Caenorhabditis elegans for preclinical drug discovery

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Given the high expenditure (which may run in millions of dollars) and the time (many years) to identify and develop a drug against any disease, a faster and less expensive system of drug discovery will be ideal. The model organism, *Caenorhabditis elegans* fits here well. Already the *C. elegans* disease models have significantly contributed to the identification of new drugs and validation or finding novel functions of the known drugs. For example, an FDA-approved antihypertensive drug, reserpine, and a common over-the-counter drug, acetaminophen, are identified to provide protection against neurodegenerative diseases like Alzheimer's disease and Parkinson's disease in the *C. elegans* model respectively. In this article, we discuss the various applications of *C. elegans* in diseases and drug discovery, viz. available disease models, high-throughput drug screening, identification/validation of drugs, toxicity evaluation and pharmacodynamics like cytochrome P_{450} induction.

We suggest that *C. elegans* could be definitely incorporated in the primary stage of drug discovery and target identification. At the secondary level, it could be used for toxicity screening to understand the mechanism of action and preclinical validation of drugs.

Keywords: *Caenorhabditis elegans*, drug discovery, lifespan, neurodegenerative disease.

CAENORHABDITIS ELEGANS is a simple model organism chosen by Sydney Brenner in 1960s to decipher the nervous system and development of a complete organism from a single cell – the zygote. *C. elegans* has turned out to be one of the most beneficial model systems for biomedical researchers as well. As a model organism it has not only greatly contributed to the understanding of the basic biology[1], but is also an ideal drug discovery system. Companies like Devgen and NemaRx use *C. elegans* for the drug discovery process. In fact, Devgen has identified leads from *C. elegans* and has now moved on to validate the same in higher organisms (pers. commun.).

Despite the phylogenetic, anatomical and physiological diversity between humans and worms, they share a lot of commonalities (Table 1). Though *C. elegans* cannot completely replace the mammalian system, it can help cut costs and save time in the drug discovery process. In addition, *C. elegans* is filling the gap between the *in vitro* cell culture and the mammalian models because of its physiological relevance. *C. elegans* is considered for drug discovery at the preclinical stage for multiple reasons (Tables 1 and 2). There are several reviews available in the literature which discuss the advantages of *C. elegans* (Figure 1), including *in vivo* green fluorescent protein (GFP) expression[2], commonalities between *C. elegans* and humans[3], and the potential of *C. elegans* to be used as a drug discovery platform[4–6]. Currently, more disease models are available in *C. elegans* (Figure 2 and Table 3) and high-throughput screens (HTS) have been conducted. More importantly, several drugs have been validated and novel classes of drugs identified against various diseases using *C. elegans* (Table 3). Moreover, *C. elegans* is being evaluated to determine the toxic effects of various chemicals which can be readily adapted to toxicology studies[7,8] and for use in the pharmacodynamics to evaluate the cytochrome P_{450} induction potential of a drug[9].

In this review, first we will discuss the clinically important pathways that have come to light because of *C. elegans*. Then, we will move on to the high-throughput drug screens followed by the available disease models and drugs identified/validated in *C. elegans*. Further, we will see how *C. elegans* is considered for pharmacodynamic studies of a drug and as an alternative model for toxicity screening. Finally, we will see how one pathway, viz. the delay in aging or lifespan extension could provide protection against various diseases, which is a unique contribution and link established using *C. elegans*.

Clinically important molecular mechanisms identified in *C. elegans*

The contribution of *C. elegans* to biomedical research is crowned by three Nobel prizes in the last five years. The fundamental biological processes are highly conserved between *C. elegans* and humans. *C. elegans* has greatly aided in deciphering the mechanism of apoptosis, synaptic transmission, development, lifespan extension, fat metabolism, addiction[10] and the world of small RNAs. Sydney Brenner and Robert Horvitz won the Nobel Prize for delineating the mechanism of programmed cell death otherwise known as 'apoptosis' in *C. elegans*, which was

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Table 1. Advantages of *Caenorhabditis elegans*

Morphology and lifestyle	• Small size, ~ 1 mm. • Short generation time (3 days); short lifespan (~ 15 days). • ~ 959 cells with fixed lineage, differentiated systems – digestive, reproductive and nervous system (Figure 1 *a*). • Transparent, vital green fluorescent protein (GFP) marking[2] (Nobel Prize for Martin Chaffie; Figure 1 *b*). • Visualization of specific cell/tissue in real-time.
Growth and broodsize	• Grown on petri dishes with *Escherichia coli* as food source. • Can be easily grown in liquid culture. • Compatible for 98-well, 384-well high-throughput screen format. • Hermophrodite single worm gives around 300 progeny. • Can be frozen in liquid nitrogen and regrown.
Molecular and genetic amenability – functional genomics	• Small genome size (97 Mb); completely sequenced genome, ~ 19,000 genes well documented in the Wormbase (www.wormbase.org). • Mutagenesis (with ethyl methane sulphonate or transposon) and high-throughput screen for scoring of specific behavioural phenotype – locomotion, chemotaxis, reduced reproduction, slow growth and stress tolerance[1]. • Large collection of genetic mutants available from *Caenorhabditis* Stock Center. • Whole genome RNAi library readily available[2,76]. • Generation of transgenic worms. Cell and tissue-specific expression with specific promoters and real-time monitoring with or without GFP. • COPAS–BIOSORT (Biometra) – an automated continuous flow system to sort and analyse *C. elegans* based on size, optical density and fluorescence.
Drug/chemical screening	• Chemical genetics – to identify functions of bioactive small molecules. (e.g. Nemadipine – a novel calcium channel antagonist[77]; Prozac – new targets[78].

Table 2. Comparison of disease model generation and high-throughput drug screens in different model systems

	Disease model generation			Highly-throughput screen		
Model system	Time scale	Location	Cost	Feasibility	Cost	Physiological relevance
C. elegans (*in vivo*)	In days	Inhouse	Minimal	Yes	Comparable	Yes
Mammalian/Drosophila cell culture (*in vitro*)	In days	Inhouse	Minimal	Yes		No
Drosophila melanogaster (*in vivo*)	In months	Inhouse	Minimal	No		Yes
Transgenic mice (*in vivo*)	Minimum 6 months	Transgenic facility	Expensive (~ US$ 3000 per microinjection)			Yes

later shown to be present in many species, including humans[11,12]. Apoptosis is an important event during development. In addition, several systems like immune system, nervous system, etc. widely utilize this apoptosis cascade for cell maintenance/exclusion. Problems in this pathway could lead to innumerable diseases, including cancer and neurodegenerative diseases (NDs) as discussed in Thompson[13].

One major key discovery in *C. elegans* is small RNA interference (siRNA)[14], which resulted in the Nobel Prize to Craig Mello and Andrew Fire. Since the identification of siRNA in *C. elegans*, many drug companies are evaluating this as a potential approach to silence the harmful genes in various diseases. A new class of small RNAs, microRNAs (miRs), which regulate gene silencing at the mRNA level was first discovered in *C. elegans*[15,16]. Now, these miRs are shown to be important during development[15], synaptic sculpturing[17], and in the most prevalent chronic disease, diabetes[18], Huntington's disease[19] and amyotrophic lateral sclerosis. Thus once identified, the basic biological mechanisms will be good drug targets against diseases.

High-throughput drug screening in *C. elegans*

C. elegans has many features required to be a suitable system for high-throughput drug screen (Tables 1 and 2). In fact, this is the only multicellular organism which is used in HTS[5]. Generally HTS is carried out in liquid culture in 96-well or 384-well format. The food source, *Escherichia coli* (OP50) is delivered robotically. Specific number of worms are dispensed using a COPAS–BIOSORT system (Biometra). The advantage with COPAS is selection and addition of specific growth stage worms. In HTS, generally worm growth or survival, locomotion, thrashing, reproduction, or change in fluorescence intensity upon drug treatment are monitored

Table 3. Identification or validation of drugs in various disease models in *C. elegans*

Disease	Gene/transgene expression	Drug	Drug target/MOA	Protection
Myopathies				
DMD (Duchenne muscular dystrophy)	Mutant dystrophin-1	Methazolamide and dichlorphenamide[28]	Inhibition of carbonic anhydrase	Protection against muscle degeneration
Spinal muscular atrophy	Mutation in *smn-1* gene[79]		Muscle degeneration and reduced lifespan	
Cancer				
RAS-dependent cancer	RAS-gain of function mutation	Geranylgeranyltransferase inhibitors[1]	Multivulva phenotype	
Ageing		Resveratrol[60,73]	Calorie restriction pathway!	Extend lifespan
		Methiopin and mianserin[72]		
		Reserpine[64]		Delays ageing and extends lifespan
		Lithium[63]		
		Ethosuximide[71] and trimethadione		
Neurodegenerative diseases				
Alzheimer's disease	P*unc54* :: Aβ/constitutive *myo-3* :: Aβ_{1-42}-inducible	*Ginkgo biloba* extract[37]	Reduction in oligomers	Delay in paralysis
		Soy isoflavone glycitein[49]	Antioxidant	
	P*unc-54* :: Aβ_{1-42}/ constitutive muscle	Reserpine[50]	Independent of decrease in toxic Aβ transcript and protein levels	
Parkinson's disease	P*dat-1*: GFP; dopaminergic neurons	Acetaminophen[43]		DA neuroprotection
	P*dat-1*: GFP; dopaminergic neurons	Bromocriptine[45] Quinpirole[45]	(DA D2 receptor agonists) receptor-independent mechanism	Neuroprotection
	MPP^+ (840 µM) treated *C. elegans*	Lisuride, apomorphine and Rottlerin[46]	Dopamine receptor agonists; protein kinase C inhibitor	Reduced lethality, amelioration of behavioural defects
Huntington's disease	*pha-1*; *rtIs11* [Htn-Q150]	Trichostatin A (TSA)[52]	Class I and Class II HDAC inhibitor	Neuroprotection
	P*mec-3* :: htt57Q128 :: GFP; touch receptor neurons	Resveratrol[54]	Sirtuin activator, *sir-2.1* and *daf-16*-dependent; does not alter 128Q transgene expression	Rescue of neuronal dysfunction, i.e. rescue of defective posterior (tail) mechanosensation
	osm-10 :: Htn-Q150/neurons	LiCl and mithramycin[53]	Independent of DAF-16 activity (insulin signalling)	Neuroprotection
Spastic paraplegia	*NIPA-1* mutation[80]		Neurodegeneration and progressive paralysis	
Infectious diseases				
Helminths/parasitic infection		Immunophilins[81]	Anti-parasitic; host–pathogen interaction	
Bacterial infection		Twenty-eight new compounds[23]		Protection against *Enterococcus faecalis*
Fungal infection		Caffeic acid phenethyl ester and enoxacin[24]		Protection against *Candida albicans*

The various promoters (P) to drive the mutant protein in the specific tissue (muscle – *unc-54* and *myo-3*; mechanosensory neurons – *mec-3*; dopaminergic neurons – *dat-1*; sensory neurons – *osm-10*) are mentioned along with the transgene.

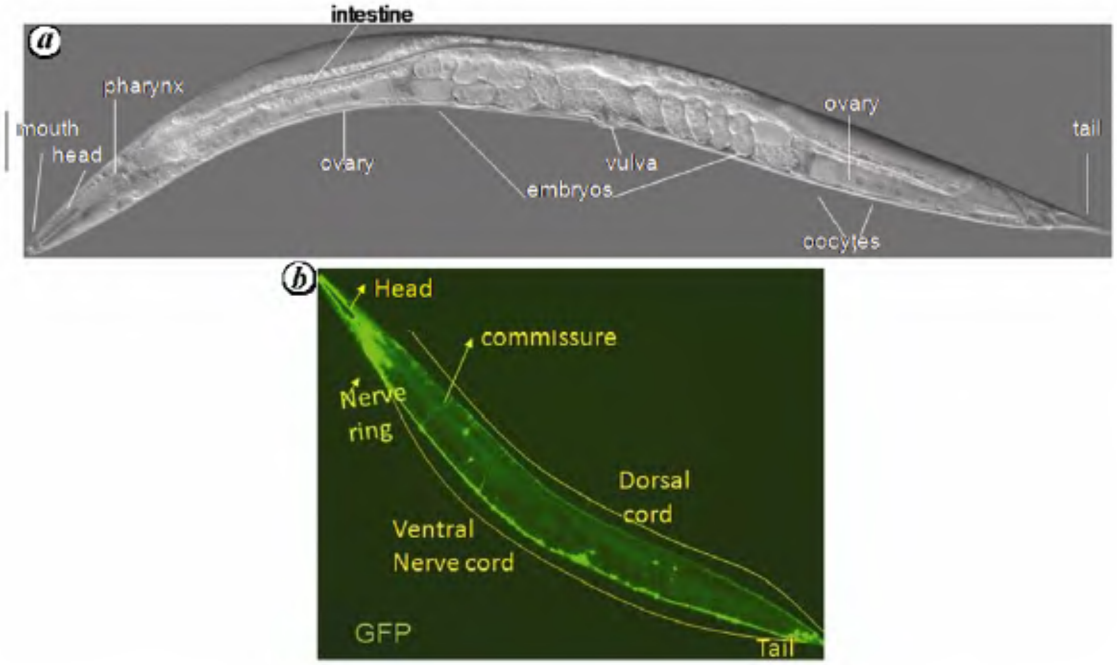

Figure 1. *Caenorhabditis elegans* – the miniature systems. ***a***, Digestive system (mouth, pharynx and intestine) and reproductive system (oocytes, ovary and embryo). ***b***, Nervous system marked by green fluorescent protein (GFP) expression (driven by *unc-18* promoter) in the motor neurons.

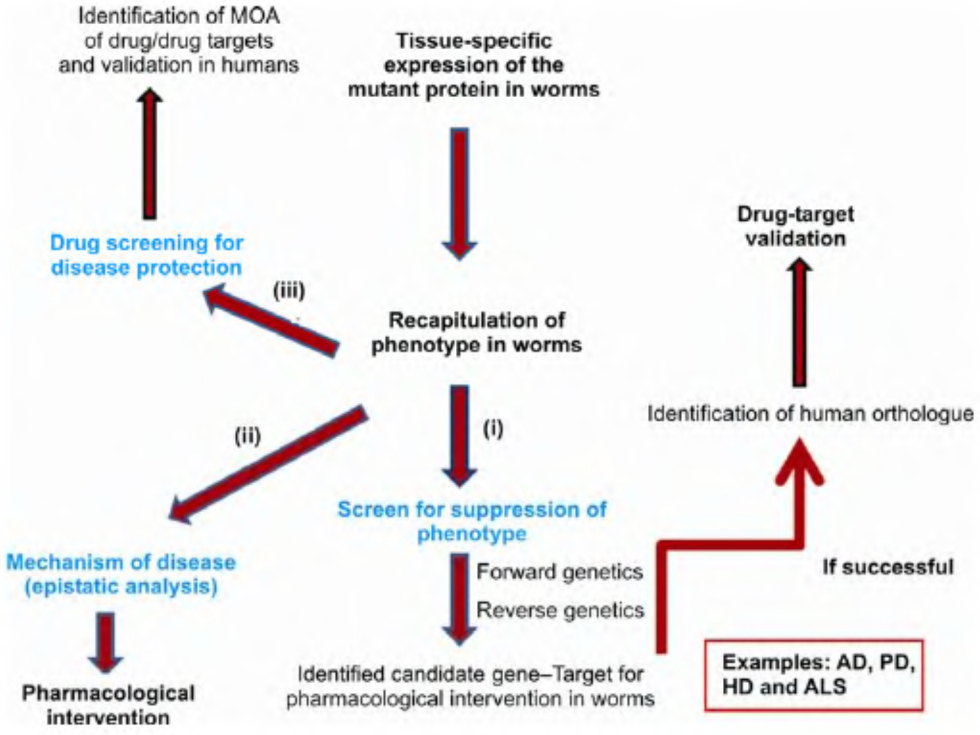

Figure 2. *C. elegans* disease models can be used (i) to identify suppressors of disease phenotype; (ii) to understand disease mechanism and (iii) for drug screening.

over a period of time, which could run into several days. The whole system can be semi-automated and the COPAS–BIOSORT system could be used for screening thousands of worms in minutes for the above-mentioned parameters. Some well-known high throughput drug screens are for antibacterials, antifungals, longevity enhancers, cancer and NDs. In addition, HTS are used for toxicity screening. For example, in a collaborative US

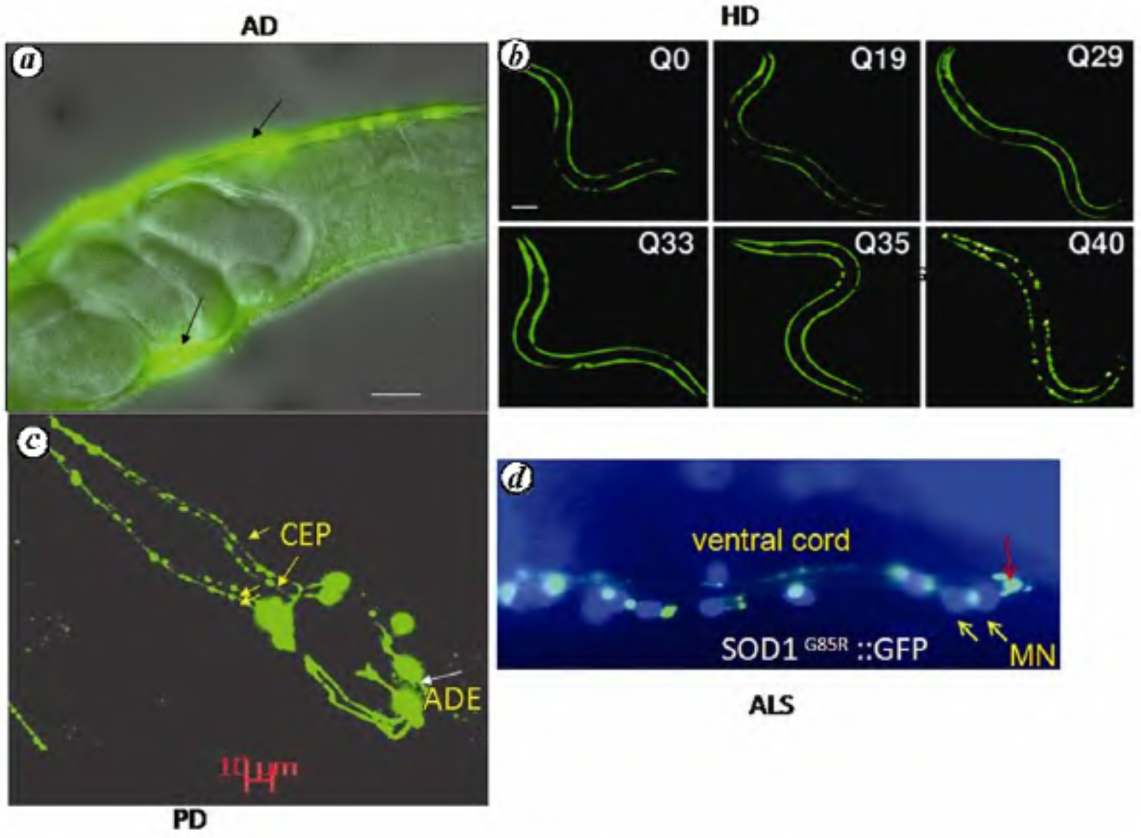

Figure 3. Different neurodegenerative disease models. ***a***, AD – Alzheimer's disease model. Aβ accumulation is shown in green by immunohistochemistry. Reproduced from Arya *et al.*[50] (copyright Elsevier). ***b***, HD – Huntington's disease. PolyQ length-dependent aggregation (copyright National Academy of Sciences, USA). ***c***, PD – Parkinson's disease. Dopaminergic neurons, CEP and ADE expressing α-synuclein, marked with GFP. The CEP neuronal processes are indicated by arrows. ***d***, ALS – Amyotrophic lateral sclerosis. Mutant SOD1 :: GFP accumulation (green) in the motor neuron cell bodies of the ventral cord in *C. elegans*. The nuclei are in blue (DAPI staining).

NIH toxicology research programme of US environmental protection agency and NIH Chemical Genomics Center, Boyd *et al.*[8] have screened thousands of neurotoxicants in two libraries by medium and high-throughput screening using COPAS–BIOSORT (in some of them), to determine the toxic effects in feeding, growth, reproduction and locomotion in worms[8].

Diseases modelled and drugs identified/validated in *C. elegans*

The last 15 years have seen considerable increase in the number of disease models developed in *C. elegans*. The various diseases that are modelled in *C. elegans* are given in Table 3. Right now, several *C. elegans* disease models have advanced to the stage of drug validation (Table 3) from identification of drug targets with the RNAi library screening and microarray analysis[2,3]. There are few reports of screening of large library of compounds to identify novel drugs in *C. elegans*. The strategies are depicted in Figure 3. Once potential drugs are identified in worms, they can be validated in the mammalian models.

C. elegans for infectious disease drug discovery

Bacterial infection: Several pathogenic bacteria (PB) could be evaluated in *C. elegans*. *Pseudomonas aeruginosa* was the first PB demonstrated to infect and kill *C. elegans*. Later several broad (*Serratia marcescens*, many *Burkholderia* sp. and *entomo-* and phytopathogens) and narrow host range PBs like *Salmonella enterica*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pneumoniae* were identified to kill the worms[21]. *C. elegans* has been used to identify drugs by HTS against the pathogen and then to study innate immunity[20], host–pathogen interaction, and virulence factors and host modifiers[21,22]. In an automated HTS, ~35,000 compounds were screened and 28 new compounds showing antibacterial effects were found[23]. In addition, these compounds did not inhibit the pathogen growth *in vitro*, but cured infection in the worms making them a new class of potential drugs different from antibiotics[23].

Antifungal drug identification: Systemic fungal infection by *Candida albicans* is a common and serious condition.

Other than bacteria, fungi can also be a food source for *C. elegans*. The pathogenic fungi can cause infection of the intestine and kill *C. elegans,* thus making the latter a good model for antifungal drug discovery. Screening of a known bioactive compound library (~1500) identified caffeic acid phenethyl ester, a major active compound of honey-bee propolis, and the fluoroquinolone agent, enoxacin[24], to have antifungal activity. The antifungal activity of these two compounds was further confirmed in the murine model. This validates *C. elegans* as a good model for novel antifungal drug discovery.

Myopathies

In Duchenne muscular dystrophy both the striated muscle and the cardiac muscle degenerate. This is caused due to mutations in the gene, dystrophin. Similarly, lack of dystrophin causes skeletal muscle degeneration in vertebrates. *C. elegans* has the homologue of dystrophin (*dys-1*) and has striated muscles. Loss of function, *dys-1* mutant, shows muscle degeneration. But this is of low penetrance. Hence, a weak mutation in the CemyoD/*hlh-1* (transcription factor required for muscle development) was combined with *dys-1*. In this, 30% of the muscles showed degeneration. This dystrophin-dependent muscle degenerating (DMD) model was screened with 100 s of bioactive compounds. The neurohormone, serotonin, was highly protective against muscle degeneration. In fact, serotonin was more protective than the palliative drug, prednisone, a steroid analogue[25] in this model[26]. In addition, carbonic anhydrase inhibitors, methazolamide and dichlorphenamide[27], which are currently used in human therapy showed the best protection.

Obesity

Obesity and its associated diseases like heart disease, diabetes and hypertension are major chronic health problems of this century. *C. elegans* helps in the understanding of the mechanism of fat accumulation[28]. In *C. elegans,* the fat is stored as droplets in the intestine instead of dedicated adipocytes or liver. The fat droplets are readily visualized because of *C. elegans* transparency either by Sudan black or vital fluorescent BODIPY staining. With this, one can readily assay for fat storage. The fat breakdown pathway is relatively well-conserved in *C. elegans*. Ciliated sensory neurons are essential for proper signalling of fat metabolism in *C. elegans*. In the mammalian system, hypothalamus controls fat storage. Interestingly, the gene mutation, tubby, in the mammalian system shows increased fat storage. The *C. elegans* homologue also does the same[29]. The fat metabolic pathway is well-conserved between *C. elegans* and humans, and details of the fat metabolic pathway enzymes are discussed in Ashrafi[30]. Thus, *C. elegans* is useful in understanding and possibly identifying therapeutics against obesity.

Neurodegenerative disease models in C. elegans

Neurodegenerative disease (ND) condition is characterized by progressive and gradual loss of neurons from affected areas in the brain, that is generally accompanied by varied symptoms and is often fatal. There are a number of well-known human NDs, including Alzheimer's (AD), Parkinson's (PD), Huntington's (HD), amyotrophic lateral sclerosis (ALS), etc., collectively referred to as protein-misfolding diseases[31]. Despite being severe and fatal, there is no cure for these diseases, and the available drugs provide palliative treatment and minimal protection. In addition, the mechanism of disease development is also poorly understood in this case. Animal models of NDs in mouse, worm, fly, etc. aid in drug discovery and deciphering the incompletely understood disease mechanism[32,33].

The genetically tractable *C. elegans* model aids in the study of NDs[33,34]. The basic, cellular, molecular and pathological aspects of these diseases can be fairly recapitulated *in vivo*[33,34]. Furthermore, though *C. elegans* contains only 302 neurons, they belong to 118 classes and the neural network has been mapped[35]. Several human NDs have been modelled in *C. elegans* (Figure 3). These models have been used to understand the disease mechanism, identify potential drug targets and also in drug screening applications.

The well-characterized *C. elegans* model of AD expresses the toxic human Aβ–amyloid beta (1–42) constitutively in the body wall muscles under the control of *unc-54* promoter[32] (Figure 3 *a*). The human Aβ expression leads to the manifestation of several pathological features, including progressive paralysis and intracellular muscle-specific Aβ deposits[32]. In another transgenic inducible line, toxic human Aβ has been expressed under the control of *myo-3* promoter and the expression induced after temperature upshift from 16 to 23°C (ref. 32). A direct correlation between Aβ expression and rate of paralysis has been reported for this line[36,37]. Further, this inducible line showed precedence of oxidative stress to Aβ deposition and manifestation of paralysis independent of Aβ deposition[36]. In addition, neuronal expression of Aβ in *C. elegans* causes hypersensitivity to exogenous serotonin[37].

Several *C. elegans* models of polyglutamine/HD which express polyQ have been independently developed. These models show polyQ length (PQL)-dependent toxicity in the worms. In one model, muscle-specific expression causes POLYQ :: YFP aggregation and toxicity (progressive paralysis) at a pathogenic threshold of 35–40 CAG repeats, but not lesser repeat length[38] (Figure 3 *b*). Another model expresses N-terminal 171 amino acid fragment of human huntingtin with varying PQL in the

neurons under the control of *osm-10* gene promoter[39]. In yet another *C. elegans* model of HD, transgenic worms express exon1-like N-terminal fragment of huntingtin in the touch receptor neurons under the control of *mec-3* promoter and show polyQ-mediated defects in mechanosensation and accumulation of polyQ fusion protein[40,41].

PD is also modelled in *C. elegans*. Dopaminergic neuron loss (accompanied by locomotor defects) was exhibited when WT/A53T mutant α-synuclein was expressed in motor neurons or in pan-neuronal manner in the worms[42]. One of the well-characterized and established *C. elegans* PD models coexpresses wild type α-synuclein and GFP under the control of individual *dat-1* promoters (P*dat-1* :: GFP and P*dat-1* :: α-synuclein; Figure 3 *c*), which leads to age-dependent neurodegeneration (~ 1 week), loss of cell body, processes and blebbing in six of the dopaminergic neurons (two ADE – anterior deirid and four CEP – cephalic) in *C. elegans*[43,44]. There are two toxin-induced PD models in which the eight dopaminergic neurons of *C. elegans* degenerate upon exposure to 6-OHDA[45] (needs dopamine transporter, DAT) or $MPTP^+$ (ref. 46).

We have developed a model for ALS by the expression of ALS-causing mutant $SOD1^{G85R}$ :: GFP in the motor neurons driven by *unc-18* promoter. Here, we notice age-dependent increase in mutant SOD1 :: GFP aggregates (Figure 3 *d*, unpublished results). Interestingly, these aggregates did not lead to any phenotype demonstrating the complexity of ALS disease development. Wang *et al.*[47] have expressed the mutant $SOD1^{G85R}$ :: YFP in all the neurons and these worms showed locomotion defects. They reported that overall synaptic vesicle number and neurotransmitter release was low in these worms.

Drug discovery against NDs: *C. elegans* models of NDs have been extensively subjected to HTS. The pharmaceutical company, NemaRx Pharmaceuticals which focuses on finding drugs for NDs has used the worm model to test drugs against important NDs such as AD[38]. Parker *et al.*[40] have reported the identification of pharmacological suppressors of neuronal dysfunction in HD worms. In this study, they screened a collection of FDA-approved compounds. Several such studies reinforce the promising use of *C. elegans* for drug discovery[48] against different NDs (Table 3).

The well-characterized AD model has aided in the identification and validation of potential therapeutic interventions for AD such as *Ginkgo biloba* EGb 761 extract[37], soy isoflavone glycitein[49] and reserpine[50]. These compounds not only delayed the toxic human Aβ-mediated paralysis, but also conferred considerable lifespan extension and stress tolerance. It is to be noted that though the neuroprotective effects of *G. biloba* EGb 761 extract were already known, the efficacy of this extract in relation to reduction of toxic Aβ oligomerization was shown *in vivo* for the first time, which correlated fairly well with the reduction of paralysis observed in Aβ-expressing animals[37].

Studies that focused on understanding the mode of action of *G. biloba*-mediated beneficial effects in *C. elegans* have shown that: (i) in addition to reduction of Aβ oligomeric species, expression of *Phsp16.2* :: GFP is also suppressed by *G. biloba* EGb 761 extract, suggesting that EGb 761 decreases cellular stress[51]; (ii) EGb 761 extract can reduce intracellular H_2O_2 levels in worms[37]; and (iii) EGb 761 extract inhibits amyloid deposit formation in AD worms[37]. Glycitein could provide protection against AD and can reduce H_2O_2 levels in worms, thus having an antioxidative effect[49]. On the other hand, the protective effects of the well-known biogenic amine downregulator and FDA-approved antihypertensive drug, reserpine (Figure 4 *c*), in Aβ-expressing animals, were found to be independent of modulation of toxic Aβ levels, as the Aβ mRNA and *in vivo* protein deposits were found to remain unaffected upon reserpine treatment[50]. Further, reserpine did not even alter the exogenous serotonin sensitivity of the neuronal Aβ worms[50]. Mode of action (MOA) studies with these drugs suggest multiple mechanisms to reduce Aβ toxicity.

The PD model has aided in the discovery and validation of bromocriptine, quinpirole (DA, D2 receptor agonists), lisuride, apomorphine, rottlerin and acetaminophen as potential pharmacological interventions for PD[45,46]. These compounds have been shown to be neuroprotective in the *C. elegans* model of PD. Braungart *et al.*[46] did a HTS with the *C. elegans* $MPTP^+$ model of PD and found that several anti-PD drugs can ameliorate $MPTP^+$-induced mobility defects in this model. Notably, lisuride (dopamine receptor agonist), apomorphine (dopamine receptor agonist) and rottlerin (protein kinase C inhibitor) were found to be considerably useful in the amelioration of behavioural defects. In addition, nomifensine (dopamine transporter inhibitor), nicotine (nACh receptor antagonist), selegiline (MAO-B inhibitor), MPEP (mGluR-5 inhibitor), amantadine, α-lipoic acid (antioxidant) and ascorbic acid were useful at higher concentrations in the amelioration of behavioural defects. In another screen, the mammalian D2R agonists, quinpirole and bromocriptine were identified to confer significant neuroprotection independent of DA receptors in 6-OHDA-induced dopaminergic neurodegeneration model of PD in *C. elegans*[45]. Similarly, Locke *et al.*[43] reported that acetaminophen can ameliorate dopaminergic neurodegeneration in *C. elegans*. Low concentration of acetaminophen was found to significantly protect dopaminergic neurodegeneration against 6-OHDA toxicity in P*dat1* :: GFP expressing worms.

In polyQ expressing HD model worms, resveratrol, trichostatin A (TSA), lithium chloride (LiCl) and mithramycin provide considerable neuroprotection and effectively alleviate neuronal dysfunction[40,52,53]. Voisine *et al.*[53] had screened candidate pharmacological com-

pounds in the HD model. In *pqe-1* genetic mutant background which enhances polyQ-mediated toxicity, both LiCl (inhibitor of GSKβ) and mithramycin alleviated neuronal cell death. The MOA of LiCl and mithramycin was found to be independent of DAF-16 activity[53]. Resveratrol rescued neuronal dysfunction in Htt57-Q128 expressing lines in a *daf-16*-dependent manner[54]. In addition, resveratrol action was dependent on Sir-2.1 activity, a *C. elegans* sirtuin family member[54]. Likewise, TSA, a class I and class II histone deacetylase (HDAC) inhibitor, provided significant neuroprotection in HD models[52]. HDAC inhibitors are known to provide protection against polyQ-mediated toxicity in vertebrate and Drosophila neurons[2]. The finding that TSA can confer neuroprotection against polyQ-mediated toxicity in *C. elegans* validates and strengthens the previous results[53].

Recently, a compound, psammaplysene A (PA), that increases nuclear localization of FOXO3a, a mammalian homologue of DAF-16 has been added to the list as a potential compound with neuroprotective effects against motor neuron disease[55]. Treatment of the worms with PA prevents neuronal cell death in a DAF-16-dependent manner.

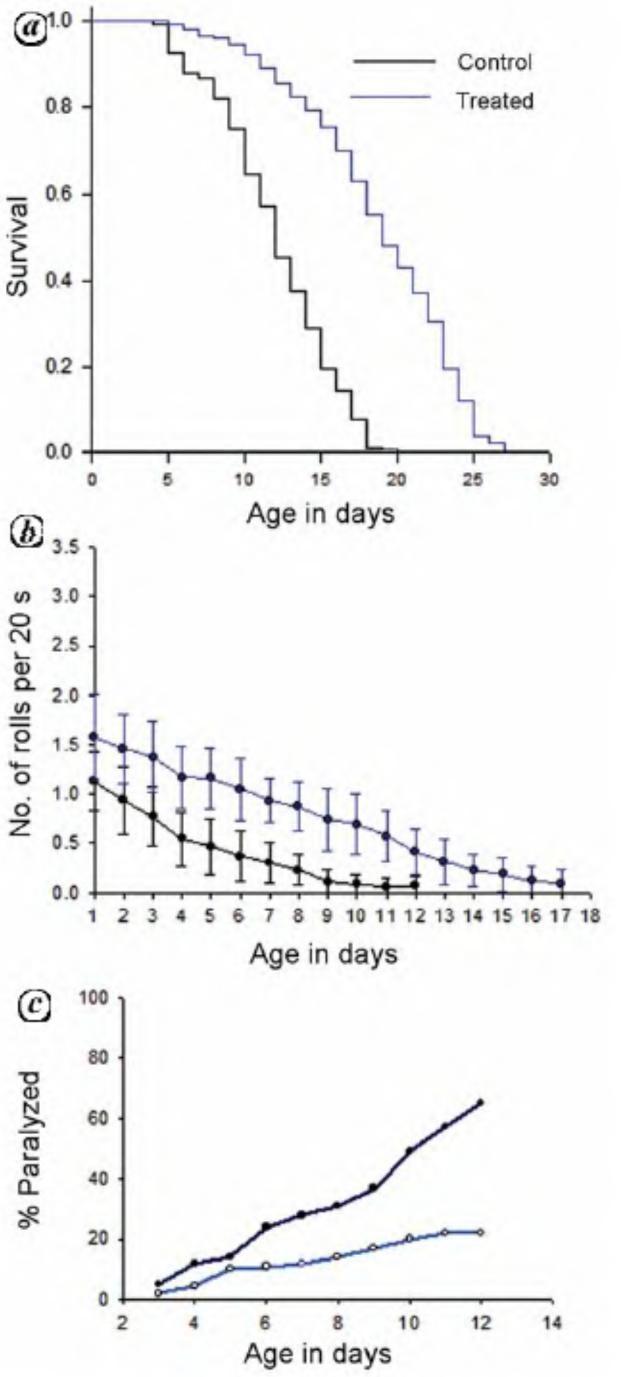

Figure 4. ***a***, Reserpine extends *C. elegans*, lifespan; ***b***, Improves and prolongs locomotion till late age; ***c***, Delays Aβ-proteotoxicity-induced paralysis in the AD model worms. Reproduced from Arya *et al.*[50] (copyright Elsevier).

Cancer

Three companies, Exelixis, Devgen and NemaRx, had used *C. elegans* extensively for drug discovery. Bristol Meyers Squibbs in collaboration with Exelixis screened for anticancer drugs and found farensyl transferase inhibitors to reverse the *ras* (the common oncogene involved in many cancers)-dependent gain of function phenotype (multivulva formation) and activate the pro-apoptotic pathway[2]. They narrowed down further to geranylgeranyl transferase inhibitors. Thus, *C. elegans* can be a good model for identifying drugs against RAS-dependent cancers.

C. elegans for pharmacodynamic studies

Once administered, all the drugs get metabolized by the detoxification system, namely the big superfamily of enzymes called cytochrome P_{450} (CYP_{450}). DevGen in collaboration with academia has characterized the CYP_{450} superfamily in *C. elegans* and shown that the *C. elegans* CYP_{450}s do respond to xenobiotics[56]. When the expression of a specific CYP_{450} is induced by the drug, the drug gets cleared from the system faster, which drastically reduces the half-life of the drug. The CYP_{450} CYP3A4 is the most highly induced CYP in humans and many drugs could bring about its induction. Chakrapani *et al.*[9] showed that *C. elegans* indeed has the homologs of the highly induced human CYPs and they in turn can be induced by the same drugs that induce the human CYPs using CYP promoter and GFP reporter expression induction assays. They have screened close to 20 xenobiotics and shown the specificity of the CYPs. Hence, *C. elegans* $Pcyp_{450}$::GFP expression induction could be used for xenobiotic screening and then validated in the mammalian systems.

C. elegans for toxicity studies

There is a huge initiative to develop *C. elegans* into an alternative animal model for toxicity screening. As described earlier, a high-throughput automated *C. elegans* drug toxicity screen has been already developed. The authors have shown that *C. elegans* growth could be a reliable parameter for high-throughput assay screening for toxicological purposes[8].

Modulation of one pathway (delay in ageing) protects against many diseases

A new paradigm or awareness against prevention of many diseases has emerged from the *C. elegans* longevity studies. Though the fountain of youth has been long sought after, we had no clue whether longevity is a random event or genetically modulated. The first long-lived genetic mutants were identified in *C. elegans*[57]. Later, Cynthia Kenyon *et al.*[58] reported that a single gene mutation in the insulin/IGF-1 receptor, *daf-2*, can extend the *C. elegans* lifespan twofold, opening up the possibility of exploring the molecular mechanism of longevity. The reduced insulin signalling (RIS) pathway-mediated lifespan extension (LE) is conserved across species from worms to mice[59]. Similarly, calorie restriction (CR)[60] is another longevity pathway which was first identified in *C. elegans* and later proved true in other species as well. Contribution of mitochondrial function modulation (mutation in the demethoxyubiquinone hydroxylase gene *clk-1*) to LE was again shown in *C. elegans*[61]. Interestingly, these pathways act in tandem with other pathways like RIS and additively increase the lifespan up to five fold[61]. In addition, negative regulation of lifespan by germline was shown by the ablation of germline cells. Ablation of germ cells in the *daf-2* mutant made the longest lived worms[62]. Lithium shown to provide protection against HD[53] also extends *C. elegans* lifespan independent of RIS or CR pathways[63]. Srivastava *et al.*[64] have reported that reserpine could extend *C. elegans* LE. Reserpine seems to act by yet another unknown pathway of lifespan extension other than CR and RIS. Reserpine provided lifespan extension (Figure 4 *a*) with high quality of life, namely locomotion till late age (Figure 4 *b*) and stress tolerance.

Longevity-enhancing mutations suppress tumour growth

Pinkston *et al.*[65] showed that the long-lived mutants, *daf-2* and *clk-1*, could suppress tumour formation in *C. elegans*. In the *gld-1* mutants, extensive germline tumour was observed. In the background of the long-lived mutant, *daf-2,* the germline tumour was drastically suppressed in the *gld-1* mutant worms. This is a remarkable insight connecting longevity and cancer. The authors further showed that the positive regulator of lifespan, the FOXO transcription factor, *daf-16*, which is in the downstream and negatively regulated by the insulin signalling cascade, does this through induction of apoptotic machinery gene expression without affecting mitosis[65].

Long-lived mutants survive pathogenic bacteria

One of the most dreaded pathogenic infections is *Pseudomonas aeruginosa*. The laboratory-grown *C. elegans* diet is *E. coli*. When these bacteria were replaced with PB, the worms were infected and died. With a mutant screen one can obtain pathogen-resistant worms and identity the genes involved or virulent factors[66]. *C. elegans* has innate immunity and mounts a defence against PB. Interestingly, in addition to being stress-tolerant, the long-lived mutant, *daf-2*, could withstand the pathogenesis of PB[67].

Delay in ageing can alleviate NDs

Though long suspected, *C. elegans* provided the first evidence that delay in ageing could slow down NDs. First, it was demonstrated with the HD model that the long-lived insulin signalling pathway mutant, *age-1*, could delay the proteotoxicity of the polyQ-huntingtin in the HD model[68]. More recently, a similar effect was demonstrated for the AD model[58]. In the AD and HD models, involvement of two downstream positive regulators of longevity, *daf-16* and *hsf-1* in delaying the disease was clearly shown[69,70].

Identification of longevity-enhancing drugs with C. elegans

Screening of currently existing drugs has led to the identification of ethosuximide[71] – an anticonvulsive drug, and lithium chloride[53], and a large screen of 33,000 active compounds identified two antidepression drugs, methiopin and mianserin, to extend lifespan in *C. elegans*[72]. In addition, there are some naturally occurring small molecules in the food we take, which can extend lifespan in *C. elegans*. They are resveratrol[60,73] – initially identified in red wine, polyphenols from blueberry[74] and the supplement, *G. biloba* extract[38,75]. Reserpine, a plant alkaloid and antihypertensive drug, known to act through down-regulation of biogenic amine neurotransmitters could extend *C. elegans* lifespan with high quality of life. Reserpine showed around 35% lifespan extension. This enhanced longevity was even more remarkable (~65%) when the treatment was started from young adult onwards[66]. In addition, reserpine rendered the worms stress-tolerant.

Drugs that enhance longevity alleviate NDs

Reserpine could extend *C. elegans* lifespan[64] and delay the proteotoxicity effect in the AD worms[50] (Figure 4 *a*). In the well-established AD worms, where Aβ toxicity was manifested as progressive paralysis, reserpine provided significant protection (Figure 4 *c*). In addition, the lifespan of AD worms was also extended. Thus, *C. elegans* has provided a platform to identify both a novel function for the already existing drug and a potential candidate drug for NDs in general, and AD in particular, from the existing repertoire of drugs. Studies have shown that the *G. biloba* extract can extend lifespan and alleviate AD in *C. elegans*[38,75]. Though resveratrol and LiCl showed protection in the HD model of *C. elegans*[75], like reserpine, only LiCl can cross the blood–brain barrier in the higher animals, including humans. Reserpine needs to be evaluated in mammalian models for protection of NDs similar to *G. biloba* extract and resveratrol.

Summary

C. elegans has already entered into the drug discovery process. Several disease models are available. *C. elegans* has moved forward from the drug target identification stage to drug validation stage. Though there are the limitations – (i) some genes not present in *C. elegans*; (ii) absence of specific organs like the eye, ear and bone and cardiovascular system, where there are homologies, *C. elegans* is a versatile platform. For example, as discussed above, brain-related NDs can be modelled in *C. elegans*; they have given considerable insights and several drugs have been validated. Moreover, the *C. elegans* models will considerably reduce the cost of drug screening and the time duration to carry them out in animal models. *C. elegans* is far superior to the *in vitro* mammalian cell culture models which are generally used for primary screening, because it is a multicellular organism with defined systems which provides physiological relevance. In antibacterial and antifungal drug identification, *C. elegans* has contributed to the discovery of novel drugs which need to be validated in humans. Hence, despite the shortcomings, where applicable, *C. elegans* can be readily used for primary drug screening.

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