Review

Caenorhabditis elegans Models Count

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Review

Amyotrophic Lateral Sclerosis Mechanism, the *Caenorhabditis elegans* Models Count

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Abstract: Amyotrophic Lateral Sclerosis (ALS) stands as a debilitating neurodegenerative condition characterized by the progressive degeneration of motor neurons. Despite extensive research in various model animals, the cellular signal mechanisms of ALS remain elusive, impeding the development of efficacious treatments. Among these models, a well-characterized and diminutive organism, *Caenorhabditis elegans* (*C. elegans*), has arisen as a potent tool for investigating the molecular and cellular dimensions of ALS pathogenesis. This review summarizes the contributions of *C. elegans* models to our comprehension of ALS, emphasizing pivotal findings pertaining to genetics, protein aggregation, cellular pathways, and potential therapeutic strategies. We analyze both the merits and constraints of *C. elegans* system in the realm of ALS research, and point towards future investigations that could bridge the chasm between *C. elegans* foundational discoveries and clinical applications.

Keywords: ALS; *C. elegans* model; cellular mechanism; therapeutic application

1. Brief introduction of Amyotrophic Lateral Sclerosis

Motor neuron disease (MND) encompasses a group of disorders, including but not limited to amyotrophic lateral sclerosis (ALS), progressive spinal muscular atrophy, primary lateral sclerosis, and progressive bulbar palsy These disorders share a common feature, the damage to upper and lower motor neurons, leading to the loss of essential motor function [1,2]. Typically, individuals diagnosed with MND display symptoms such as muscle wasting and limb weakness. Beyond motor impairments, they may also experience language and swallowing difficulties [3]. As MND advances, most patients succumb to complications like pneumonia or respiratory failure [4]. This review zeroes in on the intricate mechanisms underlying ALS, the most prevalent adult-onset neurodegenerative form of MND, insights from studies conducted with the small model animal *Caenorhabditis elegans* (*C. elegans*).

ALS is also referred to as Charcot's disease or Lou Gehrig's disease. Like most MNDs, ALS targets both upper and lower motor neurons within the central nervous system, which regulate voluntary muscle movement. Clinically, ALS is typified by muscle rigidity and the progressive weakening of limbs and bulbar muscles, leading to varying degrees of difficulty in speech, swallowing, and respiration [4,5]. It is worth noting that functions like bladder control, bowel movements, and eye movements are typically preserved until the advanced stages of the disease [6]. Beyond muscle dysfunction, 30-50% of ALS patients also exhibit cognitive and other nervous system deficits. Common cognitive symptoms in ALS patients encompass challenges in social cognition, verbal memory, language, and executive function [7]. In about 15% of cases where cognitive impairment is observed, there is visible atrophy in the frontal and/or temporal lobes, which results in behavioral changes or language impairment meeting the criteria for frontotemporal dementia (FTD) [8]. These observations suggest that ALS is a complex and progressive diseases involving functional deficits in multiple tissues.

The onset of ALS generally occurs between the ages of 40 and 70, although instances in younger patients have been documented. Patients with ALS typically succumb to the disease within 2-5 years

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after diagnosis [9,10]. The estimated incidence rate of ALS varies from 0.3 to 2.5 cases per 100,000 individuals [11]. While ALS might be perceived as relatively rare in the public's perception, it exerts substantial impact on patients' quality of life.

2. ALS associated genetic mutations modeled in *C. elegans*

In the pursuit of understanding the pivotal genetic factors underlying various forms of ALS the development of in vivo models has proven indispensable. Two overarching strategies have been employed for generating these models. The first method involves the overexpression of human wild-type or mutated proteins in model organisms, allowing researchers to scrutinize the functional and structural consequences of these proteins in specific tissues. This approach unveils the underlying pathological mechanisms of human ALS-associated molecules in animal models. The second approach revolves around the creation of loss-of-function or gain-of-function mutants through the manipulation of homologous genes in model organisms. This will help to introduce the intrinsic functions of the key molecules involved in ALS and to compare the similarities in the pathogenic mechanisms of the different species.

The *C. elegans* has emerged as a powerful tool for probing the mechanisms underpinning neurodegenerative diseases [12–15]. This is primarily due to its ease of genetic manipulation, rapid cultivation, and its capacity to serve as a whole-animal system amenable to a plethora of molecular and biochemical techniques [16]. Furthermore, *C. elegans* shares functional conservation with many critical pathogenic genes associated with ALS, reinforcing the notion that models established in this organism possess biological relevance [17,18]. Indeed, *C. elegans* has gained widespread recognition as an animal model for investigating the fundamental causal genes of ALS [19–23]. Approximately 42% of human disease-associated genes have identifiable nematode orthologs, rendering this worm an apt model for delving into the molecular mechanisms and cellular processes that drive disease onset and progression [16,24]. In Table 1 below, we provide an overview of the nematode ALS models employed in different studies.

Table 1. A list of published C. elegans models of ALS.

Model	Strain/Transgene name/Plasmid	Expression in *C. elegans*	Phenotypes
Pro-aggregant lines: *Is*[P*rab-3*::F3ΔK280 + P*myo-2*::mCherry]	BR5270, 5485, 5706, 5944	Constitutive pan neuronal	Severe locomotive impairment of adulthood at day 1; accelerating aggregate formation; severe developmental deficiency in nervous system; injury in presynaptic transmission [25]
Anti-aggregant lines: *Is*[P*rab-3*::F3ΔK280(I277P)(I308P) + P*myo-2*::mCherry]	BR5271, 5486, 6427, 6516	Constitutive pan-neuronal	No overt locomotive impairment; minimum influence on neurodevelopment [25]
Is[P*hsp-16.2*::*sod-1* (WT, A4V, G37R,G93A) + P*myo-3*::sod-1(WT, A4V)::*gfp* + *rol-6*(su1006)]	n.a.	Heat-shock inducible muscles	Oxidative stress-induced aggregate formation [26]
iwIs8[P*snb-1*::*sod-1*(WT, G85R)::*yfp*]	n.a.	Constitutive pan-neuronal	Severe motor dysfunction accompanied by both soluble oligomers and insoluble aggregate deposits [27]
Is[P*sng-1*::*sod-1*(WT, A4V, G37R, G93C)::*gfp*]	n.a.	Constitutive pan-neuronal	Compared to heterodimers, mutant homodimers demonstrate increased aggregate formation but G85R heterodimers are more toxic in functional assays [28]
Is[P*unc-54*::*sod-1*(WT, G85R, G93A,G127insTGGGstop)::*yfp*]	AM263, 265	Constitutive muscle	SOD-1 mutants demonstrate morphologically heterogenous aggregates with variety biophysical properties and mild motility defects [29]
ngIs36[P*unc-25*::*sod-1*(G93A)::*gfp*]	n.a.	GABAergic motor neurons	G93A SOD-1 animals demonstrate progressive motor dysfunction, aggregate formation, and axonal guidance defects [30]
lin-15(n765ts); [P*rgef-1*::FUS (WT, R514G, R521G, R522G, R524S, P525L) + P*pab-1*:: mCherry; *lin-15*(+)]	PJH897	Constitutive pan-neuronal	Form of cytoplasmic FUS aggregates: R522G, P525L, FUS513 and FUS501 demonstrate a significantly shorter lifespan; P525L, FUS513 and FUS501 demonstrate a partially or completely paralyses, severely shrunken by 8 days of age [31]
unc-119(ed3); *Is*[P*unc-47*::TDP-43-(WT, A315T) + *unc-119*(+)] *unc-119(ed3)*; *Is*[P*unc-47*::FUS-(WT, S57Δ) + *unc-119*(+)]	*xqIs132*, *xqIs133*, *xqIs173*, *xqIs98*	GABAergic motor neurons	Having normal lifespan, but displayed adult-onset, age-dependent damage of motility, progressive paralysis, neuronal degeneration, accumulation of highly insoluble TDP-43 and FUS proteins [32]
iwIs26 [P*snb-1*::TDP-43-YFP WT], *iwIs22*[P*snb-1*::TDP-C25-YFP], *iwEx20*[P*snb-1*::TDP-43-YFP Q331 K], *iwEx28*[P*snb-1*::TDP-43-YFP M337 V)], *iwIs27*[P*snb-1*::SOD1-YFP WT], *iwIs8*[P*snb-1*::SOD1-YFP G85R]	IW63, IW33, IW20, IW46, IW31, IW8	Constitutive pan-neuronal	Transgenic models developed robust locomotion defects and protein aggregation [33]

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3. Mechanisms investigation of protein aggregation, propagation in *C. elegans*

The misfolding and aggregation of frequently encountered proteins, such as SOD1, TDP-43, FUS, C9orf72, optineurin (OPTN), and others, occur in over 90% of ALS patients [34].

SOD-1, a ubiquitously expressed cytosolic protein, functions in the conversion of toxic superoxide anions to hydrogen peroxide, serving as a cellular defense against oxidative stress [35]. While initially associated with a loss of dismutase activity, it is now acknowledged that mutations in SOD1 primarily induce toxicity through a gain-of-function mechanism, although the precise toxic mechanisms remain to be fully elucidated [36]. Mutations in the SOD1 gene induce alterations in the enzyme's folding and stability, ultimately leading to aggregation within motor neurons and subsequent paralysis [14].

TDP-43, a protein encoded by the TARDBP gene on human chromosome I, is expressed ubiquitously and predominantly localized within neuronal nuclei [37]. TDP-43 serves as a multifunctional RNA-binding protein involved in RNA processing, including the regulation of alternative mRNA splicing and mRNA stability [38]. Notably, TDP-43 represents a key constituent of ubiquitin-positive aggregates within the motor neurons of ALS patients. These aggregates comprise C-terminally truncated and hyperphosphorylated TDP-43 [37]. Over 40 mutations linked to ALS have been identified, with G298S, A315T, M337V, G348C, and A382T being the most frequently associated mutations [39].

FUS, a DNA and RNA-binding protein that plays a role in regulating transcription and mRNA processing in neurons, experiences mutations that result in aggregated proteins, inducing motor impairment and altering synaptic functions through overexpression [40,41]. At the molecular level, mutations in FUS provoke dysregulation in RNA processes, encompassing splicing, transcription, and stabilization, ultimately leading to neuronal dysfunction [42,43].

C9ORF72, the most common genetic cause of ALS is the expansion of the hexanucleotide GGGGCC repeat in the first intron of the C9ORF72 gene [44]. The exact mechanism of this expansion remains elusive, one interpretation is that AUG individual translation of GGGGCC may lead to the formation of dipeptide repeats [45].

Optineurin (OPTN) is a highly conserved hexameric protein weighing 64 kDa, composed of 577 amino acids (aa). OPTN interacts with a multitude of proteins involved in processes such as inflammation, vesicle-based protein trafficking, and signal transduction, including the nuclear factor kappa B (NF-κB) pathway [46,47]. Linked to neurodegenerative diseases like ALS, OPTN comprises several coiled-coil domains, a ubiquitin-binding domain (UBD), a leucine-zipper kinase, and an LC3-binding domain [48]. Specific mutations in OPTN, including exon 5 deletion, Q398 nonsense, and E478G missense mutations, have been identified in ALS patients [49,50].

Several transgenic *C. elegans* strains, expressing human SOD-1 variants, have been developed to investigate the functions of mutant SOD-1 [13]. Initially, an ALS *C. elegans* model was established by employing the *hsp16.2* or *mec-3* promoter to introduce human wild-type SOD-1 and various family ALS (FALS)-related mutants (A4V, G37R, and G93A) of SOD-1. However, these transgenic nematodes did not exhibit any discernible phenotypes [26]. Another transgenic *C. elegans* model was then developed to explore differences in the aggregation and toxicity tendencies of wild-type and mutant SOD-1. This was achieved by introducing YFP-tagged wild-type or mutant (G85R and G93A) SOD-1 proteins into the body wall muscle cells under the regulation of the *unc-54* promoter [29]. Transgenic *C. elegans* with pan-neuronal expression of SOD-1(G85R) exhibited severe locomotor defects and presynaptic dysfunction, which were correlated with the insoluble aggregation of SOD-1 in neurons under the control of synaptobrevin (*snb-1*) gene promoter [51]. When mutant SOD-1(G93A) was expressed in GABAergic motor neurons, it led to age-dependent motor impairments, axon guidance failures, and significant SOD-1 accumulation [23]. The *C. elegans sod-1* gene shares functional similarities with its human counterpart. Expressing SOD-1loss of function mutants increased the levels of superoxide anions in worms, resulting in a shorter lifespan and heightened susceptibility to certain environmental stresses [52]. Conversely, over-activation of *C. elegans sod-1*, as a by-product of the catalase reaction, elevated hydrogen peroxide levels and extended lifespan [21].

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TDP-1, the *C. elegans* ortholog of TDP-43, played a pioneering role in modeling TDP-43-related ALS. Initially, this model was established by inducing pan-neuronal expression of human TDP-43 under the *snb-1* promoter's control. This resulted in a notable presentation of harmonious, slow movement and defasciculation of the GABAergic motor neurons [53]. Recent developments in transgenic *C. elegans* models have included the introduction of pan-neuronal expression of both human wild-type and mutant TDP-43 variants, including G290A, A315T, and M337V. These studies have revealed that wild-type TDP-43 induces moderate motor defects, while the mutant TDP-43 variants lead to severe motor dysfunction [54]. Intriguingly, similar phenotypes to those observed with the overactivation of wild-type TDP-43 were observed when the C-terminal fragment of human TDP-43 was pan-neuronally expressed [33].

Numerous transgenic *C. elegans* models have been generated to investigate the effects of mutated and overexpressed FUS genes. FUST-1 serves as the *C. elegans* ortholog of the human FUS gene, and studies in *C. elegans* have revealed that FUS and TDP-43 share common functions and exhibit similar outcomes when mutated variants associated with ALS are expressed [55]. Moreover, specific ALS-related mutations, R524S and P525L, in the FUS gene have been used to establish transgenic *C. elegans* models. These models exhibit impaired neuromuscular function and locomotion, mirroring characteristics of ALS [20]. Recent advancements in genetic manipulation have enabled the creation of a single-copy FUS mutant transgenic strain of *C. elegans*, which displays ALS-like phenotypes, including GABAergic neurodegeneration and progressive paralysis [56]. Furthermore, it has been demonstrated that reducing *dnc-1* (dynactin 1) levels can enhance autophagosome transport and promote motor neuron degeneration. Building on this insight, Ikenaka and their team have developed a novel *C. elegans* transgenic model with dnc-1 knockdown [57]. Notably, this behavior-based model has been employed to identify and assess potential neuroprotective drugs against motor neuron diseases, opening up new avenues for drug discovery [58].

Alfa-1, an ortholog of the ALS/FTD-associated gene C9ORF72 in *C. elegans*, offers a valuable model to investigate their association with ALS [22]. The development of a transgenic model inducing motor deficits in *C. elegans* began with a mutation in the *alfa-1* ortholog. This model revealed a synergistic toxic effect when combined with a TDP-43 mutation [19]. *C. elegans* with loss-of-function mutations in the *alfa-1* gene exhibit age-dependent motility impairments, ultimately leading to paralysis and GABAergic stress-dependent neurodegeneration [12,19]. Notably, *alfa-1* mutants display endocytosis defects, which can be partially rescued by the expression of the human wild-type C9ORF72 protein, underscoring a degree of functional conservation [59]. However, it's worth noting that transgenic nematode models are predominantly employed to investigate human C9ORF72 toxicity, as *alfa-1* does not harbor hexanucleotide repeat expansions [12]. Comparative studies using models with different repeat lengths have revealed that transgenes containing 29 GGGGCC repeats result in early-onset paralysis and increased lethality, albeit with less severe impacts than their counterparts with nine repeats [60,61].

Gene mutations encoding optineurin have been identified in ALS patients. Three distinct types of OPTN mutations—a homozygous deletion of exon 5, a homozygous Q398X nonsense mutation, and a heterozygous E478G missense mutation within its ubiquitin-binding domain—are linked to ALS pathogenesis in *C. elegans* [50]. Notably, the nonsense and missense mutations in OPTN led to the reversal of the inhibition of NF-kB activation, as evidenced through cell transfection analyses. Moreover, these mutations demonstrated that the E478G mutation resulted in a distinct cytoplasmic distribution, differing from the wild type or a primary open-angle glaucoma (POAG) mutation [49]. Hence, OPTN plays a significant role in the pathogenesis of ALS, and targeting NF-kB with inhibitors could potentially be a therapeutic approach for ALS treatment.

We summarize the ALS pathogenic mechanisms in Table 2.

Table 2. ALS pathogenic mechanisms.

Pathogenic Molecule	Normal Functions	Pathogenic Mechanism	*C. elegans* Model	Phonotype
C9orf72-SMCR8 complex subunit (C9orf72) [66]	Guanine nucleotide exchange factor (GEF) activity and regulating autophagy [45]	A hexanucleotide repeat (GGGGCC) within the first intron of C9orf72 undergoes expansion with AUG independent producing five separate dipeptide-containing proteins [19]	*alfa-1*	Motor neuron degeneration and a motility defect [59]
Superoxide dismutase (SOD1) [66]	A cytosolic enzyme, catalyzes the detoxification of superoxide [64]	Mutant alleles of SOD1 generating toxic increase of function in motor neurons; misfold and then eventually aggregate in motor neurons until in vitro; ER stress [62]	a: Pan-neuronal expression of human G85R SOD1; b: Motor neuron overexpression of a human G93A SOD1 [51]	a: Locomotor deficiency, growth of aggregates and axonal abnormalities; b: Age-dependent paralysis result in the consequence of axonal guidance defects [23]
Transactive response (TAR) DNA-binding protein 43 (TDP-43) [67]	Participate in various steps of RNA metabolism, including mRNA splicing, RNA transport, translation, and microRNA biogenesis [65]	a: Deficiency of normal function in the nucleus; b: A toxic GOF by form of cytoplasmic aggregates [63]	a: Pan-neuronal expression of humanTDP-43; b: *C. elegans* homologous gene, TDP-1 [53]	a: Within the GABAergic neurons, occur to slowed and uncoordinated movement, as well as defasciculation of the motor neurons; b: Deficiency of *tdp-1* result in lower fertility, slower growth, and a locomotor deficit [55]
Progranulin (PGRN)	Participate in a diversity of physiologic and pathological processes, consist of cell proliferation, wound healing, and modulation of inflammation	Decreasing PGRN levels result in the hexanucleotide repeat expansion in the C9orf72 gene	Stress and aging produces PGRN impairing the expression and activity of lysosomal proteases [73]	PGRN deficiency resulted in abnormal expression of multiple lysosomal, immune-related, and lipid metabolic genes lysosomal dysfunction, defects in autophagy, and neuroinflammation [70]
RNA-binding protein FUS/TLS (FUS)	DNA repair and several aspects of RNA metabolism involving in transcription alternative splicing mRNA transport, mRNA stability, and microRNA biogenesis [75]	Disturb the nuclear localization signal resulting in mis-localization of FUS to the cytoplasm with protein aggregates [74]	a: Expressing a FUS variant prone to aggregate in GABAergic neurons by the *unc-47* promoter; b: Expressing panneuronlly in FUS mutants under control of the rgef-1 promoter; c: *C. elegans* homologous gene, *fust-1* [55,71]	a: Neurodegeneration, synaptic dysfunction, paralysis and aggregation; b: Motor dysfunction; c: Achieve maximum microRNA (miRNA)-mediated gene silencing [68]
TANK-binding kinase 1 (TBK1)/optineurin gene (OPTN)	TBK1conducts to inflammatory pathways via conducting downstream of proteins that sense bacterial lipopolysaccharides and viral RNA/DNA; OPTN involves in ubiquitinated proteins and produces its role as an autophagy adaptor [76]	Malfunctions of the autophagic pathway	a: Loss of function Variants; b: In-Frame Deletions of Single Amino Acids; c: Missense Variants [69]	Neuronal protein aggregates results in malfunctions of the autophagic pathway [72]
NIMA-related serine/threonine kinase protein family (NEK), NEK1 [67]	Controlling cell cycle, DNA damage repair, and ciliogenesis splicing, RNA transport, translation, and microRNA biogenesis [65,78]	Increasing DNA damage and a compromised DNA damage response [77]	Acting on DDR signaling downstream of ATM/ATR [78]	DNA damage response and repair as well as mitochondrial function [77]

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4. Cell signaling pathways implicated in *C. elegans* based ALS

A range of pathology-related signaling pathways has been explored in ALS nematode models. Data from clinical studies show that multiple genetic mutations linked to ALS (eg, mutations in SOD1, TARDBP, and C9orf72) enhance neuroinflammation, which provides compelling evidence for immune dysregulation in the pathogenesis of ALS [50,79]. Therefore, an improved understanding of the biological processes that induce this immune dysregulation will help to identify therapeutic strategies that circumvent or ameliorate the pathogenesis of ALS. Notably, mutant ALS proteins have been found to activate an innate immune response of *C. elegans* [80–82]. In *C. elegans* strains expressing mutant TDP-43 or FUS in their motor neurons, age-dependent motility defects culminate in paralysis and motor neuron degeneration at a rate significantly higher than that observed in wild-type TDP-43 or FUS control strains. By examining the expression of immune response proteins, including NLP-29 (an antimicrobial, neuropeptide-like protein expressed in hypodermal and intestinal tissue) [83,84], it's evident that the expression of mutant proteins linked to ALS in *C. elegans* motor neurons triggers an innate immune response through TIR-1/Sarm1. Loss-of-function mutations in *tir-1*, downstream kinases, and the transcription factor *atf-7* collectively serve to suppress motor neuron degeneration. Furthermore, the neurosecretory proteins UNC-13 and UNC-31 play a vital role in response and the subsequent degeneration of motor neurons. Notably, the human orthologue of UNC-13, UNC13A, has been identified as a genetic modifier of survival in ALS [80]. Cell-based strategies that enhance the anti-inflammatory reactivity and reverse immune dysregulation offer the potential of slowing disease progression and improving quality of life of patients with ALS.

It is well known that the dysregulation of autophagy in motor neurons is a pivotal event in ALS [85,86]. Particularly, intensified immunoreactivity for microtubule-associated protein 1 light chain 3 (LC3), which is a marker of autophagosome, is often observed in the spinal motor neurons of ALS patients [87,88]. The expression of DNC-1, the homolog of dynactin 1, is specifically knocked down in motor neurons in *C. elegans*. This model exhibited severe motor defects together with axonal and neuronal degeneration and also observed impaired movement and increased number of autophagosomes in the degenerated neurons [57]. It can be seen that transport of autophagosomes is a novel and substantial therapeutic target for motor neuron degeneration. In addition, defective autophagy likely contributes to neuronal dysfunction in ALS diseases, as demonstrated in autophagy-related genes SQSTM1/p62, UBQLN2, VCP, OPTN and TBK1 [50,89–93]. Autophagy adaptor protein SQSTM1 accumulates in ALS patient motor neurons [94], which may indicate autophagy dysfunction. Consistent with this, LC3-positive autophagy vesicles are elevated in ALS FUS patient motor neurons [95]. Unlike animals lacking the endogenous FUS ortholog, ALS FUS animals exhibit impaired neuronal autophagy and an increased accumulation of SQST-1 in motor neurons. The loss of *sqst-1*, the *C. elegans* ortholog for the ALS-linked autophagy adaptor protein SQSTM1/p62, suppresses both neuromuscular and stress-induced locomotion defects in ALS FUS animals. However, it's important to note that it does not suppress neuronal autophagy defects. Consequently, it is evident that autophagy dysfunction is upstream of SQSTM1 function in ALS FUS pathogenesis, and not dependent on it. The highly conserved autophagy pathway plays a crucial role in preventing and countering pathogenic insults that can lead to neurodegeneration [96,97]

Protein homeostasis, often referred to as proteostasis, is carefully maintained through an intricately regulated and interconnected network of biological pathways. This network serves the crucial function of preventing the accumulation and aggregation of damaged or misfolded proteins. Therefore, the integrity of the proteostasis network is paramount in ensuring the longevity and overall health of an organism [98]. Conversely, when proteostasis falters, it contributes to the development of diseases like ALS, which involve the problematic aggregation of proteins [99]. Transgenic *C. elegans* models that express human TDP-43 variants in the nervous system developed severe locomotor defects associated with the aggregation of TDP-43 in neurons. The neurotoxicity and the protein aggregation of TDP-43 were regulated by environmental temperature and heat shock transcriptional factor 1 (HSF-1). Furthermore, the neurotoxicity and the protein aggregation of TDP-43 can be significantly attenuated by a deficiency in the insulin/insulin-like growth factor 1 (IGF-1)

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signaling in *C. elegans* and mammalian cells [33]. In addition, protein misfolding associated with TDP-43 underlies the aging-dependent neurodegeneration. One extensively studied mechanism for regulating longevity revolves around phosphorylation's control of the insulin/IGF-1 signaling pathway [100,101]. The downstream receptor of insulin molecules in *C. elegans*, *daf-2*, demonstrates the capacity to mitigate the shortened lifespan associated with FUS overexpression as well as well as a significant reduction in aggregates in the insoluble pellets of extracted worm homogenates [33,102]. The identification and harnessing of biological networks and molecular targets that govern aging and longevity have, therefore, become central to research, with the ultimate goal of translating these findings into therapeutic strategies.

5. Advances in therapeutic application of *C. elegans* ALS models

C. elegans has provided a valuable platform for investigating potential therapeutic strategies for ALS. Researchers have delved into the use of small molecules to modulate disease pathways, RNA-based therapies targeting specific genes, and genetic modifiers to gain deeper insights into disease mechanisms. These studies not only enhance our comprehension of ALS but also offer insights into potential treatments that may eventually progress to clinical trials for human patients. However, it is imperative to acknowledge that findings in *C. elegans* require validation in more complex models and, ultimately, in clinical trials to ensure their relevance to human ALS. In Table 3, we present a summary of the advances in therapeutic approaches in *C. elegans* ALS models.

Table 3. Advances in Therapeutic Strategies.

Therapeutic Strategies		Functions	Mechanisms of Treatment
Small molecules	Riluzole [103,104]	Decreasing glutamate release for neuroprotective	Decreasing glutamate release for neuroprotective
	Trehalose [105]	Autophagy-enhancing properties contributing to clear protein aggregates in ALS	Improving motor function and increasing the lifespan of *C. elegans* models of ALS
	Curcumin [106]	Decreasing oxidative stress and slowing disease progression	Prospective neuroprotective effects
	Methylene blue [107,108]	An aggregation inhibitor of the phenothiazine class	Protects against oxidative stress
	Bafilomycin [109]	Blocking autophagosome-lysosome fusion and inhibiting acidification and protein degradation in cell lysosomes to produce the effect of inducing apoptosis	Decreasing neurodegeneration via inhibiting autophagic vesicle maturation
	Dantrolene [110,111]	A muscle relaxant for noncompetitively inhibiting human erythrocyte glutathione reductase	Decreasing neurodegeneration by inhibition of intracellular calcium free in the ER
	Probucol [112]	Regulating blood lipid and anti-lipid peroxidation	Attenuating neurodegeneration by its antioxidant properties
	Resveratrol [113,114]	Antioxidant and anti-inflammatory properties	Mitigating ALS-like symptoms via activating cellular protective mechanisms
RNA-based therapies	RNAi (RNA Interference) [115–118]	A gene therapy for ALS and FTD because of reduction in toxicity induced by the repeat-containing C9orf72 transcripts	Aiming and knocking down genes associated with ALS-related proteins by RNAi
	Antisense Oligonucleotides (ASOs) [118,119]	Reducing, restoring, or modifying RNA and protein expression	Modulating the expression of ALS-associated genes and potentially reducing toxic protein production
Genetic modifiers	Cell division cycle kinase 7 (CDC7)	Decreasing the transactive response DNA binding protein of 43 KDa (TDP-43) phosphorylation in vitro and vivo	Decreasing phosphorylation of TDP-43 and the consequent neurodegeneration
	UNC-13A [80]	Regulates the release of neurotransmitters	UNC-13 is required for induction of the degeneration of motor neurons

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6. Limitations of *C. elegans* as ALS Models and Future Directions

ALS is a multifactorial disease, and a growing body of literature underscores the influence of comorbid processes on its pathological progression [5,120,121]. A significant challenge in the quest for effective disease-modifying therapies lies in our limited comprehension of the multifaceted pathways contributing to the development of the disease. While mammalian disease models provide valuable in vivo opportunities and share considerable similarities with the human brain, they come with their complexities. However, the relatively straightforward architecture of *C. elegans*, a microscopic nematode, also brings its own set of constraints. Notably, various tissue and organ systems crucially implicated in neurodegenerative diseases, particularly the central nervous system (CNS) and brain, are notably absent or been greatly simplified in these worms, rendering them less suitable for investigating the systemic pathogenesis of ALS. Consequently, *C. elegans* often serves as a complementary model to gain insights into the pathogenesis and therapeutic approaches for ALS. Nevertheless, it is essential to underscore that the findings derived from *C. elegans* research demand validation in mammalian models and clinical settings to ascertain their clinical relevance.

One potential approach to develop *C. elegans* ALS models involves functionally annotating human genome variants to identify factors contributing to susceptibility and resilience. The continuous growth of databases containing human gene sequence information has led to an overwhelming volume of variants with uncertain significance. Functional gene analysis in *C. elegans* can be directed towards the functional attributes of mutation data, significantly enhancing our comprehension of pathogenic mechanisms and treatment possibilities [122].

Nematodes hold great potential for expediting the development of neuroprotective drugs due to their simple genetic properties and suitability for high-throughput compound screening [58]. Both target-driven and phenotypic screening approaches can be readily implemented in these organisms, rendering *C. elegans* an ideal screening target. Unlike rodent models, worm models offer a rapid and cost-effective means to test numerous drug combinations. Additionally, the advent of technologies such as CRISPR provides the potential to swiftly generate new and more precise nematode models of ALS [123,124]. This is achieved by precisely delivering a single copy of the mutated gene identified from the patient to the desired location in the worm's genome. In the future, the amalgamation of a more accurate genetic *C. elegans* model with a high-throughput automated drug screening platform presents a potentially highly effective strategy for drug discovery in the treatment of ALS.

In summary, *C. elegans* models have made remarkable contributions in facilitating the transition from experimental research to clinical applications. The manifold advantages of *C. elegans* offer an appealing and ethically sound alternative to costlier and time-consuming in vitro or mammalian models. With a growing track record of translational outcomes arising from *C. elegans* research, this microscopic organism is poised to illuminate the remaining obscurities surrounding ALS. Indeed, ALS, as a global neurodegenerative ailment, imposes a substantial burden on tens of millions of individuals daily. Injecting urgency and innovative strategies into model systems research is imperative to expedite discoveries and advancements.

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