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REVIEW

Caenorhabditis elegans: An Emerging Model in Biomedical and Environmental Toxicology

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The nematode *Caenorhabditis elegans* has emerged as an important animal model in various fields including neurobiology, developmental biology, and genetics. 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 maintenance, short and prolific life cycle, and small body size. These same features have led to an increasing use of *C. elegans* in toxicology, both for mechanistic studies and high-throughput screening approaches. We describe some of the research that has been carried out in the areas of neurotoxicology, genetic toxicology, and environmental toxicology, as well as high-throughput experiments with *C. elegans* including genome-wide screening for molecular targets of toxicity and rapid toxicity assessment for new chemicals. We argue for an increased role for *C. elegans* in complementing other model systems in toxicological research.

***Key Words: Caenorhabditis elegans*; neurotoxicity; genotoxicity; environmental toxicology; high-throughput methods.**

Caenorhabditis elegans is a saprophytic nematode species that has often been described as inhabiting soil and leaf-litter environments in many parts of the world (Hope, 1999); recent reports indicate that it is often carried by terrestrial gastropods and other small organisms in the soil habitat (Caswell-Chen *et al*., 2005; Kiontke and Sudhaus, 2006). Although scientific reports on the species have appeared in the literature for more than 100 years (e.g., Maupus, 1900), the publication of Brenner's seminal genetics paper (Brenner, 1974) signaled its emergence as an important experimental model. Work with *C. elegans* has since led in a short time span to seminal discoveries in neuroscience, development, signal transduction, cell death, aging, and RNA interference (Antoshechkin and Sternberg, 2007). The success of *C. elegans* as a model has attracted increased attention as well in the fields of in biomedical and environmental toxicology.

Clearly, *C. elegans* will be a valuable toxicity model only if its results were predictive of outcomes in higher eukaryotes. There is increasing evidence that this is the case both at the level of genetic and physiological similarity and at the level of actual toxicity data. Many of the basic physiological processes and stress responses that are observed in higher organisms (e.g., humans) are conserved in *C. elegans*. Depending on the bioinformatics approach used, *C. elegans* homologues have been identified for 60–80% of human genes (Kaletta and Hengartner, 2006), and 12 out of 17 known signal transduction pathways are conserved in *C. elegans* and human (NRC, 2000; Table 1). We discuss specific examples in the areas of neurotoxicology and genetic toxicology in this review.

Caenorhabditis elegans has a number of features that make it not just relevant but quite powerful as a model for biological research. First of all, *C. elegans* is easy and inexpensive to maintain in laboratory conditions with a diet of *Escherichia coli*. The short, hermaphroditic life cycle (~3 days) and large number (300+) of offspring of *C. elegans* allows large-scale production of animals within a short period of time (Hope, 1999). Since *C. elegans* has a small body size, *in vivo* assays can be conducted in a 96-well microplate. The transparent body also allows clear observation of all cells in mature and developing animals. Furthermore, the intensively studied genome, complete cell lineage map, knockout (KO) mutant libraries, and established genetic methodologies including mutagenesis, transgenesis, and RNA interference (RNAi) provide a variety of options to manipulate and study *C. elegans* at the molecular level (Tables 2 and 3; for a more detailed presentation of genetic and genomic resources, see Antoshechkin and Sternberg, 2007). We address the particular power of these genetic and molecular tools in *C. elegans* at more length below.

Since reverse genetic and transgenic experiments are much easier and less expensive to conduct in *C. elegans* as compared

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TABLE 1
Signal Transduction Pathways Conserved in Nematodes and Vertebrates[a,b]

Pathways involved in early development
Wnt pathway via β-catenin
Receptor serine/threonine kinase (tumor growth factor-β receptor) pathway
Receptor tyrosine kinase pathway (small G-protein [Ras] linked)
Notch-delta pathway
Receptor-linked cytoplasmic tyrosine kinase (cytokine) pathway
Pathways involved in later development (e.g., organogenesis and tissue renewal)
Apoptosis pathway (cell death pathway)
Receptor protein tyrosine phosphatase pathway
Pathways involved in the physiological function of differentiated cells of the fetus, juvenile, and adult
G-protein–coupled receptor (large G-protein) pathway
Integrin pathway
Cadherin pathway
Gap junction pathway
Ligand-gated cation channel pathway

[a]Adapted from NRC (2000).

[b]Signal transduction pathways that are not conserved in nematodes and vertebrates include the Wnt pathway via c-Jun N-terminal kinase, the Hedgehog pathway (patched receptor protein), the nuclear factor kappa-B pathway, the nuclear hormone receptor pathway, the receptor guanylate cyclase pathway, and the nitric oxide receptor pathway.

to many other model systems, it is a useful model for molecular analyses of the response of conserved pathways to *in vivo* chemical exposure. As an *in vivo* model, *C. elegans* provides several characteristics that complement *in vitro* or cellular models. The use of whole-organism assays, first of all, allows the study of a functional multicellular unit, such as a serotonergic synapse, instead of a single cell (Kaletta and Hengartner, 2006). *Caenorhabditis elegans* also enables the detection of organism-level end points (e.g., feeding, reproduction, life span, and locomotion) and the interaction of a chemical with multiple targets in an organism. Thus, *C. elegans* complements both *in vitro* and *in vivo* mammalian models in toxicology.

Of note, these characteristics facilitate high-throughput experiments that can examine both fundamental toxicity, which are critical since so many chemicals have yet to be thoroughly tested, and the gene-gene and gene-environment interactions whose importance is just beginning to be appreciated in toxicology.

Here we review three major applications of *C. elegans* in biomedical and environmental toxicology: (1) mechanistic toxicology, with a focus on neurotoxicity and genotoxicity; (2) high-throughput screening capabilities; and (3) environmental toxicology and environmental assessment. We emphasize studies of neurotoxicity because they are the area of toxicology in which *C. elegans* has been most exploited to date. We discuss research methods, recent advances, and important considerations including limitations of the *C. elegans* model.

Caenorhabditis elegans AND NEUROTOXICITY

Caenorhabditis elegans Is Well Suited for Neurophysiology Analysis of Neurotoxicity

With 302 neurons representing 118 characterized neuronal subtypes (Hobert, 2005), *C. elegans* provides an *in vivo* model

TABLE 2
Examples of Mutational Analysis of *Caenorhabditis elegans* in Toxicology Research

Approach/toxin investigated	Mutants used	Major findings	References
A. KO mutant analysis			
Black widow spider venom	*lat-1*: KO of latrophilin	Latrophilin is the receptor responsible for the toxicity of venom	Mee *et al.* (2004)
As	*asna-1*: KO of ArsA ATPase	ArsA ATPase is important in Ar resistance in both bacteria and animals	Tseng *et al.* (2007)
Cd	*pgp-5*: KO of a ABC transporter	ABC transporter is required for resistance to Cd toxicity	Kurz *et al.* (2007)
PCB52	*cyp-35A1* to *cyp-35A5*: KOs of cytochrome P450 35A subfamily	CYP35A is required for fat storage and resistance to PCB52 toxicity	Menzel *et al.* (2007)
B. Forward genetics screen			
BPA	*bis-1*: mutant created from EMS mutagenesis	Collagen mutants are hypersensitive to BPA	Watanabe *et al.* (2005)
Phosphine	*pre-1*, *pre-7*, *pre-33*: mutants created from EMS mutagenesis	Uptake and oxidization of phosphone are directly associated with oxidative stress in cells	Cheng *et al.* (2003)
Bt toxins	*bre-1* to *bre-5*: mutants created from EMS mutagenesis	Five new genes involved in Bt toxicity are identified	Marroquin *et al.* (2000)
	bre-5: KO of β-1,3-galactosyltransferase	Carbohydrate modification is involved in Bt toxicity	Griffitts *et al.* (2001)
	bre-2 to *bre-5*: KOs of glycolipid carbohydrate metabolism	Glycolipid receptors are targets of Bt toxins	Griffitts *et al.* (2005)
	bre-1: KO of GDP-mannose 4,6-dehydratase	The monosaccharide biosynthetic pathway is involved in Bt toxicity	Barrows *et al.* (2007b)

Note. ABC, ATP-binding cassette; PCB52, polychlorinated biphenyl 52; EMS, ethane methyl sulfonate.

TABLE 3
Examples of Transgenic *Caenorhabditis elegans* Used in Toxicology Research

Field/target tagged	Reporter used	Applications	References
A. Mechanistic studies			
DAergic neurons	GFP	Detect neurodegradation caused by chemicals	Jiang *et al.* (2007)
CYP14A3 and 35A3	GFP	Detect intestinal CYP overexpression in response to PCB52 as well as other xenobiotic CYP inducers	Menzel *et al.* (2007)
GST	GFP	Measure GST induction in response to acrylamide as well as other inducers of oxidative stress	Hasegawa and van der Bliek (in press)
B. Environmental biomonitoring			
Heat shock proteins	GFP; β-galactosidase	Widely used for measuring stress response associated to toxicity of heavy metals, fungicides, pharmaceuticals, as well as field samples	Dengg and van Meel (2004); Easton *et al.* (2001); Mutwakil *et al.* (1997); Roh *et al.* (2006)
Metallothionein	β-galactosidase	Specifically used for monitoring the bioavailability of heavy metals	Cioci *et al.* (2000)
ATP level	Firefly luciferase	Measure the reduction of metabolic activity in response to environmental stressor	Lagido *et al.* 2001

Note. CYP, cytochrome P450; GST, glutathione *S*-transferase.

for studying mechanisms of neuronal injury with resolution of single neurons. It intially underwent extensive development as a model organism in order to study the nervous system (Brenner, 1974), and its neuronal lineage and the complete wiring diagram of its nervous system are stereotyped and fully described (Sulston, 1983; Sulston *et al.*, 1983; White *et al.*, 1986). Each neuron has been assigned a code name corresponding to its location. For example, ADEL describes the dopaminergic (DAergic) head neuron "anterior deirid left." This relatively "simple" nervous system is comprised of 6393 chemical synapses, 890 electrical junctions, and 1410 neuromuscular junctions (Chen *et al.*, 2006). Additionally, the main neurotransmitter systems (cholinergic, γ-aminobutyric acid (GABA)ergic, glutamatergic, DAergic, and serotoninergic) and their genetic networks (from neurotransmitter metabolism to vesicle cycling and synaptic transmission) are phylogenetically conserved from nematodes to vertebrates, which allows for findings from *C. elegans* to be extrapolated and further confirmed in vertebrate systems.

Several genes involved in neurotransmission were originally identified in *C. elegans*. This is exemplified by the GABA vesicular transporter *unc-47* and the regulatory transcription factor *unc-30* (for review on the GABAergic system [Jorgensen, 2005]), the vesicular acetylcholine (ACh) transporter *unc-17* (for review on the cholinergic system [Rand, 2007]), the glutamate-gated chloride channel subunits α1 and β (*glc-1* and *glc-2*, respectively, for review on the glutamatergic system [Brockie and Maricq, 2006]), and the synaptic proteins *unc-18*, *unc-13*, *unc-26* (for review on synaptic function [Richmond, 2005]). Experiments challenging the *C. elegans* nervous system by laser ablation of individual neurons/axons, exposure to drugs, and other external stimuli have facilitated the design of robust behavioral tests to assess the function of defined neuronal populations (Avery and Horvitz, 1990; Bargmann, 2006; Barr and Garcia, 2006; Brockie and Maricq, 2006; Chase and Koelle, 2007; Goodman, 2006; Morgan *et al.*, 2007; Rand, 2007). For example, inhibitory GABAergic and excitatory cholinergic motor functions are assessed by quantifying the sinusoidal movement (amplitude and frequency of body bends) and foraging behavior of the worm. Motor and mechanosensory functions of glutamatergic neurons are evaluated by measuring the pharyngeal pumping rate and the response to touch. Mechanosensory functions of DAergic and serotoninergic neurons are appraised by observing the ability of worms to slow down when they encounter food. Furthermore, the creation of transgenic strains expressing fluorescent proteins in defined neurons allows *in vivo* imaging of any desired neuron. While experimentally challenging in the cells of microscopic animals, electrophysiology studies can be conducted with relative ease and success in live worms and cultured *C. elegans* neurons, establishing that they are electrophysiologically comparable to vertebrate neurons in their response to various drugs (Bianchi and Driscoll, 2006; Brockie and Maricq, 2006; Cook *et al.*, 2006; Schafer, 2006). Given the relative ease with which gene KO and transgenic animals can be generated, the ability to culture embryonic or primary *C. elegans* cells offers unique perspectives for neurotoxicology applications and study designs.

Caenorhabditis elegans Is a Potent Model to Decipher Genetic Aspects of Neurotoxicity

The conservation of neurophysiologic components from nematodes to humans largely relies on shared genetic networks and developmental programs. Hence, the availability of mutants for many of the *C. elegans* genes facilitated significant progress in unraveling of evolutionarily conserved cellular and genetic pathways responsible for neuron fate specificity

(Hobert, 2005), differentiation (Chisholm and Jin, 2005), migration (Silhankova and Korswagen, 2007), axon guidance (Quinn and Wadsworth, 2006; Wadsworth, 2002), and synaptogenesis (Jin, 2002, 2005). Recently, laser axotomy in *C. elegans* has been successfully applied to identify axon regeneration mechanisms (Gabel *et al.*, 2008; Wu *et al.*, 2007), which are of utmost importance in developing treatments to reverse neurodegenerative processes and spinal cord injuries. Essential cell functions relevant to neurotoxicity studies are also conserved. This is best exemplified by the mechanistic elucidation of the apoptotic pathway in *C. elegans*, for which the 2002 Nobel Prize in Physiology or Medicine was awarded (Hengartner and Horvitz, 1994; Horvitz, 2003; Sulston, 2003). The pathway is of direct interest to neurotoxicologists since apoptosis is implicated in many neurodegenerative diseases and toxicant-induced cell demise (Bharathi *et al.*, 2006; Hirata, 2002; Koh, 2001; Mattson, 2000; Ong and Farooqui, 2005; Savory *et al.*, 2003). Pathways relevant to oxidative stress–related neuronal injuries, such as the p38 mitogen-activated protein kinase and AKT signaling cascades, the ubiquitin-proteasome pathway, and the oxidative stress response are also conserved in the worm (Ayyadevara *et al.*, 2005, 2008; Daitoku and Fukamizu, 2007; Gami *et al.*, 2006; Grad and Lemire, 2004; Inoue *et al.*, 2005; Kipreos, 2005; Leiers *et al.*, 2003; Tullet *et al.*, 2008; Wang *et al.*, 2007a).

The nematode model is also amenable to interesting genetic alterations. Hence, it is very easy to generate transgenic worms expressing any kind of mutant recombinant protein, providing means for the study of neurodegenerative diseases (see additional discussion below). Gene KO and altered function mutations are in many cases available from the Gene Knockout Consortium or the National BioResource Project of Japan (currently ~1/3 of the ~20,000 total genes in *C. elegans*; Antoshechkin and Sternberg, 2007) or alternatively are conveniently generated using chemicals, radiations, or transposons (discussed below under *Caenorhabditis elegans* and Genotoxicity). Hence, classical approaches to elucidate intracellular pathways in *C. elegans* include forward and modifier screens following random mutagenesis (Inoue and Thomas, 2000; Malone and Thomas, 1994; Morck *et al.*, 2003; Nass *et al.*, 2005; O'Connell *et al.*, 1998). Finally *C. elegans* is amenable to gender manipulation (possible generation of males, feminized males, masculinized hermaphrodites, or feminized hermaphrodites) permitting studies on sex specificity mechanisms of neurotoxicants or disorders and "rejuvenation" by forcing development through the quiescent dauer larval stage (Houthoofd *et al.*, 2002).

Neurotoxicological Studies in C. elegans

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.

Toxicity Mechanisms of Neurotoxic Metals in C. elegans

Caenorhabditis elegans has been used as a model system to elucidate the toxicity and toxicological mechanisms of various heavy metals, such as Aluminum (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Copper (Cu), Lead (Pb), Mercury (Hg), Uranium (U), and Zinc (Zn). In general, these studies focused on various toxic end points, such as lethality, reproduction, life span, and protein expression. Some focus has also been directed to the effects of these metals on the nervous system by assessing behavior, reporter expression and neuronal morphology. We provide here a few examples of these approaches.

Investigators have performed numerous studies to assess behavior-induced alterations following exposure of the worm to heavy metals. Depending on the end point assessed, neurotoxic effects on specific neuronal circuitries can be inferred.

For instance, a defect in locomotion reflects an impairment of the neuronal network formed by the interneurons AVA, AVB, AVD, and PVC providing input to the A- and B-type motor neurons (responsible for forward and backward movement) and the inhibitory D-type motor neurons involved in the coordination of movement (Riddle *et al.*, 1997). By recording short videos and subsequently analyzing them using computer tracking software, it has been possible to quantify the overall movement of *C. elegans* (distance traveled, directional change, etc.), body bends and head thrashes, upon metal treatments, allowing to further correlate the data with damages to neuron circuitries. These computer tracking studies showed that worms displayed a dose-dependent decrease in locomotory movement upon exposure to Pb (Anderson *et al.*, 2001, 2004; Johnson and Nelson, 1991) and Al (Anderson *et al.*, 2004), while an increase in locomotion was observed upon exposure to low concentrations of Hg as compared with Cu (Williams and Dusenbery, 1988). Another study showed that exposure to Ba impaired both body bend and head thrashing rates in a dose-dependent manner (Wang *et al.*, 2008), corroborating mammalian data on the effect of Ba on the nervous system attributed to its ability to block potassium channels (Johnson and Nelson, 1991).

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 and Candido, 1999).

Behavioral research studying the effect of heavy metals on *C. elegans* has also taken the route of assessing the ability of the worm to sense the toxin and alter its behavior accordingly, involving other neural circuitry, such as the amphid and phasmid neurons responsible for chemosensation (Riddle *et al*., 1997). By generating concentration gradient–containing plates, Sambongi *et al*. (1999) discovered that *C. elegans* was able to avoid Cd and Cu but not Ni and that the amphid ADL, ASE, and ASH neurons were responsible for this avoidance as their combined ablation eliminated the avoidance phenotype. Furthering the investigation into the role of ASH neurons, researchers found that a calcium (Ca^{2+}) influx could be elicited upon exposing the *C. elegans* to Cu, which may provide insight into the mechanism of the ability of the worm to display avoidance behaviors (Hilliard *et al*., 2005).

Caenorhabditis elegans exhibits both short-term and long-term learning-related behaviors in response to specific sensory inputs (Rankin *et al*., 1990), which involve defined neuronal networks. As an example, thermosensation-associated learning and memory rely on the AFD sensory neuron sending inputs to the AIY and AIZ interneurons, whose signals are integrated by the RIA and RIB interneurons to command the RIM motor neuron (Mori *et al*., 2007). When assessing the function of this circuitry, worms grown and fed at a definite temperature are moved to a food-deprived test plate exposed to a temperature gradient. The ability of the worms to find and remain in the area of the test plate corresponding to the feeding temperature reflects the functioning of the thermosensation learning and memory network aforementioned (Mori *et al*., 2007). Interestingly, worms exposed to Al and Pb exhibit poor scores at this test, indicative of a significant reduction of the worms' learning ability (Ye *et al*., in press). This recapitulates the learning deficits observed in young patients overexposed to the same metals (Garza *et al*., 2006; Goncalves and Silva, 2007).

While behavioral testing was informative of the neuronal circuitries affected by heavy metals, additional experiments uncovered the molecular mechanisms of their neurotoxic effects. For example, in the previously described study, after determining that Al and Pb induced memory deficits, the investigators showed that the antioxidant vitamin E effectively reversed these deficits, indicating a role of oxidative stress in Al and Pb neurotoxicity (Ye *et al*., in press). The involvement of oxidative stress in metal-induced toxicity was further confirmed when worms mutated in glutamylcysteine synthetase (*gcs-1*), the rate-limiting enzyme in glutathione synthesis exhibited hypersensitivity to As exposure when compared to wild-type animals (Liao and Yu, 2005).

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).

Observation of neuron morphology following heavy metal exposure was also performed using *C. elegans* strains expressing the green fluorescent protein (GFP) in discrete neuronal populations. Tests using depleted U evoked no alterations in the DAergic nervous system of *C. elegans*, an observation corroborated with data from mammalian primary neuronal cultures (Jiang *et al*., 2007). Meanwhile, *kel-8* and *numr-1*, which are involved in resistance to Cd toxicity, were upregulated upon Cd exposure. In particular, GFP levels of KEL-8::GFP and NUMR-1::GFP were increased in the pharynx and the intestine in addition to the constitutive expression observed in AWA neurons (Cui *et al*., 2007a; Freedman *et al*., 2006; Jackson *et al*., 2006; Tvermoes and Freedman, 2008). Furthermore, *numr-1* was shown to be induced in response to heavy metals, such as Cd, Cu, Cobalt (Co), Chromium (Cr), Ni, As, Zn, and Hg. NUMR-1::GFP was localized to nuclei within the intestine and the pharynx and colocalized with the stress-responsive heat-shock transcription factor HSF-1::mCherry (Tvermoes and Freedman, 2008). This indicates that these particular genes were altered in response to heavy metals and this may aid in the understanding of the toxicity of or the protection against these agents.

Toxicity Mechanisms of Neurotoxic Pesticides in C. elegans

Currently, there are over a hundred types of pesticides available and substantial efforts have been put forth to examine the neurotoxicity of these agents. Similarity in neural circuitry and the conservation in genetic makeup between *C. elegans* and humans have led to a number of recent studies on pesticide neurotoxicity in this species (summarized in Table 4). In this section, we discuss the effects of three groups of pesticides on neurological pathways in *C. elegans* and their relevance to understanding mechanisms of human neurotoxicity.

Paraquat, also known as methyl viologen (*mev*), is mainly used as an herbicide. Increased concerns for the potential human risks associated with paraquat exposure stems from studies indicating that subjects experiencing exposure to this and other herbicides/insecticides have a higher prevalence of Parkinson disease (PD) (Liou *et al*., 1997; Semchuk *et al*., 1992) (Gorell *et al*., 1998) and increased mortality from PD (Ritz and Yu, 2000). The use of *C. elegans* to study the etiology of PD will be discussed in the later section. This is due to the specificity with which these pesticides target the nigrostriatal DAergic system via an elevation of dopamine and amine turnover (Thiruchelvam *et al*., 2000a, 2000b). All forms of paraquat are easily reduced to a radical ion, which generates superoxide radical that reacts with unsaturated membrane lipids (Uversky, 2004), a likely mechanism of

TABLE 4
Pesticides that Have Been Tested Using *Caenorhabditis elegans* as a Model Organism

Compound	Strains investigated	Observations	References
Paraquat	*mev-1(kn1)*[a], *mev-2(kn2)*[a]	Hypersensitive to oxygen and paraquat, decreased SOD activity[b]	Ishii *et al.* (1990)
	rad-8(mn162)	Hypersensitive to oxygen and paraquat, reduced fecundity, decreased life span	Ishii *et al.* (1993)
	age-1(hx542), *age-1(hx546)*	Increased catalase and Cu/Zn SOD activity, increased life span	Vanfleteren (1993)
		Vitamin E (antioxidant) inhibits oxidative damage from paraquat[b]	Goldstein and Modric (1994)
	mev-1(kn1), *rad-8(mn162)*	Paraquat and high oxygen content inhibit development, inversely proportional to life span	Hartman *et al.* (1995)
	age-1(hx546), *daf-16(m26)*, *mev-1(kn1)*[a]	Increased resistance to paraquat and heat, extended life span, increased SOD, and catalase mRNA level only in *age-1* mutant, but not *daf-16 or mev-1*	Yanase *et al.* (2002)
	mev-5(qa5005)[a], *mev-6 (qa5006)*[a], *mev-7(qa5007)*[a]	Longevity and sensitivity to paraquat, UV or heat do not correlate	Fujii *et al.* (2005)
	mev-1(kn1), *gas-1(fc21)*	Overproduction of superoxide anion in submitochondrial particles upon paraquat exposure	Kondo *et al.* (2005)
	skn-1(zu67)	Activation of SKN-1 transcription factor, localizes to the nucleus following paraquat exposure	Kell *et al.* (2007)
	daf-2(e1370)	Extended animal life span and increased resistance to ROS produced by paraquat	Kim and Sun (2007); Yang *et al.* (2007)
	Overexpression of GSTO, *gsto-1* RNAi	Increased resistance to paraquat-induced oxidative stress	Burmeister *et al.* (2008)
Rotenone	*gas-1(fc21)*	Increased sensitivity to rotenone under hyperoxia	Ishiguro *et al.* (2001)
	pdr-1, *djr-1.1* RNAi	Increased vulnerability to rotenone	Ved *et al.* (2005)
	Overexpression of LRRK2, *lrk-1* RNAi	Overexpression of wild-type LRRK2 strongly protects against rotenone toxicity	Wolozin *et al.* (2008)
Ops	N2	Computer tracking system is a promising tool for assessing neurobehavioral changes associated with OP toxicity	Williams and Dusenbery 1990
		Cholinesterase inhibition associated with high behavioral toxicity	Cole *et al.* (2004)
		Absorption effects are more prominent than biodegradation in soil toxicity tests	Saffih-Hdadi *et al.* (2005)
Carbamates	N2	Rank order of toxicity of carbamate pesticides in *C. elegans* correlates well with values for rats and mice, and degree of behavioral alteration correlates with AChE inhibition	Melstrom and Williams (2007)
Bt toxin	*bre-1(ye4)*, *bre-2(ye31)*, *bre-3 (ye28)*, *bre-4(ye13)*, *bre-5(ye17)*	Extensive damage to gut, decreased fertility, and death	Marroquin *et al.* (2000)
	bre-5(ye17)	Increased resistance to *Bt* toxin	Griffitts *et al.* (2001)
	bre-2(ye31), *bre-2(ye71)*, *bre-3(ye28)*, *bre-4(ye13)*	*Bt* toxin resistance involves the loss of glycosyltransferase in the intestine	Griffitts *et al.* (2003)
	glp-4(bn2), *kgb-1(um3)*, *jnk-1(gk7)*, *sek-1(km4)*	*Bt* toxin reduces brood size and causes damage to the intestine	Wei *et al.* (2003)
		A p38 MAPK and a c-Jun N-terminal-like MAPK are both transcriptionally upregulated by *Bt* toxin	Huffman *et al.* (2004a, 2004b)
		Survival rate, infection level, and behavior differred in *C. elegans* isolated from geographically distinct strains	Schulenburg and Muller (2004)
	bre-2(ye31), *bre-3(ye28)*, *bre-4(ye13)*, *bre-5(ye17)*	*Bt* toxin resistance entails loss of glycolipid carbohydrates and the toxin directly and specifically binds to Glycolipids	Griffitts *et al.* (2005)
	bre-3(ye28)	Resistance to *Bt* toxin develops as a result of loss of glycolipid receptors for the toxin	Barrows *et al.* (2006)
	bre-1(ye4), *bre-2(ye31)*	Resistance to toxin is achieved by mutations in gylcosyltransferase genes that glycosylate glycolipid or with a loss of the monosaccharide biosynthetic pathway	Barrows *et al.* (2007a, 2007b)
	daf-2(e1370), *daf-2(e1368)*, *age-1 (hx546)*, *daf-16(mgDf50)*, *daf-2(0(m26)*	Mutations in the insulin-like receptor pathway lead to distinct behavioral responses, including the evasion of pathogens and reduced ingestion	Hasshoff *et al.* (2007)
		Reproduction and growth significantly reduced by *Bt* toxin	Hoss *et al.* (2008)
Captan	*hsp-16.48;hsp-16.1::lacZ*	Stress induction localized to muscle cells of the pharynx	Jones *et al.* (1996)
		Inhibits feeding, cessation of muscular contraction	
Dithiocarbamate fungicides	*hsp-16.48;hsp-16.1::lacZ*	Induction of stress response	Guven *et al.* (1999)
Organochlorinated pesticides	N2	Decreased sensitivity to organochlorinated pesticide in *C. elegans* than other soil invertebrates. Compared to other organic pollutants tested, organochlorinated pesticides are the most toxic substances in soil or aquatic medium	Bezchlebova *et al.* (2007); Sochova *et al.* (2007)

Note. MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species.
[a]These mutants showed defective dye filling, indicative of chemosensory neuron damage.
[b]SOD, superoxide dismutase.

neurotoxicity. *Caenorhabditis elegans*, has a well-defined, yet simple DAergic network, consisting of eight neurons in the hermaphrodite and an additional six neurons located in the tail of the male (Chase and Koelle, 2007) and four DA receptors. Dopamine is known to be required in the modulation of locomotion and in learning in *C. elegans* (Hills *et al.*, 2004; Sanyal *et al.*, 2004; Sawin *et al.*, 2000). To date, several paraquat/*mev*–altered strains have been generated to study potential pathways in which paraquat exerts its toxic effects. *mev-1* (mutated for the succinate dehydrogenase) (Hartman *et al.*, 1995; Ishii *et al.*, 1990; Kondo *et al.*, 2005) and *mev-3* (Yamamoto *et al.*, 1996) were generated first, and both strains displayed increased sensitivity to paraquat- and oxygen-mediated injury as a result of increased production of superoxide radicals (Guo and Lemire, 2003; Ishii *et al.*, 1990) and hypersensitivity to oxidative stress. *mev-4* (Fujii *et al.*, 2004), *mev-5*, *mev-6*, and *mev-7* (Fujii *et al.*, 2005) displayed resistance to paraquat. However, since the proteins that are encoded by these genes are currently unknown, future mapping of these genes will likely reveal pathways involved in paraquat toxicity.

Paraquat exerts oxidative damage in vertebrates, which has also been corroborated in *C. elegans*. Mutants that lack antioxidant enzymes such as cytosolic or mitochondrial superoxide dismutases (*sod-1* and *sod-2*) show increased sensitivity to paraquat (Yang *et al.*, 2007), whereas mutants with increased superoxide dismutase levels, such as *age-1* (encoding the catalytic subunit of phosphoinositide 3-kinase) (Vanfleteren, 1993; Yanase *et al.*, 2002) and worms over-expressing the omega-class glutathione transferase *gsto-1* (Burmeister *et al.*, 2008) display increased resistance to paraquat toxicity. Moreover, *C. elegans* mutants hypersensitive to oxygen toxicity, such as *rad-8* (Honda *et al.*, 1993; Ishii *et al.*, 1990) or those with a prolonged life span, such as *daf-2* (encoding insulin/insulin growth factor receptor) (Bardin *et al.*, 1994; Kim and Sun, 2007) show increased tolerance to paraquat. Taken together, these results provide novel information on mechanisms by which paraquat mediates its toxicity (by enhancing sensitivity to oxygen toxicity with an elevation in production of reactive oxygen species and shortening life span) and provide directions for future investigations on mechanisms that lead to DAergic neurodegeneration.

A second ubiquitous pesticide is rotenone; it is a naturally occurring and biodegradable pesticide effective in killing pests and fish (Uversky, 2004). Researchers first reported in 2000 that Iv exposure to rotenone may lead in humans to the development of PD-like symptoms accompanied by the selective destruction of nigral DAergic neurons (Betarbet *et al.*, 2000). Since rotenone acts by inhibiting mitochondrial NADH dehydrogenase within complex I (Gao *et al.*, 2003), the development of a mutant *C. elegans* strain that exhibits mitochondrial inhibition provided an experimental platform where the role of this enzyme could be directly evaluated. A mutation in a 49-kDa subunit of mitochondrial complex I in *C. elegans* mutant *gas-1* displays hypersensitivity to rotenone and oxygen (Ishiguro *et al.*, 2001), highlighting the importance of a functional complex I in rotenone resistance. Moreover, *C. elegans* with alterations in PD causative genes are highly sensitive to rotenone toxicity, suggesting the ability of these proteins to protect against rotenone-induced oxidative damage in DAergic neurons (Ved *et al.*, 2005; Wolozin *et al.*, 2008) (see neurodegenerative disease section below).

The organophosphates (OPs) are a group of insecticides that target the cholinergic system. ACh is the primary neurotransmitter involved in motor function in most organisms, including the nematode (Rand and Nonet, 1997). Due to the involvement of the neuromuscular system, a computer tracking system was used to study the neurobehavioral changes in *C. elegans* associated with two OP pesticides (malathion and vapona). *Caenorhabditis elegans* showed a remarkable decline in locomotion at a concentration below survival reduction (Williams and Dusenbery, 1990b). Comparison studies using similar behavioral analyses were later developed to assess movement alteration as an indicator of the neurotoxity of 15 OP pesticides (Cole *et al.*, 2004) and carbamate pesticides, which unlike OP pesticides are reversible AChE inhibitors (Melstrom and Williams, 2007). The LD_{50} values in *C. elegans* closely correlated with LD_{50} in both rats and mice. Pesticides (vapon, parathion, methyl parathion, methidathion, and fun-sulfothion) that showed cholinesterase inhibition were associated with pronounced behavioral toxicity (i.e., decrease in movement). A recent study has compared end points using OPs and found AChE inhibition to be the most sensitive indicator of toxicity but also the most difficult to measure (Rajini *et al.*, in press). Reduction in movement for 10 OPs was found to correlate to rat and mouse acute lethality data. Finally, simulation studies examining the rate of absorption and biodegradation of OP (parathion) also (Saffih-Hdadi *et al.*, 2005) establish the relevance and reliability of *C. elegans* as an experimental model and predictor for soil toxicity.

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).

PD is a progressive, neurodegenerative disorder afflicting ~2% of the U.S. population (Bushnell and Martin, 1999). Characteristic features include a gradual loss of motor function due to the degeneration of DAergic neurons within the *substantia nigra pars compacta* and loss of DAergic terminals in the striatum (Wilson *et al.*, 1996). At the cellular level,

deposition of cytoplasmic Lewy bodies composed of aggregated protein, such as α-synuclein, is observed. PD cases are referred as familial (FPD) or idiopathic (IPD) depending on whether the disease is hereditary (FPD) or from unknown origin, possibly due to environmental exposure to neurotoxicants (IPD) (Dauer and Przedborski, 2003; Samii *et al*., 2004). Among 11 genomic regions (PARK1 to 11) associated with FPD, 7 were narrowed down to single genes: *PARK1* (*α-SYNUCLEIN*), *PARK2* (*PARKIN*), *PARK4* (*α-SYNUCLEIN*), *PARK5* (*UCHL1*), *PARK6* (*PINK1*), *PARK7* (*DJ1*), *PARK8* (*DARDARIN*/*LRRK2*), and *PARK9* (*ATP13A2*) (Wood-Kaczmar *et al*., 2006). All but *α-SYNUCLEIN* are strictly conserved in the nematode with most residue positions mutated in PD patients encoding identical amino acids in *C. elegans* orthologues (Benedetto *et al*., 2008). Worms overexpressing wild type, mutant A30P, or A53T human α-SYNUCLEIN in DAergic neurons show differential levels of injury, including reduced DA content, DAergic neuron degeneration, motor deficits reversible by DA administration, intracellular α-SYNUCLEIN aggregates similar to Lewy bodies, and increased vulnerability to mitochondrial complex-I inhibitors, which is reversed by treatment with antioxidants (Kuwahara *et al*., 2006; Lakso *et al*., 2003; Ved *et al*., 2005). Furthermore, deletion (Springer *et al*., 2005) and knockdown of the *C. elegans PARKIN* and *DJ1* genes produce similar patterns of pharmacological vulnerability as those described above for *α-SYNUCLEIN* overexpression (Ved *et al*., 2005). Other PD genes in *C. elegans* have been investigated. For example, *ubh-1* and *ubh-3* (Chiaki Fujitake *et al*., 2004) share similar functions with the human *PARK5*/*UCHL1* orthologue. Studies on other genes have been instrumental in unraveling previously unknown functions. For example, examination of the *PARK8*/*DARDARIN* orthologue *lrk-1* showed that the protein allows the proper targeting of synaptic vesicle proteins to the axon (Sakaguchi-Nakashima *et al*., 2007) and protects against rotenone-induced mitochondrial injury (Wolozin *et al*., 2008). Recently, RNAi, genomic, and proteomic approaches using human *α-SYNUCLEIN* transgenic worms identified genetic networks linking PD to G-protein signaling, endomembrane trafficking, actin cytoskeleton, and oxidative stress (Cooper *et al*., 2006; Gitler *et al*., 2008; Hamamichi *et al*., 2008; Ichibangase *et al*., 2008; van Ham *et al*., 2008; Vartiainen *et al*., 2006), illustrating the power of this transgenic model for PD study.

Nonhereditary PD cases have also been associated with exposure to 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine, a designer drug that is converted intracerebrally (by astrocytes) to 1-methyl-4-phenylpyridinium (MPP+) by the monoamine oxygenase B. MPP+ damages the DAergic nervous system, leading to a typical Parkinsonian syndrome (Kopin and Markey, 1988; Langston *et al*., 1984). Similarly, MPP+-exposed *C. elegans* show specific degeneration of DAergic neurons and associated behavioral defects (Braungart *et al*., 2004), which is due to ATP depletion (Wang *et al*., 2007b). Exposures to rotenone (see above) or 6-hydroxydopamine also lead to PD syndromes that share similar features both in humans and worms (Cao *et al*., 2005; Ishiguro *et al*., 2001; Marvanova and Nichols, 2007; Nass *et al*., 2002, 2005; Ved *et al*., 2005). Though the nematode does not truly exhibit PD-like symptoms, results with transgenic and drug-exposed worms emphasize the relevance of *C. elegans* as a model organism that (1) permits rapid insights in the genetic pathways involved in PD and (2) enables high-throughput screening methods for the development of new anti-PD drugs (Schmidt *et al*., 2007).

Tauopathies and polyglutamine extension disorders have also been investigated in the worm using mutants and transgenic strains (Brandt *et al*., 2007; Dickey *et al*., 2006, Link, 2001; Kraemer *et al*., 2003, 2006, and Kraemer and Schellenberg, 2007). The first AD-associated proteins identified were the beta-amyloid peptide precursor (betaAPP) and the presenilins PS1 and PS2. Study of the *C. elegans* presenilin orthologues *sel-12* (Baumeister *et al*., 1997; Levitan and Greenwald, 1995) and *hop-1* (Li and Greenwald, 1997; Smialowska and Baumeister, 2006) linked AD to the apoptotic pathway (Kitagawa *et al*., 2003) and Notch signaling, which was later confirmed in vertebrates (Berezovska *et al*., 1998, 1999; Ray *et al*., 1999). Characterization of the *C. elegans* betaAPP orthologue revealed a key role for microRNA in AD gene regulation (Niwa *et al*., 2008). However, most of the knowledge about AD acquired in *C. elegans* came from two transgenic models: worms expressing the human betaAPP (Boyd-Kimball *et al*., 2006; Drake *et al*., 2003; Gutierrez-Zepeda and Luo, 2004; Wu and Luo, 2005; Wu *et al*., 2006) or TAU (Brandt *et al*., in press; Kraemer *et al*., 2003). Studies on betaAPP transgenic worms revealed toxicity mechanisms of AD by identifying two new genes, *aph-1* and *pen-2*, likely involved in the progression of the disease (Boyd-Kimball *et al*., 2006; Francis *et al*., 2002). They also allowed the characterization of oxidation processes preceding fibrillar deposition (Drake *et al*., 2003) and the identification of genes activated upon induction of betaAPP expression (Link *et al*., 2003). Furthermore, protective mechanisms were identified (Florez-McClure *et al*., 2007; Fonte *et al*., 2008) and potential therapeutic drugs for AD (ginkgolides, *Ginkgo biloba* extract EGb 761, soy isoflavone glycitein) were originally and successfully assayed in worms (Gutierrez-Zepeda *et al*., 2005; Luo, 2006; Wu *et al*., 2006). *Caenorhabditis elegans* overexpressing the human TAU or a pseudohyperphosphorylated mutant TAU were found to exhibit age-dependent motor neuron dysfunctions, neurodegeneration, and locomotor defects due to impaired neurotransmission (Brandt *et al*., 2007; Kraemer *et al*., 2003).

Likewise, while a few Huntingtin (Htt)-interacting genes were identified in *C. elegans* (Chopra *et al*., 2000; Holbert *et al*., 2003), most data came from transgenic worms expressing polyQ variants of Htt. Several groups targeted different neuronal subsets to study polyQHtt neurotoxicity in the worm. They described behavioral defects prior to neurodegeneration and protein aggregation and axonal defects and uncovered a role for apoptosis in HD neurodegeneration (Bates *et al*., 2006; Faber

et al., 1999; Holbert *et al*., 2003; Parker *et al*., 2001). Protective mechanisms of the polyQ enhancer-1 and ubiquilin were demonstrated (Faber *et al*. 2002; Wang *et al*., 2006), and pharmacological screening using polyQHtt transgenic *C. elegans* is ongoing (Faber *et al*. 2002; Wang *et al*., 2006).

A final illustration of the successful use of *C. elegans* in elucidating the genetic basis of neurodegenerative disorder is exemplified by the characterization of the genetic network implicated in DMD. DMD is mainly characterized by a progressive loss of muscular mass and function occurring in males due to mutations in the *DYSTROPHIN* gene located on the X chromosome, which commonly leads to paralysis and death by the age of 30. DYSTROPHIN is both muscular and neuronal, being required for brain architecture and neurotransmission, such that DMD patients exhibit neurodegeneration associated with motor deficits and reduced cognitive performances (average IQ is 85 in DMD boys) (Anderson *et al*., 2002; Blake and Kroger, 2000; Poysky, 2007). DYSTROPHIN is conserved in *C. elegans*, but its loss-of-function in the worm results in hypercontractility due to impaired cholinergic activity and does not affect muscle cells (Bessou *et al*., 1998; Gieseler *et al*., 1999b). Nevertheless, the observation that double mutants for *Dystrophin*/*dys-1* and *MyoD*/*hlh-1* display severe and progressive muscle degeneration in the worm (as observed in mice), set up the basis for a *C. elegans* model to study dystrophin-dependent myopathies (Gieseler *et al*., 2000). Using this model, several partners of DYSTROPHIN were characterized, establishing their role in cholinergic neurotransmission and muscle degeneration (Gieseler *et al*., 1999a, 1999b, 2001; Grisoni *et al*., 2002a, 2002b, 2003). Additionally, it was shown that the overexpression of *DYSTROBREVIN*/*dyb-1* delays neurological and muscular defects (Gieseler *et al*., 2002), and mutations in *CHIP*/*chn-1*, chemical inhibition of the proteasome, and prednisone or serotonin treatments suppress muscle degeneration in *C. elegans* (Carre-Pierrat *et al*., 2006; Gaud *et al*., 2004; Nyamsuren *et al*., 2007).

Thus, though at first glance *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.

Caenorhabditis 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

Genes and pathways involved in DNA repair in mammals are generally well conserved in *C. elegans* (Boulton *et al*., 2002; Hartman and Nelson, 1998; O'Neil and 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 translesion DNA polymerases, helicases, 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 and 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 and 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 aflatoxin 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 involving biochemical analyses of DNA repair activities (Dequen *et al*., 2005a; Gagnon *et al*., 2002; Hevelone and Hartman, 1988; Kanugula and Pegg, 2001; Munakata and Morohoshi, 1986; Shatilla *et al*., 2005a, 2005b; Shatilla and Ramotar, 2002) as well *in vivo* sensitivity to DNA damaging agents (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 phenotypes (Aoki *et al*., 2000; Kelly *et al*., 2000; Sadaie and 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.

Apoptosis and Cell Cycle Checkpoints in C. elegans

DNA damage that is not repaired can trigger cell cycle arrest and apoptosis, and these pathways are very well studied in *C. elegans*. The great progress made in understanding them mechanistically demonstrates the power of this model organism. As mentioned, the cellular mechanisms regulating apoptosis were discovered in *C. elegans*, and apoptosis and cell cycle responses to DNA damage continue to be heavily studied in *C. elegans* (Ahmed *et al*., 2001; Ahmed and Hodgkin, 2000; Conradt and Xue, 2005; Gartner *et al*., 2000; Jagasia *et al*., 2005; Kinchen and Hengartner, 2005; Lettre and Hengartner, 2006; Olsen *et al*., 2006; Schumacher *et al*., 2005; Stergiou *et al*., 2007). The short life span of *C. elegans* has especially lent itself to groundbreaking studies on the mechanisms of germ line immortality (Ahmed, 2006; Ahmed and Hodgkin, 2000). Another important advantage of *C. elegans* is the ability to easily study *in vivo* phenomena such as age- or developmental stage–related differences in DNA repair capacity. For example, Clejan *et al*. (2006) showed that the error-prone DNA repair pathway of nonhomologous end joining has little or no role in the repair of DNA double-strand breaks in germ cells but is functional in somatic cells. Holway *et al*. (2006) showed that checkpoint silencing in response to DNA damage occurs in developing embryos but not in the germ line. Both these findings are important in our understanding developmental exposure to genotoxins in that they suggest a special protection for germ line cells.

DNA Damage–Related Pathological Processes in C. elegans

DNA damage–related pathological processes including carcinogenesis (He *et al*., 2007; Kroll, 2007; Pinkston-Gosse and Kenyon, 2007; Poulin *et al*., 2004; Sherwood *et al*., 2005; van Haaften *et al*., 2004a), aging (Antebi, 2007; Brys *et al*., 2007; Hartman *et al*., 1988; Johnson, 2003; Kenyon, 2005; Klass, 1977; Klass *et al*., 1983; Murakami, 2007; Rea *et al*., 2007; Ventura *et al*., 2006), and neurodegenerative diseases (described above) are also areas of active research in *C. elegans*. This research has both established the relevance of *C. elegans* as a model for the study of genotoxic agents (due to conservation of the DNA damage response) and enormously increased its utility in such studies by providing a wealth of complementary and contextual biological information related to the pathological responses to DNA damage in this organism.

Tools for the Study of DNA Damage, Repair, and Mutation in C. elegans

Caenorhabditis elegans is an excellent model for studies of genotoxicity due to the plethora of powerful tools available. Genetic manipulation via RNAi and generation of KOs or other mutants is relatively straightforward. If suitable mutants are not already available, they can be generated by a variety of approaches. These include untargeted and targeted methods, including chemical mutagenesis, transposon insertion, and biolistic transformation (Anderson, 1995; Barrett *et al*., 2004; Berezikov *et al*., 2004; Plasterk, 1995; Plasterk and Groenen, 1992; Rushforth *et al*., 1993).

Assays for the measurement of mutagenesis, DNA damage and repair, and transcriptional activity have also been developed for genotoxicity assessment in *C. elegans* (Table 5). Some DNA damage and repair assays in *C. elegans* can be carried out with as few as one or a few individual nematodes, permitting studies of interindividual differences and permitting high-throughput screening of DNA- damaging agents or genes involved in DNA repair. It is also possible, using PCR- or Southern blot–based methods, to distinguish damage and repair in different genomic regions and genomes (i.e., mitochondrial vs. nuclear DNA; (Hyun *et al*., 2008; Meyer *et al*., 2007)). Mutagenesis has been studied by a variety of methods (Table 5) including phenotype-based genetic mutation reversion screens, an out-of-frame LacZ transgene reporter, and direct sequencing.

Genotoxin Studies in C. elegans

Unlike the case of neurotoxicology, there have so far been relatively few studies of genotoxicity *per se* 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 and 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*.,

TABLE 5
Genotoxicity Assays Available for the *Caenorhabditis elegans* Model

Endpoint	Assay	Principle	References
A. Mutagenesis	Direct sequencing	The mutation rate of a given locus is calculated using data from DNA sequencing.	Denver *et al.* (2000, 2004, 2006)
	''Big blue worms''	Transgenic *C. elegans* carrying an out-of-frame LacZ reporter gene expresses blue pigment upon frameshift or insertion/deletion mutations.	Pothof *et al.* (2003); Tijsterman *et al.* (2002)
	Reversion assay	Mutants with an easily scored phenotype (e.g., uncoordinated movement) are exposed to a chemical of interest; the restoration of a normal phenotype indicates mutagenesis.	Degtyareva *et al.* (2002); Greenwald and Horvitz (1980); Hartman *et al.* (1995)
	Lethality assay	The lethality of transgenic, mutation-sensitive *C. elegans* was measured for mutagen detection	Rosenbluth *et al.* (1983); Rosenbluth *et al.* (1985)
B. DNA damage and repair	PCR-based assay	The amount of PCR product is inversely proportional to the amount of DNA damage on a given length of template	Meyer *et al.* (2007); Neher and Sturzenbaum (2006)
	Southern blot	T4 endonuclease–sensitive sites in specific genes (identified by genomic DNA sequence) indicate the presence of UV photodimers	Hyun *et al.* (2008)
	Immunoassay	Antibodies to specific UV photoproducts are identified	Hartman *et al.* (1989)
	Enzymatic activity	A diagnostic enzymatic activity is measured *in vitro*	Shatilla and Ramotar (2002)
	Reproduction/development assay with KO mutants	Specific DNA damage (e.g., DNA adduct) can be tested using simple reproduction/development assays with mutants lacking a specific DNA repair pathway (e.g., nucleotide excision repair)	Park *et al.* (2002, 2004)
C. Transcriptional activities	RNA: DNA ratio	A decrease in RNA: DNA ratio indicates the inhibition of transcriptional activities	Ibiam and Grant (2005)

2005a; Johnson and Hartman, 1988; Stergiou *et al.*, 2007; Weidhaas *et al.*, 2006), heavy metals (Cui *et al.*, 2007b; Neher and Sturzenbaum, 2006; Wang *et al.*, 2008), methylmethanesulphonate (Holway *et al.*, 2006), polycyclic aromatic hydrocarbons (Neher and Sturzenbaum, 2006), photosensitizers (Fujita *et al.*, 1984; Hartman and Marshall, 1992; Mills and Hartman, 1998), and prooxidant compounds (Astin *et al.*, 2008; Hartman *et al.*, 2004; Hyun *et al.*, 2008; Salinas *et al.*, 2006). Studies have taken advantage of the utility of *C. elegans* as an *in vivo* model; for example, it was shown that nucleotide excision repair slowed in aging individuals (Meyer *et al.*, 2007) and that longer lived and stress-resistant strains have faster nucleotide excision repair (Hyun *et al.*, 2008) than do wild type. It has been possible to identify cases in which UV resistance was correlated to life span (Hyun *et al.*, 2008; Murakami and Johnson, 1996), and others in which it was not (Hartman *et al.*, 1988), so that theories about the relationship of DNA damage and repair with aging can be directly tested. Studies of aging populations or individuals are slow and expensive in mammalian models and impossible *in vitro*.

High-Throughput Approaches with C. elegans

High-throughput screening has two specific definitions in toxicology: (1) genome-wide screens for molecular targets or mediators of toxicity and (2) rapid, high-content chemical screens to detect potential toxicants. A genome-wide screen can serve as a hypothesis-finding tool, providing a direction for further mechanistic investigation. This approach is particularly useful for studying any toxicant with a poorly understood mechanism of action. Genome-wide screens can be done using forward genetics, DNA microarrays, or genome-wide RNAi in *C. elegans*.

High-throughput chemical screening, in comparison, has been proposed as a quicker and less expensive method for toxicity testing (Gibb, 2008). The conventional animal testing used by companies or agencies is labor intensive and time consuming, resulting in a large number of toxicants not being tested at all. It is estimated, for instance, that there are more than 10,000 environmental chemicals from several Environmental Protection Agency programs that require further testing (Dix *et al.*, 2007). The objective of high-throughput chemical screening is to shortlist chemicals showing high toxicity, thereby setting priority for regulations as well as further toxicity testing in mammalian models.

High-throughput screening is feasible with *C. elegans* due to its experimental manipulability as well as several automation technologies. *Caenorhabditis elegans* is easy to handle in the laboratory; it can be cultivated on solid support or in liquid, in Petri dishes, tubes, or 6-, 12-, 24-, 96-, or 384-well plates. It can also be exposed to toxicants acutely or chronically by injection, feeding, or soaking. Automated imaging methods for absorbance, fluorescence, movement, or morphometric

measurement have been developed since the late 1980s (Baek *et al*., 2002; Bennett and Pax, 1986; Hoshi and Shingai, 2006; Simonetta and Golombek, 2007; Tsibidis and Tavernarakis, 2007; Williams and Dusenbery, 1990b). Nowadays, cell sorters adapted to sort worms based on morphometric parameters or expression of fluorescent proteins combined with imaging platforms have been successfully used for large-scale promoter expression analyses and drug screening purposes (Burns *et al*., 2006; Dupuy *et al*., 2007; Pulak, 2006). Recently, a microfluidic *C. elegans* sorter with three dimensional subcellular imaging capabilities was developed, allowing high-throughput assays of higher complexity (Rohde *et al*., 2007).

While the simplicity and manipulability of the *C. elegans* system enables high-throughput approaches, it also leads to several potential disadvantages in toxicology studies. *Caenorhabditis elegans* exhibits important metabolic differences compared to vertebrates. For example, *C. elegans* is highly resistant to benzo[*a*]pyrene (Miller and Hartman, 1998), likely because it does not metabolize the chemical (M. Leung and J. Meyer, unpublished data). This problem can be potentially solved, however, by expressing the vertebrate cytochrome P450s in *C. elegans*. The impermeable cuticle layer as well as selective intestinal uptake, furthermore, may block the entry of chemicals, thereby necessitating high exposure doses to impact the worm's physiology. A mutant strain (*dal-1*) has recently been isolated that is healthy under laboratory conditions but exhibits altered intestinal morphology and increased intestinal absorption of a wide range of drugs (C. Paulson and J. Waddle, personal communication). The resultant-increased vulnerability of this strain to the toxic or pharmacological activities of tested compounds has the potential to increase the sensitivity of the *C. elegans* system.

Forward Genetics Screens in C. elegans

Forward genetics refers to the study of genes based on a given phenotype. In a forward genetics screen, *C. elegans* are treated with a mutagen, as described above. Mutant strains are then exposed to a toxicant and are screened for increased resistance or sensitivity. Once a resistant or hypersensitive mutant is identified, the mutation is located using two-point and three-point mapping and confirmed using single-gene rescue or RNAi phenocopying (Hodgkin and Hope, 1999). Forward genetics is efficient in *C. elegans* because the mutants can cover genes expressed in a variety of tissues. *Caenorhabditis elegans* is hermaphroditic, so homozygous mutant strains can be produced in the F_2 generation via self-crossing.

Forward genetics screens are a useful method in mechanistic toxicology. Griffitts *et al.* (2001, 2005), for instance, discovered the role of glycolipid receptors and carbohydrate metabolism in *Bacillus thuringiensis* (Bt) toxins using *C. elegans* subjected to a forward genetics screen. The mutation of glycolipid receptors prevents Bt toxin from entering intestinal epithelium in *C. elegans*. Such a tissue-specific mechanism would have been difficult to detect using *in vitro* cell cultures.

Gene Expression Analysis in C. elegans

Caenorhabditis elegans has several advantages over other species in gene expression analysis. WormBase (Harris *et al*., 2004), the information-rich central genomic database of *C. elegans*, provides an intuitive interface into a well-annotated genome. *Caenorhabditis elegans* also has a consistent system of gene identification, thereby avoiding the confusion of gene identification that is common in many species, including human. The interactome modeling of *C. elegans* is also the most developed among all animal species (Dupuy *et al*., 2007; Li *et al*. 2004, 2008; Zhong and Sternberg, 2006) and along with other genome-level bioinformatics tools (Kim *et al*., 2001) greatly facilitates system-based analysis.

The results of gene expression analysis can be validated *in vivo* using mutational or transgenic approaches in *C. elegans*. For example, the gene expression of *C. elegans* exposed to ethanol, atrazine, polychlorinated biphenyls, endocrine disrupting chemicals, and polycyclic aromatic hydrocarbons have been profiled (Custodia *et al*., 2001; Kwon *et al*., 2004; Menzel *et al*., 2007; Reichert and Menzel, 2005). Follow-up studies with transgenic *C. elegans* expressing fluorescent markers were used to detect overexpression of protein in specific tissues *in vivo* (Menzel *et al*., 2007; Reichert and Menzel, 2005). Mutant *C. elegans* were also used to confirm the role of specific molecular targets based on gene expression analysis (Menzel *et al*., 2007).

Genome-Wide RNAi Screens in C. elegans

The discovery of RNAi mechanisms in *C. elegans* for which the 2006 Nobel Prize was awarded (Fire *et al*., 1998) and the complete sequencing of the nematode genome (*C. elegans* Sequencing Consortium, 1998) led to the generation of publically available RNAi libraries covering ~90% of its genes (Fewell and Schmitt, 2006; Kamath and Ahringer, 2003). Strategies to improve RNAi efficiency, especially in neurons, were further developed (Esposito *et al*., 2007; Lee *et al*., 2006; Simmer *et al.* 2002, 2003; Tabara *et al*., 2002; Tops *et al*., 2005). RNAi can be triggered by injection of worms with interfering double-strand RNA (dsRNA), by feeding them with transgenic bacteria producing the dsRNA or by soaking them in a solution of dsRNA. The latter allow timed RNAi exposure and genome-wide screens in 96- or 384-well plates with liquid worm cultures and have contributed to discoveries of mechanisms of axon guidance as well as mitochondrial involvement in oxidative stress and aging (Ayyadevara *et al*., 2007; Hamamichi *et al*., 2008; Hamilton *et al*., 2005; Ichishita *et al*., 2008; Lee *et al*., 2003; Schmitz *et al*., 2007; Zhang *et al*., 2006).

A genome-wide RNAi screen typically assesses a number of physiological parameters at the same time, such as viability, movement, food intake, and development, thereby facilitating the interpretation of screening results. While most RNAi screens have been done in wild-type *C. elegans*, some are performed using KO mutants to provide more sensitive or selective assays (Kaletta and Hengartner, 2006). Genome-wide RNAi screens are becoming a method of choice for discovering gene function. A recent study by Kim and Sun (2007), for example, identified a number of *daf-2*-dependent and nutrient-responsive genes that are responsive to paraquat-induced oxidative stress.

High-Content Chemical Screens

The use of *C. elegans* as a predictive model for human toxicity was first proposed in the context of heavy metals (Williams and Dusenbery, 1988). The *C. elegans* assay was validated as a predictor of mammalian acute lethality using eight different metal salts, generating LC_{50} values parallel to the rat and mouse LD_{50} values. A later study investigated the acute behavioral toxicity of 15 OP pesticides in *C. elegans* (Cole *et al.*, 2004). The toxicity of these pesticides in *C. elegans* was found to be significantly correlated to the LD_{50} acute lethality values in rats and mice. Several other studies have also validated a number of *C. elegans*–based assays for predicting neurological and developmental toxicity in mammalian species (Anderson *et al.*, 2004; Dhawan *et al.*, 1999; Tatara *et al.*, 1998; Williams *et al.*, 2000).

A *C. elegans*–based, high-throughput toxicity screen was first published by the Freedman group at National Institute of Environmental Health Sciences (Peterson *et al.*, in press); additional groups including industry and government groups in the United States and elsewhere are also carrying out high-throughput toxicity screening. Screens are typically conducted on a 96-well plate with a robotic liquid handling workstation (Biosort, Union Biometrica, Inc., Holliston, MA) to analyze the length, optical density, motion, and fluorescence of *C. elegans*. *Caenorhabditis elegans* is cultured in liquid from fertilized egg to adult through four distinct larval stages. The development, reproduction, and feeding behaviors of the *C. elegans* culture in response to different chemical exposures are characterized. The screen has been validated by the Freedman group with 60 chemicals including metals, pesticides, mutagens, and nontoxic agents (Peterson *et al.*, in press).

The high-throughput toxicity screen is being further improved with additional genetics and automation techniques. The generalized stress response of *C. elegans*, for instance, was visualized with transgenic GFP constructs, providing a more sensitive end point for toxicity screens (Dengg and van Meel, 2004; Roh *et al.*, 2006). Nematode locomotion can be tracked automatically, providing a more sensitive screen of neurotoxicity (Cole *et al.*, 2004; Williams and Dusenbery, 1990b). Transgenic or mutant *C. elegans* can also be used in the high-throughput screen to detect specific modes of action, including metal response (Cioci *et al.*, 2000), oxidative stress (Hasegawa *et al.*, 2008; Leiers *et al.*, 2003), and DNA damage (Denver *et al.*, 2006). A microfluidic *C. elegans* sorter with three-dimensional subcellular imaging capabilities was recently reported, thereby allowing high-throughput assays of higher complexity (Rohde *et al.*, 2007).

Environmental Assessment of Chemical Exposure

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 Table 6 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, *C. elegans* testing has been used to assess contamination in field settings (Table 7). 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 and Jones, 1996; Chu *et al.*, 2005; Dengg and van Meel, 2004; Easton *et al.*, 2001; Mutwakil *et al.*, 1997; Roh *et al.*, 2006), growth and reproduction (Anderson *et al.*, 2001; Hoss and 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.

Two of the principal limitations in using *C. elegans* in environmental testing are concerns related to its comparison to other nematodes and reliable and simple methods for extracting them from soil and sediments. Given the almost countless variety of nematodes, it is impossible for one species to be representative of the entire Nematoda phylum. Limited studies

TABLE 6
Representative Laboratory Studies Evaluating Environmentally Relevant Toxicants

Medium	End point (test duration)	Chemicals tested/comments	References
A. Aquatic	Lethality (24–96 h)	Tested metallic salts of 14 metals (Ag, Hg, Be, Al, Cu, Zn, Pb, Cd, Sr, Cr, As, Tl, Ni, Sb). Established initial aquatic testing procedures and compared results to traditionally used aquatic invertebrates.	Williams and Dusenbery (1990a)
	Lethality and stress reporter gene induction (8–96 h)	Assessed the induction of *hsp16-lacZ* and lethality in *C. elegans* exposed to water-soluble salts of Cd, Cu, Hg, As, and Pb.	Stringham and Candido 1994
	Growth, behavior, feeding, and reproduction (4–72 h)	Compared a number of sublethal end points and found feeding and behavior to be the most sensitive. Tested metallic salts Cd, Cu, and Pb.	Anderson *et al.* (2001)
	Feeding and movement (4–24 h)	Determined changes in ingestion using microbeads and movement in the presence of metals and varying availability of food	Boyd *et al.* (2003)
	Behavior (4 h)	Tested a variety of toxicants from several categories of chemicals including metals, pesticides, and organic solvents. Established the use of a 4-h exposure period for behavioral assessments.	Anderson *et al.* (2004)
	Reproduction (96 h)	Evaluated the effects on reproduction of several endocrine disruptors.	Hoss and Weltje (2007)
B. Sediment	Growth (72 h)	$CuSO_4$ in spiked water added to whole sediments and refined method for using organism in sediments.	Hoss *et al.* (1997)
	Growth (72 h)	Spiked natural sediments with $CdCl_2$ and extracted pore water to determine effects.	Hoss *et al.* (2001)
C. Soil	Lethality (24 h)	Spiked soil with $CuCl_2$ and developed the recovery method used with *C. elegans* exposed in soil.	Donkin and Dusenberry (1993)
	Lethality (24 h)	Tested metallic salts of five metals (Cu, Cd, Zn, Pb, Ni) in artificial soil. Compared *C. elegans* data to earthworm data from same medium. Determined that 24-h exposures for the nematode had similar effects to 14-day exposures with earthworms.	Peredney and Williams (2000)
	Lethality (24–48 h)	Tested seven organic pollutants (four azarenes, one short-chain chlorinated paraffin, and two organochlorinated pesticides) in soil, aquatic, and agar and compared results across media.	Sochova *et al.* (2007)

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.

TABLE 7
Examples of Field Studies Using *Caenorhabditis elegans* to Assess Environmental Samples

Field site	Environmental medium	Overview	References
Carnon River system (England)	Water	Transgenic strains of *C. elegans* that carry stress-inducible l*acZ* reporter genes were used to assess metal contamination of a river system.	Mutwakil *et al.* (1997)
Wastewater treatment process (Georgia)	Water discharges from industrial operations and a municipal treatment plant	The contribution of several industrial operations to the waste stream feeding a municipal wastewater treatment plant and the treatment plant's discharge were assessed to identify sources of water contamination and effectiveness of waste treatment. The 72-h mortality was used as end point.	Hitchcock *et al.* (1997)
Elbe River (Germany)	Sediments	Tested polluted sediments using growth and fertility as end points.	Traunspurger *et al.* (1997)
Twelve freshwater lakes (Germany)	Fresh water sediment	Evaluated 26 sediment samples from unpolluted lakes in southern Germany to determine the effect of sediment size and organic content on growth and fertility.	Hoss *et al.* (1999)
Middle Tisza River flood plain (Hungary)	Soil	Following a major release of cyanide and heavy metals from a mine waste lagoon in Romania, soil contamination was assessed following a 100-year flood event using mortality as end point.	Black and Williams (2001)
Agricultural soil (Germany)	Soil	Assessed the toxicity of soil from fields cultivated with transgenic corn (*Bt* corn; MON810) compared to isogenic corn. Growth and reproduction used as end points.	Hoss *et al.* (2008)

Much progress has been made to develop better methods to extract the worm from soil and sediments. The initial method developed by Donkin and Dusenbery (1993) has led to a standardized soil toxicological testing method adopted in 2001 by the American Society for Testing and Materials (ASTM, 2002) and recently the International Standards Organization in Europe (ISO 2007). The initial extraction method has been improved through the use of transgenic strains of nematodes (Graves *et al*., 2005) which allows for GFP-labeled worms to be used that distinguishes the worms being tested in soils from the large numbers of indigenous species that are similar in size and appearance. It also makes easier removal from soil with high organic content. All this work has led to more interest in using *C. elegans* in environmental studies.

CONCLUSION: THE ROLE OF *C. elegans* IN TOXICOLOGY RESEARCH

The unique features of *C. elegans* make it an excellent model to complement mammalian models in toxicology research. Experiments with *C. elegans* do not incur the same costs as experiments with *in vivo* vertebrate models, while still permitting testing of hypotheses in an intact metazoan organism. The genetic tools available for *C. elegans* make it an excellent model for studying the roles of specific genes in toxicological processes and gene-environment interactions, while the life history of this organism lends itself to high-throughput analyses. Thus, *C. elegans* represents an excellent complement to *in vitro* or cell culture–based systems and *in vivo* vertebrate models.

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