Proper development of the nervous system is critical for its function, and deficits in neural development have been implicated in many brain disorders. A precise and predictable developmental schedule requires highly coordinated gene expression programs that orchestrate the dynamics of the developing brain. Especially, recent discoveries have been showing that various mRNA chemical modifications can affect RNA metabolism including decay, transport, splicing, and translation in cell type- and tissue-specific manner, leading to the emergence of the field of epitranscriptomics. Moreover, accumulating evidences showed that certain types of RNA modifications are predominantly found in the developing brain and their dysregulation disrupts not only the developmental processes, but also neuronal activities, suggesting that epitranscriptomic mechanisms play critical post-transcriptional regulatory roles in development of the brain and etiology of brain disorders. Here, we review recent advances in our understanding of molecular regulation on transcriptome plasticity by RNA modifications in neurodevelopment and how alterations in these RNA regulatory programs lead to human brain disorders. [BMB Reports 2020; 53(11): 551-564]

INTRODUCTION

During development of the central nervous system (CNS), a wide variety of unique cell types are generated with temporal and spatial precision. In the early embryo, neural stem cells sequentially produce neurons and glial cells that orderly migrate to assemble into neural circuits (1). These processes are accurately curated by dynamic gene expression programs that guide the patterning of the differentiation potential of progenitors and the divergence of neuronal/glial lineages. Epigenetic mechanisms, such as DNA methylation, histone modification, and changes in chromatin architecture, have been extensively investigated in neural development over the last decades (2). In addition, post-transcriptional regulation mediated by chemical modification on RNA provides an additional control of fine-tuned gene expression patterns requiring for the proper development and activity of the nervous system (3). Especially, the advance of high-throughput sequencing approaches and quantitative mass spectrometric analysis revealed the existence of more than 160 types of RNA modifications, including N6-methyladenosine (m^6^A), 5-methylcytosine (m^5^C), pseudouridine (ψ), N6,2′-O-dimethyladenosine (m^2′^Am), N1-methyladenosine (m^1^A) and N4-acetylcytidine (ac^4^C) (4, 5). Different RNA species, such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), messenger RNAs (mRNAs), and non-coding RNAs (ncRNAs) are often post-transcriptionally modified by chemical modifications.

The transcriptomic plasticity, conferred by post-transcriptional regulation including RNA modification, editing, and alternative splicing, is recognized as a fundamental mechanism driving proteomic diversity (6). Notably, recent studies suggested that RNA modifications influence almost all aspects of RNA metabolism, including stability, splicing, localization, and translation. These regulations by RNA modifications have physiologically important functions in many different biological contexts in cell- and/or tissue type-specific manners, eventually opening a newly emerging field, known as the epitranscriptomics.

Several epitranscriptome mapping studies showed that the embryonic and adult tissues that build up the mammalian CNS contain relatively higher abundance of RNA modifications than other organs. For example, higher level of m^6^A and m^1^Am in mRNA exist both in human and mouse brain tissues compared to non-brain tissues (7, 8), and it was also reported that either m^5^C or ψ level was significantly higher in the brain than those in other tissues (9, 10). These results imply that fine-tuning of gene expression by RNA modifications may regulate development and function of the CNS. Therefore, dysregulation of these post-translational regulatory programs often manifests as malformation or dysfunction of the normal CNS, which is implanted in various human brain disorders. In this review, we will overview the recent advances in our understanding of the transcriptome plasticity by RNA modifications in neurodevelopment, and how the alterations in these RNA regulatory programs lead to human brain disorders.
REGULATORY MACHINERIES OF THE EPITRANSCRIPTOME: WRITERS, ERASERS AND READERS

RNA modifications are tightly regulated by specialized RNA-binding proteins. Similar to epigenetic regulatory proteins, epitranscriptomic "writers" catalyze the installation of chemical modifications on RNA, "erasers" reverse the modified chemical groups into the original form, and "readers" recognize the modified RNAs to affect various aspects of RNA metabolism (Fig. 1).

First of all, we will discuss the major epitranscriptomic marks predominantly found in the CNS and their regulatory machineries, and then focus on recent progress to highlight the physiological roles of epitranscriptome in development and disorders of the nervous system.

N6-methyladenosine (m6A)

m6A is the most abundant internal modification in mRNA and noncoding RNA, that affects various aspects of RNA metabolism, including stability, splicing, translation, localization, and biogenesis of specific small regulatory RNAs (4, 5). Genome-wide mapping studies showed that m6A modification generally prefers to be installed at the DRACH (D = A, U or G; R = Purine; H = A, U or C) consensus sequence, and is highly enriched either in the 3' UTR around stop codons and long exons (7, 11), though sometimes it can be also found in 5' UTR region with lesser levels (12). The m6A modification is catalyzed by a core methyltransferase complex which is composed of two heterodimeric subunits, corresponding to Methyltransferase like-3 (METTL3) and Methyltransferase like-14 (METTL14), both of which are essential for precise action of the complex (13). Furthermore, other regulatory proteins, including Wilms tumor 1-associating protein (WTAP), Vir like m6A methyltransferase associated (VIRMA), KIAA1429, zinc finger CCCH-type containing 13 (ZC3H13), and RNA binding motif protein 14 (RBM14/14B) interact with core METTL3-METTL14 complex to contribute to RNA binding specificity and nuclear localization of the core complex (5). On the other hand, two major Fe2+-α-ketoglutarate-dependent m6A demethylases, Fat mass and obesity-associated protein (FTO) and alkB homolog 5 (ALKBH5) are known for their function in erasing m6A marks from target RNAs (14, 15), although the substrate preference of FTO is still controversial due to its demethylase activity either to internal m6A and 5' cap-specific terminal m6Am modification in different contexts (16, 17). Until now, a dozen of m6A reader proteins have been identified and characterized. Among them, YT521-B homology (YTH)-domain containing protein family, including three YTHDF proteins (YTHDF1/2/3) plus two YTHDC proteins (YTHDC1/2) selectively recognize and directly binds to m6A tag on RNA. YTHDF1 is well-known to directly promote translation of target mRNAs by the recruitment of elf3, a key component of translation initiation complex, to m6A-modified transcripts (18). On the other hand, YTHDF2 has been known for its significant role in accelerating mRNA degradation through direct recruitment of the CCR4-NOT deadenylase complex (19) or HRSP12-RNase P/MRP complex (20).

Next, it was reported that YTHDF3 not only promotes protein synthesis in synergy with YTHDF1, but also affects methylated mRNA decay mediated through YTHDF2 (21). However, a common theme of YTHDF proteins in recent studies is that all three YTHDF proteins would have similar functions and compensate for each other, which is supported by their highly conserved sequence similarity, similar localization in the cytoplasm, as well as their tendency to bind the same targets (22, 23). Thus, the functions of all three YTHDF proteins should be carefully re-examined in order to resolve the controversy whether YTHDF proteins serve distinct or redundant roles in different biological contexts.

Next, YTHDC1 is widely distributed in the nucleus and appears to regulate alternative splicing, by recruiting RNA splicing factor SRSF3, while blocking SRSF10 from binding to mRNAs (24). Moreover, it was also reported that YTHDC1 interacts with SRSF3 and NXF1 to promote the m6A-dependent mRNA nuclear export (25), as well as regulates the transcrip-
tional inactivation of X chromosomal genes mediated by XIST (26). On the other hand, YTHDC2, a putative RNA helicase, has been characterized as a key protein to enhance the translation efficiency (27).

Apart from YTH proteins, a number of other m^6^A readers have been identified. For instance, HNRNPC/G and HNRNPA2B1, which bind target RNAs through m^6^A-mediated destabilization of secondary structure, have been shown to regulate pri-miRNA processing and translation (28). Additionally, insulin-like growth factor 2 mRNA-binding proteins 1-3 (IGF2BP1/2/3), as well as Fragile X mental retardation protein (FMRP) were also shown to preferentially bind to m^6^A-modified mRNAs through evolutionarily conserved RNA recognition element, such as KH homology (KH), RNA recognition motif (RRM) and arginine/glycinereich (RGG) domains (29). Lastly, proline rich coiled-coil 2A (Prc2a) has more recently been identified as a novel m^6^A reader protein, which regulates oligodenndroglial specification and myelination (30). Collectively, multiple types of m^6^A reader proteins are specifically recruited by m^6^A-tagged RNAs to regulate their metabolism. How the similar m^6^A moieties in RNA are differentially interpreted by reader proteins in specific biological contexts require additional studies to be understood.

(2'-O)-methylation (Nm)

Unlike other modification, Nm does not require a specific nucleotide rather it occurs on any kind of bases by adding a methyl group to the 2'-hydroxyl of the ribose molecule (31). In general, Nm can influence RNAs in different ways as it can increase hydrophobicity, protect RNAs from nuclelease attacks, stabilize helical structures and affect interaction between modified RNAs and proteins (32). Nm is frequently deposited at internal region of rRNAs and small regulatory RNAs, though tRNAs and mRNAs also have considerable sites for this modification (33). To date, several Nm methyltransferases have been identified. For example, FTS1 is a 2'-O-methyltransferase that targets the C_2_ and N_2_3_ positions in the anticodon loop of tRNA^Thr^ and tRNA^Glu^ (34). TRMT44 is a putative 2'-O-methyluridine methyltransferase predicted to methylate residue 44 in tRNA^Asn^ (35). In addition, Fibrillarin (FBL) is localized in the dense fibrillar component (DFC) of the nucleolus where newly synthesized pre-ribosomal RNAs reside and methylates specific RNA targets with help from C/D box family snoRNA (36). Last, CMTR1 is a 2'-O-methyltransferase that modifies the first transcribed nucleotide of the mRNA (37).

Previous studies have reported that m^5^C is related to nuclear export (39) and translation efficiency (40) for certain target RNAs, but general regulatory functions of m^5^C on gene expression and its precise mechanism still need more further investigation. Additionally, deposition patterns of m^5^C on RNA are enriched at CG dinucleotides adjacent to transcription initiation sites of mRNA (38). Among several eukaryotic m^5^C methyltransferases, two key writer proteins, DNMT2 and NSUN2, have been separately focused on their functions. Originally identified as eukaryotic cytosine-5-quinone DNA methyltransferase, DNMT2 also serves as a RNA m^5^C methyltransferase, mainly affecting stability and biogenesis of tRNA (41). Conversely, NSUN2 shows broader target specificity, including mRNAs, long non-coding RNAs (lncRNAs) and other small regulatory RNAs, such as vault RNAs, 7SK and Y-RNAs with non-overlapping manner to DNMT2 (38). Very similar to removal pathway of DNA, RNA m^5^C can be oxidized by ten-eleven translocator (TET) family proteins, giving rise to 5-hydroxymethylcytosine, then 5-formylcytosine and 5-carboxycytosine (42). Although it should be addressed whether oxidized form of cytosine in RNA can be changed to uracil that is suitable for appropriate base-excision repair process, the fact that C-to-U conversion is common phenomenon in RNA and the emergence of key enzyme, SMLUG1, which remove 5-methyluracil in RNA support the possibility that reversible m^5^C metabolism is likely to be similar to that of DNA (43). Unfortunately, less about exact reader protein for m^5^C is known so far, except for ALYREF nuclear exporter (39), leaving a question to be addressed.

Pseudouridine (ψ)

ψ is known as one of the most prevalent RNA modifications dominantly found in non-coding RNA, though it is also detectable in a subset of mRNAs at a low level (44). Intrinsically, isomerization of uridine to ψ, in which additional hydrogen bonds are adopted, enhances the stability of RNAs by increasing base stacking interactions, resulting in stable secondary structure formation (45). Moreover, ψ is thought to be a key molecular regulator that affects translation efficiency, processing of rRNA and siRNA, as well as telomerase activities (44). Installation of ψ is modulated by two different pathways: guide RNA-dependent, in which the guide H/ACA box snoRNA is required for base pairing to target RNA to be modified, and guide RNA-independent pathway, in which stand-alone protein guides target RNAs and catalyzes the formation of ψ. For instance, human Dyskerin (DKC1) belongs to guide RNA-dependent writers of ψ that is also involved in sno-ribo nucleoprotein complex (46), whereas pseudouridine synthases (PUS) family proteins, including PUS1, PUS3, PUS7 and PUS10, are included in guide RNA-independent writers (45) and some of these PUS proteins directly recognize target RNAs in structure-dependent manner (47). Unfortunately, there are no direct eraser and reader proteins identified so far, leaving the reversibility of pseudouridylation and the downstream mechanisms unclear. Taken together, further studies to screen regulatory pro-
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...teins will be required for better understanding of this epitranscriptomic mark.

ROLES OF EPITRANSCRIPTOMIC REGULATION IN NEURODEVELOPMENT

The neurodevelopment of animals is composed of a lot of biological events, including early fate decision of neural stem/progenitor cells, generation of neurons and glial cells, migration of post-mitotic immature neurons, formation of synapses, programmed cell death, synaptic rearrangement, as well as maintenance and reconstruction of neurons throughout the postnatal stages. In this section, we will overview the dynamic changes of epitranscriptomic modifications and their functional contribution of transcriptome plasticity in different steps of neurodevelopment (Fig. 2).

**Embryonic neurogenesis**

**m6A**: During embryonic cortical development, m6A modification regulates proliferation and differentiation of multipotent neural stem cells (2). For example, a study with nervous system-specific conditional knockout (cKO) mice of Mettl14 revealed that m6A temporally controls transcriptional transition from maintenance of neural stem cell/progenitor cells (NSPCs) to pro-neurogenic, and its dysregulation led to loss of differentiation capability, prolonged cell cycle and extended cortical neurogenesis into postnatal stages (48). The m6A-modified mRNAs in the developing cortex were highly enriched for gene ontologies corresponding to cell cycle, stem cells and neuronal differentiation, exhibiting markedly reduced half-life compared to mRNAs without m6A modification. These results suggest that m6A is responsible for destabilization of target mRNAs that are involved in either self-renewal or differentiation of NPCs in the cortex, which is required for the rapid transition of gene expression profiles for proper temporal progres-

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**Fig. 2.** The roles of epitranscriptome in neurodevelopment. During the embryonic and postnatal development, RNA modifications play important roles in regulation of transcriptome plasticity, by which different developmental programs of the CNS, such as embryonic neurogenesis, gliogenesis, cerebellar development, adult neurogenesis, neuronal maturation, and synapse formation are precisely progressed. Bottom panels comprehensively summarize the physiological functions of m6A, Nm, and m5C in neurodevelopment revealed by loss- and/or gain-of-function studies about related epitranscriptomic machinery: RGC, radial glial cell; IPC, intermediate progenitor cell; CLN, cortical layer neuron; AST, astrocyte; ODC, oligodendrocyte; EGL, external granular layer; IGL, inner granule cell layer; PCL, Purkinje cell layer; aNSC, adult neural stem cell; Mm, Mus musculus; Dr, Danio rerio.
sion of cortical neurogenesis (48). Interestingly, loss of m^A- 
-tagging in Mettl14 cKO cortex led to aberrant misexpression of 
neuronal lineage genes, such as Tbr2 and Neurod1, in NPCs, 
suggesting that NPCs are transcriptionally "pre-patterned" for 
differentiation by actively transcribing neuronal lineage genes, 
which are rapidly degraded through m^A-mediated mRNA degra-
dation (2).

In addition, m^A was shown to also regulate histone modifi-
cations at genome-wide level (49). In detail, NPCs derived from 
Mettl14 cKO mice exhibited globally increased histone H3 
acetylation at lysine 27 (H3K27ac), histone H3 trimethylation 
at lysine 4 (H3K4me3) and histone H3 trimethylation at lysine 
27 (H3K27me3). In this study, the stability of several trans-
scripts encoding histone remodeling proteins, such as CBP and 
p300, were regulated in m^A-dependent manner, leading to 
global alterations of histone modifications by dysregulated 
chromatin remodelling proteins (49). Moreover, a recent study 
reported that m^A-tagged nascent transcript on chromatin 
recruited H3K9me2 demethylase KDM3B via YTHDC1, 
resulting in the removal of repressive histone mark H3K9me2 
and enhancement of downstream gene expression (50). Given 
that the dynamic epigenome controlling mechanisms are 
critical for appropriate gene expression in embryonic develop-
ment (51), these studies indicate that the linkage between the 
epigenetic and epitranscriptomic marks is also important for 
proper progression of the embryonic neurogenesis.

In addition, m^A reader proteins also play a significant role 
in brain development. For example, YTHDF2 mediated the 
decay of certain transcripts involved in JAK-STAT signaling 
pathway, which significantly contributes to normal neuropro-
tection and neurite outgrowth. Consistently, Ythdf2-depleted 
mice exhibited impaired proliferation and differentiation of 
NPCs similar with Mettl14 cKO mice, again highlighting the 
esential function of m^A in NPC maintenance and neuronal 
differentiation during cortical development (52). 

Nm: Several studies suggested that Nm modification on tRNA 
and rRNA are likely to be related to neurodevelopment. For 
example, an tRNA 2’-O-methyltransferase, FTSJ1, was shown 
to be highly expressed in human fetal brain compared to other 
tissues, and FTSJ1 mutations have been identified as a risk 
factor of non-syndromic X-linked intellectual disability (ID) 
(53). Not only to tRNA, but Nm modification is also added to 
rRNAs, which requires the site-specific guides of small nucleo-
lar RNAs (snoRNAs). Until now, two different families of sno-
RNAs have been well classified by their structural and func-
tional features. Among them, C/D box snoRNAs are known to 
mediate 2’-O-methylation of rRNAs, while H/ACA box sno-
RNAs are responsible for pseudouridylation (33). Intriguingly, 
it was shown that loss of three different snoRNAs in zebrafish 
causd the failure of normal rRNA methylation, eventually 
leading to severe malformation of head region (54) and, con-
sistently, zebrafish that lacks FBL, an essential nucleolar Nm 
writer protein responsible for proper pre-rRNA processing, 
exhibited abnormal optic tectum and the eye development 
due to impaired neural differentiation (55). Furthermore, C/D 
box snoRNAs also have been shown to be associated with 
Prader-Willi syndrome (PWS), a complex genetic condition that 
affects many parts of the body, especially the central nervous 
system (56). Interestingly, previous studies have demonstrated 
that PWS is likely to be caused by the loss of a group of 
imprinted snoRNAs (56, 57), which suggests that Nm modifi-
cation may govern the neuronal differentiation and tissue de-
development. 

m^C: The roles of m^C on embryonic neurogenesis are differ-
et across species. For instance, morpholino-based knockdown of 
Dnmt2 in zebrafish embryo exhibited reduced early pro-
nuclear marker Neurog1 (ngr-1) in hypothalamus and dienceph-
alon, together with severe defects in neurogenesis (58). How-
ever, Dnmt2-deficient mice did not show severe developmental 
phenotypes because of the compensation by another major 
m^C methyltransferase in mouse, Nsun2 (59). Interestingly, it 
was shown that Nsun2-depletion solely caused microcephaly 
phenotypes both in human and mice (60), unlike the case of 
Dnmt2. This observation was further supported by a study 
demonstrating that Nsun2-mediated m^C modification regulates 
nearl stem cell differentiation and motility in mouse (61). 
Consistently, Dnmt2 and Nsun2 double knockout, which lacks 
90% of total m^C, exhibited significantly undeveloped cortex 
(62), suggesting that m^C modification is required for normal 
mouse brain development and cellular differentiation.

ψ: Although exact functions of ψ in neurodevelopment have 
yet to be investigated, several recent studies have suggested that 
pseudouridine could play putative biological roles in proper 
formation of the central nervous system. Among the TruA 
family proteins, for instance, PUS3 transcripts are exclusively 
enriched in the nervous system of developing mouse embryo 
(63). In addition, a homozygous truncation mutation in PUS3 
in human patients with a history of global developmental delay 
and severe ID showed decreased levels of pseudouridine in 
RNA (64). Similar to PUS3, H/ACA box-mediated pseudouridine 
synthase, DKC1 also showed enhanced expression level of 
transcripts restricted to the mitral cell layer of the olfactory 
bulb and neuronal tissues in telencephalon region (65), sug-
gesting that pseudouridylation is likely to have important roles 
in neurodevelopment.

Gliogenesis: Gliogenesis proceeds not only in a prenatal but also in 
a postnatal development. Mettl14 cKO displayed severe defects 
in neurogenic/gliogenic transition and astrocyte differentiation 
(48). For oligodendrocyte differentiation and myelination, m^A 
mRNA methylation regulates splicing pattern of paranode 
component NF155, and its dysregulation led to attenuation of 
the differentiation of oligodendrocytes and myelinations (66). 
In addition, Prc2a, newly identified m^A reader, stabilized 
Olig2 mRNA in oligodendrocyte, and promoted subsequent 
specification and myelination of oligodendrocytes (30).
Cerebellar development
Cerebellum proceeds its development until postnatal period in chronological order (67). Mettl3-depleted cerebellum showed the enhancement of mRNA stability related to apoptosis, leading to premature cerebellar granule cell death in external granular layer and subsequent cerebellar hypoplasia. In addition, the neuronal layer and structures of Purkinje cell and Bergmann glia cells malformed, which can be resulted from dysregulation of splicing of synapse-associated genes like Grin1 (68). The other study uncovered that balanced expression of m^A writers and readers fine-tunes mRNA methylation in a time-specific way, which leads to normal cerebellar development (69). In addition, under hypoxia condition, Alkbh5-deletion caused abnormal cell proliferation and differentiation in the cerebellum by impaired nuclear export of the hypermethylated RNAs, suggesting that the dynamic regulation of m^A epitranscriptome by Alkbh5-mediated demethylation has important physiological roles depending environmental conditions in vivo (69).

Adult neural stem cell
After birth, adult neural stem cells (aNSCs) reside in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and subventricular zone (SVZ) of the lateral ventricles in mouse (70). aNSCs are also regulated in a genetic and epigenetic mechanism upon environmental stimuli and neuronal activity (70), as well as epitranscriptomic regulation. For example, Mettl3 depletion in aNSCs led to reduced proliferation of aNSCs and alteration of differentiation potential toward glial lineage (71). Depletion of an eraser protein, Fto, in aNSC also resulted in reduced proliferation and differentiation of aNSCs in the SGZ, leading to impairment of learning and memory (72). Interestingly, m^A was present on the transcripts of histone methyltransferase Ezh2, and its protein level and consequent H3K29me3 level were markedly dysregulated upon Mettl3 knockdown (71), suggesting highly interconnected regulation between epigenome and epitranscriptome in neurodevelopment. As we mentioned already, interactions between epigenetic and epitranscriptomic regulations were also identified in other systems during neurodevelopment (8, 49).

Neuronal maturation and synapse formation
Complex tasks in the CNS including learning, memory, and cognition require the precise and accurate formation of neural networks through synapse formation and function (73). m^A modification has been suggested to play a role in neuronal maturation and synapse formation (74) as well as in synaptic plasticity (75). Mettl14 depletion led to downregulation of m^A level on mRNAs encoding synapse-specific proteins (76). In cerebellum, Mettl3-mediated m^A regulation affects alternative splicing of synapse-associated pre-mRNAs (66). Ythdf1 regulates activity-dependent neural responses related to learning and memory, and depletion of Ythdf1 showed impairment of synaptic transmission and long-term potentiation in the mouse hippocampus (77). In addition, depletion of Ythdf1 or Ythdf3 in cultured hippocampal neurons led to dysregulation of excitatory synaptic transmission as well as immature spine morphology (78). In another study, Mettl3-depletion in adult mouse brain caused impaired long-term memory formation without morphological alteration of the brain (79). These findings imply that m^A-mediated regulations have crucial roles at synapse for both development and activity-dependent modulation of neural networks.

Taken together, several types of RNA modifications appear to play important roles in controlling RNA metabolisms, such as splicing, degradation, translation, as well as crosstalk with epigenetic mechanisms to regulate gene expression, all of which are essential for proper development of the CNS.

Dysregulation of epitranscriptome in human neuronal disorders
Neurodegenerative disorders
Neurodegenerative disorders, such as Alzheimer’s Disease (AD) and Parkinson’s Disease (PD), are highly correlated with aging (80). While several factors including genomic instability, malfunction of mitochondria, and cellular senescence are hallmarks of aging that affect neurodegeneration (80), it was also reported that mRNA modifications such as m^A and m^C regulate stability of mRNAs which encode senescence-associated proteins such as AGO2 (81, 82). In addition, another study showed that defect in ALKBH8, tRNA methyltransferase was related to senescence with downregulation of selenoprotein synthesis and elevation of reactive oxygen species in mouse embryonic fibroblasts (83). Regarding these points, the perturbation of epitranscriptomic regulation might be a possible mechanism of neurodegenerative disorders (Fig. 3).

Lesion in the sciatic nerve induces the elevation of m^A levels on regeneration-associated genes and protein translation machinery components in adult mouse dorsal root ganglion (DRG) to enhance injury-induced protein translation essential for axon regeneration. Indeed, loss of Mettl14 or Ythdf1 attenuated protein translation related to axon regeneration in adult DRGs and subsequent axon regeneration (84). Furthermore, Pten deletion-induced axon regeneration of retinal ganglion neurons in the adult CNS was attenuated upon Mettl14 knockdown, suggesting m^A-dependent post-transcriptional regulation is important for neuronal regeneration in both the CNS and the peripheral nervous system. Because the failure of neuronal regeneration is tightly connected to neurodegenerative disorders, it is interesting to examine how m^A-dependent regeneration pathways contributes to the protection of neurodegenerative disorders in aging.

Stroke is an acute focal injury in the CNS, but if not cured immediately, causes poststroke neurodegeneration and further AD (85). Using transient middle cerebral artery occlusion (MCAO), a popular model of stroke in mouse, a study found that global level of m^A on transcripts related to inflammation, apoptosis, and transcriptional regulation increased after 12 to
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Fig. 3. Epitranscriptomic regulation in major brain disorders. Epitranscriptomic RNA modifications are involved in multiple types of brain disorders. Human genetic analysis and animal model studies revealed that various RNA modifications and their regulatory machineries have critical roles in etiology of neurodegenerative disorders, intellectual disability, mental disorders, and brain cancers. ADHD, attention deficit hyperactivity disorder; MLASA, mitochondrial myopathy, lactic acidosis, and sideroblastic anemia; XLMR, X-linked mental retardation; m6A, N6-methyladenosine; ψ, pseudouridine; m5C, 5-methylcytosine; Nm, 2′-O-methylation.

24 hours of MCAO (86). The m6A writers were unaltered, but the m6A eraser Fto decreased significantly after stroke, but the functional contribution of m6A in stroke and injury response is yet unclear.

Alzheimer’s disease (AD) is one of the most common neurodegenerative disease in the old, and progresses into dementia and cognitive impairment (87). In human genetic studies, different genetic variants of FTO have been reported to be associated with AD risk (88, 89). It was shown that Fto activated the phosphorylation of Tau in a mTOR-dependent manner, and conditional knockout of Fto in the neurons reduced the cognitive deficits in AD model mice (90). APP/IP51 double transgenic mouse, which is well-known for AD model, displayed elevated m6A and Mettl3 level as well as reduced Fto level in the cortex and the hippocampus (91). Moreover, a recent study showed that FMRP, a m6A reader protein, bound to Adam9 and Psen1 which are APP secretases to regulate their nuclear export. When FMRP was absent, cytoplasmic level of Adam9 and Psen1 were downregulated, leading to aberrant APP processing (92). Additionally, a study showed that Cmtr1, a Nm writer, was increasingly upregulated by the age of AD model mouse (5xFAD line), suggesting other epitranscriptomic modifications beyond m6A would be also involved in neurodegenerative diseases (93).

Parkinson’s Disease (PD) is one of the most commonly diagnosed neurodegenerative movement disorder with the symptoms such as tremor and rigidity (94). Several genetic factors and mechanisms are known to be associated with PD, such as loss of dopaminergic neurons and accumulation of misfolded alpha-synuclein (94). FTO overexpression or m6A reduction in dopaminergic neurons upregulated GRIN1 expression, leading to subsequent elevation of oxidative stress and Ca2+ influx and apoptosis (95). On the other hand, Fto depletion in dopaminergic neurons caused impaired dopamine receptor type 2 mediated signaling, which led to attenuated activity of G protein-coupled inwardly-rectifying potassium channel (GIRK channel) and dysregulation of behaviors controlled by dopaminergic transmission (96). Recently, entacapone, known as catechol-o-methyltransferase inhibitor used for PD treatment, has been suggested as FTO inhibitor (97). Entacapone directly bound to and deactivated FTO, then inhibited the activity of transcription factor forkhead box protein O1 (FOXO1). As genetic and epigenetic factors have been suggested to play a role in regulating neurodegenerative diseases, specific molecular and cellular mechanisms in epitranscriptomic way are yet to be more investigated.

Intellectual disability

Intellectual disability (ID) is a complicated developmental disorder concluding cognitive impairment, learning failure, maladjustment to social environment, and so on. Several genetic factors including Fmr1 mutation in Fragile X syndrome.
were found to be cause of ID, and autosomal dominant and recessive genes related to ID have been increasingly accumulated (98). In addition to genetic factors, epigenetic alterations in histone modification and chromatin structure affect ID occurrence (99). Mutations in a number of X-linked genes such as ATRX and MeCP2 dysregulated chromatin structure and subsequent gene expression pattern (99).

Among X-linked genes, Fragile X Mental Retardation Protein (FMRP) is related to synaptic function, and depletion of this protein caused degradation of its target mRNAs (100). Interestingly, FMRP interacts with YTHDF2, m^4A reader protein to enhance stability of its target mRNAs. Moreover, frameshift mutation on METTL5, which is 18S ribosomal RNA methyltransferase (101) abundantly expressed in the hippocampus, caused ID with symptoms in moderate to severe level accompanied with microcephaly (102).

Homozygous mutation of truncation form in PUS3 is linked to ID with decreased levels of pseudouridine in tRNA (64). In the case of homozygous mutation on PUS7 in human caused ID as well as microcephaly (103). These patients with PUS7 mutation showed decreased level of pseudouridylation in mRNA as well as tRNA. In addition, mutations in Dyskerin, rRNA pseudouridine synthase, are linked to X-linked recessive dyskeratosis congenita and Hoyeraal-Hreidarsson syndrome, which both show symptoms of microcephaly and some extent of mental deficiency related to ID (65). Moreover, missense mutation in PUS1 was found to be the cause of Myopathy-lactic acidosis-sideroblastic anemia (MLASA) with altered expression in muscle and brain (104). In other research, two different genetic mutations on PUS1 were found to cause MLASA and consequent ID (105).

NSUN2, m^6C writer, was also suggested as the cause of ID. For example, mutations in NSUN2 are the risk factor of MLASA syndrome, leading to delayed response of sensory signals and mild cognitive impairment (106). Through whole-exome sequencing, it was shown that miss-spliced mutants in NSUN2 caused Dubowitz Syndrome that displays microcephaly and ID (107). In another study, mutation in NSUN2 was suggested to be a risk of ID by genetic analysis of human pedigree. Also, mutation in NSUN2 caused the mislocalization of NSUN2 protein in the nucleus of Purkinje cells in the cerebellum (108).

From 1990s, FTSJ1, Nm writer, has been reported for its mutation to be associated with nonsyndromic X-linked mental retardation (NS-XLMR) in human genetic studies (109, 110). Moreover, single nucleotide polymorphisms (SNPs) (111, 112) or copy number variations (113) of FTSJ1 were associated with the risks of intellectual ability and NS-XLMR. Firstly, it was reported that FTSJ1 has a function of methyltransferase which targets Cm and Gm of tRNA^{58}, and its malfunction methylating Gm can be associated to NS-XLMR (53). While investigating molecular mechanism, one study found that FTSJ1 interacts with WDR6 in cytoplasm, so FTSJ1 can methylate Gm34 in tRNA^{58} with the prerequisite of m^1G37 (114). Recently, based on gene trapped stem cell line, Ftsj1 deficient mouse line showed ID symptoms as well as alteration in metabolism and immune function (115). Also, TRMT44, a putative Nm writer, was suggested to be associated with partial epilepsy with pericentral spikes (PEPS) through methylating on tRNA^{58} (35). The exact role and precise mechanism of each writers and other epitranscriptomic factors in ID pathogenesis should be investigated further.

**Mental disorders**

FTO, which demethylates both m^4A and m^6Am (m^4A/m, collectively), has been actively investigated in mental disorders even before generally known as being related to RNA modification. Exposure to arsenite led to decrease of Fto expression, which increased m^4A level and subsequent dysregulation related to deficits in dopaminergic neurotransmission (116). Several SNPs of FTO were associated with a risk for ADHD with symptoms of memory and cognitive defects (117). Even though specific mechanism is unclear, ZC3H13 polymorphism has been reported to be associated with schizophrenia risk (118). These results imply that m^4A regulation affects dopaminergic midbrain circuits related to mental disorders such as schizophrenia.

Glucocorticoid response upon chronic psychological stress, which underlies several mental disorders, induces profound time-specific alteration of m^4A/m landscape (75, 119). Deletion of Mettl3 and Fto in adult neurons altered m^4A/m epitranscriptome, increased fear memory, and changed transcriptome response to fear and synaptic plasticity (75), suggesting that dysregulation of the m^4A/m response may contribute to the pathophysiology of stress-related mental disorders. Regarding m^6Am level is dysregulated in major depressive disorder patients (75, 120), and certain m^6A regulator like ALKBHS is highly associated with the risk of major depressive disorder (121), the detail roles of m^6Am in the etiology of major depressive disorder need to be addressed further. Moreover, Mettl14 deletion in dopaminergic neurons led to dysregulation of neuronal excitability and impairment of striatal-mediated learning (76), indicating that epitranscriptomic regulators can be involved in mental diseases related to dysfunction of the dopaminergic system.

**Brain cancer**

Neuroblastoma is known to be the most common solid tumor found in early children and derived from several genetic aberrations. Interestingly, polymorphisms in METTL3 and METTL14 are highly associated with neuroblastoma susceptibility (122, 123). In a study using human neuroblastoma cells, m^6A modification on 3′-UTR of MYCN interacted with miR-98, which inhibited cell proliferation, migration, and invasion of neuroblastoma cells (124). In another study, several m^6A writer proteins (METTL14, WTAP) and reader proteins (YTHDF1, HNRNPC, and IGF2BP2) were suggested to be hallmarks of tumor malignancy using a hundred of human neuroblastoma tissues (125).
Glioma, which is one of the most common malignant tumor of astrocytes in brain, has four different grades defined by WHO (I-IV): low-grade and anaplastic astrocytoma (WHO grades II-III) and glioblastoma (WHO grade IV) (126). The prognosis of glioma patients is quite poor even its treatment has been evolved (127). Among the m^6^A-related proteins, Wilms’ tumor 1-associating protein (WTAP) is implicated for the marker of glioblastoma (128). WTAP was over-expressed in glioblastoma with 169 clinical samples with glioma patients, and the higher expression of WTAP was correlated with poorer prognosis (128), suggesting m^6^A regulatory proteins may have important roles in tumor progression.

Glioblastoma stem cells (GSCs) were suggested as a primary source that contributes to tumor propagation, maintenance, and treatment resistance (129), which m^6^A-mediated regulation also takes part in. For example, GSC-mediated tumorigenesis was markedly promoted by knockdown of METTL3 or METTL14, by inducing changes in m^6^A profiles and subsequently altering mRNA expression of genes with critical biological functions in GSCs, such as ADAM19 (130). Meanwhile, ALKBH5 is highly expressed in GSCs and demethylases 3’s UTR of FOXM1 nascent RNAs to enhance FOXM1 expression and tumorigenesis of GSCs (131). On the other hand, it was also reported that elevated expression of METTL3 in GSCs enhanced the maintenance of GSCs through SOX2 stabilization compared to elevation of that in differentiated glioma cells. Moreover, SOX2-dependent DNA repair was more activated in METTL3 expression-elevated GSCs, which led to radioresistance (132). These results suggest m^6^A-mediated regulations govern various steps for genesis and progression of glioma.

As altered histone modification and their modifiers are related to glioma genesis, these epigenetic regulators have been regarded as therapeutic targets and applicable biomarkers (133). Similarly, epitranscriptomic factors have been suggested as applicable biomarkers and therapeutic targets in gastrointestinal cancer (134) and renal cancer (135). To identify biomarkers of glioblastoma, m^6^A-IncRNA co-expression networks were constructed through statistical analysis of primary glioblastoma patients, which screened four IncRNAs with their co-expressed functional genes. In that the IncRNA expression was correlated with the effect of m^6^A modification, it is suggested that posttranscriptional regulation of noncoding RNAs may have a significant role in dynamic gene expression control of glioblastoma (136). In addition, twenty-four IncRNAs were explored to be prognostic m^6^A-related IncRNAs, which shows the distinct m^6^A status between low- and high-risk subgroups of lower-grade glioma patients (137). Moreover, it was reported that FTO inhibition enhanced effectiveness of chemotherapy of glioma (138), and a newly synthesized inhibitor of ALKBH5 successfully kept specific glioblastoma cell line from migration and invasiveness (139), suggesting that epitranscriptomic regulators will be considered as important targets for development of potential therapeutic intervention of brain cancers in the future.

**CONCLUSION AND FUTURE DIRECTIONS**

In summary, we have reviewed overall mechanisms of epitranscriptomic gene regulation and endeavors to identify the physiological functions of various epitranscriptomic modifications and their mechanisms. Indeed, the dynamic regulations of transcriptome plasticity via RNA modifications have been demonstrated to be involved in prenatal and postnatal neurodevelopment ranging from neural stem cell establishment to adult neurogenesis. However, only few of writer, eraser, and reader proteins modulating these neurodevelopmental processes have been investigated in detail. Further studies on regulator proteins of epitranscriptome and cell-type specific reader proteins will be necessary to appreciate the whole picture of dynamic transcriptome plasticity in development and physiology of the brain. In addition, it is still challenging to detect a specific RNA modification with high resolution and low background signals by current epitranscriptome mapping technologies that majorly rely on the sensitivity and the specificity of modification-specific antibodies. Therefore, recently developed antibody-independent approaches (140, 141) will greatly improve our understanding on the landscape of epitranscriptome upon various cellular and environmental context at single base-pair resolution.

In addition, we examined several brain disorders including neurodegenerative diseases, intellectual disability, mental disorder, and brain cancer in the context of epitranscriptomic regulation. Accumulating evidences suggest that the dysregulated epitranscriptome is a potent pathological mechanism of brain disorders, together with genetic and epigenetic factors. From this perspective, novel biomarkers to predict disease progression as well as new therapeutics are currently being developed by investigating and modulating epitranscriptomic regulation. Through continued efforts to advance epitranscriptome mapping technologies, to uncover functional mechanisms of key regulatory proteins of RNA modification, and to develop novel reagents to control disease-related features of transcriptome plasticity, we believe our expanding understanding of epitranscriptome will significantly contribute to deciphering the daunting complexity of brain disorders.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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