synapses and co-localized with m^{6}A-modified RNAs at active local ribosomes, demonstrating that m^{6}A demethylation of RNAs by ALKBH5 is involved in synaptic translation during early synaptic plasticity. As m^{6}A methylation has previously been associated with proliferation and differentiation in embryonic stem cells and oligodendrocyte lineage progression (Xu et al., 2020), the authors examined changes in m^{6}A-RNAs and effector protein machinery at synapses during differentiation of human neuronal progenitor stem cells. They found that m^{6}A-modified transcripts were scarce throughout the cytoplasm in undifferentiated and early differentiated cells but in fully differentiated, synaptically mature cells, m^{6}A-modified RNAs were dramatically increased. Furthermore, YTHDF readers exhibited differential roles during developmental stages during synaptic maturation suggesting that their temporal and subcellular abundance may determine specific functions.

The same study also performed high throughput sequencing of m^{6}A sites (m^{6}A-seq) of human parahippocampal grey and white matter tissue and fetal brain tissue from non-diseased individuals to assess m^{6}A methylation patterns across individual transcripts and to characterize modified transcripts belonging to functional categories. They reported that transcripts encoding known m^{6}A writer, reader and eraser, and other RNA binding proteins were themselves m^{6}A-modified and hence provided the first evidence for m^{6}A autoregulation within a RNA methylation system. When assessing differences between the parahippocampal grey and white matter and fetal brain tissue, they observed that only one third of significantly enriched gene ontology terms were shared across all tissues. In particular, the grey and white matter parahippocampus tissue reveal distinct m^{6}A methylome profiles indicating that cellular context was a fundamental factor dictating regulated pathways in the brain. However, both grey and white matter brain tissue did show commonality for m^{6}A enriched transcripts belonging to the disease classification terms, 'psychiatric disease' and 'neurological disease' as well as transcripts associated with other cognitive disease pathologies or brain states, such as addiction disorders, and which have the potential to be reversed.

How changes to m^{6}A-modified mRNAs may be contributing to synaptic pathology and disease is as yet unknown. However, Martinez De La Cruz et al. (2021) hypothesized that m^{6}A mRNA methylation and demethylation by ALKBH5 is integral to a "synaptic tagging and capture" mechanism (Frey and Morris, 1997), i.e. a process proposed to explain how mRNAs are localized to, and available at, specifically activated synapses. Such a tagging mechanism would not only involve the regulation of mRNAs locally but also control the spatiotemporal timing of local synaptic protein synthesis. A second postulated mechanism proposed by the same authors arose from the recent observations that m^{6}A multi-modified mRNAs act as a multivalent scaffold for the YTHDF reader proteins and hence promote liquid-liquid phase separation and the formation of highly condensed membraneless macromolecular aggregates (Gao et al., 2019; Ries et al., 2019; Liu et al., 2020; Wang et al., 2020a). Given that postsynaptic densities and presynaptic active zone regions are recognized to be dynamic molecular assemblies (Zeng et al., 2016), Martinez De La Cruz et al. (2021) hypothesized that during early phase synaptic plasticity, m^{6}A multi-modified RNA-YTHDF interaction contributes to the formation of dynamic postsynaptic and presynaptic nanodomains and as a consequence could moderate distinct neurotransmitter system activity. Disruption to such biological condensates mechanisms could have important consequences both for early-stage synaptopathy as well as the accumulation of large pathological protein aggregates, e.g. amyloid plaques, tau aggregates, or Lewy bodies, which are the hallmark of several neurodegenerative diseases.

To date, few studies have examined directly the localization of m^{6}A-modified RNAs within cells and cell subcompartments in brain tissue and changes in m^{6}A RNA abundance in clinical populations with the disease. Recently, using an antibody specific for m^{6}A, microscopy, and a machine learning approach, Martinez De La Cruz et al. (2023) performed cellular profiling of m^{6}A-RNA abundance and YTHDF1, YTHDF3 reader expression within four regions of the human brain (the cerebellum, and frontal and cingulate cortices and the hippocampus) from aged non-affected individuals and individuals with mild cognitive impairment or Lewy body diseases such as Parkinson's disease and dementia with Lewy bodies (Martinez De La Cruz et al., 2023). In disease tissue, m^{6}A abundance was found to be significantly altered in all brain regions examined but showed consistent opposing patterns across neurodegenerative disorders. In Parkinson's disease tissue, the abundance of m^{6}A-modified RNAs was significantly decreased except in the cerebellum where modified RNAs were significantly more abundant than in healthy tissue. However, within the cerebellum, Purkinje cells showed no, or reduced, m^{6}A-RNAs and YTHDF3 abundance within the cytosol. In comparison, dementia with Lewy bodies tissue showed a significant increase in both modified RNAs and YTHDF3 expression across all regions although, in some areas such as the hippocampus, the pattern of m^{6}A-RNA subcellular localization did not differ from unaffected tissue. Brain tissue from individuals with mild cognitive impairment also showed variability with both significant increases and decreases in m^{6}A abundance across brain areas and similar to dementia with Lewy bodies, in brain areas where there was an overall increase in m^{6}A-RNAs, modified RNAs within dendritic processes were found to be reduced. In the same study, the analysis of mass spectrometry proteomic data from prefrontal tissue (Yu et al., 2020; Johnson et al., 2022), substantiated that the abundance of the reader YTHDF3 and additional m^{6}A effector machinery such as m^{6}A "anti-reader proteins" that preferentially interacting with unmodified m^{6}A RNA binding sequences are significantly changed in individuals with AD, asymptomatic AD/mild cognitive impairment or individuals with have high cognitive resilience. Complementary to these findings, Jiang et al. (2021) reported that the m^{6}A reader HNRNPA2B1 complexes with oligomeric aggregated tau, to regulate RNA translation. Furthermore, using an m^{6}A antibody approach similar to the above study, in late-stage AD temporal cortical tissue, the group found that global HNRNPA2B1, tau, and m^{6}A-RNA modifications are increased in abundance. However, the cytoplasmic distribution of m^{6}A immunoreactivity in severe AD cases was found much broader than Tau or HNRNPA2B1, suggesting that alterations in the m^{6}A modification system begin earlier and hence may be independent of tau pathology. Together, these newly emerging findings suggested that there may be independent molecular switches that govern changes in m^{6}A-modified RNA abundance within cell compartments and functional subcellular structures, and which differ between neurodegenerative diseases and potentially, stage of disease pathology. Moreover, the disrupted localization and abundance of m^{6}A RNAs in such diseases are consistent with evidence for alterations in the control of RNA translation contributing to the formation of protein aggregate neurotoxic structures and suggest that manipulation of epitranscriptomic processes influencing translational control may lead to new therapeutic approaches for neurodegenerative diseases.

Advances in Technologies and Future Outlook

Our ability to investigate RNA modification profiles has experienced significant growth with the advancement of experimental, computational, and high-throughput technologies. Early work that aimed to detect and quantify RNA methylation types was based on the principle that modified nucleotides have distinct physicochemical properties. Several methods were established and routinely used including thin-layer chromatography, high-performance liquid chromatography, and mass spectrometry (Thuring et al., 2016). Even though high sensitivity and low starting material were an advantage to performing these methods on tissues from clinical samples, the lack of sequence information has been a limitation. The current technologies for the identification of transcriptome-wide m^{6}A modifications rely on three main approaches: one, antibody-based techniques; two, chemical-based techniques; and three, enzyme/protein-based techniques. Antibody-based sequencing methods including m^{6}A-Seq and MeRIP-Seq, use antibodies that specifically bind to modified ribonucleotides (Dominissini et al., 2012; Linder et al., 2015; Molinie et al., 2016; Koh et al., 2019). However, the lack of single base resolution and quantitative data indicating how many transcripts are modified at specific sites is a drawback of many such antibody-based approaches. Chemical-based sequencing methods such as m^{6}A-SEAL-Seq and m^{6}A-Label-Seq utilize chemical compounds that specifically react with modified bases (Shu et al., 2020; Wang et al., 2020b). These methods have the advantage of high accuracy and direct identification of modified bases but they have the limitation of lacking stoichiometric information, i.e., the ability to quantify the proportion of adenosine bases that are modified and occupancy at each site. Techniques such as DART-Seq, Mazter-Seq/m^{6}A-REF-Seq, and m^{6}A-SAC-Seq, scDART-Seq utilize special enzymes or RNA modification-related proteins to selectively discriminate modified and unmodified bases (Garcia-Campos et al., 2019). These methods also have the advantage of high accuracy as they are at the single nucleotide resolution, but can have methodological drawbacks such as m^{6}A binding motif preference, biased sequence context, and low sensitivity for low-abundance m^{6}A sites.

Recently, two additional m^{6}A mRNA mapping methods have been established by two independent research teams. Liu et al. (2022) reported GLORI, a transcriptome-wide identification technology based on glyoxal and nitrite-assisted deamination of unmethylated adenosine that enables an unbiased absolute quantification method of m^{6}A at a single-base resolution. Using GLORI, the authors reported more than 175,000 m^{6}A sites in human HEK293T cells and that highly m^{6}A-modified sites occur in clusters across the transcriptome, and such m^{6}A clustered sites are associated with significantly lower expression (Liu et al., 2022). Similarly, in 2023, Xiao et al. developed TadA-assisted N6-methyladenosine sequencing that also employs an enzymatic global A deamination method by adenosine-to-inosine conversion using a hyperactive transfer RNA adenosine deaminase (TadA) variant. Using TadA-assisted N6-methyladenosine sequencing, site-specific transcriptome-wide distribution of mRNA m^{6}A in HeLa and mouse embryonic stem cells was performed using low input amounts (~50 ng) and circa 25,000 m^{6}A sites were reported suggesting that TadA-assisted N6-methyladenosine sequencing is less sensitive to sites of low methylation levels.

Advances in sequencing technologies have also led to a significant increase in the number of studies examining m^{5}C RNA methylation profiles. Various methods have been developed to detect RNA m^{5}C methylation, including m^{5}C RNA immunoprecipitation sequencing, 5-azacytidine-mediated RNA immunoprecipitation, methylation-individual nucleotide resolution crosslinking immunoprecipitation (miCLIP), and RNA bisulfite sequencing (Edelheit et al., 2013; Hussain et al., 2013; Khoddami and Cairns, 2013). Among these approaches, m^{5}C RNA immunoprecipitation sequencing, 5-azacytidine-mediated RNA immunoprecipitation, and miCLIP are based

on immunoprecipitation technique, primarily using anti-m^5C antibodies for detection followed by high throughput sequencing. 5-Azacytidine-mediated RNA immunoprecipitation and miCLIP target the specific catalytic sites of RNA m^5C methyltransferases by immunoprecipitation and provide a transcriptome-wide view of mRNA m^5C at a single nucleotide resolution. However, 5-azacytidine is highly toxic for cells and since miCLIP utilizes mutated RNA m^5C methyltransferases, it is time-consuming and expensive (Khoddami and Cairns, 2013; George et al., 2017). RNA bisulfite sequencing is the gold standard and the most used method for m^5C modification detection and can provide a transcriptome-wide landscape of m^5C profile with high specificity at a single nucleotide resolution. However, it still requires technical improvement as experimental temperature and pH can lead to RNA damage, and, owing to bisulfite-induced damage, this method needs higher amounts of input RNA. For such reasons, and to the authors' knowledge, as yet no studies have assessed single base transcriptome-wide mRNA m^5C methylation using RNA bisulfite sequencing in cohorts with neurocognitive diseases.

The current technical limitations of both m^6A and m^5C mapping techniques may be overcome by third-generation sequencing such as direct long-read RNA Oxford Nanopore Sequencing Technologies and Pacific Biosciences of California (Garalde et al., 2018; Mahmoud et al., 2023). Nanopore sequencing, as opposed to next-generation RNA sequencing, can sequence RNA without the need for reverse transcription and PCR amplification. However, there are still challenges for Oxford Nanopore Sequencing Technologies such as significant constraints of the current software tools available to call modification sites accurately (Leger et al., 2021; Mateos et al., 2023).

To conclude, deleterious mutations identified within m^5C and m^6A effector protein genes provided the first evidence that RNA modification systems contribute to brain function and cognitive dysfunction. Quantifying m^6A modification abundance and alterations in RNA and protein expression of the m^5C and m^6A writer, reader, and erasers in brain tissue and across cognitive disease forms, indicate significant changes at the tissue, cell, and subcompartment level. However, the contrasting aberrant patterns identified across conditions suggest that there are differences in disrupted molecular mechanisms driving disease. With such accumulating evidence that alterations in RNA methylation systems are associated with the occurrence of disease, it has been anticipated that RNA modifications could also serve as promising biomarkers for early clinical diagnosis, targeted drug therapies, or prediction of treatment responses. The next-generation sequencing methods such as Oxford Nanopore Sequencing Technologies will play a central part in understanding changes in clinical populations including individuals with neurocognitive diseases.

Author contributions: *Manuscript preparation, manuscript editing, and manuscript review: APS, MDÖ, and HMK. All authors approved the final version of the manuscript.*
Conflicts of interest: *The authors declare no conflicts of interest.*
Data availability statement: *Not applicable.*

References

Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, Wieczorek D, Motazacker MM, Esmaeeli-Nieh S, Cremer K, Weissmann R, Tzschach A, Garshasbi M, Abedini SS, Najmabadi H, Ropers HH, Sigrist SJ, Kuss AW (2012) Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 90:847-855.

Aguilo F, Li S, Balasubramaniyan N, Sancho A, Benko S, Zhang F, Vashisht A, Rengasamy M, Andino B, Chen CH (2016) Deposition of 5-methylcytosine on enhancer RNAs enables the coactivator function of PGC-1α. Cell Rep 14:479-492.

Arango D, Sturgill D, Yang R, Kanai T, Bauer P, Roy J, Wang Z, Hosogane M, Schiffers S, Oberdoerffer S (2022) Direct epitranscriptomic regulation of mammalian translation initiation through N4-acetylcytidine. Mol Cell 82:2912.

Arguello AE, DeLiberto AN, Kleiner RE (2017) RNA chemical proteomics reveals the N6-methyladenosine (m6A)-regulated protein–RNA interactome. J Am Chem Soc 139:17249-17252.

Blaze J, Navickas A, Phillips HL, Heissel S, Plaza-Jennings A, Miglani S, Asgharian H, Foo M, Katanski CD, Watkins CP, Pennington ZT, Javidfar B, Espeso-Gil S, Rostandy B, Alwaseem H, Hahn CG, Molina H, Cai DJ, Pan T, Yao WD, et al. (2021) Neuronal Nsun2 deficiency produces tRNA epitranscriptomic alterations and proteomic shifts impacting synaptic signaling and behavior. Nat Commun 12:4913.

Boccaletto P, Machnicka MA, Purta E, Piatkowski P, Baginski B, Wirecki TK, de Crécy-Lagard V, Ross R, Limbach PA, Kotter A, Helm M, Bujnicki JM (2018) MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res 46:D303-d307.

Bourgeois G, Ney M, Gaspar I, Aigueperse C, Schaefer M, Kellner S, Helm M, Motorin Y (2015) Eukaryotic rRNA modification by yeast 5-methylcytosine-methyltransferases and human proliferation-associated antigen p120. PLoS One 10:e0133321.

Braak E, Strotkamp B, Braak H (1991) Parvalbumin-immunoreactive structures in the hippocampus of the human adult. Cell Tissue Res 264:33-48.

Brown JA, Kinzig CG, DeGregorio SJ, Steitz JA (2016) Methyltransferase-like protein 16 binds the 3′-terminal triple helix of MALAT1 long noncoding RNA. Proc Natl Acad Sci U S A 113:14013-14018.

Brzezicha B, Schmidt M, Makałowska I, Jarmołowski A, Pieńkowska J, Szweykowska-Kulińska Z (2006) Identification of human tRNA: m5C methyltransferase catalysing intron-dependent m5C formation in the first position of the anticodon of the pre-tRNA Leu (CAA). Nucleic Acids Res 34:6034-6043.

Chen CY, Gherzi R, Andersen JS, Gaietta G, Jurchott K, Royer HD, Mann M, Karin M (2000) Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. Genes Dev 14:1236-1248.

Chen H, Yang H, Zhu X, Yadav T, Ouyang J, Truesdell SS, Tan J, Wang Y, Duan M, Wei L, Zou L, Levine AS, Vasudevan S, Lan L (2020) m5C modification of mRNA serves a DNA damage code to promote homologous recombination. Nat Commun 11:2834.

Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, Stone EF, Chen C, Fak JJ, Chi SW, Licatalosi DD, Richter JD, Darnell RB (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146:247-261.

Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL (1993) The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. Nat Genet 4:335-340.

Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, Cesarkas K, Jacob-Hirsch J, Amariglio N, Kupiec M, Sorek R, Rechavi G (2012) Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 485:201-206.

Dube U, Del-Aguila JL, Li Z, Budde JP, Jiang S, Hsu S, Ibanez L, Fernandez MV, Farias F, Norton J, Gentsch J, Wang F, Allegri R, Amtashar F, Benzinger T, Berman S, Bodge C, Brandon S, Brooks W, Buck J, et al. (2019) An atlas of cortical circular RNA expression in Alzheimer disease brains demonstrates clinical and pathological associations. Nat Neurosci 22:1903-1912.

Dufu K, Livingstone MJ, Seebacher J, Gygi SP, Wilson SA, Reed R (2010) ATP is required for interactions between UAP56 and two conserved mRNA export proteins, Aly and CIP29, to assemble the TREX complex. Genes Dev 24:2043-2053.

Edelheit S, Schwartz S, Mumbach MR, Wurtzel O, Sorek R (2013) Transcriptome-wide mapping of 5-methylcytidine RNA modifications in bacteria, archaea, and yeast reveals m5C within archaeal mRNAs. PLoS Genet 9:e1003602.

Eyler DE, Franco MK, Batool Z, Wu MZ, Dubuke ML, Dobosz-Bartoszek M, Jones JD, Polikanov YS, Roy B, Koutmou KS (2019) Pseudouridinylation of mRNA coding sequences alters translation. Proc Natl Acad Sci U S A 116:23068-23074.

Falnes PO, Bjoras M, Aas PA, Sundheim O, Seeberg E (2004) Substrate specificities of bacterial and human AlkB proteins. Nucleic Acids Res 32:3456-3461.

Feng C, Liu Y, Wang G, Deng Z, Zhang Q, Wu W, Tong Y, Cheng C, Chen Z (2014) Crystal structures of the human RNA demethylase Alkbh5 reveal basis for substrate recognition. J Biol Chem 289:11571-11583.

Finet O, Yague-Sanz C, Kruger LK, Tran P, Migeot V, Louski M, Nevers A, Rougemaille M, Sun J, Ernst FGM, Wacheul L, Wery M, Morillon A, Dedon P, Lafontaine DLJ, Hermand D (2022) Transcription-wide mapping of dihydrouridine reveals that mRNA dihydrouridylation is required for meiotic chromosome segregation. Mol Cell 82:404-419.

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533-536.

Gao Y, Pei G, Li D, Li R, Shao Y, Zhang QC, Li P (2019) Multivalent m(6)A motifs promote phase separation of YTHDF proteins. Cell Res 29:767-769.

Garalde DR, Snell EA, Jachimowicz D, Sipos B, Lloyd JH, Bruce M, Pantic N, Admassu T, James P, Warland A, Jordan M, Ciccone J, Serra S, Keenan J, Martin S, McNeill L, Wallace EJ, Jayasinghe L, Wright C, Blasco J, et al. (2018) Highly parallel direct RNA sequencing on an array of nanopores. Nat Methods 15:201-206.

Garcia-Campos MA, Edelheit S, Toth U, Safra M, Shachar R, Viukov S, Winkler R, Nir R, Lasman L, Brandis A, Hanna JH, Rossmanith W, Schwartz S (2019) Deciphering the "m(6)A code" via antibody-independent quantitative profiling. Cell 178:731-747.

Gardner OK, Wang L, Van Booven D, Whitehead PL, Hamilton-Nelson KL, Adams LD, Starks TD, Hofmann NK, Vance JM, Cuccaro ML, Martin ER, Byrd GS, Haines JL, Bush WS, Beecham GW, Pericak-Vance MA, Griswold AJ (2019) RNA editing alterations in a multi-ethnic Alzheimer disease cohort converge on immune and endocytic molecular pathways. Hum Mol Genet 28:3053-3061.

George H, Ule J, Hussain S (2017) Illustrating the epitranscriptome at nucleotide resolution using methylation-iCLIP (miCLIP). Methods Mol Biol 1562:91-106.

Haag S, Sloan KE, Ranjan N, Warda AS, Kretschmer J, Blessing C, Hubner B, Seikowski J, Dennerlein S, Rehling P, Rodnina MV, Hobartner C, Bohnsack MT (2016) NSUN3 and ABH1 modify the wobble position of mt-tRNAMet to expand codon recognition in mitochondrial translation. EMBO J 35:2104-2119.

Han Z, Niu T, Chang J, Lei X, Zhao M, Wang Q, Cheng W, Wang J, Feng Y, Chai J (2010) Crystal structure of the FTO protein reveals basis for its substrate specificity. Nature 464:1205.

Heissenberger C, Liendl L, Nagelreiter F, Gonskikh Y, Yang G, Stelzer EM, Krammer TL, Micutkova L, Vogt S, Kreil DP, Sekot G, Siena E, Poser I, Harreither E, Linder A, Ehret V, Helbich TH, Grillari-Voglauer R, Jansen-Dürr P, Koš M, et al. (2019) Loss of the ribosomal RNA methyltransferase NSUN5 impairs global protein synthesis and normal growth. Nucleic Acids Res 47:11807-11825.

Huang T, Chen W, Liu J, Gu N, Zhang R (2019) Genome-wide identification of mRNA 5-methylcytosine in mammals. Nat Struct Mol Biol 26:380-388.

Hussain S, Sajini AA, Blanco S, Dietmann S, Lombard P, Sugimoto Y, Paramor M, Gleeson JG, Odom DT, Ule J, Frye M (2013) NSun2-mediated cytosine-5 methylation of vault noncoding RNA determines its processing into regulatory small RNAs. Cell Rep 4:255-261.

Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, Dickson DW, Duyckaerts C, Frosch MP, Masliah E, Mirra SS, Nelson PT, Schneider JA, Thal DR, Thies B, Trojanowski JQ, Vinters HV, Montine TJ (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 8:1-13.

Jiang L, Lin W, Zhang C, Ash PEA, Verma M, Kwan J, van Vliet E, Yang Z, Cruz AL, Boudeau S, Maziuk BF, Lei S, Song J, Alvarez VE, Hovde S, Abisambra JF, Kuo MH, Kanaan N, Murray ME, Crary JF, et al. (2021) Interaction of tau with HNRNPA2B1 and N6-methyladenosine RNA mediates the progression of tauopathy. Mol Cell 81:4209-4227.

Johnson ECB, Carter EK, Dammer EB, Duong DM, Gerasimov ES, Liu Y, Liu J, Betarbet R, Ping L, Yin L, Serrano GE, Beach TG, Peng J, De Jager PL, Haroutunian V, Zhang B, Gaiteri C, Bennett DA, Gearing M, Wingo TS, et al. (2022) Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA level. Nat Neurosci 25:213-225.

Kawarada L, Suzuki T, Ohira T, Hirata S, Miyauchi K, Suzuki T (2017) ALKBH1 is an RNA dioxygenase responsible for cytoplasmic and mitochondrial tRNA modifications. Nucleic Acids Res 45:7401-7415.
Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V, Vincent AK, Malli R, Ali G, Khan FS, Ishak GE, Doherty D, Weksberg R, Ayub M, Windpassinger C, Ibrahim S, Frye M, Ansar M, Vincent JB (2012) Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. Am J Hum Genet 90:856-863.
Khoddami V, Cairns BR (2013) Identification of direct targets and modified bases of RNA cytosine methyltransferases. Nat Biotechnol 31:458-464.
Koh CWQ, Goh YT, Goh WSS (2019) Atlas of quantitative single-base-resolution N6-methyl-adenine methylomes. Nat Commun 10:5636.
Kumar R, Corbett MA, van Bon BW, Woenig JA, Weir L, Douglas E, Friend KL, Gardner A, Shaw M, Jolly LA, Tan C, Hunter MF, Hackett A, Field M, Palmer EE, Leffler M, Rogers C, Boyle J, Bienek M, Jensen C, et al. (2015) THOC2 mutations implicate mRNA-export pathway in X-linked intellectual disability. Am J Hum Genet 97:302-310.
Leger A, Amaral PP, Pandolfini L, Capitanchik C, Capraro F, Miano V, Migliori V, Toolan-Kerr P, Sideri T, Enright AJ, Tzelepis K, van Werven FJ, Luscombe NM, Barbieri I, Ule J, Fitzgerald T, Birney E, Leonardi T, Kouzarides T (2021) RNA modifications detection by comparative Nanopore direct RNA sequencing. Nat Commun 12:7198.
Lentini JM, Bargabos R, Chen C, Fu D (2022) Methyltransferase METTL8 is required for 3-methylcytosine modification in human mitochondrial tRNAs. J Biol Chem 298:101788.
Li Y, Bedi RK, Moroz-Omori EV, Caflisch A (2020) Structural and dynamic insights into redundant function of YTHDF proteins. J Chem Inf Model 60:5932-5935.
Linder B, Grozhik AV, Olarerin-George AO, Meydan C, Mason CE, Jaffrey SR (2015) Single-nucleotide-resolution mapping of m6A and m6Am throughout the transcriptome. Nat Methods 12:767-772.
Liu C, Sun H, Yi Y, Shen W, Li K, Xiao Y, Li F, Li Y, Hou Y, Lu B, Liu W, Meng H, Peng J, Yi C, Wang J (2022) Absolute quantification of single-base m(6)A methylation in the mammalian transcriptome using GLORI. Nat Biotechnol 41:355-366.
Liu J, Huang T, Zhang Y, Zhao T, Zhao X, Chen W, Zhang R (2021) Sequence- and structure-selective mRNA m(5)C methylation by NSUN6 in animals. Natl Sci Rev 8:nwaa273.
Liu SY, Feng Y, Wu JJ, Zou ML, Sun ZL, Li X, Yuan FL (2020) m(6) A facilitates YTHDF-independent phase separation. J Cell Mol Med 24:2070-2072.
Ma CJ, Ding JH, Ye TT, Yuan BF, Feng YQ (2019) AlkB homologue 1 demethylates N(3)-methylcytidine in mRNA of mammals. ACS Chem Biol 14:1418-1425.
Maden BE (1986) Identification of the locations of the methyl groups in 18 S ribosomal RNA from Xenopus laevis and man. J Mol Biol 189:681-699.
Maden BE (1988) Locations of methyl groups in 28 S rRNA of Xenopus laevis and man. Clustering in the conserved core of molecule. J Mol Biol 201:289-314.
Mahmoud M, Huang Y, Garimella K, Audano PA, Wan W, Prasad N, Handsaker RE, Hall S, Pionzio A, Schatz MC, Talkowski ME, Eichler EE, Levy SE, Sedlazeck FJ (2023) Utility of long-read sequencing for All of Us. bioRxiv doi:10.1101/2023.01.23.525236.
Marques-Coelho D, Iohan LdCC, Melo de Farias AR, Flaig A, Letournel F, Martin-Négrier M-L, Chapon F, Faisant M, Godfraind C, Maurage CA, Deramecourt V, Duchesne M, Meyronnet D, Streichenberger N, de Paula AM, Rigau V, Vandenbos-Burel F, Duyckaerts C, Seilhean D, Milin S, et al. (2021) Differential transcript usage unravels gene expression alterations in Alzheimer's disease human brains. NPJ Aging Mech Dis 7:2.
Martinez De La Cruz B, Markus R, Malla S, Haig MI, Gell C, Sang F, Bellows E, Sherif MA, McLean D, Lourdusamy A, Self T, Bodi Z, Smith S, Fay M, Macdonald IA, Fray R, Knight HM (2021) Modifying the m(6)A brain methylome by ALKBH5-mediated demethylation: a new contender for synaptic tagging. Mol Psychiatry 26:7141-7153.
Martinez De La Cruz B, Gell C, Markus R, Macdonald I, Fray R, Knight HM (2023) m(6) A mRNA methylation in human brain is disrupted in Lewy body disorders. Neuropathol Appl Neurobiol 49:e12885.
Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, Frye M, Al-Gazali L, Gleeson JG (2012) Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a Dubowitz-like syndrome. J Med Genet 49:380-385.
Mateos PA, Sethi AJ, Ravindran A, Guarnacci M, Srivastava A, Xu J, Woodward K, Yuen ZWS, Mahmud S, Kanchi M, Zhou Y, Sneddon A, Hamilton W, Gao J, Starrs LM, Hayashi R, Wickramasinghe V, Zarnack K, Preiss T, Burgio G, et al. (2023) Simultaneous identification of m6A and m5C reveals coordinated RNA modification at single-molecule resolution. bioRxiv doi:10.1101/2022.03.14.484124.
Mattioli F, Isidor B, Abdul-Rahman O, Gunter A, Huang L, Kumar R, Beaulieu C, Gecz J, Innes M, Mandel JL, Piton A (2019) Clinical and functional characterization of recurrent missense variants implicated in THOC6-related intellectual disability. Hum Mol Genet 28:952-960.
Mattioli F, Worpenberg L, Li CT, Ibrahim N, Naz S, Sharif S, Firouzabadi SG, Vosoogh S, Saraeva-Lamri R, Raymond L, Trujillo C, Guex N, Antonarakis SE, Ansar M, Darvish H, Liu RJ, Roignant JY, Reymond A (2023) Biallelic variants in NSUN6 cause an autosomal recessive neurodevelopmental disorder. Genet Med 25:100900.
Merkurjev D, Hong WT, Iida K, Oomoto I, Goldie BJ, Yamaguti H, Ohara T, Kawaguchi SY, Hirano T, Martin KC, Pellegrini M, Wang DO (2018) Synaptic N(6)-methyladenosine (m(6)A) epitranscriptome reveals functional partitioning of localized transcripts. Nat Neurosci 21:1004-1014.
Metodiev MD, Spahr H, Loguercio Polosa P, Meharg C, Becker C, Altmueller J, Habermann B, Larsson NG, Ruzzenente B (2014) NSUN4 is a dual function mitochondrial protein required for both methylation of 12S rRNA and coordination of mitoribosomal assembly. PLoS Genet 10:e1004110.
Miller JA, Guillozet-Bongaarts A, Gibbons LE, Postupna N, Renz A, Beller AE, Sunkin SM, Ng L, Rose SE, Smith KA, Szafer A, Barber C, Bertagnolli D, Bickley K, Brouner K, Caldejon S, Chapin M, Chua ML, Coleman NM, Cudaback E, et al. (2017) Neuropathological and transcriptomic characteristics of the aged brain. Elife 6:e31126.
Molinie B, Wang J, Lim KS, Hillebrand R, Lu ZX, Van Wittenberghe N, Howard BD, Daneshvar K, Mullen AC, Dedon P, Xing Y, Giallourakis CC (2016) m(6)A-LAIC-seq reveals the census and complexity of the m(6)A epitranscriptome. Nat Methods 13:692-698.
Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, Hosseini M, Behjati F, Haas S, Jamali P, Zecha A, Mohseni M, Puttmann L, Vahid LN, Jensen C, Moheb LA, Bienek M, Larti F, Mueller I, Weissmann R, et al. (2011) Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature 478:57-63.
Nakano S, Suzuki T, Kawarada L, Iwata H, Asano K, Suzuki T (2016) NSUN3 methylase initiates 5-formylcytidine biogenesis in human mitochondrial tRNA(Met). Nat Chem Biol 12:546-551.
Ougland R, Zhang CM, Liiv A, Johansen RF, Seeberg E, Hou YM, Remme J, Falnes PO (2004) AlkB restores the biological function of mRNA and tRNA inactivated by chemical methylation. Mol Cell 16:107-116.
Paramasivam A, Meena AK, Venkatapathi C, Pitceathly RDS, Thangaraj K (2020) Novel biallelic NSUN3 variants cause early-onset mitochondrial encephalomyopathy and seizures. J Mol Neurosci 70:1962-1965.
Pendleton KE, Chen B, Liu K, Hunter OV, Xie Y, Tu BP, Conrad NK (2017) The U6 snRNA m(6)A methyltransferase METTL16 regulates SAM synthetase intron retention. Cell 169:824-835.
PerezGrovas-Saltijeral A, Rajkumar AP, Knight HM (2023) Differential expression of m(5)C RNA methyltransferase genes NSUN6 and NSUN7 in Alzheimer's disease and traumatic brain injury. Mol Neurobiol 60:2223-2235.
Raffetseder U, Frye B, Rauen T, Jurchott K, Royer HD, Jansen PL, Mertens PR (2003) Splicing factor SRp30c interaction with Y-box protein-1 confers nuclear YB-1 shuttling and alternative splice site selection. J Biol Chem 278:18241-18248.
Richard EM, Polla DL, Assir MZ, Contreras M, Shahzad M, Khan AA, Razzaq A, Akram J, Tarar MN, Blanpied TA, Ahmed ZM, Abou Jamra R, Wieczorek D, van Bokhoven H, Riazuddin S, Riazuddin S (2019) Bi-allelic variants in METTL5 cause autosomal-recessive intellectual disability and microcephaly. Am J Hum Genet 105:869-878.
Ries RJ, Zaccara S, Klein P, Olarerin-George A, Namkoong S, Pickering BF, Patil DP, Kwak H, Lee JH, Jaffrey SR (2019) m(6)A enhances the phase separation potential of mRNA. Nature 571:424-428.
Schumann U, Zhang HN, Sibbritt T, Pan A, Horvath A, Gross S, Clark SJ, Yang L, Preiss T (2020) Multiple links between 5-methylcytosine content of mRNA and translation. BMC Biol 18:40.
Shu X, Cao J, Cheng M, Xiang S, Gao M, Li T, Ying X, Wang F, Yue Y, Lu Z, Dai Q, Cui X, Ma L, Wang Y, He C, Feng X, Liu J (2020) A metabolic labeling method detects m(6)A transcriptome-wide at single base resolution. Nat Chem Biol 16:887-895.
Sun S, Chen L, Wang Y, Wang J, Li N, Wang X (2020) Further delineation of autosomal recessive intellectual disability syndrome caused by homozygous variant of the NSUN2 gene in a chinese pedigree. Mol Genet Genomic Med 8:e1518.
Thuring K, Schmid K, Keller P, Helm M (2016) Analysis of RNA modifications by liquid chromatography-tandem mass spectrometry. Methods 107:48-56.
Van Haute L, Dietmann S, Kremer L, Hussain S, Pearce SF, Powell CA, Rorbach J, Lantaff R, Blanco S, Sauer S, Kotzaeridou U, Hoffmann GF, Memari Y, Kolb-Kokocinski A, Durbin R, Mayr JA, Frye M, Prokisch H, Minczuk M (2016) Deficient methylation and formylation of mt-tRNA(Met) wobble cytosine in a patient carrying mutations in NSUN3. Nat Commun 7:12039.
Van Haute L, Lee SY, McCann BJ, Powell CA, Bansal D, Vasiliauskaite L, Garone C, Shin S, Kim JS, Frye M, Gleeson JG, Miska EA, Rhee HW, Minczuk M (2019) NSUN2 introduces 5-methylcytosines in mammalian mitochondrial tRNAs. Nucleic Acids Res 47:8720-8733.
van Tran N, Ernst FGM, Hawley BR, Zorbas C, Ulryck N, Hackert P, Bohnsack KE, Bohnsack MT, Jaffrey SR, Graille M, Lafontaine DLJ (2019) The human 18S rRNA m6A methyltransferase METTL5 is stabilized by TRMT112. Nucleic Acids Res 47:7719-7733.
Wang J, Wang L, Diao J, Shi YG, Shi Y, Ma H, Shen H (2020a) Binding to m(6)A RNA promotes YTHDF2-mediated phase separation. Protein Cell 11:304-307.
Wang Y, Xiao Y, Dong S, Yu Q, Jia G (2020b) Antibody-free enzyme-assisted chemical approach for detection of N(6)-methyladenosine. Nat Chem Biol 16:896-903.
Warda AS, Kretschmer J, Hackert P, Lenz C, Urlaub H, Hobartner C, Sloan KE, Bohnsack MT (2017) Human METTL16 is a N(6)-methyladenosine (m(6)A) methyltransferase that targets pre-mRNAs and various non-coding RNAs. EMBO Rep 18:2004-2014.
Xiao YL, Liu S, Ge R, Wu Y, He C, Chen M, Tang W (2023) Transcriptome-wide profiling and quantification of N(6)-methyladenosine by enzyme-assisted adenosine deamination. Nat Biotechnol doi: 10.1038/s41587-022-01587-6.
Xu H, Dzhashiashvili Y, Shah A, Kunjamma RB, Weng YL, Elbaz B, Fei Q, Jones JS, Li YI, Zhuang X, Ming GL, He C, Popko B (2020) m(6)A mRNA methylation is essential for oligodendrocyte maturation and CNS myelination. Neuron 105:293-309.
Xue S, Xu H, Sun Z, Shen H, Chen S, Ouyang J, Zhou Q, Hu X, Cui H (2019) Depletion of TRDMT1 affects 5-methylcytosine modification of mRNA and inhibits HEK293 cell proliferation and migration. Biochem Biophys Res Commun 520:60-66.
Yang X, Yang Y, Sun BF, Chen YS, Xu JW, Lai WY, Li A, Wang X, Bhattarai DP, Xiao W, Sun HY, Zhu Q, Ma HL, Adhikari S, Sun M, Hao YJ, Zhang B, Huang CM, Huang N, Jiang GB, et al. (2017) 5-methylcytosine promotes mRNA export- NSUN2 as the methyltransferase and ALYREF as an m(5)C reader. Cell Res 27:606-625.
Yang Y, Wang L, Han X, Yang WL, Zhang M, Ma HL, Sun BF, Li A, Xia J, Chen J, Heng J, Wu B, Chen YS, Xu JW, Yang X, Yao H, Sun J, Lyu C, Wang HL, Huang Y, et al. (2019) RNA 5-methylcytosine facilitates the maternal-to-zygotic transition by preventing maternal mRNA decay. Mol Cell 75:1188-1202.
Yu L, Tasaki S, Schneider JA, Arfanakis K, Duong DM, Wingo AP, Wingo TS, Kearns N, Thatcher GRJ, Seyfried NT, Levey AI, De Jager PL, Bennett DA (2020) Cortical proteins associated with cognitive resilience in community-dwelling older persons. JAMA Psychiatry 77:1172-1180.
Zeng M, Shang Y, Araki Y, Guo T, Huganir RL, Zhang M (2016) Phase transition in postsynaptic densities underlies formation of synaptic complexes and synaptic plasticity. Cell 166:1163-1175.
Zhang F, Kang Y, Wang M, Li Y, Xu T, Yang W, Song H, Wu H, Shu Q, Jin P (2018) Fragile X mental retardation protein modulates the stability of its m6A-marked messenger RNA targets. Hum Mol Genet 27:3936-3950.
Zhang X, Liu Z, Yi J, Tang H, Xing J, Yu M, Tong T, Shang Y, Gorospe M, Wang W (2012) The tRNA methyltransferase NSun2 stabilizes p16INK(4) mRNA by methylating the 3'-untranslated region of p16. Nat Commun 3:712.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y