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Review

Genetic screens in *Caenorhabditis elegans* models for neurodegenerative diseases☆

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ABSTRACT

Caenorhabditis elegans comprises unique features that make it an attractive model organism in diverse fields of biology. Genetic screens are powerful to identify genes and *C. elegans* can be customized to forward or reverse genetic screens and to establish gene function. These genetic screens can be applied to "humanized" models of *C. elegans* for neurodegenerative diseases, enabling for example the identification of genes involved in protein aggregation, one of the hallmarks of these diseases. In this review, we will describe the genetic screens employed in *C. elegans* and how these can be used to understand molecular processes involved in neurodegenerative and other human diseases. This article is part of a Special Issue entitled: From Genome to Function.

1. Introduction

1.1. Caenorhabditis elegans as a model organism

Sydney Brenner first introduced the nematode *C. elegans* as a genetic model organism in 1965 and since then the model has been extensively used in very diverse fields of research, from developmental biology to ecotoxicology, aging and neuroscience [1]. This has resulted in several breakthroughs in biomedical science, which include the discovery of genetic regulators of programmed cell death, the use of the green fluorescent protein as a protein marker, and the discovery of RNA interference. Indeed, this nematode combines a number of characteristics that make it an advantageous model, anatomically and genetically, which are summarized in Box 1. Moreover, the characteristics of this invertebrate make it an easy experimental model to study biological processes in a relatively cheap, quick, and easy way.

C. elegans is a small, free-living nematode of about 1–1.5 mm in length that can be found in temperate soil environments feeding on different bacteria, including *Escherichia coli*. It exists in two sexual forms, as a hermaphrodite or as a male. The former is self-fertile, able to produce its own sperm and eggs and is the predominant adult form. Although males are rare (about 0.02%), their abundance in the offspring can be increased to 50% by mating with hermaphrodites [2]. The length of the life cycle of wild type N2 *C. elegans* strains and its lifespan depends on the growth temperature. Grown at 20 °C, hermaphrodites usually lay 300–350 eggs and once the eggs hatch, it takes about three days to develop from a larva to an adult. The average lifespan of this organism can vary between 18 and 20 days [3,4]. At higher temperatures, the life cycle is shortened and the lifespan decreased. One major advantage of *C. elegans* is that it has a well-dissected and predetermined anatomy. The adult hermaphrodite has exactly 959 somatic cells and 302 neurons [1,5,6]. Its transparent body enables one to easily follow cell fate or expression of fluorescently tagged proteins of interest in the living animal. Moreover, *C. elegans* was the first multicellular organism to have the complete genome sequenced and this gave rise to several databases and resources that are currently available online for the scientific community ([7], see "Online links" at the end of this article).

Genetic screens are widely used in *C. elegans* to discover gene function. It can be easily applied to discover which gene mutations are responsible for a specific phenotype of interest (forward genetics) or, conversely, the gene function can be purposely altered to assess what is the consequence in terms of development, behavior or alterations in specific biological processes (reverse genetics). The two major genetic screens employed are ethyl methane sulfate (EMS) screens and (genome-wide) RNAi screens. They have been fundamental not only to dissect nematode genetics but also to identify genes involved in aging, development, DNA damage response, and signal transduction, amongst other biological processes [8–13].

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Box 1

Advantages of using *Caenorhabditis elegans* as a model organism

1. Small size (1–1.5mm long)
2. Short reproductive cycle
3. Short lifespan
4. Translucent body
5. Precise, predetermined anatomy
6. Ease of culture
7. Small genome
8. Whole genome sequenced
9. RNAi library available
10. Deletion mutant database

1.2. C. elegans homology to humans

For the scope of this review, we shall explore the rationale and the basic procedures for both methods not only highlighting their advantages but also pinpointing the drawbacks. Next, we will explore how genetic screens can help us gain insight into the molecular and cellular mechanisms of human diseases. Specifically, we will focus on the application of genetic screens to discover potential disease-modifier genes by exemplifying studies on *C. elegans* models for neurodegenerative diseases.

There are a significant number of proteins that are evolutionary conserved between *C. elegans* and humans. At the time that the *C. elegans* genome sequencing was complete, 36% of *C. elegans* proteins (from a set composed of 18,891 protein sequences) were found to have homologs in humans (set composed of 4979 protein sequences), by pairwise comparison (The *C. elegans* Sequencing Consortium, 1998 [7]). Thereafter, this percentage was increased to 83% due to the much larger human gene dataset available to perform the comparison [14]. A more recent study estimated that 38% of the 20,250 *C. elegans* protein-coding genes had unique corresponding functional orthologs in humans (7663 unique hits) [15]. In a nutshell, biological processes unraveled in the invertebrate *C. elegans* can provide insight into human biology.

2. Genetic screens

Genetic screens in *C. elegans* are well-established and commonly used to assess gene function in any biological process of interest. High-throughput (semi-) automatized setups and screening methods enable hundreds of parallel experiments in microtiter plates. In a screen, wild type animals are mutagenized or treated with RNAi and then scored for phenotypical changes. Below we describe two types of genetic screens that are most frequently used: EMS mutagenesis and RNA interference (RNAi). The characteristics of both type of screens are summarized in Table 1.

Table 1
Features of EMS mutagenesis versus RNA interference.

EMS mutagenesis	RNA interference
Inactivation or alteration of gene function	Reduction or depletion of gene function
Requires identification of gene mutation	Candidate gene is known
Permanent mutation	Possible to select developmental stage for depletion
	No effects on embryos in the first generation
Can select for non-essential genes	Can identify roles of essential genes in a post-developmental process
	Limited penetrance to neurons
	Limited efficiency if the protein that is encoded by the targeted gene is very stable

2.1. EMS mutagenesis

The most commonly used method to mutate the genome of *C. elegans* is the treatment with EMS. The mutagen induces mutations in the sperm and oocytes of hermaphrodites. Sydney Brenner tested systematically different mutagens, but researchers are mostly using EMS because of its relatively low toxicity and relatively good efficiency (summarized in [16]). The hermaphroditism of *C. elegans* allows easy maintenance of a mutation as a homozygous worm will pass it to all the progeny through self-fertilization.

Mutations can be identified using a simple F2 screen firstly described by Brenner in 1974 [1]. Thousands of copies of any particular gene can be analyzed in a typical EMS screen. The frequency of a null mutation at any particular locus of the genome is one for every 2000 copies by using standard concentrations (50 mM) of the mutagen. That means that one can expect to identify 6 mutations per particular gene in a typical experiment of 12,000 haploid genomes (reviewed in [16]). The mutagenized worms are placed on Petri dishes and grown for two generations to produce homozygous mutants (Fig. 1). Worms from the F2 generation showing a specific phenotype of interest are further singled to new plates to determine whether the phenotype is transmitted to the next generation.

Once a worm with a specific phenotype is isolated, the responsible mutation needs to be identified. By using single nucleotide polymorphisms (SNPs) of the Hawaiian wild type strain in comparison to the Bristol strain (natural variation wild type) it is possible to

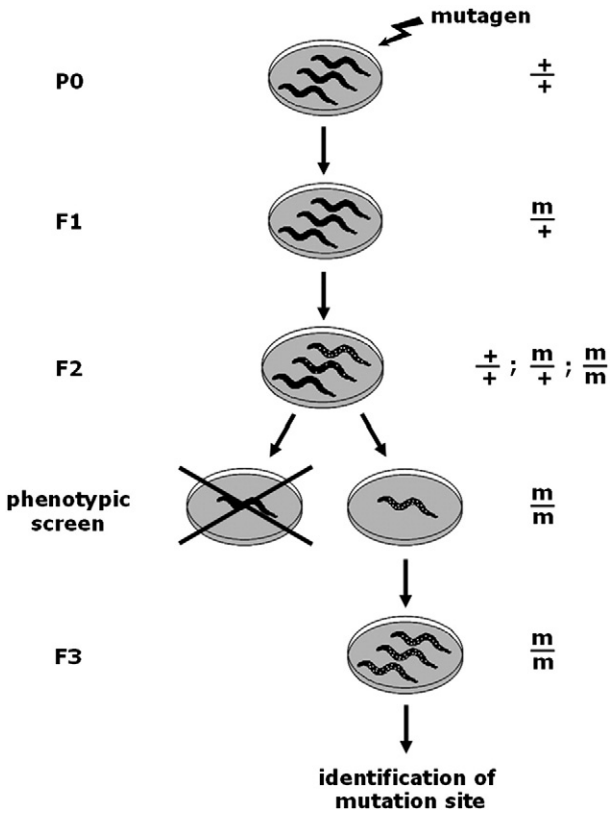

Fig. 1. High-throughput mutagenesis screen in *C. elegans*: Animals (P0) are treated with a mutagen (e.g. EMS) to produce progeny (F1) containing mutations in alleles of different genes. These animals get progeny (F2) of different genotypes by self-fertilization which are further scored for a specific phenotype. Positive evaluated animals are singled to validate a homozygous mutation by breeding through of the phenotype to the next generation (F3). Once a mutant is isolated, the mutation site needs to be mapped to a specific genomic site to continue with functional studies (adapted from [16]).

map a mutation first to a certain chromosome [17] and then in several steps to a specific region on that chromosome. When a mutation is mapped to a gene region, sequencing or the specific knockdown of every single gene in that area by RNAi can be used to identify the mutated gene. The development of new sequencing methods like deep sequencing in the last decade facilitates the identification of mutations and can save laborious fine mapping [18–20]. It is important to keep in mind that an isolated, mutated animal can have several mutations at different loci. For further interpretation, it is therefore necessary to backcross the animals several times with wild type strains. The importance for controlling the genetic background was shown by Burnett and colleagues. They demonstrated that a described lifespan extension [21] by overexpression of SIR-2 disappeared after several backcrossings [22]. Tissenbaum and Guarente further showed that the overexpression of SIR-2.1 slightly increases lifespan but to a much lesser extent than the transgenic animals used in their first publication [23]. Deep sequencing methods can be used to monitor mutations in the background. Preferentially, one should use independent mutant or deletion alleles of a gene to confirm results.

In the first EMS screens, 619 mutants were identified with visible phenotypes especially from the uncoordinated class [1]. This group of genes impairs wild type movements when mutated. Under laboratory conditions proper moving is not essential as food is plentiful and sex is dispensable so therefore maintenance and characterization of mutants that may not survive in non-laboratory conditions are possible. Many of these mutants have revealed important information about molecules and mechanisms involved in human disease. For example, one gene of the uncoordinated class is *unc-2* (uncoordinated 2) and encodes for a homolog of the voltage-sensitive calcium-channel alpha-1 subunit (human P/Q calcium channel CACNA1A) [24]. Missense mutations in the CACNA1A calcium channel in humans are associated with a rare form of migraine [25] which is often associated with low levels of serotonin [26]. *unc-2* mutants show neuronal migration defects similar to serotonin-deficient mutants [27] and UNC-2 is required for the desensitization to the two neurotransmitters dopamine and serotonin [28]. Studies using *C. elegans* showed that UNC-2 interacts with the transforming growth factor (TGF)-β, a pathway that is required for movements through regulation of serotonin levels probably through the modulation of the expression of *tph-1* (tryptophan hydroxylase), the enzyme that converts tryptophan into serotonin [29]. There are elevated levels of TGF-β1 in migraine patients compared to those of pain-free individuals [30].

In addition to mutations in the uncoordinated class, Brenner also identified mutants with aberrant appearance like animals with small bodies, blistered cuticles, twitching muscles, rolling locomotion, long bodies, dumpy bodies, forked heads or bent heads [1].

EMS screens are often used to identify different mutations with the same phenotype to further investigate if those genes function in the same processes. Using this approach, John Sulston and H. Robert Horvitz searched e.g. for mutants that show defects in the differentiation of a vulva from epidermal cells [31]. Molecular follow-up studies revealed that animals that lack a vulva had mutations in two signaling pathways: the epidermal growth factor (EGF)/RAS pathway and the Notch signaling pathway (reviewed in [32–34]), both having major roles in cell fate determination. These studies in *C. elegans* have increased the understanding of these molecular pathways involved in oncogenesis in humans (reviewed in [35,36]).

Another possibility to find genes of the same genetic pathways are enhancer or suppressor screens. In this case the mutagenesis occurs on a non-wild type strain whose genetic composition is known and causes a defined phenotype. Like this, one can screen for mutations in this genetic background that enhance or suppress (reverse) that phenotype. With this approach one is able to show that two genes not only act in the same pathway but also their hierarchy which means that one is acting upstream of the other (summarized in [16]). However, one should still keep in mind that it might also be possible that some proteins result in the same phenotype when mutated even though they do not necessarily function in the same pathway.

Although EMS mutagenesis is a powerful tool to generate a high number of mutations and high-throughput screens to identify mutants with a specific phenotype it also has some limitations. It has been estimated that about 30% of the genes in *C. elegans* can be mutated to a visible phenotype [37] (some mutations might result e.g. in a lethal phenotype as it is the case for a number of developmental genes) and it needs to be mentioned that the identification of the same mutations which indicates a saturation of the screen is no guarantee that some other genes might be missed in this screen. High-throughput screens are only a starting point for further detailed experiments at molecular levels.

2.2. RNA interference

RNA interference was first discovered and investigated in *C. elegans* and published in 1998 by Andrew Fire et al. [38] (Nobel Prize in Physiology and Medicine in 2006). The discovery of dsRNA-mediated gene silencing has revolutionized genetic studies in *C. elegans*, as well as in other model organisms. Similar to EMS screens, RNAi screens can be used to identify genes that, when depleted, result in a certain phenotype or enhance or suppress a mutant phenotype.

RNAi in *C. elegans* is systemic, which, to date, is not the case for any other animal models. Therefore it is sufficient to introduce dsRNA into one specific tissue to get RNA silencing also in distant cells because of an amplification process called transitive RNAi [39]. This systemic effect is advantageous for large-scale genome-wide RNAi screens in *C. elegans*.

There are different methods that describe how to silence gene expression in *C. elegans*. The dsRNA can be delivered into the worm by (1) injection into any region [38], (2) feeding with dsRNA-producing bacteria [40], (3) soaking in dsRNA [41] or (4) in vivo production of dsRNA from transgenes under the control of specific promotors [42]. Cell-specific factors seem to regulate thereby the entry and export of dsRNA [39,43,44]. Some cell types (e.g. neurons) seem not to respond well to systemically delivered RNAi [42]. The use of RNAi enhanced mutants (e.g. *eri-1* mutant or mutants of the retinoblastoma pathway that are described to enhance RNAi especially in nervous tissue) might circumvent this problem [45,46]. In addition, Calixto and colleagues generated transgenic animals overexpressing the transmembrane protein SID-1 which is an essential component for systemic RNAi in the neurons. This modification increased the response to dsRNA delivered by feeding. It seemed that the expression of SID-1 in the neurons, on the other hand, decreased the RNAi effect on non-neuronal cells which might be useful for studying the function of essential genes in the neurons. This effect could be even increased when using a *sid-1* mutant background [47]. Durieux and colleagues further used this mutant that is insensitive for systemic RNAi to investigate the knockdown of one of the cytochrome c oxidase-1 subunits in specific tissues by controlling the expression of dsRNA via tissue-specific promotors [48]. The tissue-specific expression of SID-1 (not only in neurons) in a *sid-1* knockout background probably also enables to study tissue-specific effects especially of essential genes.

Especially the possibility to feed animals with dsRNA-producing bacteria enables to perform high-throughput RNAi screens in *C. elegans* (Fig. 2) [49,50]. For efficient induction of RNA interference the choice of the dsRNA-coding region is essential. In *C. elegans*, long dsRNA fragments (more than 100 bp) trigger gene silencing via RNAi. For most genes dsRNA is about 200–1000 nucleotides or even longer and covers exon-regions of the targeted gene. The fragment should only target one gene. Once the coding region is chosen it can be cloned into a specific vector encoding the production of the specific dsRNA (summarized in [51]). The L4440 vector contains two bacteriophage T7 RNA polymerase promotors flanking the multiple cloning site in which the cDNA of a specific gene has been inserted. The construct can be transformed into *E. coli* strain HT115 (ED3). This strain is deficient for the bacterial

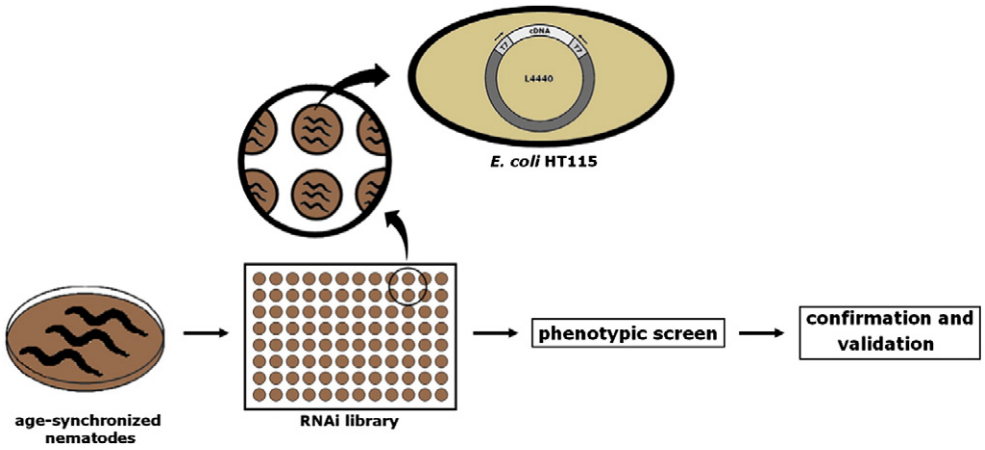

Fig. 2. High-throughput RNAi screen in *C. elegans*: Age-synchronized animals are transferred to microtiter plates containing different clones of HT115 *E. coli* bacteria. Every clone produces a specific dsRNA which is taken up by the nematodes and induces a knockdown of the corresponding gene. Positive hits in the phenotypic screen are finally confirmed by sequencing the bacterial clone and repeating the specific knockdown in single experiments. Starting point of the RNAi feeding (possible at any developmental stage) and time point of the phenotypic scoring depend on the experiment setup.

RNA polymerase III and its production of bacteriophage T7 polymerase from the construct can be induced by the addition of isopropyl β-D-1-thiogalactopyranoside (IPTG). The bacteria are then synthesizing two complementary RNA strands that form a duplex RNA which can mediate RNAi [38].

RNAi libraries are commercially available which includes one library of bacteria clones containing cDNAs of 17,575 genes which represents about 87% of the *C. elegans* genome [49] and one library including clones of 11,800 *C. elegans* genes [52]. Positive scored clones subsequently can be sequenced to confirm that they target the predicted gene. To prevent any further off-target effects of the dsRNA and therefore false-positive results, one should consider generating a second RNAi construct targeting the mRNA of the same gene [52]. Besides the coding regions, the 3′UTR of mRNA might as well be a suitable target as RNA localization elements for the transport of the mRNA or regulation elements of eukaryotic gene expression are typically located in this region (summarized in [53,54]).

Dissolving adult hemaphrodites with hypochloride (bleach) will yield only fertilized eggs and can be used to age-synchronize the animals for a screen.

Gene knockdown by RNAi can be induced at different developmental stages in contrast to EMS mutagenesis, which generates stable mutations that are present at all stages. Thus, the examination of the function of a gene that is transcribed at different developmental stages is possible. This is especially interesting when an active gene is essential at early developmental stages [55]. To investigate the effect of gene depletion by RNAi during embryonic development it is necessary to feed the parental worm with the specific bacterial strain.

Another possibility to study the effect of gene depletion at a certain timepoint was previously described by Calixto and colleagues, by performing a temperature-sensitive conditional knockdown. They could induce the knockdown of a gene by controlling via temperature the expression of RDE-1, a *C. elegans* argonaut protein which is required for RNA interference. This resulted in active RNAi at 15 °C but not at 25 °C. Furthermore, they observed that the switching ON and OFF is much faster than transferring animals from RNAi-mediating bacteria to non-RNAi inducing bacteria and vice-versa [56].

The dilution of bacteria containing a specific RNAi construct with non-RNAi mediating bacteria may decrease the efficiency of knocking down a certain gene. In that case of mild RNA interference, lethality effects and other very strong phenotypes are reduced and it might still be possible to study the function of these special genes.

The target of RNAi is known. This is one major difference to EMS mutagenesis that cannot be directed to specific genes. Therefore, besides genome-wide RNAi screens, one can also screen in a smaller subset of candidate genes for example based on previous microarray data, GWAS data, interactome studies, etc. Colaiácovo and colleagues performed for example a RNAi screen to check for germline phenotypes in a subset of genes that were generated by a previous microarray analysis of Reinke et al. that was focusing on germline-enriched gene expression [57,58]. In another screen we were looking for genes that, when knocked down, increased the number of alpha-synuclein inclusions in a Parkinson's disease *C. elegans* model [59]. This group of 80 genes was then further used for a second RNAi screen in order to find candidates that, when knocked down, induced motility changes in the disease background [60].

RNAi efficiency of bacterial clones in the library can differ. Whereas some dsRNAs induce gene silencing closely to a knockout of a gene, others only generate a mild knockdown. One should always be aware that RNAi is only silencing gene activity and that it is not a full knockout of a gene. It is estimated that about 10–30% of candidates are scored as false negatives as the RNAi is not efficient enough to result in an obvious phenotype. On the other hand the percentage of false positives is relatively low (0.4%) [61]. It is also important to keep in mind that RNA interference is acting at the mRNA level and therefore only influencing the expression of a protein. That means that the stability of a protein is highly influencing the RNAi effect as already generated proteins and their activity are not affected anymore.

Results can also differ from one experiment to the other using the same bacteria clone to silence a specific gene. For example the freshness of the material can be crucial (IPTG, Ampicillin, RNAi construct containing bacteria) [51]. In contrast, a knockout mutant e.g. by EMS mutagenesis has the advantage that it results in a stable genotype. Results of the RNAi screen should therefore be confirmed by single experiments with the candidate genes, preferentially with a gene mutant strain.

A clear phenotype for scoring is mandatory for any successful screen. An obvious easy-to-recognize phenotype as well as automation of scoring facilitates the screening process.

The small size of the animals, the variety of simple phenotypes that are often results of one single gene disruption or silencing, the hermaphroditic reproduction, the homology to higher organisms (see above) and the knowledge of the *C. elegans* genome, cell-distribution and nematode anatomy make this animal an optimal model organism to identify the function of genes via any kind of high-throughput screen.

3. From genome to function: what have genetic screens taught us?

One of the advantages of *C. elegans* is that it is amenable to generate "humanized" models of human diseases. For the purpose of this review, we will describe as an example *C. elegans* models of neurodegenerative

diseases. Neuropathological hallmarks found in the human brain can be successfully recapitulated in the nematode, such as protein aggregation [62]. Indeed, one of the common features in neurodegenerative diseases is the presence of protein aggregates in the brains of affected patients. These structures originate from protein misfolding and aggregation of so-called "aggregation-prone proteins". To name a few, these can be the amyloid-beta in Alzheimer's disease (AD), mutant huntingtin in Huntington's disease (HD) and alpha-synuclein in Parkinson's disease (PD) [63]. By mechanisms that are still to be unraveled, these aggregation-prone proteins adopt a distinct conformation, which is thought be a toxic gain-of-function [64,65]. The general understanding is that aggregation (or inclusion formation) renders cellular protection by sequestering misfolded proteins, therefore preventing potentially toxic protein–protein interactions [65,66].

Several nematode models have been generated to recapitulate molecular aspects of diseases, including HD, PD, AD and muscular dystrophy [59,67–72]. Although they do not feature clinical aspects of the disease, they provide the means to understand the molecular mechanisms in these diseases. Genetic screens performed in some of these models represent quick, unbiased methods that have enabled insights into the underlying mechanisms of neurodegeneration. Indeed, many of the disease modifiers discovered in *C. elegans* were found to be reproducible in human cell-based models and other animal models such as mice, strengthening the validity of using this small organism to study complex human diseases, as summarized in Table 2 [69,73–78].

3.1. C. elegans models for polyglutamine diseases

Polyglutamine diseases comprise a subset of neurodegenerative disorders that include HD, spinocerebellar ataxias (−1, −2, −6, −7, −17), Machado–Joseph disease (also know as spinocerebellar ataxia 3) and spinobulbar muscular atrophy [79]. The common characteristic of polyglutamine diseases is an abnormal expansion of CAG triplets (which encode glutamine) in the coding region of the disease gene. Although the length of the CAG repeat may vary from individual to individual, the threshold to develop disease is around 40 CAG repeats (except for SCA6), which cause a polyglutamine expansion in the protein that is prone to aggregate. The larger the CAG repeat the earlier onset will occur and the more severe the disease phenotype will be. A more detailed and complete information on polyglutamine diseases is reviewed elsewhere [79].

In *C. elegans*, several different models have successfully recapitulated protein aggregation. Similarly to what occurs in humans, the length of the CAG repeats also determines the aggregation phenotype in *C. elegans*. At least three models have been generated to induce polyglutamine-associated toxicity in neurons by expressing expanded polyglutamine stretches in ASH sensory neurons, touch receptor neurons or the entire nervous system of *C. elegans* [68,80–82]. Polyglutamine aggregation has been modeled in the body wall muscle cells of *C. elegans* [68]. In this model, expanded polyglutamine stretches are fused to a yellow fluorescent protein (YFP) under the *unc-54* promotor, which is specific to the body wall muscle. The aggregation and toxicity phenotype is polyglutamine length-dependent. As the animal ages, the accumulation of protein aggregates increases, which is associated with toxicity [68]. This model has been widely used for genetic screens to discover enhancers and/or suppressors of polyglutamine proteotoxicity. Two genome-wide RNAi screens revealed modifier genes and classified them according to their biological function [83,84]. In the first screen, Q35 animals were fed with dsRNA-producing bacteria and scored for genes that, when downregulated, provoked premature polyglutamine aggregation [83]. The major functional classes included RNA synthesis and processing, protein synthesis, folding, transport and degradation and components of the proteasome. In the second screen, the authors sought for genes that drive aggregation in Q35 animal and therefore the selection was made for genes that suppressed polyglutamine-induced aggregation when downregulated [84]. With this study, a new subset of modifier genes was recently found to belong to broader biological functions, namely cell cycle, cell structure, protein transport and energy and metabolism [83,84]. Therefore, proteotoxicity is not derived only from protein-related processes but rather a more diverse spectrum of biological functions that also have an effect on protein misfolding and aggregation. Interestingly, nine of these recently identified modifier genes were able to fold misfolded proteins back into the native state when constitutively expressed in misfolding mutants [84].

Forward genetics have also been used to identify modifiers of proteotoxicity. One such screen consisted in treating Q40-expressing worms with EMS. The aim was to find positive regulators of aggregation by selecting genes that suppressed protein aggregation when chemically mutated by EMS. The screen revealed MOAG-4 (modifier of aggregation) as a general aggregation-promoting factor in polyglutamine, Parkinson's and Alzheimer's disease models [76]. Inactivating MOAG-4 alleviated from polyglutamine-induced aggregation and toxicity; moreover, this effect was functionally conserved in the human orthologs SERF1A and SERF2. A recent follow-up on one of these orthologs showed that SERF1A is a specific aggregation promoting factor, since it was able to bind specifically to amyloidogenic proteins, including alpha-synuclein, prion protein, amyloid-beta and huntingtin, but not to non-amyloidogenic proteins [85].

Genetic screens have also been used to find regulators of proteotoxicity using the *C. elegans* neuronal system. An RNAi screen performed in a *C. elegans* model expressing 128 polyQ stretches in the touch receptor neurons resulted in 662 genes that either enhanced or suppressed neuron toxicity, as measured by loss of touch response [77]. Comparison of these disease modifier genes to gene expression data in two mouse models of HD showed that there was an overlap of 49 genes that were dysregulated in the striatum of either model, emphasizing the power of using *C. elegans* to find novel regulators of proteotoxicity relevant in human diseases.

3.2. C. elegans models for Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease (after Alzheimer's disease) that affects 1% of the population over the age of 50. Clinically, it is characterized by resting tremors, rigidity, bradykinesia and postural instability [86,87]. The defects in the motor system result from the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc), which project and innervate the neurons in the caudate and putamen. Consequently, there is a reduction of dopamine levels, which is the neurotransmitter that plays a role in the coordination of body movements. Besides motor disabilities, PD patients can experience non-motor symptoms such as autonomic dysfunction, sleep disturbances and neuropsychiatric symptoms [88]. Most cases of PD are sporadic (about 95%) with unknown etiology. It has been suggested that disease can result from the accumulation of toxins (pesticides and heavy metals) over the years. Only 5% of PD has a familial origin and is associated with genetic mutations [88]. However, there are neuropathological hallmarks common to both sporadic and familial forms of PD. These are the loss of dopaminergic neurons in the SNpc, that result from the degeneration of the nigrostriatal pathway which leads to the motor symptoms described earlier as well as the formation of intraneuronal protein aggregates known as Lewy bodies and Lewy neurites in the surviving neurons, which contain alpha-synuclein.

Alpha-synuclein is a small (140 amino acids) natively soluble, monomeric protein that is predominantly expressed in the brain and is enriched in presynaptic terminals [89]. Although the precise function of this protein remains unclear, it is thought to be involved in the regulation of dopamine neurotransmission, vesicular trafficking and modulation of synaptic function and plasticity [90–92]. Three different mutations in the alpha-synuclein gene (A53T, A30P and E46K) cause autosomal-dominant PD [93–95] and genomic duplications and triplications of the gene have also been identified; suggesting that

Table 2
Summary of genetic screens performed in *C. elegans* models of neurodegenerative diseases.

Model	Transgene	Tissue	Genetic screen	Modifier hits	Mammalian ortholog	Cellular process(es)	Transposed to	Reference
PolyQ diseases	Q35-YFP	Body wall muscle	Genome-wide RNAi screen	186 genes	n.a.	RNA synthesis and processing, protein synthesis, folding, transport, degradation, components of the proteasome	n.a.	[83]
PolyQ diseases	Q35-YFP	Body wall muscle	Genome-wide RNAi screen	88 genes	n.a.	Cell cycle, cell structure, protein transport and energy and metabolism	n.a.	[84]
PolyQ diseases	Q40-YFP	Body wall muscle	EMS screen	MOAG-4	SERF1A and SERF2	Unknown	Human cell models	[76]
PD	Alpha-synuclein-YFP	Body wall muscle	Genome-wide RNAi screen	80 genes	n.a.	Vesicle trafficking, lipid metabolism, lifespan	n.a.	[59]
PD	Alpha-synuclein-YFP	Body wall muscle	RNAi	30 genes, including TDO-2	TDO	Tryptophan metabolism	n.a.	[60]
PD	Alpha-synuclein-GFP	Body wall muscle and DA neurons	Hypothesis-based RNAi screen	20 genes, including ATGR7	ATG7	Autophagy	Mice	[69,78]
PD	Alpha-synuclein (WT, A53T, A30P)	Whole nervous system	RNAi screen	10 genes, including APA-2; APS-2	n.a.	Vesicular trafficking	n.a.	[70]
AD	Tau	Whole nervous system	Genome-wide RNAi screen	60 genes	38 have human homologs	Kinases and phosphatases; protein folding, stress response and degradation; transcription; proteolysis; neurotransmission and signaling; neuronal regeneration	n.a.	[75]

overproduction of wild type alpha-synuclein is sufficient to cause disease [96,97].

Genetic screens performed with this model have been supporting an important relationship between alpha-synuclein and vesicle transport. The "humanized" model of *C. elegans* for PD expresses the human alpha-synuclein fused to YFP in the body wall muscle. Phenotypically, immobile YFP-positive foci can be seen in the muscle cells and these foci increase in number and correlate with age-dependent toxicity. An unbiased genome-wide RNAi screen with this model showed 80 modifier genes that, when suppressed, provoked premature alpha-synuclein inclusion formation [59]. A follow-up on those modifier genes revealed *tdo-2*, a gene involved in tryptophan degradation, as a general regulator of protein homeostasis during aging [60]. Moreover, 49 of the original 80 modifier genes had human homologs, which were enriched for genes related to vesicular trafficking functions. In another screen using a similar model, nematode genes orthologous to human familial PD genes were preselected to perform a hypothesis-based RNAi screen [69]. A subset of candidate genes from the initial screen was then further analyzed in another *C. elegans* model, expressing alpha-synuclein in the dopamine neurons, in order to assess their relevance at the neuronal level. This study revealed five candidate genes that were able to protect from alpha-synuclein-induced dopaminergic neurodegeneration. Again, the most representative class of genes here was associated with vesicular trafficking, with the exception of the autophagy-related gene *Atgr7*, of which the mammalian ortholog (*Atg7*) was previously implicated in neurodegeneration in mice [78]. Also, a serine/threonine kinase involved in axonal elongation, UNC-51, was found to be homologous to the previously associated risk factor ULK-2, as revealed by a genome-wide association study performed in PD patients [98]. Parallel to these findings, Kuwahara et al. were able to pinpoint two genes, *apa-2* and *aps-2*, that when knockdown by RNAi increase alpha-synuclein-induced neurotoxicity in a *C. elegans* model expressing the transgene in the whole nervous system [70]. These two genes encode for subunits of the AP-2 adaptor complex, which mediates the internalization of cargo into the cell from the extracellular space via clathrin-mediated endocytosis [99].

3.3. C. elegans models for Alzheimer's disease

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, which is predicted to affect 66 million people worldwide by 2030 [100]. It represents the most common form of dementia, leading to clinical symptoms such as memory loss and mood swings. Aging and lifestyle are risk factors for development of AD, but 70% of the cases are attributable to genetics [101]. The main neuropathological features are the presence of extracellular amyloid-beta plaques, which consist of an accumulation of aggregated amyloid-beta, and intraneuronal tangles of hyperphosphorylated tau. Mutations in several genes can lead to the development of AD, including mutations in genes encoding for the amyloid-precursor protein (APP), presenelin 1 (PSEN1) and presenelin 2 (PSEN2). These genes are part of the APP cleavage pathway and mutations in these genes promote the processing of APP towards the amyloidogenic pathway, promoting the formation of amyloid-beta. Amyloid-beta peptides can have different lengths, including 40 or 42 amino acids. Amyloid-beta 42 is the most common species found in the amyloid plaques, indicating its propensity to rapidly aggregate in comparison to amyloid-beta 40.

Tau is encoded by the microtubule-associated tau protein (MAPT) gene and predominantly expressed in the nervous system. As to its function, it is known to associate and stabilize microtubules. It has been already classified as one of the risk genes for developing AD by at least two independent studies [102,103].

There are several models in *C. elegans* that express either human amyloid-beta or tau. In the first case, the worms express amyloid-beta 3–42 in the body wall muscles which causes the progressive accumulation of amyloid-beta 3–42 in the muscle cells and paralysis, which worsens with aging [71,104]. There have been several variations to this model, either combined with inducible systems, driving expression in neurons or, more recently, expressing full-length amyloid-beta 1–42 [105–107]. On the other hand, tau-expressing models have been specific to neuronal cells and the phenotype is either worsening of uncoordinated movement or insensitivity to the touch response due to transgene expression [75,108,109].

Genetic screens in *C. elegans* models for AD have been scarce. So far, there has been no genetic screen performed in any of the models expressing amyloid-beta. There is only one report on genome-wide RNAi done in a tau-expressing model [75]. Sixty modifier genes were discovered to belong to several functional classes including, kinases, chaperones, proteases and phosphatases. Of these, 38 had homologs in humans but, more importantly, 6 had already been associated with disease, either in humans or other animal models. One of these modifiers was the nicotinic acetylcholine receptor alpha-7 (nAchR), a ligand-gated ion channel expressed in the human brain and known to contribute to tau phosphorylation [110].

4. Final considerations

Genetic screens are powerful means to find genes involved in a certain biological process of interest and their function. The fact that *C. elegans* is a tractable system to model human diseases further allows one to perform genome-wide screenings in a relatively quick and unbiased manner. Genetic screens can have two outcomes, both being equally informative. On the one hand, new genes are discovered and therefore novel pathways are implicated, giving fresh perspectives on the biological process being studied. On the other hand, genetic screens that reveal genes already known to be associated with disease strengthen the importance of those genes in pathogenesis. Many screens that start with a genome-wide approach end up with an extensive list of candidate genes that are classified according to their functional class. From here, a selection of these genes should be refined and prioritized in order to study further their individual contribution to pathogenesis. One of the critical points is considering those that might have significance at the mammalian level. Additionally, if the human gene can replace the function of the endogenous one, it demonstrates evolutionary conservation of function and enables one to extrapolate findings from small organisms to complex human diseases. It is, therefore, essential to validate the genes from the screen in higher organisms. However, it is also often that a screen might reveal genes that do not have a direct sequence homolog in mammals. Nevertheless, they may be indicators of other genes that may be functional orthologs or otherwise regulators of genes with a role in human disease (e.g. transcription factors).

Another contribution of genetic screens may be to provide novel targets for drug development [111]. Although not all features of a complex human disease are fully recapitulated in the nematode, one can argue that its simplicity can be advantageous. Especially, because analysis of the expression of the causative gene and its interactors or modifiers can be done without other confounding factors inherent to the complexity of the human biology.

Genome-wide association studies (GWAS) have gained importance in the last ten years, becoming one of the forefront strategies to find common genetic factors associated with susceptibility to develop disease. One challenge now is to establish the functional consequences of these genetic variations. Coupling genetic screens or candidate gene approach in *C. elegans* to find causative genes may represent a quick and inexpensive way to assess functional relevance of associated variations and consequently obtain concrete targets to act upon. For instance, a genome-wide toxicity screen in yeast revealed 6 modifiers of amyloid-beta toxicity that were previously identified as risk factors in GWAS [112]. Importantly, those modifiers were functionally conserved from yeast, to *C. elegans* and to rat. Another study by Shulman et al. showed, for the first time, a link between an AD risk factor and a causative gene by functional screening in the fly [113].

Although this review focused in *C. elegans* models for neurodegenerative diseases, it should be noted that *C. elegans* is a model organism for other human diseases as well. *C. elegans* has been used to model certain aspects of cancer, diabetes, obesity, polycystic kidney disease, muscular dystrophy and innate immunity, to name a few. A more complete view of these different disease models is summarized elsewhere [111].

All in all, genetic screens in small organisms such as *C. elegans* can not only aid to dissect fundamental biological questions but also have the versatility of being adapted to model complex human diseases, such as neurodegenerative diseases. Moreover, its attributes make it a tractable system to drug target discovery and compound screening, emphasizing the potential of this organism to extrapolate findings from small organisms to higher vertebrates.

Online links

- Textpresso, a full text literature searches of *C. elegans* (http://www.textpresso.org/)
- Worm Interactome Database (http://interactome.dfci.harvard.edu/C_elegans/index.php)
- The *Caenorhabditis* Genetic Center, with an extensive list of strains (http://www.cbs.umn.edu/CGC/)
- Wormbase, a complete database of genetics, genomics and biology of *C. elegans* (www.wormbase.org)
- Wormbook, a comprehensive, open-access collection of original, peer-reviewed chapters covering topics related to the biology of *C. elegans* and other nematodes (http://wormbook.org)
- *C. elegans* Gene Knockout Consortium, which creates knockout strains (http://celeganskoconsortium.omrf.org/)
- National Bioresource Project, which generates, collects, stores and distributes deletion mutants of *C. elegans* (http://www.shigen.nig.ac.jp/c.elegans/index.jsp)
- Wormatlas, a database of behavioral and structural anatomy of *C. elegans* (http://www.wormatlas.org)

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