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 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...
TABLE 1
Signal Transduction Pathways Conserved in Nematodes and Vertebratesa,b

| Pathways involved in early development | Mutants used | Major findings | References |
|----------------------------------------|--------------|----------------|------------|
| Wnt pathway via β-catenin               | lat-1: KO of latrophilin | Latrophilin is the receptor responsible for the toxicity of venom | Mee et al. (2004) |
| Receptor serine/threonine kinase (tumor growth factor-β receptor) pathway | asna-1: KO of ArsA ATPase | ArsA ATPase is important in Ar resistance in both bacteria and animals | Tseng et al. (2007) |
| Notch-delta pathway | pgp-5: KO of a ABC transporter | ABC transporter is required for resistance to Cd toxicity | Kurz et al. (2007) |
| Receptor-linked cytoysine kinase (cytokine) pathway | 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) |

aAdapted from NRC (2000).
bSignal 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 for understanding nervous system function.

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) |
| Phoshine                    | pre-1, pre-7, pre-33: mutants created from EMS mutagenesis | Uptake and oxidation of phosphine 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 | Griffiths et al. (2001) |
|                              | bre-2 to bre-5: KOs of glycolipid carbohydrate metabolism | Glycolipid receptors are targets of Bt toxins | Griffiths 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.
for studying mechanisms of neuronal injury with resolution
of single neurons. It initially 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

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

for 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 neuro-
muscular junctions (Chen, 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 phylogenet-
ically 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 x1 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 [Richardson,
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 mechanosen-
sory functions of glutamatergic 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 con-
ducted 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.

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**TABLE 3**

Examples of Transgenic *Caenorhabditis elegans* Used in Toxicology Research

| Field/target tagged | Reporter used | Applications | References |
|---------------------|---------------|--------------|------------|
| A. Mechanistic studies | | | |
| DAergic neurons CYP14A3 and 35A3 | GFP | Detect neurodegradation caused by chemicals | Jiang et al. (2007) |
| GST | GFP | Measure GST induction in response to acrylamide | Hasegawa and van der Bliek (in press) |
| Heat shock proteins | GFP, β-galactosidase | Widely used for measuring stress response | 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 | Cioci et al. (2000) |
| ATP level | Firefly luciferase | Measure the reduction of metabolic activity in response to environmental stressor | Lagido et al. 2001 |
| B. Environmental biomonitoring | | | |
| | | | |
| CAENORHABDITIS ELEGANS IN TOXICOLOGY RESEARCH

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


| Compound | Strains investigated | Observations | References |
|----------|----------------------|--------------|------------|
| Paraquat | mev-1(kn1), mev-2(kn2)<sup>a</sup> | Hypersensitive to oxygen and paraquat, decreased SOD activity<sup>b</sup> | Ishii et al. (1990) |
|          | rad-8(mn162)         | Hypersensitive to oxygen and paraquat, reduced fecundity, decreased life span | Ishii et al. (1993) |
|          | age-1(hs542), age-1(hs546) | Increased catalase and Cu/Zn SOD activity, increased life span | Vanfleteren et al. (1995) |
|          | mev-1(kn1), rad-8(mn162) | Paraquat and high oxygen content inhibit development, inversely proportional to life span | Hartman et al. (1995) |
|          | age-1(hs546), daf-16(m26), mev-1(kn1)<sup>a</sup> | Increased resistance to paraquat and heat, extended life span, increased SOD, and catalase mRNA levels only in age-1 mutants, but not daf-16 or mev-1 | Yanase et al. (2002) |
|          | mev-5(qa5005)<sup>a</sup>, mev-6<sup>(qa5006f, mev-7(qa5007f))</sup>, mev-1(kn1), gas-1(fc21) | Longevity and sensitivity to paraquat, UV or heat do not correlate | Fujii et al. (2005) |
|          | skn-1(zu67) | Overproduction of superoxide anion in submitochondrial particles upon paraquat exposure | Kondo et al. (2005) |
|          | daf-2(e1370) | Activation of SKN-1 transcription factor, localizes to the nucleus following paraquat exposure | Kell et al. (2007) |
|          | Overexpression of GSTO, gsto-1 RNAi | Increased resistance to paraquat-induced oxidative stress | Burmeister et al. (2008) |
|          | gas-1(fc21) | Increased sensitivity to rotenone under hyperoxia | Ishiguro et al. (2001) |
|          | pdr-1, daf-1 RNAi | Increased vulnerability to rotenone | Ved et al. (2005) |
|          | 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 Dusenberg 1990 |
|          | | Cholinesterase inhibition associated with high behavioral toxicity | Cole et al. (2004) |
|          | | Absorption effects are more prominent than biodegradation in soil toxicity tests | Safih-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-2(ye31), bre-2(ye71), bre-3(ye28), bre-4(ye13) | Increased resistance to Bt toxin | Griffitts et al. (2001) |
|          | gfp-4(b22), kgb-1(um3), juk-1(gk7), sek-1(km4) | Bt toxin resistance involves the loss of glycosyltransferase in the intestine | Griffitts et al. (2003) |
|          | bre-2(ye31), bre-3(ye28), bre-4(ye13), bre-5(ye17) | Bt toxin reduces brood size and causes damage to the intestine | Wei et al. (2003) |
|          | bre-3(ye28) | 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 differed in C. elegans isolated from geographically distinct strains | Schulenberg and Muller (2004) |
|          | daf-2(e1370), daf-2(e1368), age-1(hs546), daf-16(m697), daf-20(m26) | Bt toxin resistance entails loss of glycolipid carbohydrates and the toxin directly and specifically binds to Glycolipids | Griffitts et al. (2005) |
| Captan   | hsp-16:48, hsp-16:1::lacZ | Resistance to Bt toxin develops as a result of loss of glycolipid receptors for the toxin | Barrows et al. (2006) |
| Dithiocarbamate fungicides | hsp-16:48, hsp-16:1::lacZ | Resistance to toxin is achieved by mutations in glycosyltransferase genes that glycosylate glycolipid or with a loss of the monosaccharide biosynthetic pathway | Barrows et al. (2007a, 2007b) |
| Organochlorinated pesticides | N2 | Mutations in the insulin-like receptor pathway lead to distinct behavioral responses, including the evasion of pathogens and reduced ingestion | Hasshoef et al. (2007) |
|          | hsp-16:48, hsp-16:1::lacZ | Reproduction and growth significantly reduced by Bt toxin | Hoss et al. (2008) |
|          | | Stress induction localized to muscle cells of the pharynx | Jones et al. (1996) |
|          | | Inhibits feeding, cessation of muscular contraction | |
|          | | Induction of stress response | Guven et al. (1999) |
|          | | 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) |

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Note. MAPK, mitogen-activated protein kinase; ROS, reactive oxygen species.

<sup>a</sup> These mutants showed defective dye filling, indicative of chemosensory neuron damage.

<sup>b</sup>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 oxidative-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 neurotoxicity 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 (vapmon, 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 neurotoxins (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 4-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 (Spriniger 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) show 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 oxidase B. MPP+ damages the DAergic nervous system, leading to a typical Parkinsonian syndrome (K opin 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; Marvanov a 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).

Taufopathies 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-l (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 Huntington (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 apoptos is in HD neurodegeneration (Bates et al., 2006; Faber et al., 2005).
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/ldmd/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 Haften 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 as 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 Haften 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.,
C. Transcriptional activities

RNA: DNA ratio

A decrease in RNA: DNA ratio indicates the inhibition of transcriptional activities.

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.

Southern blot

T4 endonuclease–sensitive sites in specific genes (identified by genomic DNA sequence) indicate the presence of UV photodimers.

Immunoblot

Antibodies to specific UV photoproducts are identified.

Enzymatic activity

A diagnostic enzymatic activity is measured in vitro.

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).

Lethality assay

The lethality of transgenic, mutation-sensitive C. elegans was measured for mutagen detection.

C. Transcriptional activities

RNA: DNA ratio

A decrease in RNA: DNA ratio indicates the inhibition of transcriptional activities.

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 F2 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 Friedman 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 Friedman 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; Mutwikil et al., 1997; Roh et al., 2006), growth and reproduction (Anderson et al., 2001; Hess 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
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 6

| 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, Ti, 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 hisp16-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)                                                                            | CuSO₄ 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 CdCl₂ and extracted pore water to determine effects.        | Hoss et al. (2001)                            |
| C. Soil       | Lethality (24 h)                                                                          | Spiked soil with CuCl₂ 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)                         |

### TABLE 7

| Field site                                      | Environmental medium          | Overview                                                                                                                    | References                                      |
|------------------------------------------------|------------------------------|----------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Cannon River system (England)                   | Water                        | Transgenic strains of C. elegans that carry stress-inducible lacZ 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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