Chapter 1
Values of *C. elegans* in Toxicological Study

Abstract The model animal of nematode *Caenorhabditis elegans* has become an important in vivo alternative assay system for toxicological study of different environmental toxicants or stresses. We here introduced the several important values of *C. elegans* in the toxicological study for environmental toxicants or stresses. Meanwhile, we also discussed the limitations of nematodes for the toxicological study of environmental toxicants or stresses.

Keywords Alternative assay system · Value · Limitation · *Caenorhabditis elegans*

1.1 Introduction

So far, the model animal of nematode *Caenorhabditis elegans* has gradually become an important in vivo alternative assay system for both the toxicity assessment and the toxicological study of different environmental toxicants or stresses [1–3]. *C. elegans* is a free-living nematode mainly found in the liquid phase of soils. *C. elegans* is one of the most thoroughly studied model animals and has the typical properties of model organisms, such as well-defined anatomy, short life cycle, short lifespan, small size, perfect reproductive capacity, availability of many useful genetic sources, and ease in handling [4]. Moreover, the nematodes can be easily cultivated in a laboratory and reproduced in thousands of individuals, which allow the offer of an advantage assay system suitable for asking the in vivo underlying mechanisms for the observed toxicity of environmental toxicants or stresses. *C. elegans* has the ecological significance due to its important roles in the nutrient cycling in the soil. Especially, *C. elegans* has been proven to be very sensitive to the adverse effects at different aspects induced by different environmental toxicants or stresses [1–3].

In this chapter, we discussed the several important values of *C. elegans* in the toxicological study for environmental toxicants or stresses. The mainly introduced values are:

1. Raise of a series of useful sublethal endpoints for toxicity assessment of environmental toxicants
2. High-throughput screen and identification of chemicals

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3. Toxicity assessment of environmental toxicants under susceptible genetic backgrounds
4. Toxicity assessment of environmental toxicants at environmentally relevant concentrations
5. Understanding the in vivo physicochemical, cellular, and physiological mechanisms of toxicity induced by environmental toxicants
6. Elucidation of toxicological mechanisms of environmental toxicants in certain targeted organs
7. Elucidation of underlying molecular mechanisms of toxicity induced by environmental toxicants
8. Distribution and translocation of environmental toxicants
9. Confirmation of chemical with low-toxicity or non-toxicity property

Moreover, we further discussed the limitations of nematodes for the toxicological study of environmental toxicants or stresses.

1.2 Raise of a Series of Useful Sublethal Endpoints for Toxicity Assessment of Environmental Toxicants

In nematodes, besides the endpoint of lethality, many important sublethal endpoints associated with development, reproduction, neuronal development and function, intestinal development and function, epidermal development, innate immune response, lifespan, metabolism, oxidative stress, and transgenic strains reflecting stress response or oxidative stress have been further employed and raised [5–9]. Among these raised sublethal endpoints, the endpoints associated with the development and the reproduction are widely used ones involved in the evaluation of possible toxicity of environmental toxicants or stresses on the growth and the development of nematodes [6–9]. Additionally, the endpoints associated with the oxidative stress and the transgenic strains reflecting stress response or oxidative stress are also widely used endpoints to reflect the possible toxicity of environmental toxicants or stresses on nematodes [10–15]. Especially, the raised some useful transgenic strains reflecting stress response or oxidative stress can help us directly detect the potential toxicity induction of environmental toxicants or stresses on nematodes based on the noticeable induction of fluorescent signals [13–15]. The endpoints associated with the lifespan and innate immune response are important to detect the potential long-term effects of certain environmental toxicants or stresses on nematodes [16–18]. More importantly, some useful endpoints associated with the possible damage on the functions of primary targeted organs, such as the intestine and the epidermal, of certain environmental toxicants have been raised in nematodes [19–21]. The useful endpoints associated with the possible damage on the functions of secondary targeted organs, such as the reproductive organs and the neurons, have also been raised in nematodes [8, 9, 22]. These useful sublethal endpoints will largely open some new windows for us in understanding the underlying toxicological mechanisms of environmental toxicants and environmental stresses in organisms.

1.3 High-Throughput Screen and Identification of Chemicals

Due to the important properties of small size and easy cultivation in the laboratory of nematodes, this model animal is very valuable for high-throughput screen and identification of chemicals. Using *C. elegans* as an in vivo assay system, one of the values in the high-throughput screen is the high-throughput toxicity assessment of environmental toxicants or chemicals. For example, *C. elegans* was used in the high-throughput evaluation of possible toxicity of 20 engineered nanomaterials (ENMs) at 4 concentrations using body length, locomotion speed, and lifespan as the toxicity assessment endpoints [23]. Using *C. elegans* as an in vivo assay system, another important value in the high-throughput screen is to identify the susceptible or resistant genetic loci affecting the toxicity formation of certain environmental toxicants. For example, *C. elegans* was used in the high-throughput identification of genetic loci affecting the toxicity and the translocation of graphene oxide (GO), an important carbon-based ENMs, based on the screen of 20 strains with mutations of genes required for stress response or oxidative stress [24]. Seven genes were identified, and their mutations altered both the translocation and toxicity of GO in nematodes [24]. Mutations of the *hsp-16.48*, *gas-1*, *sod-2*, *sod-3*, or *aak-2* resulted in greater GO translocation into the body and toxic effects on both primary and secondary targeted organs; however, mutations of the *isp-1* or *clk-1* caused significantly decreased GO translocation into the body and toxicity on both primary and secondary targeted organs [24].

1.4 Toxicity Assessment of Environmental Toxicants Under Susceptible Genetic Backgrounds

Due to the role of classic model animal, so far, there are many useful genetic mutants that are available for researchers in the related fields. Meanwhile, it is easy to perform RNAi knockdown of any interested gene in the nematodes. These research backgrounds provide a solid foundation to systematically perform the toxicity assessment of environmental toxicants under susceptible genetic backgrounds. With the TiO_2-nanoparticles (TiO_2-NPs) as an example, *sod-2*, *sod-3*, *mtl-2*, and *hsp-16.48* mutants were susceptible for TiO_2-NP toxicity on reproduction and locomotion behavior; *sod-2*, *sod-3*, and *mtl-2* mutants were susceptible for TiO_2-NP toxicity on survival and intestinal development; and *mtl-2* mutant was susceptible for TiO_2-NP toxicity on development [25]. Mutations of these genes, together with sensitive endpoints, will have the potential in assessing the possible TiO_2-NP toxicity at the concentration of 0.0001 μg/L [25].

1.5 Toxicity Assessment of Environmental Toxicants at Environmentally Relevant Concentrations

Considering the sensitivity property of *C. elegans* to environmental toxicants or stresses, *C. elegans* has been gradually used in the toxicity assessment of environmental toxicants at environmentally relevant concentrations [26–30]. In nematodes, at least acute exposure, prolonged exposure, chronic exposure, one-generation exposure, and transgenerational exposure have been raised as the useful exposure routes for toxicity assessment of environmental toxicants. Among these exposure routes, at least the prolonged exposure and the chronic exposure have the potential in assessing the possible toxicity of certain environmental toxicants at environmentally relevant concentrations. Further with TiO_2-NPs as an example, it has been shown that TiO_2-NPs at the concentration of 0.01 μg/L could cause the significant reduction in brood size, decrease in locomotion behavior, and induction of intestinal autofluorescence in nematodes after prolonged exposure from L1-larvae to adult day-1 [25]. Moreover, after chronic exposure from adult day-1 to adult day-8, TiO_2-NPs (4 and 10 nm) at concentrations more than 0.01 μg/L could significantly decrease the locomotion behavior in nematodes [30].

1.6 Understanding the In Vivo Physicochemical, Cellular, and Physiological Mechanisms of Toxicity Induced by Environmental Toxicants

As a model animal, *C. elegans* provide an important in vivo assay system to systematically examine the potential roles of different physicochemical properties of certain environmental toxicants, such as the ENMs, in the toxicity formation in organisms. In nematodes, the important contribution of physicochemical properties, such as size, surface charge, shape, surface groups, and impurity, in the toxicity formation of ENMs have been examined [5, 12, 17, 27–29, 31–35]. Moreover, the underlying chemical mechanism for the oxidative stress induced by ENMs has also been elucidated in nematodes [36].

Besides this, several aspects of cellular mechanisms of toxicity formation of certain environmental toxicants, such as ENMs, have been examined in nematodes. These raised cellular mechanisms of toxicity formation of environmental toxicants include release of metal ion, oxidative stress, intestinal permeability, defecation behavior, bioavailability to targeted organs, acceleration in aging process, innate immune response, mitochondrial damage and DNA damage, developmental fate, and deficit in cellular endocytosis in intestinal cells [5, 10, 16–19, 37–46]. Moreover, several aspects of physiological mechanisms of toxicity formation of certain environmental toxicants, such as ENMs, have also been determined. These raised physiological mechanisms of toxicity formation of environmental toxicant include environmental factors, exposure, physiological state of nematodes, developmental stages, and hormesis of nematodes [5, 26, 31, 34–36, 47, 48].

1.7 Elucidation of Toxicological Mechanisms of Environmental Toxicants in Certain Targeted Organs

With ENMs as the example, the ENMs such as the GO could be distributed and translocated into both the primary targeted organs, such as the intestine, and the secondary targeted organs, such as the reproductive organs of gonad and spermatheca and the neurons, in nematodes [18, 42]. Using series of tissue-specific RNAi knockdown tools, we can perform the RNAi knockdown of certain genes in certain tissues in nematodes. Meanwhile, there are different tissue-specific promoters available in nematodes, which can help us to express certain genes in certain tissues. With the aid of these techniques, we can systematically elucidate the possible toxicological mechanisms of environmental toxicants in certain targeted organs in nematodes.

1.8 Elucidation of Underlying Molecular Mechanisms of Toxicity Induced by Environmental Toxicants

In nematodes, their many basic physiological processes, stress responses, signal transduction pathways, and epigenetic marks are conserved compared with those in mammals and humans. Additionally, the completion of *C. elegans* genome has approximately 45% of the genes with the human homologues, including numerous disease-related genes. So far, some important signaling pathways, such as insulin, p38 MAPK, Wnt, ERK, and oxidative stress-related signaling, have been identified to be involved in the regulation of response of nematodes to environmental toxicants, such as carbon-based ENMs, in nematodes [13, 15, 24, 49–51]. Moreover, some important microRNAs and long noncoding RNAs have also been identified to be involved in the regulation of response of nematodes to carbon-based ENMs in nematodes [16, 52–56].

1.9 Distribution and Translocation of Environmental Toxicants

Distribution and translocation of environmental toxicants is one of the crucial cellular contributors for the toxicity induction of certain environmental toxicants. The property of transparent body of nematodes allows us directly visualize the distribution and the translocation of certain environmental toxicants, such as some ENMs. Some powerful techniques have already been employed to determine the distribution and the translocation of ENMs, and the distribution and the translocation patterns of some important ENMs have been well described in nematodes with the aid of these powerful techniques [57–64]. Moreover, using these techniques, the behavior and the regulation of distribution and translocation of different ENMs in the

primary or the secondary targeted organs, as well as the patterns of transgenerational translocation of ENMs, have been systematically investigated in nematodes [7, 13, 18–20, 24, 28, 29, 35, 42, 43, 60]. *C. elegans* is also helpful for the elucidation of dynamic cellular, molecular, and chemical metabolisms of environmental toxicants, such as the ENMs, in the body of nematodes [18, 21, 24, 29, 60, 65, 66].

1.10 Confirmation of Chemical with Low-Toxicity or Non-toxicity Property

Due to the sensitivity of *C. elegans* to environmental toxicants, *C. elegans* not only acts as a wonderful in vivo assay model for assessing ecotoxicological effects of certain environmental toxicants but also serves as a useful assay model for the confirmation of low-toxicity or relative non-toxicity property of environmental chemicals. With ENMs as the example, the relative non-toxicity property of some important ENMs, such as graphite, graphene quantum dots (GQDs), carboxyl-functionalized graphene (G-COOH), $Gd@C_{82}(OH)_{22}$, and fluorescent nanodiamond (FND), has been confirmed in nematodes [7, 21, 64, 67].

1.11 Limitations of *C. elegans* in the Toxicological Study

Although *C. elegans* has many important values in both the toxicity assessment and the toxicological study of environmental toxicants or stresses, the limitations of nematodes in the toxicological study still exist. One of the important limitations is that the nematodes do not have some important organs, such as the heart, liver, lung, and kidney, which exist in the mammals, since the nematodes do not have the related developmental process of mesoderm during the development. Another important limitation is that the genome for human or mammals may be more complex information and structure than that of nematodes. Therefore, some important molecular signaling pathways may be not able to be detected in the in vivo assay system of *C. elegans*.

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