**Review**

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# Circadian rhythm in neurodegenerative disease: the role of RNA modifications and potential application of RNA-based therapeutics

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## Abstract

Neurodegenerative diseases usually present as progressive impairment of the motor or mental functions of the central or peripheral nervous system, which is often linked to genetic and biochemical factors. The main features include synaptic and neuronal deficits, abnormal protein homeostasis, DNA and RNA defects, inflammation, and pathological protein aggregation. Clinical evidence suggests that circadian rhythms affect different neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and Huntington's disease, through oxidative stress, neuroinflammation, and other mechanisms. Disruptions in circadian rhythms, which are often linked to alterations in RNA modifications, contribute to disease progression. This review provides an overview of current research progress on neurodegenerative diseases and outlines their relationship in terms of aberrant circadian rhythm, highlights the role of RNA modifications in circadian rhythm-regulated

neurodegenerative diseases, and presents the potential applications of RNA-based drugs for treating neurodegenerative diseases.

## INTRODUCTION

The term circadian rhythm originated from the Latin words circa and diem, which respectively mean "about" and "day", with a cycle of approximately 24 h. Circadian rhythms are physiological and behavioral oscillations that influence diverse physiological processes. The suprachiasmatic nucleus, acting as a circadian pacemaker, regulates circadian rhythms, enabling human adaptation to Earth's axial rotation and environmental changes. Circadian rhythms are usually characterized by several features: self-sustaining, rhythmic, synchronously regulated, and measurable. Among these is self-maintenance because it exists without exogenous time signals (including the dark light cycle), indicating an intrinsic timekeeping mechanism (i.e., a biological clock). In addition, disruption in the circadian system can negatively affect various functions, including sleep, alertness, cognition, mental functioning, motor control, and metabolism. Alterations in the balance of circadian rhythms have been increasingly recognized in neurodegenerative disorders, including Parkinson's disease (PD), Huntington's disease (HD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD).

Extensive research has demonstrated that numerous RNA editing processes during RNA processing, transportation, and degradation are governed in a circadian fashion, thereby significantly contributing to the regulation of circadian gene expression. These include crucial steps such as messenger RNA (mRNA) capping, alternative splicing (AS), alterations in splicing efficiency, and adjustments in RNA stability, which are modulated by factors such as the tail length of polyadenylation or the utilization of alternative polyadenylation sites. In addition, altered RNA editing patterns such as adenosine to inosine (A-to-I) and cytidine to uridine (C-to-U) RNA editing and clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 13 (Cas13)-mediated editing have been observed in various neurological pathologies. In addition to RNA editing, other RNA modifications related to circadian rhythms have also been implicated in neurodegenerative diseases. A recent comprehensive investigation, encompassing both mouse models and cultured human cells, revealed the involvement of $N^6$-methyladenosine RNA-methylation, among the common RNA modifications, in the intricate regulation of the circadian clock mechanism[1].

In this review, we first examine the pathogenesis and abnormal protein aggregation of common neurodegenerative diseases, the clinical features of neurodegenerative diseases regulated by circadian rhythm [Tables 1 and 2], and epigenetic inheritance involving circadian rhythm genes. Next, we review common RNA modifications, including methylation of adenosine at position 6 [namely, the $N^6$-methyladenosine (m⁶A)], $N^1$-methyladenosine (m¹A), 5-methylcytosine (m⁵C), pseudouridine, and RNA editing, including A-to-I and C-to-U RNA editing and CRISPR-Cas13. Then, we introduce the potential and application prospects of drugs based on RNA modifications for treating neurodegenerative diseases.

## NEURODEGENERATIVE DISEASES

Neurodegenerative diseases are a group of neurological disorders with heterogeneity that involve the neurons' progressive loss in the central nervous system (CNS) or peripheral nervous system (PNS). The deterioration of neural networks' structure and function and the inability of neurons to regenerate efficiently due to their terminal differentiation disrupt fundamental communication pathways, ultimately resulting in impaired memory, cognition, behavior, and sensory and/or motor function[2].

**Table 1. The protein aggregation and main pathogenesis in neuropathology of common neurodegenerative diseases**

| | Disease type | Protein aggregates | Main pathogenesis in neuropathology |
|---|---|---|---|
| Amyloid diseases | Alzheimer's disease | Aβ-42<br>3R+4R tau | Aβ-42 aggregation leads to amyloid plaques<br>Neurofibrillary tangles |
| | Familial British dementia | ABRI | Amyloid angiopathy<br>Parenchymal amyloid plaques |
| Prion diseases | Creutzfeldt-Jakob disease | PRNP | Spongiform changes<br>PRNP accumulation |
| | Gerstmann-Sträussler-Scheinker disease | PRNP | Spongiform change<br>Multicentric PRNP plaques |
| Synucleinopathies | Parkinson's disease | SNCA | The presence of Lewy bodies<br>Substantia nigra neuronal loss |
| | Multiple system atrophy | SNCA | Glial cytoplasmic inclusions |
| TDP-43 proteinopathies | Amyotrophic lateral sclerosis | TDP-43<br>FUS | Protein misfolded and inclusion formation with TDP-43 and FUS |
| | Primary lateral sclerosis | TDP-43 | The loss of upper motor neuron<br>Corticospinal tract degeneration |
| | Frontotemporal lobar degeneration | TDP-43 | Neuronal cytoplasmic inclusions<br>Neuronal nuclear inclusions<br>Dystrophic neurites |
| | Progressive muscular atrophy | TDP-43 | Lower loss of motor neuron<br>Swollen motor neurons |
| Others | Huntington disease | Huntingtin | A CAG trinucleotide repeat expansion dominantly inherited in the huntingtin gene on chromosome 4 |

Aβ: Amyloid-β; ABRI: British amyloid; PRNP: prion protein; SNCA: α-synuclein; TDP-43: TAR-DNA-binding protein 43 kDa; FUS: fused in sarcoma.

**Table 2. Clinical features of common neurodegenerative diseases regulated by circadian rhythms**

| Type of neurodegenerative diseases | Neuroendocrine markers | Neuropathological markers | Main clinical features/Functional indicators of disruption |
|---|---|---|---|
| AD | (i) Melatonin (main)<br>(ii) Histamine<br>(iii) 5-HIAA | (i) Aβ aggregation<br>(ii) Tau aggregation<br>(iii) Level of MT1, AVP and VIP in SCN | (i) Rest-activity rhythms<br>(ii) Alterations in the autonomic nervous system (including blood pressure, heart rate variability, and body temperature rhythm) |
| HD | (i) Melatonin in serum<br>(ii) Cortisol in serum and urine | (i) Level of AVP and VIP in SCN<br>(ii) Hypothalamic grey matter volumes | (i) Sleep disturbance (including insomnia, excessive daytime sleep, rapid eye movement sleep behavior disorder, sleep-disordered breathing)<br>(ii) Movement disorders (including involuntary movements and some nonmotor symptoms) |
| PD | (i) Melatonin<br>(ii) Dopamine | (i) Lewy bodies and Lewy neurites in pinealocytes | (i) Alterations in the autonomic nervous system (including blood pressure, heart rate variability and body temperature rhythm)<br>(ii) Sleep disturbance (including insomnia, excessive daytime sleep, rapid eye movement sleep behavior disorder, sleep-disordered breathing)<br>(iii) Movement disorders (including involuntary movements and some nonmotor symptoms)<br>(iv) Cognitive impairment |
| FTD | (i) Orexin receptor type 2<br>(ii) Casein kinase 1 epsilon<br>(iii) FUS | (i) Dipeptide repeat protein in pinealocytes and SCN<br>(ii) Cortical atrophy | (i) Sleep disturbance (including insomnia, excessive daytime sleep, rapid eye movement sleep behavior disorder, sleep-disordered breathing)<br>(ii) Rest-activity rhythms |

AD: Alzheimer's disease; 5-HIAA: 5-hydroxyindoleacetic acid; MT1: melatonin receptor 1; AVP: arginine vasopressin; VIP: vasoactive intestinal peptide; SCN: suprachiasmatic nuclei; HD: Huntington's disease; PD: Parkinson's disease; FTD: frontotemporal dementia; FUS: fused in sarcoma.

Recent studies have identified the hallmarks of neurodegenerative diseases in terms of genetic factors and biochemical pathways. There are eight significant and interactive hallmarks of neurodegenerative diseases

with relatively complete evidence: pathological protein aggregation, synaptic and neuronal network dysfunction, aberrant proteostasis, cytoskeletal abnormalities, altered energy homeostasis, DNA and RNA defects, inflammation, and neuronal cell death [Figure 1][2]. Despite similarities in hallmarks, their mechanism varies in different diseases. For example, the aberrant amyloid-β (Aβ) and tau proteins can cause AD[3,4], while numerous inflammatory cell mediators can cause neuronal degeneration, thus leading to inflammation and then AD[5]. Disease development likely involves genetic and environmental factors, specific neuronal populations, brain regions, or affected cell types. Therefore, it is crucial to identify the primary factors driving disease pathways and the subsequent secondary effects in each concrete neurodegenerative condition, including its subcategories.

Most neurodegenerative diseases share a common protein basis, such as the accumulation and deposition of aberrant proteins whose physicochemical properties are partially or wholly altered, including AD, tauopathy, PD[6,7], HD, ALS[8,9], spinal muscular atrophy, and spinocerebellar ataxia[10-12]. While many factors and hallmarks of neurodegenerative diseases are mutually connected, the feature, namely one of the eight hallmarks, pathological protein aggregation, accounts for the largest proportion. The most frequent proteins involved in the pathogenesis of neurodegenerative diseases are Aβ, prion protein (PRNP), tau, α-synuclein (SNCA), TAR-DNA-binding protein 43 kDa (TDP-43), superoxide dismutase 1 (SOD1), and fused in sarcoma (FUS).

As indicated above, some aberrant proteins indeed cause neurodegenerative disease. One of the most commonly diagnosed neurodegenerative diseases is AD, also one type of senile dementia. Specifically, AD refers to a clinical syndrome linked to a specific neuropathological process characterized by two key features: the aggregation of 42-amino-acid Aβ and tau proteins. The formation of extracellular neuritic plaques, primarily consisting of 42-amino-acid Aβ originating from the amyloid precursor protein (APP), involves the sequence of α-secretase, β-secretase, and γ-secretase[13]. Conversely, the accumulation of intracellular neurofibrillary tangles consists of hyperphosphorylated species of microtubule-associated protein tau (MAPT)[14].

The degeneration of dopaminergic neurons in the substantia nigra pars compacta constitutes the primary pathological feature of PD. PD is microscopically characterized by Lewy bodies (LBs), intracytoplasmic neuronal inclusions containing insoluble, fibrillated aggregates of SNCA and ubiquitin. While motor symptoms are typically attributed to neuronal loss in the substantia nigra, this area is not the initial site of pathology[15]. The distribution and progression of LBs in the CNS were detailed by Braak[16], starting in the dorsal motor nuclei of the vagus nerve and then spreading through the brainstem to reach the cortex[17]. In PD, SNCA is also observed in neuronal processes (Lewy neurites), astrocytes, and oligodendroglial cells.

## CIRCADIAN RHYTHMS
### Circadian rhythms
The circadian rhythms function as an intrinsic biological clock that orchestrates various aspects of life processes, from genetic activity and cellular metabolism to overall behavioral patterns. These rhythms, constituting roughly 24-h cycles in the physiological functions of most organisms, are internally generated and prone to external influences. A typical circadian cycle shows characteristics such as being self-sustained, rhythmic, synchronized, and measurable[18]. These rhythms are self-sustaining, maintaining a consistent oscillation independent of external cues, such as light-dark cycles, indicating the presence of an inherent timekeeping mechanism. The rhythmic quality stems from the consistent 24-h cycle of circadian processes. Additionally, circadian cycles can synchronize with external factors; hence, the term is synchro-tuned. Disruptions in circadian rhythms can adversely affect various functions such as sleep, alertness, cognition,
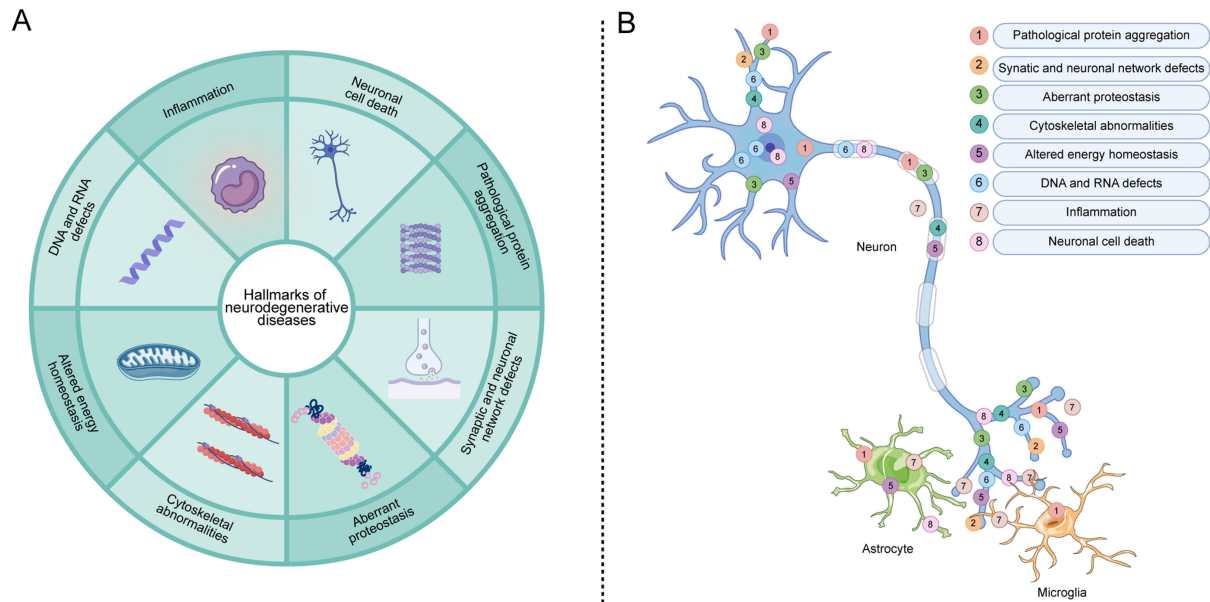
**Figure 1.** (A) Hallmarks of neurodegenerative diseases. As identified and showcased on the left side, eight key characterized hallmarks have been uncovered through extensive research in genetics, biochemistry, and clinical studies. These hallmarks encompass pathological protein aggregation, synaptic and neuronal network dysfunction, aberrant proteostasis, cytoskeletal abnormalities, altered energy homeostasis, DNA and RNA defects, inflammation, and neuronal cell death. Over decades, researchers have unearthed genetic factors and biochemical pathways linked to these diseases, fostering a deeper comprehension of their underlying mechanisms; (B) The neurodegenerative diseases' hallmarks and their distribution within a recapitulative presentation. As visually depicted on the right side, there are the distinctive hallmarks and precise locations of neurodegenerative diseases. Each hallmark is numbered and showcased within the modeled neuron and/or supportive astrocyte and microglia.

mental acuity, motor skills, and metabolism. The importance of maintaining a balanced circadian rhythm is increasingly emphasized for neurodegenerative disorders.

Circadian neurons work together to build a fine-grained regulatory system by forming a complex neural network that interconnects with other biological clock-related nuclei[19]. These neurons regulate circadian rhythms in organisms by releasing neurotransmitters and hormones that influence the function of downstream target cells. The circadian system consists of interconnected clock oscillators in the suprachiasmatic nuclei (SCN) of the hypothalamus and certain active peripheral organs in terms of metabolism. It regulates daily behavioral and physiological rhythms encompassing sleep/wake cycles, fasting/feeding patterns, metabolic activities, body temperature, and endocrine functions. This system significantly influences daily feeding habits[20], sleep-wake patterns regulation[21], neuroendocrine hormonal secretion[22], and maintaining homeostasis and metabolic processes, including the autonomic control of blood pressure and body temperature. In its intrinsic state, the circadian rhythm follows a natural period known as tau, slightly exceeding the length of the 24-h light-dark cycle (24.18 h) observed in humans[23].

**Relative genes of circadian rhythms**
The hypothalamic SCN, acting as central circadian pacemakers in mammals, communicates synchronized signals to various circadian oscillators within the brain, tissues, and organs. Specialized cells containing intrinsic photosensitive melanopsin (MOP) receive light signals necessary for circadian regulation. Melanopsin is found in a small subset of retinal ganglion cells, which project toward the SCN through the retino-hypothalamic bundle[24,25]. Neuropeptides such as vasoactive intestinal peptide (VIP), arginine vasopressin (AVP), and gastrin-releasing peptide (GRP) primarily synchronize oscillatory neurons within the SCN[26-31]. Peripheral tissues' rhythmicity is predominantly coordinated by glucocorticoid signaling

within the SCN[32-34]. While the hypothalamic SCN serves as the primary circadian pacemaker, most peripheral organs and tissues can exhibit circadian oscillations independently despite their interactions with each other and the system as a whole[35]. At a molecular level, the generation of circadian oscillations involves a complex network of genes referred to as "clock genes," which include the clock circadian regulator (*CLOCK*), aryl hydrocarbon receptor nuclear translocator-like (*ARNTL*, also known as *BMAL1*), period circadian regulators 1 (*PER1*) and 2 (*PER2*), and cryptochrome circadian regulators 1 (*CRY1*) and 2 (*CRY2*). These encoded proteins have critical functions in regulating circadian rhythmicity.

The core oscillator exhibits high consistency in function and compositional principles across eukaryotes. In *Drosophila*, the positive edges of the clock are formed by *CLOCK* and *CYCLE*, which bind to E-box-containing enhancers upstream of the period (*per*) and timeless (*tim*) genes to induce their transcription[36-38]. When they reach critical levels in the cytoplasm, PER and TIM interact physically, translocating into the nucleus where they inhibit CLOCK-CYCLE function, thus repressing their own transcription[39,40]. TIM undergoes photodegradation throughout the morning via the cytochrome (CRY)-dependent pathway[41]. When TIM is experiencing a loss, the direct homolog of mammalian casein kinase 1ε (CSNK1E) destabilizes PER, leading to its ubiquitination and subsequent proteasomal degradation[42,43]. The simultaneous degradation of PER and TIM initiates a new circadian cycle. Independent peripheral circadian oscillators exist in some non-mammalian vertebrates and numerous invertebrates, such as zebrafish and *Drosophila*[44,45]. Multi-oscillatory systems, found in non-mammalian vertebrates like birds and lizards[46], involve direct responses to environmental cues by the retina and pineal gland.

Molecular clock in transcriptional autoregulatory feedback loop begins with the activation of BMAL1 and CLOCK, prompting the expression level of PER and CRY genes at the beginning of a cycle. In a negative feedback mechanism, the PER/CRY repressor complex translocates into the nucleus to inhibit BMAL1/CLOCK activity[47]. This loop operates with a genetically predetermined period lasting approximately 24 h and is primarily entrained by environmental light cues. Additionally, CLOCK-BMAL1 controls a myriad of target genes downstream, referred to as clock-regulated genes. The circadian system, an intricately regulated network, involves the oversight of PER and CRY protein stability by E3 ubiquitin ligase complexes known as S-phase kinase-associated protein 1 (SKP1)-cullin (CUL1)-F-box protein. Furthermore, casein kinase 1ε/δ (CSNK1E/D) and AMP kinase (AMPK) influenced the phosphorylation of PER and CRY proteins. Moreover, the respective E3 ubiquitin ligase complexes and the 26S proteasome complex, respectively, led to their subsequent polyubiquitination and degradation.

### Aging and circadian rhythm

Circadian rhythms in mammals govern daily (approximately 24-h) oscillations in behavior and physiology, harmonizing internal processes with environmental timing cues to ensure optimal adaptation. Recognized is the attribution of circadian dysfunction in older adults, in part, to the SCN's degradation, recognized as the mammals' core circadian clock. The central clock is located in the hypothalamus' SCN, orchestrating the synchronization of peripheral clocks in body tissues through both humoral and neural signals. These peripheral clocks operate autonomously at the cellular level, are influenced by the central clock, and do not necessitate SCN inputs to generate rhythmic patterns. The endocrine system also demonstrates circadian regulation. During aging, circadian rhythms show reduced amplitude and phase shifts, which may impact the organism's ability to adapt to environmental changes and potentially affect tissue homeostasis, sleep regulation, behavior, health status, and lifespan[48].

The aging process is closely linked to changes in circadian rhythm, which can accelerate aging[49]. There is an increasing indication of the interplay between changes in circadian rhythmicity and aging naturally.

Chronic conditions such as obesity, diabetes, cancer, cardiovascular disease, and neurodegenerative disorders are significantly influenced by aging. In aged mice, epidermal and muscle stem cells continue to exhibit circadian rhythmicity at a cellular level. However, the oscillatory transcriptome of aged muscle stem cells undergoes reprogramming[50], shifting the focus of gene expression toward stress responses rather than maintaining homeostasis. Likewise, aging alters the circadian function of the liver in mice, resulting in a notable decrease in protein acetylation oscillations[51]. Calorie restriction has been shown to reverse the aging-related reprogramming in both scenarios, hinting at potential benefits. Additionally, therapeutic interventions such as oxidized nicotinamide adenine dinucleotide (NAD+) precursors can modulate the circadian effects of aging by deacetylating Lys680 on PER2, thereby enhancing the binding of BMAL1 to chromatin across the genome[52]. This observation suggests that it is feasible to intervene in the circadian output tied to natural aging through methods such as calorie restriction and specific treatments.

**Epigenetic modification of circadian rhythms**
Core clock genes are not the sole factor in modulating circadian transcription. It is also important to notice the genetic and epigenetic factors. Therefore, the feedback loop of the core circadian loop fails to explain all results solely, particularly those related to human behavioral traits and disorders[53,54]. The key mechanism in epigenetic factors is translating environmental influences into the expression level of the circadian rhythm gene. Throughout the day-night cycle, dynamic shifts in the epigenetic landscape occur[55]. Gene expression oscillations are collectively controlled by the collaboration between circadian transcription and chromatin modifications of circadian rhythms. Promoter regions of circadian clock genes (CCGs) exhibit rhythmic histone acetylation (AcH3K9 and AcH3K14)[56], connecting the histone acetyltransferase (HAT) p300 (EP300) with CLOCK's intrinsic HAT activity[56,57]. Nonetheless, the NAD+-dependent deacetylase sirtuin 1 (SIRT1) counterbalances this activity[58,59], dynamically regulating the circadian acetylation of histone and non-histone proteins.

CDC-like kinases (CLKs) utilize histone acetylation/deacetylation as a crucial epigenetic mechanism to regulate circadian rhythms. The regulation of histone H3 acetylation at the *CRY* and *PER* promoters is controlled by the EP300 in cooperation with the CLOCK/BMAL1 complex, impacting gene expression[60]. HAT activity is also exhibited by CLK within the CLK-BMAL1 complex, leading to the recruitment of CRY1 by acetylated BMAL1, resulting in transcriptional repression[56,61]. During the transcriptionally active phase, there is a peak in histone H3 acetylation levels in *PER1*, *PER2*, and *CRY1* promoters[62]. Circadian rhythms, addiction-related behaviors, memory formation, and metabolism are significantly influenced by histone deacetylases (HDACs)[57,62]. The recruitment of HDAC3 by nuclear receptor corepressor 1 (NCOR1) represses the expression level of *BMAL1*, thereby influencing circadian rhythms. Fluctuations in recruiting HDAC3, along with nuclear receptor subfamily 1 group D member 1 and NCOR1, lead to the formation of an HDAC3/NR1D1/NCOR1 complex that correlates with the oscillatory transcription of numerous genes[63,64]. Treatment with HDAC inhibitors results in increased acetylation of H3 and impacts the expression of *PER2*. SIRT1, a NAD+-dependent HDAC, binds to CLK-BMAL1 to directly interact with clock genes, promoting the deacetylation and degeneration level of PER2. This connection links the metabolic state to the circadian system. Besides its role in circadian regulation, SIRT1 is also involved in various brain functions such as aging, neurodegeneration, synaptic plasticity, and memory formation.

The regulation level of circadian rhythm gene expression is significantly influenced by histone methylation and demethylation processes[65-67]. Various histone methyltransferases and demethylases participate in the rhythmic methylation of Histone H3 Lysine 4, 9, 27, 36 (H3K4, H3K9, H3K27 and H3K27)[66,67]. The suppressor of variegation 3-9 homolog 1 (SUV39H1) is key in the rhythmic di-methylation of H3K9 (H3K9me2) and is responsible for recruiting CLOCK-BMAL1 to the E-box regions of clock-controlled gene promoters [Figure 2][65,68]. The interaction between SUV39H and PER2 could potentially regulate the
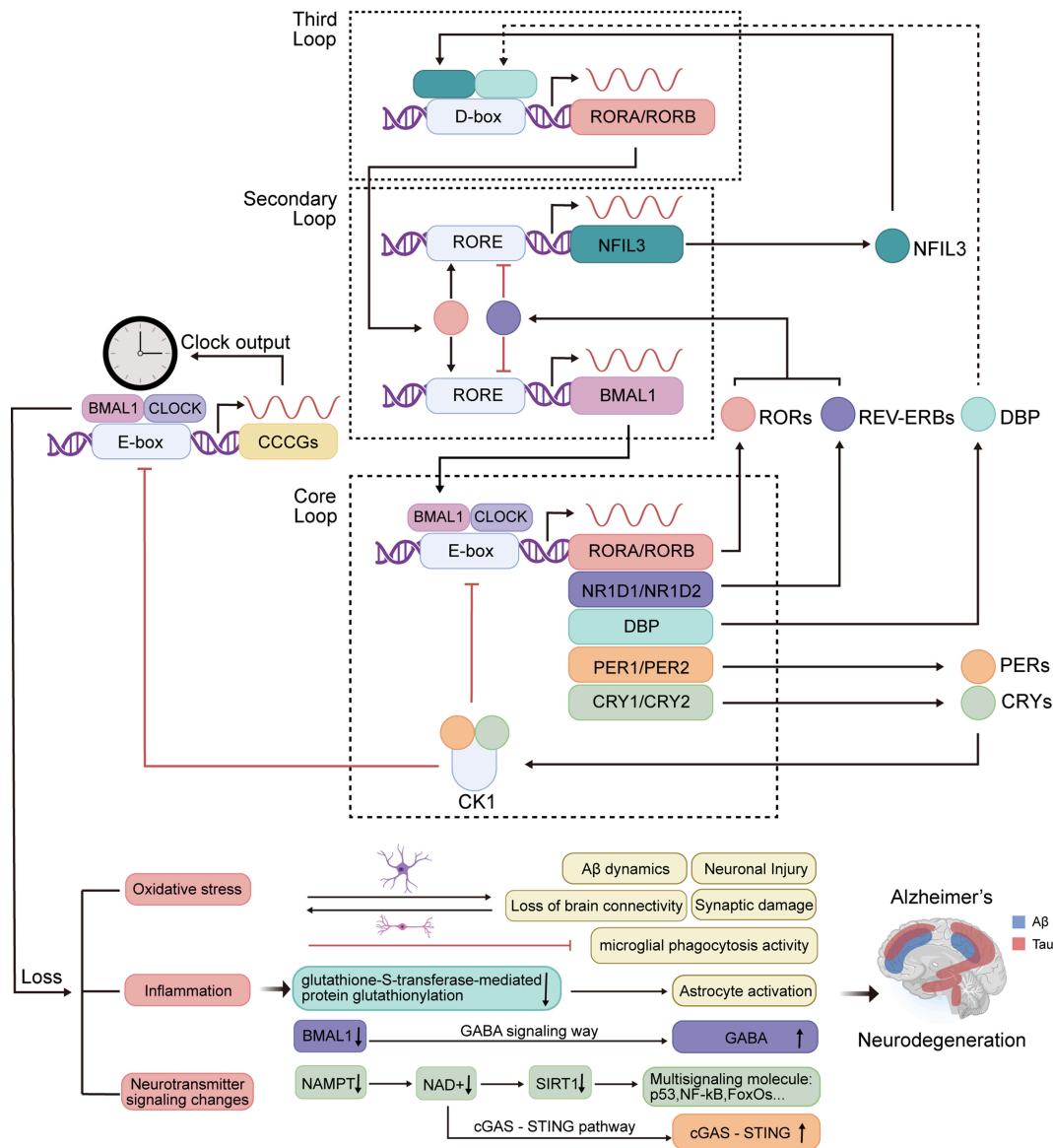
**Figure 2.** The core components of the circadian oscillator within the cell autonomously regulate the 24-h gene expression cycle, and a series of pathological alterations are caused by the loss of BMAL1. The 24-h rhythm of gene expression and the absence of BMAL1 are governed by the cell-autonomous core components of the circadian oscillator. Within the primary loop, as well as the core loop, the heterodimerization of CLOCK and BMAL1 drives the expression of PER and CRY genes in the morning, mediated by E-box elements. Subsequently, the PER and CRY proteins constitute a repressor complex that inhibits the transactivation of CLOCK and BMAL1. In the secondary loop, REV-ERBα, REV-ERBβ, and RORα, RORβ, RORγ subfamilies of nuclear receptors act as repressors and activators to antagonistically regulate BMAL1 and other target genes at RORE promoter elements at nighttime. The D-box, activated by PAR-bZIP proteins like DBP and repressed by E4BP4, plays a crucial role in the transcriptional regulation of circadian clock-controlled genes. Various ligands and small molecules have been identified to influence core clock components and circadian functions. Loss of BMAL1 is associated with neurodegeneration in AD and other age-related dementias. The absence of BMAL1 in the brain adversely affects astrocytes, microglia, neurons, and pericytes, resulting in increased oxidative stress and inflammation, as well as alterations in neurotransmitter signaling. These changes may contribute to astrogliosis and astrocyte activation, impaired microglial phagocytosis, and neuronal injury, which, in turn, lead to synaptic damage, alterations in Aβ dynamics, and loss of brain connectivity - key pathological features of AD and related dementias. Potential signaling pathways involved include GST-mediated protein glutathionylation, the SIRT1-BMAL1 axis, GABA signaling, NAD$^+$ depletion-related cyclic GMP-AMP synthase, and interferon gene stimulating factor activation, all of which play potential roles in AD. BMAL1: Aryl hydrocarbon receptor nuclear translocator-like; CLOCK: clock circadian regulator; PER: period circadian regulators; CRY: cryptochrome circadian regulators; DBP: D-box binding PAR bZIP transcription factor; AD: Alzheimer's disease; Aβ: amyloid-β; GST: glutathione S-transferase; GABA: gamma-aminobutyric acid; NAD$^+$: nicotinamide adenine dinucleotide; GMP-AMP: guanosine monophosphate - adenosine monophosphate.

formation of rhythmic heterochromatin during the repressive phase through H3K9me2 and chromobox 5 (CBX5) binding at D-box binding PAR bZIP transcription factor (*DBP*), *PER1*, and *PER2*[54,66]. Enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) methyltransferase contributes to histone methylation, including di- and tri-methylation of H3K27 (H3K27me2 and H3K27me3), influencing the circadian gene expression of *PER1* and *PER2*. The lysine demethylase 1A (KDM1A), lysine demethylase 5A (KDM5A), and ARID domain-containing histone lysine demethylase 1a (JARID1A) are crucial partners of CLOCK-BMAL1, enhancing its transcriptional activity. KDM1A removes methyl groups from H3K4 and H3K9, interacting with CLOCK and BMAL1, playing a pivotal role in the circadian clock mechanism and the regulation of clock-controlled gene expression[69]. In contrast, JARID1A interacts directly with CLOCK-BMAL1 and is involved in regulating circadian gene expression, with additional functions impacting HDAC1 recruitment without altering H3K4me3 methylation. Furthermore, the histone demethylase, lysine demethylase 8 (KDM8)[70], demethylates H3K36 and is responsible for recruiting various modifications, playing a role in modulating heterochromatin on a circadian timescale.

## CIRCADIAN RHYTHMS IN NEURODEGENERATIVE DISEASES

The circadian system exerts a profound influence on a multitude of physiological processes within the human body, including sleep, alertness, and cognitive performance. Disruptions in circadian rhythms can have adverse effects on humans. In addition, neurodegenerative diseases present a relatively diverse range of symptoms, numerous of which show differently frequent and intense daily changes. A bidirectional relationship exists between circadian rhythm homeostasis and neurodegenerative diseases, indicating that circadian rhythms may exert a significant influence on the progression of neurodegenerative diseases.

Current indicators of circadian disruption include (i) sleep disturbances, (ii) cognitive impairment, (iii) uncontrolled mood or behavior, (iv) motor control, and (v) metabolic regulation [Table 2][71]. Circadian rhythm disruptions are more severe in neurodegenerative diseases than in age-related disruptions. These disruptions can present as increased nighttime activity, decreased daytime activity, or a complete reversal of the 24-h rest-activity pattern. Importantly, these circadian disruptions may serve as early indicators of neurodegeneration and could potentially heighten the risk of developing neurodegenerative diseases in healthy adults aged over 60 years. Circadian rhythms may intervene in the clinical features of neurodegenerative diseases by disrupting the above indicators, and understanding the relationship between circadian disruption and the clinical features of neurodegenerative diseases is critical for the early detection and treatment of these diseases.

### AD and related dementias

The relationship between sleep and disease-specific pathways, particularly regarding Aβ, a pathogenic protein associated with AD, has been robustly documented. Aβ, known to form amyloid plaques, aggregates in the brain of patients with AD years before cognitive impairment occurs, serving as an early AD biomarker[72]. Aβ concentrations fluctuate in the brain's extracellular space following a circadian rhythm, with an increase during waking periods and a decrease during rest, irrespective of light cycles. These oscillations in Aβ are closely tied to changes in neuronal metabolic activity during sleep and wakefulness[73]. Preclinical research using transgenic mouse models with Aβ deposition has shown that sleep deprivation accelerates Aβ plaque formation, whereas sleep promotion through orexin antagonist drugs inhibits Aβ plaque deposition. Genetic deletion of the hypocretin neuropeptide precursor (*HCRT*) gene, which modulates wakefulness and regulates feeding and metabolism, results in modest increases in sleep duration and effectively suppresses Aβ plaque formation in AD mouse models[74]. Aβ deposition in the brains of transgenic mice gradually increased with age, especially peaking in old age[73]. This result suggests that age is an important factor in Aβ deposition and is closely related to the risk of developing neurodegenerative diseases, such as AD.

Epidemiological studies have corroborated these findings, identifying fragmented or deficient sleep as a risk factor for future symptomatic AD in cognitively normal individuals[75]. Moreover, individuals with Aβ plaque pathology experience noticeable declines in sleep quality prior to the onset of cognitive symptoms. Studies in humans have also shown a correlation between the levels of orexin and tau protein, another hallmark of AD, in cerebrospinal fluid (CSF). Higher levels of orexin-A, promoting wakefulness, are linked with increased levels of phosphorylated tau, a well-established marker of neurodegeneration in AD, in the CSF[76]. However, the relationship between sleep and tau pathology is yet to be fully elucidated. In summary, sleep deprivation contributes to Aβ plaque formation, while orexin modulation and improved sleep quality show promise in inhibiting Aβ plaque deposition. Further research is needed to obtain a deeper comprehension of the intricate relationship between sleep, Aβ, tau, and neurodegeneration in AD.

Patients with moderate-to-severe AD experience more pronounced disruptions in their circadian rhythms than healthy adults of a similar age. These disruptions manifest as increased sleep fragmentation, reduced amplitude, and phase delay rather than the typical advanced circadian phase seen in healthy aging[77]. The phase delay of temperature and hormone rhythms in AD may partly contribute to the phenomenon of "sundowning", characterized by heightened behavioral and neuropsychiatric symptoms in patients with AD around sunset[78]. Commonly, sleep-wake disorder observed in AD patients is irregular sleep-wake rhythm disorder (ISWRD), differing from the advanced sleep-wake rhythm disorder commonly seen in older adults without cognitive impairment[79]. In cases of severe AD, the lack of a distinct 24-h sleep-wake cycle, manifested by prolonged periods of wakefulness at night and erratic episodes of sleep throughout the day, serves to define ISWRD.

In recent times, there has been a notable increase in the number of studies examining patients with varying degrees of cognitive impairment. These studies have identified discrepancies in their circadian patterns compared to previous research that predominantly focused on patients with moderate to severe AD[77]. This discrepancy may be attributed to the disparate types or degrees of cognitive impairment documented in these more recent studies. The included studies encompassed patients with preclinical AD[80], mild cognitive impairment[81], mild AD[82,83], moderate to severe AD[83], global AD[84], and early onset dementia[85]. All these studies reported disruptions in rest-activity rhythms and sleep timing as behavioral markers of circadian rhythm disruption. Overall, these studies found that rest-activity rhythm fragmentation was high[80,85]; however, a modest decrease or absence of alteration was observed in the amplitude of rest-activity or melatonin rhythms[80,82,84-86]. Therefore, there is a need to focus on patients with different severities of AD.

## PD

PD, a progressive neurodegenerative movement disorder, affects 2%-3% of individuals aged over 65 globally. The primary diagnostic markers of PD are motor function abnormalities, primarily caused by a reduction in dopamine levels within the basal ganglia system. Moreover, circadian rhythms also exert influence over inflammation and oxidative stress, both of which are pivotal pathologies linked with PD-specific neurodegeneration. More critically, neurodegeneration and pathological protein aggregation resulting from various etiologies can, conversely, cause sleep disturbance and disruptions of circadian rhythms, thereby establishing a deleterious feedback loop.

PD exhibits disruptions in the typical 24-h rhythms of motor and non-motor symptoms. Unlike those with AD, individuals with PD and circadian rhythm disturbances experience a decrease in the amplitude of their circadian rhythm, albeit lacking a statistically significant shift in circadian phases[87-92]. Indeed, there is

evidence to suggest that fluctuations in the non-motor symptoms of PD do occur on a seasonal basis. These findings indicate that individuals with PD experience a greater burden of symptoms during the winter months than during the summer months[93]. Some have proposed that these seasonal fluctuations in PD symptoms may be attributed to changes in circadian regulation. It is well-established that dopamine metabolism is closely intertwined with circadian homeostasis. In a male *Drosophila* model, disruption of circadian rhythms exacerbated the degeneration of dopaminergic neurons within them[94].

Furthermore, circadian rhythms of microglia have a potential role in PD and other neurodegenerative diseases[95]. A substantial body of evidence from numerous studies indicates that the circadian clock in microglia may regulate several processes, including the release of inflammatory cytokines, autophagy, phagocytosis, and redox homeostasis, under physiological conditions. However, some theories require further investigation[96,97]. Consistently, disturbances in circadian rhythms or sleep disorders may exacerbate PD progression through mechanisms involving microglia-mediated neuroinflammation, oxidative stress, and protein homeostasis[98,99].

Disruptions to the sleep-wake rhythm are extensively documented symptoms in neurodegenerative diseases such as PD. Sleep-wake disturbances, impacting up to 80% of patients with PD[100], represent the most common non-motor symptom in PD. Various studies have reported excessive daytime sleepiness (EDS) and changes in sleep timing among patients with PD[89,101-103], indicating that they are more than twice as likely to experience EDS than healthy older adults[87,88]. To date, limited evidence suggests a slightly delayed sleep onset in patients with PD compared to healthy controls[89], with some studies not finding significant differences[90,92]. An Australian study revealed a notable decrease in the mesor and amplitude of the core body temperature rhythm in patients with PD compared to age-matched controls[90]. Research on melatonin secretion rhythms in patients with PD using different samples, such as plasma, serum, and saliva, indicates lower circulating levels of melatonin in patients with PD than their healthy counterparts, although the timing of melatonin onset remains similar[87,89,92]. Additionally, patients with PD may lack the typical nocturnal decline in blood pressure, leading to an increased risk of cardiovascular issues, including nocturnal hypertension[91]. For example, one study conducted in Spain found that 71.1% of patients with PD did not demonstrate the expected decrease in blood pressure throughout a 24-h period, as recorded by ambulatory blood pressure monitoring[91].

## ALS
Sleep-related breathing disorders represent the most prevalent sleep disturbance among patients with ALS, with a substantial body of documented evidence[104]. Modifications in respiration during the sleep cycle may foreshadow the onset of respiratory symptoms upon waking, even in patients with unimpaired respiratory function. However, as ALS progresses, the weakening of respiratory and upper airway muscles and the diaphragm precipitates nocturnal hypoxia and hypoventilation[105,106]. Sleep disorders in ALS encompass reduced sleep quality and EDS, with the latter being more common and linked to disease severity and cognitive decline[102]. Insomnia, featured by difficulties with initiating or maintaining sleep or with waking up at an early hour, is frequently observed in patients with ALS[104,107]. Nonetheless, sleep disorders that are not associated with respiratory issues have been less extensively investigated in the context of ALS.

## HD
HD is a progressive neurodegenerative disorder that is typified by a range of symptoms, including motor deficits, cognitive decline, and psychiatric manifestations. Despite the predominance of sleep disturbances in HD, they have not been as thoroughly investigated as in other neurodegenerative conditions[108]. Recent findings suggest the presence of circadian dysregulation in HD, with flattened melatonin secretion rhythms and delayed circadian phases observed in HD patients[109]. Circadian disruption has been observed in animal

models of HD, exemplified by transgenic R6/2 mice showing increased daytime activity and decreased nighttime activity[110]. Large animal models of HD, like transgenic sheep expressing the mutant Huntingtin protein, display significant circadian abnormalities, including behaviors resembling human sundowning[111]. The observed behavioral changes coincide with abnormal expression of the clock genes *BMAL1* and *PER2* in the striatum, motor cortex, and SCN.

While the observed circadian abnormalities provide a basis for considering circadian-based interventions for individuals with HD, systematic investigations of such interventions in patients with HD are lacking. Limited studies examining the effects of exercise on sleep in HD have not consistently demonstrated substantial improvements in sleep metrics[112]. Pharmacological interventions have shown efficacy in restoring circadian rhythms in R6/2 mice[113]. Other zeitgebers, besides light, such as feeding schedules, have demonstrated positive impacts on rest-activity cycles in the HD model of R6/2 mice. These effects are likely mediated through the activation of the food-entrainable oscillator, which operates independently of the SCN[114]. Exploring sleep disturbances in HD may further help to inform future therapeutic strategies to slow disease progression[115]. A recent study showed promise in restoring locomotor function and normalizing disrupted circadian gene expression in the HD model of *Drosophila* using melatonin and curcumin[116], a polyphenol derived from turmeric known to activate BMAL1 and potentially modulate circadian clock processes[116].

### Underlying mechanisms between circadian rhythms and neurodegenerative diseases

The impact of neurodegenerative pathology on circadian function varies across different diseases. In AD, research has demonstrated a reduction in crucial neuronal populations in the SCN, specifically those that express AVP or VIP[84]. The reduction in the number of neurons expressing VIP in the SCN is associated with the circadian dysfunction observed before death. However, the precise mechanisms underlying this neuronal loss remain uncertain. Unlike the regions heavily affected by Aβ plaque or neurofibrillary tangles, the SCN does not show significant pathology. Transgenic mouse models of AD expressing mutant human APP, tau, or both also exhibit circadian abnormalities, but establishing a direct link with pathology has proven challenging, hindering mechanistic understanding[117-119]. The Aβ peptide has been proposed to contribute to circadian dysfunction by promoting the degeneration of BMAL1 in cultured cells[120,121]. Nevertheless, at present, there is no indication to imply that Aβ directly interacts with the circadian clock in animals or humans. Disrupted circadian rhythms, attributed to dysregulated methylation of the *BMAL1* promoter, have been observed in fibroblasts from patients with AD and post-mortem brain samples, indicating an epigenetic mechanism underlying the disruptions of circadian rhythms in AD.

Conversely, potential mechanisms through which circadian rhythms impact neurodegenerative diseases have been postulated[122]. Disruption of circadian rhythms may influence the advancement of neurodegenerative diseases by changing sleep duration, resulting in disjointed nighttime sleep and increased daytime napping. Sleep deprivation can potentially modify Aβ dynamics in humans and exacerbate Aβ and tau pathology in mouse models[123-125]. Furthermore, it can escalate levels of inflammatory and neuron-specific injury biomarkers in the human CSF[126]. Moreover, sleep deprivation has been implicated in triggering protein clearance from the brain in mouse models, promoting inflammation, and impacting synaptic homeostasis associated with neurodegenerative diseases by modulating immune responses[127,128]. Interventions aimed at enhancing sleep quality are generally intended to counteract the consequences of circadian rhythm disturbances. Nonetheless, research has revealed that mutations in clock genes can provoke neuropathology resembling astrocyte proliferation in mice, even without changes in sleep patterns. This observation suggests that the impacts of circadian terminals on the brain cannot be solely attributed to alterations in sleep.

Circadian dysfunction may impact neurodegeneration through the regulation of immune responses. The timing of exposure to inflammogens significantly influenced the peripheral immune response in mice. Circadian rhythms strongly affect the level of inflammation[129,130]. In a mouse model of experimental autoimmune encephalitis, disease severity at later stages was notably influenced by the timing of immunization. Rodents with *Bmal1* deleted in myeloid cells experienced exacerbated pathology[131,132]. In addition, global and brain-specific deletion of *Bmal1* in neurons leads to cell-autonomous dopaminergic neurodegeneration[133]. Microglia and astrocytes, the key innate immune cells in the brain, have functional circadian clocks that control inflammatory responses[96,134]. Disruption of the circadian clock function in the mouse brain by deleting *Bmal1* led to widespread activation of astrocytes and synaptic degeneration. These findings underscore the pivotal function of the core clock function in preserving immune equilibrium within the brain[135]. Disturbances in circadian rhythms induced by non-24-h light-dark cycles have been demonstrated to elevate the activation level of glial and inflammation levels of neurons, exacerbating disease progression in mouse models of ALS and PD[136,137]. Therefore, circadian dysfunction is likely involved in certain aspects of neuroinflammation that can impact neurodegeneration in distinct disease states.

The circadian clock's influence on protein homeostasis and quality control may affect protein deposits in neurodegenerative diseases. Diurnal variations in Aβ levels have been observed in human CSF and mouse interstitial fluid[73,120,138]. Furthermore, disrupting the circadian clock in a mouse model of β-amyloidosis and AD accelerated the accumulation of amyloid plaques[138]. In a broader context, regulating circadian rhythms within protein quality control mechanisms, such as autophagy, may also lead to the circadian influence on protein aggregation[139,140]. The glymphatic system, a perivascular fluid clearance system mediated by glial cells, eliminates aggregated proteins from the brain in bulk. While this process is associated with sleep, its relationship with the circadian system and the involvement of glial clocks remains unclear[127]. Therefore, various identified and unknown mechanisms could link the circadian clock to neurodegenerative conditions.

## RNA MODIFICATIONS IN CIRCADIAN RHYTHM AND NEURODEGENERATIVE DISEASES

The advent of genome-wide technologies, including microarray analysis and deep RNA sequencing, has made it possible to identify the pervasive circadian regulation of transcription at both the species and individual tissue levels. The circadian control of gene expression shows organ-specific patterns, highlighting the complexity of biological timekeeping[141]. While mRNA abundance in the cytosol governs the possibility and timing of protein synthesis for most proteins, the transcription rate and mRNA export to the cytoplasm determines protein accumulation[142]. However, this does not extend to the numerous genes that are subject to post-transcriptional regulation by the circadian clock. While rhythmic transcription significantly contributes to the rhythmic abundance of proteins, the levels and activity of proteins can cycle independently of the rhythms in mRNA transcript abundance[143,144]. Numerous examples of circadian regulation impacting various phases of mRNA lifespan exist, encompassing mRNA processing, capping, translation initiation, elongation, and decay rates[144]. Disruptions in circadian homeostasis have detrimental effects on human health, particularly in neurodegenerative diseases that exhibit diurnal symptom fluctuations. These disorders disturb the circadian balance, impacting symptom manifestation and overall quality of life. Recent findings propose a mutual correlation between circadian equilibrium and neurodegeneration, suggesting a potential involvement of circadian functionality in the progression of neurodegenerative conditions. Consequently, the circadian rhythm system associated with RNA modifications has become a promising focus for neurodegenerative disease research and clinical applications.

## RNA modifications

Chemical alterations to the bases or ribose sugar in RNA molecules are known as RNA modifications. Over 150 different changes have been found to date[145]. These modifications exert a pivotal influence on the nervous system, regulating cellular heterogeneity and molecular complexity through the modulation of gene expression. The chemical modifications of RNA bases and sugar residues constitute the neuronal epitranscriptome. These RNA modifications are regulators of RNA stability, transport, and translation. They affect diverse downstream signaling pathways. Specific chemical modifications of biological components are an effective way to regulate molecular activity. Several pathogenic diseases, including cancer, cardiovascular abnormalities, and neurodegenerative disorders, can result from perturbations in the external transcriptome.

Commonly observed RNA modifications can contribute to various neurodevelopmental and neurodegenerative disorders, including methylation and isomerization of adenine and cytosine bases. Representative post-transcriptional processing steps for eukaryotic RNA include 5' capping, intron excision or splicing, and the insertion of a 3' polyadenylated tail. Another significant pathway of post-transcriptional RNA modification involves the chemical alteration of RNA bases and sugar residues on the RNA backbone.

The functional impact of the epitranscriptome is evident and pervasive, manifesting in nearly all tissues. It especially contributes to the complexity of the nervous system by modulating various levels of RNA metabolism. Recent studies have emphasized the contribution of dysregulated RNA transport, splicing, stabilization, translation, or microRNA (miRNA) biogenesis to neurodegenerative diseases. RNA metabolism is crucial for maintaining proper brain function and consolidating learning-based memory in the adult brain.

While 170 distinct RNA modifications have been detected so far, only a few have been well characterized and particularly associated with neurologic disorders. Three groups of protein factors with substrate-specific enzymatic activities, termed writers, readers, and erasers, collectively edit epitranscriptome. They are collectively called RNA-modifying proteins (RMPs) and are responsible for depositing, reading, and removing RNA modification marks[145]. Readers, which frequently alter RNA metabolism, recognize specific RNA modification marks, binding to these sites and activating downstream pathways. Mutations and variations in RMP abundance are associated with several diseases, including infertility, obesity, neurodegenerative and neurodevelopmental disorders, and cancer.

RNA metabolic mechanisms modulate the expression of proteins involved in developing, structuring, and functioning the brain over time. RNA-binding proteins (RBPs) adjust RNA metabolism, leading to complex gene expression patterns in different parts of the neuron. Other RBPs may counteract the functions of expression-mediated RBPs to balance RNA metabolism and gene expression in neurons throughout the brain. Maladjusted functioning and enrichment of RBPs can seriously disrupt control mechanisms, which can lead to neurodevelopmental or neurological diseases.

Quite many RNA modifications have been confirmed on coding and non-coding RNAs (ncRNAs), with only a limited number extensively studied or linked to diseases. Among the well-studied RNA modifications are m⁶A, m¹A, m⁵C, pseudouridine, and RNA editing [Table 3].

## RNA modifications and neurodegenerative diseases

### *m⁶A*

The dynamic and reversible RNA modification known as m⁶A plays a crucial role in dendritic structure,

**Table 3. Related proteins and function of common RNA modifications**

| Name | Role | Proteins | Function | Ref. |
|---|---|---|---|---|
| $m^6A$ | Writer | METTL3 | Forms a heterodimer with METTL14 to convert A to $m^6A$ on mRNA<br>METTL3-mediated mRNA circularization promotes translation initiation | [146] |
| | | METTL14 | METTL14 plays a crucial structural role in catalysis facilitation | [146] |
| | | WTAP | Catalyzes the formation of $m^6A$ and involved in pre-mRNA splicing | [147] |
| | Eraser | ALKBH5 | Responsible solely for catalyzing $m^6A$ removal on ssRNAs<br>Localizes to nuclear speckles and contributes to the assembly of mRNA-processing factors | [148] |
| | | FTO | Removes $m^6A$ modification | [149] |
| | Reader | YTHDF1 | Promotes mRNA translation | [150] |
| | | YTHDF2 | Reduces mRNA stability | [151] |
| | | YTHDF3 | Functions synergistically with YTHDF1 to enhance translation and with YTHDF2 to promote mRNA decay | [151] |
| | | IGF2BP1/2/3 | Protect target mRNAs in the P-body from degradation | [152] |
| $m^1A$ | Writer | TRMT6/61A | Methylate cyto-tRNA and mRNAs that have a T-loop-like structure | [153] |
| | | TRMT61B | Functions as the $m^1A$ writer for mitochondrial mRNAs | [154] |
| | Eraser | ALKBH1 | Inhibits the translation initiation and translation efficiency | [153] |
| | | ALKBH3 | Inhibits the translation initiation and translation efficiency | [153] |
| | Reader | YTHDF3 | Demonstrates lower binding affinity with $m^1A$ sites compared to $m^6A$ | [153] |
| $m^5C$ | Writer | NSUN1-7 | Forms complex with a full-length tRNA substrate and catalyzes tRNA $m^5C$ modification | [155] |
| | | DNMT2 | Catalyzing methylation of cytosine-5 | [154] |
| | | TRDMT | Catalyzing methylation of cytosine-5 | [154] |
| | Eraser | TET | Mediates the demethylation of written RNA | [155] |
| | Reader | ALYREF | The first found in the nucleus<br>$m^5C$ recognition site of K171 | [155] |
| | | YBX1 | Cytoplasmic mRNA $m^5C$ reading protein | [155] |
| Pseudouridine | Writer | PUS | Catalyzes the conversion of uridine to pseudouridine in their targets without any accessory RNA contribution | [154] |
| RNA editing | Writer | ADAR2 | ADAR2 edits the AMPA receptor pre-mRNA to regulate its function<br>Reduced editing and functional defects of AMPAR occur when ADAR2 is downregulated in disease conditions | [156] |

$m^6A$: $N^6$-methyladenosine; METTL3: methyltransferase like 3; METTL14: methyltransferase-like 14; WTAP: WT1-associated protein; ALKBH5: alkB homolog 5; YTHDF: YTH N6-methyladenosine RNA binding protein; IGF2BP: insulin-like growth factor-2 mRNA-binding protein; $m^1A$: $N^1$-methyladenosine; ALKBH: alkB homolog; FTO: alpha-ketoglutarate dependent dioxygenase; $m^5C$: 5-methylcytosine; NSUN1-7: NOP2/Sun domain family members 1-7; TRDMT: tRNA aspartic acid methyltransferase; ALYREF: Aly/REF export factor; YBX1: Y-box binding protein 1; PUS: pseudouridine synthase; ADAR: adenosine deaminase.

spinogenesis, learning memory, neurogenesis, axon regeneration, and brain development[157-161]. It is present in various RNA types, including mRNAs, transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), ncRNAs, circular RNAs, and microRNAs (miRNAs). Within mRNAs, $m^6A$ marks predominantly occur at the beginning of the final exons, situated either in the 3' untranslated region (3'UTR) or near the stop codons. The reversible nature of $m^6A$ modification is regulated bidirectionally by the $m^6A$ methyltransferase and demethylase in collaboration with RNA $m^6A$ methylation-binding proteins to influence RNA fates[162-164]. Methyltransferase complexes, such as methyltransferase like 3 (METTL3) and 4 (METTL4) and WT1-associated protein (WTAP), act as writers of $m^6A$ marks[165,166]. Demethylases, known as erasers, including FTO and alkB homolog 5 (ALKBH5), catalyze the removal of $m^6A$ modifications from RNA substrates[165,166]. The $m^6A$ binding proteins, referred to as readers, in $m^6A$ methylation, selectively bind to $m^6$ A methylation sites to disrupt homologous RNA-protein interactions and alter RNA secondary structure, thereby affecting protein-RNA interactions. Various readers, such as YT521-B homology (YTH) domain family members, fragile X messenger ribonucleoprotein 1 (FMR1), heterogeneous nuclear ribonucleoproteins, eukaryotic initiation factor 3, insulin-like growth factor-2 mRNA-binding protein (IGF2BP), and proline-rich coiled-coil 2A, have been confirmed as critical players in $m^6A$ RNA methylation modification.

The capacity for chemical modifications to occur in a dynamic manner is a fundamental aspect of neural system functionality. Extensive m⁶A methylation alterations are present in various mammalian organs, with the brain being a prominent example. Interestingly, m⁶A's adaptability has been confirmed across several types of brain regions, including the frontal cortex, hippocampus, and amygdala[167]. m⁶A plays a crucial role in early and late brain development in a spatial-temporal manner. Disruptions in circadian rhythms, seen in neurodegenerative diseases, may be linked to changes in the expression or activity of enzymes responsible for m⁶A modification[168]. Moreover, oxidative stress and inflammatory reactions, characteristic features of neurodegenerative diseases, can influence m⁶A modification and circadian rhythms[169]. The manifestation of neurological disorders is rooted in the dysregulation of the m⁶A pathway, caused by disease-specific mutations or alterations in the levels of various m⁶A components.

Relationships between m⁶A and AD have been examined in relatively high detail. m⁶A modifications play a pivotal role in AD, influencing the transcripts' protein levels which are important in determining disease phenotypes[170]. RNA m⁶A methylation is recognized as a significant epigenetic marker associated with AD disturbances. In PD models, the m⁶A level is significantly reduced in dopaminergic neurons, while the m⁶A demethylase FTO is markedly increased, both *in vivo* and *in vitro*[171]. As a novel diagnostic biomarker for PD, methyltransferase-like 14 (METTL14) modifies pathogenic SNCA protein through an m⁶A-YTH N6-methyladenosine RNA binding protein F2 (YTHDF2)-dependent mechanism[172]. Furthermore, m⁶A modification influences RNA binding by TDP-43, indicating its importance in TDP-43-related neurodegeneration in ALS[173]. Highlighting m⁶A as a novel hallmark of HD, a distinctive landscape of RNA methylation contributes to cognitive symptoms associated with HD[174]. The combined loss of TDP-43 function and altered m⁶A modification represent a novel mechanism underlying AS or unannotated exon usage in HD[175]. Crucial for the establishment and development of the mammalian CNS and PNS, proper m⁶A modification is essential overall.

*m¹A*
The m¹A modifications predominantly occur in the 5'UTR and coding sequence (CDS) of mRNAs. tRNA methyltransferase 6 non-catalytic subunit (TRMT6)/tRNA methyltransferase 61A (TRMT61A), a non-catalytic subunit of tRNA methyltransferase 6, is the most abundant cytosolic m¹A writer. Responsible for mitochondrial mRNA, the homodimeric tRNA methyltransferase 61B (TRMT61B) functions as the m¹A writer. As m¹A erasers, alkB homologs 3 (ALKBH3) and 1 (ALKBH1) are involved in demethylating the m¹A mark on mRNAs[153]. Among the recently identified m¹A readers are nine recognized ones, including ribonucleoproteins and YTH family members[176].

A solid relationship between m¹A marks and neurodegenerative diseases has yet to be established. The primary function of m¹A mRNA modifications is to control mRNA translation and degradation, probably significantly impacting appropriate brain growth and function. The m¹A modification is abundant in neuron mRNAs and subject to dynamic regulation during oxygen-glucose deprivation/re-oxygenation induction[177]. Furthermore, mutations in hydroxysteroid 17-beta dehydrogenase 10 (HSD17B10) have been shown to cause a disease characterized by progressive neurodegeneration and cardiomyopathy, termed HSD10 disease[178]. A consensus exists that m¹A marks modify tRNA stability and translation to regulate neurological disorders.

*m⁵C*

DNA m⁵C has traditionally been seen as a crucial epigenetic modification that impacts various physiological processes, including brain development. Notably, m⁵C modifications have been detected in rRNAs, tRNAs, mRNAs, and other less common RNA species[179]. In humans, the creation of m⁵C marks is attributed to NOP2/Sun domain family members 1-7 (NSUN1-7) and tRNA aspartic acid methyltransferase 1 (TRDMT1)[180]. Reader proteins Aly/REF export factor (ALYREF) and Y-box binding protein 1 (YBX1) recognize and bind to m⁵C, playing roles in mRNA stability and nucleocytoplasmic shuttling. The impact of m⁵C modification on mRNA translation varies depending on the site of m⁵C deposition, with translation efficiency impaired by m⁵C in the 5'UTR or CDS. In contrast, NSUN2-mediated m⁵C modification in the 3' untranslated region (3'UTR) enhances translation efficiency, which is intriguing.

Dysregulated expression of genes encoding m⁵C methyltransferases, given their significant functional roles in RNA metabolism, may be implicated in various diseases. Mutations in genes encoding NSUN proteins have been linked to several neurodevelopmental disorders. NSUN6 and NSUN7, two RNA methylation writers, exhibit different patterns: NSUN6 shows a notable decrease in the hippocampus and temporal gyrus of individuals with AD and traumatic brain injury, while NSUN7 is elevated in the hippocampus of individuals with AD or high neuropathology[181]. Genetic mutations in the m⁶A writer gene methyltransferase 5 (*METTL5*); m5C writer genes *NSUN2*, *NSUN3*, *NSUN5*, and *NSUN6*l and THO complex subunits 2 (*THOC2*) and 6 (*THOC6*), which form a protein complex with the m5C reader ALYREF, have been identified as causative factors in intellectual developmental disorders[182]. Impaired m⁵C methylation leading to tRNA cleavage restricts translation in diseased neurons.

*Pseudouridylation*

Pseudouridine, a uridine isomer, is primarily found in ncRNAs, including rRNAs, tRNAs, and small nuclear RNAs (snRNAs). Two distinct mechanisms have been proposed for RNA pseudouridylation. In the first mechanism, guide RNA-dependent pseudouridylation occurs, wherein H/ACA-box snRNAs bind to target RNAs via specific interactions, resulting in uridine modification catalyzed by specific enzymes [e.g., dyskerin pseudouridine synthase 1 (DKC1) in humans or pseudouridine synthase CBF5 in yeast] within the H/ACA-box snoRNA ribonucleoprotein complex. The second mechanism, guide RNA-independent pseudouridylation, relies on pseudouridine synthases (PUSs) directly converting uridine to pseudouridine in their targets without needing accessory RNAs[183-185].

Pseudouridylation abnormalities in *PUS* genes, which are linked with neuronal dysfunction, are being considered as a likely therapeutic target for neurodegenerative diseases. *Pus3* expression in the nervous system of mouse embryos suggests its involvement in neuronal development, a notion supported by reports that link PUS3 truncation to intellectual disability in humans[186,187]. Patients exhibiting intellectual disability, microcephaly, speech delay, and aggressive behavior have been found to harbor mutations in *PUS7*[188,189]. While the precise mechanism remains elusive, it is probable that the existence of pseudouridine results in a reduction in mRNA stability and impedes translation.

## RNA modifications and circadian rhythm

*Circadian RNA processing*

The circadian regulation of RNA processing is pivotal in the expression of circadian genes. These steps involve processes such as mRNA capping, AS, and modulating RNA stability by altering the length of the polyadenylation tail or using selective polyadenylation sites[190]. The onset of pre-mRNA processing in mammals commences with establishing the cap structure within the 5'UTR. This process involves the combination of eukaryotic translation initiation factor 4E (eIF4E) with the cap structure, with observations

indicating that it can modulate cap-dependent translation in a circadian manner through a phosphorylation pathway under the influence of circadian rhythms[191]. Pre-mRNA splicing is another key aspect of gene expression that can impact the circadian clock. This process can generate alternative mRNAs by selecting different regions, known as AS. The circadian regulation of AS may have significant implications for circadian control, as demonstrated by a recent study in mouse liver using a microarray-based approach[192].

Co-transcriptional addition of the 3' poly(A) tail is a crucial feature of eukaryotic mRNAs, playing a role in their nuclear export, translation, and stability. While long poly(A) tails generally stabilize mRNAs, poly(A) tail shortening typically triggers mRNA degradation. The length of the poly(A) tail may be under the influence of the circadian clock. Furthermore, the polyadenylation site within the 3'UTR region of the mRNA may also be subject to circadian control[190]. Variations in mRNA 3'UTR lengths can result from utilizing multiple polyadenylation sites, a process known as alternative polyadenylation[193]. eIF4E, a vital component of the eukaryotic translation initiation machinery, is a limiting factor for translation initiation due to its binding to the 5' cap structure of the mRNA, which recruits ribosomes for translation initiation and is tightly regulated. Research has indicated that eIF4E plays a role in circadian rhythm translation regulation through phosphorylation[194]. The mitogen-activated protein kinase (MAPK)/MAPK-interacting kinase (MNK) pathway, regulated by light and the circadian clock, induced eIF4E's phosphorylation in the mouse SCN of the hypothalamus[191]. Rhythmic phosphorylation of the CCG protein BMAL1 is facilitated by ribosomal protein S6 kinase B1 (RPS6KB1). Following phosphorylation, BMAL1 interacts with the translation mechanisms via eIF4E which acts as the binding protein to combine cap[195]. eIF4E's involvement in the regulation of circadian translation is facilitated by phosphorylation governing its cap-dependent translation control, beginning with eIF4E's recognition of the 5' cap of the mRNA, thus allowing it to contribute to circadian translation regulation.

*Interaction between RNA modifications and circadian rhythm*
Both RNA methylation and editing have potential interactions with the circadian clock[196]. Research indicates that the timing and pace of circadian oscillators can be modulated by RNA methylation[1]. The inhibition of RNA methylation has been observed to extend circadian rhythms. Furthermore, transcripts of several core CCGs, including *CLOCK*, *PER1*, *PER2*, *PER3*, *DBP*, and nuclear receptor subfamily 1 group D members 1 (*NRLD1*) and 2 (*NRLD2*), feature multiple methylation sites[1]. Liver-specific *Mettl3* knockout mice showed reduced levels of m⁶A and several metabolic transcripts crucially regulated by circadian rhythm. The nuclear protein levels of the core clock transcription factors BMAL1 and CLOCK were also reduced[197].

The importance of RNA methylation in circadian rhythm has been confirmed. Overall, the negative feedback loop of clock genes in circadian rhythm is regulated by m⁶A RNA modification. Circadian rhythms also govern the regulation of RNA methylation. Studies have demonstrated the control exerted by circadian regulators over m⁶A RNA modification[198]. A decrease in global m⁶A methylation levels occurs after knockdown of *CRY1/2*[198]. Studies have shown the presence of circadian rhythmic-dependent m⁶A methylation of mRNA[199]. The specific knockout of *Bmal1* in the liver of mutant mice increased m⁶A methylation levels in the mRNA of the nuclear receptor peroxisome proliferator-activator alpha (PPARα)[199]. In conclusion, there is indeed an interaction between RNA methylation and circadian rhythm.

RNA editing converts adenosine to inosine via adenosine deaminases (ADARs) that act on RNA, adding guanosine residues to the translation process and further increasing the protein diversity of individual genes. In mammals, ADAR1 and ADAR2 are mainly involved in RNA editing. In the liver, ADAR2 exhibits a circadian rhythm of mRNA and protein abundance under constant conditions and causes diurnal changes

in A-to-I RNA editing[200]. In addition, the circadian cycle length of motor activity in *Adar2* knockout mice was significantly shorter than that of wild-type mice[200]. Subsequent studies have also indicated the essential role of ADAR2 in light-induced circadian clock phase shifts[201]. These studies propose a mutual interaction between RNA editing and the circadian system. It has been found that ADAR1-mediated AS regulation of the ATP binding cassette subfamily B member 1 (*ABCB1*) gene is the key mechanism of its circadian expression in renal proximal tubule epithelial cells[202]. Post-transcriptional regulation is key to the function of circadian oscillators and the timing of circadian output.

**Linkage among RNA modifications, circadian rhythms, and neurodegenerative diseases**

Studies have demonstrated that RNA modification affects the development and progression of degenerative neurological diseases. In the post-transcriptional modification of mRNA, A-to-I RNA editing, m⁶A RNA methylation, and AS assume pivotal roles across all stages of the neuronal cell life cycle, with alterations in their mechanisms significantly contributing to aging and neurodegeneration[203]. In the AD brain, neurons show decreased m⁶A levels and expression of m⁶A METTL3, and *METTL3* knockdown leads to decreased m⁶A modification of neurons in the hippocampus, leading to significant memory deficits, suggesting that METTL3-mediated m⁶A dysregulation may lead to neurodegeneration in AD[204]. The overexpression of *METTL3* rescues synaptic damage and cognitive impairment *in vivo* induced by Aβ[204]. Inactivation of *FTO* has been demonstrated to alter the delivery of neuronal dopamine[205], implicating adenosine RNA methylation in regulating dopaminergic signaling pathways and suggesting its potential involvement in the etiology of PD. These studies suggest a link between RNA methylation and neurodegenerative diseases.

The co-occurrence of TDP-43 aggregation and decreased expression level of ADAR2 in the same motor neurons is a characteristic of sporadic ALS[206]. ADAR2 primarily targets the Q/R site in glutamate ionotropic receptor AMPA type subunit 2 (GRIA2) pre-mRNA. The absence of ADAR2 leads to unedited GRIA2, resulting in heightened intracellular calcium influx and consequent slow motor neuron demise[207]. ADAR2 deficiency triggers calpain activation, initiating the progressive formation of TDP-43 aggregates and subsequent disease progression[206]. In patients with AD, GRIA2-Q/R editing was less efficient at 30 different sites in the brain[182]. The decrease in ADAR2 levels was associated with diminished Q/R site editing, potentially contributing to the initiation or progression of ALS. Within the realm of neurodegenerative diseases, several have been predominantly associated with alterations in AS, including AD, PD, ALS, FTD, and familial dysautonomia (FD).

A comprehensive transcriptomic analysis conducted on the dorsolateral prefrontal cortex of 450 subjects from two distinct aging cohorts revealed the presence of 84 genes exhibiting aberrant pre-mRNA splicing events, which were found to be significantly associated with AD[208]. In PD, the 112-amino-acid *SNCA* transcript is generated by the removal of exon 5, resulting in the absence of S129, a pivotal phosphorylation site crucial for SNCA clearance, aggregation, and toxicity[209]. Removal of exon 3 leads to the production of the 126-amino-acid *SNCA* transcript, which is elevated in stages 3 and 4 in patients with PD[210]. Isoforms lacking exons 3-5 or 2-7 are upregulated in PD, and AS variants of parkin RBR E3 ubiquitin protein ligase (PRKN) lacking exon 4 are upregulated in sporadic PD[211]. Mutations in elongator acetyltransferase complex subunit 1 (*ELP1*) in FD disrupt the 5' splice site within intron 20, causing exon 20 to be skipped, resulting in a frameshift that generates premature termination codons in exon 21, triggering nonsense-mediated decay of *IKBKAP* transcripts. The absence of these transcripts contributes to the progressive degeneration of autonomic and sensory neurons[212]. Therefore, RNA modifications linked with circadian rhythms, including A-to-I RNA editing, m⁶A RNA methylation, and AS, are intricately linked to the pathogenesis of neurodegenerative diseases, influencing key processes in neuronal function and survival.

## RNA-BASED THERAPIES

### Methods

RNA-based therapeutics have become increasingly popular in treating various neurological diseases, offering diverse potential applications. Various therapeutic approaches, including RNA editing, antisense oligonucleotides (AONs), small interfering RNAs (siRNAs), RNA aptamers, RNA-based vaccines, and mRNA drugs, are proving effective in treating diverse conditions. Among these modalities, various studies have extensively researched the design, chemistry, and utilization of AONs[213-215]. Despite advancements in rare genetic diseases and neurodegenerative disorders, overcoming the challenge of safely and effectively delivering these therapies to the brain remains a significant obstacle. Furthermore, the safety concerns associated with RNA-based therapies encompass not only challenges in delivery but also issues related to suboptimal pharmacological properties, cell-specific targeting, immune-related toxicities, and potential long-term adverse effects. The development of personalized RNA-based treatments for ultra-rare diseases is being explored alongside their application in more common conditions such as AD and stroke. The potential of RNA-based therapeutics in addressing behavioral disorders and CNS tumors has not been fully realized. However, with advancements in the field, their transformative impact on neurology is promising. There are three groups of RNA-based therapies classified based on different mechanisms: those that act on nucleic acids [such as AONs or via the RNA interference (RNAi) pathway], those targeting proteins (utilizing RNA aptamers), and mRNA drugs designed to produce proteins.

Currently, the predominant form of RNA-based therapeutics is AONs. AONs consist of DNA or RNA sequences, usually 15-20 bp in length, and are modified to enhance stability. The selection of specific chemicals relies on the intended application, with all clinically approved AONs belonging to either oligomer of phosphorodiamidate morpholino or 2'-O-methoxyethyl[216]. Moreover, splice-switching AONs (ssAONs) obstruct intronic or exonic cis-regulatory elements to manipulate the target exon's inclusion or exclusion. These ssAONs target splice sites, exonic splicing enhancers or silencers, and their respective intronic counterparts. Furthermore, ssAONs can be effective in inhibiting the use of cryptic splice sites or introducing new splice sites for donors or acceptors[217].

RNAi, a regulatory mechanism in most eukaryotic cells for direct control of gene activity, has emerged as a mechanism for drug action in developing RNAi-based therapies [Figure 3]. Researchers have been familiar with RNAi for over twenty years, a natural process where short RNA strands, like siRNAs, induce specific gene suppression. siRNAs are double-stranded RNA (dsRNA) molecules that separate into single strands and selectively bind to target mRNA sequences. This binding initiates a series of events leading to the degradation and cutting of the target mRNA, halting translation and subsequent gene expression and function. siRNAs offer significant therapeutic potential by enabling the specific targeting and silencing of mRNAs from genes previously deemed challenging to target pharmacologically.

Almost all human protein-coding genes exhibit AS, which involves processes such as exon inclusion or exclusion, switching between different splice sites, retaining introns, selecting mutually exclusive exons, and other complex patterns of splice site selection[218]. The regulation of these splicing events relies on the spliceosome, a large molecular complex responsible for catalyzing splicing reactions[219]. Spliceosome assembly necessitates a coordinated interplay among trans-acting factors, encompassing small nuclear ribonucleoproteins, such as U1 and U2, constituting snRNAs and highly related proteins, in conjunction with approximately 150 accessory proteins. Recent breakthroughs in cryo-electron microscopy have provided detailed insights into the structure and mechanics of spliceosomes, revealing their structural foundation with unprecedented clarity [Figure 4].

The CRISPR-Cas13 system can potentially modify RNA, allowing for programmable regulation of AS, A-to-I, and C-to-U RNA editing, and $m^6A$ modifications[220]. Catalytically inactive Cas13 (dCas13b) is combined with the ADAR's deaminase domain in humans, which naturally deaminates adenosine to
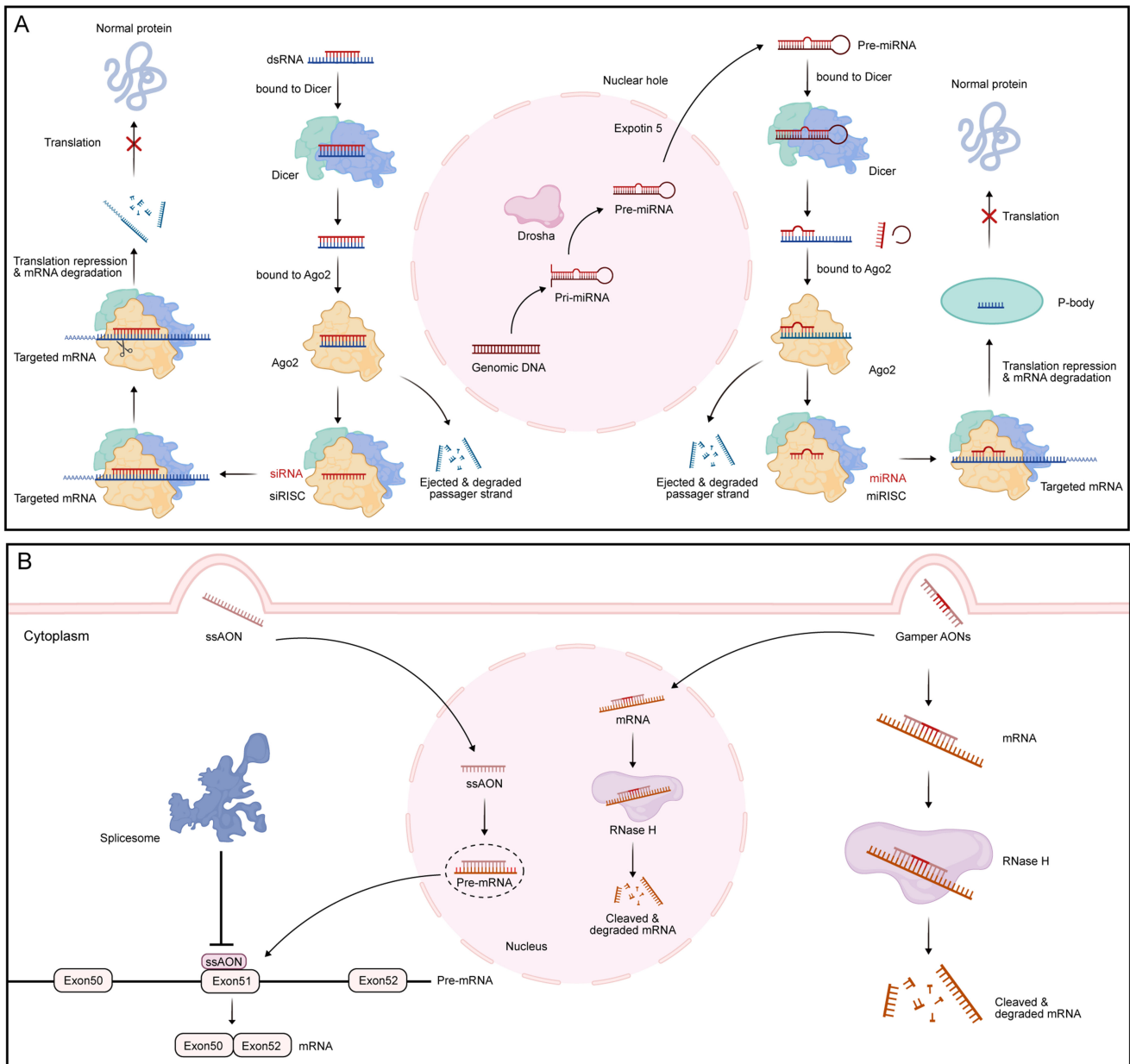
**Figure 3.** Overview of RNAi and AONs. (A) RNAi pathways (siRNAs on the left side; miRNAs on the right side). siRNAs are usually of exogenous origin, while miRNAs are typically derived from transcripts possessing internal hairpins. Following Dicer-mediated processing, the RNA is recruited into AGO2 to constitute the RISC. siRNAs target mRNAs with quite precise sequence complementarity, resulting in mRNA cleavage by AGO2. In contrast, miRNAs recognize target sites with imperfect complementarity, predominantly in the 3'UTR of mRNAs, resulting in translational repression, deadenylation, and destabilization of the target. Additionally, miRNAs with precise complementarity can induce endonucleolytic cleavage by AGO2. Drosha, an RNase III enzyme, cleaves miRNA precursors in the initial stages of miRNA production by processing pri-miRNAs into pre-miRNAs, which are further processed by Dicer to produce mature miRNAs. AGO proteins play a central role in the RISC complex, which combines siRNAs with AGO proteins and Dicer enzymes to achieve gene silencing through the cleavage of target mRNA by the antisense strand of siRNAs; (B) AONs pathways (ssAONs on the left side; Gapmer AONs on the right side). ssAONs are directed to the nucleus to bind to their target pre-mRNA, which disrupts the splicing process by blocking the spliceosome. The specific example highlighted involves an AON targeting exon 51 of the dystrophin gene, leading to exon skipping. On the other hand, Gapmer AONs, shown on the right side, can trigger RNase H-mediated cleavage of the target mRNA in both the nucleus and the cytoplasm. RNAi: RNA interference; AONs: antisense oligonucleotides; siRNAs: small interfering RNAs; miRNAs: microRNAs; AGO2: Argonaute protein 2; RISC: RNAi induced silencing complex; 3'UTR: 3' untranslated region; ssAONs: splice-switching AONs.
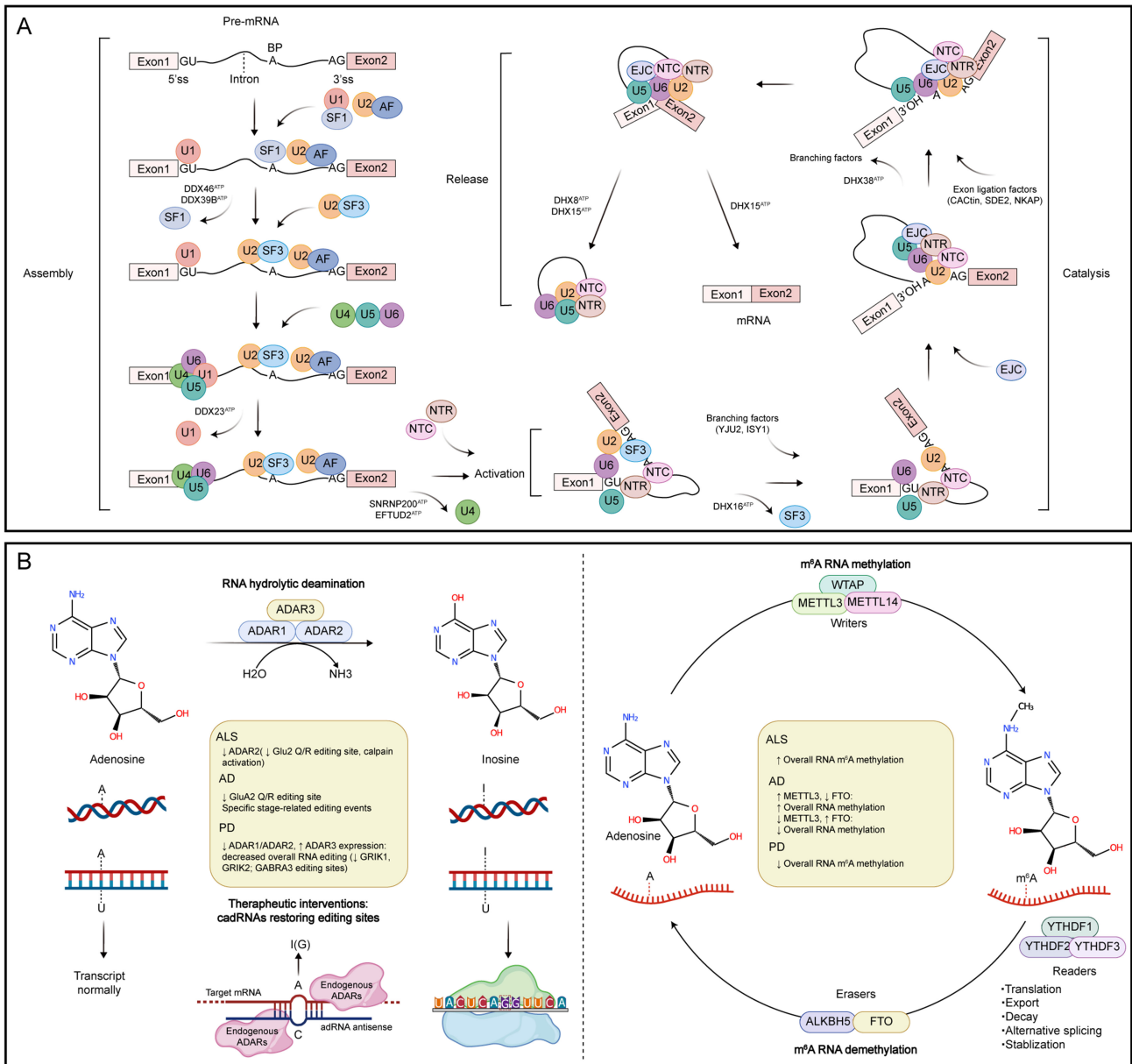
**Figure 4.** Overview of AS and RNA editing. (A) In pre-mRNA splicing, boundaries between intron and exon are recognized as the splice sites, and two consecutive transesterification reactions occur. Internal introns are eliminated from precursor mRNA transcripts through a two-step splicing process. The spliceosome, consisting of 5 snRNP complexes and over 150 accessory proteins, serves to recognize, juxtapose, and facilitate intron excision. As depicted in this figure, ATP-consuming RNA helicases (in humans), namely DDX46, DDX39B, DDX23, SNRNP200, EFTUD2, DHX16, DHX38, DHX8, and DHX15, orchestrate the dynamic process of spliceosome assembly and facilitate the exchanges of snRNPs and other factors. These helicases navigate through kinetic barriers to drive spliceosome activation, catalysis, and product release; (B) A-to-I RNA editing, illustrated on the left, plays a role in neurodegenerative diseases like ALS, AD, and PD, while the right side focuses on m⁶A RNA methylation and its impact on these conditions. A-to-I RNA editing is a post-transcriptional process that alters dsRNA structures through the deamination of specific adenosines (A) to inosines (I) by the ADAR enzyme family. This modification has an impact on both coding and non-coding transcripts. The schematic diagram represents the m⁶A RNA modification process and its involvement in neurodegenerative disorders like AD, PD, and ALS. The methylation of RNA m⁶A is carried out by the methyltransferase complex (writers), including the core subunit heterodimer METTL3/METTL14, along with cofactors like WTAP. Furthermore, erasers such as FTO and ALKBH5 can reversibly remove m⁶A RNA modifications. Specific readers like YTHDF1-3 recognize and bind to m⁶A-modified RNAs, influencing RNA processing, transportation, translation, and stability. AS: Alternative splicing; A-to-I: adenosine to inosine; ALS: amyotrophic lateral sclerosis; AD: Alzheimer's disease; PD: Parkinson's disease; m⁶A: N⁶-methyladenosine; dsRNA: double-stranded RNA; ADAR: adenosine deaminase; WTAP: WT1-associated protein; FTO: alpha-ketoglutarate dependent dioxygenase; ALKBH5: alkB homolog 5.

inosine in dsRNA, with CRISPR RNA guiding the editing process by creating a dsRNA structure around the target adenosine[221]. For specific C-U exchange, the cytidine deaminase from the adenine deaminase domain of ADAR2 has been engineered and fused with dCas13b to create programmable RNA editing in mammalian cells[222]. Cas13-based tools have been developed for the m⁶A of mRNA, utilizing YTH N6-methyladenosine RNA binding protein F1 (YTHDF1) and YTHDF2 fused to catalytically inactive Cas13 ortholog from *Prevotella* sp. (dPspCas13b) proteins to modulate mRNA transcripts within cells[223]. Fusing dPspCas13b with the methyltransferase domain of m⁶A writer METTL3 offers the possibility of cytoplasmic adenine N⁶ methylation, while similar tools target m⁶A demethylation at specific sites[224,225]. The potential for site-specific Cas13-mediate[d RNA editing to correct disease-causing mutations presents new therapeutic avenues for addressing neurodegenerative diseases resulting from abnormal RNA methylation or editing.

RNA therapeutics that are currently in development to effectively target neurodegenerative diseases are examined and summarized in Table 4. While not exhaustive, it is hoped that these promising drugs will provide readers with useful information.

**Current concerns**

In recent years, advancements in RNA design and delivery have facilitated the development of RNA-based medicines across diverse applications, including therapeutics, vaccines, and diagnostics. However, before exploring the potential of RNA-based therapies, it is imperative to elucidate the latent safety concerns associated with these approaches. These concerns encompass not only fundamental challenges in accurate delivery to the designated location but also issues related to poor pharmacological properties, cell-specificities, immune-related toxicities, and potential long-term adverse effects.

Oligonucleotide (OGN)-based therapeutics represent the emerging class of agents that utilize nucleic acids to treat various gene diseases. OGNs include AONs, siRNAs, aptamers, and miRNA inhibitors. Two of the bright and extensively utilized platforms in *OGN* gene therapies are AONs and siRNAs, which both frequently target similar molecular entities. Given two clinical safety reports of drug-related severe thrombocytopenia associated with AONs and exacerbation of peripheral neuropathy with the siRNA revusiran, we must take steps to emphasize the safety of these OGN-based therapies[234]. More specifically, it needs to be considered whether OGN-based drug toxicity is (i) only present in AONs *vs.* siRNAs, (ii) determined by platform specificity, (iii) caused by certain modifications or sequences independent of class, and (iv) based only on relative distinctions in potency while allowing for the use of the lowest therapeutically-effective dose.

While mRNA therapies have demonstrated advantages in safety, programmability, flexibility, and cost-effectiveness in design and production, several inherent properties of mRNA and technological limitations pose significant challenges. The primary issues include (i) instability (they are single-stranded, negatively charged, and degrade rapidly, necessitating strict cold-chain operation), (ii) innate immunity (stimulation of innate and adaptive immune responses and low immunogenicity are required for protein-replacement therapies), (iii) delivery (highly efficient and cell/tissue-specific delivery).

In the past few years, significant advancements have been achieved in utilizing RNA aptamers for therapeutic and diagnostic applications. Notably, selection strategies have been refined, and post-modification techniques to enhance the biostability of RNA aptamers have been developed. However, as nucleic acid biopolymers, the therapeutic potency *in vivo* of RNA aptamers is critically limited by their inherent physicochemical traits. These properties affect the pharmacokinetic properties, thus exposing the aptamers to challenges such as the susceptibility of unmodified aptamers to degradation by nucleases, the

**Table 4. Proven RNA therapeutic agents for related neurodegenerative diseases**

| Type of RNA | Related neurodegenerative diseases | Drug name/Lab code | Target | Company | Development stage | Ref. |
|---|---|---|---|---|---|---|
| siRNA | hATTR amyloidosis-polyneuropathy | Onpattro | Transthyretin mRNA | Alnylam | Approved by FDA in 2018 | [226] |
| AON | Duchenne muscular dystrophy | Exondys 51 | Exon 51 dystrophin | Sarepta therapeutics | Approved by FDA in 2016 | [227] |
| | | Vyondys 53 | Exon 53 dystrophin | Sarepta therapeutics | Approved by FDA in 2019 | [228] |
| | | Viltepso | Exon 53 dystrophin | NS pharma | Approved by FDA in 2020 | [229] |
| | | SRP-5044 | Exon 44 dystrophin | Sarepta therapeutics | Preclinical | [230] |
| | | SRP-5050 | Exon 50 dystrophin | Sarepta therapeutics | Preclinical | [230] |
| | Spinal muscular atrophy | Spinraza | Survival of motor neuron 2 mRNA | Ionis pharmaceuticals | Approved by FDA in 2016 | [231] |
| | hATTR amyloidosis-polyneuropathy | Tegsedi | Transthyretin mRNA | Ionis pharmaceuticals | Approved by FDA in 2018 | [232] |
| | Amyotrophic lateral sclerosis | Tofersen | SOD1 | Ionis pharmaceuticals/Biogen | Phase III | [233] |

siRNA: Small interfering RNA; FDA: U.S. Food and Drug Administration; AON: antisense oligonucleotide; SOD1: superoxide dismutase 1.

susceptibility of small aptamers to rapid excretion by renal filtration, and potential physiological toxicities such as polyanionic effects, unintentional tissue accumulation, dense accumulation of chemical modifications or conjugates, and nonspecific immune activation[235-237].

Advancing technology in RNA offers a creative approach to developing novel therapeutics targeting those rare diseases and other challenging-to-cure ones. As RNA-based therapeutics have evolved, their challenges have become increasingly prominent, and in addition to focusing on the most notable delivery issues, potential side effects, cytotoxicity, and pharmacological properties cannot be ignored.

**Promising potential**

The use of ssAONs is attracting attention as potential therapeutics for dementia-associated diseases, as the role of RNA metabolism deficiencies becomes increasingly evident[238]. Targeting RNA directly at the source of pathogenesis holds promise for more effective outcomes than downstream interventions through alternative therapies. Due to the crucial involvement of APP proteolytic processing in neurodegenerative processes, ssAONs have been investigated for their impact on APP regulation. Notably, ssAONs have been developed to trigger APP exon 17 skipping, which contains the cleavage site of γ-secretase essential for Aβ production, showing effectiveness both *in vitro* and *in vivo*[239,240]. Additionally, ssAONs targeting tau, another significant factor in neurodegeneration, are also in development. Tauopathies manifest as the aberrant accumulation of the MAPT encoded by the *MAPT* gene.

RNA editing offers a multitude of appealing therapeutic possibilities. Neurological and neurodegenerative disorders are underpinned by A-to-I RNA editing. The dysregulation of RNA editing in AMPA receptors leads to epilepsy and related conditions. Several *in vivo* proof-of-concept studies have demonstrated the potential of ADAR-mediated RNA editing to rectify missense and nonsense mutations in support of therapeutic RNA editing. ADAR naturally participates in splicing regulation[241], and the editing of splice sites in the genome can influence splicing[242-244], bolstering the argument for therapeutic targeting of splice sites.

In conclusion, RNA modifications, encompassing various molecular techniques such as RNA editing, RNA aptamers, RNA biosensors, and *in vitro* transcribed mRNA therapeutics, hold significant promise as a therapeutic approach for a wide array of challenging neurodegenerative diseases. They have become a vital component of the molecular toolkit in biotechnology.

## CONCLUSION AND PERSPECTIVE

As the demographic landscape shifts toward an aging population, the prevalence of neurodegenerative disorders will likely escalate. Comprehensively comprehending these conditions is imperative to advance diagnostic and therapeutic strategies. Neurodegenerative diseases have multifaceted etiologies, encompassing disruptions in circadian rhythms, among other factors. Circadian rhythms can interact with disease-relevant biomarkers to generate multidimensional predictive or diagnostic patterns, enabling more precise diagnostics. Moreover, the progression of such maladies is governed by intricate molecular mechanisms, prominently featuring transcriptional regulation. Through scrutinizing these dynamics, it becomes evident that RNA modification is crucial in circadian rhythms and neurodegenerative diseases, underscoring its potential as a pivotal target for therapeutic intervention.

The potential for advancing RNA-based therapeutic modalities for treating neurological diseases seems boundless. While RNA therapy is emerging as a potent therapeutic modality, its application in neurodegenerative disorders remains limited due to persisting hurdles requiring resolution. Among the pivotal avenues for advancing RNA therapies lie in RNA modifications, encompassing $m^6A$, $m^1A$, $m^5C$, pseudouridylation, and others, which may serve as linchpins in this endeavor. Furthermore, the synergy of artificial intelligence, brain-computer interfaces, CRISPR, biosensors, and liquid biopsy is poised to catalyze research efforts to support RNA therapy for neurodegenerative diseases. One significant obstacle is how to deliver such therapies safely and efficiently. With rapid research developments, there is palpable optimism for leveraging these modalities to ameliorate or decelerate the progression of neurodegenerative diseases in the foreseeable future.

## DECLARATIONS

### Authors' contributions
Participated in different parts of writing: Jiang G, Yuan L, Liu X, Wu H, Yu H, Huang Y
Composed and edited the manuscript: Jiang G, Yuan L, Liu X, Wu H, Zhang W, Zhang S, Huang Y
Illustrated the figure and artwork in consultation with co-authors: Jiang G, Liu X, Huang Y
The article has received approval from all authors.

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Not applicable.

**Conflicts of interest**
All authors declared that there are no conflicts of interest.

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Not applicable.

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Not applicable.

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