Review

Modeling human diseases in *Caenorhabditis elegans*

Maria Markaki and Nektarios Tavernarakis

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas, Heraklion, Crete, Greece

Genes linked to human diseases often function in evolutionarily conserved pathways, which can be readily dissected in simple model organisms. Because of its short lifespan and well-known biology, coupled with a completely sequenced genome that shares extensive homology with that of mammals, *Caenorhabditis elegans* is one of the most versatile and powerful model organisms. Research in *C. elegans* has been instrumental for the elucidation of molecular pathways implicated in many human diseases. In this review, we introduce *C. elegans* as a model organism for biomedical research and we survey recent relevant findings that shed light on the basic molecular determinants of human disease pathophysiology. The nematode holds promise of providing clear leads towards the identification of potential targets for the development of new therapeutic interventions against human diseases.

1 Introduction to *Caenorhabditis elegans* biology

The roundworm *Caenorhabditis elegans* was selected as a simple metazoan model in the early 1960s by Sydney Brenner for pursuing research in developmental biology and neurobiology. Since its introduction as a model organism, *C. elegans* has been widely used to investigate important biological processes most of which have remained essentially unchanged during evolution [1]. *C. elegans* is a free-living, non-parasitic nematode, with a life cycle of 3.5 days at 20°C and a lifespan of about 2–3 weeks under suitable living conditions. The adult is about 1 mm in length and 80 μm in diameter; it feeds on bacteria such as *Escherichia coli* in liquid medium or on agar plates, and can be easily cultivated in large numbers. *C. elegans* has five pairs of autosomes and one pair of sex chromosomes. It has two sexes, hermaphrodites and males; the ratio of sex chromosomes to autosomes determines its sex. A single hermaphrodite produces ~300 progeny by self-fertilization, and more if it mates with males, which arise occasionally at a frequency of 0.1%. Wild-type individuals consist of 959 somatic cells, 302 of which are neurons. The animal body is transparent so it is easy to track cells and follow cell lineages and biological processes [2]. The genome of *C. elegans*, comprising about a hundred million base pairs, is completely sequenced and surprisingly similar to that of humans; it is estimated that 60–80% of the genes have a human counterpart [3]. Viable mutant strains, strains that overexpress a gene or lack a gene function can be efficiently generated and the resulting phenotypes can be rapidly identified [4]. Comprehensive information concerning gene structure, expression patterns, protein-protein interactions, mutant or RNA interference (RNAi) phenotypes and microarray

Correspondence: Dr. Nektarios Tavernarakis, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas, N. Plastira 100, Vassilika Vouton, PO Box 1385, Heraklion 70013, Crete, Greece
E-mail: tavernarakis@imbb.forth.gr
Fax: +30-2810-391067

Abbreviations: Aβ, β-amyloid peptide; **AD**, Alzheimer's disease; **ALS**, amyotrophic lateral sclerosis; **APP**, amyloid precursor protein; **α-syn**, α-synuclein; **CHIP**, C terminus of Hsc70 interacting protein; **DA**, dopaminergic; **DMD**, Duchenne muscular dystrophy; **DR**, dietary restriction; **ER**, endoplasmic reticulum; **Htt**, Huntingtin; **HD**, Huntington's disease; **LRRK2**, leucine-rich repeat kinase 2; **miRNAs**, microRNAs; **MSP**, major sperm protein; **OPMD**, oculopharyngeal muscular dystrophy; **PD**, Parkinson's disease; **SMA**, spinal muscular atrophy; **SMN**, survival motor neuron gene; **SOD1**, Cu/Zn superoxide dismutase 1; **YFP**, yellow fluorescent protein

data, is available in Wormbase, the online resource for nematode-related information (http://www.wormbase.org/) [5]. Nematode strains can be stored indefinitely in liquid nitrogen allowing large mutant collections and public mutant repositories to be set up. *C. elegans* is amenable to unbiased forward and reverse genetic screens and is particularly susceptible to gene inactivation by RNAi [6, 7]. Due to its value as a research tool, a large set of methods that include advanced high-resolution imaging techniques have been developed.

C. elegans has emerged as a powerful experimental system to study the molecular and cellular aspects of human disease *in vivo*. It has been estimated that about 42% of the human disease genes have an ortholog in the genome of *C. elegans*, including those genes associated with Alzheimer's disease (AD), juvenile Parkinson's disease (PD), spinal muscular atrophy (SMA), hereditary non-polyposis colon cancer, and many others age-related disorders [8–10]. Modeling a human disease in a simple invertebrate, such as *C. elegans*, allows the dissection of complex molecular pathways into their component parts, thus providing a meaningful insight into the pathogenesis of a complex disease phenotype. Here, we survey nematode models of human disease, highlighting recent discoveries that shed light on the molecular mechanisms underlying disease pathogenesis.

2 *C. elegans* models of neurodegenerative disorders

Neurodegeneration has a profound effect on human health, yet the mechanisms underlying neuronal injury and death remain unclear. *C. elegans* models of neuronal dysfunction have been established for a number of neurodegenerative diseases, including AD, PD and polyglutamine-expansion disorders. These models typically involve the transgenic expression of human disease genes.

2.1 Alzheimer's disease

AD is a progressive neurological disease that results in the irreversible loss of neurons particularly in the neocortex. The brain of AD-affected patients is characterized by the accumulation of intracellular neurofibrillary tangles (NFTs) composed of the microtubule-associated protein tau that stabilizes microtubules when phosphorylated [11] and of extracellular senile plaques primarily composed of β-amyloid peptide (Aβ) [12]. Compelling evidence supports the causative role of $A\beta_{1-42}$, which derives from the proteolysis of amy-

loid precursor protein (APP) by β- and γ- secretase, in AD. Similarly, mutations in the tau gene lead to familiar tauopathies.

Autosomal dominant mutations in APP are correlated with rare cases of early-onset AD. Determining the *in vivo* functions of APP is difficult because mammals have an *APP* gene family containing two *APP*-related genes. *C. elegans* has a single *APP*-related gene, *apl-1* mapped on the X chromosome. *The apl-1* gene is expressed in multiple cell types and is necessary for many developmental processes, including proper molting and morphogenesis. Loss of *apl-1* causes larval lethality, which can be rescued by neuronal expression of the extracellular domain of APL-1. Similarly, the overexpression of APL-1 causes defects in movement, brood size and larval viability of transgenic nematodes. *The apl-1* overexpression-induced lethality is partially rescued by the reduced activity of *sel-12*, a *C. elegans* homologue of the human γ-secretase gene component presenilin 1, suggesting that SEL-12, like mammalian PS1, regulates the activity of APL-1 either directly or indirectly [13].

C. elegans AD models were developed that express Aβ variants under the control of male-specific, body wall muscle-specific and neuronal-specific promoters. Only transgenic lines carrying the unc-54/$A\beta_{1-42}$ minigene accumulate intracellular Aβ, and show a progressive paralysis beginning in young adulthood. Co-immunoprecipitation studies coupled with mass spectrometry reveal that chaperone-related proteins (two human HSP70, three HSP-16 homologous to αB-crystallin and a putative negative regulator of HSP70 function) interact with Aβ *in vivo*. This interaction is considered as part of a cellular protective response since the overexpression of either HSP-16.2 or a human HSP70 partially suppresses the Aβ-induced paralysis in worms [14]. Heat shock treatment reduces Aβ oligomeric protein and delays paralysis in transgenic Aβ worms, probably through the activation of heat shock proteins regulated by the heat shock transcription factor 1 (HSF-1) [15]. HSF-1 has been coupled to normal ageing and age-related diseases [16]. Previous studies showed that constituents of *Ginkgo biloba* leaf extract (EGb 761 and ginkgolides) delay β-amyloid-induced paralysis in transgenic *C. elegans* [17]. This effect was not additive to protective heat shock, suggesting a shared mechanism of action by the two treatments [15]. Microarray analysis in a transgenic worm strain that accurately expresses a temperature-inducible $A\beta_{42}$ in body wall muscle, reveals that the gene encoding the arsenite-inducible protein (*aip-1*) is up-regulated as part of a cellular protective response. Worms overexpressing AIP-1 show decreased ac-

cumulation of $A\beta_{42}$ peptide and attenuated paralysis. The AIP-1 human homologue, AIRAPL, but not AIRAP when expressed under the control of *myo-3* promoter also confers protection against Aβ toxicity [18]. Both AIP-1 and AIRAPL contain a predicted farnesylation site known to be critical for the AIP-1-mediated longevity in *C. elegans* [19]. AIP-1 and AIRAPL enhance at least partially general protein turnover by acting as positive regulators of proteosomal function, and thus protect against Aβ-induced toxicity [18]. The effects of tetracycline and its analogues doxycycline and minocycline on $A\beta_{42}$-induced toxicity were assayed [20] using the worms expressing a temperature-inducible $A\beta_{1-42}$ transgene [21]. Tetracyclines successfully protected transgenic worms from the Aβ insult by reducing the concentration of oligomers considered to be responsible for the toxic phenotype. These effects were specific, dose-dependent and not linked to any antibiotic activity. Furthermore, tetracyclines protect Aβ-expressing nematodes from oxidative stress by reducing the superoxide production, suggesting a potential use of these drugs in targeting Aβ aggregates.

2.2 Tauopathies

Abnormal phosphorylation, aggregation and ultimately functional alteration of the main microtubule-associated protein, tau, is the major cause of neurodegenerative disorders known as tauopathies [22, 23]. Tau aggregates are found in AD, Pick's disease, corticobasal degeneration, Down's syndrome and frontotemporal dementia with parkinsonism chromosome 17 type (FTDP-17) [24]. The most common tauopathy is AD. To analyze the role of tau modification during AD progression, human tau and a pseudohyperphosphorylated tau (PHP tau; in which ten serine/threonine residues were changed to glutamates), which mimics AD-relevant tau modification, were brought under the control of a pan neuronal (a 3.4 kb upstream of the gene F25B3.3) promoter. Transgenic worms expressing either tau or PHP tau developed a phenotype of uncoordinated (*Unc*) locomotion, characteristic of a variety of *C. elegans* nervous system defects. However, only PHP tau-expressing worms showed a defective pattern of motor neuron development, suggesting that PHP tau may better mimic the stable disease-like tau modification that interferes with mechanisms of axonal outgrowth and path finding [25]. To identify specific modifiers for the tau-induced pathological phenotype, a genome-wide RNAi screen was conducted using transgenic worms expressing mutations in tau (FTDP-17 tau) driven by the *aex-3* pan neuronal promoter. Sixty

enhancer genes modified only the tau-induced *Unc* phenotype. These genes normally function in protein phosphorylation, protein folding, stress response, nucleic acid function, proteolysis and neurotransmission [26]. In addition, random mutational screens on these transgenic animals resulted in the identification of two novel tauopathy-associated genes, *sut-1* and *sut-2* (suppressor of tauopathy) [27, 28]. SUT-1 requires the activity of UNC-34, which plays a role in the correct neuronal cell migration and axonal guidance. SUT-2 encodes a CCCH zinc finger protein, primarily expressed in the nucleus of neuronal cells, which binds ZYG-12, a protein of the HOOK family. In turn, ZYG-12 acts as a linker connecting membrane compartments with the microtubule cytoskeleton protein.

2.3 Polyglutamine-expansion disorders

Numerous human neurodegenerative disorders including Huntington's disease (HD), several spinocerebellar ataxias and Kennedy's disease [29, 30] are associated with misfolded and aggregation-prone proteins that interfere with protein homeostasis, leading to cellular dysfunction. Polyglutamine diseases are caused by a CAG triplet expansion in the coding regions of seemingly unrelated genes, resulting in a pathogenic protein with abnormally expanded track of glutamines (Fig. 1) [31].

To address the mechanisms underlying the impact of aggregation-prone proteins on cellular function, transgenic *C. elegans* lines expressing different lengths of polyQ-YFP (yellow fluorescent protein) or CFP (cyan fluorescent protein) from integrated arrays in muscle (polyQm) [32] or neuronal (polyQn) [33] cells have been utilized in genetic screens. These polyglutamine aggregation models show polyQ-length-dependent aggregation and toxicity. *C. elegans* polyQm or polyQn strains were crossed with animals expressing temperature-sensitive mutations considered to be sensitive indicators of a disruption in protein homeostasis. These experiments have shown that chronic expression of an aggregation-prone protein interferes with the function of multiple structurally and functionally unrelated proteins in a polyQ-length-dependent manner. PolyQ expansions uncover the folding defect of proteins harboring mildly destabilizing temperature-sensitive mutations, which in turn modify polyQ aggregation and toxicity and enhance the disruption of cellular folding homeostasis. Thus, mild folding mutations in the genetic background can modify the toxic phenotypes of polyglutamine diseases [34].

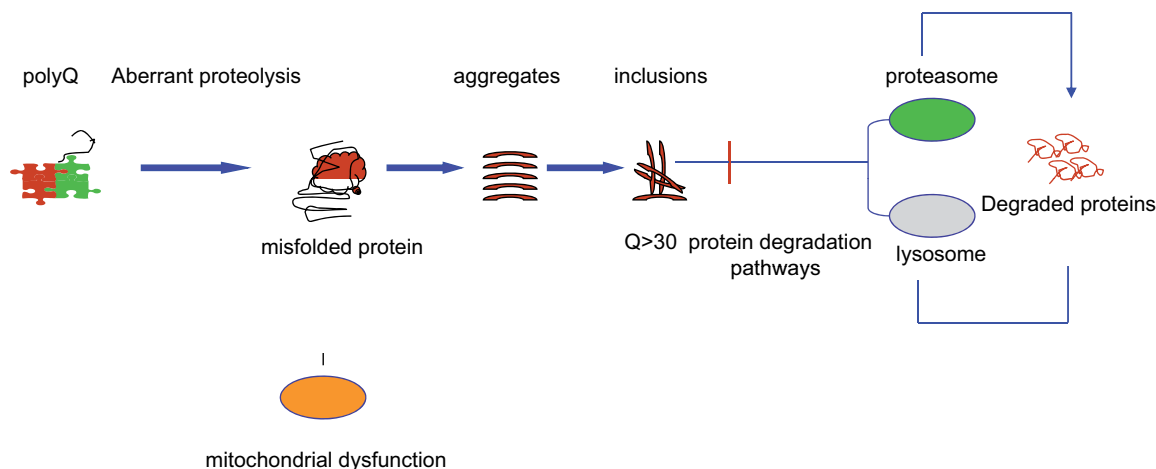Protein aggregation and cellular dysfunction can occur at a threshold of approximately 40 gluta-

Figure 1. Pathogenesis of polyglutamine diseases. Polyglutamine expansion disorders are caused by abnormally expanded tracks of polyglutamine residues (polyQ) in seemingly unrelated proteins. In many cases, abnormal proteolytic cleavage of the affected proteins could result in toxic folding intermediates, oligomers and aggregates, which ultimately form intracellular inclusions. Damaged proteins cannot be degraded through the ubiquitin (proteosomal) and lysosomal degradation pathways. Thus, the accumulation of unwanted proteins may lead to neurodegeneration. Mitochondrial dysfunction has also been linked to polyglutamine-expansion proteotoxicity.

mine residues. However, the threshold for polyQ aggregation and toxicity is dynamic and age-dependent in *C. elegans*. At 3 days of age or less, only worms expressing Q40 or greater exhibit aggregates, while in older animals aggregates appear even in Q33- and Q35-expressing worms [32].

A link between molecular determinants of ageing and aggregation-mediated proteotoxicity was established in *C. elegans*. Polyglutamine toxicity was dependent on the activity of *daf-16*, a forkhead transcription factor of the FOXO family functioning downstream of *age-1* (a phosphoinositide-3 kinase) in the insulin-like signaling pathway [35]. Work on polyQ suppression shows that TOR-2, the worm ortholog of the mammalian torsinA, is an endoplasmic reticulum (ER)-associated protein with chaperone activity that suppresses polyglutamine aggregation in *C. elegans* [36].

HD is the most frequent autosomal dominant inherited polyQ disorder caused by an expansion of a CAG trinucleotide sequence in the huntingtin (Htt) protein. Normal Huntington alleles encode up to 37 CAG repeats, whereas HD-affected individuals have more than 40.

Previous work has shown that ubiquilin is an interactor of presenilin, a protein associated with AD. Ubiquilin proteins contain multiple ubiquitin-related domains and are linked to the ubiquitin-proteasome system of protein degradation as both their ubiquitin-like domain and the ubiquitin-associated domain have been shown to bind the proteosomal subunit S5a [37]. The role of ubiquilin in HD pathogenesis was investigated using *C. elegans*

expressing GFP-Htt-polyQ fusion proteins in body wall muscle. Transgenic worms display a polyQ-length-dependent motility defect that is suppressed by ubiquilin overexpression through an mRFP-tagged ubiquilin fusion protein. Conversely, knockdown by RNAi of *C. elegans* ubiquilin gene exacerbated the motility defect associated with the expression of GFP-Htt (Q55) fusion protein. These findings show that, similar to AD, ubiquilin protects against polyglutamine-induced toxicity, and they suggest a general role for ubiquilin in regulating a number of different neurodegenerative disorders [38]. The motility defect that these *C. elegans* HD models display is reduced by the knockdown of the gene encoding the dynamin-related protein 1 (Drp-1), which controls mitochondrial fission. This result establishes a link between HD pathogenesis and mitochondrial dysfunction [39]. A role for insulin-like signaling pathway in the amelioration of HD-associated proteotoxicity has been reported [16].

Two different *C. elegans* models of human disease were used to explore the effects of dietary restriction (DR) on polyglutamine-associated age-related diseases. An HD model in which 35 glutamine residues were fused to YFP (Q35YFP) and expressed in the body wall muscles [32], and a transgenic model expressing a 42-amino acid Aβ (Aβ$_{42}$) under the control of the *unc-54* promoter, were used in this investigation [40]. The results demonstrate that DR suppresses age-associated paralysis in these worms. To test if DR acts as a general suppressor of proteotoxicity, the effects of food depri-

vation on transgenic worms expressing an aggregation-prone form of GFP (GFP-degron) were also examined. It was shown that both environmental and genetic (represented by the RNAi inhibition of *eat-2* and the knockdown of *rab-10*, *sams-1* or *drr-1* genes that act downstream of food consumption) models of DR confer protection against proteotoxicity in *C. elegans*. Compelling evidence was presented that the general protective effect of DR is mediated by the activity of the heat shock transcription factor, *hsf-1* [41].

2.4 Parkinson's disease

PD is the second most common neurodegenerative disorder characterized by involuntary movements including bradykinesia and difficulty in balance and muscle rigidity due to the progressive loss of dopaminergic (DA) neurons from the substantia nigra. At the cellular level, PD is characterized by cytoplasmic protein inclusions called Lewy bodies, which are the pathological feature of PD and other neurodegenerative disorders known as synucleopathies [42]. α-Synuclein (α-syn), a major constituent of Lewy bodies, is causatively related to PD since missense mutations in the α-syn gene or duplications and triplications of the α-syn locus cause familiar forms of PD [43–45]. Sporadic PD affecting 1–4% of those over 65 years of age appears to be unrelated to mutations or multiplications of the α-syn locus, and implicates a combination of environmental factors. How α-syn inclusions are formed and how these are linked to disease are poorly understood. Synucleopathies have been associated with mitochondria dysfunction, defective autophagy, impairment of the ubiquitin-proteasome system, production of reactive oxygen species (ROS), and stress within the ER [46].

The worm genome includes orthologs of all established familiar PD genes (*Parkin, DJ-1, PINK1, UCHL-1, LRRK2, PARK9* and *NURR1*) except the α-syn. A transgene bearing Rab1 guanosine triphosphate, a GTPase involved in transport from the ER to Golgi, under the control of the dopamine transporter (DAT-1) gene promoter was used to drive the expression of Rab1 along with α-syn in DA neurons in a worm PD model. Human α-syn overexpression in *C. elegans* leads to the formation of misfolded α-syn aggregates that increase with age. Coexpression of Rab1 significantly rescued the neurodegeneration caused by the expression of α-syn alone. Considering that Rab1 plays an essential role in ER-Golgi vesicle trafficking and its enhanced expression protects against α-syn pathology, these combined results indicate that the accumulation of α-syn inhibits the ER-Golgi trafficking [47]. Once

coexpressed, TOR-2 [48] ameliorated the formation of these aggregates by associating with α-syn in Lewy bodies [49].

Worms expressing both α-syn::GFP and TOR-2 in body wall muscles were used in a large-scale RNAi screen for modifiers of α-syn misfolding and neurodegeneration. In all, 868 candidate genes potentially associated with existing PD genes and pathways were targeted for knockdown; 20 gene products that enhance misfolding of human α-syn upon depletion were isolated. The neuroprotective genes, F32A6.3 (the *C. elegans* ortholog of VPS41, a conserved vesicular protein, essential for lysosomal biogenesis), W08D2.5 (the worm ortholog of PARK9), and F55A4.1 (ortholog of Sec22p, a well characterized vesicular trafficking protein in yeast), were among those identified. The identification of *C. elegans* ATG7 as a neuroprotective gene indicates the significant role of autophagy and lysosomal function in preventing the DA neuron loss caused by excess α-syn [48]. To further characterize the processes involved in inclusion body formation, a genome-wide RNAi screen was performed in transgenic worms expressing the α-synuclein-YFP chimera driven by the *unc-54* promoter. The screen identified 80 suppressors, of which 49 have an established human ortholog. Most of the genes are involved in protein quality control and vesicle trafficking between the ER-Golgi as well as other vesicular compartments. Moreover, this screen uncovered several regulators of lifespan (the NAD^+-dependent protein deacetylase *sir-2.1*, the sphingolipid synthase *lagr-1*) as modifiers of inclusion formation, suggesting a link between the molecular mechanisms of ageing and α-syn pathology [50].

Mutations in GTPase (R1441C/G) and kinase (G2019S) domains of leucine-rich repeat kinase (LRRK2) are the most frequent cause of autosomal dominant and idiopathic PD [51, 52]. Expression of human LRRK2 in *C. elegans* neurons modifies the response to mitochondrial inhibition, inferred by the increased survival of worms exposed to rotenone or paraquat, which are agents that impair mitochondrial function. These findings demonstrate the power of the nematode as a model to study the possible gain-of-function phenotype acquired by LRRK2 mutations. *lrk-1* is the sole *C. elegans* ortholog of LRRK2. *lrk-1* and LRRK2 seems to share similar functions since LRRK2 complements the sensitization to rotenone caused by the *lrk-1* knockdown. These results indicate a role for LRRK2 and its worm ortholog in regulating mitochondrial dysfunction [53]. R1441C/G and G2019S mutations increase kinase activity of LRRK2 *in vitro* [54, 55]. The role of LRRK2 in PD pathogenesis

was investigated *in vivo* using transgenic *C. elegans* overexpressing human LRRK2 wild type, R1441C/G and G2019S mutations and a LRRK2-inactive mutation K1347A in DA neurons. All transgenic worms show an age-dependent degeneration of DA neurons, locomotor dysfunction, behavioral abnormalities and dopamine deficiency. In contrast, loss of endogenous LRK-1 and the GTP-binding defective mutation, K1347A, ameliorated LRRK2-associated phenotypes [56].

2.5 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), the most common motor neuron disorder, is characterized by dysfunction or death of motor neurons in the motor cortex, brain stem and spinal cord. Approximately 10% of ALS cases are familiar with an autosomal dominant inheritance [57] and ~20% of familiar ALS (FALS) are associated with mutations in the gene coding for Cu/Zn superoxide dismutase (SOD1) [58], which catalyzes the conversion of highly reactive superoxide anions into hydrogen peroxide [59]. While it is accepted that toxic gain-of-function mutations in SOD1 gene evoke the disease phenotype, the mechanisms of toxicity remain unclear.

Transgenic *C. elegans* expressing FALS-related mutant (A4V, G37R, G93A) human SOD1 are more sensitive to paraquat-induced oxidative stress than control worms expressing wild-type human SOD1. Moreover, the transgenic worms that harbor gain-of-function alleles show an aberrant accumulation and aggregate formation of mutant proteins under oxidative stress. Thus, oxidative damage during ageing might cause FALS by inhibiting degradation and enhancing aggregate formation of mutant SOD1 proteins [60].

G85R, an ALS-associated human mutant SOD1, causes a severe locomotor defect in transgenic *C. elegans* when expressed pan-neuronally under the control of the synaptobrevin (*snb-1*) gene promoter. Paralyzed animals have soluble oligomers and insoluble aggregates in neurons. The movement defect results from the dysfunction of synapses, the connections between neurons and between neurons and muscles. An RNAi screen identified molecular chaperones as critical modifiers of protein aggregation-mediated toxicity [61]. Expression of three distinct SOD1 mutations (G85R, G93A, 127X) in the body muscle cells of wild-type (N2) worms causes only mild cellular toxicity. However, when SOD1 mutants are expressed on the background of destabilized protein polymorphisms, their toxicity is enhanced. These results suggest that the genetic background may intensify the specific toxic pheno-

types of misfolded and aggregation-prone proteins that characterize conformational diseases [62].

A recent study identified the gene ALS8 as a novel cause of ALS [63]. Mutations in ALS8, as in SOD, result in a wide range of defects that vary in the age of onset, the speed of progression and the motor neurons affected. This variation may be due to genetic modifiers, redundancy or environmental contribution. ALS8 codes for VAMP (synaptobrevin)-associated protein B (VAPB), which is closely related to VAPA, a protein associated with the cytoplasmic face of the ER and the Golgi apparatus [64–66]. Many species have VAP proteins characterized by the major sperm protein domain (MSP) of about 125 residues at their N-terminal end [67], a central region of coiled-form motif and a hydrophobic C terminus, which acts as a membrane anchor. The MSP domain is named from its similarity to *C. elegans* MSPs, the most abundant proteins in the worm sperm [68].

MSP functions in oocyte maturation and sheath contraction in *C. elegans* [69]. After secretion, extracellular MSP directly binds to the VAB-1 Eph receptor and other yet-to-be-identified receptors on oocyte and ovarian cell surfaces [70].

The biological function of VAPs and the mechanisms underlying their impact in ALS pathogenesis is not well understood. To define the role of VAPs, *C. elegans* was used together with other model systems. *C. elegans* has a single VAP homolog, the VPR-1, and VAP MSP domains share a 25% identity in their primary sequences with MSPs. Injection of recombinant VPR-1 MSP stimulated oocyte maturation and sheath contraction in the gonads of worms lacking MSP and sperm, suggesting that *C. elegans* MSP and VAP MSP domains have evolutionary conserved extracellular signaling activity. Microinjection of *Drosophila* VAP (dVAP) and human VAP also induced both responses. VAPs have been shown to regulate Eph receptor signaling *in vivo*. MSP domains of VAP proteins are cleaved and provide ligands for Eph receptors. Mutations in VAPs may result in a failure to secrete the MSP domain, accumulation of inclusions in the ER and an unfolded protein response. These findings provide insight into the disease mechanism [71].

2.6 Spinal muscular atrophy

SMA, the most common genetic cause of infant mortality, is an autosomal recessive neuromuscular disorder. SMA patients suffer from degeneration of lower motor neurons in the anterior horn of the spinal cord leading to atrophy of the corresponding muscles [72]. The survival motor neuron gene (SMN) has been causatively linked with SMA, but

the mechanisms underlying the disease pathogenesis are poorly understood. Given that SMN is widely expressed both within and outside the nervous system [73], the specific motor neuron defect that the SMA patients face may be rather linked to the gene function [74]. *C. elegans smn-1* gene has been identified as the ortholog of human SMN [75] with a widespread expression in various tissues including the nervous system and body wall muscles. The mutant *smn-1 (ok355)* harbors a deletion allele that removes most of the *smn-1*, including the translational start codon. Having reduced SMN-1 levels throughout the organism, *smn-1 (ok355)* is used for modeling aspects of SMA. Mutant animals proceed through embryogenesis due to maternally contributed SMN-1, but showed late larval arrest, decreased lifespan, defects in motility and pharyngeal pumping. Neuronal, but not muscle-directed expression of full-length *smn-1* partially rescues the *smn-1 (ok355)* phenotype [76]. A genome-wide RNAi screen and behavioral studies in the loss of function *smn-1 (ok355)* mutant strain provide preliminary evidence that the TGF-β/BMP signaling pathway plays a role in the SMN-mediated neuromuscular pathology in *C. elegans* [77].

3 Stroke – Excitotoxicity

Necrotic cell death underlies the pathology of numerous human degenerative conditions [78]. In *C. elegans*, non-apoptotic cell death (necrosis) is triggered by specific genetic lesions or environmental conditions such as energy depletion, toxin exposure or extreme temperatures. Severe deprivation of energy resources can rapidly develop during ischemic or hypoglycemic episodes. Prolonged hypoxia, a condition of oxygen deprivation that takes place in ischemic episodes and stroke, also induces cell death in *C. elegans* [79]. The most thoroughly characterized necrosis-induced mutations in the nematode are gain-of-function mutations in specific ion channel genes, such as degenerin genes *deg-1* and *mec-4*, the acetylcholine receptor channel subunit gene *deg-3* and the Gs protein a-subunit gene *gsa-1*, which result in necrotic cell death of neurons expressing the mutant proteins [80]. Execution of necrotic cell death requires the activity of calcium-regulated CLP-1 and TRA-3 calpain proteases and ASP-3 ASP-4 aspartyl proteases. Perturbation of intracellular concentrations of calcium, either by extracellular calcium influx or by release of ER stores, activates calpain proteases, which in turn engage executioner aspartyl proteases leading to cell destruction [81]. In mammalian models of excitotoxicity, calpains increase Ca^{2+}

concentration by mediating the cleavage of specific glutamate receptors [82]. Aspartyl proteases are either cytoplasmic or lysosomal, being released into the cytoplasm as a result of lysosomal rupture. Acidification of the cytoplasm due to the action of hydrolytic enzymes liberated during the lysosomal rupture requires the vacuolar H^+-V-ATPase (V-ATPases), a proton pump that acidifies endocytic compartments. Similar mechanisms may underlie the extreme acidosis that is observed during stroke in humans [83]. Evidence suggests that lysosomes contribute to execution of necrosis. Suppression of lysosome biogenesis and of lysosome-mediated cytoplasmic acidification, which develops during necrosis and is required for cell death, affects necrosis in *C. elegans* [84]. A working model for necrotic cell death and cellular defense pathways such as autophagy that may underlie diverse aspects of development, tissue homeostasis and disease pathogenesis in *C. elegans* is outlined in Fig. 2.

4 Muscle-associated disorders

Dystrophin is a huge cytosolic protein that links the intracellular F-actin filaments to the members of the dystrophin-glycoprotein complex (DGC) [85, 86]. Genetic defects in the complex components cause a variety of pathological conditions, including muscular dystrophy, cardiomyopathy and vasospasm. Determining the molecular function of the complex will increase our understanding of muscle diseases pathogenesis. *C. elegans* has a dystrophin complex (DAPC) and mutations in its components result in head bending and hypercontraction [87, 88]. Several lines of evidence couple defects in the DAPC with calcium homeostasis disruption through a mechanism involving the ISLO-1, which mediates the interaction of SLO-1 (the ortholog of the mammalian BK channel) with DAPC. A defect in either DAPC or ISLO-1 impacts SLO-1 channel localization resulting in muscle hyperactivity [89].

4.1 Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is one of the most severe X-linked childhood diseases, characterized by progressive muscle wasting and weakness due to mutations in the dystrophin gene [90]. A *C. elegans* DMD model carrying mutations in *dys-1* and *hlh-1*, the worm orthologs of the human dystrophin and MyoD, respectively, was used to investigate the effects of *chn-1* on muscle degeneration. *chn-1* encodes the homololog of the human E3/E4 ubiquitylation enzyme CHIP (C terminus of Hsc-70
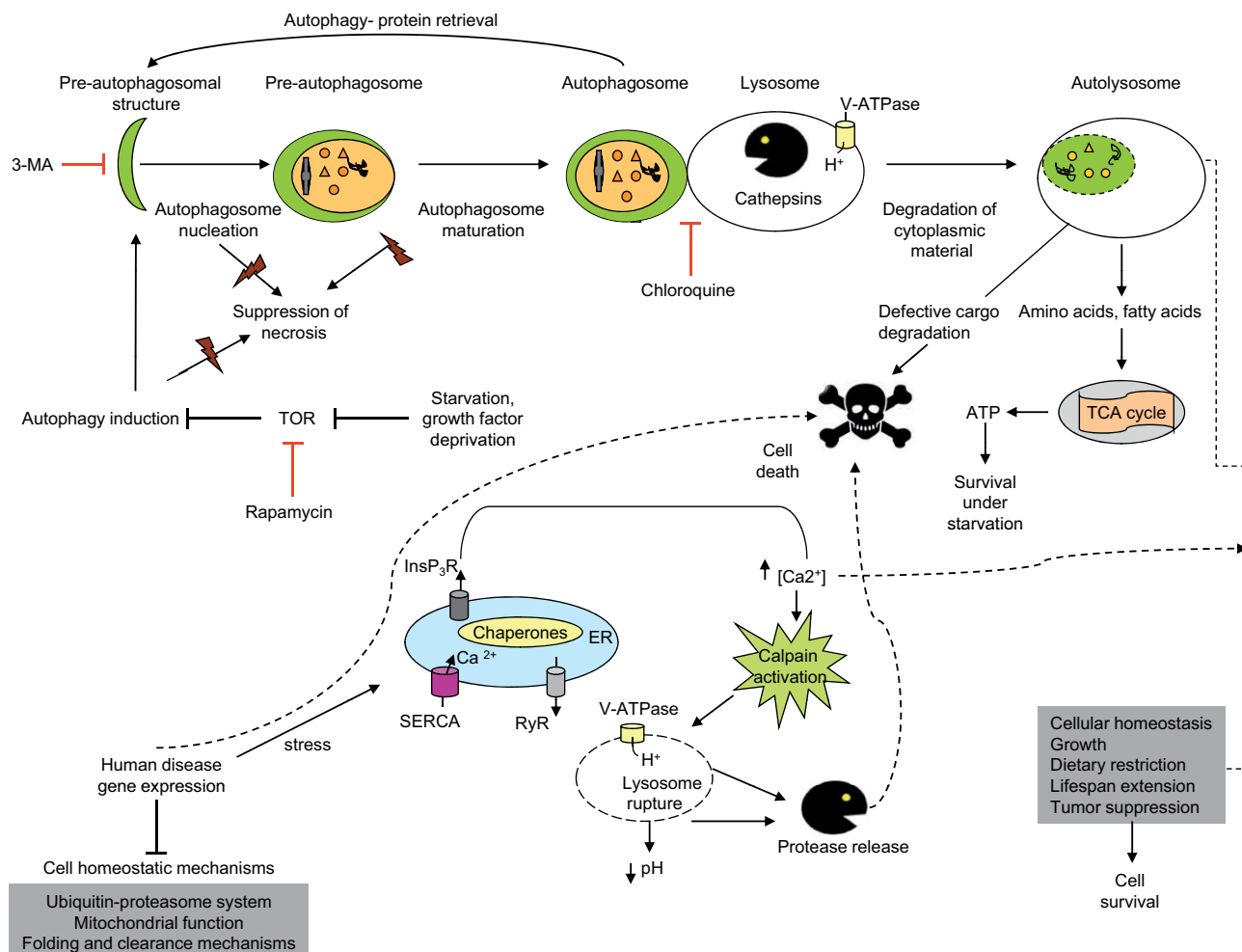
Figure 2. The complex interplay between autophagy, cell survival and cell death. Autophagy is a catabolic process responsible for the degradation of long-lived proteins, molecules and organelles. Cellular material to be degraded is engulfed in double-membrane vesicles, called autophagosomes, which undergo fusion with lysosomes to form autolysosomes where hydrolases degrade the cytoplasmic material. Free fatty acids and amino acids produced by this process can be reutilized by the cell to maintain ATP levels and protein synthesis, contributing to cell survival. Starvation or growth factor deprivation induces autophagy by inhibiting TOR signaling. Autophagy contributes to lifespan extension under caloric restriction in many organisms including yeast, nematodes, flies and mammals. Mutations in genes functioning in various steps of the autophagy pathway suppress cell death triggered by diverse stimuli. Pharmacological inhibitors block distinct steps of the autophagic pathway (red blocking lines). Autophagy is implicated in neurodegeration in *C. elegans*. Diverse genetic or environmental insults lead to elevated cytoplasmic calcium concentration disturbing calcium homeostasis. Increased calcium concentration activates cytoplasmic calpain proteases causing lysosomal membrane rupture and release of lysosomal components into the cytoplasm, which becomes acidified. The vacuolar H+ ATPase (V-ATPase) pump is responsible for lysosomal and subsequent cytoplasmic acidification. Low pH conditions favor the activation of cathepsin proteases, contributing to cell death. Autophagy is up-regulated upon induction of necrosis directly and/or through calpain activation, and synergizes with lysosomal cathepsin proteases to mediate cell death. Similarly impaired autophagy can be deleterious by allowing damaged organelles and proteins to accumulate. Expression of human disease genes in worms disturbs cellular homeostatic mechanisms and induces stress, which beyond a certain threshold, becomes detrimental. TCA: tricarboxylic acid; 3-MA: 3-methyladenine; [Ca^{2+}]: cytoplasmic calcium concentration; V-ATPase: vacuolar H+-ATPase; ER: endoplasmic reticulum; SERCA: sarco-endoplasmic reticulum; InsP3R: inositol triphosphate receptor; RyR: ryanodine receptor.

interacting protein) that is strongly expressed in both neurons and muscles [91]. Down-regulation of *chn-1* by mutation, as well as by pharmacological treatment with a proteosomal inhibitor (MG132) improves the motility of DMD worms. Muscle-specific decrease of CHN-1 activity is sufficient to control muscle degeneration in dystrophin mutant animals. These data suggest a specific role of

CHN-1/CHIP in the control of muscle degeneration through the ubiquitin-proteasome pathway [92].

4.2 Oculopharyngeal muscular dystrophy

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant myopathy characterized by eyelid ptosis, dysphagia and limb-girdle

muscle weakness [93]. A short polyalanine (GCG-triplet) stretch of 10–13 alanines or more in the gene encoding polyalanine-binding protein 1 (PABPN1) on chromosome 14q causes gain-of-function mutations leading to misfolding, aggregate formation and dysfunction of the ubiquitin proteasome system [94–96]. However, the pathogenesis of disease remains unclear.

In a *C. elegans* OPMD model, loss-of-function mutations in *sir-2.1*/SIRT1 and *daf-16* protect transgenic worms from the muscle decline and abnormal motility induced by expression of mutant (13 alanines) PAPBN1 protein [97].

5 Cancer

Cancer is a major cause of human mortality and typically evolves as a multistep process from mutations in key cellular genes. Although *C. elegans* does not form tumors *per se*, other mutant phenotypes are linked to worm homologues of human cancer genes, which often function at distinct steps within evolutionarily conserved pathways. Thus, identifying *C. elegans* genes that disrupt processes relevant to cancer could contribute importantly to our understanding of the cancer gene functions.

Several studies show that c-Met, a receptor tyrosine kinase (RTK) is overexpressed and amplified in many human cancers [98–102]. Mutations in the c-Met juxtamembrane domain as well as germline mutations have been identified in small cell lung cancer [103] and in non-small cell lung cancer [104] cases, respectively.

Transgenic worms expressing wild-type c-Met or the frequent human lung cancer specific c-Met mutants (R988C and T1010I) were generated to investigate cancer gene function in a whole organism context. Mutant transgenic worms show locomotion defects, low fecundity and vulval hyperplasia, phenotypes that were intensified upon exposure to nicotine [105].

The RTK/Ras/Raf/MAPK signaling pathway is highly conserved and interfaces with many aspects of animal development. Work in *C. elegans* has greatly contributed in our understanding of this pathway and its regulation [106]. In *C. elegans*, Ras controls vulval induction and its hyperactivation interferes with vulval cell differentiation, leading to a multivulval phenotype [107]. Vulval development provides a model system to understand cell proliferation and differentiation, deregulation of which is causatively linked to human tumors.

Recently, the role of microRNAs (miRNAs) in human cancer has been extensively studied. miRNAs are small non-protein coding RNA molecules of ~21–23 nucleotides, which control gene expression at the post-transcriptional level acting as developmental timing regulators [108]. Many of the 112 miRNAs identified so far in *C. elegans* are conserved across phylogeny. Compelling evidence suggests that miRNAs are involved in the regulation of ageing and metabolism and provides insight into their function as tumor suppressor genes or oncogenes.

Several miRNAs act in the Notch and Ras signaling pathways [109–111]. miR-84, a *let-7* family miRNA, targets *let-60*, a *C. elegans* ortholog of HRAS, KRAS and NRAS, the human ras oncogenes. All of the human ras genes have complementary sites for human *let-7* family miRNAs in their 3′UTRs, suggesting a similar mode of regulation to that of the worm *let-60* [110]. Gain-of-function mutations in *let-60* result in a multivulval phenotype that is partially suppressed by mir-84 overexpression [112]. Accumulating evidence suggests that *let-7* acts as a tumor suppressor gene. Similarly, miR-61, which targets *vav-1*, a homologue of the Vav oncogene family in vertebrates [113], may influence cancer through the regulation of Notch signaling [111].

The role of apoptosis in cancer treatment sensitivity is well established. Apoptosis is a genetically regulated cell death mechanism that is required for normal development and homeostasis. Defects in key apoptotic proteins can interfere with tumorigenesis and chemotherapy [114]. Therefore, understanding the events that affect DNA damage-induced apoptosis at the molecular level is important for cancer research. The apoptotic molecular pathway is highly conserved [115].

In *C. elegans*, germline apoptosis limits the number of oocytes competing for nutrients in the gonad. It can also be activated in response to pathogenic microorganism invasion or DNA-damaging agents and genotoxic stress [116]. The p53/CEP-1, CED-9 (Bcl-2), CED-4 (ApafI) and CED-3 (caspase) proteins function in the checkpoint machinery of DNA damage-induced germline apoptosis. A recent study revealed that the hypoxia-inducible transcription factor (HIF-1) antagonizes the function of CEP-1 in DNA-damage induced apoptosis. HIF-1 inhibits apoptosis by transcriptionally upregulating the tyrosinase family member TYR-2 in the ASJ neurons. TYR-2 is then secreted and, on entering the gonad, it blocks apoptosis. These findings yield insight into a novel pathway through which two single neurons regulate apoptotic fate in another tissue. Ultimately, this pathway links hypoxia to apoptosis. Knockdown of the TYR-2 homologue TRP2 in human melanoma cells increases basal and cisplatin-induced p53 apoptosis, indicat-

ing an evolutionary conserved function. TYR-2 would be a candidate target in new therapeutic strategies [117].

6 Metabolic diseases

The *O*-GlcNAc signaling pathway, one of the most evolutionary ancient pathways, couples the nutrient-dependent synthesis of UDP-GlcNAc to *O*-GlcNAc modification of Ser/Thr residues of a large number of nuclear and cytoplasmic protein targets. Accumulating data suggest the involvement of *O*-GlcNAc cycling in age-associated human diseases such as insulin resistance, X-linked dystonia parkinsonism, cancer and disease of the immune system.

Two highly conserved enzymes are responsible for the cycling of *O*-GlcNAc on Ser/Thr residues of target proteins: glysosyltransferase, OGT, and the *O*-GlcNAcase, which mediate the addition and the removal of *O*-GlcNAc, respectively. The identification of the enzymes involved in *O*-GlcNAc cycling led to the detection of their interacting partners. Two-hybrid screens reveal diverse proteins associated with vesicle and organelle movements in neurons, mitochondrial movement, or factors involved in the control of gene expression that interact with OGT. In *C. elegans*, two-hybrid analysis indicates the interaction of OGT-1 with the MAP kinase, PMK-1. The highly conserved hexosamine biosynthetic pathway is upstream of *O*-GlcNAc cycling. Accumulating findings suggested a link between the hexosamine biosynthetic pathway, *O*-GlcNAc cycling and mammalian insulin resistance [118].

C elegans strains with putative null alleles of *ogt-1* (ortholog of *O*-GlcNAc transferase) or *oga-1* (ortholog of *O*-GlcNAcase) provide versatile platforms for studying the role of *O*-GlcNAc in cellular signaling and metabolism. Nuclear transport of transcription factors is normal in homozygous *ogt-1(ok430)* [119] and *oga-1(ok1207)* knockout animals [120], yet the *ogt-1(ok430)* mutant completely lacks any detectable *O*-GlcNAc on nuclear pore proteins, whereas the *oga-1(ok1207)* accumulated *O*-GlcNAc. Both mutant strains show increased glycogen and trehalose levels and decreased lipid storage. The link of human OGT and OGA to insulin resistance prompted the examination of the impact of *ogt-1* and *oga-1* deletions on the insulin- like-signaling pathway controlling nutrient storage, dauer formation, longevity and stress responses in *C. elegans*. *oga-1(ok1207)* knockout enhances dauer formation induced by a temperature-sensitive allele of the insulin-like receptor *daf-2 (e1370)* [120] under conditions in which the *ogt-1(ok430)* null al-

lele lowers dauer formation [119]. *C. elegans* is the first animal with viable and fertile knockout alleles of OGA or OGT, which provide genetically amenable models of non-insulin-dependent diabetes. Use of *O*-GlcNAc cycling mutants in high-throughput RNAi screening revealed the interaction of *ogt-1* with essential components of important signaling pathways such as β-catenin. In mammals, β-catenin triggers key developmental switches including the formation of T cells and B cells in adaptive immunity [121].

Accumulating evidence suggests that the enzymes of *O*-GlcNAc cycling may play a role in modulating transcription [122, 123]. The impact of null alleles of *O*-GlcNAc cycling genes on chromatin structure and gene expression can be globally examined in *C. elegans*. Whole-genome chromatin immunoprecipitation on- chip tiling arrays revealed over 800 promoters in which *O*-GlcNAc cycling occurs, including miRNA genes and multigene operons. Transcriptional profiling of *O*-GlcNAc knockout mutants confirmed the deregulation of numerous genes associated with insulin-like signaling, ageing, stress response, innate immunity and carbohydrate/lipid metabolism [124]. These findings suggest that altered *O*-GlcNAc cycling has important implications in the diseases associated with ageing, including obesity, type II diabetes mellitus, cardiovascular diseases, cancer and neurodegenerative diseases.

In *C. elegans*, insulin and its signaling pathway are implicated in the regulation of fat storage and reproduction and in modulating lifespan. *daf-2*, the homologue of the mammalian insulin receptor, is part of a signal transduction cascade homologous to the insulin pathway in mammals. DAF-2 knockout induces dauer formation and extends lifespan. Restoration of DAF-2 function specifically in neurons is sufficient to restore lifespan and reproduction of *daf-2* knockouts to wild-type levels. Growing evidence suggests that impaired insulin signaling in the central nervous system plays a fundamental role in the pathogenesis of common metabolic diseases, including obesity and type II diabetes [125].

C. elegans provides a versatile platform for dissecting the molecular mechanisms underlying glucose-mediated impact on cellular and mitochondrial function in diabetes. An intracellular glucose concentration of 14 mmol/L, which is within the range observed in poorly controlled diabetes patients, results in methylglyoxal-derived modifications of mitochondrial proteins and increase in ROS formation, thus reducing lifespan in *C. elegans*. These effects are independent of osmotic pressure and the pathways activated by caloric re-

Table 1. Selected human disease models in *Caenorhabditis elegans*.

Disease	Disease-associated protein	Characteristic pathology in *C. elegans*	*C. elegans* findings with potential therapeutic relevance
AD	β-amyloid peptide	Muscle-associated human Aβ$_{1-42}$ deposits [14] Neuronal Aβ expression induces defect in chemotaxis [17]	Delay of Aβ$_{1-42}$-mediated paralysis by heat shock treatment [14] and ginkgolide A [17] AIRAPL/AIP-1 suppresses Aβ toxicity [18] Tetracycline and its analogues protect from β-amyloid- induced toxicity [20]
	APP/APL-1	Severe developmental defects induced by inactivation or overexpression of *apl-1* [13]	
AD-relevant tau	PHP-tau	Insoluble highly phosphorylated tau aggregates in neurons linked to a defective pattern of motor neuron development [25]	
PG	polyQ aggregates	Muscle (polyQm)[32] or neuronal (polyQn) [33] induced toxicity	TOR-2 mediated suppression of polyQ aggregation [36]
HD	Expression of human Htt (Q>40) in body wall muscle	Motility defects [38]	Rescue of disease phenotype by ubiquilin overexpression[38] Amelioration of the motility defect by Drp-1 knockdown [39] Suppression of proteotoxicity by DR [41]
PD	Human α-syn expression in dopaminergic neurons W08D2.5/PARK9, B0432.2/DJ-1, EEED8.9/PINK1 LRRK2/LRK-1	Lewy body formation, selective degeneration of dopaminergic neurons [47] Knockdown by RNAi enhances misfolding of human a-syn [48] Enhanced loss of dopaminergic neurons [53]	Co-expression of Rab1 rescue the degeneration [47] Co-expression of TOR-2 ameliorates the aggregate formation [48] *sir-2.1*/SIRT1, *lagr-1*/LASS2 suppress a-syn inclusion formation [50] Autophagy confers neuroprotection [48]
ALS	SOD1 ALS8/VPR-1	Pan-neuronal expression caused severe locomotion defect [61] Mild body muscle toxicity influenced by the genetic background [62]	
SMA	SMN/SMN-1	Late larval arrest, decreased lifespan, defects in motility and pharyngeal pumping [76]	*smn-1(ok355)* deletion mutant as a useful platform for functional analysis of human SMN protein [77]
Stroke – Excitotoxicity	Specific ion channels (DEG-1, MEC-4, DEG-3, GSA-1 in nematode) Specific proteases (calpains CLP-1, TRA-3 and aspartyl proteases ASP-3, ASP-4 in nematode)	Neurodegeneration [80, 81]	Inhibition of executioner proteases (ASP-3, ASP-4) suppresses necrosis [81] Alterations of lysosome biogenesis and function by genetic or pharmacological treatment could affect necrotic cell death [84]
DMD	Dystrofin/dys-1	Muscle degeneration [90, 91]	*chn-1*/CHIP elimination in the musculature suppresses muscle degeneration [92]
OPMD	PABPN1	Muscle cell decline and abnormal motility [97]	Treatment with sirtuin inhibitors protect against mutant PABPN1 [97, 128]

Table 1. Continued

Disease	Disease-associated protein	Characteristic pathology in *C. elegans*	*C. elegans* findings with potential therapeutic relevance
Cancer	c-Met	Locomotion defects, low fecundity and vulval hyperplasia [105] Anti-apoptotic factor, antagonizes p53/CEP-1, interferes with tumor progression and therapy [117]	
	let-60/ras oncogenes *vav-1*/Vav	Multivulval phenotype [109–111]	
	cep-1/p53	Apoptotic defects interfere with tumorigenesis and resistance to chemotherapeutic drugs [114]	
	HIFa/HIF-1	Anti-apoptotic factor, antagonizes p53/*cep-1*, interferes with tumor progression and therapy [117]	Target TYR-2/TRP2 [117]
Metabolic diseases (insulin resistance, type II diabetes)	*ogt-1, oga-1*	Null mutations cause alterations in carbohydrate and lipid metabolism [119, 120, 124]	Overexpression or inhibition of specific miRNAs may be a future tool for targeting diabetes [127]

striction in *eat-2* mutants and the insulin-like receptor *daf-2/daf-16* pathway. Conversely, high glucose-mediated lifespan reduction in *C. elegans* is dependent on glyoxalase-1-controlled mitochondrial complexes II and III. Further studies should delineate the relevance of these mechanisms described in *C. elegans* to the situation in diabetic patients [126].

miRNAs have also been implicated in the regulation of glucose and lipid metabolism, and hence in the initiation and progression of diabetes and its specific complications. miRNAs that play a critical role in the pathogenesis of the disease may serve as potential biomarkers for prognosis and diagnosis of diabetes. Furthermore, the altered expression profiles of miRNAs in the pancreas and insulin-targeted tissues in diabetic patients could provide insights for treating this complex metabolic disease [127].

7 Concluding remarks

Accumulating evidence demonstrates that most of the important pathways such as insulin signaling, Ras/Notch signaling, p53, and many miRNAs have remained essentially unchanged during evolution. Many human diseases, including cancer, originate from mutations in genes functioning in pathways conserved in *C. elegans*. Although nematode disease models might not perfectly recapitulate the pathophysiology of human disease, the small,

transparent worm enables the use of powerful genetic and molecular approaches to dissect the toxicity mechanisms and determine the *in vivo* factors that exacerbate or cure pathological conditions. Table 1 summarizes a selection of human disease models in *C. elegans*. Besides being an excellent model for drug target identification, *C. elegans* is amenable to high-throughput screens for rapid and inexpensive drug evaluation before embarking on expensive and elaborate animal models.

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Nektarios Tavernarakis is a Research Director at the Institute of Molecular Biology and Biotechnology, and Professor of Molecular Systems Biology at the Medical School of the University of Crete in Heraklion, Greece. He earned his PhD at the University of Crete, studying gene expression in yeast, and trained in *Caenorhabditis elegans* biology at Rutgers University, New Jersey, USA. His research focuses on neuronal function and dysfunction. His main interests are the molecular mechanisms of necrotic cell death and neurodegeneration, the molecular mechanisms of sensory transduction and integration by the nervous system, the interplay between cellular metabolism and ageing, and the development of novel genetic tools for *C. elegans* research. He is the recipient of a European Research Council (ERC) Advanced Investigator grant award, a European Molecular Biology Organization (EMBO) Young Investigator award, the Alexander von Humboldt Foundation, Friedrich Wilhelm Bessel research award, and is member of EMBO.

Maria Markaki is a staff scientist in the *Caenorhabditis elegans* molecular genetics laboratory at the Institute of Molecular Biology and Biotechnology, in Heraklion, Crete, Greece. She earned her PhD degree at the University of Crete studying the ecophysiology of *Oxalis pes-caprae* L. Her main interests are the molecular mechanisms that underlie the effects of autophagy on ageing.

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