Chapter 2
Molecular Basis for Reduced Lifespan Induced by Environmental Toxicants or Stresses

Abstract What's the potential basic principle for the toxicity induction on different endpoints in nematodes exposed to environmental toxicants or stresses? To answer such a question, we here focus on the endpoint of lifespan to discuss the potential basic principle for toxicity induction from environmental toxicants or stresses. In this chapter, we will discuss how the environmental toxicants or stresses reduce lifespan by affecting the molecular basis for longevity, and how the innate immune response is involved in the regulation of longevity reduction in nematodes exposed to environmental toxicants or stresses. We will further introduce the genetic identification of genes and signaling cascade in the regulation of toxicity of environmental toxicants or stresses. We will also discuss how the environmental toxicants or stresses reduce lifespan by affecting signaling pathways associated with the stress response.

Keywords Molecular basis · Lifespan reduction · Environmental exposure · *Caenorhabditis elegans*

2.1 Introduction

In nematodes, many useful sublethal endpoints have been raised and will be further raised to be used for toxicity assessment and toxicological study of various environmental toxicants and stresses. The already raised sublethal endpoints in nematodes are at least associated with development, reproduction, neuronal development and function, intestinal development and function, epidermal development, innate immune response, lifespan, metabolism, and oxidative stress [1–17]. With the concern on these valuable endpoints, we are facing upon an important scientific question. That is, what's the potential basic principle for the toxicity induction on these sublethal endpoints in nematodes exposed to environmental toxicants or stresses?

Among the widely used toxicity assessment endpoints in *C. elegans*, lifespan is an endpoint to reflect the long-term effects of certain environmental toxicants or stresses on animals [18–20]. Both lifespan and aging-related phenotypes can be

D. Wang, *Molecular Toxicology in Caenorhabditis elegans*,
https://doi.org/10.1007/978-981-13-3633-1_2

used to determine the aging process in nematodes. In this chapter, we will focus on the lifespan to discuss the potential basic principle for the toxicity induction in nematodes exposed to environmental toxicants or stresses. We first introduced the molecular basis for the longevity control, especially the important signaling pathways required for the control of longevity (insulin signaling pathway, dietary intake signaling pathway, mitochondrial respiration signaling pathway, and germline signaling pathway). After that, we discussed how the environmental toxicants or stresses reduce lifespan by affecting the molecular basis for longevity, and how the innate immune response is involved in the regulation of longevity reduction in nematodes exposed to environmental toxicants or stresses. Moreover, we introduced the genetic identification of genes and signaling cascade in the regulation of toxicity on lifespan by environmental toxicants or stresses. Finally, we tried to discuss how the environmental toxicants or stresses reduce lifespan by affecting signaling pathways associated with the stress response.

2.2 Molecular Basis for Longevity Control

Over 30 years ago, it has been found that certain genetic or environmental manipulations could result in the *C. elegans* to live over twice as long as that in wild-type nematodes [21–23]. Since this discovery, many more genes and these genes-mediated signaling pathways have been successfully identified and elucidated to explain the underlying molecular mechanisms for the aging process or longevity in *C. elegans* [24–30]. The widely described signaling pathways involved in the regulation of longevity in *C. elegans* contain insulin, dietary intake, mitochondrial respiration, and germline signaling pathways [24–30]. More importantly, these signaling pathways described in *C. elegans* can also be shared by other organisms, including the mammals [24–30], which implies that the underlying molecular mechanisms for longevity are conserved among different organisms.

2.2.1 Insulin Signaling Pathway

In 1997, it was reported that the lifespan extension can be achieved by mutations of *daf-2* encoding the sole insulin/IGF-1 receptor in *C. elegans* [31]. The insulin signaling pathway is the most well-described signaling pathway in the regulation of longevity in *C. elegans*.

2.2.1.1 The Basic Information for Insulin Signaling Pathway

During the control of aging process, the insulin signals activated DAF-2 can initiate a subsequent signaling cascade by activating AGE-1, a phosphatidylinositol 3-kinase (PI3K), to produce the PIP3 and the AKT family kinases in the dependent of a PDK-1 kinase [32–35]. The activated signaling cascade of AKT family kinases will phosphorylate the forkhead transcription factor DAF-16 in order to prevent the entering of DAF-16 into the nucleus to promote or inhibit the transcription of its downstream genes [36–40]. Inactivation of insulin signaling pathway occurs at least partially via the activity of DAF-18, a PIP3 phosphatase [40, 41].

2.2.1.2 Nucleus-Cytoplasmic Translocation of DAF-16

It has been widely considered that the nucleus-cytoplasmic translocation of DAF-16 plays a major role in the regulation of longevity and stress response [24–30, 42]. Nevertheless, a constitutive nuclear localization of DAF-16 did not potentially increase the lifespan of nematodes [43]. Additionally, some important regulators, such as SIR-2.1, HSF-1, LIN-14, and SMK-1, have been identified to be capable of modulating DAF-16's phenotypes once translocated into the nucleus [43–46].

2.2.1.3 Targets of DAF-16

To understand the underlying mechanisms for DAF-16 in extending the lifespan and modulating the resistance to various stressors, some groups have identified the downstream targets of DAF-16 [47–52]. Transcriptional profiling analyses by comparing wild-type, dauer, and long-lived insulin/IGF-1 signaling- *daf-2* mutant identified heat shock proteins and mitochondrial Mn-SOD protein SOD-3 as the most DAF-16-responsive downstream targets [47–50], implying the important roles of proteostasis and reactive oxygen species (ROS) detoxification in the regulation of longevity and resistance to environmental toxicants or stresses. The further more in-depth expressional profiling analysis by comparing *daf-2* and wild-type adult nematodes found that, besides the heat shock proteins, many neuropeptide-like proteins and antimicrobial proteins associated with the innate immune response could be upregulated [51]. After the profiling comparisons of *daf-2* and *age-1* mutants, or of *daf-2* gene inactivation with and without *daf-16* mutation or inactivation, totally 254 upregulated and 243 downregulated genes were identified to act as the possible targeted genes of *daf-16* [52]. Among the identified possible downstream targets for DAF-16, the potential functions of heat shock proteins (HSP-16.1, HSP-16.2, HSP-12.3, and HSP-12.6), catalases and superoxide dismutases (CTL-1, CTL-2, and SOD-3), and antimicrobial proteins (LYS, SPP, CLEC, and NLP proteins) in the regulation of longevity and stress response need to be further carefully considered [52].

2.2.2 Dietary Intake Signaling Pathway

In 1998, it was reported that the *eat-2* mutants can exhibit a significant increase in lifespan, which may be largely due to the result of their decreased rates of feeding [53]. Dietary restriction (DR) is defined as the reduction of particular or total nutrient intake without causing the malnutrition.

2.2.2.1 mTOR Signaling Pathway Is a Major Effector Regulating the Dietary Restriction

In nematodes, loss-of-function mutation of *let-363* encoding the TOR results in an increased lifespan [54, 55]. Moreover, the TOR adaptor protein rap-TOR (DAF-15) can be directly suppressed by the active DAF-16 [54, 55]. Meanwhile, loss-of-function mutation of *let-363* in combination with *daf-2* mutation could not further extend the lifespan, although the extended lifespan in *let-363* mutant could not be suppressed by *daf-16* mutation [55]. In nematodes, TOR and *daf-2* extension of longevity may share the same or similar downstream mechanisms and targets.

2.2.2.2 Important Role of Autophagy

In *C. elegans*, inhibition of several autophagy-related genes (*unc-51*/ATG1/Ulk1, *bec-1*/ATG6/Beclin1, *vps-34*, *atg-18*/Wipi, or *atg-7*) could effectively shorten the long lifespan phenotype of *eat-2* mutants [56–59], suggesting the potential directly link of modulation in autophagy to dietary restriction-mediated longevity. Additionally, the nematodes with the mutations of *daf-15*/raptor also require the autophagy genes for their lifespan extension [59]. Recently, it has been further observed that intestine-specific RNAi knockdown of autophagy genes *atg-18*/Wipi or *lgg-1*/Atg8 in *eat-2* mutants was sufficient to shorten their extended lifespan and disrupt the improved intestinal barrier function [56].

2.2.2.3 Transcriptional Factors Involved in the Regulation of Dietary Restriction-Mediated Lifespan Extension

So far, at least the transcriptional factors of FOXA ortholog PHA-4, nuclear hormone receptor NHR-62, and TFEB ortholog HLH-30 have been identified to be required for dietary restriction- or mTOR-mediated lifespan extension [60, 61].

2.2.3 *Mitochondrial Respiration Signaling Pathway*

If the reactive oxygen species (ROS) production can be properly controlled by modulating the mitochondrial activity, the longevity is predicted to be increased. The mitochondria are primary sites producing ROS. Based on this assumption, the mitochondrial electron transport and ATP synthase have been identified as the important regulators of longevity in *C. elegans* [62–64].

2.2.3.1 Complex I (NADH:Ubiquinone Oxidoreductase)

gas-1 encodes a 49 kDa subunit of complex I. The *gas-1(fc21)* mutant has shortened lifespans and few offspring and grows slowly [65]. The increased oxidative damage onto mitochondrial protein was further observed in *gas-1* mutant nematodes [66].

nuo-1 encodes a NDUFV1 subunit of complex I. The *nuo-1* mutant cannot develop into the adulthood (arrested at the L3 stage) [67], implying that the *nuo-1* mutant can be technically considered as long-lived.

nuo-6 encodes a complex I subunit orthologous to mammalian NUDFB4 complex I subunit. The missense mutation of *nuo-6* decreased the complex I function and increased the lifespan [68].

2.2.3.2 Complex II (Succinate:Ubiquinone Oxidoreductase)

mev-1 encodes a cytochrome b large subunit of complex II [69]. The *mev-1(kn1)* mutant shows a decreased mitochondrial activity (as indicated by the reduced mitochondrial respiratory rates) and short lifespan [69–71]. In addition, the *mev-1(kn1)* mutant has a higher level of oxygen-free radicals compared with wild type [72].

2.2.3.3 Complex III

isp-1 encodes an iron–sulfur component of complex III. Mutations of *isp-1* could increase the longevity by decreasing the oxygen consumption [62, 63].

ctb-1 encodes a cytochrome b subunit of complex III. Missense mutation of *ctb-1* could suppress the slow developmental rate of *isp-1* mutant, but not the prolonged lifespan [63]. *ctb-1* mutant alone has no aging phenotype [63].

2.2.3.4 Complex IV

RNAi knockdown any of the three complex IV subunits, including the COX IV, could increase the lifespan in nematodes [64, 73].

2.2.3.5 Complex V

Loss-of-function mutation or RNAi knockdown of *atp-2* caused the extended lifespan [62, 67].

2.2.3.6 Coenzyme Q (Ubiquinone, CoQ) Synthesis

clk-1 encodes a ubiquinone biosynthesis protein COQ7. Mutation of *clk-1* could lengthen the lifespan [74]. More importantly, the long-lived phenotype of *daf-2(e1370)* mutant could be further extended by *isp-1* or *clk-1* mutation or RNAi of respiratory chain components [62–64, 74].

2.2.3.7 Mitochondrial Mn-SODs

Mitochondrial superoxide dismutases contain SOD-2 and SOD-3. SOD-2 is localized to the I:III:IV super complex.

sod-2 encodes a constitutively expressed mitochondrial dismutase. It was reported that mutation of *sod-2* could lengthen the lifespan, and the *clk-1;sod-2(ok1030)* could live longer than *clk-1* mutant, although the increased oxidative damage could be detected [75].

sod-3 encodes an inducible mitochondrial superoxide dismutase. The *sod-2;gas-1sod-3* triple mutant is synthetically lethal [76], which implies the importance of SOD-3 for the normal survival of *sod-2;gas-1*.

2.2.4 Germline Signaling Pathway

Certain cells and tissues, such as the gonad, in the body can act as an endocrine source to regulate metabolism, behavior, development, and longevity. In nematodes, prevention of germline stem cell proliferation can induce approximately 60% extension of lifespan [77]. So far, at least steroid nuclear receptor DAF-12, FOXO transcription factor DAF-16, FOXA transcription factor PHA-4, and HNF-4-like nuclear receptor NHR-80 have been identified to be required for the control of gonadal longevity in *C. elegans* [77].

2.2.4.1 DAF-12

The longevity of nematodes lacking the germline was found to be dependent of a steroid hormone signal encoded by the nuclear hormone receptor DAF-12 [78]. After response to ligands (bile acid-like steroids or dafachronic acids (DA)), DAF-12 extends the adult lifespan when the germline stem cells are removed [78], which suggests that the DAF-12 acts as an important link between the developmental progression and the longevity. That is, when the gonad is intact, signals from the germline may impinge on the somatic gonad to inhibit the DA/DAF-12 signaling. However, when the germline is removed, the DA/DAF-12 signaling may be suppressed.

2.2.4.2 DAF-16

It has been considered that the DAF-16/FOXO can act as a central regulator of gonadal longevity [78]. Additionally, it has been shown that the *daf-2;glp-1* double mutants live four- to five-fold longer than wild-type nematodes [78]. This implies that the longevity of reduced IIS may be additive with that of gonadal longevity, and DAF-16/FOXO may respond to the germline ablation differently from the reduced IIS.

2.2.4.3 TOR Signal

Germline removal can also trigger the downregulation of TOR signal, which will in turn stimulate the PHA-4 and the autophagy to promote a healthful state through the effects on certain metabolisms (such as fats, sterols, amino acids, carbohydrates) or other stress signals [77].

2.2.4.4 NHR-80

Besides the fat metabolism, the nuclear hormone receptor NHR-80 has been found to be involved in the regulation of longevity [79]. Loss-of-function mutation of *nhr-80* could abrogate the lifespan extension of germline-less nematodes, but has little effect on the lifespan in wild-type nematodes [79]. The *nhr-80* expression could be induced upon germline ablation in a manner independent of the *daf-12* or the *daf-16* [79], which implies that this observed extended longevity may be associated with the reduced IIS, the reduced mitochondrial function, and the dietary restriction. The increase in NHR-80 expression was observed specifically within the intestine upon the germline ablation; however, loss-of-function mutation of *nhr-80* has no effect on the DAF-16/FOXO nuclear localization [79]. Additionally, RNAi knockdown of *nhr-80* could further shorten the lifespan of double mutant of *daf-16;glp-1* [79].

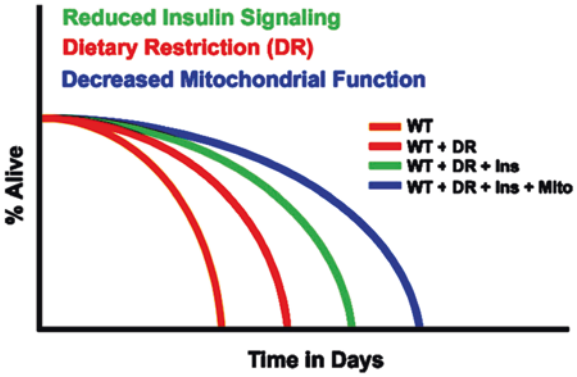

Fig. 2.1 Modulation of the three core signaling pathways that affect the aging act synergistically with each other [82]. Dietary restriction (red line), reduced insulin signaling (green line), or impairment of the mitochondrial electron transport chain (blue line) can extend the lifespan of a wild-type animal (orange line). Modulation of any two of these three pathways has an additive effect on lifespan, supporting arguments for their mechanistic autonomy. Knockdown of all three pathways should create an animal that is far longer lived than an animal with reduced function in just two of the three pathways

2.2.5 Interaction Among Different Signaling Pathways

It has been observed that the simultaneous manipulation of more than one of the signaling pathways required for the control of longevity could result in longer lifespan than that through the manipulation of a single signaling pathway [41, 53, 62, 80, 81]. Meanwhile, the combination of mutations in multiple genes downstream of IIS or of an IIS mutation in combination with the *daf-2(RNAi)* yielded the results only in the lifespan extension comparable to the single mutations or to stronger *daf-2* mutant [41, 53, 62, 80, 81]. Therefore, these synergistic effects between different signaling pathways required for the control of longevity may not be simply due to the further downregulation of insulin signaling in nematodes (Fig. 2.1).

2.3 Environmental Toxicants or Stresses Reduce Lifespan by Affecting the Molecular Basis for Longevity

2.3.1 Environmental Toxicants or Stresses Reduce Lifespan by Affecting the Insulin Signaling Pathway

Illegal or unsuitable use of weight loss agents is an important public health concern [83, 84]. Clenbuterol is a typical weight loss agent by acting as a β2-adrenergic agonist. It was illegally used as a feed additive to improve production performance and a carcass composition. Ractopamine is another synthetic β2-adrenoceptor

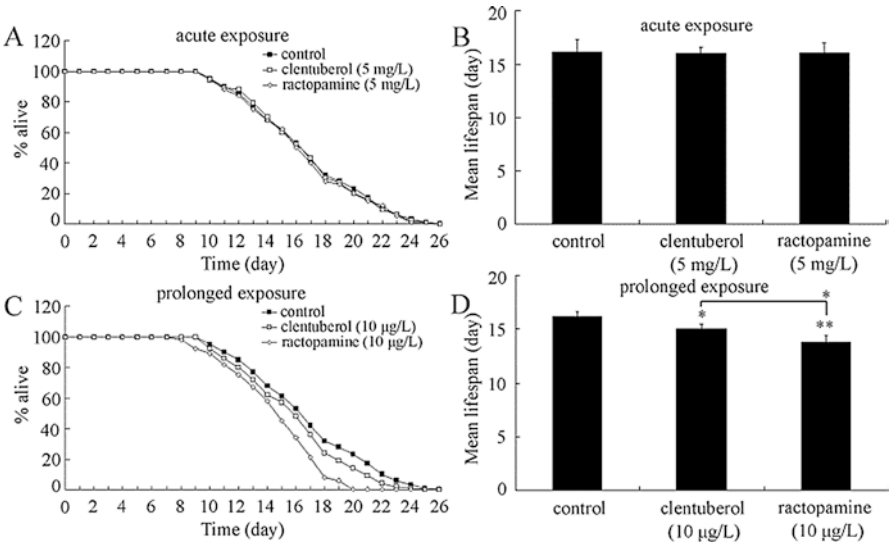

Fig. 2.2 Comparison of lifespan in nematodes exposed to clenbuterol or ractopamine [86]. (**a**, **c**) Lifespan curves of nematodes exposed to clenbuterol or ractopamine. (**b**, **d**) Comparison of mean lifespans in nematodes exposed to clenbuterol or ractopamine. Exposures were performed from the young adult for 24 h (acute exposure) or from L1-larvae to adult (prolonged exposure). Thirty nematodes were examined per treatment. Bars represent mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$

agonist and is also widely used as a feed additive to promote the reduction in body fat and to enhance the muscle growth [85]. Here, we selected the ractopamine as an example to explain the effect of certain environmental toxicants or stresses in reducing the lifespan by affecting the insulin signaling pathway in nematodes.

In nematodes, acute exposure to the ractopamine at the concentration of 5 mg/L could not significantly alter the lifespan (Fig. 2.2) [86]. In contrast, prolonged exposure to the ractopamine at the concentration of 10 mg/L could significantly reduce the lifespan (Fig. 2.2) [86]. Additionally, prolonged exposure to the ractopamine at the concentration of 10 mg/L could more severely inhibit the lifespan than that from the clenbuterol (Fig. 2.2) [86].

To determine the underlying molecular mechanism for the ractopamine to reduce the lifespan, the expression patterns of genes encoding the insulin signaling pathway were examined in the ractopamine-exposed nematodes. After prolonged exposure, ractopamine (10 mg/L) could significantly decrease the transcriptional expressions of *daf-16*, *skn-1*, and *aak-2* and increase the transcriptional expressions of *daf-2* and *age-1* [86], which implies that prolonged exposure to the ractopamine (10 mg/L) may reduce the lifespan by altering the molecular basis for insulin signaling pathway in nematodes.

To further confirm the functions of the dysregulated genes in the insulin signaling pathway in the regulation of ractopamine toxicity, the lifespans of some corresponding mutants exposed to the ractopamine were examined. It was found that the

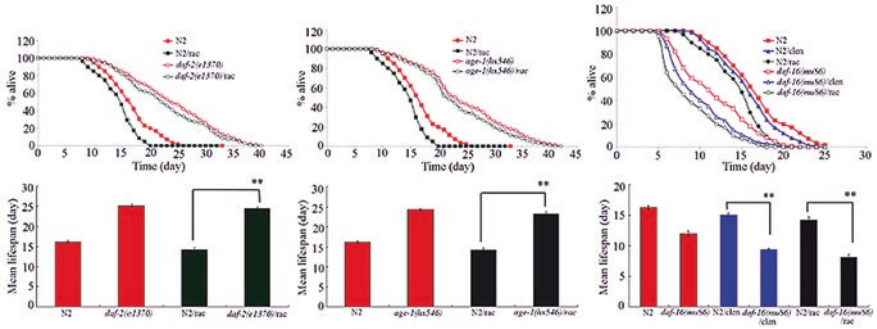

Fig. 2.3 Lifespans in wild type and mutants exposed to ractopamine [86]. Exposures were performed from L1-larvae to adult (prolonged exposure) at the concentration of 10 mg/L. Thirty nematodes were examined per treatment. rac, ractopamine. Bars represent mean ± S.E.M. $^{**}P < 0.01$

daf-16(mu86) mutant nematodes showed the susceptibility to the toxicity of ractopamine (Fig. 2.3) [86]. In contrast, the *daf-2(e1370)* or the *age-1(hx546)* mutants showed the resistance to the toxicity of ractopamine (Fig. 2.3) [86]. These results further confirm the involvement of the insulin signaling pathway in the regulation of ractopamine toxicity in nematodes.

2.3.2 *Environmental Toxicants or Stresses Reduce Lifespan by Affecting the Mitochondrial Respiration Signaling Pathway*

Graphene oxide (GO), one of the derivatives of graphene, can be potentially applied in different areas, including drug, gene carrier, and bioimaging [87–89]. In nematodes, GO exposure could cause the toxicity on the functions of both primary targets organs (such as the intestine) and secondary targeted organs (such as the neurons and reproductive organs) [90–92]. After prolonged exposure from L1-larvae to adult day 1, it was further observed that GO at concentrations of 10–100 mg/L could significantly reduce the lifespan, although GO at concentrations of 0.1–1 mg/L did not significantly affect the lifespan (Fig. 2.4) [93]. Moreover, the aging-related properties were also affected by GO exposure in nematodes. After prolonged exposure, GO (1–100 mg/L) obviously resulted in the intestinal autofluorescence caused by lysosomal deposits of lipofuscin and the intestinal ROS production, although GO (0.1 mg/L) still could not induce the noticeable intestinal autofluorescence and intestinal ROS production (Fig. 2.4) [93]. Additionally, from adult day 4 to adult day 12, prolonged exposure to of GO (10 mg/L) could significantly decrease both the head thrash and the body bend (Fig. 2.4) [93]. Therefore, prolonged exposure to GO can not only reduce the lifespan but also alter the aging-related properties in nematodes.

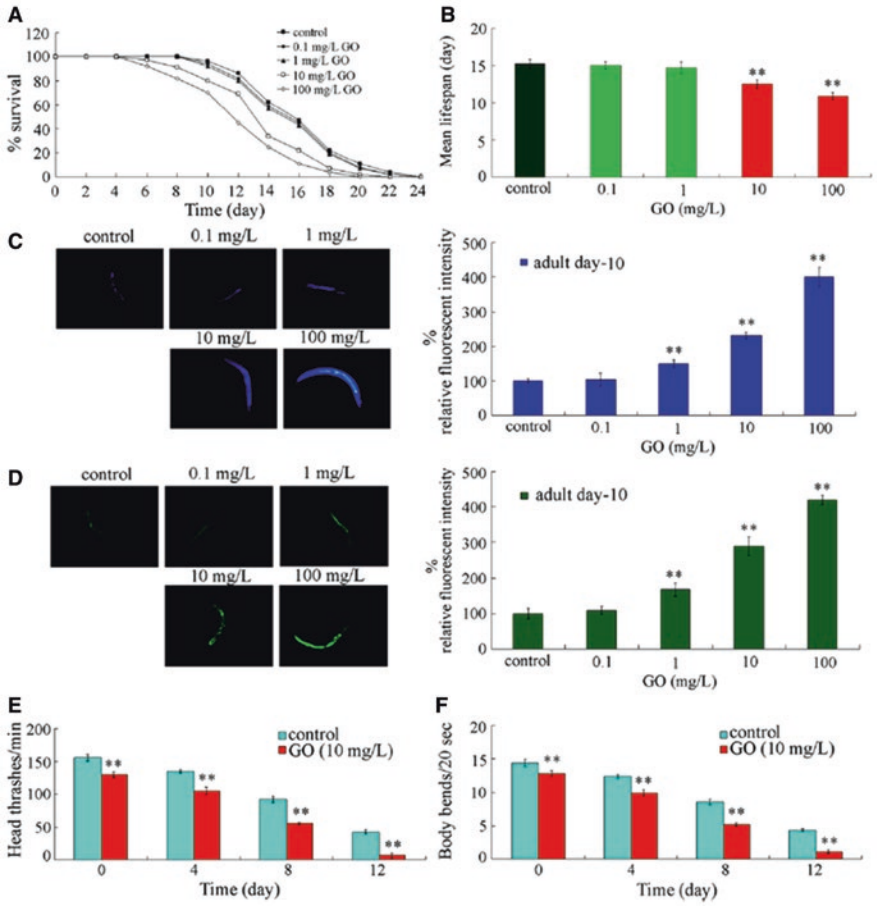

Fig. 2.4 Toxicity assessment of GO [93]. (**a**) Effects of GO exposure on lifespan. (**b**) Comparison of mean lifespans. Forty nematodes were examined per treatment. (**c**) Effects of GO exposure on intestinal autofluorescence. Forty nematodes were examined per treatment. (**d**) Effects of GO exposure on intestinal ROS production. Fifty nematodes were examined per treatment. (**e–f**) Effects of GO exposure on locomotion behavior as indicated by endpoints of head thrash and body bend. Thirty nematodes were examined per treatment. Bars represent mean ± S.E.M. $^{**}P < 0.01$

To understand the molecular basis for the observed GO toxicity, the expression patterns of genes required for the control of oxidative stress were examined. After prolonged exposure, GO (100 mg/L) caused the significant decrease in transcriptional expression of *gas-1* and the significant increase in transcriptional expressions of *isp-1* and *clk-1* [94]. *isp-1* encodes a "Rieske" iron–sulfur protein, *gas-1* encodes a subunit of mitochondrial complex I, and *clk-1* encodes a ubiquinone biosynthesis protein COQ7. In *C. elegans*, the *gas-1(fc21)* mutant has a shortened lifespan [65], whereas mutations of *isp-1* or *clk-1* could increase the longevity [62, 63, 74].

Therefore, these results suggest that prolonged exposure to GO may reduce the lifespan by at least partially modulating the function of mitochondrial respiration signaling pathway in nematodes.

2.3.3 *Environmental Toxicants or Stresses Reduce Lifespan by Affecting Certain MicroRNAs-Mediated Molecular Signals*

With the aid of SOLiD sequencing, the GO-induced miRNA profiling was examined in nematodes. Total 23 upregulated and 8 downregulated miRNAs were identified in GO-exposed nematodes [93]. The upregulated miRNAs were *mir-259*, *mir-1820*, *mir-36*, *mir-82*, *mir-239*, *mir-246*, *mir-247*, *mir-392*, *mir-4806*, *mir-2217*, *mir-360*, *mir-4810*, *mir-4807*, *mir-1822*, *mir-4805*, *mir-800*, *mir-1830*, *mir-236*, *mir-244*, *mir-235*, *mir-4937*, *mir-4812*, and *mir-43*, and the downregulated miRNAs were *mir-1834*, *mir-800*, *mir-231*, *mir-5546*, *mir-42*, *mir-2214*, *mir-2210*, and *mir 73* [93]. With the aid of TargetScan database, the possible targeted genes for the dysregulated miRNAs by GO exposure we further predicted [93].

To further determine the role of dysregulated miRNAs in the regulation of GO toxicity, the available mutants for the identified dysregulated miRNAs were employed to further determine their role in regulating the lifespan in GO-exposed nematodes. After prolonged exposure, it was found that GO exposed *mir-244* or *mir-235* mutants showed the significantly decreased lifespan compared with that in GO exposed wild-type N2 (Fig. 2.5) [93]. In contrast, GO exposed *mir-247/797*, *mir-73/74*, or *mir-231* mutants exhibited the significantly increased lifespan compared with that in GO exposed wild-type N2 (Fig. 2.5) [93]. The GO exposed other miRNA mutants had the similar lifespan to that in GO exposed wild-type N2 (Fig. 2.5) [93]. These results suggest that only a limited number of miRNAs are involved in the control of GO toxicity in reducing the lifespan in nematodes.

Meanwhile, it has been shown that mutations of some miRNAs could also alter the aging-related properties in GO-exposed nematodes. GO-exposed *mir-244* or *mir-235* mutants showed the significantly increased induction of intestinal autofluorescence and intestinal ROS production compared with that in GO-exposed wild-type N2; however, GO-exposed *mir-247/797*, *mir-73/74*, or *mir-231* mutants exhibited the significantly decreased intestinal autofluorescence and intestinal ROS production compared with that in GO-exposed wild-type N2 (Fig. 2.6) [93]. Additionally, GO-exposed *mir-244* or *mir-235* mutants showed the significantly decreased locomotion behavior compared with that in GO-exposed wild-type N2, whereas GO-exposed *mir-247/797*, *mir-73/74*, or *mir-231* mutants had the significantly increased locomotion behavior compared with that in GO-exposed wild-type N2 (Fig. 2.6) [93].

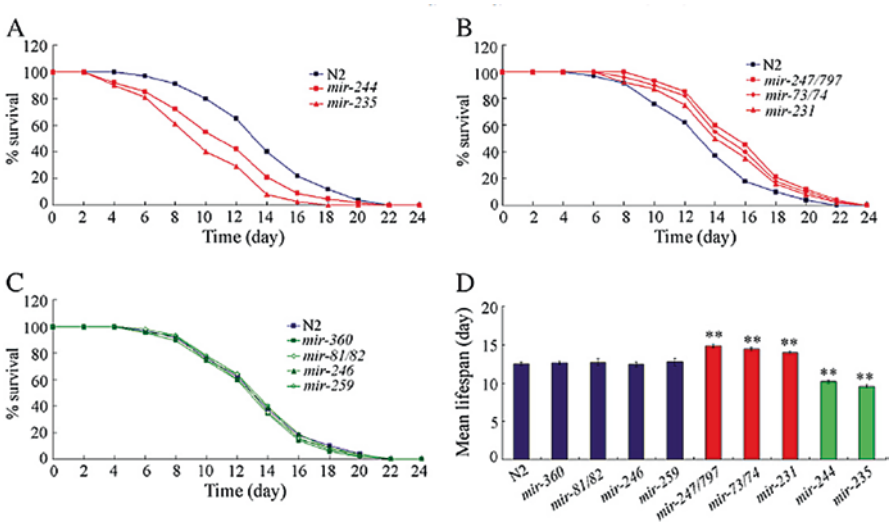

Fig. 2.5 Lifespan of mutants for some dysregulated miRNAs exposed to GO [93]. (**a**) Lifespan of wild-type, *mir-244*, and *mir-235* mutants exposed to GO. (**b**) Lifespan of wild-type, *mir-247/797*, *mir-73/74*, and *mir-231* mutants exposed to GO. (**c**) Lifespan of wild-type, *mir-360*, *mir-81/82*, *mir-246*, and *mir-259* mutants exposed to GO. Forty nematodes were examined per treatment. Exposure concentration was 10 mg/L. Bars represent mean ± S.E.M. $^{**}P < 0.01$

To understand the underlying molecular mechanism for the candidate miRNAs in the regulation of GO toxicity in reducing lifespan, their corresponding targeted genes with function involved in the control of longevity were searched. In nematodes, *daf-16*, *daf-18*, *pdk-1*, *akt-2*, *sgk-1*, *smk-1*, *hcf-1*, *aak-2*, *unc-51*, *daf-15*, *raga-1*, *rheb-1*, *pha-4*, *daf-9*, *daf-12*, and *kri-1* were possible targeted genes for dysregulated miRNAs in GO-exposed nematodes [93], and meanwhile these genes are involved in the molecular control of longevity [30].

Moreover, based on the quantitative analysis, it was found that the expression patterns of *daf-16*, *daf-18*, *pdk-1*, *sgk-1*, *smk-1*, *daf-15*, and *kri-1* could be significantly altered in nematodes exposed to GO (10 mg/L) (Fig. 2.7) [93]. After GO (10 mg/L) exposure, transcriptional expressions of *pdk-1* and *daf-15* were significantly increased; however, transcriptional expressions of *daf-16*, *daf-18*, *sgk-1*, *smk-1*, and *kri-1* were significantly decreased (Fig. 2.7) [93]. In *C. elegans*, *daf-16*, *daf-18*, *pdk-1*, and *sgk-1* encode the insulin/IGF signaling pathway, *smk-1* encodes a DAF-16 transcriptional coregulator, *daf-15* encodes a component of TOR signaling pathway, and *kri-1* encodes a component of germline signaling pathway [30]. Therefore, a hypothesis was raised that the GO may reduce the lifespan through influencing the functions of insulin/IGF signaling, TOR signaling, and germline signaling pathways controlled by a limited number of miRNAs in nematodes.

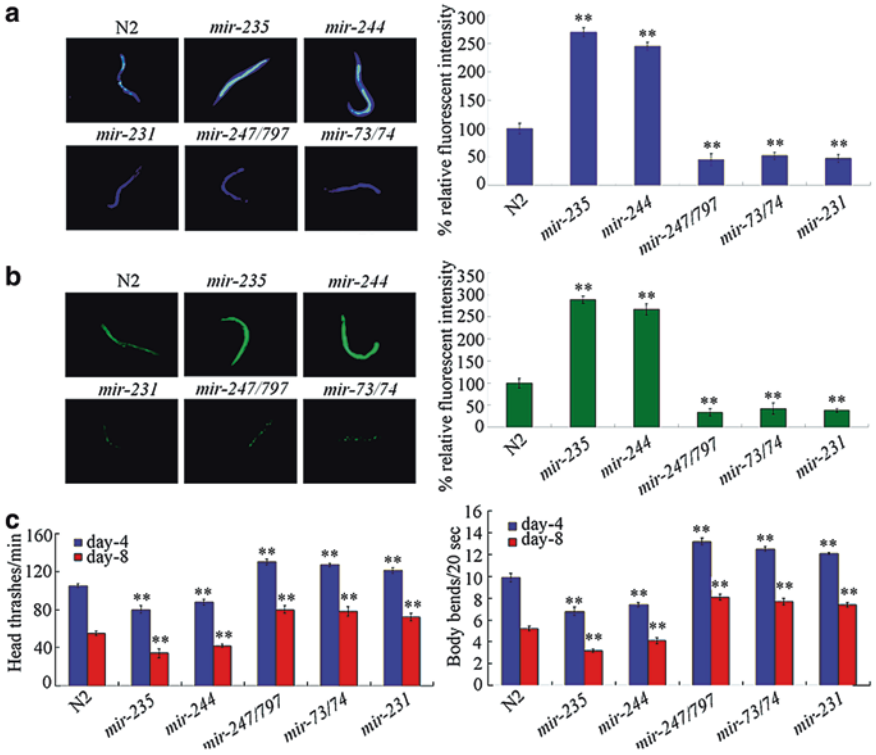

Fig. 2.6 Aging-related properties in mutants for some dysregulated miRNAs exposed to GO [93]. (**a**) Intestinal autofluorescence at day 10 in wild type and mutants of some dysregulated miRNAs exposed to GO. Fifty nematodes were examined per treatment. (**b**) Intestinal ROS productions at day 10 in wild type and mutants of some dysregulated miRNAs exposed to GO. Fifty nematodes were examined per treatment. (**c**) Locomotion behaviors in wild type and mutants of some dysregulated miRNAs exposed to GO. Thirty nematodes were examined per treatment. Exposure concentration was 10 mg/L. Bars represent mean ± S.E.M. $^{**}P < 0.01$

2.4 Innate Immune Response Is Involved in the Regulation of Longevity Reduction in Nematodes Exposed to Environmental Toxicants or Stresses

In nematodes, the effects of chronic exposure to GO on different aspects have also been examined. Chronic exposure to GO (1 mg/L) from L1-larvae to adult day 8 induced the death of nematodes (Fig. 2.8) [95], implying the formation of lifespan reduction in GO-exposed nematodes. Moreover, although exposure to GO at the concentration of 0.001 mg/L from L1-larvae to adult day 8 could not affect the functions of both primary and secondary targeted organs, exposure to GO at concentrations of 0.01–1 mg/L from L1-larvae to adult day 8 significantly decreased the locomotion behavior and induced both the intestinal autofluorescence and the

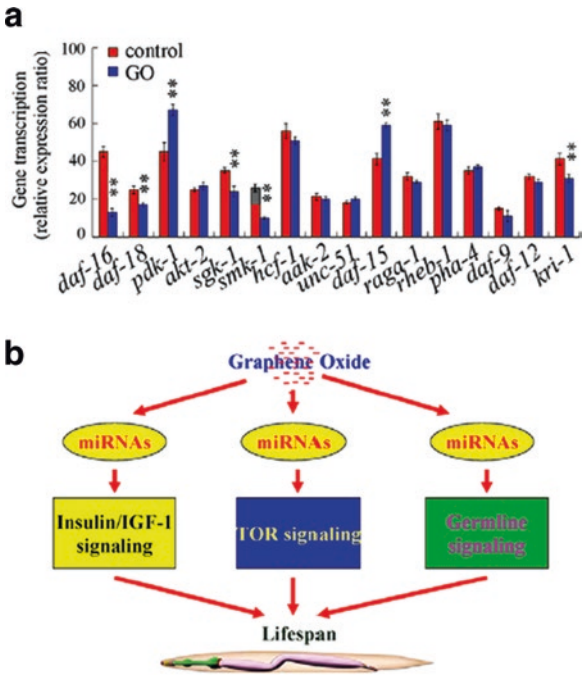

Fig. 2.7 Expression of possible targeted genes for dysregulated miRNAs in GO-exposed nematodes [93]. (**a**) Expression patterns of targeted genes for dysregulated miRNAs in GO-exposed nematodes. Exposure concentration was 10 mg/L. Bars represent means ± S.E.M. $^{**}P < 0.01$. (**b**) Model for miRNAs in regulating the GO-induced lifespan reduction in nematodes. miRNAs could regulate the GO-induced lifespan reduction through influencing the insulin/insulin-like growth factor (IGF), target of rapamycin (TOR), and germline signaling pathways in nematodes

intestinal ROS production (Fig. 2.8) [95]. These results also suggest that exposure to GO from L1-larvae to adult day 8 may potentially result in the more severe toxicity on the functions of both primary and secondary targeted organs than exposure to GO from adult day 1 to adult day 8 in nematodes [95].

In *C. elegans*, the animals can survive with *E. coli* OP50 as their food source. With an increase of GO exposure duration, a severe accumulation of OP50 in the intestine was observed in nematodes at least after adult day 4 (Fig. 2.9) [95], suggesting that chronic GO exposure can induce the OP50 proliferation in the intestine of nematodes. In contrast, in day 8 adults, only a moderate accumulation of OP50 could be observed in control nematodes (Fig. 2.9) [95]. Moreover, in the day 8 adults, an obvious colocalization of GO with OP50 was observed in GO-exposed nematodes (Fig. 2.9) [95]. In the day 6 adults, this colocalization of GO with OP50 was mainly observed in the anterior region of the intestine, and the accumulation of both GO and OP50 in the tail region was seldom observed (Fig. 2.9) [95]. In the day 8 adults, this colocalization of GO with OP50 could be further formed at both the anterior region and the posterior region (Fig. 2.9) [95]. Especially, in the day 8 adults, this colocalization of GO with OP50 could be observed at the region of the defecation structure in the tail (Fig. 2.9) [95], implying the possible disrupted defecation behavior in nematodes.

In *C. elegans*, innate immune response serves as the first line of defense for animals against the infection from pathogens, including the pathogenic microbial food OP50 [96]. To further understand the results from the severe accumulation of

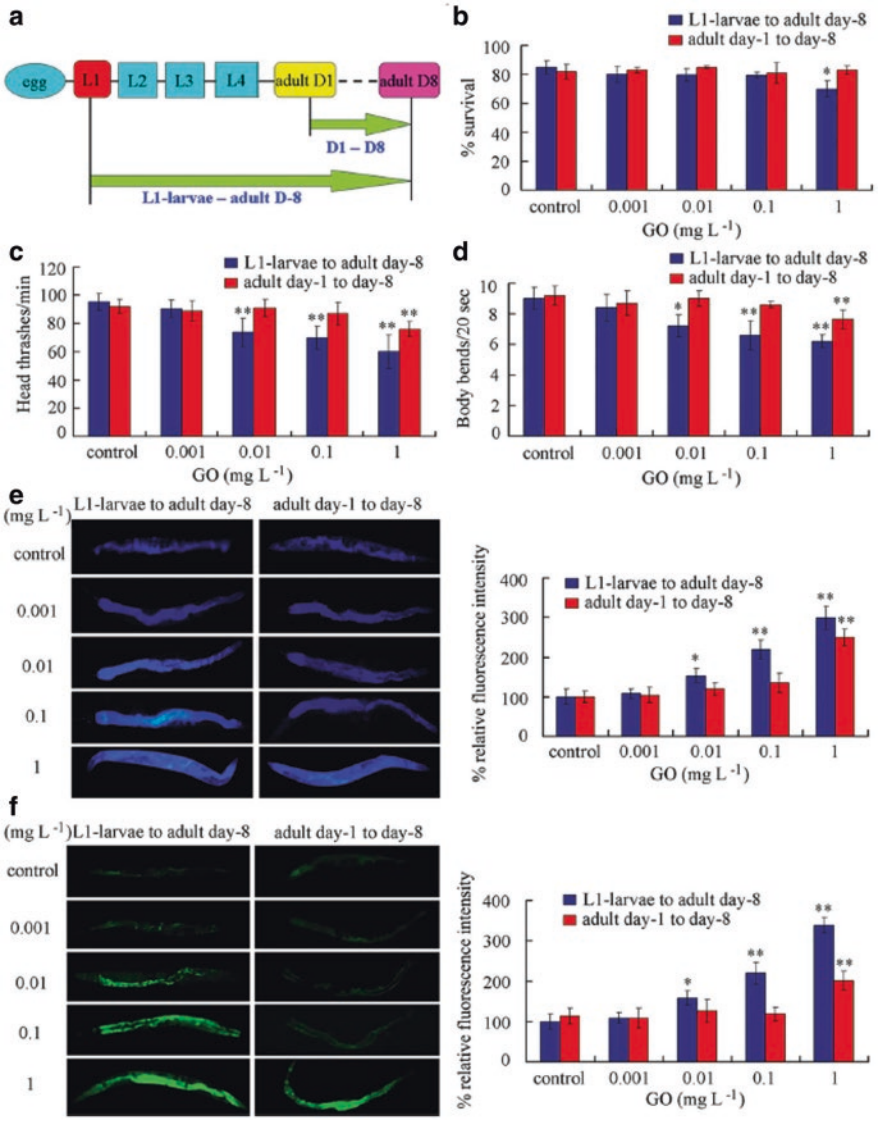

Fig. 2.8 Chronic toxicity assessment of GO using two different assay systems [95]. (**a**) Diagram of two assay systems for chronic GO exposure. (**b**) Effects of chronic GO exposure on survival of nematodes at the stage of adult day 8. (**c**) Effects of chronic GO exposure on head thrash. (**d**) Effects of GO chronic exposure on body bend. (**e**) Effects of chronic GO exposure on intestinal autofluorescence. (**f**) Effects of chronic GO exposure on intestinal ROS production. GO exposure was performed from L1-larvae to adult day 8 or from adult day 1 to adult day 8. Bars represent means ± S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$

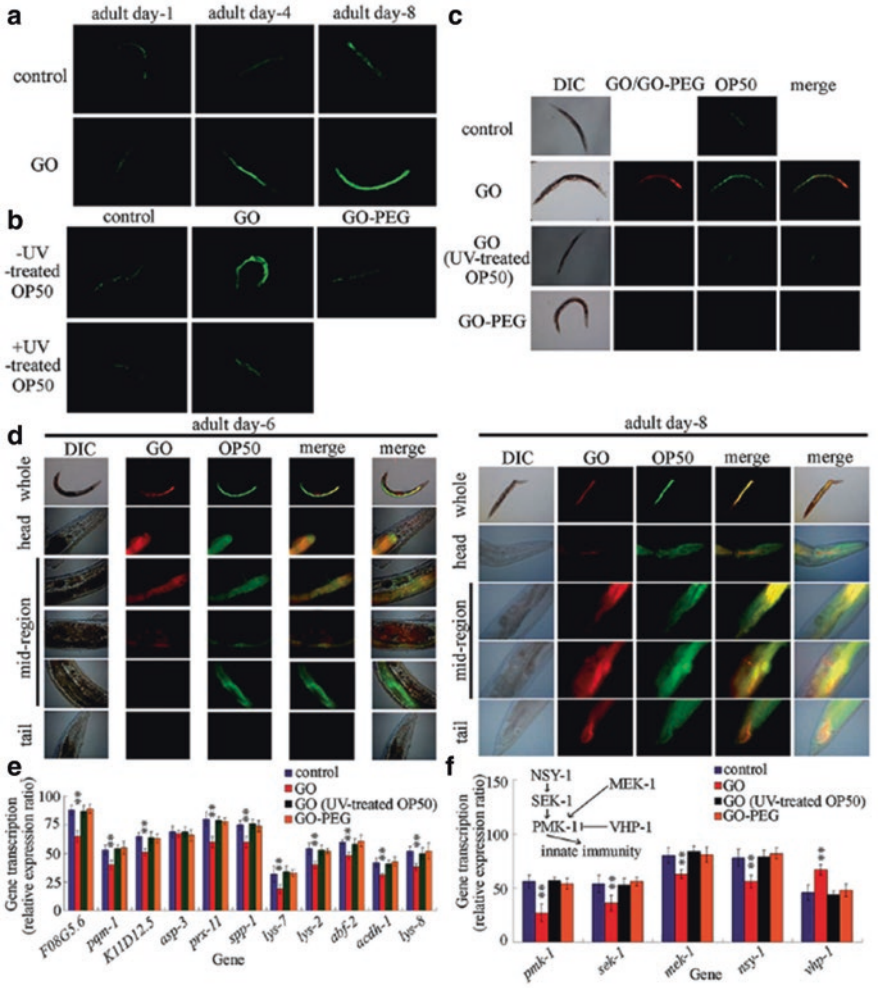

Fig. 2.9 Chronic GO exposure altered the immune response in nematodes [95]. (**a**) Chronic exposure to GO (1 mg/L) induced the accumulation of OP50 in the intestine. (**b**) Effects of UV-treated OP50 feeding or PEG surface modification on OP50 accumulation in GO (1 mg/L)-exposed nematodes. (**c**) Effects of UV-treated OP50 feeding or PEG surface modification on GO distribution and OP50 accumulation in the intestine of nematodes exposed to GO (1 mg/L). (**d**) Colocalization of GO with OP50 in GO (1 mg/L)-exposed nematodes at adult day 6 or day 8. (**e**) Quantitative real-time polymerase chain reaction assay showing effects of UV-treated OP50 feeding or PEG surface modification on expression patterns of genes encoding antimicrobial peptides in GO (1 mg/L)-exposed nematodes at adult day 8. (**f**) Quantitative real-time polymerase chain reaction assay showing effects of UV-killed OP50 feeding or PEG surface modification on expression patterns of genes encoding the p38 MAPK signaling pathway in GO (1 mg/L)-exposed nematodes at adult day 8. GO exposure at a concentration of 1 mg/L was performed from L1-larvae to adult day 8. Bars represent means ± S.E.M. $^{**}p < 0.01$

OP50 in the intestine, the possible involvement of innate immunity in the control of chronic GO toxicity was further analyzed. In nematodes, *F08G5.6*, *pqm-1*, *K11D12.5*, *asp-3*, *prx-11*, *spp-1*, *lys-7*, *lys-2*, *abf-2*, *acdh-1*, and *lys-8* encode antimicrobial proteins [97, 98]. Chronic GO exposure could induce a significant decrease in the transcriptional expressions of *F08G5.6*, *pqm-1*, *K11D12.5*, *prx-11*, *spp-1*, *lys-7*, *lys-2*, *abf-2*, *acdh-1*, and *lys-8* (Fig. 2.9) [95]. *nlp-29* encodes another antimicrobial peptide in nematodes. The expression of P*nlp-29::GFP* also exhibited a similar pattern in the transgenic nematodes after chronic exposure to GO [95].

In *C. elegans*, p38 MAP kinase (MAPK) pathway is one of the key signaling pathways required for the control of innate immune response to pathogen infection [97, 98]. It was further observed that chronic exposure to GO could significantly decrease the transcriptional expressions of *nsy-1*, *sek-1*, *pmk-1*, and *mek-1* and increase the transcriptional expression of *vhp-1* in the p38 MAPK signaling pathway (Fig. 2.9) [95]. Together, the innate immune response may be suppressed by chronic exposure to GO through inducing the severe accumulation of OP50 in the intestine of nematodes. That is, the suppression in certain protective response(s) may contribute greatly to the lifespan reduction in nematodes exposed to environmental toxicants or stresses.

2.5 Genetic Identification of Genes and Signaling Cascade in the Regulation of Toxicity on Lifespan by Environmental Toxicants or Stresses

Ultraviolet (UV) light is a ubiquitous environmental stress with the potential induction of DNA damage and deleterious somatic mutations by inducing pyrimidine dimmers [99, 100]. UV irradiation also potentially caused the alterations in various cellular components through formation of free radicals [99, 100].

In *C. elegans*, the Age mutants were employed to test their possible involvement in the control of resistance to UV light (Uvr). It has been shown that the *age-l (hx546)* survived significantly longer than wild type after UV irradiation (Fig. 2.10) [101]. The mean lifespan of the other possible allele, *age-l(hx542)*, was also longer than wild type after UV irradiation (Fig. 2.10) [101]. Meanwhile, all other Age mutants, *daf-2*, *daf-23*, *daf-28*, *spe-26*, and *clk-1*, were also UV resistant (Fig. 2.10) [101]. In contract, in the TGF-β signaling pathway, the *daf-7* mutant was indistinguishable from wild type for UV resistance, and *daf-4* mutant was more sensitive than wild type [101].

Some evidence was further raised to prove the role of *daf-16* mutation in suppressing the resistance of Age mutant to UV irradiation. The increased UV resistance of all the recessive mutants *(age-1*, *daf-2*, *daf-23*, *spe-26*, and *clk-1)* was obviously suppressed by *daf-16* mutation (Fig. 2.11) [101]. Based on the observation above, a genetic pathway model for Age genes in the regulation of the resistance to UV irradiation and the dauer formation was provided in Fig. 2.12.

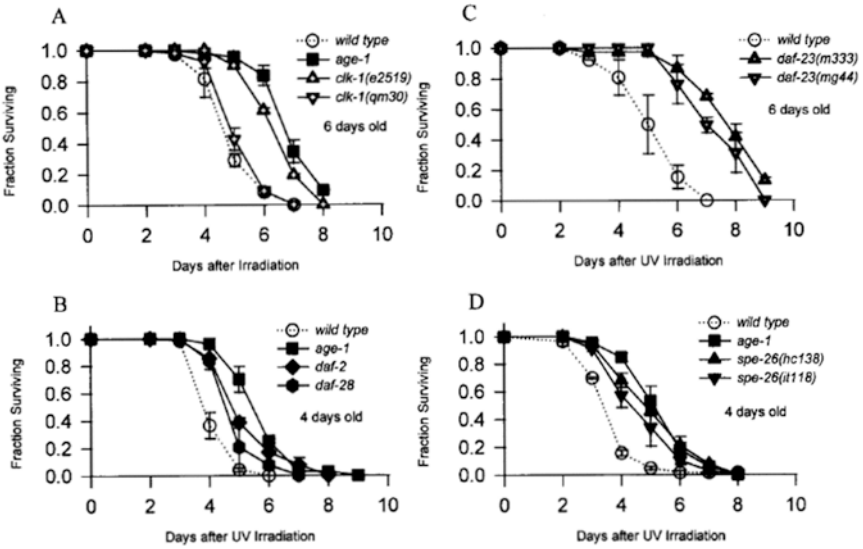

Fig. 2.10 Increased UV resistance of Age mutants in one experiment after UV irradiation [101]. Survival of the Age strains was significantly longer than wild type, N2 ($p < 0.0001$). The Uvr of the non-Age mutant, *ck-l(qm30)*, was not increased. (**a**) Survival of *age-l(hx546)*, *ck-l(e2519)*, and *clk-l(qm30)*. (**b**) Survival of *age-I(hx546)*, *daf2(e1370)*, and *daf28(sa191)*. (**c**) Survival of *daf23(m333)* and *daf23(mg44)*. (**d**) Survival of *age-l(hx546)* and the *hc138* and *it118* alleles of *spe-26*

Mean life span of various mutants and combinations

Expt	*Genotype*	Mean life span (days)	Ratio *vs.* wt[a]	Maximum life span (days)[b]	*N*	*P vs.* wt[c]
1	*wild type*	19.8 ± 4.5		26, 33	50	
	daf-16(m26)	19.7 ± 3.1	0.99	26, 26	59	0.678
	age-1(hx546) fer-15(b26)	38.3 ± 9.5	1.93	57, 57	39	<0.0001
	age-1(hx546) fer-15(b26); daf-16(m26)	23.0 ± 4.4	1.16	33, 33	71	<0.0001[d]
2	*wild type*	21.2 ± 8.9		37, 37	53	
	daf-16(m26)	20.3 ± 8.0	0.95	40, 40	54	0.536
	age-1(hx546)	30.2 ± 11.8	1.42	50, 56	49	<0.001
	age-1(hx546); daf-16(m26)	20.7 ± 8.0	0.97	28, 37	41	0.652
3	*wild type*	19.3 ± 6.4		28, 31	47	
	daf-16(m26)	18.9 ± 5.9	0.98	28, 31	60	0.473
	spe-26(hc138)	28.0 ± 6.9	1.45	38, 38	33	<0.0001
	spe-26(hc138); daf-16(m26)	19.9 ± 4.6	1.03	25, 31	41	0.675
4	*wild type*	19.8 ± 5.0		24, 27	36	
	daf-16(m26)	19.2 ± 4.8	0.97	24, 24	31	0.583
	daf-16(m27)	20.7 ± 3.4	1.05	24, 27	36	0.705
	age-1(hx542) fer-15(b26)	42.0 ± 7.9	2.12	45, 45	37	<0.0001
	clk-1(e2519)	33.6 ± 13.0	1.70	40, 40	24	<0.001
	spe-26(it118)	28.9 ± 12.7	1.46	40, 40	45	0.005
	age-1(hx542) fer-15(b26); daf-16(m27)	22.1 ± 4.6	1.12	27, 27	43	0.019
	clk-1(e2519); daf-16(m26)	21.4 ± 5.2	1.08	30, 30	46	0.047
	clk-1(e2519); daf-16(m27)	20.7 ± 5.6	1.05	27, 30	51	0.338
	spe-26(it118); daf-16(m27)	16.7 ± 5.0	0.84	24, 24	39	0.008

Fig. 2.11 Mean lifespan of various mutants and combination [101]. Daf-16 mutants represses UV resistance and life extension

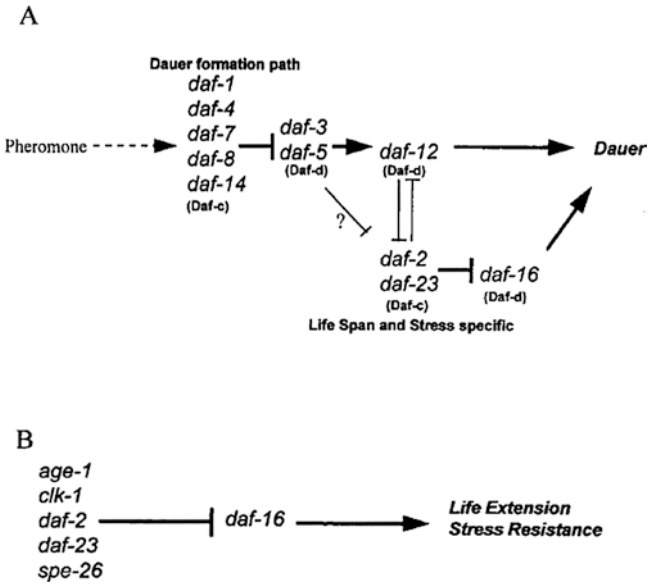

Fig. 2.12 A genetic pathway model for Age genes in the regulation of resistance to UV irradiation [101]. (**a**) A partial genetic pathway for dauer formation. (**b**) A genetic pathway for the resistance to UV irradiation

2.6 Environmental Toxicants or Stresses Reduce Lifespan by Affecting Signaling Pathways Associated with the Stress Response

In nematodes, p38 MAPK signaling pathway is at least required for the control of pathogen response and stress response [102, 103]. In the p38 MAPK signaling pathway, the core signaling pathway contains components of *pmk-1* encoded MAPK, *sek-1* encoded MAPK kinase (MAPKK), and *nsy-1* encoded MAPK kinase kinase (MAPKKK). We here selected the p38 MAPK signaling pathway to explain the important role of signaling pathways associated with the stress response in the regulation of toxicity from certain environmental toxicants or stresses in reducing the lifespan in nematodes.

In nematodes, after prolonged exposure, GO (100 mg/L) could significantly increase the transcriptional expression of *pmk-1*, *sek-1*, and *nsy-1* (Fig. 2.13) [104]. PMK-1/MAPK is predominantly expressed in the intestine. Moreover, exposure to GO (100 mg/L) could significantly increase the PMK-1::GFP expression in the intestine and the percentage of PMK-1::GFP nucleus localization in intestinal cells (Fig. 2.13) [104]. Activation of the p38 MAPK signaling pathway requires the phosphorylation of p38 MAPK/PMK-1. Exposure to GO (100 mg/L) obviously increased the expression of phosphorylated PMK-1 compared with control (Fig. 2.13) [104].

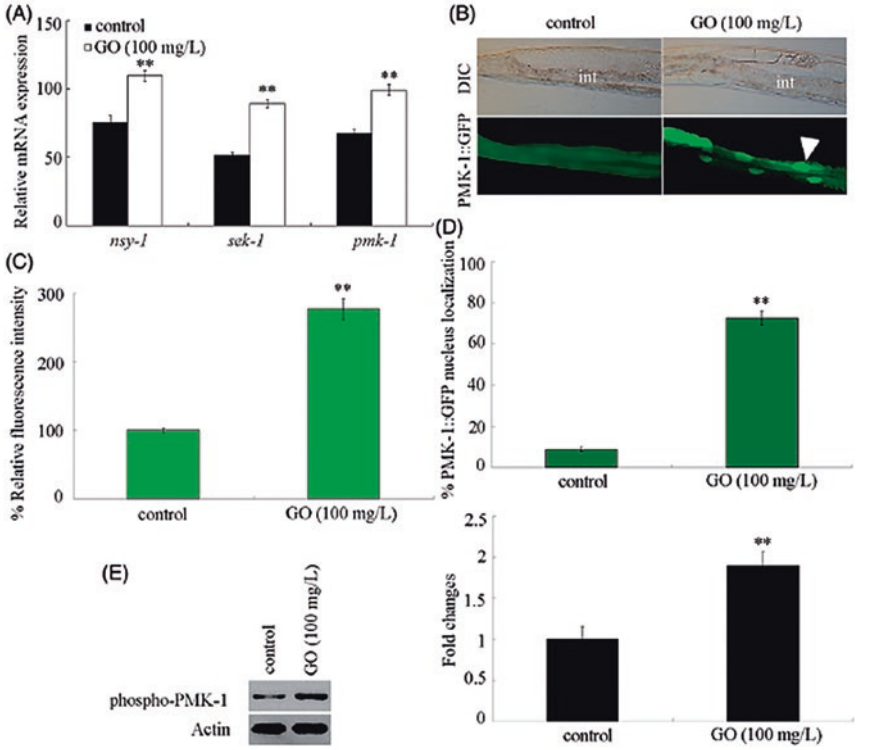

Fig. 2.13 Effects of GO exposure on expression pattern of genes encoding p38 MAPK signaling pathway in wild-type nematodes [104]. (**a**) GO exposure altered the transcriptional expression of genes encoding p38 MAPK signaling pathway. (**b**) GO exposure affected PMK-1::GFP expression in the intestine. int, intestine. Arrowhead indicates the localization of PMK-1::GFP in nucleus of intestinal cells. (**c**) Comparison of relative fluorescence intensity of PMK-1::GFP in the intestine. (**d**) GO exposure influenced nucleus translocation of PMK-1::GFP. (**e**) Western blotting analysis of the effect of GO exposure on expression level of phosphorylated PMK-1. Actin protein was used as the loading control. Prolonged exposure was performed from L1-larvae to young adults. Bars represent means ± SEM. $^{**}p < 0.01$ vs control

More importantly, it has been recently observed that chronic exposure to GO (10 mg/L) from L1-larvae to adult day 8 could further significantly decrease the transcriptional expression of *pmk-1*, *sek-1*, and *nsy-1* (Shao and Wang, unpublished data). Therefore, GO exposure can potentially dysregulate the expression of p38 MAPK signaling pathway in nematodes.

To confirm the function of genes encoding the core p38 MAPK signaling pathway in the regulation of GO toxicity, the GO toxicity in mutants for genes encoding the core p38 MAPK signaling pathway was investigated. *pmk-1(km25)*, *sek-1(ag1)*, and *nsy-1(ag3)* mutants have the normal lifespan (Fig. 2.14) [104]. It was observed that, after prolonged exposure, GO (100 mg/L) exposure induced the more reduced

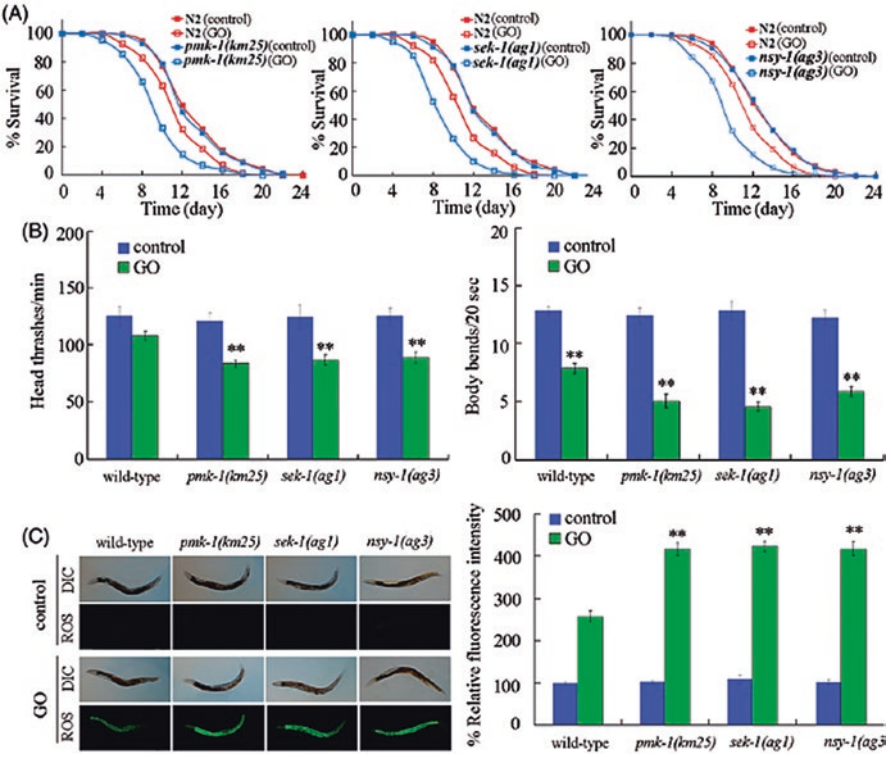

Fig. 2.14 Toxicity assessment of GO exposure on mutants of genes encoding p38 MAPK signaling pathway [104]. (**a**) Toxicity assessment of GO exposure on lifespan of mutants of genes encoding p38 MAPK signaling pathway. (**b**) Toxicity assessment of GO exposure on locomotion behavior of mutants of genes encoding p38 MAPK signaling pathway. Locomotion behavior was assessed by the endpoints of head thrash and body bend. (**c**) Toxicity assessment of GO exposure in inducing ROS production in mutants of genes encoding p38 MAPK signaling pathway. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 100 mg/L. Bars represent means ± SEM. $^{**}p < 0.01$ vs wild type

lifespan in *pmk-1(km25)*, *sek-1(ag1)*, or *nsy-1(ag3)* mutant than that in wild-type N2 nematodes (Fig. 2.14) [104]. Similarly, GO exposure at the concentration of 1 or 100 mg/L also resulted in the more decreased locomotion behavior in *pmk-1(km25)*, *sek-1(ag1)*, or *nsy-1(ag3)* mutant than that in wild-type N2 nematodes (Fig. 2.14) [104]. Additionally, GO (100 mg/L) exposure also induced a more significant induction of intestinal ROS production in *pmk-1(km25)*, *sek-1(ag1)*, or *nsy-1(ag3)* mutant than that in wild-type N2 nematodes (Fig. 2.14) [104]. Therefore, mutations of genes encoding core p38 MAPK signaling pathway may induce a susceptibility to GO toxicity, including the lifespan reduction.

As indicated above, prolonged exposure to GO (100 mg/L) could significantly increase the expression of *isp-1* and decrease the expression of *gas-1* in nematodes [94]. Moreover, after GO (100 mg/L) exposure, it was found that mutation of *pmk-1*,

sek-1, or *nsy-1* could more severely increase the expression of *isp-1* and decrease the expression of *gas-1* compared with those in wild-type nematodes [104]. These results imply that certain environmental toxicants or stresses may reduce the lifespan by affecting signaling pathways associated with stress response to dysregulate the mitochondrial respiration signaling.

2.7 Perspectives

In this chapter, we focused on the toxicity assessment endpoint of lifespan to discuss the potential basic principle for the toxicity induction on different endpoints in nematodes exposed to certain environmental toxicants or stresses. One of the important principles is that exposure to the environmental toxicants or stresses can reduce the lifespan by altering molecular basis for longevity. Another important principle is that exposure to the environmental toxicants or stresses may reduce the lifespan by suppressing protective response(s), such as the innate immune response. Besides these, exposure to the environmental toxicants or stresses can also reduce the lifespan by affecting signaling pathways associated with the stress response, such as the p38 MAPK signaling pathway, which can further dysregulate at least the mitochondrial respiration signaling. Nevertheless, with the concern on different toxicity assessment endpoints, the detailed principles may be somewhat or very different. Thus, more efforts are still needed to elucidate the detailed underlying molecular mechanisms for toxicity on other aspects in nematodes.

References

1. Zhao Y-L, Yang J-N, Wang D-Y (2016) A microRNA-mediated insulin signalling pathway regulates the toxicity of multi-walled carbon nanotubes in nematode *Caenorhabditis elegans*. Sci Rep 6:23234
2. Wu Q-L, Zhi L-T, Qu Y-Y, Wang D-Y (2016) Quantum dots increased fat storage in intestine of *Caenorhabditis elegans* by influencing molecular basis for fatty acid metabolism. Nanomedicine 12:1175–1184
3. Zhi L-T, Fu W, Wang X, Wang D-Y (2016) ACS-22, a protein homologous to mammalian fatty acid transport protein 4, is essential for the control of toxicity and translocation of multi-walled carbon nanotubes in *Caenorhabditis elegans*. RSC Adv 6:4151–4159
4. Shakoor S, Sun L-M, Wang D-Y (2016) Multi-walled carbon nanotubes enhanced fungal colonization and suppressed innate immune response to fungal infection in nematodes. Toxicol Res 5:492–499
5. Yang R-L, Rui Q, Kong L, Zhang N, Li Y, Wang X-Y, Tao J, Tian P-Y, Ma Y, Wei J-R, Li G-J, Wang D-Y (2016) Metallothioneins act downstream of insulin signaling to regulate toxicity of outdoor fine particulate matter ($PM_{2.5}$) during Spring Festival in Beijing in nematode *Caenorhabditis elegans*. Toxicol Res 5:1097–1105
6. Wu Q-L, Han X-X, Wang D, Zhao F, Wang D-Y (2017) Coal combustion related fine particulate matter ($PM_{2.5}$) induces toxicity in *Caenorhabditis elegans* by dysregulating microRNA expression. Toxicol Res 6:432–441

7. Zhi L-T, Ren M-X, Qu M, Zhang H-Y, Wang D-Y (2016) Wnt ligands differentially regulate toxicity and translocation of graphene oxide through different mechanisms in *Caenorhabditis elegans*. Sci Rep 6:39261
8. Zhao L, Rui Q, Wang D-Y (2017) Molecular basis for oxidative stress induced by simulated microgravity in nematode *Caenorhabditis elegans*. Sci Total Environ 607–608:1381–1390
9. Zhao L, Qu M, Wong G, Wang D-Y (2017) Transgenerational toxicity of nanopolystyrene particles in the range of μg/L in nematode *Caenorhabditis elegans*. Environ Sci Nano 4:2356–2366
10. Xiao G-S, Zhao L, Huang Q, Yang J-N, Du H-H, Guo D-Q, Xia M-X, Li G-M, Chen Z-X, Wang D-Y (2018) Toxicity evaluation of Wanzhou watershed of Yangtze Three Gorges Reservoir in the flood season in *Caenorhabditis elegans*. Sci Rep 8:6734
11. Chen H, Li H-R, Wang D-Y (2017) Graphene oxide dysregulates Neuroligin/NLG-1-mediated molecular signaling in interneurons in *Caenorhabditis elegans*. Sci Rep 7:41655
12. Qu M, Xu K-N, Li Y-H, Wong G, Wang D-Y (2018) Using *acs-22* mutant *Caenorhabditis elegans* to detect the toxicity of nanopolystyrene particles. Sci Total Environ 643:119–126
13. Dong S-S, Qu M, Rui Q, Wang D-Y (2018) Combinational effect of titanium dioxide nanoparticles and nanopolystyrene particles at environmentally relevant concentrations on nematodes *Caenorhabditis elegans*. Ecotoxicol Environ Saf 161:444–450
14. Zhao L, Kong J-T, Krasteva N, Wang D-Y (2018) Deficit in epidermal barrier induces toxicity and translocation of PEG modified graphene oxide in nematodes. Toxicol Res 7(6):1061–1070. https://doi.org/10.1039/C8TX00136G
15. Ding X-C, Wang J, Rui Q, Wang D-Y (2018) Long-term exposure to thiolated graphene oxide in the range of μg/L induces toxicity in nematode *Caenorhabditis elegans*. Sci Total Environ 616–617:29–37
16. Xiao G-S, Chen H, Krasteva N, Liu Q-Z, Wang D-Y (2018) Identification of interneurons required for the aversive response of *Caenorhabditis elegans* to graphene oxide. J Nanbiotechnol 16:45
17. Wang D-Y (2018) Nanotoxicology in *Caenorhabditis elegans*. Springer, Singapore
18. Li W-J, Wang D-Y, Wang D-Y (2018) Regulation of the response of *Caenorhabditis elegans* to simulated microgravity by p38 mitogen-activated protein kinase signaling. Sci Rep 8:857
19. Yang R-L, Ren M-X, Rui Q, Wang D-Y (2016) A *mir-231*-regulated protection mechanism against the toxicity of graphene oxide in nematode *Caenorhabditis elegans*. Sci Rep 6:32214
20. Zhuang Z-H, Li M, Liu H, Luo L-B, Gu W-D, Wu Q-L, Wang D-Y (2016) Function of RSKS-1-AAK-2-DAF-16 signaling cascade in enhancing toxicity of multi-walled carbon nanotubes can be suppressed by *mir-259* activation in *Caenorhabditis elegans*. Sci Rep 6:32409
21. Johnson TE, Wood WB (1982) Genetic analysis of life-span in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 79:6603–6607
22. Klass MR (1977) Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. Mech Ageing Dev 6:413–429
23. Klass MR (1983) A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. Mech Ageing Dev 22:279–286
24. Kapahi P, kaeberlein M, Hansen M (2017) Dietary restriction and lifespan: lessons from invertebrate models. Ageing Res Rev 39:3–14
25. Smith-Vikos T, Slack FJ (2012) MicroRNAs and their roles in aging. J Cell Sci 125:7–17
26. Martins R, Lithgow GJ, Link W (2016) Long live FOXO: unraveling the role of FOXO proteins in aging and longevity. Aging Cell 15:196–207
27. Hekimi A, Guarente L (2003) Genetics and the specificity of the aging process. Science 299:1351–1354
28. Pan H, Finkel T (2017) Key proteins and pathways that regulate lifespan. J Biol Chem 292:6452–6460
29. Kenyon CJ (2010) The genetics of ageing. Nature 464:504–512
30. Lapierre LR, Hansen M (2012) Lessons from *C. elegans*: signaling pathways for longevity. Trends Endocrinol Metab 23:637–644

31. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G (1997) *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science 277:942–946
32. Morris JZ, Tissenbaum HA, Ruvkun G (1996) A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature 382:536–539
33. Hertweck M, Gobel C, Baumeister R (2004) *C. elegans* SGK-1 is the critical component in the Akt/PKB kinase complex to control stress response and life span. Dev Cell 6:577–588
34. Paradis S, Ruvkun G (1998) *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. Genes Dev 12:2488–2498
35. Paradis S, Ailion M, Toker A, Thomas JH, Ruvkun G (1999) A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. Genes Dev 13:1438–1452
36. Henderson ST, Johnson TE (2001) *daf-16* integrates developmental and environmental inputs to mediate aging in the nematode *Caenorhabditis elegans*. Curr Biol 11:1975–1980
37. Lee RY, Hench J, Ruvkun G (2001) Regulation of *C. elegans* DAF-16 and its human ortholog FKHRL1 by the *daf-2* insulin-like signaling pathway. Curr Biol 11:1950–1957
38. Lin K, Hsin H, Libina N, Kenyon C (2001) Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat Genet 28:139–145
39. Lin K, Dorman JB, Rodan A, Kenyon C (1997) *daf-16*: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. Science 278:1319–1322
40. Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature 389:994–999
41. Dorman JB, Albinder B, Shroyer T, Kenyon C (1995) The *age-1* and *daf-2* genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. Genetics 141:1399–1406
42. Zhao Y-L, Yang R-L, Rui Q, Wang D-Y (2016) Intestinal insulin signaling encodes two different molecular mechanisms for the shortened longevity induced by graphene oxide in *Caenorhabditis elegans*. Sci Rep 6:24024
43. Boehm M, Slack F (2005) A developmental timing microRNA and its target regulate life span in *C. elegans*. Science 310:1954–1957
44. Hsu AL, Murphy CT, Kenyon C (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 300:1142–1145
45. Tissenbaum HA, Guarente L (2001) Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. Nature 410:227–230
46. Wolff S, Ma H, Burch D, Maciel GA, Hunter T, Dillin A (2006) SMK-1, an essential regulator of DAF-16-mediated longevity. Cell 124:1039–1053
47. Shore DE, Ruvkun G (2013) A cytoprotective perspective on longevity regulation. Trends Cell Biol 23:409–420
48. Honda Y, Honda S (1999) The *daf-2* gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. FASEB J 13:1385–1478
49. Cherkasova V, Ayyadevara S, Egilmez N, Shmookler Reis R (2000) Diverse *Caenorhabditis elegans* genes that are upregulated in dauer larvae also show elevated transcript levels in long-lived, aged, or starved adults. J Mol Biol 300:433–481
50. Yu H, Larsen P (2001) DAF-16-dependent and independent expression targets of DAF-2 insulin receptor-like pathway in *Caenorhabditis elegans* include FKBPs. J Mol Biol 314:1017–1045
51. Halaschek-Wiener J, Khattra JS, McKay S, Pouzyrev A, Stott JM, Yang GS, Holt RA, Jones SJ, Marra MA, Brooks-Wilson AR, Riddle DL (2005) Analysis of long-lived *C. elegans daf-2* mutants using serial analysis of gene expression. Genome Res 15:603–615
52. Murphy C, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature 424:277–360

53. Lakowski B, Hekimi S (1998) The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 95:13091–13096
54. Jia K, Chen D, Riddle DL (2004) The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development 131:3897–3906
55. Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F (2003) Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature 426:620
56. Gelino S, Chang JT, Kumsta C, She X, Davis A, Nguyen C, Panowski S, Hansen M (2016) Intestinal autophagy improves healthspan and longevity in *C. elegans* during dietary restriction. PLoS Genet 12:e1006135
57. Toth ML, Sigmond T, Borsos E, Barna J, Erdelyi P, Takacs-Vellai K, Orosz L, Kovacs AL, Csikos G, Sass M, Vellai T (2008) Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. Autophagy 4:330–338
58. Jia K, Levine B (2007) Autophagy is required for dietary restriction-mediated lifespan extension in *C. elegans*. Autophagy 3:597–599
59. Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C (2008) A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet 4:e24
60. Heestand BN, Shen Y, Liu W, Magner DB, Storm N, Meharg C, Habermann B, Antebi A (2013) Dietary restriction induced longevity is mediated by nuclear receptor NHR-62 in *Caenorhabditis elegans*. PLoS Genet 9:e1003651
61. Lapierre LR, De Magalhaes Filho CD, McQuary PR, Chu CC, Visvikis O, Chang JT, Gelino S, Ong B, Davis AE, Irazoqui JE, Dillin A, Hansen M (2013) The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in *Caenorhabditis elegans*. Nat Commun 4:2267
62. Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) Rates of behavior and aging specified by mitochondrial function during development. Science 298:2398–2401
63. Feng J, Bussiere F, Hekimi S (2001) Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. Dev Cell 1:633–644
64. Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G (2003) A systematic RNAi screen identifies a critical role for mitochondria in *C. elegans* longevity. Nat Genet 33:40–48
65. Hartman PS, Ishii N, Kayser EB, Morgan PG, Sedensky MM (2001) Mitochondrial mutations differentially affect aging, mutability and anesthetic sensitivity in *Caenorhabditis elegans*. Mech Ageing Dev 122:1187–1201
66. Kayser EB, Sedensky MM, Morgan PG (2004) The effects of complex I function and oxidative damage on lifespan and anesthetic sensitivity in *Caenorhabditis elegans*. Mech Ageing Dev 125:455–464
67. Tsang WY, Sayles LC, Grad LI, Pilgrim DB, Lemire BD (2001) Mitochondrial respiratory chain deficiency in *Caenorhabditis elegans* results in developmental arrest and increased life span. J Biol Chem 276:32240–32246
68. Yang W, Hekimi S (2010) Two modes of mitochondrial dysfunction lead independently to lifespan extension in *Caenorhabditis elegans*. Aging Cell 9:433–447
69. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394:694–697
70. Ishii N, Takahashi K, Tomita S, Keino T, Honda S, Yoshino K, Suzuki K (1990) A methyl viologen-sensitive mutant of the nematode *Caenorhabditis elegans*. Mutat Res 237:165–171
71. Hosokawa H, Ishii N, Ishida H, Ichimori K, Nakazawa H, Suzuki K (1994) Rapid accumulation of fluorescent material with aging in an oxygen-sensitive mutant *mev-1* of *Caenorhabditis elegans*. Mech Ageing Dev 74:161–170
72. Senoo-Matsuda N, Yasuda K, Tsuda M, Ohkubo T, Yoshimura S, Nakazawa H, Hartman PS, Ishii N (2001) A defect in the cytochrome b large subunit in complex II causes both superox-

ide anion overproduction and abnormal energy metabolism in *Caenorhabditis elegans*. J Biol Chem 276:41553–41558
73. Hamilton B, Dong Y, Shindo M, Liu W, Odell I, Ruvkun G, Lee SS (2005) A systematic RNAi screen for longevity genes in *C. elegans*. Genes Dev 19:1544–1555
74. Wong A, Boutis P, Hekimi S (1995) Mutations in the *clk-1* gene of *Caenorhabditis elegans* affect developmental and behavioral timing. Genetics 139:1247–1259
75. Van Raamsdonk JM, Hekimi S (2009) Deletion of the mitochondrial superoxide dismutase *sod-2* extends lifespan in *Caenorhabditis elegans*. PLoS Genet 5:e1000361
76. Dancy BM, Sedensky MM, Morgan PG (2014) Effects of the mitochondrial respiratory chain on longevity in *C. elegans*. Exp Gerontol 56:245–255
77. Antebi A (2013) Regulation of longevity by the reproductive system. Exp Gerontol 28:596–602
78. Hsin H, Kenyon C (1999) Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature 399:362–366
79. Goudeau J, Bellemin S, Toselli-Mollereau E, Shamalnasab M, Chen Y, Aguilaniu H (2011) Fatty acid desaturation links germ cell loss to longevity through NHR-80/HNF4 in *C. elegans*. PLoS Biol 9:e1000599
80. Arantes-Oliveira N, Berman JR, Kenyon C (2003) Healthy animals with extreme longevity. Science 302:611
81. Larsen PL, Albert PS, Riddle DL (1995) Genes that regulate both development and longevity in *Caenorhabditis elegans*. Genetics 139:1567–1583
82. Wolff S, Dillin A (2006) The trifecta of aging in *Caenorhabditis elegans*. Exp Gerontol 41:894–903
83. Chan TY (1999) Health hazards due to clenbuterol residues in food. J Toxicol Clin Toxicol 37:517–519
84. Yen M, Ewald MB (2012) Toxicity of weight loss agents. J Med Toxicol 8:145–152
85. Yaeger MJ, Mullin K, Ensley SM, Ware WA, Slavin RE (2012) Myocardial toxicity in a group of greyhounds administered ractopamine. Vet Pathol 49:569–573
86. Zhuang Z, Zhao Y, Wu Q, Li M, Liu H, Sun L, Gao W, Wang D (2014) Adverse effects from clenbuterol and ractopamine on nematode *Caenorhabditis elegans* and the underlying mechanism. PLoS ONE 9:e85482
87. Shi X, Gong H, Li Y, Wang C, Cheng L, Liu Z (2013) Graphene-based magnetic plasmonic nanocomposite for dual imaging and photothermal therapy. Biomaterials 34:4786–4793
88. Park S, Ruoff RS (2009) Chemical methods for the production of graphenes. Nat Nanotechnol 4:217–224
89. Yang K, Li Y, Tan X, Peng R, Liu Z (2013) Behavior and toxicity of graphene and its functionalized derivatives in biological systems. Small 9:1492–1503
90. Ren M-X, Zhao L, Ding X-C, Krasteva N, Rui Q, Wang D-Y (2018) Developmental basis for intestinal barrier against the toxicity of graphene oxide. Particle Fibre Toxicol 15:26
91. Ren M-X, Zhao L, Lv X, Wang D-Y (2017) Antimicrobial proteins in the response to graphene oxide in *Caenorhabditis elegans*. Nanotoxicology 11:578–590
92. Xiao G-S, Zhi L-T, Ding X-C, Rui Q, Wang D-Y (2017) Value of *mir-247* in warning graphene oxide toxicity in nematode *Caenorhabditis elegans*. RSC Adv 7:52694–52701
93. Wu Q-L, Zhao Y-L, Zhao G, Wang D-Y (2014) microRNAs control of in vivo toxicity from graphene oxide in *Caenorhabditis elegans*. Nanomedicine 10:1401–1410
94. Wu Q-L, Yin L, Li X, Tang M, Zhang T, Wang D-Y (2013) Contributions of altered permeability of intestinal barrier and defecation behavior to toxicity formation from graphene oxide in nematode *Caenorhabditis elegans*. Nanoscale 5:9934–9943
95. Wu Q-L, Zhao Y-L, Fang J-P, Wang D-Y (2014) Immune response is required for the control of in vivo translocation and chronic toxicity of graphene oxide. Nanoscale 6:5894–5906
96. Hahm J, Kim S, Paik Y (2011) GPA-9 is a novel regulator of innate immunity against *Escherichia coli* foods in adult *Caenorhabditis elegans*. Aging Cell 10:208–219

97. Kawli T, Tan M (2008) Neuroendocrine signals modulate the innate immunity of *Caenorhabditis elegans* through insulin signalling. Nat Immunol 9:1415–1424
98. Pukkila-Worley R, Ausubel FM (2012) Immune defense mechanisms in the *Caenorhabditis elegans* intestinal epithelium. Curr Opin Immunol 24:3–9
99. Black HS (1987) Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. Photochem Photobiol 46:213–221
100. Friedberg EC (1985) DNA repair. W.H. Freeman and Company, New York
101. Murakami S, Johnson TE (1996) A genetic pathway conferring life extension and resistance by UV stress in *Caenorhabditis elegans*. Genetics 143:1207–1218
102. Hayakawa T, Kato K, Jayakawa R, Hisamoto N, Katsumoto K, Takeda K, Ichijo H (2011) Regulation of anoxic death in *Caenorhabditis elegans* by mammalian apoptosis signal-regulating kinase (ASK) family proteins. Genetics 187:785–792
103. Kim DH, Ausubel FM (2005) Evolutionary perspectives on innate immunity from the study of *Caenorhabditis elegans*. Curr Opin Immunol 17:4–10
104. Zhao Y-L, Zhi L-T, Wu Q-L, Yu Y-L, Sun Q-Q, Wang D-Y (2016) p38 MAPK-SKN-1/Nrf signaling cascade is required for intestinal barrier against graphene oxide toxicity in *Caenorhabditis elegans*. Nanotoxicology 10:1469–1479