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Applications of the Free-Living Nematode, Caenorhabditis Elegans: A Review

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ABSTRACT

The free-living nematode, Caenorhabditis elegans, has been suggested as an excellent model organism in ecotoxicological studies. It is a saprophytic nematode species that inhabits soil and leaf-litter environments in many parts of the world. It has emerged to be an important experimental model in a broad range of areas including neuroscience, developmental biology, molecular biology, genetics, and biomedical science. Characteristics of this animal model that have contributed to its success include its genetic manipulability, invariant and fully described developmental program, well-characterized genome, ease of culture and maintenance, short and prolific life cycle, and small and transparent body. These features have led to an increasing use of C. elegans for environmental toxicology and ecotoxicology studies since the late 1990s. Although generally considered a soil organism, it lives in the interstitial water between soil particles and can be easily cultured in aquatic medium within the laboratory. It has been successfully used to study toxicity of a broad range of environmental toxicants using both lethal and sub lethal endpoints including behavior, growth and reproduction and feeding. In this work we review the choice, use and applications of this worm as an experimental organism for biological and biomedical researches that began in the 1960s.

Keywords: *Caenorhabditis elegans, Biomedical Science, Molecular Biology, Environmental Toxicology.*

INTRODUCTION

Animals are used to understand basic biology, as "models" for studying human biology and disease, and as test subjects for the development and testing of drugs, vaccines, and other biological (i.e. antibodies, hormones, ingredients in vaccines, etc.) to improve and advance human health. As models, scientists aim to produce artificially, a condition in an animal in a laboratory that may resemble the human equivalent of a medical disease or injury (Gawrylewski, 2007). A wide variety of animals are used in basic research, with mice being the most common. Rats, birds, amphibians, and fish are also used, and invertebrates such as fruit flies and worms are heavily used in genetic research (Greek& Shanks, 2009).

Nematodes are the most abundant animal in soil ecosystems and also found in aquatic and sediment environments. They serve many important roles in nutrient cycling and in maintaining environmental quality. From the late 1970s, a variety of nematode species have been used to study environmental issues. During the late 1990s, Caenorhabditis elegans began to emerge as the nematode species of choice based on the tremendous body of knowledge developed by basic scientists using this model organism for biological and ecotoxicological studies (Hope, 1990). Although scientific reports on this species have appeared in the literature for more than one century, extensive studies on C. elegans was not started until Brenner's seminal genetics paper was published in 1974 (Brenner, 1974). Since then it has emerged to be an important experimental model in a broad range of areas including neuroscience, developmental biology, molecular biology, genetics, and biomedical science. Characteristics of this animal model that have contributed to its success include its genetic manipulability, invariant and fully described developmental program, well-characterized genome, ease of culture and maintenance, short and prolific life cycle, and small and transparent body (Leung et al., 2008).

THE NATURAL HISTORY OF *CAENORHABDITIS ELEGANS* RESEARCH

Over a century ago, the zoologist Emile Maupas first identified the nematode, Rhabditis elegans, in the soil in Algiers. Subsequent work and phylogenic studies renamed the species Caenorhabditis elegans or more commonly

referred to as C. elegans; (Caeno meaning recent; rhabditis meaning rod; elegans meaning nice). In 1963, Sydney Brenner suggested the future of biological research lay in model organisms through his work on DNA, RNA, and the genetic code and he believed that biological research required a model system that could grow in vast quantities in the lab, were cheap to maintain and had a simple body plan, and he chose the nematode C. elegans to fulfill such a role. Since that time, C. elegans has emerged as one of the premiere model systems for aging research (Tissenbaum, 2014).

From 1967 until the early 1970s, over 300 mutagen-induced mutations were identified; many of them affected behaviour, primarily resulting in worms that were defective in movement ("uncoordinated")(Brenner, 1974).Although at least the first five years of work on C. elegans focused on developing the genetics of the organism, Brenner maintained his long-term interests in exploring the nervous system. However, it soon became clear that the precise connections between neurons would ultimately be necessary to understand the relationship between the genetic "programme" and the behavior of the animal. By using serial electron micrograph sections it was possible to perfectly determine neural connectivity although through a painfully long-term project done by John White together with Eileen Southgate, J. Nichol Thomson and Brenner. This project was completed in ~1984, resulting in an enormous article known in short as "The Mind of a Worm". The result of this project was the complete mapping of the architecture of the nervous system. It was concluded that C. elegans has 302 neurons and form a total of ~8,000 synapses throughout the hermaphrodite. By comparing the nervous systems of genetically identical individuals, the researchers found essentially the same connections, with minor differences in cell morphology, position and connectivity.

In 1974, a little more than a decade after his first thoughts about working on a model organism, Brenner published four manuscripts, including one entitled 'The genetics of Caenorhabditis elegans' (Brenner, 1974) and a new field began. In this influential paper, Brenner outlined methodology for isolation, complementation, and mapping of worm mutants. Importantly, the publication also included the successful isolation of several hundred mutants affecting behavior and morphology, a discussion of the number of defined genes, and an estimation of mutation frequency.

Brenner's choice of C. elegans seems to have been partially influenced by the desire to do something no one had been able to do (or that none of his peers was likely to be able to do with the organisms they had selected): to achieve a complete understanding of a simple organism. He quickly noted that the organism he had selected had certain attributes that allowed what some have viewed as an extreme approach (Horace Judson has term edit the "brute force" approach, namely the complete description of all detectable genetic mutants, as well as of the structure of the nervous system. Others biologists soon took a similar tack with the developmental processes in the organism, observing and documenting the complete cell lineages in the worm. The first of this kind of study was done by John Sulston on the post-embryonic developmental lineages in the ventralcord (Sulston, 1976).Since that time, many discoveries including dissection of programmed cell death (Coulson et al., 1986; Ellis et al., 1991). Mutants published by Brenner (1974),post embryonic cell lineages determined (Sulston and Horwitz, 1976), programmed cell death(Horwitz, 1982), complete embryonic cell lineages determined (Sulston, 1983), complete connectivity of nervous system was established (1986),the systematic cloning of the genome (Coulson et al., 1986; Crawford, 2001), RNAi and miRNA discovered in worms (1991-1998)(Nobel Prize: Fire and Mello, 2006), micro RNAs (Lee et al., 1993; Reinhart et al., 2000),RNA interference (Fire et al., 1998), the deciphering of the entire DNA sequence (Consortium, 1998), and the first use of GFP (green fluorescent protein) in animals (1994) (Nobel Prize: Chalfie, 2008) (Chalfie et al., 1994)have been done in C. elegans(the first animal whose whole genome was sequenced (97Mb,now 100.3Mb)which has led to an expansion in the number of researchers working with C. elegans.

The Nobel Prize in Physiology or Medicine was awarded in 2002to Sydney Brenner, H. Robert Horvitz and John Sulston for their work on C. elegans (Fig. 1) and the apoptosis and in 2006 to Andrew Fire and Craig C. Mello for their discovery of RNA interference. In 2008, Martin Chalfie shared a Nobel Prize in Chemistry for his work on green fluorescent protein (GFP) (Fig. 2).

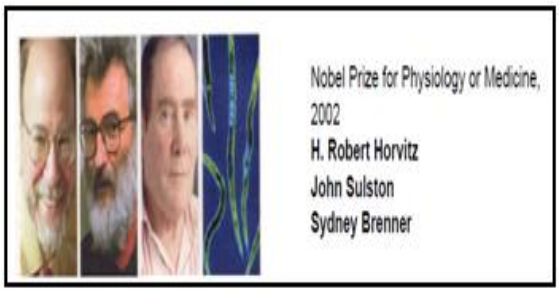

Figure1. *Nobel Prize for Physiology of Medicine, 2002*

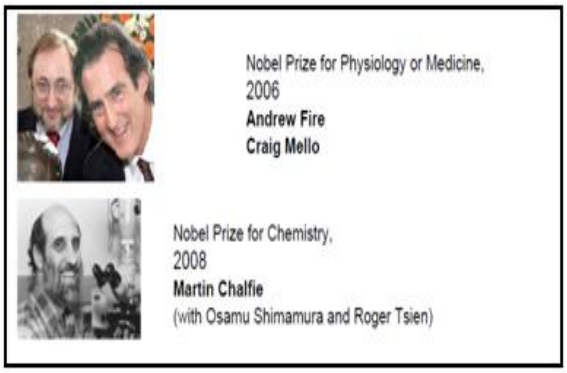

Figure2. *Nobel Prize for Physiology or Medicine, 2006 & Nobel Prize for Chemistry, 2008*

Why are Caenorhabditis Elegans Important to Biology?

Caenorhabditis elegans is important to study developmental biology and genetics for a variety of reasons (Jinel Shah, 2015): 1- One of the simplest organisms with a nervous system, 2- Genetic manipulations are relatively easy (You can silence any gene by actually feeding them RNAi bacteria! Yes! it's that simple), 3- Transparent body for easy developmental studies, 4- Short life span to study aging and neurodegeneration, 5- Each of their germ cells are completely mapped to final differentiation, 6- easy to grow in bulk populations, 7- Whole Genome has been sequenced and 8- About 35% Genes have Human Homologs.

Key Benefits of the Worm

- C. elegans can be grown cheaply and in large numbers on plates containing bacteria
- Healthy cultures of C. elegans can be frozen and then defrosted and revived when needed
- C. elegans produce over 1,000 eggs every day
- They have a short life cycle of only two weeks, which is useful for studying their development
- C. elegans is a very small organism so is convenient to keep in the lab
- The worm is transparent throughout its life so the behavior of individual cells can be followed through its development
- The anatomy and development of C. elegans can be examined easily under a microscope
- A unique feature of C. elegans is that their development is very specific, cells divide and specialize in a characteristic way, so each cell can be traced back to the embryo
- Although C. elegans is a relatively simple organism, many of the molecular signals controlling its development are also found in more complex organisms, like humans, 9- Mutant forms of C. elegans, where specific genes are altered, can be produced very easily to closely study gene function
- Many of the genes in the C. elegans genome have functional counterparts in humans which makes it an extremely useful model for human diseases
- C. elegans mutants provide models for many human diseases including neurological disorder s, congenital heart disease and kidney disease
- C. elegans mutants can be screened with thousands of potential drugs for important diseases and
- Studying cell death or 'apoptosis' in the C. elegans could hold the key to counteracting the effects of ageing in humans as well as providing clues about cancer, diabetes and other diseases (Figs. 3- 5).

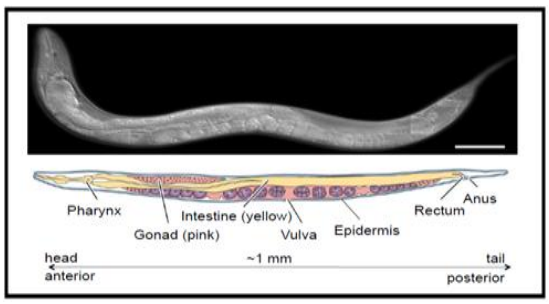

Figure3. *Caenorhabditis elegans*

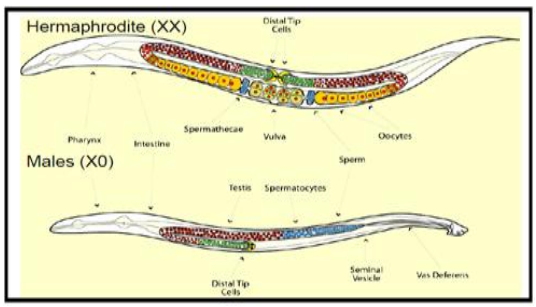

Figure4. *Two sexes, A self-fertilizing hermaphrodite (XX) and A male(X0)*

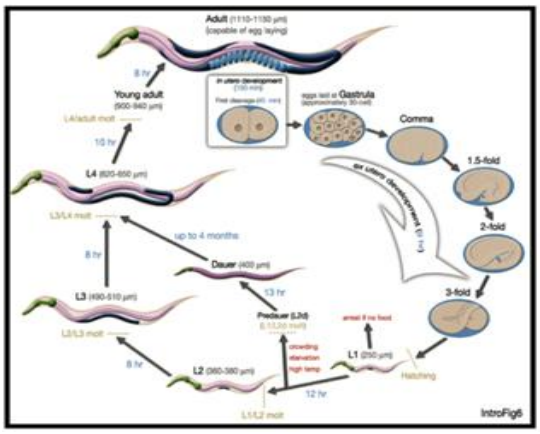

Figure5. *Caenorhabditis elegans have a three day life cycle at room temperature (A), can be maintained on agar plates containing bacteria as their food (B) and are easily visible under the light microscope (C).*

Disadvantages of the Worm

Despite all the excellent advantages of working with *C. elegans* for aging research, there are also several disadvantages for *C. elegans* as a model for human aging. First, *C. elegans* have a simple body plan, and lack many defined organs/tissues including a brain, blood, a defined fat cell, internal organs, and is evolutionarily distant from humans. Second, *C. elegans* are also only 1 mm in length which makes biochemistry more difficult. Typically, all biochemistry, microarray, immune precipitation, and chromatin immune precipitation is performed on whole worm extracts of either mixed-stage animals or animals at a similar growth stage. This may lead to limited understanding of any tissue-specific signaling such as whether a gene is expressed in the hypodermis or the intestine. Finally, *C. elegans* cell culture is limited with no system equivalent to Drosophila S2 cells.

C. elegans is no ordinary worm: it's a small, free-living, non-parasitic, multicellular, unsegmented, eukaryotic, bilaterally-symmetri cal, vermiform nematode that has elongated cylindrical bodies, tapered at both ends, with smooth skin, and no append ages with a life cycle of 3.5days at 20°C and a lifespan of ab out 2–3 weeks under suitable living conditions. Adults grow to approximately 1mm in length and 80 µm in diameter; it feeds on bacteria such as *Escherichia coli* in liquid medium or on agar plates, and can be easily cultivated in large numbers **(Fig. 6)**. *C.elegans* has five pairs of autosomes and one pair of sex chromosomes. Exactly 959 cells compose *Caenorhabditis elegans*, and their bodies are transparent; therefore, individual cells are easily observed with a microscope **(Edgley, 1999)**.

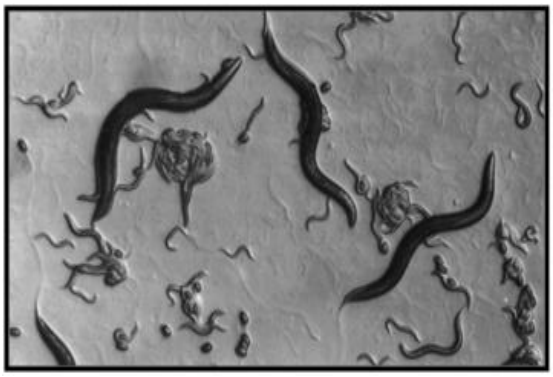

Figure6. *Caenorhabditis elegans is a free-living nematode, ~1 mm in length, with relatively simple behaviours and structures.(Photograph by Henri van Leunen, kindly provided by Jonathan Hodgkin, Genetics Unit, Department of Biochemistry, University of Oxford).*

Applications on *Caenorhabditis Elegans* in Various Biological Research Areas

Developmental Biology and Cell Biology Neurobiology

Transparency helps to view anatomy and development, Individual cell lineages can be easily traced and directly inspected by DIC light microscopy and programmed cell death can also be visualized.

Neurobiology Toxicity Mechanisms of Neurotoxic Metals in C. elegans

Act as a model for neuronal development and function, No brain per say, but have sophisticated nervous system (302neurons/959 cells) and it responds to chemo attarctants/repellants. Laser beams (selective cutting of neurons) and electrophysiology studies can be conducted.

Caenorhabditis Elegans in the Study of Neurodegeneration

As previously stated, the C. elegans nervous system functionally recapitulates many of the characteristics of the vertebrate brain. In particular, it can undergo degeneration through conserved mechanisms and is thus a powerful model for uncovering the genetic basis of neurodegenerative disorders. In this section, we will focus on PD, Alzheimer disease (AD),

Huntington disease (HD), and Duchenne muscular dystrophy (DMD).

Toxicity Mechanisms of Neurotoxic Metals in C. elegans

C. elegans appears quite different from vertebrates, its nervous circuitry and the cellular processes guiding neuronal development, neuronal death or survival, neurotransmission, and signal integration rely on the same neuronal and molecular networks as vertebrates. Combined with the advantages of a small and fast-growing organism, these properties make C. elegans a perfect system for rapid genetic analysis of neurotoxicity mechanisms neurotoxicological studies in C. elegans (Riddle et al., 1997).

Feeding behavior has also been shown to be affected upon heavy metal exposure. Feeding requires a different neuronal circuitry including M3 (involved in pharyngeal relaxation), MC (control of pumping rate), M4 (control of isthmus peristalsis), NSM (stimulate feeding), RIP, and I neurons (Riddle et al., 1997). A decrease in feeding was observed when worms were exposed to Cd or Hg (Boyd et al., 2003; Jones & Candido, 1999).

Studies conducted in mammalian models found that Hg is able to block Ca^{2+} channels. In neurons, this blockage can induce spontaneous release of neurotransmitters (Atchison, 2003). In C. elegans, the Ca^{2+} channel blocker verapamil was found to protect against Hg exposure, suggesting that Ca^{2+} signaling plays a role in the toxicity of Hg in this model organism as in mammals (Koselke et al., 2007).

The nematode Caenorhabditis elegans has previously been used as a biosensor for ecotoxicological studies (David et al., 2003; Jones et al., 1996; Stringham & Candido, 1994). The use of this animal model has several advantages: it represents a multicellular organism, is a self-fertilizing hermaphrodite, has a high progeny rate, a short life cycle, and can be easily maintained in the laboratory in microtiter plates (Brenner, 1974; Candido & Jones, 1996).

Genotoxin Studies in C. Elegans

Unlike the case of neurotoxicology, there have so far been relatively few studies of genotoxicity per using C. elegans. One exception has been the study of UV radiation, typically as a model genotoxin that introduces bulky DNA lesions (Astin et al., 2008; Coohill et al., 1988; Hartman, 1984; Hartman et al., 1988; Hyun et al., 2008; Jones & Hartman, 1996; Keller et al., 1987; Meyer et al., 2007; Stergiou et al., 2007; Stewart et al., 1991). However, other classes of genotoxins have been studied, including ionizing radiation (Dequen et al., 2005a; Johnson & Hartman, 1988; Stergiou et al., 2007; Weidhaas et al., 2006), heavy metals (Cui et al., 2007b; Neher & Sturzenbaum, 2006; Wang et al., 2008), methyl methane sulphonate (Holway et al., 2006), polycyclic aromatic hydrocarbons (Neher & Sturzenbaum, 2006), photosensitizers (Fujita et al., 1984; Hartman & Marshall, 1992; Mills & Hartman, 1998), and prooxidant compounds (Astin et al., 2008; Hartman et al., 2004; Hyun et al., 2008; Salinas et al., 2006).

C. Elegans and Genotoxicity

As is the case for neurotoxicity, C. elegans provides a cost-effective, in vivo, genetically manipulable and physiological model for the study of the toxicological consequences of DNA damage. As described below, the machinery that responds to DNA damage in C. elegans is very similar genetically to the corresponding machinery in higher eukaryotes. Many processes related to DNA damage have been extensively studied in C. elegans, providing an important biological context and clear relevance to mechanistic studies. Finally, powerful tools for the study of DNA damage, DNA repair, and mutations have been developed in this organism.

DNA Damage Response Proteins are Conserved between C. Elegans and Higher Eukaryotes DNA Repair in C. elegans

Genes and pathways involved in DNA repair in mammals are generally well conserved in C. elegans (Boulton et al., 2002; Hartman & Nelson, 1998; O'Neil & Rose, 2005). Proteins involved in nucleotide excision repair, mismatch repair, homologous recombination, and nonhomologous end joining, for instance, are almost entirely conserved between C. elegans, mouse, and human based on nucleotide sequence homology.

(http://www.niehs.nih.gov/research/atniehs/labs/lmg/dnarmd/docs/Cross-species-comparison-of-DNA-repair-genes.xls)

This is also true for proteins involved in many DNA repair–related processes, such as translation DNA polymerases, helices, and nucleases. Base excision repair proteins, interestingly, show somewhat less conservation. While this conservation is based in some cases only on sequence homology, many of these

proteins have now been biochemically or genetically characterized. Critically, proteins involved in other DNA damage responses including apoptosis and cell cycle arrest are also conserved in C. elegans and mammals (Stergiou & Hengartner, 2004).

DNA Repair in C. Elegans

Early studies on DNA repair in C. elegans were carried out by Hartman and colleagues, who identified a series of radiation-sensitive mutants (Hartman, 1985; Hartman & Herman, 1982) and used an antibody-based assay to measure induction and repair of ultraviolet (UV) radiation–induced damage (Hartman et al., 1989). These and more recent studies (Hyun et al., 2008; Meyer et al., 2007) have shown that nucleotide excision repair is similar in C. elegans and humans both in terms of conservation of genes and kinetics of repair. Nucleotide excision repair is a critical pathway in the context of exposure to environmental toxins since it recognizes and repairs a wide variety of bulky, helix-distorting DNA lesions, including polycyclic aromatic hydrocarbon metabolites, mycotoxins such as a flatoxin B1, UV photoproducts, cisplatin adducts, and others (Friedberg et al., 2006; Truglio et al., 2006).

While nucleotide excision repair has been the best-studied DNA repair pathway in C. elegans, significant progress has been made in the study of genes involved in other DNA repair pathways as well. The role of specific C. elegans gene products in DNA repair has been studied both via high-throughput and low-throughput methods. High-throughput methods including RNAi knockdown and yeast two-hybrid analysis of protein-protein interaction have been used to identify a large number of genes coding for proteins involved in responding to DNA damage (Boulton et al., 2002; van Haaften et al., 2004a, 2004b). Lower throughput studies in ol veing biochemical analyses of DNA repair activities (Dequen et al., 2005a; Gagnon et al., 2002; Hevelone & Hartman, 1988; Kanugula & Pegg, 2001; Munakata & Morohoshi,1986; Shatilla et al., 2005a & 2005b; Shatilla & Ramotar, 2002) as well in vivo sensitivity to DNA damaging ag-ents (Astin et al., 2008; Boulton et al., 2004; Dequen et al., 2005b; Lee et al., 2002 & 2004; Park et al., 2002 & 2004; St-Laurent et al., 2007) or other DNA damage–related pheno types (Aoki et al., 2000; Kelly et al., 2000; Sadaie & Sadaie, 1989; Takanami et al., 1998) have supported the sequence similarity–based identification of C. elegans homologues of DNA repair genes in higher vertebrates, as well as in some cases permitting identification of previously unknown genes involved in these pathways.

Environmental Assessment of Chemical Exposure Model for Toxicity Testing of Nano-Materials

Several reports describe C. elegans as model system for environmental toxicity studies a (David et al., 2003; Jones et al., 1996; Stringham & Candido, 1994; Williams et al., 2000). A simple signal for toxicity can be the measurement of lethality of the nematodes. However, more subtle indicators of toxicity would be desirable.

Nematodes are the most abundant animal in soil ecosystems and also found in aquatic and sediment environments. They serve many important roles in nutrient cycling and in maintaining environmental quality. These features have supported their use in ecotoxicological studies and, from the late 1970s, a variety of nematode species have been used to study environmental issues.

During the late 1990s, *C. elegans* began to emerge as the nematode species of choice based on the tremendous body of knowledge developed by basic scientists using this model organism for biological studies. Although generally considered a soil organism, *C. elegans* lives in the interstitial water between soil particles and can be easily cultured within the laboratory in aquatic medium. The majority of environmental studies have been performed in an aquatic medium, given its ease of use, and as toxicological end points have been developed, the assessment tools have been applied to sediment and soil medium which allows for a more relevant direct environmental comparison.

The environmental toxicological literature using *C. elegans* is extensive and the next table provides an overview of laboratory-based studies where a toxicant of environmental interest has been added to a medium (water, sediment, or soil) followed by exposure to *C. elegans* and the assessment of an adverse effect. In a limited number of situations. Much of the early work explored metal toxicity and used lethality as an endpoint. Over time, a wider variety of toxicants have been tested and more sophisticated sublethal end points have been developed including the use of transgenic strains with specific biomarkers (Candido & Jones,

1996; Chu *et al.*, 2005;Dengg& van Meel, 2004; Easton *et al.*, 2001; Mutwakil *et al.*, 1997; Roh *et al.*, 2006), growth and reproduction (Anderson *et al.*, 2001; Hoss & Weltje, 2007), feeding (Boyd *et al.*, 2003), and movement (Anderson *et al.*, 2004). These types of end points developed through environmental studies are directly applicable to the use of the organism as an alternative for mammalian testing.

Limited studies comparing the toxicological effects between nematodes species indicate that *C. elegans* is as representative as any of the ones commonly used and, in many cases, little difference in response has been found between species (Boyd and Williams, 2003; Kammenga *et al.*, 2000). Further, this organism is much more thoroughly understood and benefits from its ease of use.

Model for Toxicity Testing of Nano-Materials C. elegans as model of aging

C. elegans is a useful because it has been applied widely for toxicological studies for conventional compounds and because several ecologically relevant endpoints can be easily measured in the same exposure system.

Years before the latest technologic developments (RNAi and high-throughput techniques), C. elegans was used to study toxicity mechanisms of environmental factors affecting the nervous system. The following section provides a synopsis of the available literature on neurotoxicity-related issues addressed in C. elegans. It is not meant to be exhaustive but rather to illustrate typical studies that are amenable in the C. elegans platform. We highlight studies with exposure outcomes to various metals and pesticides, as well as general considerations on studies of neurodegenerative diseases. We emphasize the utility of C. elegans in addressing hypothesis-driven mechanisms of neurotoxicity and extrapolations to vertebrate systems.

C. Elegans as Model of Aging Human disease studies

During the last three decades the soil nematode C. elegans has become a prominent model organism for studying aging. Initially research in the C. elegans aging field was focused on the genetics of aging and single gene mutations that dramatically increased the life span of the worm. Undoubtedly, the existence of such mutations is one of the main reasons for the popularity of the worm as model system for studying aging. However, today many different approaches are being used in the C. elegans aging field in addition to genetic manipulations that influence life span. For example, environmental manipulations such as caloric restriction and hormetic treatments, evolutionary studies, population studies, models of age-related diseases, and drug screening for compounds that extend life span are now being investigated using this nematode.

Adult C. elegans worms are self-fertilizing hermaphrodites with a 3-day life cycle and a mean life span of approximately 18–20 days when cultured at 20°C. This nematode displays a number of age-related changes reminiscent of those observed in other organisms. With advanceng age worms are less active, display uncoordinated movements, and eventually they stop moving. This appears to be the result of muscle degeneration rather than neuronal defects as the cellular integrity of the nervous system is preserved till very late in life (Herndon et al., 2002). Other age-related changes include accumulation of lipofuscin, dark pigments, presence of vacuole-like structures,1 and increased levels of oxidized proteins(Adachi et al., 1998; Nakamura et al., 1999; Yasuda et al., 1999).

Life-Span Prediction Models of age-related diseases

Individual worms of an isogenic population display considerable variation in life span, paralleling the heterogeneity observed in physiological markers of aging.1 why is it that, given that all worms have the same genetic makeup and have been exposed to the same environment, some worms may die on day 14 while others die as late as on day 34? A recent study has attempted to answer this question **(** REA, et al. 2005**)**A large population of worms were subjected to a sub-lethal heat shock to induce expression of hsp-16 and were subsequently grouped based upon levels of HSP-16. The life span of these different groups was then measured. Worms with high levels of HSP-16 early in life were found to be significantly longer-lived and stress-resistant than worms with lower levels of HSP-16. Thus, levels of HSP-16 appear to reflect some physiological status that is linked to longevity. Although HSP-16 may only be one of the many proteins involved, it does provide a biomarker that early in life can be used to predict life span.

Models of Age-Related Diseases

The C. elegans genome contains homologs of approximately two-thirds of all human disease

genes (Fay et al., 1998).Thus, the worm has become a popular organism for modeling human diseases including age-related diseases. To study Alzheimer's disease, transgenic animals were engineered to express human-amyloid peptide (A) and these worms form intracellular -amyloid aggregates (Fay et al., 1998; LINK & JOHNSON, 2002). Parkinson's disease and degeneration of dopaminergic neurons can also be studied using transgenic nematodes (Nass et al., 2002). Another very elegant example of disease modeling in the worm is the Huntington's disease model that addresses the effects of polyglutamine expansions (Morley et al., 2002; Satyal et al., 2000; Faber et al., 1999; Parker et al., 2001).

Human Disease Studies

C. elegans has emerged as a powerful experimental system to study the molecular and cellular spects of human disease in vivo. It has been estimated that about 42% of the human disease genes have an ortholog in the genome of C. elegans, including those genes associated with Alzheimer's disease(AD),juvenile Parkinson's disease (PD),spinal muscular atrophy (SMA), hereditary non-polyposiscolon cancer, and many others age-related disorders (Kenyon, 2005).

Modeling a human disease in a simple invertebrate, such as C. elegans, allows the dissection of complex molecular pathways in to their component parts, thus providing a meaningful insight into the pathogenesis of a complex disease phenotype. Here, we survey nematode models of human disease, highlighting recent discoveries that shed light on the molecular mechanisms underlying (Markaki & Tavemarak is, 2010) disease pathogenesis. ~75% of human disease genes have potential C. elegans homologs and ~40-50% have a C. elegans ortholog.

C. Elegans as Model of Dmd

Duchenne Muscular Dystrophy (DMD) (Fig. 7) is an X-linked recessive disease characterized by progressive muscle loss and weakness. This disease arises from a mutation that occurs on a gene that encodes for dystrophin, which results in observable muscle death and inflammation; however, the genetic changes that result from dystrophin's functionality remain unknown. The dystrophin gene is highly conserved; homologues have been identified not only in vertebrates (mammals, birds and fish) but also in the popular invertebrate laboratory models Caenorhabditis elegans (Bessou et al. 1998; Chamberlain & Benian 2000) and Drosophila melanogaster (Neuman et al., 2001)

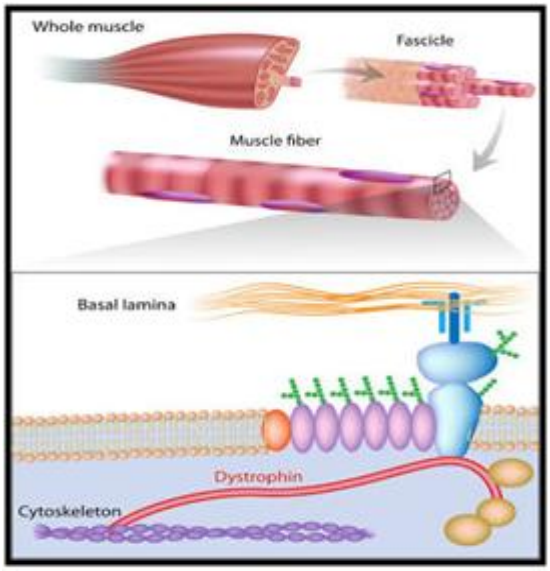

Figure7. *Duchenne Muscular Dystrophy*

Current DMD research uses mdx mice as a model, and while very useful, does not allow the study of cell-autonomous transcriptome changes during the progression of DMD due to the strong inflammatory response, perhaps hiding important therapeutic targets (Hoffman et al., 1987).

C. elegans, which has a very weak inflammatory response compared to mdx mice and humans, has been used in the past to study DMD with some success.The worm ortholog of the dystrophin gene has been identified as dys-1 since its mutation phenocopies the progression of the disease and a portion of the human dystrophin gene alleviates symptoms. Importantly, the extracted RNA transcriptome from dys-1 worms showed significant change in gene expression, which needs to be further investtigated with the development of a more robust model (Bessou et al., 1998).

The presence of dys-1 and other members of the dystrophin complex in the body muscle were supported by the development of a resulting phenotype due to RNAi knockdown of each component in the body muscle; however, further experimentation is needed to reinforce this conclusion. Thus, the constructed chimeric C. elegans strain possesses unique characteristics that will allow the study of genetic changes, such as transcriptome rearrangements and dysregulation of miRNA, and how they affect

the progression of DMD (Baumeister & Ge, 2002).

SUMMARY AND CONCLUSION

In summary, our review on Caenorhabditis elegans present that this model organism, is suitable to study potential toxicities and neuroscience, developmental biology, molecular biology, genetics, and biomedical science. Advantages of a nematode model are that it represents a short-living, self-fertilising multicellular organism, which can be kept easily in the laboratory. Further applications may include precise analyses of the cells or organs involved in the stress responses induced by compound.

Consequently, studies with C. elegans could provide a rapid and low-cost assessment of toxicity of pharmaceutical compounds. Furthermore, the application of this model allowed the determination of a rank order of potency for toxicity among compounds. This ranking of compounds may facilitate a more rational choice of the best candidate compounds for further in vivo tests. C. elegans has proved to be an invaluable animal for aging research.

Early work with C. elegans is best understood as part of a descriptive tradition in biological practice. Although the resources that have been generated by the C. elegans community have been revolutionary, they were produced by traditional methods and approaches. Here, we review the choice and use of the worm as an experimental organism for genetics and neurobiology that began in the 1960s and it is well known to practising biologists as a model organism.

RECOMMENDATION SUMMARY AND CONCLUSION

In view of these conclusions, some suggestions for future research on relevant topics are outlined below: Study the application of the bioassay to field settings, i.e., water samples contaminated with transition metals, study its use as bio-indicator of pollution, evaluate the impact of particle size on toxicity of nanoparticles and examine the influence of sample preparation method on toxicity of nanoparticles, future studies should focus on understanding the connection between longevity and how an animal ages, with a focus on health, and we should strive to use model systems to reveal this systemic coordination on a molecular and genetic level, and how this leads to healthy aging rather than simply lifespan extension.

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