The nematode *Caenorhabditis elegans*: A versatile model for the study of proteotoxicity and aging

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**ABSTRACT**

Toxicity arising from protein misfolding and aggregation (proteotoxicity) is tightly mechanistically linked to the emergence of late-onset neurodegenerative disorders such as Alzheimer’s and Parkinson’s diseases. Why these maladies manifest in late stages of life and what mechanisms protect the young organism from disease are key enigmas. The nematode *Caenorhabditis elegans* offers key advantages that enable systematic exploration of many cell biological and functional aspects of neurodegeneration-linked proteotoxicity. Here we review the abundantly used nematode-based proteotoxicity models and delineate common techniques for the measurement of protein aggregation and rate of proteotoxicity. We also discuss the advantages offered by the worm for genetic screening, drug development and for the exploration of the links between proteotoxicity and the aging process.

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**1. Introduction**

**1.1. Caenorhabditis elegans offers important advantages for the study of aging and proteotoxicity**

The study of aging is a challenging, time-consuming task that requires a suitable model organism which exhibits short lifecycle, high reproduction profile and is amenable to aging manipulations. Due to the important advantages offered by the nematode *C. elegans*, this round worm became the most abundant model organism used in laboratories that study different aspects of the aging process, including its links with the toxic effects of aberrant protein aggregation (proteotoxicity). The short lifespan exhibited by the nematode is a major advantage; the wild-type larva completes its development within three days and the mean lifespan of an adult worm is merely 18–19 days when cultured in 20°C. This short lifecycle can be divided into three well-defined post-developmental stages; (i) reproductive adulthood, which lasts about 6 days during which the worm lays approximately 300 eggs, (ii) midlife, a stage in which the worm lays no eggs but only minimal mortality is observed within the population, and (iii) late adulthood, when mortality starts manifesting. From day 12 on, the worms die until the whole population perishes about 27 days after hatching. Importantly, the nematode’s aging process shares fundamental similarities with those of mammals; among other features, aged worms suffer from muscle atrophy (sarcopenia) [1], exhibit reduced agility and accumulate the autofluorescent molecule lipofuscin [2], resemblances that enable the study of key aspects of aging in the nematode.

An additional major advantage offered by the nematode is the relative ease to specifically and effectively knockdown gene expression. This can be achieved by utilizing RNA interference (RNAi), a technique that was developed in the nematode and is based on the generation of a double stranded RNA that, once introduced in a system, is processed into small interfering RNA molecules (siRNAs). These molecules are then targeted to their complementary mRNA sequences, inducing the silencing of the gene to which they were specifically designed [3]. In fact, gene knockdown can be achieved simply by feeding the worms with bacteria that express the dsRNA towards the gene of interest. However, while this feeding technique efficiently knocks down the expression of genes in non-neuronal tissues, its competence is limited when one is interested in reducing the expression of a neuronal gene. This problem has been partially solved by the creation of worm strains that were engineered to be RNAi-sensitive in neurons [4,5]. The availability of RNAi libraries that cover the majority of the worm’s genome, along with the easy applicability of this gene-silencing technique, makes *C. elegans* a preferred organism for RNAi-based genetic screens, a feature that allows deciphering the mechanisms that regulate the aging process.

The transparency of worms is an additional key advantage which enables the visualization of fluorescently-tagged proteins within an intact animal. This unique feature enables the study of...
gene expression and protein deposition in both temporal- and tissue-specific fashions. Antibody-based labeling in whole worms when the proteins of interest are not fluorescently tagged also relies on the animal’s transparency; however, this technique requires fixation of the animal and thus, only enables to visualize protein distribution at the moment of death.

Finally, since it is technically simple to create transgenic worm strains various worm models that express neurodegeneration-associated, aggregative proteins in different tissues have been generated. These include worms that express the Alzheimer’s disease-linked peptide Aβ [6], fluorescently-tagged, Huntington’s disease-associated poly-glutamine stretches [7] or α-synuclein, a protein whose aggregation is accountable to the development of Parkinson’s disease [8]. The worm’s protein content and rates of aggregation can then be analyzed using standard biochemical techniques [9]. Here we describe widely used proteotoxicity worm models and techniques.

1.2. Manipulating the aging process of C. elegans

Aging was seen for a long time as a stochastic process resulting from inevitable random events that lead to the accumulation of damage over time. The way we perceive and study this process was however revolutionized by the identification and characterization of genetic and metabolic pathways that regulate the pace of aging in different species. Several evolutionary conserved, aging and lifespan modifying mechanisms have been unveiled. The first to be discovered was dietary restriction (DR) [10], a reduction of food intake without malnutrition. DR was shown to elevate resistance to various stress conditions including oxidative stress and to increase both mean and maximal lifespan in invertebrates and mammals (reviewed in [11]). DR-mediated longevity was found to be dependent on the activity of several molecules including the transcription factors PHA-4 [12] and the NRF-like factor SKN-1 [13], the neuropeptide-like protein NLP-7, the ion channel forming protein CUP-4 [14] and the E3 ubiquitin ligase WWP-1 [15].

Perhaps the most prominent and best characterized pathway that regulates lifespan and youthfulness in worms, flies and mammals is the insulin/IGF-1 signaling (IIS) pathway [16]. In the nematode, this endocrine pathway is regulated by the sole insulin/IGF-1 receptor DAF-2 that controls the activity of at least three transcription factors; the forkhead (FOXO) transcription factor DAF-16 [17], the heat shock transcription factor HSF-1 [18] and SKN-1 [19]. When activated, this pathway renders all three transcription factors inactive in the cytoplasm. In contrast, by interfering with DAF-2 signaling they translocate into the nucleus and regulate the expression of their target gene networks that in turn elevate stress resistance and extend the animal’s lifespan.

Reducing the activity of the mitochondrial electron transport chain (ETC) has also been found to alter the aging process. Perturbations in nuclear genes encoding ETC components such as clk-1 [20] and isp-1 [21] were reported to delay the aging process of C. elegans.

Finally, removal of the nematode’s germ cells have been shown to extend lifespan [22], mitigate proteotoxicity [23] and preserve protein homeostasis (proteostasis) [24].

The discovery and characterization of these aging-regulating pathways along with the development of proteotoxicity-model worm strains enabled the elucidation of the mechanistic links between the aging process, proteotoxicity and neurodegeneration.

1.3. Proteotoxicity underlies the development of human neurodegenerative maladies

Due to the importance of proteostasis for functionality and survival, cells developed surveillance mechanisms that monitor the folding integrity of newly synthesized and mature proteins [25]. Misfolded proteins are either refolded or targeted for degradation. Occasionally, however, aggregation-prone proteins escape these quality control mechanisms and form potentially hazardous aggregates. These protein aggregates can lead to the development of diseases termed “conformational disorders” [26] that include the late-onset neurodegenerative maladies such as Alzheimer’s disease (AD), Parkinson’s disease (PD) [27], Huntington’s disease (HD) [28] and amyotrophic lateral sclerosis (ALS) [29].

According to the amyloid cascade hypothesis, Alzheimer’s disease (AD) emanates from the accumulation and aggregation of the Aβ family of peptides that derive from the proteolytic digestion of the amyloid precursor protein (APP) by two proteases, the β- and γ-secretases. These events initiate cellular cascades that culminate with synaptic dysfunction and neuronal injury that lead to neurodegeneration [30]. Poly-glutamine diseases are another group of late-onset pathologies that result from the expansion of CAG repeats, coding for glutamine tracts (polyQ), in the causative proteins. When above a certain threshold, these tracts render the proteins prone to aggregate [31]. The group of polyQ-associated disorders includes Huntington’s disease (HD), which stems from the aggregation of the protein huntingtin [28] and Machado–Joseph disease (MJD) (or spinocerebellar ataxia type 3), which originates from the aggregation of ataxin-3 [32]. Parkinson’s disease (PD) is typically associated with neuronal loss in the substantia nigra and with the appearance of inclusions containing aggregates of the presynaptic protein α-synuclein (α-syn) [33].

The links of these maladies to proteotoxicity and their common feature of late-onset proposed that the aging-associated decline in the competence of the proteostasis network enables the emergence of these disorders. This theme raised the prospect that the manipulation of aging could be used to delay their emergence and slow their progression. The advantages of C. elegans for the study of aging along with the wealth of applicable genetic and molecular tools designated this animal as a preferred model for the study of proteotoxicity and prompt the creation of various nematode strains that express neurodegeneration-linked aggregative proteins (reviewed in [34]).

2. Nematode models and laboratory assays for the study of proteotoxicity

A successful nematode model for studying the links between proteotoxicity and aging has to meet a critical requirement: it has to exhibit a protein-aggregation-associated progressive phenotype that can be measured within the lifespan of the nematode. This can be achieved by successfully combining an aggregation-prone protein and a promoter that regulates its expression. Three parameters have to be considered when generating such model: (i) the nature of the aggregative, disease-linked protein, (ii) its expression levels and (iii) the tissue of expression. A combination of a highly toxic, aggregative protein and a promoter that exhibits high rate of activity will probably lead to an aggressive phenotype which will not allow progressive measurement, while a weak promoter together with a moderately toxic aggregating protein will not produce a clear phenotype within the short lifespan of the nematode. Due to the difficulties of achieving such a delicate balance, not all proteotoxicity models exhibit similar phenotypes and, thus, several assays have been developed to measure different outcomes of toxicity exhibited by distinct models. Here we describe commonly used worm proteotoxicity models and techniques (see Table 1).

2.1. CL2006, Aβ expressing worms

A well-characterized model for studying the proteotoxicity of the AD-associated, Aβ peptide is the CL2006 strain. These animals express the human Aβ42 [35] peptide under the regulation of the
unc-54 promoter, which drives the peptide’s expression in the worm’s body wall muscles [36]. The Aβ expression and subsequent aggregation in the nematodes’ muscles lead to a progressive paralysis of the worm population which can be monitored and quantified by performing a paralysis assay [9] (information regarding additional nematode-based, AD-proteotoxicity models can be found in [34]).

2.1.1. Paralysis assay

Place synchronized CL2006 eggs (obtainable by the bleach of fertile worms) on plates seeded with the desired bacteria (HT115, possibly expressing dsRNA towards a gene of interest, or OP50 bacteria, when RNAi treatment is not needed) and allow the worms to develop to adulthood. At day 1 of adulthood transfer 12 healthy worms onto small NG (nematode growth) plates seeded with bacteria as needed. It is advised to start from 120 animals per treatment as a relatively high rate of bagging is expected in CL2006 worm populations. Score paralyzed worms daily by tapping on the worms’ noses with a platinum wire or with an eyelash. A paralyzed animal is defined as an animal that can move its head but is unable to crawl away (“windshield wiper” phenotype). Censor worms that accumulate eggs within their bodies (“bagged” phenotype) and dead animals which are unresponsive to touch by the wire. During the reproductive period, the worms should be transferred daily onto fresh plates to separate the original cohort from their offspring. The assay should be terminated at the age of 12 or 13 days of adulthood, when wild-type animals start exhibiting age-related paralysis. The data can be plotted either as the cumulative number of paralyzed worms per day (Fig. 1A and B) or as the number of non-paralyzed worms per day. Aβ RNAi can be used as a control for the association between the expression of this peptide and the paralysis phenotype. Using this assay we have shown that the alteration of aging by the knockdown of daf-2 protects from Aβ-associated proteotoxicity [9] and that this manipulation alleviates proteotoxicity when it can no longer extend lifespan [37].

The rate of Aβ aggregation can be examined by biochemical, microscopic and in vitro assays.

2.1.2. Analysis of Aβ aggregation using Western blot, CL2006 worms and Aβ antibodies

Place synchronized CL2006 eggs on plates seeded with the appropriate bacteria and allow hatching and development. At the desired age collect the animals from the plates using M9 buffer and wash them three more times. Aspirate as much M9 as possible, wash again using cold PBS, let the worms settle and add protease inhibitor cocktail. Transfer the worms to a Dounce homogenizer, place it on ice while allowing the worms to settle and aspirate the PBS leaving twice the volume of the worm pellet. Homogenize the worms on ice (20 strokes) and transfer the homogenates to clean test tubes. Sediment the worm debris by centrifugation using a desktop centrifuge (3,000 rpm, 3 min), transfer the resulting supernatants into new tubes, add protease inhibitor cocktail (Roche, cOmplete tablets) and measure total protein concentrations. Prepare a stock solution of monomeric Aβ_{1-40} (300 μM) [39] and dilute to a final concentration of 20 μM in phosphate buffer (300 mM NaCl, 50 mM Na₂HPO₄, pH 7.4) containing ThT, (20 μM). Sonicate the PDS samples of CL2006 worms for 10 min and add to the assay at a final total protein concentration of 0.3 mg/ml. Load three aliquots (100 μl) of the solutions into wells of a 96-well microplate for each reaction, seal and measure rates of ThT fluorescence (excitation at 440 nm, emission at 485 nm) over time at 37 °C in 10 min intervals, with 5 s of shaking before each reading.

2.1.3. Analysis of Aβ aggregation using an in vitro kinetic aggregation assay

The in vitro kinetic aggregation assay is based on the intrinsic propensity of Aβ peptides to aggregate in the test tube and on the observation that the dye Thioflavin T (ThT) solely fluoresces (at the appropriate wave length) when it is bound to β sheets, a hallmark of amyloids. Thus, ThT enables to detect the rate of Aβ aggregation [38]. However, the spontaneous aggregation of Aβ shows a lag phase during which micro-aggregates (seeds) are formed. This lag phase can be shortened by the addition of Aβ seeds from an external source. We used CL2006 worm as an external source of seeds to measure the amounts of Aβ aggregates contained in the animals. This assay, when combined with RNA interference (RNAi), enabled us to examine the functional roles of specific genes in Aβ dis-aggregation and hyper-aggregation [9].

For the in vitro kinetic aggregation assay grow CL2006 worms from hatching to the chosen age on the desired bacterial strains and wash twice with M9 and once more with PBS. Resuspend the animals in 300 μl ice cold PBS, transfer to a 2 ml tissue grinder (885482, Kontes, Vineland, NJ), homogenize on ice and transfer the homogenates to clean test tubes. Sediment the worm debris by centrifugation using a desktop centrifuge (3,000 rpm, 3 min), transfer the resulting supernatants into new tubes, add protease inhibitor cocktail (Roche, cOmplete tablets) and measure total protein concentrations. Prepare a stock solution of monomeric Aβ_{1-40} (300 μM) [39] and dilute to a final concentration of 20 μM in phosphate buffer (300 mM NaCl, 50 mM Na₂HPO₄, pH 7.4) containing ThT, (20 μM). Sonicate the PDS samples of CL2006 worms for 10 min and add to the assay at a final total protein concentration of 0.3 mg/ml. Load three aliquots (100 μl) of the solutions into wells of a 96-well microplate for each reaction, seal and measure rates of ThT fluorescence (excitation at 440 nm, emission at 485 nm) over time at 37 °C in 10 min intervals, with 5 s of shaking before each reading.

2.1.4. Visualizing Aβ in CL2006 animals

A direct visualization of CL2006 worms not only supports semi-quantitative results obtained by Western blot but also enables to analyze Aβ distribution within worms that were exposed to different treatments (Fig. 1D). Grow synchronized CL2006 worms on the desired bacteria, wash three times with M9 buffer and fix them with 4% paraformaldehyde in MRWB (80 mM KCl, 20 mM NaCl, 10 mM EGTA, 5 mM Spermidine, 50% Methanol). Freeze the worms on dry ice and incubate on ice for 1 h. Wash the worms twice with Tris-Triton buffer (100 mM Tris pH7.4, 1% TX-100, 1 mM EDTA) and incubate for 2 h at 37 °C in Tris-Triton buffer supplemented with 1% β-Mercaptoethanol. Wash the worms with PBS buffer (25 mM H₂BO₃, 12.5 mM NaOH), incubate for 15 min in BO₃ buffer supplemented with 10 mM DTT, wash three times with BO₃ buffer and incubate the worms in BO₃ buffer supplemented with 0.3% H₂O₂ for 15 min (room temperature (RT)). Block with BO₃-blocking buffer (PBSX1, 1% BSA, 0.5% TX-100, 1 mM EDTA) and incubate over-night at 4 °C with 4G8, Aβ antibody in staining buffer (PBSX1, 0.1% BSA, 0.5% TX-100, 1 mM EDTA) (according to our experience the 4G8 antibody is more suitable for this application than 6E10). Wash the worms in blocking buffer and probe for 30 min (RT) with a fluorescein-containing secondary antibody (it is recommended to use a secondary antibody that is labeled with a red fluorophore as C. elegans exhibit relatively high green auto-fluorescence). Wash the worms three times with blocking buffer, mount and visualize by a fluorescent microscope.

2.2. Poly-glutamine (polyQ) nematode models

To study poly glutamine (poly-Q)-aggregation-mediated toxicity strains that express polyQ stretches in specific tissues including
subsets of neurons [40], all of the neurons [41] and body wall muscles were created [7]. To study the correlation between the length of polyQ stretches and proteotoxicity and to visualize aggregation in intact animals, a series of strains that express polyQ stretches of different lengths (ranging from 0 to 82 repeats) fused to the yellow fluorescent protein (polyQ-YFP) all driven by theunc-54 promoter were prepared [7]. Due to the expression of aggregation-prone peptides in their body wall muscles, these animals exhibit motility impairment that depends on two parameters; the length of polyQ-YFP stretch expressed by the worm and age. Animals that express stretches of either 35 or 40 polyQ repeats are most adequate for these assays as they exhibit moderate, yet progressive motility impairments [7]. Utilizing these strains, it was discovered that aging is a major contributor to proteotoxicity and that aging manipulation by IIS reduction alleviates proteotoxic phenotypes. While fluorescent foci were observable in animals that express 40 or more polyQ repeats at day 1 of adulthood, they could not be seen until day 3 of adulthood in worms that express 35 CAG repeats of less [7].

To follow this phenotype over time a motility assay has been developed.

2.2.1. Motility and thrashing assays

Place synchronized eggs on plates seeded with the appropriate bacteria and incubate until the worms reach the appropriate age. Transfer eight worms onto a fresh plate and record a short video clip using a stereo scope equipped with a camera. Record clips of 10 plates per treatment (total of 80 worms). The average distance crawled by the worms in a given time (typically 60 s) is calculated using an adequate software. Several software packages are used by different laboratories to track motility however, “worm tracker” (the software can be found in here: www.mrc-lmb.cam.ac.uk/wormtracker) and multi worm tracker (MWT) are commonly used for this assay [42]. Due to the age-associated decline in motility that is observed in wild-type worms, the motility assay is only applicable up until day 13 of adulthood.

An alternative method to measure the decline in muscle function is the “thrashing assay”. This technique is based on the observation that worms bend their bodies multiple times when placed in liquid. This phenotype can be utilized to assess muscle function in worms that express disease-linked, aggregative proteins as a measurement of proteotoxicity.

To perform a thrashing assay, transfer a single worm from a plate into a drop of M9 that was placed on a cover slip. Observe the animal by a stereo scope and count the number of body bends per 30 s, right after transferring. Use at least 15 animals per treatment. This method was used to measure proteotoxicity of various proteins including that of the mutated trans-activating response element DNA binding protein-43 (TDP-43) which was expressed in neurons [43]. This study unveiled that IIS reduction can rescue worms from TDP-43-mediated proteotoxicity.
2.2.2. Mechanosensory response assay

An additional technique that was developed to compare the proteotoxic effects of polyQ-YFP stretches of different lengths in touch receptor neurons is the mechanosensory response assay. While a healthy animal is expected to back away upon touching its head with a fine hair (e.g. an eyelash), a nematode whose touch receptor neurons malfunction is less responsive [40]. In order to minimize adaptive touch effects, transfer 20–30 animals onto a fresh plate 24 h prior to the assay. Using a stereoscope, touch each animal 5 times and score the number of backing responses. At least 120 animals per group should be used.

2.2.3. Fluorescent visualization of polyQ-YFP foci in the whole worm

Although the size and number of polyQ-YFP containing foci correlates with the rate of aggregation, counting dots is not a direct assessment of aggregation as the possibility that the foci are dynamic quality control deposition sites (for reference see [44]) should not be excluded. Yet, the rate of aggregation of a deposition site-resident fluorescent protein can be assessed by the fluorescent recovery after photo-bleaching (FRAP) assay (described in Section 2.3.1 below). To visualize foci, place synchronized worms on plates seeded with the desired bacteria and let them grow from hatching to adulthood. Prepare sticky agar pads (containing 2% agarose in DDW and 15 mM sodium azide) on slides, let them air dry and drop 2 µl water containing 20 mM sodium azide on the pads (cooling the agar plates can be used as an alternative technique to reduce the worms’ motility). Transfer the worms onto the pads and visualize. The worms’ transparency enables to view and count the foci using a fluorescent microscope (Fig. 1E).

2.2.4. Native agarose gel electrophoresis (NAGE)

It is possible to assess the rate of polyQ-YFP aggregation using native agarose gels. This method relies on the ability of YFP to fluoresce and on the differential migration of aggregates of different sizes in the native gel which preserves these protein assemblies. To perform this assay, place synchronized eggs of polyQ-YFP expressing worms on plates seeded with the desired bacteria and treat as needed (RNAi or compound). Grow the worms to the appropriate age, wash twice with M9 and homogenize them using a Dounce homogenizer of a bead-based mechanical homogenizer [45]. Transfer the homogenates into fresh tubes and separated debris from PDS by low speed centrifugation (3,000 rpm for 3 min in a desktop centrifuge). Supplement the PDS with native loading buffer (625 mM Tris, 10% glycerol, and 1% bromophenol blue), load on a horizontal 1% agarose gel and separate (running buffer: 25 mM Tris, 0.19 M glycine [pH 8.3]) for 14–18 h, 50 V at 4 °C. YFP signal can be detected using a fluorescent laser scanner [46].

2.2.5. Filter trap assay

The rate of polyQ aggregation can be also evaluated by a filter trap assay [23]. Homogenize polyQ expressing worms that were treated as desired and grown to the appropriate age using a non-denaturing lysis buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100) supplemented with a protease inhibitors cocktail. Clear the homogenates by low speed centrifugation, transfer supernatant into a new tube and supplement with SDS to a final concentration of 1%. Load the samples onto a cellulose acetate membrane installed in a slot blot device. After blotting wash the membrane with PBS containing 0.1% SDS and immuno-blot with the appropriate antibody.

2.3. ALS models in worms

While most ALS emerge late in life the minority of cases manifest as early-onset familial, mutation-linked disorder. Among other causes, mutations in the genes encoding for the superoxide dismutase 1 (sod-1) [47] and for the TDP-43 (reviewed in [48]), underlie the development of the disorder. C. elegans models that express YFP-tagged, TDP-43 [43] or SOD-1 [49] harboring ALS-causing mutations were created. The expression of these fluorescently-tagged, aggregative proteins, which is driven by the pan-neuronal snb-1 promoter, results in uncoordinated movement of the worm.

Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Corresponding disease</th>
<th>Applicable methods</th>
<th>Key discoveries obtained using this model</th>
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| Aβ expressing worms (CL2006) | Alzheimer’s disease | - Paralysis assay  
- Motility assay  
- Western blot analysis using Aβ antibodies  
- Immunofluorescence  
- Motility assay  
- Thrashing assay  
- Western blot analysis using GFP antibody  
- Native agarose-gel electrophoresis (NAGE)  
- Filter trap assay  
- Mechanosensory response assay (for worms that express polyQ stretches in these neurons) | The alteration of aging by knocking down the activity of the insulin/IGF signaling pathway protects from proteotoxicity by regulating opposing activities; disaggregation and protective hyper-aggregation |
| Poly-glutamine-YFP models (polyQ35,40) | This group of disorders encompasses at least nine heritable disorders, among them Huntington’s disease (HD), Machado–Joseph disease (MJD) and the spinocerebellar ataxia type 3 (SCA3), caused by ataxin-3 | - Western blot analyses  
- Mechanosensory response assay (for worms that express the aggregative proteins stretches in these neurons) | The threshold number of CAG repeats (encoding for poly glutamine) required for aggregation, declines with age. Reduction of IIS activity protects worms expressing an abnormally long poly-glutamine stretch from aggregation and from motility impairments |
| TDP-43 and SOD-1 model strains | Amyotrophic lateral sclerosis (ALS) | - Motility assay  
- Western blot analyses  
- Fluorescent foci visualization  
- FRAP  
- Mechanosensory response assay (for worms that express the aggregative proteins stretches in these neurons) | Mutations in the trans-activation response DNA binding protein 43 (TDP-43) and in the superoxide dismutase 1 (SOD1) increase the aggregation rate of these proteins which in turn leads to the development of familial ALS. IIS reduction rescued the worms that expressed the mutated form of TDP-43 |
2.3.1. Fluorescent recovery after photo-bleaching (FRAP)

Motility and biochemical assays, as well as fluorescent visualization, are applicable in these animals; however, it is also possible to test the rate of aggregation in worms that express fluorescently-tagged, aggregative proteins using a kinetic assay known as fluorescent recovery after photo-bleaching (FRAP). This technique is based on the utilization of a high-power laser pulse which bleaches the fluorescent signal of affected molecules within a limited area followed by a kinetic analysis of the signal recovery in the examined zone [50]. Highly aggregated material exhibits low rate of molecular mobility and thus, a slow signal recovery. In contrast, low rate of aggregation enables high degree of mobility that results in a relatively rapid signal renewal.

For FRAP analysis, place healthy adult worms on an agar pad as described above, anesthetize the animals by exposing them to levamisole (10 mM), bleach an area that contains aggregated fluorescently-tagged proteins and follow the rate of fluorescence recovery in time intervals (mostly 10 s), using an adequate microscopic system equipped with a video camera. The changes in fluorescent signal intensities over time should be measured in the bleached area and in a control zone of identical size using adequate software (we use the freely available ImageJ software for this purpose). The fluorescence intensities measured at the affected area divided by the rates quantified at the control region should be plotted against time to illustrate the rate of signal recovery over time. In addition to the worms strains expressing mutated TDP-43 [51] and SOD-1 [49], FRAP was also used to examine the rate of aggregation of mutated, fluorescently-tagged α-synuclein [8].

3. Summary and conclusions

The nematode *C. elegans* emerges as a preferred organism for the study of neurodegeneration-linked proteotoxicity and a large variety of models is available [34]. These nematodes are foremost useful for three purposes: (i) studying the links of proteotoxicity and aging, (ii) searching for proteostasis modifying genes and (iii) drug screening.

Indeed, major breakthroughs in these research fields were made while employing *C. elegans* and, more importantly, shown to be transferrable to mammals.

3.1. Aging enables protein aggregation to become toxic late in life

The study of proteotoxicity in worms whose aging programs have been altered unveiled that genetic manipulations can mitigate proteotoxicity associated with various neurodegeneration-causing aggregative proteins. Reducing the activity of the IIS was shown to protect worms from the proteotoxicity of Aβ [9], polyQ-YFP [7], ataxin-3 [52], TDP-43 [43] and additional disease-linked proteins. Similarly, DR protects worms from Aβ and polyQ aggregation [59]. These discoveries highlight the importance of *C. elegans* proteotoxicity models for drug screening and set the basis for a focused evaluation of novel neurodegeneration therapies prior to their examination in mammalian systems.

3.2. Exploring proteostasis regulators

How the proteostasis network is orchestrated and what components play roles in the maintenance of a pristine proteome are queries that were studied in *C. elegans* proteotoxicity models. To explore new components of the proteostasis network, these model worms and RNAi screening techniques were employed and 23 genes that suppressed motility defects of polyQ35-YFP worms were identified [56]. Similarly, genes that modify α-synuclein aggregation have been characterized [8]. The worm models and techniques described herein combined with the available genetic tools that enable to modify gene expression, designate the nematode as an advantageous system for systematic classification of gene networks.

3.3. Counter-proteotoxic drug screening using *C. elegans*

The ability of worms to live in liquid and the relatively efficient penetration of compounds to these animals are key advantages for drug screening aimed to identify counter-proteotoxic substances. Indeed, several compounds have been found to be proteostasis stabilizers in worm proteotoxicity models [57,58]. Testing whether IIS inhibitors can protect from proteotoxicity we found that PT219, a newly developed, highly efficient IIS inhibitor elevates the expression of IIS target genes and protects model worm from Aβ and polyQ aggregation [59]. These discoveries highlight the importance of *C. elegans* proteotoxicity models for drug screening and set the basis for a focused evaluation of novel neurodegeneration therapies prior to their examination in mammalian systems.

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