Chapter 5
Functions of Insulin and the Related Signaling Pathways in the Regulation of Toxicity of Environmental Toxicants or Stresses

Abstract In nematodes, the insulin signaling pathway can potentially participate in the regulation of various biological processes. In this chapter, we further discussed the involvement and possible pivotal function of core insulin signaling pathway in the regulation of toxicity of environmental toxicants or stresses. Moreover, we introduced the related information on the potential targets for DAF-16 and the possible upregulators for the insulin signaling pathway in regulating the toxicity of environmental toxicants or stresses. The possible formation of a large physical interaction surrounding the DAF-16 in regulating the toxicity of environmental toxicants or stresses needs to be paid more attention in nematodes.

Keywords Insulin and the related signaling pathways · Molecular regulation · Environmental exposure · *Caenorhabditis elegans*

5.1 Introduction

The nematode *Caenorhabditis elegans* has been successfully used in both the toxicity assessment and the toxicological study of various toxicants or stresses [1]. In nematodes, it has been shown that exposure to environmental toxicants or stresses can lead to the toxicity on many aspects of animals as reflected by a series of toxicity assessment endpoints [2–10]. Meanwhile, the insulin signaling pathway has been widely proven to participate in the regulation of various biological processes in organisms [11–18]. More and more data have implied the possible or even the potential pivotal role of insulin signaling pathway in the regulation of stress response in nematodes exposed to environmental toxicants or stresses.

In *C. elegans*, in the core insulin signaling pathway, insulin ligands bind to DAF-2/IGF-1 receptor (InR) to activate tyrosine kinase activity, which will allow to initiate the cascade of several kinases (AGE-1/phosphatidylinositol 3-kinase (PI3K), PDK-1/3-phosphoinositide-dependent kinase 1, AKT-1/2/serine/threonine kinase Akt/PKB, and SGK-1/serine or threonine-protein kinase). AKT and the SGK-1 will further phosphorylate and inactivate the transcription factor DAF-16/FOXO, which thereby blocks the transcription of its multiple target genes to regulate various biological processes

D. Wang, *Molecular Toxicology in Caenorhabditis elegans*,
https://doi.org/10.1007/978-981-13-3633-1_5

[19, 20]. We here first introduced the involvement of core insulin signaling pathway in the regulation of toxicity of environmental toxicants or stresses. We also introduced and discussed the potential targets for DAF-16 in the insulin signaling pathway in the regulation of toxicity of environmental toxicants or stresses. Moreover, we further introduced and discussed the possible upregulators for insulin signaling pathway in the regulation of toxicity of environmental toxicants or stresses. So far, the obtained data imply the possible formation of a large physical interaction surrounding the DAF-16 in the regulation of toxicity of environmental toxicants or stresses in nematodes.

5.2 Environmental Toxicants or Stresses Dysregulate the Expression of Insulin Signaling Pathway

Graphene oxide (GO), an important carbon-based engineered nanomaterials, can cause several aspects of toxicity, including adverse effects on the function of both primary (such as the intestine) and secondary (such as the neurons and the reproductive organs) targeted organs, on nematodes [21–27]. With the GO as an example, prolonged exposure to GO (100 mg/L) could result in a significant increase in the expression levels of *daf-2*, *age-1*, *akt-1*, and *akt-2* and decrease in the expression levels of *daf-18* and *daf-16* in wild-type nematodes [28]. Additionally, a significant increase in DAF-16:GFP expression in the nuclei of GO-exposed (100 mg/L) nematodes was also observed [28], which suggests that long-term exposure to GO not only affects the transcriptional activities of genes encoding the core insulin signaling pathway but also influences the nucleus-cytoplasm translocation of DAF-16. The induction of nucleus-cytoplasm translocation of DAF-16 and/or decrease in *daf-16* expression could also be observed in nematodes exposed to heavy metals (such as Mn or As) or traffic-related $PM_{2.5}$ [29–32]. Therefore, exposure to certain environmental toxicants or stresses may potentially dysregulate the expression of insulin signaling pathway in nematodes (Fig. 5.1).

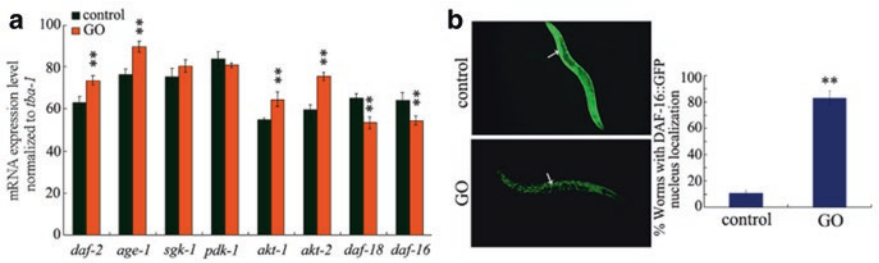

Fig. 5.1 Effects of GO exposure on the expression patterns of genes encoding insulin signaling pathway in wild-type nematodes [28]. (**a**) GO exposure altered expression levels of some genes encoding insulin signaling pathway in wild-type nematodes. (**b**) GO exposure influenced the nucleus translocation of DAF-16::GFP. Arrowheads indicate the DAF-16 expression in the intestine. GO exposure concentration was 100 mg/L. Prolonged exposure was performed from L1-larvae to young adults. Bars represent means ± SEM. $^{**}P < 0.01$ vs control

5.3 The Insulin Signaling Pathway Regulates the Toxicity of Environmental Toxicants or Stresses

Further with GO as an example, it has been shown that mutation of *daf-16* or *daf-18* could induce a susceptibility to GO toxicity in decreasing locomotion behavior and in reducing lifespan, whereas mutation of *daf-2*, *age-1*, *akt-1*, or *akt-2* could induce a resistance to GO toxicity in decreasing locomotion behavior and in reducing lifespan (Fig. 5.2) [28]. In nematodes, mutation of *daf-16* could also induce a susceptibility to the toxicity of heavy metals or traffic-related $PM_{2.5}$ in inducing intestinal ROS production [31, 33–36]. Additionally, mutation of *daf-2* or *age-1* could also suppress the toxicity of Hg in inducing deficits in development of male-specific structures, of heavy metals (Cd or Ca) or hypoxic stress in reducing lifespan, or of traffic-related $PM_{2.5}$ in inducing intestinal ROS production [31, 33–36]. These studies performed on different environmental toxicants or stresses demonstrate the important function of insulin signaling pathway in regulating the toxicity of environmental toxicants or stresses in nematodes.

5.4 Genetic Interactions of Genes in the Insulin Signaling Pathway in Regulating the Toxicity of Environmental Toxicants or Stresses

In nematodes, genetic interaction analysis demonstrated that DAF-16 acted downstream of DAF-2, AGE-1, AKT-1, or AKT-2 to regulate the GO toxicity in reducing the longevity, because mutation of *daf-16* could effectively decrease the lifespan in *daf-2*(*e1370*), *age-1*(*hx546*), *akt-1*(*ok525*), or *akt-2*(*ok393*) mutant nematodes exposed to GO (Fig. 5.3) [28]. Therefore, a signaling cascade of DAF-2-AGE-1-AKT-1/2-DAF-16 in the insulin signaling pathway was identified to be involved in the control of GO toxicity. Meanwhile, it was found that mutation of *daf-18* could effectively reduce the lifespan in *age*(*hx546*) mutant exposed to GO (Fig. 5.3) [28], which suggests the suppressor role of DAF-18 on the function of AGE-1 in the regulation of GO toxicity. The raised signaling cascade in the insulin signaling in regulating toxicity of environmental toxicants or stresses was further supported or confirmed by other toxicological studies performed in nematodes. It was also observed that mutation of *daf-16* could suppress the resistance of *daf-2* mutant nematodes to the traffic-related $PM_{2.5}$ toxicity in inducing intestinal ROS production or enhancing intestinal permeability, to the combined Ca/Cd toxicity in reducing the lifespan, or to the As toxicity in inducing ROS production [31, 32, 35].

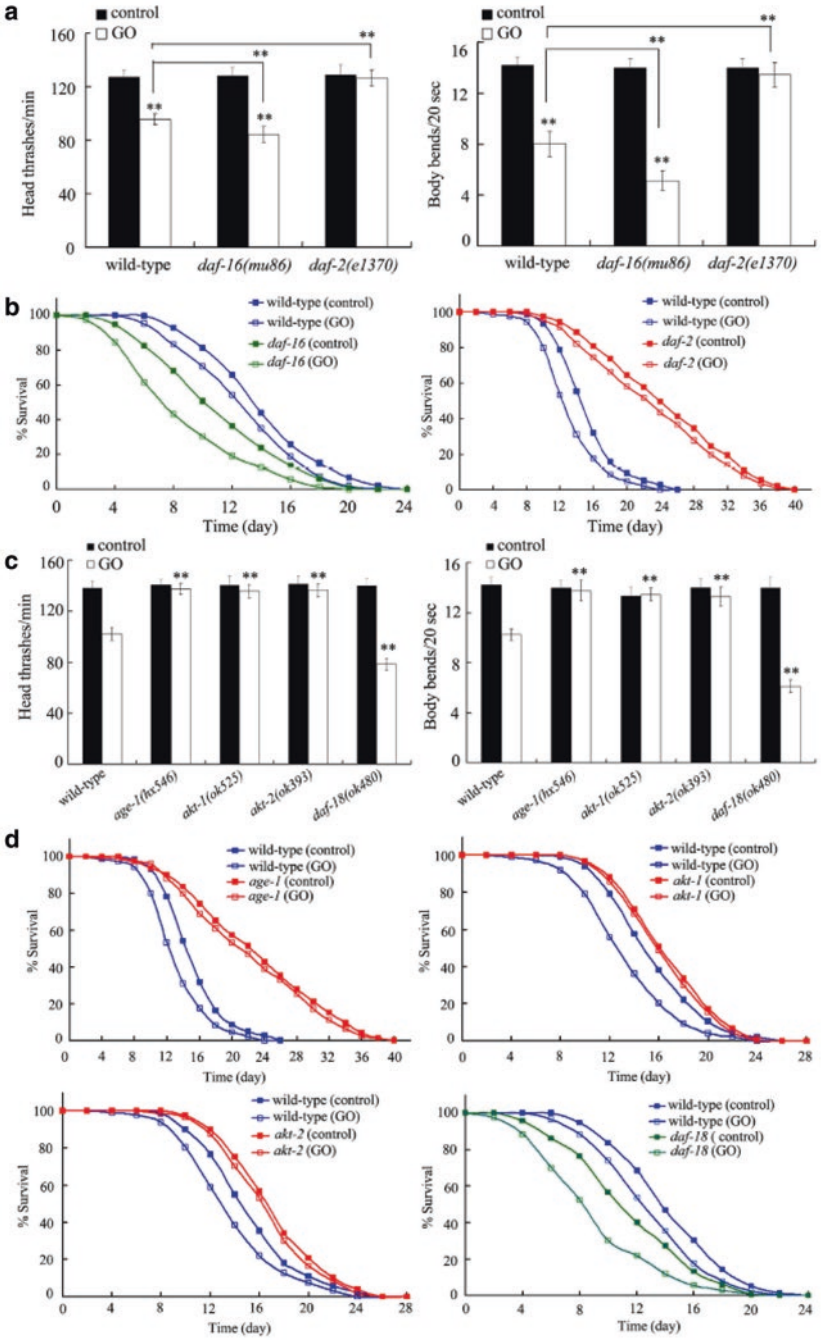

Fig. 5.2 Effects of *daf-16*, *daf-2*, *age-1*, *akt-1*, *akt-2*, or *daf-18* mutation on nematodes exposed to GO [28]. (**a**) Effects of *daf-16* or *daf-2* mutation on locomotion behavior in nematodes exposed to GO. (**b**) Effects of *daf-16* or *daf-2* mutation on lifespan in nematodes exposed to GO. (**c**) Mutations of *age-1*, *akt-1*, *akt-2*, or *daf-18* affected GO toxicity on locomotion behavior in nematodes. (**d**) Mutations of *age-1*, *akt-1*, *akt-2*, or *daf-18* affected GO toxicity on lifespan in nematodes. GO exposure concentration was 100 mg/L. Prolonged exposure was performed from L1-larvae to young adults. Bars represent means ± SEM. $^{**}P < 0.01$ vs control (if not specially indicated)

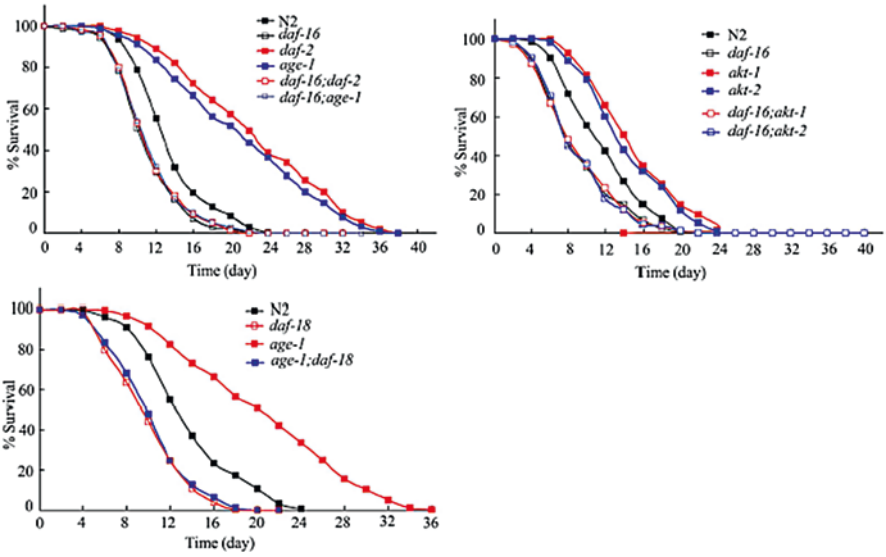

Fig. 5.3 Genetic interactions of genes in the insulin signaling pathway in regulating the GO toxicity on lifespan in nematodes [28]. GO exposure concentration was 100 mg/L. Prolonged exposure was performed from L1-larvae to young adults

5.5 Targets of DAF-16 in Regulating the Toxicity of Environmental Toxicants or Stresses

5.5.1 SOD-3

In *C. elegans*, *daf-16* is expressed in almost all tissues, including the intestine, the neurons, the muscle, and the pharynx. Nevertheless, expression of the *daf-16* in neurons, muscle, or pharynx could not significantly affect the GO toxicity in decreasing locomotion behavior and in reducing lifespan in *daf-16*(*mu86*) mutant nematodes [28]. In contrast, intestinal expression of *daf-16* could effectively augment the decreased locomotion behavior or reduced lifespan in GO-exposed *daf-16*(*mu96*) mutant nematodes [28], which demonstrating that the DAF-16 acts primarily in the intestine to regulate the toxicity of environmental toxicants or stresses. Actually, the core insulin signaling pathway can act in the intestine to regulate the GO toxicity in nematodes [28].

SOD-3, a mitochondrial iron/manganese superoxide dismutase, is expressed in the pharynx in the head, the intestine, the muscle, the vulva, and the tail. Intestinal RNAi knockdown of *sod-3* could induce a susceptibility to GO toxicity in reducing lifespan [28]. Genetic interaction analysis suggested that DAF-16 acted upstream of SOD-3 to regulate the GO toxicity, because the resistance of transgenic strain of *Ex*(*Pges-1-daf-16*) overexpressing intestinal DAF-16 to GO toxicity in reducing lifespan and in inducing intestinal ROS production could be inhibited by *sod-3*

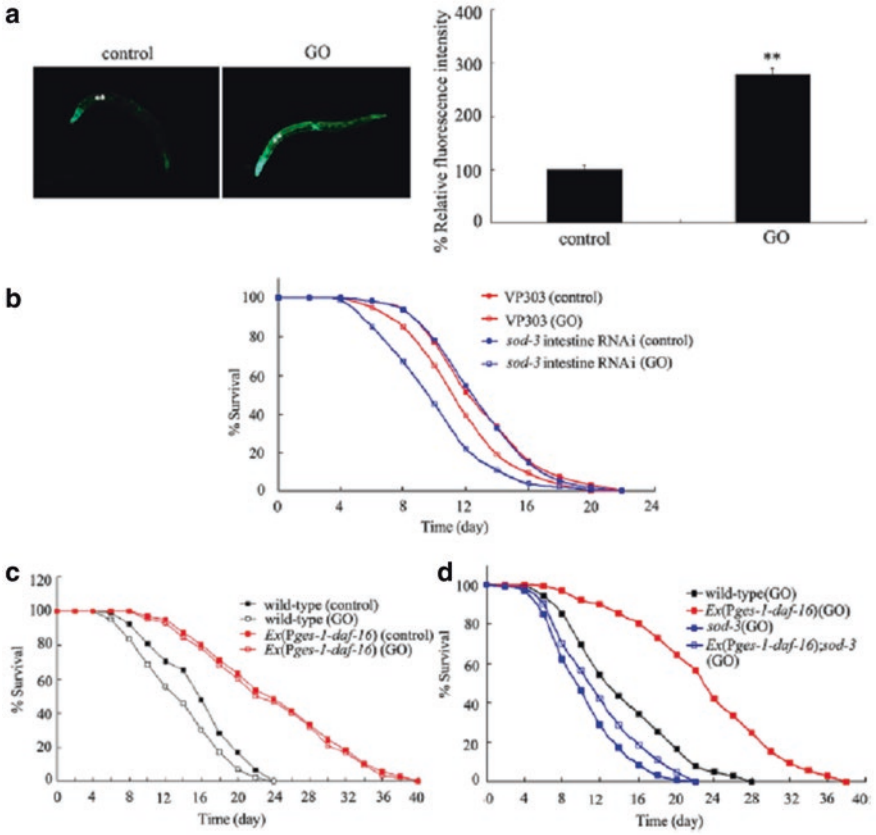

Fig. 5.4 Role of SOD-3 in regulating the GO toxicity in nematodes [28]. (**a**) Effects of GO exposure on SOD-3::GFP expression. The left shows images for SOD-3::GFP expression, and the right shows comparison of relative fluorescence intestine of SOD-3::GFP in the intestine of nematodes. Asterisks indicate the intestine of nematodes. (**b**) Effects of intestine-specific RNAi of *sod-3* gene on lifespan in GO-exposed nematodes. (**c**) Effects of intestinal overexpression of *daf-16* gene on GO toxicity on lifespan in nematodes. (**d**) Effects of *sod-3* mutation on lifespan in GO-exposed nematodes overexpressing *daf-16* gene in the intestine in nematodes. GO exposure concentration was 100 mg/L. Prolonged exposure was performed from L1-larvae to young adults. Bars represent means ± SEM. $^{**}P < 0.01$ vs wild type

mutation (Fig. 5.4) [28]. This observation also implies that prolonged exposure to GO could inhibit the function of DAF-16 within the insulin signaling and, thereby, result in the further suppression of the function of SOD-3, which plays an important role in defense against oxidative stress in nematodes [28].

5.5.2 *Antimicrobial Proteins*

In nematodes, among the candidate targeted genes for DAF-16, some genes (*lys-1*, *lys-7*, *lys-8*, *dod-6*, *F55G11.4*, *spp-1*, *spp-12*, and *dod-22*) encoding potential antimicrobial proteins have also been identified to act as the targeted genes for *daf-16* in regulating the toxicity of GO toxicity [37–40]. Among these genes encoding potential antimicrobial proteins, RNAi knockdown of *lys-1*, *dod-6*, *F55G11.4*, *lys-8*, or *spp-1* could significantly suppress the resistance of nematodes overexpressing intestinal *daf-16* to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior (Fig. 5.5) [41], suggesting that LYS-1, DOD-6,

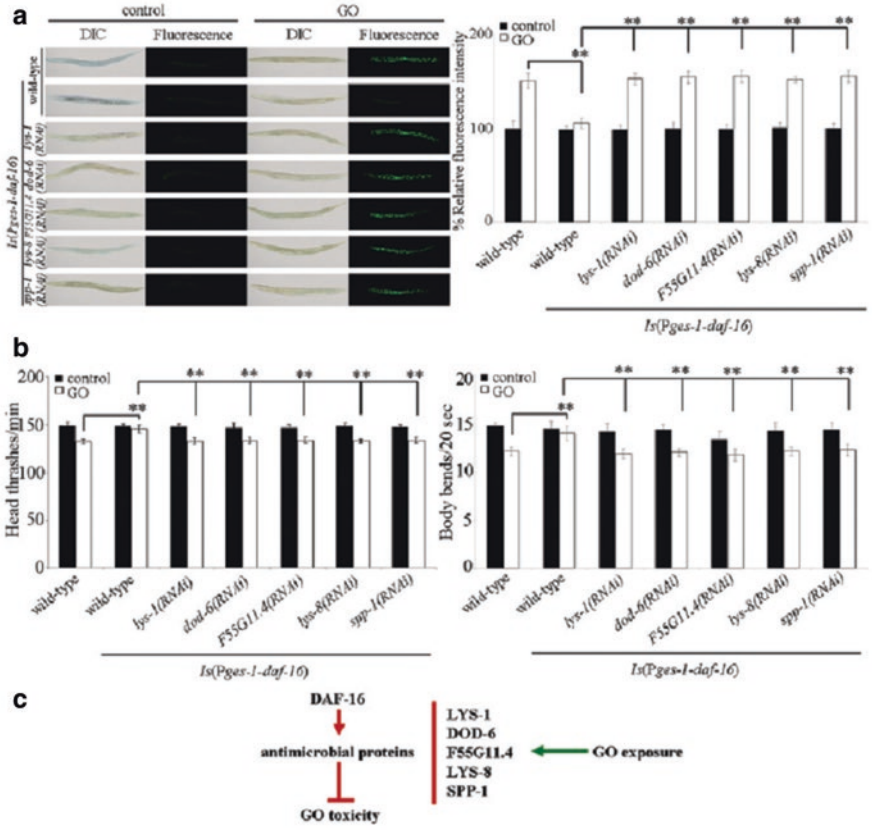

Fig. 5.5 Antimicrobial genes acted downstream of *daf-16* to regulate the GO toxicity [41]. (**a**) Antimicrobial genes acted downstream of *daf-16* to regulate the GO toxicity in inducing intestinal ROS production. (**b**) Antimicrobial genes acted downstream of *daf-16* to regulate the GO toxicity in decreasing locomotion behavior. (**c**) A diagram showing the interaction between DAF-16 and antimicrobial proteins in the regulation of GO toxicity. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 10 mg/L. Bars represent means ± SD. $^{**}p < 0.01$

F55G11.4, LYS-8, and SPP-1 act as downstream targets for intestinal DAF-16 in regulating the toxicity of environmental toxicants or stresses.

Among LYS-1, DOD-6, F55G11.4, LYS-8, and SPP-1, F55G11.4 and SPP-1 acted further downstream of SOD-3 in the regulation of GO toxicity, because RNAi knockdown of *F55G11.4* or *spp-1* could significantly suppress the resistance of nematodes overexpressing intestinal *sod-3* to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior [41]. The antimicrobial proteins of F55G11.4 and SPP-1 affected the expression of *gas-1* encoding a subunit of mitochondrial complex I that is required for the oxidative phosphorylation in GO-exposed nematodes [41], implying the important effect of F55G11.4 and SPP-1 on GAS-1-mediated molecular basis for oxidative stress in GO-exposed nematodes.

5.5.3 MTL-1 and MTL-2

In nematodes, *mtl-1* and *mtl-2* encode metallothioneins and can be expressed in the intestine. Exposure to the outdoor $PM_{2.5}$ could induce the significant expression of MTL-1 and MTL-2 [42]. Meanwhile, mutation of the *mtl-1* or *mtl-2* resulted in a susceptibility to the outdoor $PM_{2.5}$ toxicity [42], because a more severe decrease in locomotion behavior and a more significant induction of intestinal ROS production were observed in *mtl-1(tm1770)* or *mtl-2(gk125)* mutants exposed to outdoor $PM_{2.5}$ (10 mg/L) compared with those in wild-type nematodes [42]. After $PM_{2.5}$ exposure, the head thrash and body bend in the double mutant of *daf-16(mu86);mtl-1(RNAi)* were similar to those in *daf-16(mu86)* or *mtl-1(RNAi)* nematodes, and the head thrash and body bend in the double mutant of *daf-16(mu86);mtl-2(RNAi)* were similar to those in *daf-16(mu86)* or *mtl-2(RNAi)* nematodes (Fig. 5.6) [42]. These observations suggest that MTL-1 or MTL-2 acted in the same genetic pathway with DAF-16 in regulating the toxicity of environmental toxicants or stresses. Moreover, it was observed that the outdoor $PM_{2.5}$ exposed *daf-2(e1370);mtl-1(RNAi)* mutant exhibited a similar head thrash and body bend to those in outdoor $PM_{2.5}$ exposed *mtl-1(RNAi)* nematodes, and the outdoor $PM_{2.5}$ exposed *daf-2(e1370);mtl-2(RNAi)* mutant exhibited a similar head thrash and body bend to those in outdoor $PM_{2.5}$ exposed *mtl-2(RNAi)* nematodes (Fig. 5.6) [42]. That is, MTL-1 and MTL-2 may act further downstream of the DAF-16 to regulate the toxicity of environmental toxicants or stresses in nematodes.

5.5.4 NATC-1

In nematodes, *natc-1* encodes an evolutionarily conserved subunit of the N-terminal acetyltransferase C (NAT) complex, and the N-terminal acetylation is a useful modification for eukaryotic proteins. NATC-1 is expressed in many cells and tissues

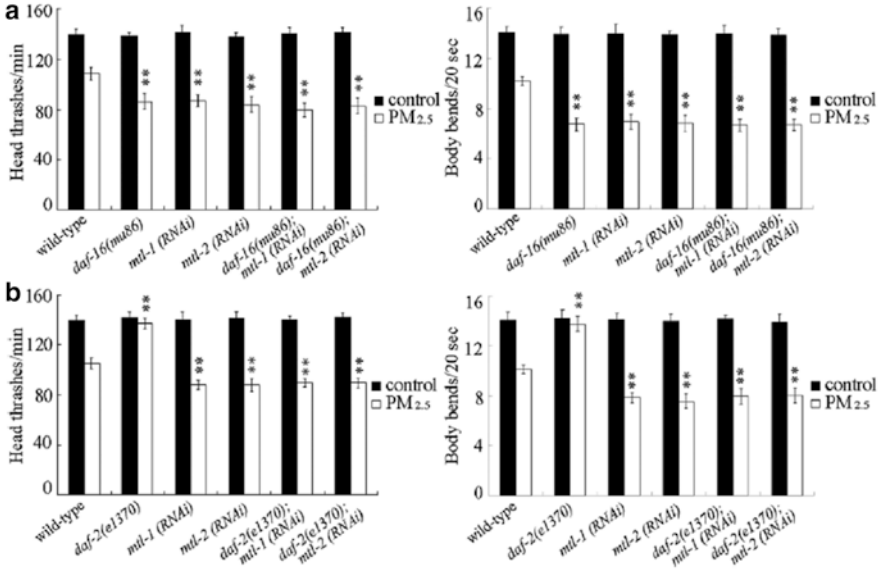

Fig. 5.6 Genetic interaction between *daf-16* or *daf-2* and genes encoding metallothioneins in regulating outdoor $PM_{2.5}$ toxicity on locomotion behavior [42]. (**a**) Genetic interaction between *daf-16* and *mtl-1* or *mtl-2* in regulating outdoor $PM_{2.5}$ toxicity on locomotion behavior. (**b**) Genetic interaction between *daf-2* and *mtl-1* or *mtl-2* in regulating outdoor $PM_{2.5}$ toxicity on locomotion behavior. Exposure concentration of outdoor $PM_{2.5}$ was 10 mg/L. Acute exposure was performed from young adults for 24 h. Bars represent mean ± SEM. $^{**}P < 0.01$ vs wild type

and localizes to the cytoplasm [43]. Loss-of-function mutation of *natc-1* caused a resistance to a broad-spectrum of physiologic stressors, including multiple metals (such as Zn), heat stress, and oxidation stress [43]. DAF-16 was predicted to directly bind the *natc-1* promoter, and *natc-1* mRNA levels were repressed by DAF-16 activity, indicating the role of *natc-1* as a physiological target of DAF-16 (Fig. 5.7) [43]. Additionally, the *daf-2* mutants displayed a twofold decrease in *natc-1* expression compared to wild-type nematodes [43]. Genetic interaction analysis demonstrated that *natc-1(am138)* could enhance the dauer formation in *daf-2(e1370)* mutant nematodes, and the *daf-2(e1370);natc-1(am138)* double mutant nematodes displayed enhanced stress resistance compared to either single mutant animal (Fig. 5.7) [43]. Moreover, the *daf-16(mu86);natc-1(am138)* double mutant animals displayed heat stress resistance similar to *natc-1* single mutant animals, although the *daf-16(mu86)* mutant displayed a mild sensitivity to the heat stress (Fig. 5.7) [43]. Therefore, NATC-1 functions downstream of DAF-16 to mediate the resistance of nematodes to environmental toxicants or stresses.

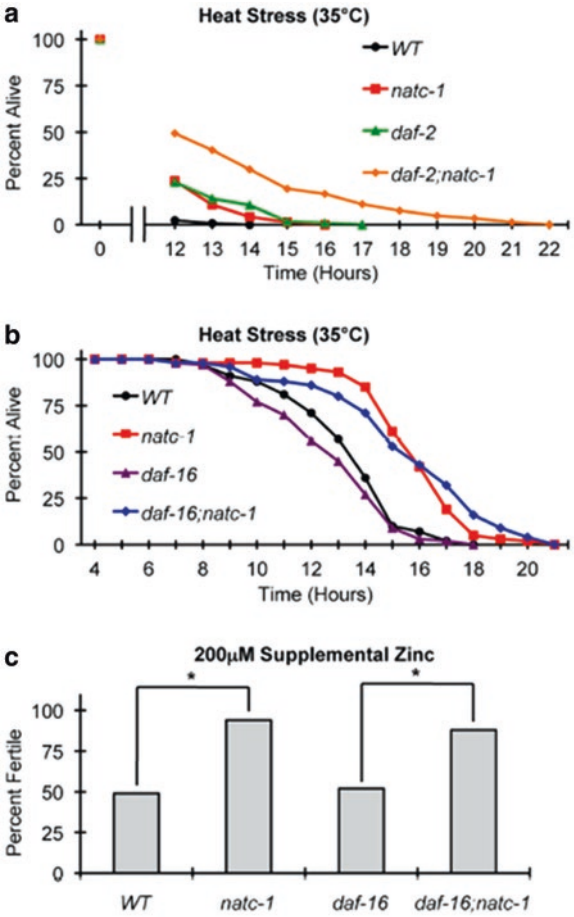

Fig. 5.7 *natc-1* is epistatic to *daf-16* in resistance to heat and zinc stress [43]. (**a**) Wild-type (WT), *natc-1(am138)*, *daf-2(e1370)*, and *daf-2(e1370);natc-1(am138)* animals were cultured at 15 °C on NGM, shifted to 35 °C as day 1 adults, and assayed for survival hourly beginning at 12 h (N = 39–61). (**b**) Wild-type (WT), *natc-1(am138)*, *daf-16(mu86)*, and *daf-16(mu86);natc-1(am138)* animals were cultured at 15 °C on NGM, shifted to 35 °C as day 1 adults, and assayed for survival hourly. (**c**) Embryos were cultured on NAMM with 200 mM supplemental zinc. Bars indicate the percentage of embryos that generated fertile adults. Genotypes were wild type (WT), *natc-1(am138)*, *daf-16(mu86)*, and *daf-16(mu86);natc-1(am138)* (N = 49–54). *daf-16(mu86)* animals were similar to wild-type animals, and *natc-1(am138)* caused significant zinc resistance in wild-type and *daf-16(mu86)* mutant animals

5.5.5 *HSF-1*

Pseudomonas aeruginosa is a commonly considered bacterial pathogen for human beings [44–47]. In nematodes, both the *daf-2*(*e1370*) mutant nematodes and the nematodes carrying additional *daf-16* gene copies were resistant to *P. aeruginosa*

and showed higher levels of HSP90 than wild-type animals (Fig. 5.8) [48], suggesting that a higher activity of HSF-1 may be in part responsible for the increased resistance of nematodes to *P. aeruginosa*. In contrast, this enhanced resistance of *daf-2*(*e1370*) and *daf-16*::*gfp* animals to *P. aeruginosa* was reduced by the RNAi knockdown of *hsf-1* (Fig. 5.8) [48]. Additionally, the heat-shock protection was not detected in *daf-16* RNAi nematodes (Fig. 5.8) [48]. Therefore, the HSF-1-regulated proteins may be effectors for the signaling cascade of DAF-2-DAF-16 required for the pathogen resistance in nematodes.

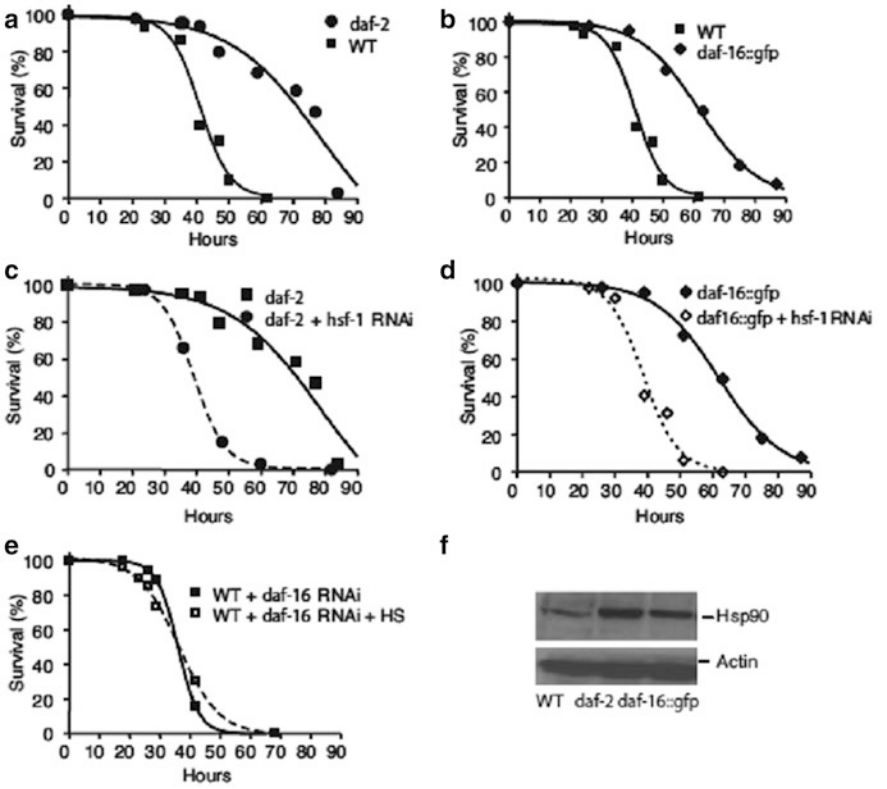

Fig. 5.8 The enhanced resistance phenotype of *daf-2*(*e1370*) and *daf-16*::*gfp* animals to *P. aeruginosa* requires HSF-1 activity [48]. (**a**, **b**) Wild-type, *daf-2*(*e1370*), and *daf-16*::*gfp* animals were exposed to *P. aeruginosa*. (**c**) *daf-2*(*e1370*) grown on *E. coli* carrying a vector control or expressing *hsf-1* double-stranded RNA were exposed to *P. aeruginosa*. (**d**) *daf-16*::*gfp* grown on *E. coli* carrying a vector control or expressing *hsf-1* double-stranded RNA were exposed to *P. aeruginosa*. (**e**) Wild-type animals grown on *E. coli* expressing *daf-16* double-stranded RNA were untreated or HS-treated and exposed to *P. aeruginosa*. For each condition, 80–100 animals were used. (**f**) Immunological detection of Hsp90 in WT, *daf-2*(*e1370*), and *daf-16*::*gfp* animals

5.5.6 *Genetic Interaction Between SOD-3 and Antimicrobial Proteins in the Regulation of Toxicity of Environmental Toxicants or Stresses*

In nematodes, it has been further observed that RNAi knockdown of *sod-3* could not affect the resistance of nematodes overexpressing intestinal *lys-1* or *lys-8* to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior, although RNAi knockdown of *sod-3* could suppress the resistance of nematodes overexpressing intestinal *dod*-6 to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior (Fig. 5.9) [41]. These results imply that LYS-1 and LYS-8 acted in a different genetic pathway from the DAF-16-SOD-3 signaling cascade, and DOD-6 acted upstream of SOD-3 to regulate the toxicity of environmental toxicants or stresses.

LYS-1 and LYS-8 are two members of the lysozyme family. Genetic interaction analysis demonstrated that LYS-1 and LYS-8 functioned redundantly in the regulation of GO toxicity, because the GO-exposed double mutant of *lys-8(ok3504);lys-1(ok2445)* exhibited the more severe induction of intestinal ROS production and decrease in locomotion behavior than that in GO-exposed *lys-1(ok2445)* mutant or in GO-exposed *lys-8(ok3504)* mutant [41]. Meanwhile, after GO (10 mg/L) exposure, mutation of *lys-1* or *lys-8* did not influence the expressions of *clk-1*, *gas-1*, and *isp-1*, which are required for the control of oxidative stress [41].

Moreover, it was found that mutation of *lys-1* significantly decreased the transcriptional expression of *tub-2* in GO (10 mg/L) exposed nematodes, and intestine-specific RNAi of *tub-2* significantly suppressed the resistance of nematodes overexpressing intestinal LYS-1 to GO toxicity in inducing ROS production and in decreasing locomotion behavior (Fig. 5.10) [41]. *tub-2* encodes an ortholog of human tubby like protein 4, and intestine-specific RNAi of *tub-2* could cause a susceptibility to GO toxicity [41]. Besides this, it was also observed that loss-of-function mutation of *lys-8* significantly decreased the transcriptional expression of *daf-8* and increased the transcriptional expression of *daf-5* in GO (10 mg/L) exposed nematodes [41]. In nematodes, *daf-5* encodes a transcriptional factor, and *daf-8* encodes a R-Smad protein in the TGF-b signaling pathway. Intestine-specific RNAi of *daf-8* could cause a susceptibility to GO toxicity in inducing ROS production, whereas intestine-specific RNAi of *daf-5* caused a resistance to GO toxicity in inducing ROS production [41]. Furthermore, intestine-specific RNAi of *daf-8* could significantly inhibit the resistance of transgenic strain overexpressing intestinal *lys-8* to GO toxicity in inducing ROS production and in decreasing locomotion behavior (Fig. 5.10) [41]. Therefore, these results suggest the formation of signaling cascades of DAF-16-LYS-1-TUB-2 and DAF-16-LYS-8-DAF-8-DAF-5 in regulating the toxicity of environmental toxicants or stresses in nematodes.

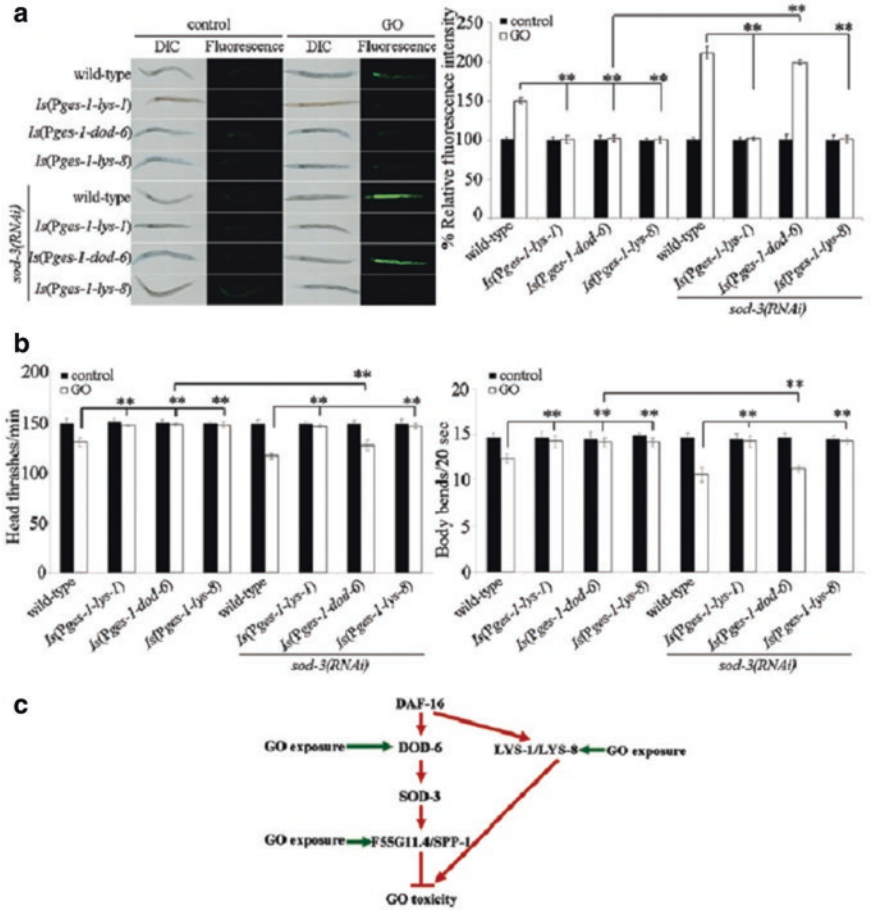

Fig. 5.9 Genetic interaction between SOD-3 and LYS-1, DOD-6, or LYS-8 in the regulation of GO toxicity [41]. (**a**) Genetic interaction between SOD-3 and LYS-1, DOD-6, or LYS-8 in the regulation of GO toxicity in inducing intestinal ROS production. (**b**) Genetic interaction between SOD-3 and LYS-1, DOD-6, or LYS-8 in the regulation of GO toxicity in decreasing locomotion behavior. (**c**) A diagram showing the unique role of LTS-1 and LYS-8 in the regulation of GO toxicity. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 10 mg/L. Bars represent means ± SD. $^{**}p < 0.01$

5.6 Upregulators of Insulin Signaling Pathway in Regulating the Toxicity of Environmental Toxicants or Stresses

So far, many upregulators of insulin signaling pathway in regulating the biological processes, especially the longevity, have been raised. However, only limited upregulators of insulin signaling pathway in regulating the toxicity of environmental toxicants or stresses have been identified.

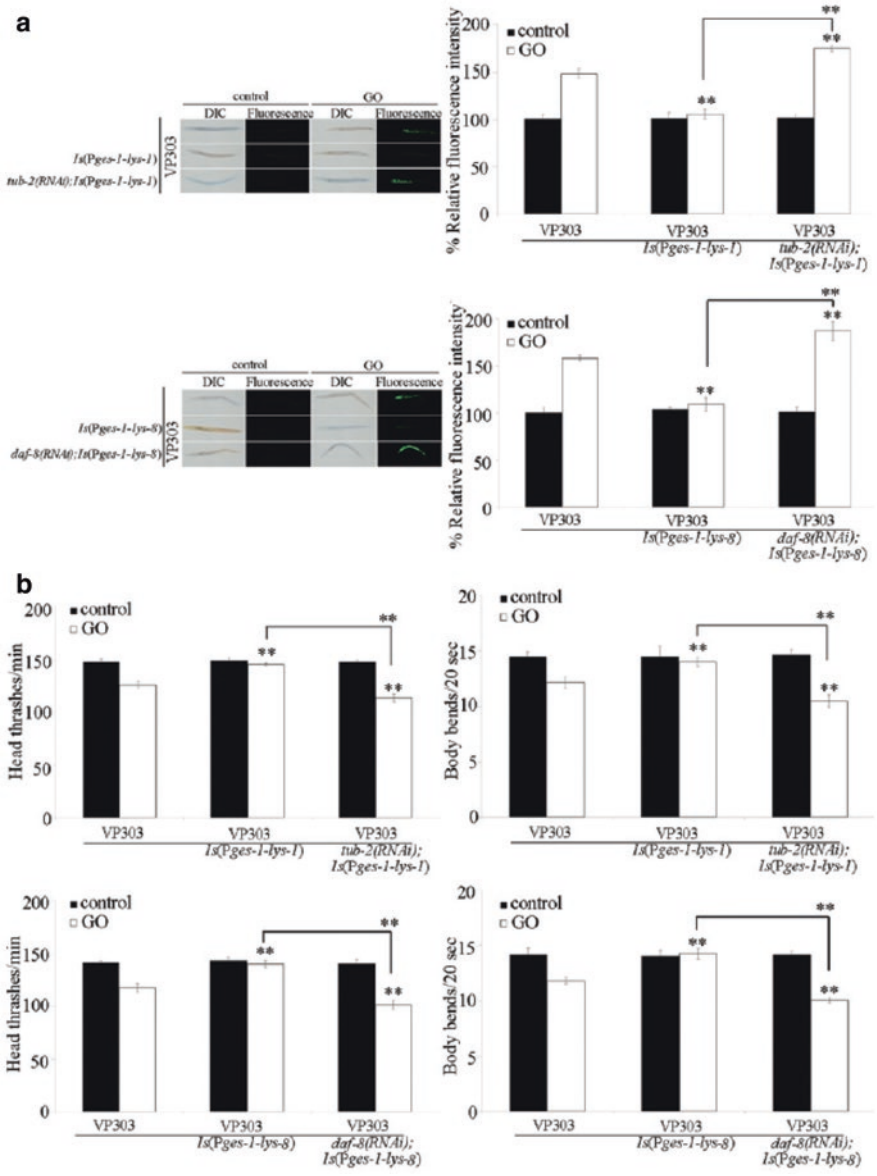

Fig. 5.10 Genetic interaction assays between LYS-1 or LYS-8 and their target(s) in the regulation of GO toxicity [41]. (**a**) Genetic interaction assays between LYS-1 or LYS-8 and their target(s) in the regulation of GO toxicity in inducing intestinal ROS production. (**b**) Genetic interaction assays between LYS-1 or LYS-8 and their target(s) in the regulation of GO toxicity in decreasing locomotion behavior. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 10 mg/L. Bars represent means ± SD. $^{**}p < 0.01$ vs VP303 (if not specially indicated)

5.6.1 SMK-1

In nematodes, *smk-1* encodes a homolog to mammalian SMEK. Under the normal conditions, the *smk-1*(*mn156*) mutant nematodes have a reduced lifespan, normal locomotion behavior, and no significant induction of intestinal ROS production [49]. After prolonged exposure to GO (100 mg/L), the *smk-1*(*mn156*) mutant showed the more severe reduction in lifespan, decrease in locomotion behavior, and induction of intestinal ROS production than wild-type nematodes [49]. Similarly, the *smk-1*(*mn156*) mutant also exhibited the susceptibility to the toxicity of coal combustion-related fine particulate matter ($PM_{2.5}$) in inducing intestinal ROS production and in decreasing locomotion behavior [50]. These results suggest the potential susceptibility of *smk-1*(*mn156*) mutant nematodes to the toxicity of environmental toxicants or stresses.

In nematodes, *smk-1* is expressed in the intestine, pharynx, neurons, muscle, and hypodermis. The tissue-restricted expression of *smk-1* in the pharynx, the neurons, the muscle, or the hypodermis did not affect the GO toxicity in reducing lifespan and in decreasing locomotion behavior in *smk-1*(*mn156*) mutant nematodes; however, expression of *smk-1* in the intestine could significantly suppress the GO toxicity in reducing lifespan and in decreasing locomotion behavior in *smk-1*(*mn156*) mutant nematodes [49]. That is, SMK-1 acted in the intestine to regulate the GO toxicity. Moreover, it was observed that the transgenic strain of *Is*(P*ges-1-smk-1*) overexpressing intestinal *smk-1* had a resistance to the GO toxicity in reducing lifespan and in decreasing locomotion behavior [49], which further confirmed the tissue-specific activity of SMK-1 in the intestine in the regulation of GO toxicity.

Moreover, it has been shown that the lifespan and the locomotion behavior in GO (100 mg/L) exposed double mutant of *daf-16*(*RNAi*)*;smk-1*(*mn156*) were similar to those in GO (100 mg/L) exposed single mutant of *smk-1*(*mn156*) or *daf-16*(*RNAi*) nematodes [49], suggesting that SMK-1 and DAF-16 may act in the same genetic pathway to regulate the toxicity of environmental toxicants or stresses. More importantly, it was found that RNAi knockdown of *daf-16* could significantly suppress the protective effects of *smk*-1 overexpression on both the lifespan and the locomotion behavior in GO-exposed nematodes (Fig. 5.11) [49], which demonstrates that SMK-1 acts upstream of DAF-16 to regulate the toxicity of environmental toxicants or stresses in nematodes.

In nematodes, it has been further found that the expressions of *sod-3*, *sod-4*, and *ctl-3* were significantly decreased in *smk-1* mutant nematodes compared to wild-type N2 after coal combustion-related $PM_{2.5}$ exposure (Fig. 5.12) [50]. SOD-3 (an iron/manganese superoxide dismutase), SOD-4 (an extracellular Cu^{2+}/Zn^{2+} superoxide dismutase), and CTL-3 (a catalase) are considered as the possible downstream targets of DAF-16. After exposure to coal combustion-related $PM_{2.5}$, *sod-3(RNAi)*, *sod-4(RNAi)*, or *ctl-3(RNAi)* nematodes had a significantly higher induction of intestinal ROS production and decrease in locomotion compared to the wild-type N2 (Fig. 5.12) [50], suggesting the susceptibility of *sod-3(RNAi)*, *sod-4(RNAi)*, or *ctl-3(RNAi)* nematodes to the toxicity of coal combustion-related

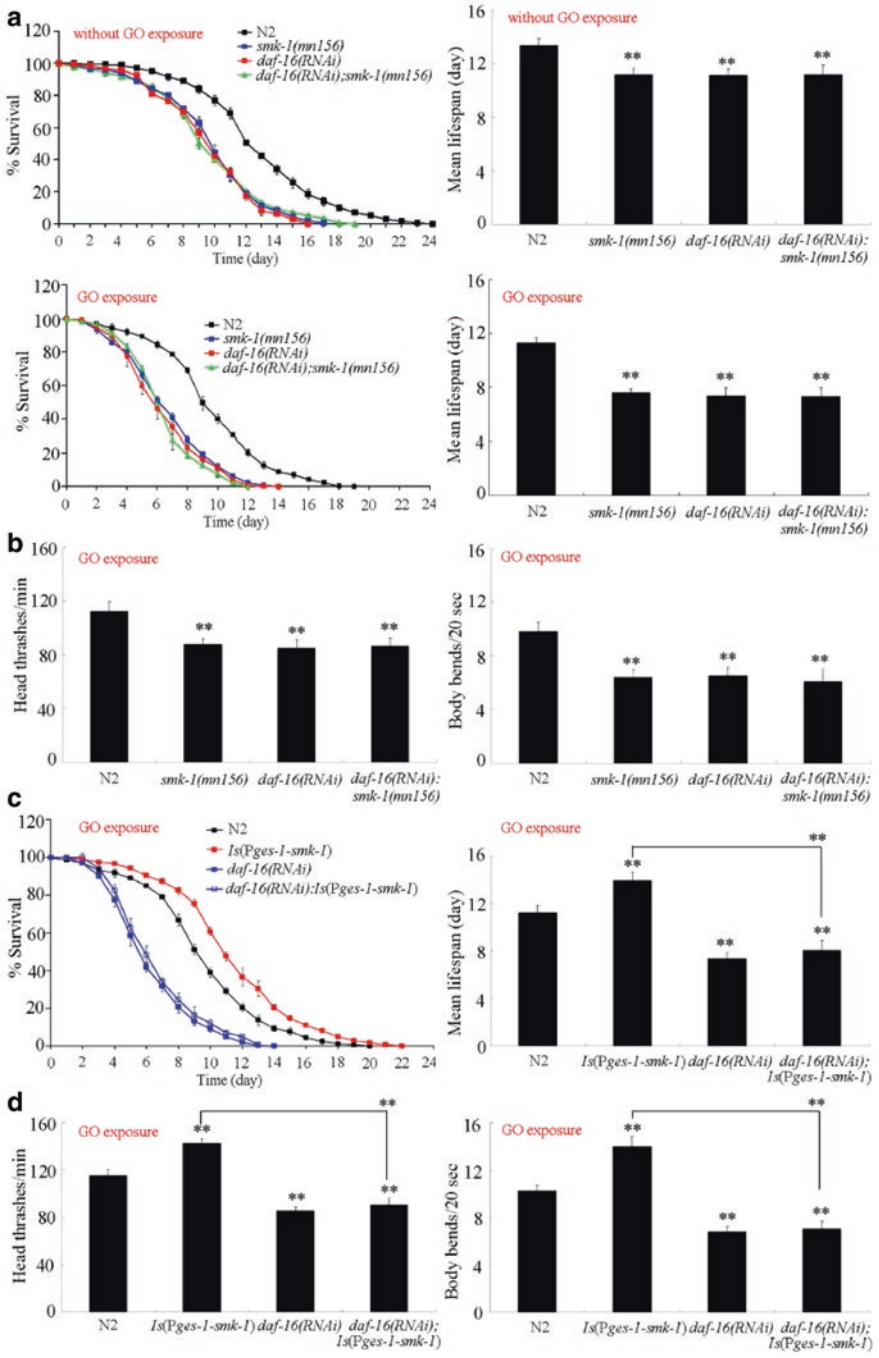

Fig. 5.11 Genetic interaction between *smk-1* and *daf-16* in regulating GO toxicity in nematodes [49]. (**a**) Genetic interaction between *smk-1* and *daf-16* in regulating GO toxicity in reducing lifespan in nematodes. (**b**) Genetic interaction between *smk-1* and *daf-16* in regulating GO toxicity in decreasing locomotion behavior in nematodes. (**c**) Effect of RNAi knockdown of *daf-16* gene on

$PM_{2.5}$. These results imply that the possible signaling cascade of SMK-1-DAF-16-SOD-3/SOD-4/CTL-3 may exist in regulating the toxicity of environmental toxicants or stresses in nematodes.

5.6.2 AAK-2

In nematodes, *aak-2* encodes a catalytic alpha subunit of AMP-activated protein kinases (AMPKs). Multi-walled carbon nanotubes (MWCNTs) is another important carbon-based nanomaterials widely used in different fields [51–54]. Previous study has indicated that AAK-2 may function upstream of DAF-16 in insulin signaling pathway to regulate the longevity [19]. In nematodes, mutation of *aak-2* induced a susceptibility to the toxicity of both MWCNTs and GO [51, 55]. Moreover, it was observed that the lifespan and the locomotion behavior at adult day-8 in MWCNTs (1 mg/L) exposed double mutant of *daf-16*(*mu86);aak-2*(*om524)* were similar to those in MWCNTs (1 mg/L) exposed single mutant of *aak-2*(*om524)* or *daf-16*(*mu86)* nematodes (Fig. 5.13) [51], implying that AAK-2 can further act together with DAF-16 in the same genetic pathway to form a signaling cascade of AAK-2-DAF-16 to regulate the toxicity of environmental toxicants or stresses in nematodes.

5.6.3 JNK-1

JNK-1 is a core component in the JNK signaling pathway. We have introduced the related detailed information in the Chap. 4. It was further observed that the loss-of-function mutation of *jnk-1* could significantly suppress the nuclear translocation of DAF-16 caused by heat stress or ROS stress (induced by H_2O_2) (Fig. 5.14) [56]. The degree of nuclear translocation of DAF-16::GFP was generally and statistically significantly lower in the *jnk-1* mutant than in the wild type after exposure to heat stress or ROS stress (Fig. 5.14) [56]. Moreover, loss-of-function mutation of *jnk-1* could further significantly inhibit the increase in SOD-3::GFP (a direct target of DAF-16) induced by heat stress [56]. Therefore, JNK-1 may modulate the environmental toxicant- or stress-induced translocation of DAF-16 from the cytosol into the cell nucleus in nematodes.

Fig. 5.11 (continued) lifespan in GO-exposed transgenic nematodes overexpressing *smk-1* in the intestine. (**d**) Effect of RNAi knockdown of *daf-16* gene on locomotion behavior in GO-exposed transgenic nematodes overexpressing *smk-1* in the intestine. GO exposure concentration was 100 mg/L. Prolonged exposure was performed from L1-larvae to young adults. Bars represent means ± SD. $^{**}P < 0.01$ vs N2 (if not specially indicated)

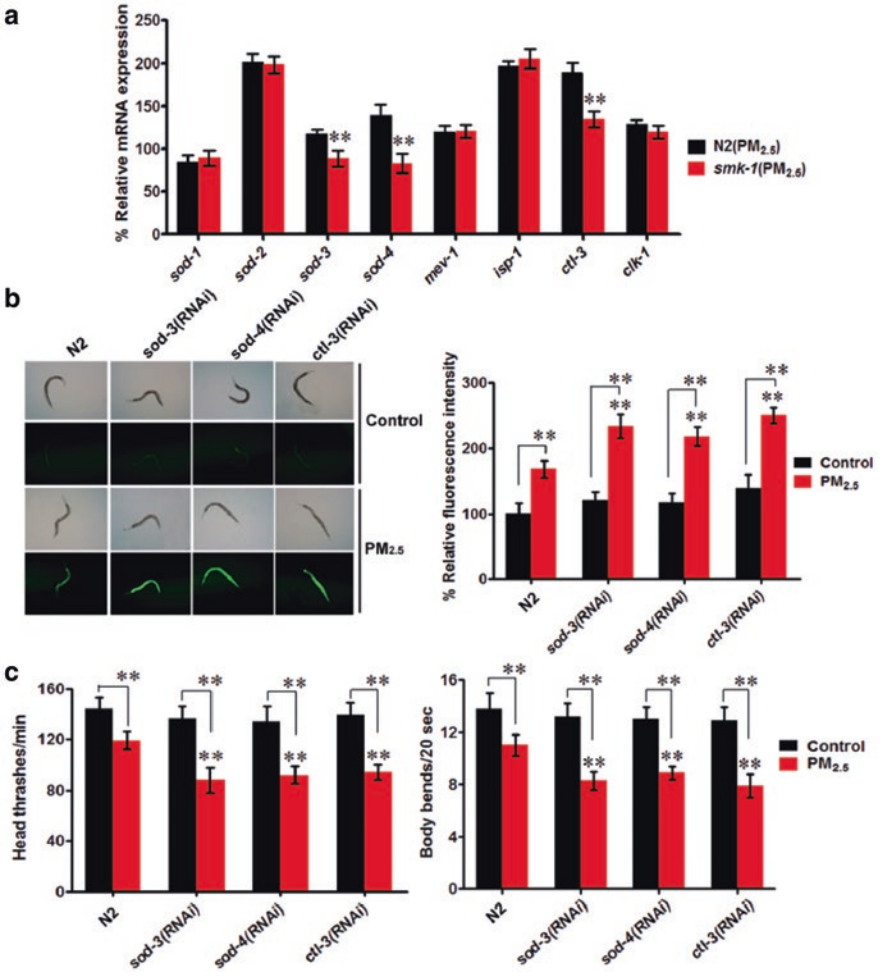

Fig. 5.12 Oxidative stress-related genes acted as downstream regulators of *smk-1* in the regulation of coal combustion-related $PM_{2.5}$ toxicity [50]. (**a**) Expression pattern of genes required for the control of oxidative stress in coal combustion-related $PM_{2.5}$ exposed wild-type and *smk-1* mutant nematodes. (**b**) Effect of RNAi knockdown of *sod-3*, *sod-4*, or *ctl-3* on toxicity of coal combustion-related $PM_{2.5}$ in inducing intestinal ROS production. (**c**) Effect of RNAi knockdown of *sod-3*, *sod-4*, or *ctl-3* on toxicity of coal combustion-related $PM_{2.5}$ in decreasing locomotion behavior. The concentration of coal combustion-related $PM_{2.5}$ was 1 mg/L. Prolonged exposure was performed from L1-larvae to young adults at 20 °C in the presence of food. Bars represent mean ± SD. $^{**}P < 0.01$ vs N2 (if not specially indicated)

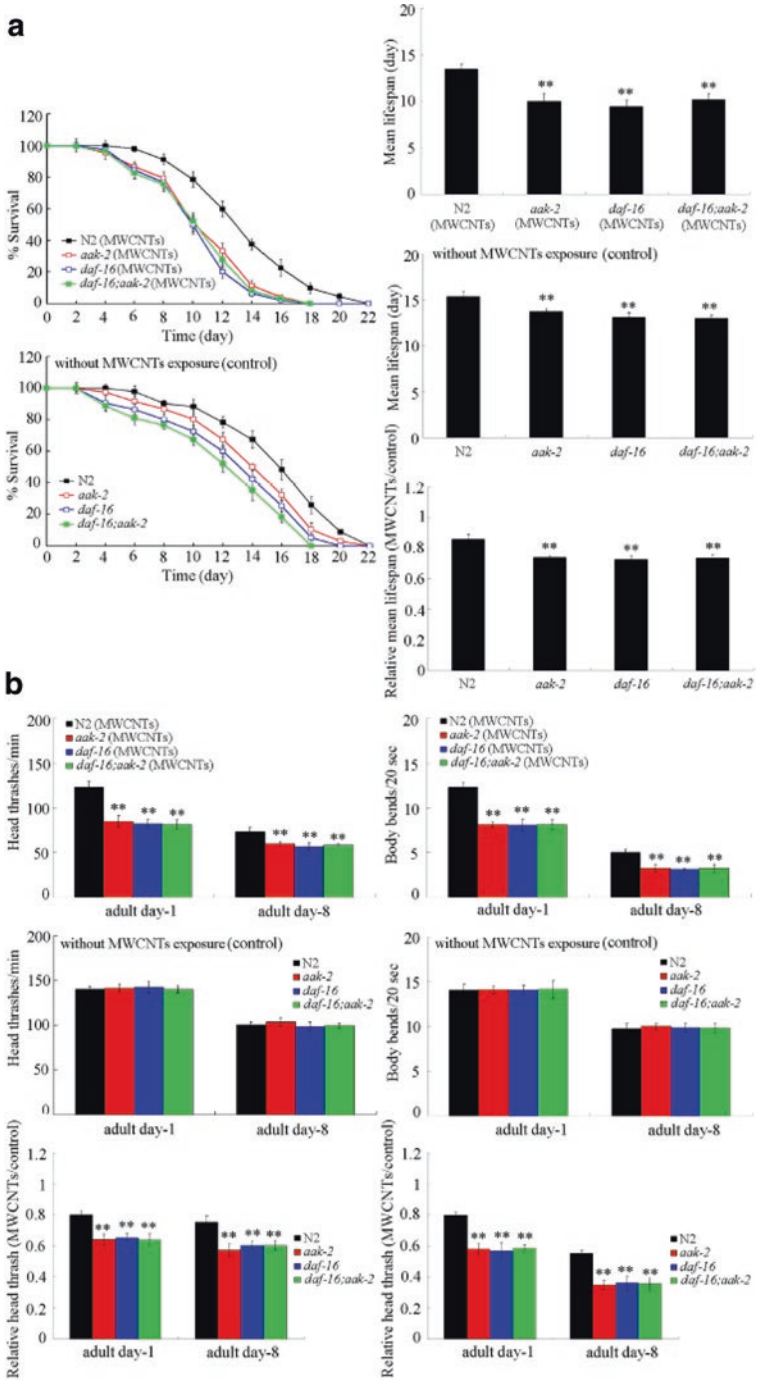

Fig. 5.13 Genetic interaction between *aak-2* and *daf-16* in regulating MWCNTs toxicity in nematodes [51]. (**a**) Genetic interaction between *aak-2* and *daf-16* in regulating MWCNTs toxicity in reducing lifespan in nematodes. (**b**) Genetic interaction between *aak-2* and *daf-16* in regulating MWCNTs toxicity in decreasing locomotion behavior in nematodes. Exposure concentration of MWCNTs was 1 mg/L. Prolonged exposure was performed from L1-larvae to young adults. Bars represent means ± SD. $^{**}P < 0.01$ vs N2

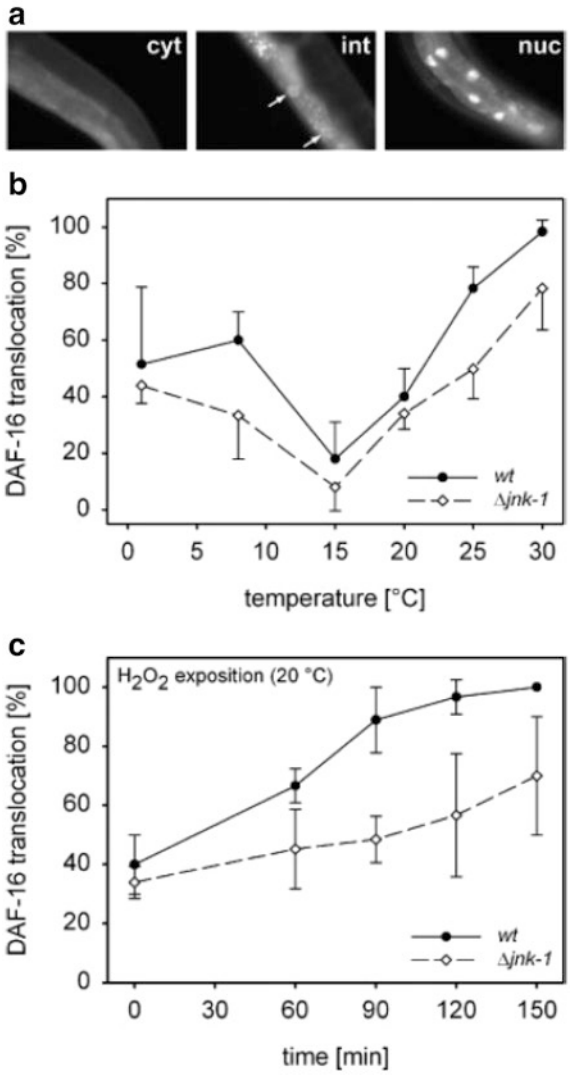

Fig. 5.14 The temperature- and H_2O_2-induced nuclear translocation of DAF-16 within intestinal cells of *C. elegans* is lower in a *jnk-1* deletion mutant than in the wild-type nematodes [56]. (**a**) Depending on the degree of nuclear GFP fluorescence, three different states of translocation of DAF-16::GFP from the cytoplasm into the cell nuclei of intestinal cells can be distinguished: cytoplasmic location (cyt; no nuclear GFP fluorescence), intermediate location (int; weak nuclear GFP fluorescence), and nuclear location (nuc; strong nuclear GFP fluorescence). (**b**) After incubation at different ambient temperatures, the degree of nuclear DAF-16 translocation within intestinal cells was minimal at 15 °C and increased toward lower and higher temperatures both in wild-type and mutant worms. In the mutant, however, DAF-16 translocation was significantly reduced in comparison to the wild type. (**c**) The degree of nuclear DAF-16 translocation also increased with the incubation period (0–150 min) on NGM plates containing 1 mM H_2O_2. Again, this cellular response was significantly lower in the mutant than in the wild type

5.6.4 HCF-1

In nematodes, *hcf-1* encodes a conserved homolog of host cell factor 1. The *hcf-1(pk924)* mutant nematodes showed the resistant to the paraquat treatment compared to wild-type nematodes at multiple time points (Fig. 5.15) [57]. Moreover, it has been found that this paraquat resistance of the *hcf-1(pk924)* mutants was dependent on *daf-16*, as the *daf-16(mgDf47);hcf-1(pk924)* double mutant was sensitive to paraquat, similar to that of the *daf-16(mgDf47)* single mutant (Fig. 5.15) [57]. Similarly, the *hcf-1(pk924)* mutant nematodes were resistant to the cadmium exposure compared to wild-type nematodes at multiple time points, and the cadmium resistance of the *hcf-1(pk924)* mutant was also *daf-16*-dependent (Fig. 5.15) [57]. It has been found that the RNA levels of *sod-3*, *mtl-1*, and *F21F3.3* encoding a farnesyl cysteine carboxyl methyltransferase were significantly elevated in both the

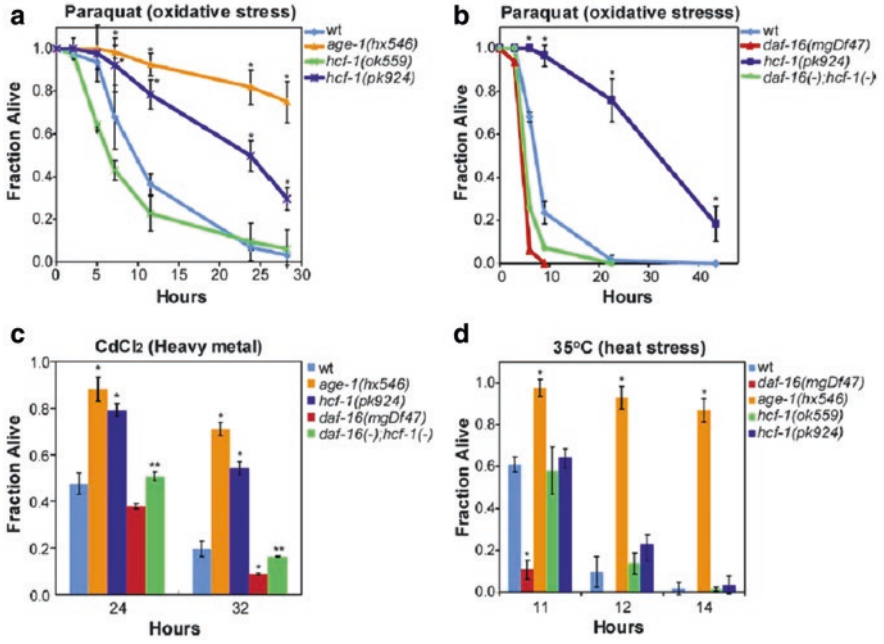

Fig. 5.15 Loss of *hcf-1* results in heightened resistance to specific environmental stresses [57]. (**a**) The *hcf-1(pk924)* mutant worms exhibited increased survival in 200 mM paraquat compared to wild-type worms. (**b**) The enhanced paraquat resistance of *hcf-1(pk924)* was dependent on *daf-16*. (**c**) The *hcf-1(pk924)* mutant worms showed increased survival in $CdCl_2$ (18 mM) that was *daf-16* dependent. (**d**) The *hcf-1(pk924)* and *hcf-1(ok559)* mutants and wild-type worms showed similar survival kinetics when cultured at 35 °C. For the stress assays, duplicate to quadruplicate samples were examined for each strain. Mean fraction alive indicates the average survival among the multiplicates and error bars represent the standard deviation of the multiplicates. *p*-Value was calculated using Student's *t*-test. $^{*}p < 0.05$ when compared to wild type (wt). $^{**}p < 0.05$ when compared to *hcf-1(pk924)*. Each of the stress assays was repeated at least two independent times with similar results, and the data of representative experiments are shown

hcf-1(ok559) and the *hcf-1(pk924)* mutants as compared to wild-type nematodes [57]. This elevated expression of *sod-3*, *mtl-1*, and *F21F3.3* in the *hcf-1* mutant nematodes was also dependent on *daf-16*, since the levels of *sod-3*, *mtl-1*, and *F21F3.3* in the *daf-16(mgDf47);hcf-1(ok559)* double mutant remained low and was similar to that seen in the *daf-16(mgDf47)* single mutant nematodes [57]. Meanwhile, among the DAF-16-repressed genes, the expression level of *C32H11.4* showed a greater than twofold downregulation in *hcf-1* mutant nematodes compared to wild-type nematodes, and this repressed expression of *C32H11.4* could also be partially dependent on *daf-16* [57]. Therefore, HCF-1 may act upstream of DAF-16 and suppress the function of DAF-16 to regulate the toxicity of environmental toxicants or stresses in nematodes.

5.6.5 SIR-2.1/SIRT1

In nematodes, overexpressing *sir-2.1* can confer a lifespan extension phenotype that is dependent on the DAF-16 [19]. Under the paraquat or t-BOOH exposure conditions, the *sir-2.1(ok434)* mutant nematodes were sensitive, and the *hcf-1(pk924)* mutant nematodes were resistant to the treatments (Fig. 5.16) [58]. It has been further observed that, under the paraquat or t-BOOH exposure conditions, mutation of *hcf-1* could suppress the susceptibility of *sir-2.1(ok434)* mutant nematodes to the toxicity of paraquat or t-BOOH (Fig. 5.16) [58]. These observations imply that SIR-2.1 can act upstream of the insulin signaling pathway to regulate the toxicity of environmental toxicants or stresses by suppressing the function of HCF-1 in nematodes.

In nematodes, it was further found that the DAF-16 and the SIR-2.1 can interact even under the stress condition, and this interaction depended on the 14-3-3 proteins as the SIR-2.1 binding partners (Fig. 5.17) [59]. The 14-3-3 proteins were also required for the SIR-2.1-induced transcriptional activation of DAF-16 and the stress resistance [59]. Following the heat stress, SIR-2.1 will bind DAF-16 in a 14-3-3-dependent manner (Fig. 5.17) [59]. In contrast, the low insulin-like signaling did not promote the SIR-2.1/DAF-16 interaction, and thereby *sir-2.1* and the *14-3-3* were not required for the regulation of lifespan by the insulin-like signaling pathway [59]. Therefore, very large physical interactions surrounding the DAF-16 may be formed during the regulation of toxicity of environmental toxicants or stresses in nematodes.

5.6.6 PRDX-2

In nematodes, PRDX-2 is a single cytosolic 2-Cys Prx. Loss-of-function mutation of *prdx-2* increased the arsenite resistance by increasing both SKN-1 and DAF-16 activities [60]. Under the normal conditions, there was a significant increase in the

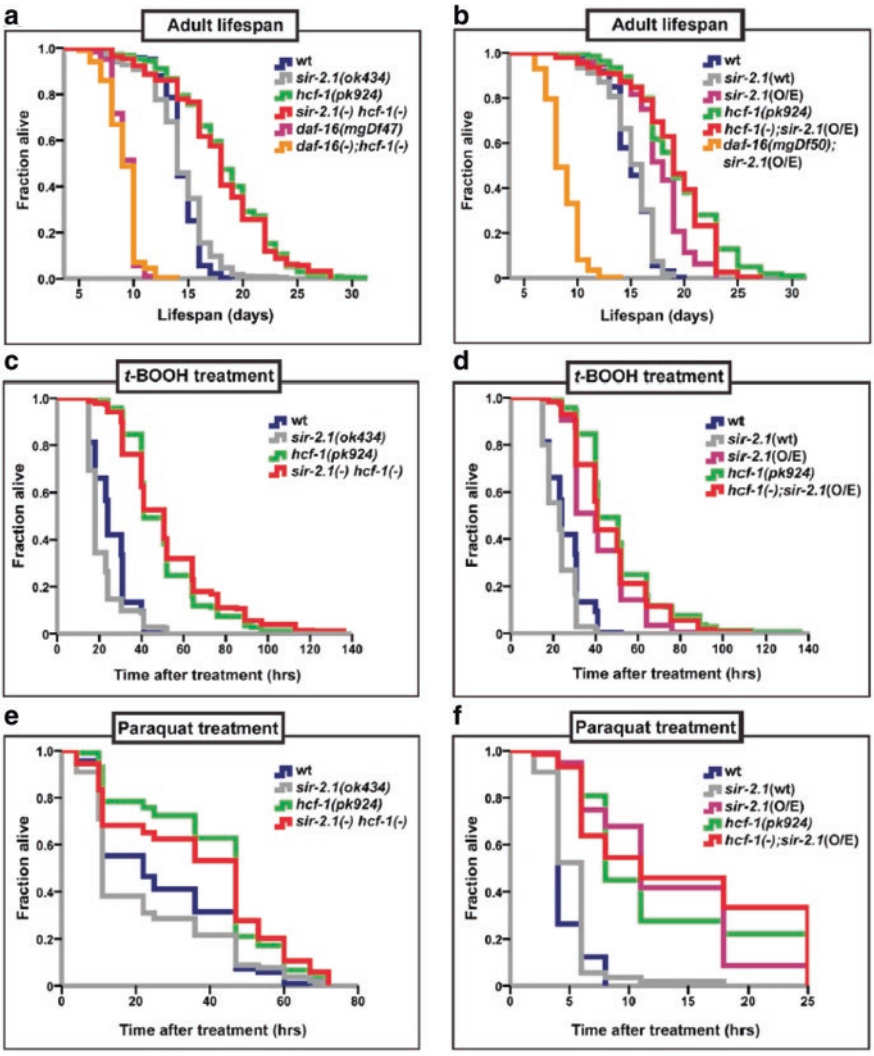

Fig. 5.16 *hcf-1* acts downstream of *sir-2.1* to modulate lifespan and oxidative stress response [58]. (**a**, **b**) Lifespans of synchronized adult populations of indicated genotypes. (**a**) Data pooled from four independent experiments are plotted. (**b**) Pooled data from three independent experiments are displayed. (**c**–**f**) Oxidative stress response of adult worms. (**c**, **d**) Day 1 adult worms were exposed to 6 mM t-BOOH on plates and their survival monitored through time. The survival curves represent pooled data from two independent experiments. (**e**, **f**) Day 2 adult worms were exposed to 150 mM (**e**) or 200 mM (**f**) paraquat in M9 buffer and their survival monitored through time. Survival curves are generated using pooled data from two independent experiments (**e**) or data from one of two representative experiments (**f**)

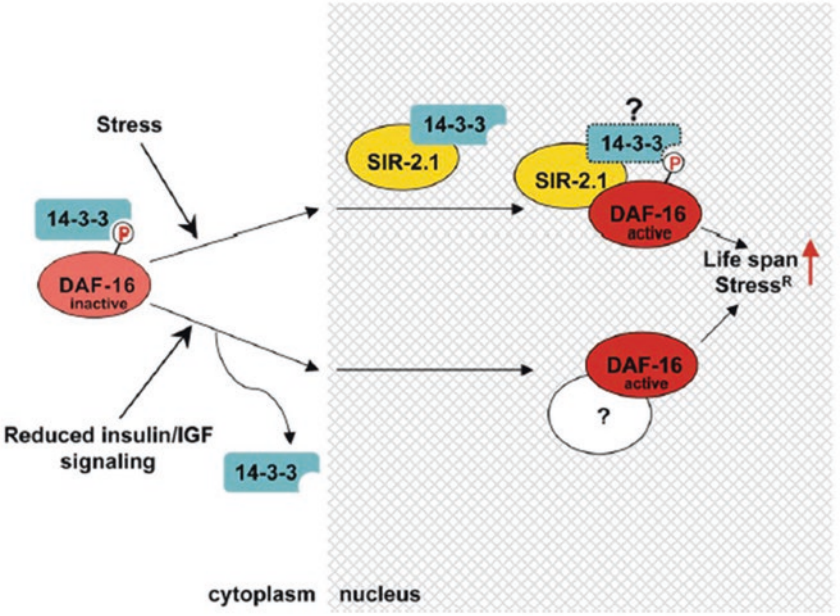

Fig. 5.17 A model for the roles of SIR-2.1 and 14-3-3 in DAF-16 regulation of stress resistance and lifespan [59]. It is proposed that, following the stress, SIR-2.1 binds DAF-16 in the nucleus in a 14-3-3-dependent manner, and the resulting complex participates in transcriptional activation of DAF-16 target genes. 14-3-3 may promote the interaction between SIR-2.1 and DAF-16 either by scaffolding the complex or through a modification of DAF-16 or SIR-2.1 following stress. Under the low insulin-like signaling conditions, DAF-16 is not phosphorylated at the Akt sites, becomes dissociated from 14-3-3, and accumulates in the nucleus. Nuclear DAF-16 produced by low insulin-like signaling does not bind SIR-2.1 and does not require *sir-2.1* and 14-3-3 function for activation

nuclear localization of DAF-16::GFP in *prdx-2* mutant nematodes (Fig. 5.18) [60]. Meanwhile, the expressions of several DAF-16-activated genes (*mtl-1*, *sod-3*, *gst-7*), as well as the expression of *sod-3p::gfp*, were also increased in *prdx-2* mutant nematodes (Fig. 5.18) [60]. More importantly, it was observed that mutation of *daf-16* or *skn-1* could suppress the resistance of *prdx-2(RNAi)* nematodes to the toxicity of arsenite in reducing the lifespan (Fig. 5.18) [60], which suggests that PRDX-2 acts upstream of both the DAF-16 and the SKN-1 to regulate the toxicity of environmental toxicants or stresses in nematodes.

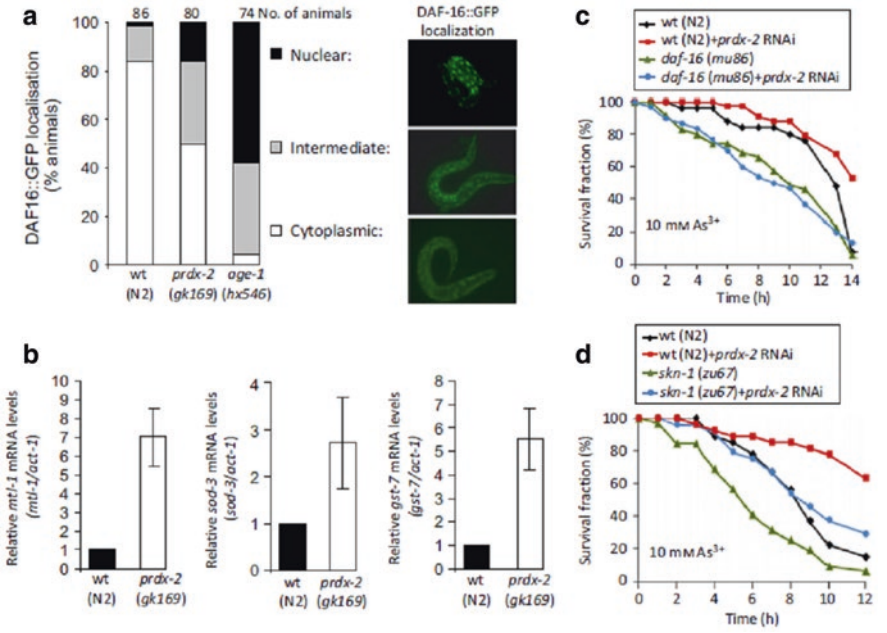

Fig. 5.18 Loss of PRDX-2 increases arsenite resistance by increasing both SKN-1 and DAF-16 activities [60]. (**a**) Loss of *prdx-2* causes nuclear accumulation of DAF-16. The localization of a DAF-16::GFP fusion protein was assessed in L2/L3 larval stage wild-type and *prdx-2 (gk169)* and *age-1 (hx584)* mutant animals expressing daf-16a-16::GFP. PRDX-2 deficiency caused nuclear accumulation of *daf-16a::GFP* in the intestinal nuclei. n refers to the number of worms examined in each group in the representative experiment shown. (**b**) *prdx-2* mutant animals contain increased levels of mRNA for *mtl-1*, *sod-3*, and *gst-7* compared with wild-type (N2) animals. mRNA levels were calculated relative to control (*act-1*) mRNA in at least six independently prepared RNA samples. Each panel depicts the levels of a particular mRNA in *prdx-2* mutant normalized to wild type (N2). Error bars represent the SEM. (**c**, **d**) The survival of L4 larval stage wild-type (N2) and *daf-16(mu86)* and *skn-1(zu67)* mutant animals microinjected with *prdx-2* dsRNA was monitored on NGM-L plates containing 10 mM sodium arsenite at indicated time points. (**c**) Loss of *prdx-2* significantly increased the arsenite resistance of wild-type but not *daf-16(mu86)* mutant animals. (**d**) *prdx-2* RNAi produces a greater increase in the arsenite resistance of wild-type than *skn-1(zu67)* mutant animals

5.7 Genetic Interaction Between SKN-1 and DAF-16 or DAF-2 in Regaling the Toxicity of Environmental Toxicants or Stresses

In nematodes, both the FOXO transcriptional factor DAF-16 and the FOXO transcriptional factor SKN-1/Nrf can act downstream of the insulin receptor DAF-2 in the insulin signaling pathway to regulate various biological processes, such as the stress response [28, 61]. Using intestinal ROS production as the toxicity assessment endpoint, it has been shown that the GO toxicity in inducing intestinal ROS

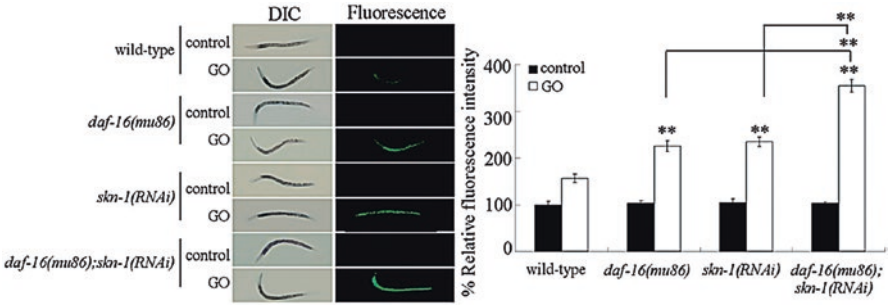

Fig. 5.19 Genetic interaction between DAF-16 and SKN-1 in the regulation of response to GO exposure [22]. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 10 mg/L. Bars represent means ± SD. $^{**}p < 0.01$ versus wild type (if not specially indicated)

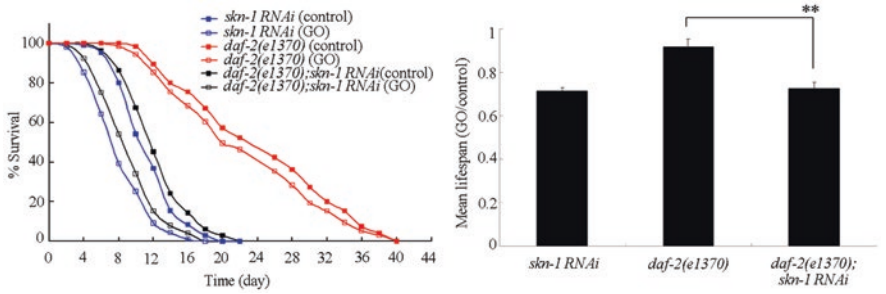

Fig. 5.20 Genetic interaction between DAF-2 and SKN-1 in regulating GO toxicity in nematodes [61]. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 100 mg/L. Bars represent means ± SEM. $^{**}P < 0.01$

production in *daf-16(mu86);skn-1(RNAi)* was more severe than that in *daf-16(mu86)* or in *skn-1(RNAi)* (Fig. 5.19) [22], suggesting that the SKN-1 and the DAF-16 may act in parallel signaling pathways to regulate the toxicity of environmental toxicants or stresses in nematodes.

Besides this, the genetic interaction between DAF-2 in insulin signaling pathway and SKN-1 in p38 MAPK signaling pathway in regulating GO toxicity was also examined. Prolonged exposure to GO (100 mg/L) could cause the similar toxicity on lifespan in double mutant of *daf-2(e1370);skn-1(RNAi)* to that in *skn-1(RNAi)* nematodes (Fig. 5.20) [61], suggesting that RNAi knockdown of *skn-1* may potentially suppress the resistance of *daf-2* mutant to the GO toxicity. Therefore, both the core signaling cascade of p38 MAPK signaling pathway and the insulin receptor DAF-2 in the insulin signaling pathway can act upstream of SKN-1 to regulate the toxicity of environmental toxicants or stresses in nematodes.

5.8 Perspectives

So far, a large amount of data has highlighted the possible pivotal function or role of the core insulin signaling pathway in the regulation of environmental toxicants or stresses in nematodes. Nevertheless, the detailed insulin signaling pathway involved in the control of toxicity from different environmental toxicants or stresses may be different. At least for the kinase cascade in the insulin signaling pathway, different environmental toxicants or stresses may affect different components. More importantly, among the large amount of targeted genes (predicted) for *daf-16*, only several genes have been proven to act as the downstream targeted genes for *daf-16* in regulating the toxicity of environmental toxicants or stresses. That is, it is still unclear whether the rest of predicted genes can also act as the targeted genes for *daf-16* in the regulation of toxicity of environmental toxicants or stresses.

As introduced above, the obtained data so far may imply the formation of a large physical interaction surrounding the DAF-16 in regulating the toxicity of environmental toxicants or stresses. The identification of exact scaffold molecules in this large complex may provide an important basis for further screen of related components and the thorough elucidation of the underlying mechanism for insulin signaling pathway in regulating the toxicity of environmental toxicants or stresses in nematodes.

References

1. Wang D-Y (2018) Nanotoxicology in *Caenorhabditis elegans*. Springer, Singapore
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. 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
4. Zhao L, Wan H-X, Liu Q-Z, Wang D-Y (2017) Multi-walled carbon nanotubes-induced alterations in microRNA *let-7* and its targets activate a protection mechanism by conferring a developmental timing control. Part Fibre Toxicol 14:27
5. 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
6. 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
7. Yin J-C, Liu R, Jian Z-H, Yang D, Pu Y-P, Yin L-H, Wang D-Y (2018) Di (2-ethylhexyl) phthalate-induced reproductive toxicity involved in DNA damage-dependent oocyte apoptosis and oxidative stress in *Caenorhabditis elegans*. Ecotoxicol Environ Saf 163:298–306
8. 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

9. 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
10. 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
11. Christopoulos PF, Corthay A, Koutsilieris M (2018) Aiming for the insulin-like growth factor-1 system in breast cancer therapeutics. Cancer Treat Rev 63:79–95
12. Haeusler RA, McGraw TE, Accili D (2018) Biochemical and cellular properties of insulin receptor signalling. Nat Rev Mol Cell Biol 19:31–44
13. Bryan MR, Bowman AB (2017) Manganese and the insulin-IGF signaling network in Huntington's disease and other neurodegenerative disorders. Adv Neurobiol 18:113–142
14. Guo CA, Guo S (2017) Insulin receptor substrate signaling controls cardiac energy metabolism and heart failure. J Endocrinol 233:R131–R143
15. Das D, Arur S (2017) Conserved insulin signaling in the regulation of oocyte growth, development, and maturation. Mol Reprod Dev 84:444–459
16. Stanley M, Macauley SL, Holtzman DM (2016) Changes in insulin and insulin signaling in Alzheimer's disease: cause or consequence? J Exp Med 213:1375–1385
17. Riera CE, Merkwirth C, De Magalhaes Filho CD, Dillin A (2016) Signaling networks determining life span. Annu Rev Biochem 85:35–64
18. Soultoukis GA, Partridge L (2016) Dietary protein, metabolism, and aging. Annu Rev Biochem 85:5–34
19. Kenyon C (2010) The genetics of ageing. Nature 464:504–512
20. Lapierre LR, Hansen M (2012) Lessons from *C. elegans*: signaling pathways for longevity. Trends Endocrinol Metab 23:637–644
21. 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
22. Qu M, Li Y-H, Wu Q-L, Xia Y-K, Wang D-Y (2017) Neuronal ERK signaling in response to graphene oxide in nematode *Caenorhabditis elegans*. Nanotoxicology 11:520–533
23. 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
24. 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
25. 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
26. Ding X-C, Rui Q, Wang D-Y (2018) Functional disruption in epidermal barrier enhances toxicity and accumulation of graphene oxide. Ecotoxicol Environ Saf 163:456–464
27. 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. Part Fibre Toxicol 15:26
28. 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
29. Gubert P, Puntel B, Lehmen T, Bornhorst J, Avila DS, Aschner M, Soares FAA (2016) Reversible reprotoxic effects of manganese through DAF-16 transcription factor activation and vitellogenin downregulation in *Caenorhabditis elegans*. Life Sci 151:218–223
30. Avila DS, Somlyai G, Somlyai I, Aschner M (2012) Anti-aging effects of deuterium depletion on Mn-induced toxicity in a *C. elegans* model. Toxicol Lett 211:319–324
31. Yang R-L, Zhao Y-L, Yu X-M, Lin Z-Q, Xi Z-G, Rui Q, Wang D-Y (2015) Insulin signaling regulates toxicity of traffic-related $PM_{2.5}$ on intestinal development and function in nematode *Caenorhabditis elegans*. Toxicol Res 4:333–343

32. Wang S, Teng X, Wang Y, Yu H, Luo X, Xu A, Wu L (2014) Molecular control of arsenite-induced apoptosis in *Caenorhabditis elegans*: roles of insulin-like growth factor-1 signaling pathway. Chemosphere 112:248–255
33. Barsyte D, Lovejoy DA, Lithgow GJ (2001) Longevity and heavy metal resistance in *daf-2* and *age-1* long-lived mutants of *Caenorhabditis elegans*. FASEB J 15:627–634
34. Liu P-D, He K-W, Li Y-X, Wu Q-L, Yang P, Wang D-Y (2012) Exposure to mercury causes formation of male-specific structural deficits by inducing oxidative damage in nematodes. Ecotoxicol Environ Saf 79:90–100
35. Wang D-Y, Liu P-D, Yang Y-C, Shen L-L (2010) Formation of combined Ca/Cd toxicity on lifespan of nematode *Caenorhabditis elegans*. Ecotoxicol Environ Saf 73:1221–1230
36. Scott BA, Avidan MS, Crowder MC (2002) Regulation of hypoxic death in *C. elegans* by the insulin/IGF receptor homolog DAF-2. Science 296:2388–2391
37. Jensen VL, Simonsen KT, Lee Y-H, Park D, Riddle DL (2010) RNAi screen of DAF-16/FOXO target genes in *C. elegans* links pathogenesis and dauer formation. PLoS ONE 5:e15902
38. McElwee J, Bubb K, Thomas JH (2003) Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. Aging Cell 2:111–121
39. Murphy CT, McGarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature 424:277–284
40. Tepper RG, Ashraf J, Kaletsky R, Kleemann G, Murphy CT, Bussemaker HJ (2013) PQM-1 complements DAF-16 as a key transcriptional regulator of DAF-2-mediated development and longevity. Cell 154:676–690
41. 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
42. 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
43. Warnhoff K, Murphy JT, Kumar S, Schneider DL, Peterson M, Hsu S, Guthrie J, Robertson JD, Kornfeld K (2014) The DAF-16 FOXO transcription factor regulates *natc-1* to modulate stress resistance in *Caenorhabditis elegans*, linking insulin/IGF-1 signaling to protein N-terminal acetylation. PLoS Genet 10:e1004703
44. Yu Y-L, Zhi L-T, Wu Q-L, Jing L-N, Wang D-Y (2018) NPR-9 regulates innate immune response in *Caenorhabditis elegans* by antagonizing activity of AIB interneurons. Cell Mol Immunol 15:27–37
45. Zhi L-T, Yu Y-L, Li X-Y, Wang D-Y, Wang D-Y (2017) Molecular control of innate immune response to *Pseudomonas aeruginosa* infection by intestinal *let-7* in *Caenorhabditis elegans*. PLoS Pathog 13:e1006152. (9)
46. Zhi L-T, Yu Y-L, Jiang Z-X, Wang D-Y (2017) *mir-355* functions as an important link between p38 MAPK signaling and insulin signaling in the regulation of innate immunity. Sci Rep 7:14560
47. Yu Y-L, Zhi L-T, Guan X-M, Wang D-Y, Wang D-Y (2016) FLP-4 neuropeptide and its receptor in a neuronal circuit regulate preference choice through functions of ASH-2 trithorax complex in *Caenorhabditis elegans*. Sci Rep 6:21485
48. Singh V, Aballay A (2006) Heat-shock transcription factor (HSF)-1 pathway required for *Caenorhabditis elegans* immunity. Proc Natl Acad Sci U S A 103:13092–13097
49. 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
50. 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

51. 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
52. Zhao Y-L, Wu Q-L, Li Y-P, Nouara A, Jia R-H, Wang D-Y (2014) In vivo translocation and toxicity of multi-walled carbon nanotubes are regulated by microRNAs. Nanoscale 6:4275–4284
53. Nouara A, Wu Q-L, Li Y-X, Tang M, Wang H-F, Zhao Y-L, Wang D-Y (2013) Carboxylic acid functionalization prevents the translocation of multi-walled carbon nanotubes at predicted environmental relevant concentrations into targeted organs of nematode *Caenorhabditis elegans*. Nanoscale 5:6088–6096
54. Wu Q-L, Li Y-X, Li Y-P, Zhao Y-L, Ge L, Wang H-F, Wang D-Y (2013) Crucial role of biological barrier at the primary targeted organs in controlling translocation and toxicity of multi-walled carbon nanotubes in nematode *Caenorhabditis elegans*. Nanoscale 5:11166–11178
55. Wu Q-L, Zhao Y-L, Li Y-P, Wang D-Y (2014) Molecular signals regulating translocation and toxicity of graphene oxide in nematode *Caenorhabditis elegans*. Nanoscale 6:11204–11212
56. Wolf M, Nunes F, Henkel A, Heinick A, Paul RJ (2008) The MAP kinase JNK-1 of *Caenorhabditis elegans*: location, activation, and influences over temperature-dependent insulin-like signaling, stress responses, and fitness. J Cell Physiol 214:721–729
57. Li J, Ebata A, Dong Y, Rizki G, Iwata T, Lee SS (2008) *Caenorhabditis elegans* HCF-1 functions in longevity maintenance as a DAF-16 regulator. PLoS Biol 6:e233
58. Rizki G, Iwata TN, Li J, Riedel CG, Picard CL, Jan M, Murphy CT, Lee SS (2011) The evolutionarily conserved longevity determinants HCF-1 and SIR-2.1/SIRT1 collaborate to regulate DAF-16/FOXO. PLoS Genet 7:e1002235
59. Berdichevsky A, Viswanathan M, Horvitz HR, Guarente L (2006) *C. elegans* SIR-2.1 interacts with 14-3-3 proteins to activate DAF-16 and extend life span. Cell 125:1165–1177
60. Olahova M, Veal EA (2015) A peroxiredoxin, PRDX-2, is required for insulin secretion and insulin/IIS-dependent regulation of stress resistance and longevity. Aging Cell 14:558–568
61. 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