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Molecular dysregulation in Amyotrophic-lateral sclerosis: investigating TDP-43, target genes, and therapeutic strategies

Charani Nakka
charaninakka.17@gmail.com

ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the selective loss of motor neurons, leading to paralysis and eventual death. TAR DNA-binding protein 43 (TDP-43) has emerged as a central pathological hallmark in both sporadic and familial ALS cases. Current research suggests that abnormal TDP-43 localization disrupts critical RNA-binding functions, leading to both a loss of nuclear RNA-binding function and a gain of toxic cytoplasmic aggregation, driving ALS's progressive neurodegeneration, synaptic dysfunction, and neuronal death This narrative review evaluates the molecular mechanisms by which TDP-43 contributes to neurodegeneration, emphasizing RNA dysregulation, protein aggregation, and gene interactions such as those involving Ataxin-2. Furthermore, TDP-43 dysfunction disrupts RNA splicing, transport, and translation, emphasizing its downstream effects on genes such as *STMN2* and *UNC13A*, which are crucial for axonal integrity and synaptic transmission. By synthesizing recent findings, this review highlights promising therapeutic directions, including antisense oligonucleotides targeting Ataxin-2, small molecules inhibiting TDP-43 aggregation, and CRISPR-based gene-editing strategies designed to restore homeostatic RNA regulation, offering a path toward disease-targeting interventions for ALS TDP-43 pathology. Despite these advances, translation from preclinical to clinical efficacy remains limited, reflecting the need for integrative therapeutic approaches that address the multifactorial nature of ALS pathology. Collectively, this review underscores TDP-43's pivotal role in disease progression and identifies molecular pathways that may inform the next generation of targeted therapies.

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that primarily affects the function of motor neurons of the brain and the spinal cord, weakening muscles of the upper and lower body, and escalating to paralysis and eventual death as a result of respiratory muscle failure. In the United States, the incidence of ALS between 2014 and 2016 was estimated at 1.6 to 1.8 cases per 100,000 individuals, with higher rates observed among white populations, males, and individuals between 60 and 79 years of age (Mehta et al., 2022).

ALS is characterized by both motor and non-motor neurodegenerative symptoms, with a median survival of approximately 34.7 months from the onset of symptoms (Kiernan et al., 2011). Motor symptoms include muscle weakness and atrophy, typically beginning in the legs and arms, and progressing to apparent thinner limbs due to loss of muscle mass. Patients frequently exhibit fasciculations and increased muscle stiffness, which contribute to impaired mobility. Additional motor symptoms involve dysarthria (difficulty speaking) and dysphagia (difficulty swallowing). As the disease advances, respiratory muscles including the diaphragm become compromised, leading to dyspnea and respiratory failure, a leading cause of death in ALS patients. Non-motor symptoms involve cognitive and behavioral impairments, as well as sleep disorders such as insomnia, obstructive sleep apnea, and nocturnal hypoventilation. While less prevalent, others have also reported experiencing autonomic dysfunction, including orthostatic hypotension and fluctuation in blood pressure (Kiernan et al., 2011).

ALS is classified into two types: familial and sporadic. Through the evaluation of family history, it is determined whether an individual may have sporadic ALS (sALS) or familial ALS (fALS). sALS, the predominant form of ALS, refers to cases without a family history and accounts for approximately 90% of all ALS cases. The exact cause of sporadic ALS has yet to be identified. However, several biological processes have been linked to it, including pathological mechanisms such as protein misfolding and aggregation, as well as environmental and genetic factors, including autoimmune components, and disruptions in molecular and cellular pathways (Ajroud-Driss & Siddique, 2015). fALS accounts for 5-10% of all ALS cases and is caused by inherited genetic mutations passed down through families. Unlike sALS, fALS is linked to genetic predisposition which elevates their risk of developing the disease. These mutations often follow an autosomal dominant pattern, where inheriting a single copy of the mutated gene is enough to increase the likelihood of developing ALS. (Ajroud-Driss & Siddique, 2015).

The neurodegeneration observed in ALS results from a complex interplay of genetic, molecular, and cellular mechanisms. These include regulated forms of cell death such as apoptosis and necroptosis, mitochondrial dysfunction leading to oxidative stress, glutamate excitotoxicity, and disruptions in protein homeostasis (Suk & Rousseaux, 2020). A hallmark of ALS is the accumulation of misfolded proteins. These aggregates disrupt essential cellular functions and contribute significantly to disease progression. One of the key proteins implicated in the pathology of ALS is TAR DNA-binding protein 43 (TDP-43), whose abnormal localization and aggregation are increasingly associated with impaired RNA processing in ALS pathology along with other proteins such as superoxide dismutase 1 (SOD1) (Suk & Rousseaux, 2020). Although this review centers on TDP-43, other major ALS-associated genes, including C9orf72, SOD1, and FUS, contribute substantially to disease heterogeneity. Notably, C9orf72 repeat expansions can likewise lead to TDP-43 pathology, whereas SOD1 and many FUS cases lack TDP-43 aggregates entirely, underscoring that TDP-43 represents a dominant but not universal disease mechanism. However, TDP-43 remains the most broadly relevant pathological feature, making it a useful anchor for understanding RNA-related mechanisms that span multiple forms of ALS.

TDP-43 has emerged as a central pathogenic factor in both sporadic and familial ALS, with recent studies emphasizing its role in RNA processing and regulation. TDP-43 is an RNA/DNA binding protein that has a critical role in various cellular processes, especially in RNA metabolism. It plays a central role in RNA processing, including regulating alternative splicing, maintaining mRNA stability, and supporting microRNA production. Additionally, TDP-43 binds to RNA and ensures accurate pre-mRNA splicing, particularly by preventing the inclusion of cryptic exons, short segments normally found within introns, the non-coding regions of a gene (Butti & Patten, 2019). Occasionally, they are mistakenly included in the final mRNA during the splicing process. This can disrupt the proper reading of the mRNA, leading either to the production of faulty proteins or to the degradation of the mRNA through a process known as nonsense-mediated decay (NMD). In ALS, TDP-43 is abnormally relocated from the nucleus to the cytoplasm, where it forms insoluble aggregates. This mislocalization impairs its normal nuclear roles, including RNA splicing and mRNA regulation, resulting in widespread disruptions in RNA processing and contributing to ALS pathogenesis (Butti & Patten, 2019).

Importantly, these molecular abnormalities do not necessarily arise at the same point in disease progression. TDP-43 mislocalization, cryptic exon inclusion, and STMN2 reduction may emerge at different stages, suggesting that early functional defects and later structural degeneration reflect distinct temporal phases of ALS pathology.

This narrative review aims to explore the role of TDP-43 dysfunction in disrupting RNA regulatory mechanisms that contribute to ALS pathogenesis. It will examine the specific genes and molecular pathways affected by TDP-43 mislocalization and aggregation, and discuss current and emerging therapeutic strategies targeting these RNA-related abnormalities. This review synthesizes peer-reviewed studies published between roughly 2010 and 2024, prioritizing data from human post-mortem tissue, iPSC-derived motor neurons, and pre-clinical ALS models. Because RNA dysregulation has emerged as a central theme in ALS research, this review intentionally focuses on TDP-43–dependent RNA processing rather than broader clinical or environmental factors.

TDP-43 FUNCTION AND PATHOLOGY

TDP-43 in Normal Physiology

TDP-43 is vital for controlling gene expression and RNA processing in healthy cells. As a multifunctional protein that binds both RNA and DNA, it participates in many RNA-related processes, including transcription, splicing, transport, and translation. This ability to interact with nucleic acids enables TDP-43 to regulate key gene expression pathways and maintain cellular balance. Proper TDP-43 function is crucial for normal cell activity, and when its regulation is disrupted, it is strongly associated with neurodegenerative diseases such as ALS, Frontotemporal Lobar Degeneration (FLTD) and Alzheimer's Disease (AD) (Hou et al., 2024). The sections that follow will explore the specific roles TDP-43 plays in gene expression and RNA metabolism.

TDP-43 in Gene Expression Regulation and RNA Processing

TDP-43 plays a critical role in regulating gene expression by modulating the interplay between R-loops and 5-hydroxymethylcytosine (5hmC) within gene bodies and at enhancer-promoter regions, a mechanism essential for maintaining genomic stability and transcriptional homeostasis (Hou et al., 2024). Additionally, TDP-43 engages in a negative feedback mechanism by binding to its own 3' untranslated region (UTR), promoting mRNA instability and degradation to prevent its overexpression (Imaizumi et al., 2022). TDP-43 also plays a key role in pre-mRNA splicing by modulating the inclusion or exclusion of specific exons, the coding segments of a gene that are joined together to form the final mRNA. (Buratti & Baralle, 2010). TDP-43 oversees RNA splicing and polyadenylation, the addition of a poly(A) tail, a stretch of adenine nucleotides, to the 3′ end of an mRNA molecule, which protects it from degradation and aids in nuclear export and translation. Both are vital for proper mRNA maturation and stability. Mutations in TDP-43 have been shown to cause missplicing and abnormal polyadenylation, contributing to the pathology of neurodegenerative disorders (Imaizumi et al., 2022). Additionally, TDP-43 is involved in microRNA biogenesis, influencing the production and regulation of microRNAs that affect gene expression post-transcriptionally (Buratti & Baralle, 2010).

Role of TDP-43 in RNA Transport and Translation

Beyond RNA processing, TDP-43 plays a significant role in directing the transport of messenger RNA molecules to specific subcellular locations, a process fundamental for localized protein synthesis required for normal cellular functions (Bjork et al., 2022). This mechanism is particularly critical in neurons, where the spatial regulation of protein production, the localized synthesis of proteins at specific sites within the cell, occurs at synapses. This localized control underlies key processes such as synaptic plasticity, the activity-dependent strengthening or weakening of connections important for learning and memory, and neural adaptability, the nervous system's ability to adjust in response to experience or change (Bjork et al., 2022). Moreover, TDP-43 participates in the regulation of protein translation by interacting with ribonucleoprotein complexes, this results in the translation efficiency of select mRNA targets, which is vital for maintaining protein homeostasis and overall cellular function (Morato et al., 2023). At the molecular level, TDP-43 undergoes liquid-liquid phase separation by interacting with RNA, forming ribonucleoprotein condensates that help regulate gene expression. These condensates have liquid-like properties crucial for normal cell function. When this process is disrupted, it can lead to the formation of harmful protein aggregates, a hallmark of neurodegenerative diseases (Grese et al., 2021). Although essential for gene regulation and RNA processing, TDP-43 malfunction is strongly linked to disorders like ALS and FTLD (Grese et al., 2021). Mutations often cause TDP-43 to mislocalize from the nucleus to the cytoplasm, where it aggregates and loses its normal function, driving disease progression. Therefore, understanding how TDP-43 is normally regulated in cells is crucial for developing effective therapies for these diseases.

TDP-43-MEDIATED RNA DYSREGULATION IN ALS

TDP-43-mediated RNA dysregulation plays a critical role in the progression of ALS by interfering with the cellular processes that regulate RNA stability, localization, and translation. TDP-43 is a DNA and RNA-binding protein present in almost all ALS cases, where mutations or mislocalization from the nucleus to the cytoplasm lead to widespread disturbances in RNA metabolism. Because RNA metabolism includes processes such as RNA splicing (cutting and rearranging RNA) and microRNA biogenesis (producing small RNAs that regulate gene expression), its disruption can impair neuron survival and function. The sections below outline the key mechanisms through which TDP-43-mediated RNA dysregulation contributes to ALS pathology.

MicroRNA Biogenesis and Cytotoxicity

As illustrated in Figure 1, TDP-43 mislocalization in ALS shifts its role from a nuclear RNA processing regulator to a source of toxic cytoplasmic interactions, leading to both loss of normal nuclear functions and impaired axonal messenger RNA (mRNA) transport. Mutations in TDP-43, including A315T and M337V, interfere with Dicer, an enzyme essential for converting precursor molecules into mature microRNAs (miRNA). miRNAs are short RNA molecules that help fine-tune protein production by targeting specific mRNAs for degradation or translation inhibition. Findings from Drosophila models, in vitro biochemical assays, and iPSC-derived motor neurons indicate that these mutations may alter Dicer's localization or reduce its activity, and in these model systems, changes in Dicer function have been associated with shifts in miRNA profiles that may contribute to cytotoxic stress (Long et al., 2024). Because direct evidence from human ALS tissue remains limited, these effects should be interpreted as model-supported observations rather than confirmed causal mechanisms. Studies using iPSC-derived motor neurons and rodent models have shown that certain TDP-43 mutations, such as M337V, may lead to altered transposable element (TE) regulation, rather than fully disrupting it. In these controlled experimental systems, mutant TDP-43 appears to weaken normal TE repression, which has been associated with changes in nearby gene expression connected to extracellular-matrix organization and RNA metabolism (Valdebenito-Maturana et al., 2022). While these findings suggest a potential role for TE activity in contributing to neuronal vulnerability, evidence in human ALS tissue remains limited, and TE dysregulation should be interpreted as a model-supported mechanism rather than a confirmed causal process in patients.

In the case of the M337V mutation, TDP-43 forms aggregates that physically reorganize Dicer but do not completely block its activity, which suggests that different mutations may influence ALS pathology through mutation-specific mechanisms observed in experimental models (Long et al., 2024). While normally regulated, TEs can influence nearby gene expression, sometimes activating genes that should remain silent or silencing genes that are needed for normal function. Motor neurons may be particularly vulnerable to TDP-43 dysfunction because they rely heavily on long-distance axonal transport, localized mRNA translation, and high metabolic output. These demands create a low tolerance for RNA-processing errors, making even subtle disruptions in TDP-43 function disproportionately harmful in these cells. In motor neurons, altered TE activity affects genes involved in maintaining the extracellular matrix, the

supportive network surrounding cells, and RNA processing, both of which are vital for neuron health (Valdebenito-Maturana et al., 2022). Together, these findings suggest that TE changes could increase neuronal vulnerability, although most of the current data come from experimental models.

RNA Splicing and Polyadenylation

TDP-43 mutations also cause widespread splicing defects, including exon skipping, leaving out necessary coding segments, and cryptic exon inclusion, accidentally adding non-coding RNA into the transcript. These splicing errors can change the resulting protein's structure or stop its production entirely, which is linked to neurodegeneration in ALS models (Arnold et al., 2024). Additionally, TDP-43 loss from the nucleus alters polyadenylation site selection, the choice of where to add the protective poly(A) tail on mRNA. This change can destabilize transcripts or alter protein expression, as seen with *MARK3*, a gene whose increased expression promotes tau protein phosphorylation, a feature observed in ALS pathology (Arnold et al., 2024).

Stress Granules and Aggregates

Under cellular stress, TDP-43 can form stress granules, which are temporary RNA-protein complexes that store mRNAs until stress subsides. However, in ALS, these granules can persist and transition into insoluble aggregates that trap RNA and prevent its translation into proteins (Coyne et al., 2017). This aggregation not only removes TDP-43 from the nucleus, where it's needed, but also sequesters essential mRNAs, disrupting protein production. Therapeutically, disrupting TDP-43's harmful interactions within stress granules, abnormal associations with RNA and other RNA-binding proteins that impair mRNA metabolism, translation, and stress response pathways, has shown promise, with small molecules reducing motor neuron toxicity in experimental ALS models (François-Moutal et al., 2019).

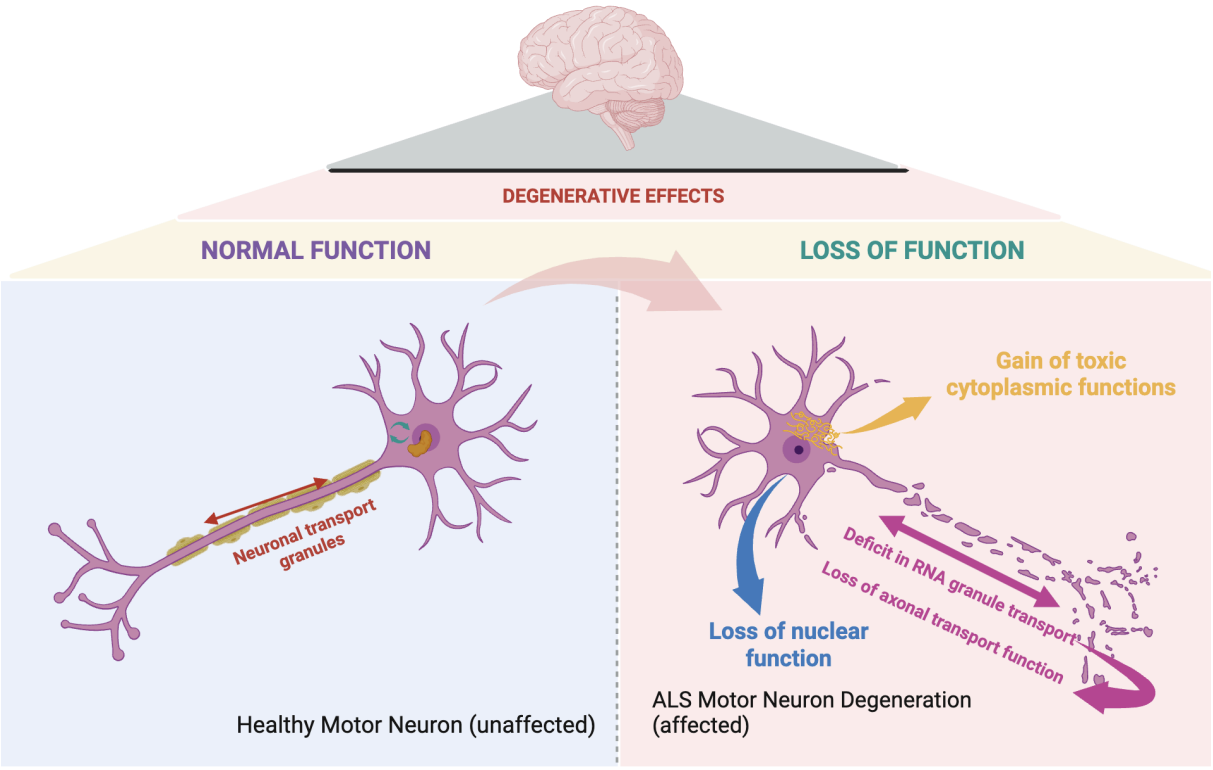

Figure 1. *TDP-43 in healthy versus ALS-affected motor neurons (adapted from Babazadeh et al., 2023),* In healthy motor neurons (left), TDP-43 is localized primarily in the nucleus, supporting normal RNA processing and assembly of neuronal transport granules, which shuttle mRNA and associated proteins along axons to support local translation at synaptic sites. In ALS-affected motor neurons (right), nuclear depletion of TDP-43 results in loss of its normal RNA processing functions, while cytoplasmic mislocalization promotes gain of toxic interactions within stress granules. These changes lead to impaired RNA granule transport, reduced axonal transport function, and ultimately degeneration of motor neuron networks.

Neuromuscular Junction (NMJ) Disruption

TDP-43 dysfunction also impacts the neuromuscular junction, the communication point between motor neurons and muscles. Mutations lead to morphological changes and impaired signal transmission at the NMJ, ultimately causing denervation, loss of nerve supply, and progressive motor impairment (Lépine et al., 2022). Because NMJ integrity is essential for movement, this link ties TDP-43 pathology directly to ALS's defining motor symptoms.

While TDP-43-mediated RNA dysregulation is a central driver of ALS pathology, it acts within a broader network of disease processes. Environmental toxins, additional genetic mutations (such as those in *SOD1*), and other cellular stressors can enhance TDP-43 dysfunction, leading to the multifaceted and

variable disease course seen in ALS patients (Mitra & Hegde, 2020). Understanding these interactions will be crucial for designing therapies that target multiple aspects of ALS simultaneously.

ROLE OF STMN2 IN NEURONAL FUNCTION

Stathmin-2 (*STMN2*) has been a key target in understanding and potentially treating ALS. Its expression is tightly regulated by TDP-43 and is essential for motor neuron maintenance, axonal outgrowth, and regeneration. In ALS, the mislocalization and loss of nuclear TDP-43 function leads to aberrant splicing of *STMN2* mRNA, generating a truncated transcript, a shortened version lacking essential coding regions, that cannot produce functional protein, ultimately reducing *STMN2* levels (Klim et al., 2019; Ritsma et al., 2023). This reduction has been consistently observed in ALS patients and is strongly associated with disease progression. *STMN2* loss exacerbates motor neuron degeneration by impairing axonal stability and regeneration, which in turn contributes to NMJ denervation and motor deficits. Notably, mouse models with *STMN2* depletion exhibit motor neuropathy without obvious motor neuron death, highlighting that early dysfunction can occur independently of cell loss (Krus et al., 2022). Clinically, *STMN2* expression levels correlate with ALS disease duration, and increased levels of truncated *STMN2* transcripts are consistently detected in ALS cases compared to healthy controls (Mehta et al., 2023). *STMN2* loss leads to neurofilament-dependent axonal collapse, contributing to both motor and sensory deprivation, key hallmarks of ALS pathology (López-Erauskin et al., 2022). This makes the interaction between TDP-43 dysfunction and *STMN2* loss a critical axis in ALS progression.

Beyond axonal maintenance, *STMN2* appears to play a crucial role in preserving mitochondrial structure and function. Mice lacking *STMN2* show abnormal mitochondrial morphology and progressive motor decline, suggesting that mitochondrial impairment may be a downstream consequence of *STMN2* deficiency (Krus et al., 2024). Although initial studies proposed that certain dinucleotide repeat expansions in the *STMN2* gene might increase ALS risk, these findings have not been consistently replicated, and some longer CA repeats are even found in unaffected individuals (Ross et al., 2022). Thus, while genetic variability in *STMN2* appears unlikely to drive disease susceptibility, reduced expression of the functional protein remains a promising biomarker and therapeutic target.

UNC13A: LINKING TDP-43 DYSFUNCTION TO SYNAPTIC FAILURE IN ALS

UNC13A has emerged as a key mediator of TDP-43-dependent RNA splicing dysfunction in ALS with evidence coming from human post-mortem motor cortex samples, iPSC-derived motor neurons, and rodent models where TDP-43 depletion is experimentally induced. Under normal conditions, TDP-43 plays a protective role by suppressing the inclusion of a cryptic exon within the *UNC13A* gene during RNA splicing, a critical step in converting pre-mRNA into mature transcripts (Brown et al., 2022; Ma et al., 2022). When TDP-43 is mislocalized from the nucleus, an effect documented in human ALS tissue

and reproduced in both iPSC-derived neurons and TDP-43 knockdown mouse models, this cryptic exon becomes more likely to be included in *UNC13A* mRNA. Since this exon contains premature stop codons, its inclusion results in nonsense-mediated decay, a quality control mechanism that degrades faulty mRNA. The consequence is a significant reduction in *UNC13A* protein levels, and has been associated with synaptic impairments across these model systems (Brown et al., 2022; Keuss et al., 2024).

Importantly, common genetic variants in *UNC13A*, particularly the ALS-associated SNP rs12608932, increase the likelihood of cryptic exon inclusion when TDP-43 function is reduced. These risk variants lie within or near the cryptic exon and appear to weaken the ability of TDP-43 to bind the RNA, effectively sensitizing it to splicing errors in model systems and human genetic data (Brown et al., 2022). Other RNA-binding proteins, like hnRNP L, hnRNP A1, and hnRNP A2B1, also influence cryptic exon inclusion, but TDP-43 remains the primary regulator (Koike, 2024). Together, these findings position UNC13A as a genetically and functionally vulnerable target within TDP-43–driven pathology, although the precise contributions across different ALS subtypes continue to be investigated.

Functionally, *UNC13A* is essential for synaptic vesicle priming, the process that prepares neurotransmitters for release at the presynaptic terminal (Willemse et al., 2023). Loss of *UNC13A*, due to cryptic exon inclusion, causes widespread deficits in neurotransmission, which are evident as abnormal synaptic firing and reduced synaptic strength (Keuss et al., 2024; Willemse et al., 2023). Figure 2 demonstrates how the absence of functional nuclear TDP-43 permits cryptic exons to be incorporated into *UNC13A* mRNA. This aberrant splicing produces defective transcripts that are rapidly degraded, ultimately diminishing *UNC13A* protein levels and contributing to neuronal dysfunction (Lipstein et al., 2022). These changes are especially damaging at NMJs, where transmission breakdown leads to motor neuron denervation, one of the earliest and most consequential events in ALS (Brown et al., 2022; Willemse et al., 2023). Moreover, *UNC13A* dysfunction disrupts both excitatory signaling, nerve cell communication that increases the likelihood of the receiving neuron firing an electrical impulse, and inhibitory signaling, communication that decreases that likelihood, within central circuits, the interconnected networks of neurons in the brain and spinal cord that process and transmit information. This imbalance further destabilizes motor and cognitive networks (Willemse et al., 2023).

Clinically, the impact of *UNC13A* dysfunction is reflected in patient outcomes. Individuals carrying two copies of the high-risk C allele (rs12608932 C/C genotype) experience a more severe disease course, characterized by bulbar-onset symptoms, increased cognitive impairment, and shorter survival (Manini et al., 2023; Tan et al., 2020; Willemse et al., 2023). These patients also show more upper motor neuron involvement and reduced lung function at diagnosis (Manini et al., 2023). Neuroimaging studies link this genotype to brain hypometabolism in regions critical for motor and cognitive processing, including the frontal and precentral cortices (Calvo et al., 2022). In line with this, cognitive assessments reveal poorer performance in tasks involving executive function and social cognition among C/C carriers (Calvo et al., 2022; Tan et al., 2020). The mechanism appears to be direct: reduced *UNC13A* impairs synaptic signaling and neuromuscular communication, leading to denervation, atrophy, and progressive motor decline (Lépine et al., 2022).

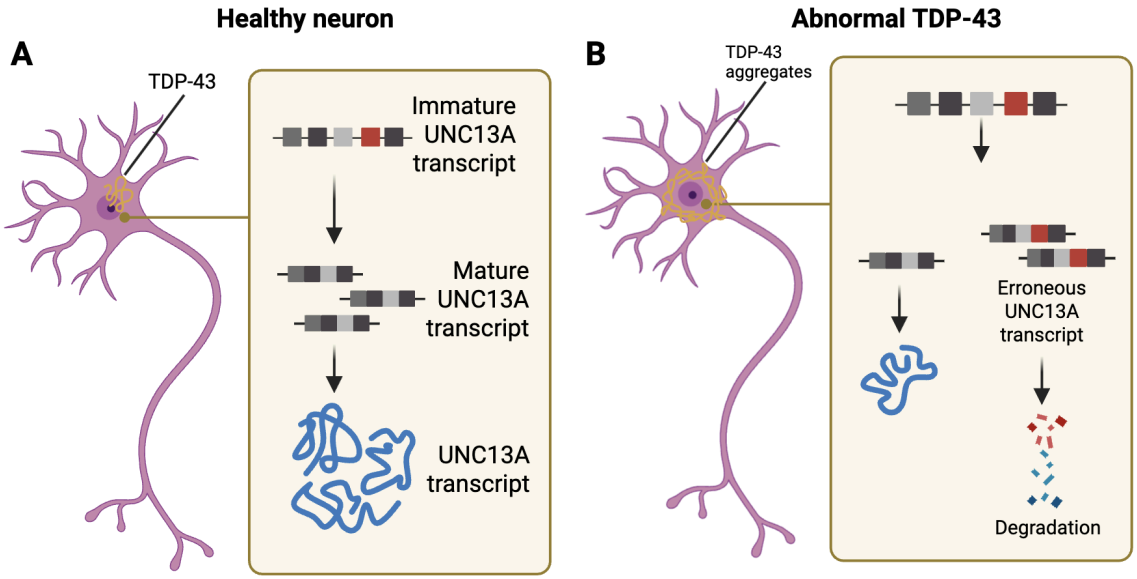

Figure 2. *TDP-43 regulation of UNC13A transcript processing in healthy versus ALS-affected neurons (adapted from Lipstein, 2022)*. In healthy neurons (A), nuclear TDP-43 binds pre-mRNA to suppress cryptic exon inclusion, enabling correct splicing and production of mature *UNC13A* transcripts, which are translated into functional protein. In ALS (B), abnormal TDP-43 aggregation and nuclear depletion lead to inclusion of cryptic exons in *UNC13A* mRNA, producing erroneous transcripts that are recognized and degraded by quality-control pathways. The loss of functional *UNC13A* protein contributes to synaptic dysfunction and neurodegeneration.

The connection between TDP-43 pathology and *UNC13A* cryptic splicing represents one of the clearest mechanistic links in ALS, direct cause-and-effect relationships at the molecular or cellular level that explain how specific changes drive the disease process. Antisense oligonucleotides (ASOs) targeting the cryptic exon successfully restore *UNC13A* protein levels and normalize synaptic transmission in disease models, demonstrating a causative role for the splicing defect and offering a compelling therapeutic strategy (Keuss et al., 2024). This also underscores a broader convergence in ALS pathology: multiple ALS-associated RNA-binding proteins, including MATR3, FUS, and hnRNPA1, have been shown to affect *UNC13A* regulation, highlighting its centrality in maintaining synaptic integrity (Watanabe et al., 2024). Together, these findings suggest that *UNC13A* misregulation is not only a consequence of TDP-43 dysfunction but also a common downstream effector of broader RNA-binding protein dysregulation in ALS.

THERAPIES TARGETING TDP-43 INDUCED ALS PATHOLOGY: CRISPR & ASOS

Clustered Regularly Interspaced Short Palindromic Repeats, or, CRISPR is a groundbreaking gene-editing technology derived from an adaptive immune system found in bacteria. This system uses CRISPR-associated (Cas) proteins to identify and cut foreign DNA, such as viral genomes, protecting bacteria from infection. In biomedical research, this mechanism has been adapted to serve as a molecular tool, allowing scientists to target and modify specific genes with precision and efficiency (Derry, 2021; Wollert, 2020). Recent advances in CRISPR technology have opened new avenues for therapeutic intervention in ALS, particularly in regulating pathological TDP-43 activity. Unlike traditional CRISPR systems that target DNA, newer RNA-targeting CRISPR effectors, such as Cas13 and Cas7-11, have shown the potential to directly bind and degrade disease-associated RNA transcripts without altering the genome. These tools offer a more flexible and reversible approach to gene regulation, making them especially suitable for neurodegenerative conditions like ALS.

Targeting Ataxin-2 to Modulate TDP-43 Pathology

A key strategy involves targeting Ataxin-2. Ataxin-2 is a protein encoded by the *ATXN2* gene in humans, playing a key role in various neurodegenerative disorders, particularly spinocerebellar ataxia type 2 (SCA2) (Scoles & Pulst, 2018). At the cellular level, ataxin-2 affectsRNA maturation, the process of modifying precursor RNA molecules into their final functional forms, and translation, the synthesis of proteins from messenger RNA, both of which are essential for maintaining proper cell function (Becherel et al., 2015; Magaña et al., 2013). This RNA-binding protein is known to modulate TDP-43 toxicity. Elevated Ataxin-2 expression enhances the formation of stress granules, temporary RNA-protein aggregates formed under cellular stress, and facilitates the abnormal aggregation of TDP-43, contributing to neuronal dysfunction. By directing CRISPR-Cas13 systems to suppress Ataxin-2 mRNA, researchers have demonstrated reduced TDP-43 mislocalization, diminished stress granule formation, and decreased neurotoxicity in cellular and animal models of ALS (Zeballos et al., 2023).

Mechanism and Therapeutic Benefits

Cas13 proteins are guided by custom-designed RNA sequences to bind and cleave specific target RNAs, in this case, Ataxin-2 transcripts. This selective degradation has been shown in cellular and animal models to prevent downstream effects that normally exacerbate TDP-43 pathology. In vivo studies in mouse models have shown that CRISPR-mediated Ataxin-2 suppression can lead to improved motor function, reduced TDP-43 aggregates, and extended survival (Zeballos et al., 2023). Furthermore, newer high-fidelity versions of Cas13 minimize off-target activity, enhancing both safety and therapeutic potential. This level of precision is essential, as TDP-43 is involved in tightly regulated feedback loops that control its own expression. Disrupting this autoregulation can inadvertently worsen pathology by increasing levels of toxic TDP-43 isoforms (Dykstra et al., 2024).

While these findings are preclinical, they underscore the potential of RNA-targeted CRISPR therapy to modulate key drivers of ALS progression. Most evidence to date comes from rodent studies and cultured

neuronal systems, and the degree to which these results translate to human ALS remains under investigation. Continued refinement of delivery systems and specificity will be critical in providing this approach to human patients.

From a clinical standpoint, CRISPR-based approaches for ALS are still in the early research stage, but they show promising potential. RNA-targeting systems like Cas13 are especially interesting because they act on RNA rather than DNA, which means their effects are reversible and may carry fewer long-term risks (Zeballos et al., 2023). However, a major challenge is delivering CRISPR tools safely and effectively into motor neurons. Current methods, such as viral vectors or lipid nanoparticles, can trigger immune responses or lead to longer-than-intended expression (Derry, 2021; Wollert, 2020). Researchers also need to make sure CRISPR tools avoid cutting the wrong RNA molecules. Even though CRISPR targeting of Ataxin-2 has improved TDP-43–related problems in both iPSC-derived neuronal models and mouse models (Zeballos et al., 2023), additional work is needed to optimize delivery, minimize immune activation, and ensure controlled, transient expression before this approach can move toward human trials.

Antisense Oligonucleotides (ASOs) as a Therapy for ALS

Antisense technology presents a powerful therapeutic platform for addressing genetic disorders by leveraging short, synthetic strands of nucleotides known as antisense oligonucleotides (ASOs). These molecules are specifically engineered to bind complementary sequences of mRNA, the molecule that carries genetic instructions from DNA to produce proteins. As shown in Figure 3, antisense oligonucleotides bind to target RNA transcripts, enabling the modulation of splicing or stability to adjust protein production levels (Hasting, 2022). By binding to mRNA, ASOs can block the production of disease-causing proteins, offering particular promise for life-threatening conditions. In the context of ALS, ASOs are being investigated for their potential to modulate TDP-43. In the pathology of ALS, TDP-43 becomes mislocalized to the cytoplasm, where it forms insoluble aggregates. This pathological shift leads to both a toxic gain of function, due to the buildup of aggregates, and a loss of nuclear function, resulting in widespread RNA dysregulation (Bisset et al., 2015). TDP-43 regulates the splicing of its own mRNA, encoded by the *TARDBP* gene, as well as other key transcripts such as *UNC13A*. ASOs can be strategically designed to restore normal splicing patterns disrupted by TDP-43 dysfunction (Koike, 2024). Additionally, age-related DNA demethylation, the gradual loss of methyl groups from DNA over time, which can alter gene expression patterns without changing the DNA sequence, can impair TDP-43 autoregulation, leading to excessive *TARDBP* mRNA production. ASOs targeting these splicing errors may help reestablish balanced protein expression levels (Koike, 2024).

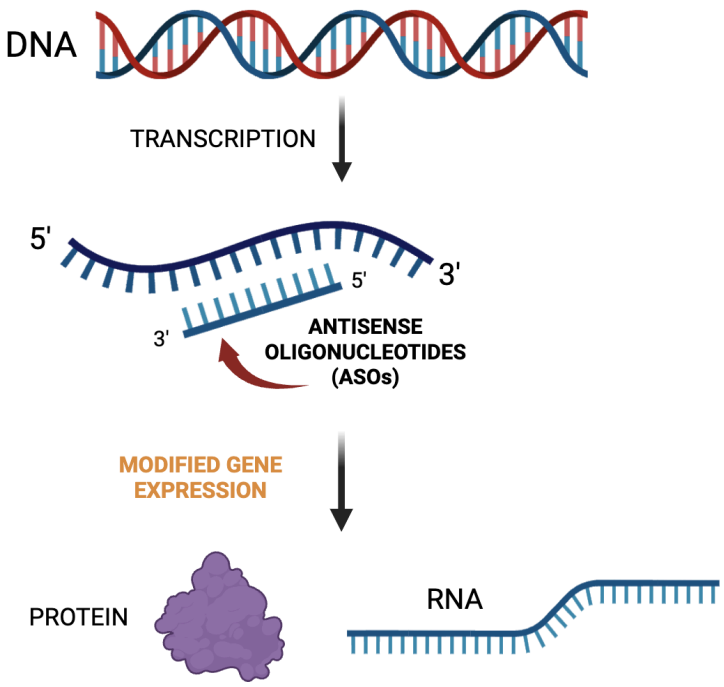

Figure 3. *Mechanism of antisense oligonucleotide (ASO) therapy in modifying gene expression (adapted from Hasting, 2022).* Antisense oligonucleotides are short, synthetic strands of nucleic acids designed to bind specific RNA sequences through complementary base pairing. By attaching to their target RNA, ASOs can alter splicing, block translation, or promote RNA degradation, thereby modifying gene expression and influencing the amount or form of protein produced.

Regulation of TDP-43 Splicing and Exitron Control

One key feature of this regulation involves an exitron, a cryptic exon-like element within a coding region that can be included or excluded during splicing. In ALS, improper splicing of this exitron leads to the production of aggregation-prone TDP-43 isoforms, different molecular forms of the same protein that arise from variations in RNA splicing or other post-transcriptional modifications, often altering the protein's function or stability.

ASOs have been shown to bind specific RNA regions to block proteins like HNRNPA1 and HNRNPC, which normally repress the splicing of the *TARDBP* exitron (Yamagishi et al., 2024). By inhibiting these repressors, ASOs enhance the correct splicing of the exitron, shifting TDP-43 production toward IDR-spliced-out isoforms (IDRsTDP**)** that are less prone to aggregation and more effectively cleared through chaperone-mediated autophagy, a cellular process that degrades misfolded proteins (Yamagishi et

al., 2024). This strategy directly mitigates TDP-43 proteinopathy, one of ALS's central pathological features.

Inhibition of TDP-43 Aggregation

Emerging evidence suggests that computationally designed molecules can bind specific regions of TDP-43 to prevent its aggregation into harmful oligomers and fibrils, structured protein assemblies linked to neuronal damage. ASOs may be engineered to target these same regions, disrupting aggregation pathways and preserving neuronal function (Liu et al., 2023).

While ASOs offer a promising route to address TDP-43-related toxicity in ALS, the multifaceted nature of TDP-43 dysfunction underscores the need for combination therapies. Interventions may need to address both the loss of nuclear function and the toxic effects of cytoplasmic aggregates. Moreover, TDP-43's impact on RNA processing, splicing, and cellular transport suggests that future ASO strategies will require precise molecular targeting, supported by a deeper understanding of TDP-43 biology (Chou et al., 2017; Smethurst et al., 2015).

Clinically, ASOs are one of the most developed RNA-based treatment approaches, and several ASO drugs are already approved for other neurological diseases, showing that this strategy can likely be successful in humans (Hastings, 2022). In ALS, ASOs are normally delivered through intrathecal injection so they can reach the spinal cord and brain, but this method requires repeated dosing since ASOs gradually get cleared over time. ASOs are very specific, which makes them useful for correcting problems like UNC13A cryptic exon inclusion or disrupted TARDBP regulation (Keuss et al., 2024; Yamagishi et al., 2024). At the same time, there are limitations, including possible off-target binding, uneven distribution across motor neurons, and dose-dependent immune responses (Hastings, 2022; Bisset et al., 2015). Although ASOs seem closer to real clinical use than many newer approaches, researchers still need long-term studies to officially confirm that they remain safe, effective, and consistently reach the right cells in ALS patients.

Implications and Future Directions

By targeting both splicing regulation and toxic protein isoform production, ASOs provide a highly specific approach to counteracting TDP-43-related pathology in ALS. Their ability to influence multiple aspects of TDP-43 biology, splicing, aggregation, and clearance, makes them a promising therapeutic candidate. However, because ALS is a complex and multifactorial disease, combining ASOs with other therapeutic strategies could potentially enhance their effectiveness.

CONCLUSION

TDP-43 plays an essential role in healthy cells by regulating RNA splicing, transport, and stability. However, in ALS, this same protein mislocalizes from the nucleus to the cytoplasm, where it aggregates

and disrupts normal gene regulation. TDP-43 is central to ALS progression, driving neurodegeneration through a dual mechanism: the loss of its normal nuclear RNA-binding function and the gain of toxic cytoplasmic aggregation. Modifiers like *ATXN2* were shown to influence TDP-43 toxicity and splicing errors, such as the inclusion of cryptic exons and exitrons, emerged as major contributors to disease progression.

This pathology underlies the disruption of critical RNA processing pathways and contributes to selective neuronal vulnerability. By integrating findings across cellular, molecular, and animal model studies, it becomes evident that TDP-43 dysregulation affects not only RNA splicing and transport but also broader gene regulatory mechanisms, allowing for widespread RNA misprocessing and neuron degeneration, especially in motor neurons. Understanding how this transformation occurs, from a regulatory protein to a pathological marker, is key to identifying effective intervention points.

Emerging targeted therapies offer a promising approach to mitigate TDP-43 toxicity. Therapies such as antisense oligonucleotides and CRISPR-mediated therapies aim to restore normal gene regulation and cellular homeostasis, highlighting the potential of targeting molecular mechanisms with precision. However, despite promising preclinical results, translation to clinical efficacy has been limited. Successful therapies will likely require combined or integrative approaches that address both triggers and consequences of TDP-43 dysregulation.

Beyond therapeutic possibilities, TDP-43 serves as a critical lens for understanding ALS physiology and pathology. Mapping its interactions identifies vulnerabilities in its mechanisms that may guide the development of upcoming interventions. Future studies should focus on investigating cell-type specific susceptibilities and the temporal dynamics of TDP-43 mislocalizations. Collectively, this review underscores the pivotal role of TDP-43 in disease progression, highlights the mechanistic basis for RNA-targeted therapies, and provides a roadmap for translating insights into interventions that may one day stop or slow ALS progression.

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