

Review

A novel model organism in the field of medicine: Nematode *Caenorhabditis elegans*

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Abstract

Caenorhabditis elegans free-living, soil nematode has been an extensively important system for investigating various aspects in life science including medicinal sciences. This round worm is richly contributed to our understanding the neurodegenerative diseases, aging pattern, analysis of toxicants. It is the organism transited from research toll as testing toll in many aspects. It is a future model organism in the field of medicine. The utility of the *C.elegans* resides in two resources: its small genome which resembles more or less human genome for various diseases, and its powerful molecular tools as a model system.

Keyword: *C.elegans*, nematode, diseases, model system.

INTRODUCTION

Today, most of scientific investigations depend on the model systems or organisms. These systems or organisms have been made man to remove the word "Impossible" from his Dictionary of Science. Over few decades so many model systems were explored to answer some of the complicated questions. The *Caenorhabditis elegans* is one among them which was established by Sydney Brenner (1963) at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England. In the letter to Max Perutz, The head of the laboratory, Brenner quoted that "it is widely realized that nearly all the 'Classical' problems of molecular biology have either been solved or will be solved in the next decade" and that the future of molecular biology lies in the extension of research to other fields of biology such as embryology and developmental and nervous system (Brenner, 1988). Presently, *Caenorhabditis elegans* is a likely one of the common used metazoans in terms of its cell biology genetics, embryology, toxicology, and behavior.

Systematic Classification of *Caenorhabditis elegans*

Kingdom: Animalia
Phylum: Nematoda
Class: Secernentea
Order: Rhabditida
Family: Rhabditidae
Genus: *Caenorhabditis*
Species: *elegans*

C.elegans being simple, unsegmented, multicellular and soil nematode organism, belongs to member of the phylum Nematoda. This nematode *C.elegans* occurs in two sexes: 1. males and 2. Self-fertilizing hermaphrodites. Adult hermaphrodites have 959 somatic cells and males with 1031 cells (Sulston et al., 1983). This difference is due to the extra structure found in the male which is associated with mating and also presence of the tail muscles, and hypodermal cells (Sulston et al., 1983). Sex in *C. elegans* is based on an XO determination system and they have five pairs of somatic chromosomes and one pair of sex chromosomes. Hermaphrodite *C. elegans* has XX system and male has XO system. The sperm of *C. elegans* is ameboid. Even though there are a limited

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number of cells, the body is differentiated into digestive system, reproductive system muscular and nervous system. (Riddle, 1998). *C. elegans* has a short life span (3 weeks at 22°C under optimal conditions), small size (1mm in length) easy to cultivate, and quick generation time, commonly *C. elegans* grown in the lawn of the bacteria (*Escherichia coli*) on the petri dishes or liquid media called S-media, self fertilization ability to frozen, measurable behavior genetic tractability and relevance to mammalian toxicology due to the high degree of conservation of gene sequence with small genome. The genome of the nematode *C. elegans* was the first animal genome (multicellular) sequenced in 1998. Moreover this worm is fully transparent one can visualize all 959 cells using sophisticated microscopy techniques. *C. elegans* develops from a fertilized egg to a gravid (adult) with in 3days at 20°C (Hali and Altun, 2008; Wood, 1998). Each hermaphrodite produces around 300 offspring. These offspring mature through four larval stages. L1-L4, growing in spurts between stages after molting old cuticles (Byerly et al., 1976; Knight et al, 2002). At two points in the life cycle if food is not available, Nematode growth will arrest. Animals will survive for several days to several months. When it is in the starved condition the larvae is called Dauer larvae (Lewis, 1995; Riddle, 1988).

Recently, with available microscopy techniques helps *C. elegans* to study each cells, cell lineages and fate during the developmental stage along with the database on Anatomy of this worm (Sulston et al., 1983 Hall and Altun, 2008;). This database maps the development of every cell from the fertilized egg to the adult (Sulston et al, 1983) and neuronal pathways and connections (White, 1986). The availability of the complete gene sequence (20,000 genes) in the database led to develop the transgenic nematodes (Mello and Fire, 1995). The "Mind of the worm" authorized by White et al., 1988 describes the detail map of complete *C. elegans* nervous system, physical location of all of the hermaphrodite neurons and all neuronal connections (Neuron–Neuron and neuromuscular). The adult hermaphrodite contains 302 neurons in two independent nervous systems: 20 neurons in pharyngeal nervous system and 282 neurons in somatic nervous system (Sulston et al., 1983; Sulston et al., 1977). There are approximately more than 6000 chemical synapses, 900 gap junctions and 1500 neuromuscular junctions (Haliland, 2008). These above structures control locomotion, feeding, defecation, reproduction and environmental sensing. *C. elegans* has the ability to sense differences in temperature, chemicals, odorants and food. The male contains 473 neurons there descriptions of the neuroanatomy can be found in (Hall and Altun, 2008, White et al., 1988). The most important aspects of *C. elegans* as a model

organism are the high degree of evolutionary conservation in the form of the gene sequence. Along with evolutionary conservation in basic biological functions, Such as DNA, RNA and protein synthesis; orthologs for many of the proteins and regulatory pathways induced as part of the stress respons in vertebrates have been identified in the *C. elegans*. According to Culetto and Sattelle 2000, O'Kane 2003 *C. elegans* suffer from many diseases which are similar to human ones, including cancer, neurodegeneration, infectious diseases and disorders of physiological control, and ageing, but their organic physiology differs from ours in many respects

Preparation of nematode cultures

The worms were cultivated on NGM plates (3 g l⁻¹ NaCl, 2.5 g l⁻¹ proteose peptone, 5 mg l⁻¹ cholestrol, 1 mmol l⁻¹ CaCl₂, 1 mmol l⁻¹ MgSo₄, 25mmol l⁻¹ Potassium phosphate, P^H 6.0, 17g l⁻¹ agar) on an established lawn of *Escherichia coli* strain OP50 (Brenner, 1974) and maintained at 20°C. To obtain synchronized culture, gravid hermaphrodites were lysed in an alkaline hypochlorite solution (Sultson *et al.*, 1988) and the eggs were transferred in fresh NGM plates. The culture was grown for 3 days at 20°C until the newly hatched worms reached adulthood. The L₄ stage worms/gravid worms were washed with K-medium (53mM NaCl, 32mM KCl) (Williams and Dusenbery, 1990), peleted by centrifugation (3000g, 5 min), washed again thrice with cold K-medium and finally suspended in K-medium to obtain 30-50 nematodes per 10 µl.

Role of *C. elegans* in Neuropharmacology

C. elegans, provides more advantages for the identification and characterization of genes required for the function of the nervous system. there are many neurons express specific neurotransmitters (Rand and Nonet, 1997) and associated receptors which are similar to higher eukaryotes including human. These include dopamine, acetylcoline, γ-aminobutyric acid (GABA), glutamate and serotonin, In addition, to this there are several neuropeptides encoding genes have been discovered which code for Insulin- like and FMRF amide (Phe-Met-Phe-NH₂) like neuropeptides. The Tools such robotics, image acquisition, electrophysiological studies in *C. elegans* have made easy to identify the rhythmic contractions of the worm pharynx during feeding (Windy et al., 2009). The electropharyngeograms (EPGs) measure the extracellular current flow in the muscle transmembrane potential during the contractions. The screening the mutants, ion channels and gap junctions have been

characterized by the EPG recordings. The Patch-clamping methods enable the highest resolution studies of the electrical activity of neurons and muscles this method is very useful to study the electrical properties of the small neurons. According to the Goodman et al (1998) Worms are glued in place and small incisions is made in the cuticle allowing a group of the neurons to emerge. With the aid of a green fluorescent protein marker, Individual neurons in this group can be easily identified for recording. This techniques are highly modified in terms of the touch receptor neurons (Hagan et al., 2005) inter-neurons of the locomotory control circuit (Brockie, 2001). From the electrophysiological studies one can characterized synaptic currents, analysis of Ach receptors, and analysis of neurotransmitter release at the neuromuscular junction.

In future the variety of postsynaptic receptor subtypes that need to be isolated using the pharmacological agents. According to the Kevin Strange in his book "*C.elegans* methods and application" Specific drugs such as agonists and antagonists can be used to acutely perturb synaptic function in a controlled manner such that the functional roles of specific neuronal inputs and specific neurotransmitter receptors can be explored. The well defined nervous system, evolutionary conservation in neuronal structure and function, and the ability to utilize medium and high throughput technologies make *C.elegans* excellent candidate for the rapid detection of the many drugs in the field of the medicine.

***C.elegans* as Toxicological model**

The European center for the validation of alternative methods as decided and accepted to use *C.elegans* as alternative method (Festing et al., 1998) which reduce or replace the use of other laboratory animals. for this purpose so many laboratory use this round worm for study the impact which are manmade and natural occurring toxins and chemicals. Toxicology is one of the important aspects in the field of the medical sciences. The easy culturing, well studied biology, short developmental time, large brood size makes the round worm *C.elegans* has a superb model organisms for toxicological and pathogenic studies. These above few lines allow large numbers of animals to be studied in a cheaper condition and gain fast results. This animal has proven itself good model system for biology which is relevant to higher animals in some of the core subject as cell death, neuroscience, and embryology. From the above points toxicological science results in *C.elegans* are likely relevant to higher animals. For example, there are so many investigations are performed on *C.elegans* using the metals and organic compounds to study toxicity of lethal dose, brood size, body length and

movement (Peredney and Williams, 2000). With the knowledge of quantifiable parameters such as growth, development, reproduction, life span and behavior one can extensively use *C.elegans* to screen the effects and modes of action for a variety of pharmacological agents (Rand and Johnson, 1995) for instance, the antiparasitic drug ivermectin was compared in wild-type and resisitant *C.elegans* strains by use of a development inhibition assay as measure of toxicity (Dent et al., 2000).

C.elegans has been used in the study of interactions between the nematode and bacteria (13-15), which worm usually consumes. In some examples there is a clear infection process by the micro-organisms; due to the transparency of animal from this one can access the pathogenicity of the specific toxins. The toxicity of pesticides, insecticides, fungicides which are well studied as neurotoxicants is also analyzed using *C.elegans* (Cole et al., 2004, Jadhav and Rajini, 2008) along with this ecotoxicological studies also had been studied sing this animal model by Williams and Dusenbery, (1990). These toxicological studies are conducted to monitor physiological parameters such mortality (Donkin, 1995) movement (Anderson et al., 2001), feeding, growth (Boyd and Williams, 2003) lifespan and expression of the small heat shock proteins (Jadhav and Rajini, 2008) with help of the LC₅₀ of the particular chemicals and drugs. Moreover, *C.elegans* has been exposed to study the oxidative stress inducing the potential of several xenobiotics such as paraquat and juglone (Hartman et al., 1995; Wu et al., 2002). In *C.elegans*, the end points of movement rate, head thrash, body bend and forward turn to evaluate locomotion behaviours have already been successfully explored to assess neurotoxicity from the heavy metal (Anderson et al., 2001, 2004; Dhawan et al., 2000). The severe deficits in memory behaviors were recorded in nematodes when exposed to Pb and Al (Ye et al., 2008). The locomotion behavior test has been further explored for soil toxicity evaluation with the aid of transgenic nematodes (Graves et al., 2005). Some authors confirmed the severe deficits in the neuronal survival and synaptic function in the larvae of the *C.elegans* when exposed to lead and mercury (Xing et al., 2009). From above all experiments one can suggest that *C.elegans* is a excellent model organism in the field of toxicological science and pathology.

***C.elegans* and Bioinformatics**

The genome of *C.elegans* has become major platform in the field of bioinformatics, chemoinformatics with exploration of WormBase, WormCommunity and WormAtlas. These two communities are playing major crucial role in opening, collecting, data-mining and

Table 1. Online *C.elegans* databases and resources

Site	URL	Service provided
1. Genomics a.WormBase	http://www.wormbase.org/	Information on genetics, genomics, genome location, mutant and RNAi, phenotypes, expression pattern of <i>C.elegans</i>
2. Gene knockout a. The <i>C.elegans</i> gene knockout b.Japanese national Bioresources project	http://elegans.bcgsc.bc.ca http://shigen.lab.nig.ac.jp/c.elegans	knockout animals available through <i>C.elegans</i> center Provide knockout animals
3. Worm reagents a. <i>C.elegans</i> genetics center b. MRC genes services c. Add gene d. Sanger institute	http://biosci.umn.edu/CGC/homepage http://www.geneservice.co.uk/products/home http://www.addgene.org/pgvec1f=c&cmd=show http://www.sanger.ac.uk/projects/c.elegans	Collect, Maintain and distribute worm strains. Genome-wide RNAi library ORF clones. <i>C.elegans</i> vector kits Provide cosmid and YAC clones to investigations
4.Protein-Protein interactions a. Vidal lab INTERACTome project b. <i>C. elegans</i> Survival kits	http://vidal.dfci.harvard.edu/main_pages/interaction.html . http://members.tripod.com/C.elegans/C_elegans_introduction_value_to_Biology_medicine.html	Genome-Scale protein-protein interaction mapping Links to many useful sites for <i>C.elegans</i>

sharing of data of *C.elegans*. These above databases provides the mutant and transgenic worm strains, cosmid, yeast artificial chromosome clones and expressed sequence tags (EST) clones which are available freely from the databases. These data include the sequence and structure of the *C.elegans* and genetic maps reagents such as antibodies, polymorphism, and transgenes cellular data and complete lineage and connectivity maps. The worm community is the first community to use the internet for electronic data sharing. WormBase (<http://www.wormbase.org>) is a most powerful data sharing database which provides the most of catalog of worm including the biology, predication of genes, descriptions including genome location, mutant and RNAi phenotypes, expression pattern, microarray data, gene ontology, mutant alleles, and BLAST and FASTA matches. The WormBase is a total repository for the information on *C.elegans* and related nematodes. This has grown into an international consortium of researchers exploring methods of data analysis, warehousing and visualization in all contexts of the *C.elegans* as model organism.

The worm base consortium is composed of researchers

at four locations: 1. The California institute of technology in CA (Caltech); Genome sequencing center, Washington University in Washington; 3. Wellcome Trust Sanger Institute in Sanger; 4. Cold Spring Harbor Laboratory (CSHL). These institutes are split between the groups in analyzing, curating and presenting data. Washington University and Wellcome Trust Sanger Institute responsible for curating gene sequences The CSHL is in charge of user-interface development and management of the website and merging the local new release version of databases. This is very useful for the comparison and conversion of the sequence of *C.elegans*. The California institute of technology meant for retrieving the data from the published literature. Worm base also maintains a development server (<http://dev.wormbase.org>) where new features and datasets are tested before being made available on the primary site. The WormBase for every 3 week it is going to release new datasets and curator confirmed annotations. The new current version is generated in the homepage. Visit <http://www.wormbase.org/about/citing.html> for the most up-to-date information on citing Table 1.

WormBase.

The primary Wormbase Server	
http://www.wormbase.org/	
Mirror Sites: Caltech (CA)	
http://caltech.wormbase.org/	
Development server	
http://dev.wormbase.org/	
IMBB (Greece)	
http://imbb.wormbase.org/	
FTP site ftp://ftp.wormbase.org/	
Data mining	archive
http://www.wormbase.org/data_mining	
Installation	guide
http://www.wormbase.org/docs/INSTALL.html	
Data mining server	
http://www.aceserver.cshl.org	
Data submission, questions, help@wormbase.org	comments
Citing	WormBase
http://www.wormbase.org/about/citing.html	
Acceptable use policy	
http://www.wormbase.org/about/acceptable	

C.elegans in Biomedical Sciences

The genetical and molecular studies have provided mechanisms underlying the cell biology process from simple unicellular animals to multicellular. In every organism the genetic instructions for the regulations of programmed cell death have been remarkably conserved throughout the evolutionary process. In case of the *C.elegans*, programmed cell death is almost exclusively apoptotic. In *C.elegans* 131 cells destined to die during development, the level of EGL-1, a BH3 domain, is increased. EGL-1 this interact with the protein complex called CED-9 which is similar to the human B-cell Lymphoma protein 2 ; BCL-2 and CED-4 similar to the human apoptotic protease activating factor, CED-4 which in turn activates in turn activities CED-3 (equal to the human caspses) by Hengartner, 2000. In 1999 *C.elegans* was used as model for two proteins-polycystin-1 (PKD1) and polycystin-2 (PKD2)-that are defective in human polycystic kidney disease. This was demonstrated by Barr and Sternberg, 1999 showed that worm proteins related to human polycystins (i.e., homologues) mediate signaling in sensory neurons. Of course, in Barr's experiments, a gene mutation in the *PKD1* homologue didn't affect kidney function, because *C. elegans* don't have kidneys. Rather, this mutation affected a specific step in the mating behavior in male worms. In fact, in worms, the *PKD1* homologue is called *LOV1* (short for "location of vulva") to reflect its function. Along with this Barr also demonstrated that the worm homologue of polycystin-2

was expressed in the same neurons as the gene product of *LOV1*.

Other important aspect in *C.elegans* is 42% of the human disease genes have an orthologue in the genome of *C.elegans*. This includes some of the disease *such as* juvenile Parkinson's disease, hereditary non-polyposis colon cancer, Alzheimer disease, Spinal muscular atrophy etc (Calahorro, Ruiz-Rubio, 2011). The *C.elegans* worm model can be used to study human diseases, e.g. the human gene can be overexpressed in certain cells of *C. elegans* by means of muscle or neuron-specific promoters and the expression of genes at the RNA level in the resulting worm population can be studied by microarray analysis, which can reveal a batch of up and down-regulated genes. In simplified terms, those up or down-regulated genes that have a human homologue can provide a baseline for further studies aimed at identifying members of the signalling cascades activated in the disease and candidates for drug therapy. There are many ways to the study of human diseases is to inactivate the function of a *C.elegans* gene orthologue by double-stranded RNA interference (dsRNAi) is one among them. Attempts were made by Double-stranded RNA for the target gene is introduced into the worms either by microinjection or by soaking them in a liquid containing the target dsRNA or ingestion of bacterially expressed dsRNA (Fire *et al.* 1998, Tabara *et al.* 1998, and Timmons *et al.* 2001). There are instant of *C. elegans* which is related to human lung cancer this was noted by the work of Shahid S. Siddiqui *et al.*, 2008 where *C.elegans* used as a model organism for *in vivo* screening in cancer: effects of human c-Met in lung cancer affect *C. elegans* vulva phenotypes.

CONCLUSION

The *nematode C.elegans* has the longest history like any other model organism. This nematode is extensively used organism in various branch of biology and medicine. The powerful biological system of *C.elegans* is utilized to address fundamental questions concerning neurological disorders, due to similarities in genome between human and *C.elegans*. Like *C.elegans Drosophila* is also one of the model organism in the field of life science. Today, it is challenging the fruit fly *Drosophila* in many streams of biology even though there is a lot difference between two. *Drosophila* is meant for the genetics purpose and nematode *C.elegans* is meant for molecular biology. *C.elegans* offers great experimental advantages in cell and molecular biology. The Etiologies and pathogenesis underlying number of monogenic neurological disorders including familial Parkinson, Alzheimer's disease and cancer have been success replicated in *C.elegans*

system, when causative mutations of those disorder were transgenetically introduced or loss of function mutations were made. There are many aspects in *C.elegans* biology and physiology waiting to be explored in the field of biomedical, pharmaceutical sciences, Ayurvedic system (Ancient Indian Medicine system) and new genomic approaches. This system is also very useful in screening the the plants products which come across in our daily life. We expect the future of *C.elegans* research in turn increasingly to questions beyond the cellular level to questions of physiology maintenance and regeneration of whole organs.

The free-living, soil nematode *C.elegans* is being greatly developed as a model to assess the effects of potential developmental and neurological toxicants in the field of Toxicology. The above several issues what author have discussed make *C.elegans* a practical species not only for the assessing the diseases concern to human but it is also concerned to environmental issues like ecotoxicity of ecosystem by number of manmade chemicals. The using of this non-mammalian model in field of many branches of life sciences provides replacement, reduction, refinement of the use of the other mammalian model in the laboratory (National Toxicology Program). Nowadays the *C.elegans* is transmitted from research tool into testing tool. This nematode as become testing tool in many toxicological and pathology laboratories through out the world. This testing tool is due to the development of the sophisticated technologies such as 1. Medium throughput using computer images to analysis important questions hidden in this animal. 2. The developmental of 3-D, 4-D computer imaging software for measuring the quantitatively the effects of the toxicants and embryology of the *C.elegans* 3. Presence of *C.elegans* microarrays. 4. Its complete genome sequences 5. Detailed database available at the tip of the fingers (online) 6. Maps of all neuronal pathways 7. Less number of cells makes *C.elegans* as a best candidate in the field of medicine.

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