Chapter 4
Functions of MAPK Signaling Pathways in the Regulation of Toxicity of Environmental Toxicants or Stresses

Abstract In nematodes, there are three important mitogen-activated protein kinase (MAPK) signals (p38 MAPK, JNK MAPK, and ERK MAPK). It is well known for the crucial role of these three MAPK signaling pathways for organisms in response to environmental stresses by transducing the extracellular cues into the cells. In this chapter, we introduced and discussed the involvement and the contribution, as well as the underlying molecular mechanisms for the response, of these three important MAPK signaling pathways in the regulation of toxicity of environmental toxicants or stresses in nematodes.

Keywords MAPK signaling pathway · Molecular regulation · Environmental exposure · *Caenorhabditis elegans*

4.1 Introduction

Many important molecular signaling pathways required for the control of various biological processes, including the stress response, are conserved among different organisms. Based on this finding, *Caenorhabditis elegans* has been already widely used in the elucidation of underlying molecular mechanisms of toxicity induced by different environmental toxicants or stresses [1]. Among the molecular signaling pathways, the mitogen-activated protein kinase (MAPK) signals (p38 MAPK, JNK MAPK, and ERK MAPK) have well been known to be involved in the response of organisms to environmental toxicants or stresses. Additionally, the MAPK signals are considered to act as central signaling hubs in the regulation of various cellular processes by transducing the extracellular cues into the cells [2, 3].

In this chapter, we introduced the involvement of these three important MAPK signaling pathways (p38, JNK, and ERK) in the regulation of toxicity of environmental toxicants or stresses. Moreover, we discussed the possible underlying molecular mechanisms for the response of these three MAPK signals to environmental toxicants or stresses. The obtained information so far highlights the possible central role of MAPK signaling pathway for nematodes in response to various environmental toxicants or stresses.

D. Wang, *Molecular Toxicology in Caenorhabditis elegans*,
https://doi.org/10.1007/978-981-13-3633-1_4

4.2 p38 MAPK Signaling Pathway

In nematodes, the core p38 MAPK signaling pathway contains components of *pmk-1*-encoded MAPK, *sek-1*-encoded MAPK kinase (MAPKK), and *nsy-1*-encoded MAPK kinase kinase (MAPKKK).

4.2.1 Exposure to Environmental Toxicants or Stress Dysregulates the Expression of p38 MAPK Signal

Microgravity is a crucial contributor to the formation of adverse effects on animals and human beings during the spaceflight [4–6]. Synthecon Rotary SystemTM was used as a simulated microgravity assay system. With the simulated microgravity treatment as an example, it was observed that the control wild-type nematodes grown in liquid S medium showed the similar transcriptional expressions of genes (*nsy-1*, *sek-1*, and *pmk-1*) encoding the core p38 MAPK signaling pathway to those in control wild-type nematodes grown on normal NGM plates (Fig. 4.1) [7]. In contrast, after simulated microgravity treatment, the significant increase in transcriptional expressions of *nsy-1*, *sek-1*, and *pmk-1* was observed in wild-type nematodes (Fig. 4.1) [7]. Considering the fact that activation of p38 MAPK signaling usually requires the phosphorylation of p38 MAPK/PMK-1, the level of phosphorylated PMK-1 between control and simulated microgravity-treated wild-type nematodes was further examined. The control wild-type nematodes grown in liquid S medium had the similar expression of phosphorylated PMK-1 to that in control wild-type nematodes grown on normal NGM plates (Fig. 4.1) [7]. However, after simulated microgravity treatment, a significant increase in the expression of phosphorylated PMK-1 was detected in wild-type nematodes (Fig. 4.1) [7].

Graphene oxide (GO), a member of graphene family containing a two-dimensional carbon structure, can be potentially used in nanomedicine including drug delivery and bioimaging [8–10]. In nematodes, GO exposure could cause the toxicity on the functions of both primary (such as intestine) and secondary (such as neurons and reproductive organs) targeted organs [11–17]. Similarly, prolonged exposure to GO (100 mg/L) could significantly increase the transcriptional expressions of *pmk-1*, *sek-1*, and *nsy-1* and increase the expression of intestinal PMK-1::GFP [18]. Additionally, prolonged exposure to GO (100 mg/L) significantly increased the percentage of PMK-1::GFP nucleus localization in the intestinal cells [18]. Moreover, the Western blotting analysis demonstrated that prolonged exposure to GO (100 mg/L) obviously increased the expression of phosphorylated PMK-1 [18]. The decreased expression in PMK-1::GFP was further observed in GO (100 mg/L)-exposed *sek-1(ag1)* or *nsy-1(ag3)* mutant nematodes, and the decreased phosphorylation of p38 MAPK/PMK-1 was detected in GO (100 mg/L)-exposed *sek-1(ag1)* or *nsy-1(ag3)* mutant nematodes [18]. These observations suggest that the p38 MAPK signal may be upregulated by the GO exposure in nematodes. The

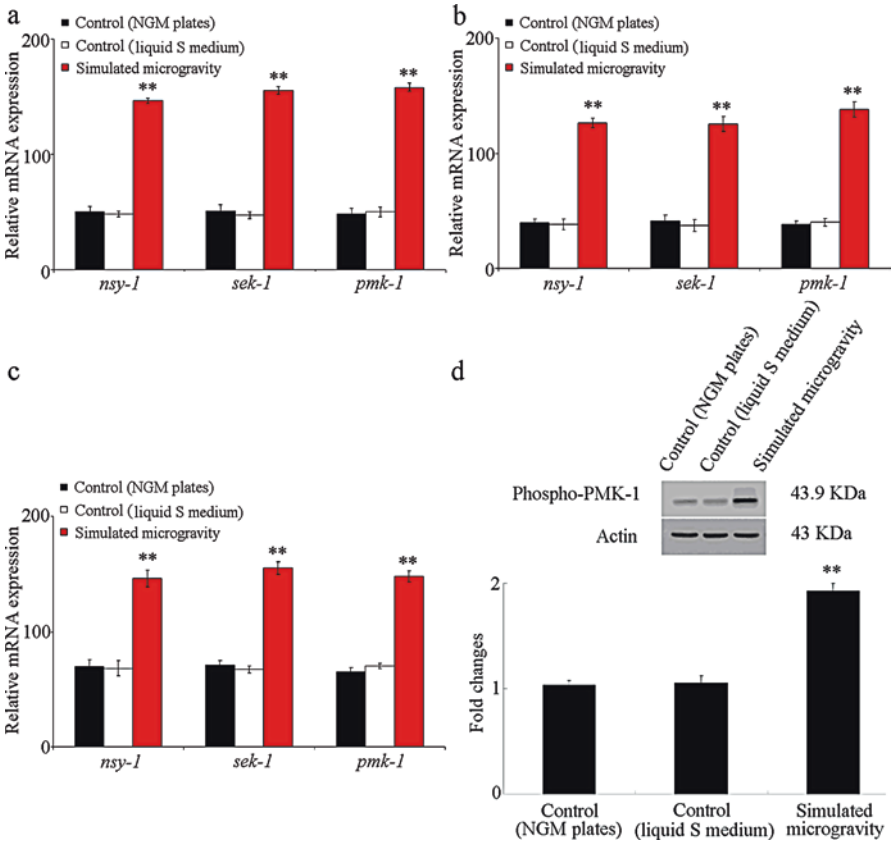

Fig. 4.1 Effect of simulated microgravity on expression of p38 MAPK signaling in wild-type nematodes [7]. (**a**) Effect of simulated microgravity on expression of p38 MAPK signaling in wild-type nematodes using *tba-1* as a reference gene. (**b**) Effect of simulated microgravity on expression of p38 MAPK signaling in wild-type nematodes using *pmp-3* as a reference gene. (**c**) Effect of simulated microgravity on expression of p38 MAPK signaling in wild-type nematodes using *act-1* as a reference gene. (**d**) Western blotting analysis of the effect of simulated microgravity on expression level of phosphorylated PMK-1. Bars represent means ± SD. $^{**}P < 0.01$ vs control (NGM plates)

upregulation of p38 MAPK signal was also observed in nematodes exposed to Ag-nanoparticles [19, 20].

Multiwalled carbon nanotubes (MWCNTs) consisting of multiple concentric graphene cylinders could also cause the toxicity on the functions of both primary and secondary targeted organs in nematodes even at environmentally relevant concentrations [21–24]. The most common fungal pathogen *Candida albicans* can invade host tissues and cause life-threatening infections when the immune system of hosts is weakened (e.g., from critical illness) or the competing bacterial flora in hosts are eliminated (e.g., from broad-spectrum antibiotic use) [25–28]. *C. elegans* has also been proven to be helpful for the study of virulence of human pathogenic

fungi [29]. In nematodes, it has been observed that pre-exposure to MWCNTs (more than 100 μg/L) could enhance the adverse effect of *C. albicans* infection in reducing the lifespan [30]. Pre-exposure could enhance the colony formation of *C. albicans* in the body of nematodes and suppress the innate immune response of nematodes by decreasing the expressions of some antimicrobial genes [30]. Different from those observed in simulated microgravity-treated or GO-exposed nematodes, it was found that MWCNTs decreased the expressions of *pmk-1*, *sek-1*, and *nsy-1* and inhibited translational expression of PMK-1::GFP in the intestine and phosphorylation of PMK-1 (Fig. 4.2) [30]. Epistasis assays further suggested that MWCNTs required the involvement of the p38 MAPK signaling pathway mediated by a NSY-1-SEK-1-PMK-1 cascade to enhance the toxicity of fungal infection, to increase fungal colony formation, and to suppress innate immune response [30]. Thus, the dysregulation pattern of p38 MAPK signaling pathway may be different under different stress conditions in nematodes.

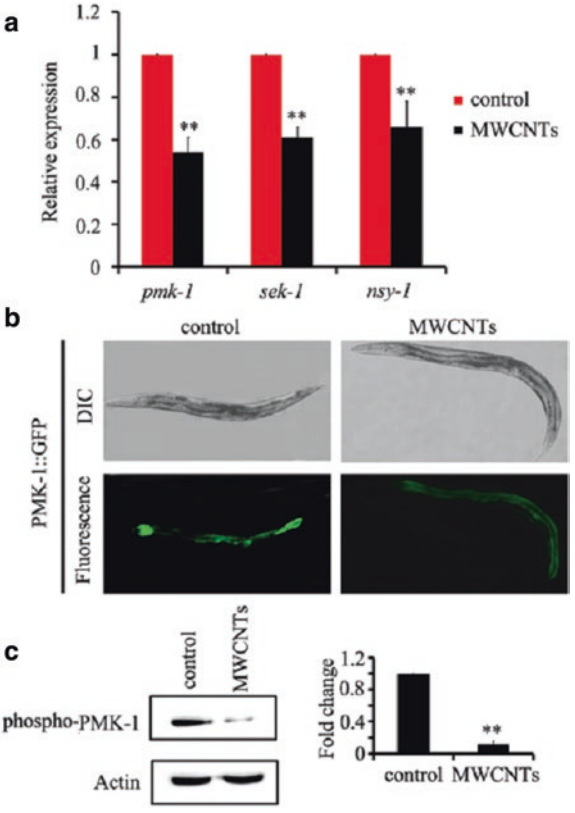

Fig. 4.2 Pre-exposure to MWCNTs altered expression patterns of genes encoding the p38 MAPK signaling pathway in wild-type nematodes [30]. (**a**) Effects of MWCNT exposure on transcriptional expression of *pmk-1*, *sek-1*, and *nsy-1*. (**b**) Effects of MWCNT exposure on expression pattern of PMK-1::GFP. (**c**) Western blot analysis of the effect of MWCNT exposure on expression level of phosphorylated PMK-1. Actin protein was used as the loading control. Nematodes were exposed to MWCNTs from L1-larvae to young adults in 12-well sterile tissue culture plates at 20 °C in the presence of food (OP50). MWCNT exposure concentration was 1000 μg/L. Bars represent means ± SEM $^{**}P < 0.01$ vs control

4.2.2 *p38 MAPK Signaling Pathway Regulates the Toxicity of Environmental Toxicants or Stresses*

With the simulated microgravity treatment as an example, intestinal ROS production and lifespan were selected as the toxicity assessment endpoints. In wild-type nematodes, simulated microgravity did not significantly affect the longevity (Fig. 4.3) [7]. The lifespan of wild-type nematodes grown in liquid S medium was similar to that on normal NGM plates under the normal conditions, and mutation of

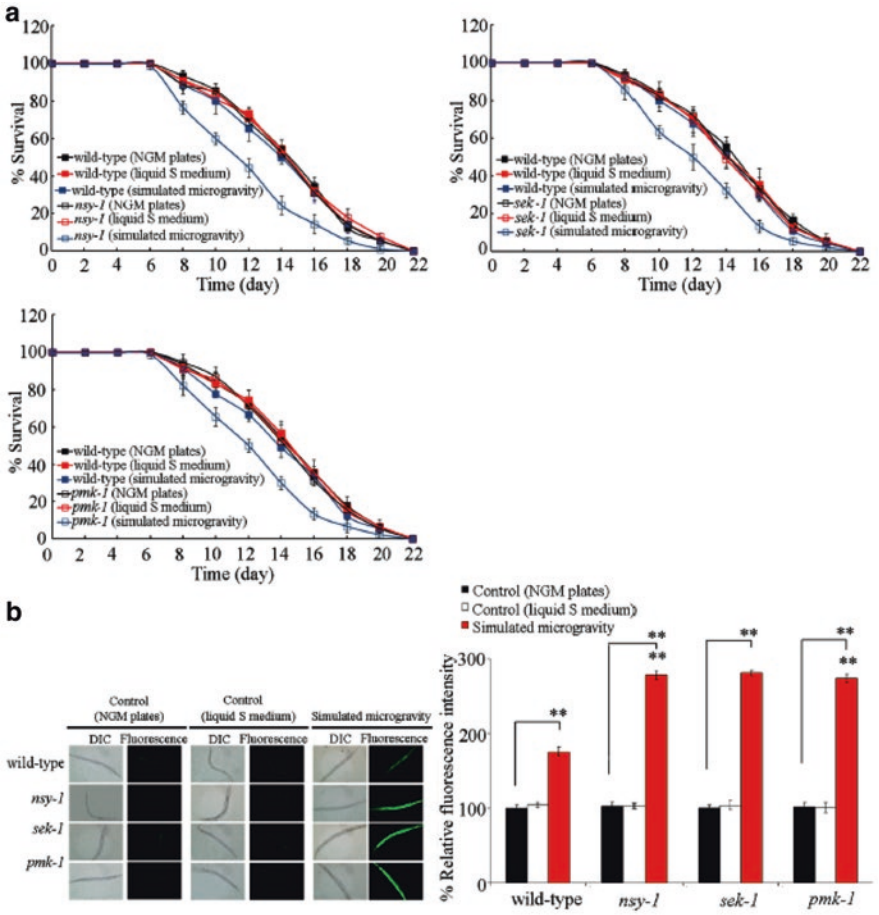

Fig. 4.3 Mutation of genes encoding p38 MAPK signaling pathway induced a susceptibility to simulated microgravity treatment in nematodes [7]. (**a**) Mutation of genes encoding p38 MAPK signaling pathway induced the reduced lifespan in simulated microgravity-treated nematodes. (**b**) Mutation of genes encoding p38 MAPK signaling pathway induced a susceptibility to simulated microgravity treatment in inducing intestinal ROS production. Bars represent means ± SD. $^{**}P < 0.01$ vs wild type (if not specially indicated)

nsy-1, *sek-1*, or *pmk-1* did not alter the longevity under the normal conditions (Fig. 4.3) [7]. However, after the simulated microgravity treatment, mutation of *nsy-1*, *sek-1*, or *pmk-1* significantly reduced the lifespan (Fig. 4.3) [7]. Meanwhile, the wild-type nematodes grown in liquid S medium or on normal NGM plates did not have the significant induction of intestinal ROS production, and the *nsy-1*, *sek-1*, and *pmk-1* mutants grown in liquid S medium or grown on normal NGM plates also did not have the significant induction of intestinal ROS production under the normal conditions (Fig. 4.3) [7]. In wild-type nematodes, simulated microgravity treatment caused the significant induction of intestinal ROS production (Fig. 4.3) [7]. Moreover, after simulated microgravity treatment, mutation of *nsy-1*, *sek-1*, or *pmk-1* could induce the more severe induction of intestinal ROS production compared with wild-type nematodes (Fig. 4.3) [7].

Under the normal conditions, *pmk-1(km25)*, *sek-1(ag1)*, or *nsy-1(ag3)* mutant also has the normal locomotion behavior [18]. Similarly, prolonged exposure to GO (100 mg/L) induced the formation of more severe reduction in lifespan, decrease in locomotion behavior, and induction of intestinal ROS production in *pmk-1(km25)*, *sek-1(ag1)*, or *nsy-1(ag3)* mutant than those in wild-type nematodes [18]. Therefore, mutations of genes encoding the core p38 MAPK signaling pathway may induce a susceptibility to toxicity of environmental toxicants or stresses, such as the GO exposure and the simulated microgravity treatment. In contrast, overexpression of *pmk-1*, *sek-1*, or *nsy-1* could significantly decrease the induction of intestinal ROS production [18]. Under normal conditions, the nematodes with the overexpression of *pmk-1*, *sek-1*, or *nsy-1* do not exhibit the obvious induction of intestinal ROS production [18]. These observations imply the important role of the p38 MAPK signaling pathway in the regulation of toxicity of environmental toxicants or stresses.

4.2.3 *Intestinal Signaling Cascade of p38 MAPK Signaling Pathway Regulates the Toxicity of Environmental Toxicants or Stresses*

pmk-1 is broadly expressed in multiple tissues including the intestine, *sek-1* is expressed in the excretory canal, the intestine, and the neurons, and *nsy-1* is expressed in the intestine, the hypodermis, and the neurons. Under the normal conditions, the nematodes with intestinal RNAi knockdown of *pmk-1*, *sek-1*, or *nsy-1* exhibited the similar lifespan to VP303 nematodes (Fig. 4.4) [18]. With the simulated microgravity as an example, it was found that neuronal expression of *pmk-1* did not affect the lifespan and the induction of intestinal ROS production in simulated microgravity-treated *pmk-1* mutant nematodes; however, intestinal expression of *pmk-1* could significantly increase the lifespan and suppress the induction of intestinal ROS production in simulated microgravity-treated *pmk-1* mutant

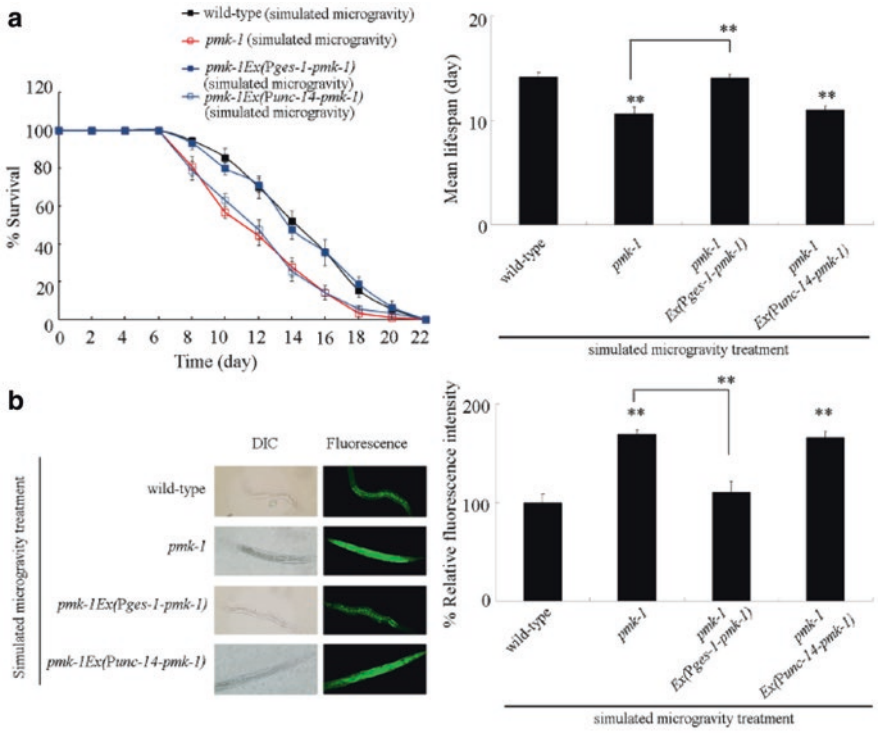

Fig. 4.4 Tissue-specific activity of PMK-1 in regulating the response of nematodes to simulated microgravity [7]. (**a**) Tissue-specific activity of PMK-1 in regulating lifespan in simulated microgravity-treated nematodes. (**b**) Tissue-specific activity of PMK-1 in regulating the induction of intestinal ROS production in simulated microgravity-treated nematodes. Bars represent means ± SD. $^{**}P < 0.01$ vs wild-type (if not specially indicated)

nematodes (Fig. 4.4) [7]. Meanwhile, after simulated microgravity treatment, intestinal RNAi knockdown of *nsy-1*, *sek-1*, or *pmk-1* could reduce the lifespan and lead to the more severe induction of intestinal ROS production compared with that in VP303 strain [7]. Moreover, intestinal overexpression of PMK-1 induced a resistance to simulated microgravity treatment, since intestinal overexpression of PMK-1 significantly suppressed the induction of intestinal ROS production observed in simulated microgravity-treated wild-type nematodes [7]. Therefore, the core p38 MAPK signaling pathway may act in the intestine to regulate the toxicity of environmental toxicants or stresses in nematodes.

Similarly, with GO as an example, it has been also found that intestinal RNAi knockdown of *pmk-1*, *sek-1*, or *nsy-1* also induced a susceptibility to GO toxicity on lifespan [18], suggesting that the signaling cascade of NSY-1-SEK-1-PMK-1 can act in the intestine to regulate the GO toxicity (Fig. 4.5) [18].

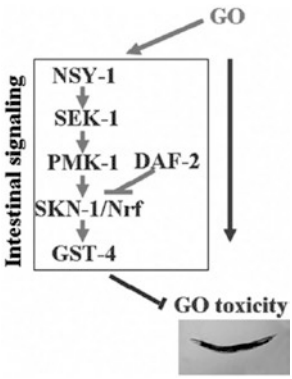

Fig. 4.5 A diagram showing the intestinal p38 MAPK-SKN-1/Nrf signaling cascade involved in the control of GO toxicity in nematodes [18]

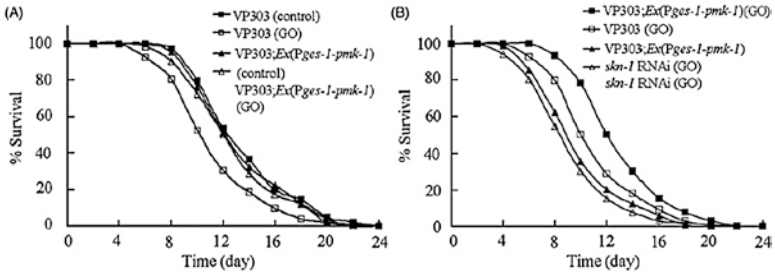

Fig. 4.6 Intestinal RNAi of *skn-1* suppressed lifespan of nematodes overexpressing *pmk-1* in intestine [18]. (**a**) Effect of overexpression of *pmk-1* in intestine on lifespan. (**b**) Effect of intestinal RNAi of *skn-1* on lifespan in nematodes overexpressing *pmk-1* in intestine. VP303 is a tool for the RNAi only in intestine. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 100 mg/L.

4.2.4 SKN-1 Acts as an Important Target for Intestinal PMK-1 in Regulating the Toxicity of Environmental Toxicants or Stresses

4.2.4.1 Conformation of the Role of SKN-1 as the Target for Intestinal PMK-1 in Regulating the Toxicity of Environmental Toxicants or Stresses

SKN-1 plays a central role in the activation of oxidative stress in nematodes [31]. Intestinal RNAi knockdown of *skn-1* could cause a susceptibility to GO toxicity in reducing lifespan and in inducing intestinal ROS production [18], suggesting that SKN-1/Nrf can act in the intestine to regulate the GO toxicity. Moreover, intestinal RNAi knockdown of *skn-1* could suppress the resistance of nematodes overexpressing intestinal *pmk-1* to GO toxicity in reducing lifespan (Fig. 4.6) [18], implying that the intestinal core p38 MAPK signaling cascade may regulate the GO toxicity by acting upstream the transcriptional factor SKN-1/Nrf (Fig. 4.5) [18].

In nematodes, prolonged exposure to GO (100 mg/L) also significantly enhanced the expression of intestinal SKN-1::GFP [18]. Additionally, prolonged exposure to GO (100 mg/L) also significantly increased the percentage of SKN-1::GFP nucleus localization in intestinal cells and the expression of intestinal GST-4::GFP [18].

4.2.4.2 Identification of Downstream Targets for SKN-1 in Regulating the Toxicity of Environmental Toxicants or Stresses

One of the identified important targets for SKN-1 is the phase II detoxification protein GST-4, a glutathione-requiring prostaglandin D synthase. GST-4 is expressed in the intestine, the pharynx, and the hypodermis. Intestinal RNAi knockdown of *gst-4* could induce a susceptibility to GO toxicity in reducing lifespan and in inducing intestinal ROS production [18], suggesting that GST-4 can act in the intestine to regulate the GO toxicity. Genetic interaction analysis indicated that the SKN-1 and the GST-4 could act in the same genetic pathway to regulate the GO toxicity, since exposure to GO (100 mg/L) caused the similar toxicity on lifespan in *skn-1(RNAi); gst-4(ok2108)* to that in *skn-1(RNAi)* strain or in *gst-4(ok2108)* mutant nematodes (Fig. 4.5) [18].

Another identified important target for SKN-1 is the GCS-1, an ortholog of γ-glutamine cysteine synthetase heavy chain [32]. *Pseudomonas aeruginosa* is normally considered to be toxic and will cause a lethal intestinal infection on nematode host [33–37]. After 24 h exposure of *P. aeruginosa* PA14, both the *gcs-1* promoter activation and the GST-4 expression were significantly suppressed by feeding with *skn-1(RNAi)*, suggesting the specific requirement of SKN-1 to elicit these responses (Fig. 4.7) [32].

4.2.5 ATF-7 Acts as Another Important Target for Intestinal PMK-1 in Regulating the Toxicity of Environmental Toxicants or Stresses

In nematodes, the simulated microgravity treatment could cause the significant increase in transcriptional expression of *atf-7* encoding a bZIP transcription factor [7]. After simulated microgravity treatment, mutation of *atf-7* caused the significant decrease in relative mean lifespan (treatment/Control(NGM plates)) in nematodes (Fig. 4.8) [7]. Meanwhile, after simulated microgravity treatment, the more severe induction of intestinal ROS production was observed in *atf-7* mutant nematodes compared with wild-type nematodes (Fig. 4.8) [7]. Moreover, RNAi knockdown of *atf-7* could further dramatically suppress the induction of GST-4::GFP expression caused by simulated microgravity treatment, and *atf-7* mutation could significantly reduce the lifespan and increase the induction of intestinal ROS production in

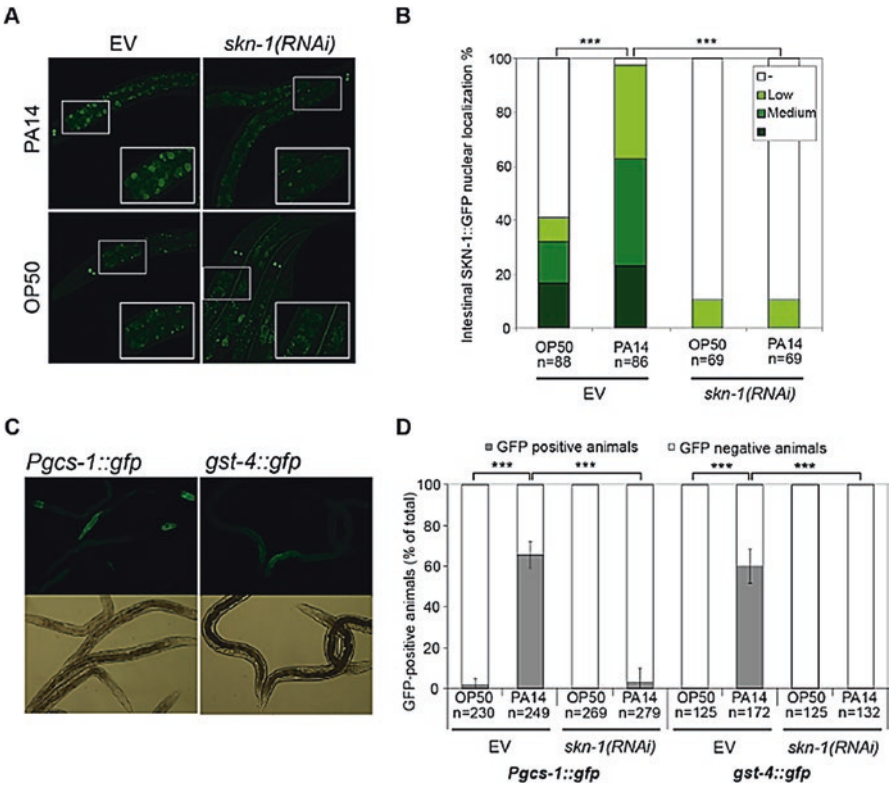

Fig. 4.7 *P. aeruginosa* infection activates SKN-1 [32]. (**a**) Representative epifluorescence image demonstrating the translocation of SKN-1::GFP in the *Is007*[SKN-1::GFP] strain to intestinal nuclei in L3 larvae, fed by the empty vector or *skn-1* dsRNA, upon a 5-h exposure to *P. aeruginosa* PA14. Note that the intestinal tissue displays autofluorescence, and in the ASI neurons, SKN-1::GFP is not silenced by *skn-1* RNAi treatment. (**b**) Quantification of SKN-1 nuclear translocation from the data shown on panel (**a**). "Low" refers to animals in which SKN-1::GFP was detected in less than five intestinal nuclei, while "high" indicates that SKN-1::GFP signal was present in more than 15 intestinal nuclei. (**c**) Representative epifluorescence microscopic image showing intestinal expression of P*gcs-1::GFP* and GST-4::GFP in L3 larvae upon a 24-h PA14 exposure. (**d**) Quantification of reporter expression demonstrating the SKN-1 dependence of the response. Data were obtained from panel (**c**) completed with the data of *skn-1(RNAi)* animals. Microscopic images are representatives of three independent experiments. EV: empty vector RNAi

transgenic strain overexpressing intestinal *pmk-1* in simulated microgravity- treated nematodes (Fig. 4.8) [7], which suggests the important role of ATF-7 as the important target for intestinal PMK-1 in regulating the toxicity of environmental toxicants or stresses.

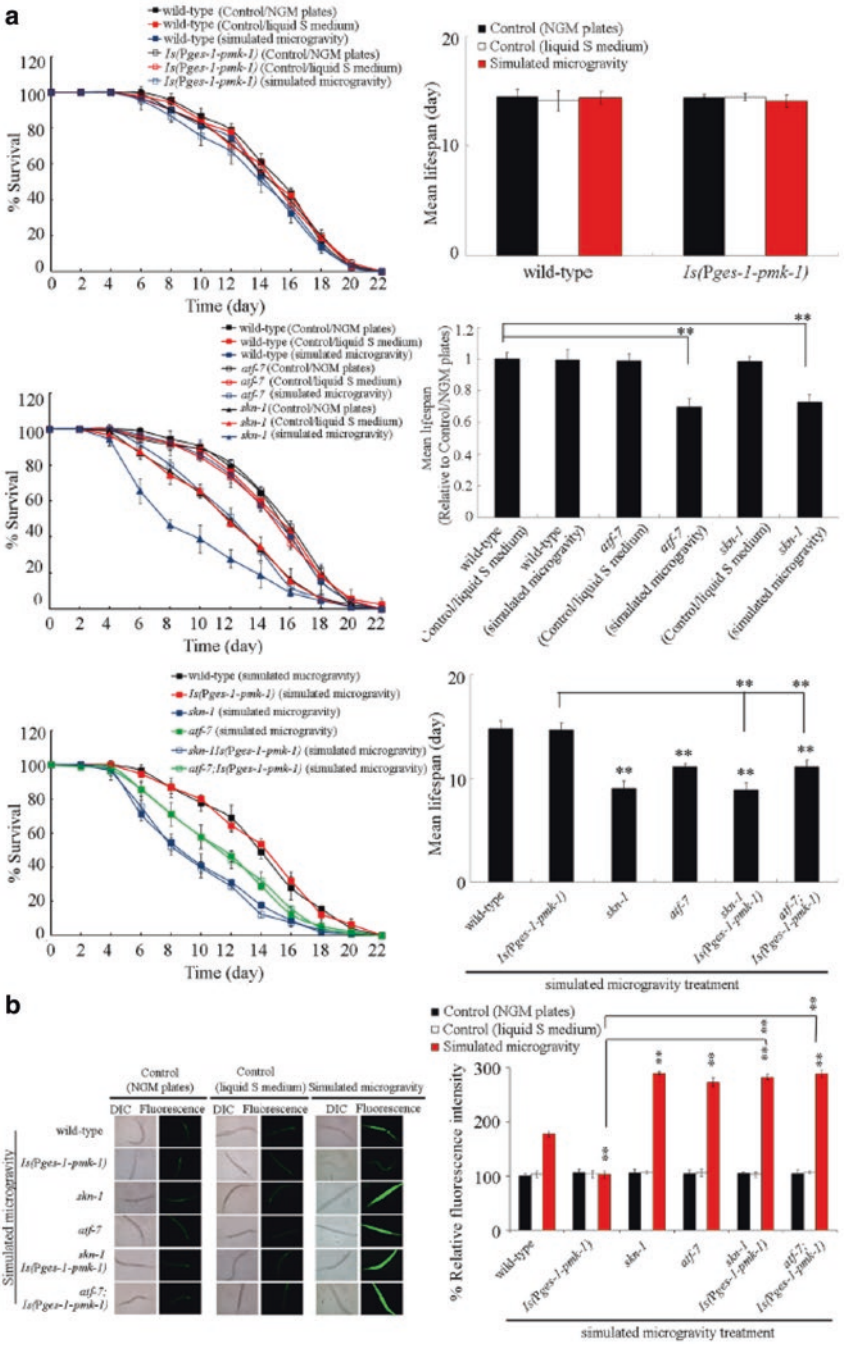

Fig. 4.8 Genetic interaction between PMK-1 and SKN-1 or ATF-7 in regulating the response of nematodes to simulated microgravity [7]. (**a**) Genetic interaction between PMK-1 and SKN-1 or ATF-7 in regulating the lifespan in simulated microgravity-treated nematodes. (**b**) Genetic interaction between PMK-1 and SKN-1 or ATF-7 in regulating the induction of intestinal ROS production in simulated microgravity treatment. Bars represent means ± SD. $^{**}P < 0.01$ vs wild type (if not specially indicated)

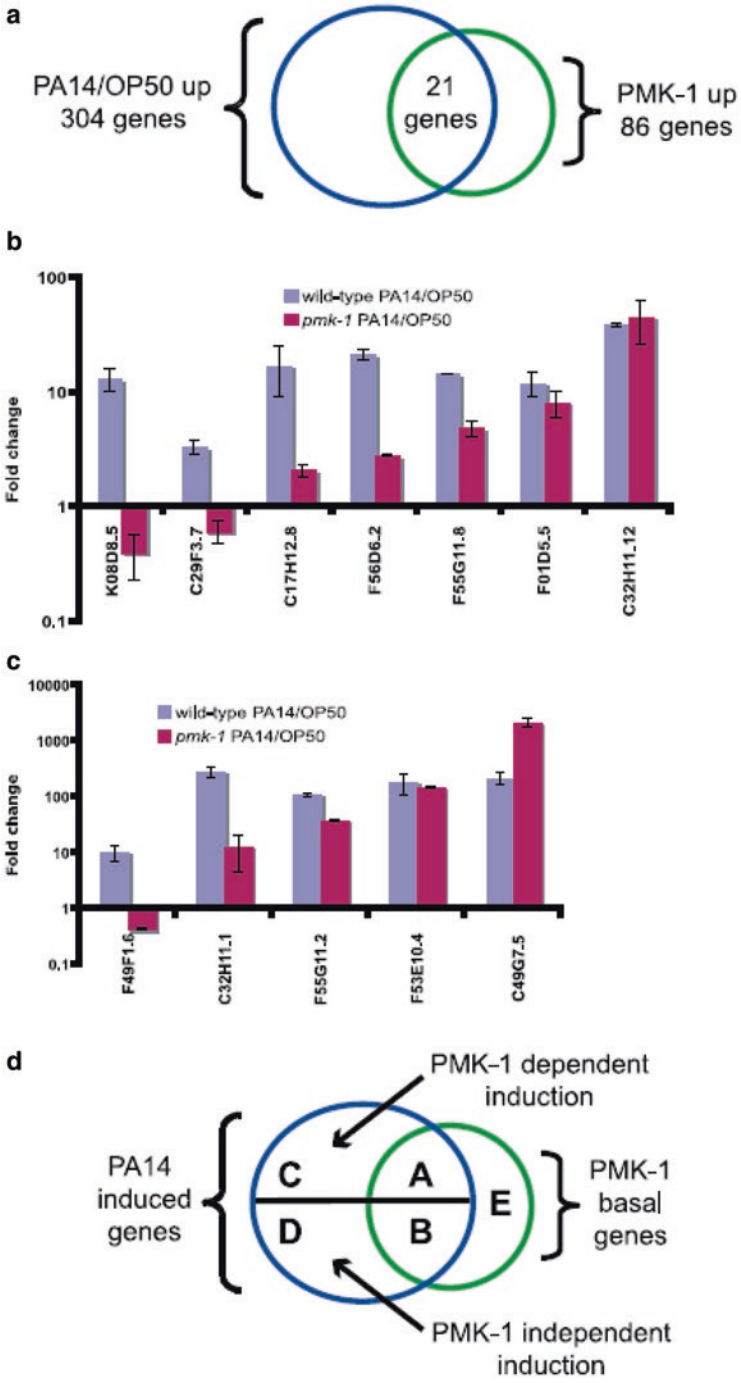

Fig. 4.9 PMK-1 regulates basal and inducible expression of *P. aeruginosa*-induced genes [38]. (**a**) Venn diagram of overlap between genes regulated by PMK-1 and *P. aeruginosa*. (**b**, **c**) qRT-PCR analysis of PA14-induced gene expression in wild-type animals and in *pmk-1* mutants. Results are

4.2.6 Role of Antimicrobial Proteins as the Targets for PMK-1 in Regulating the Toxicity of Environmental Toxicants or Stresses

In nematodes, the qRT-PCR analysis demonstrated that the basal expression of the overlap genes requires PMK-1 in animals grown on *E. coli*, and these genes could be induced by infection with *P. aeruginosa* [38]. The further examination on *P. aeruginosa*-induced gene expression in *pmk-1* mutants indicated that most of the overlap genes were not fully induced by *P. aeruginosa* in *pmk-1(km25)* mutant nematodes, suggesting that PMK-1 is required for their induction (Fig. 4.9) [38]. The microarray and qRT-PCR studies of genes regulated by PMK-1 and *P. aeruginosa* identified five classes of candidate immunity genes (Fig. 4.9) [38]. Class A genes are regulated basally by PMK-1 on *E. coli* and require PMK-1 for their induction by pathogenic *P. aeruginosa* (e.g., *K08D8.5*). Class B genes are regulated basally by PMK-1 and are induced by *P. aeruginosa*, but that induction does not require PMK-1 (e.g., *C32H11.12*). Class C genes are not regulated basally by PMK-1 based on microarray results but do require PMK-1 for induction by *P. aeruginosa* (e.g., *F49F1.6*). Class D genes are not regulated basally by PMK-1 based on microarray results and do not require PMK-1 for induction by *P. aeruginosa* (e.g., *C49G7.5*). Class E genes are regulated basally by PMK-1 but are not induced by *P. aeruginosa*.

4.2.7 Upregulators of p38 MAPK Signaling Pathway in Response to Environmental Toxicants or Stresses

4.2.7.1 Duox1/BLI-3

ROS can be generated during infection in the model host *C. elegans* by the dual oxidase Duox1/BLI-3 [39]. SKN-1 could be activated in the intestine upon exposure to the human bacterial pathogens, *Enterococcus faecalis* and *P. aeruginosa*, and a weakened response was observed in attenuated mutants of these pathogens [39]. The signaling cascade of NSY-1-SEK-1-PMK-1 was required for the SKN-1

Fig. 4.9 (continued) the average of two biological replicates, each replicate measured in duplicate and normalized to a control gene. Error bars are SEM. (**d**) Diagram of different gene classes regulated by PMK-1 and/or *P. aeruginosa*. PMK-1 is required for basal and inducible regulations of class A genes. PMK-1 is required for basal, but not inducible expression of class B genes. PMK-1 is required for inducible but not basal expression of class C genes. PMK-1 is required for neither basal nor inducible expression of class D genes. PMK-1 regulates basal expression of class E genes, but these genes are not induced by *P. aeruginosa*

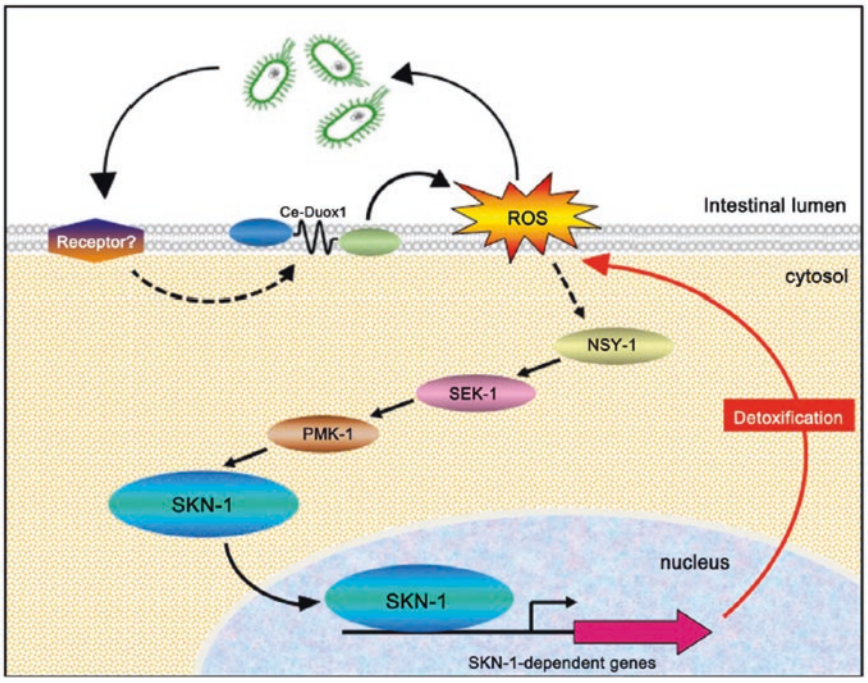

Fig. 4.10 Regulation of SKN-1 activation by Duox1/BLI-3 during infection [39]

activity during the infection [39]. Moreover, it was found that the ROS produced by Duox1/BLI-3 was just the source of SKN-1 activation via p38 MAPK signaling during the infection (Fig. 4.10) [39]. That is, the ROS generation by Duox1/BLI-3 may activate a protective SKN-1 response via p38 MAPK signaling pathway against the environmental toxicants or stresses in nematodes (Fig. 4.10) [39].

4.2.7.2 TIR-1 and VHP-1

In nematodes, silencing the *pmk-1* could entirely prevent the SKN-1-dependent activation of *gcs-1* in response to PA14 infection [32]. The PMK-1 could be inactivated by the dual specificity MAPK phosphatase VHP-1. Suppression of VHP-1 resulted in an increased PMK-1 phosphorylation and a resistance to PA14 infection, and *vhp-1(RNAi)* could significantly increase P*gcs-1::GFP* activation upon PA14 infection (Fig. 4.11) [32]. Meanwhile, TIR-1, a conserved Toll/IL-1 resistance (TIR) domain protein, has the function to activate the p38 MAPK signaling independently of the Toll-like receptor ortholog TOL-1 during the PA14 infection. RNAi knockdown or mutation of *tir-1* could prevent the P*gcs-1::GFP* fluorescence upon PA14 infection and prevent the nuclear translocation of SKN-1 induced by PA14 infection (Fig. 4.11) [32].

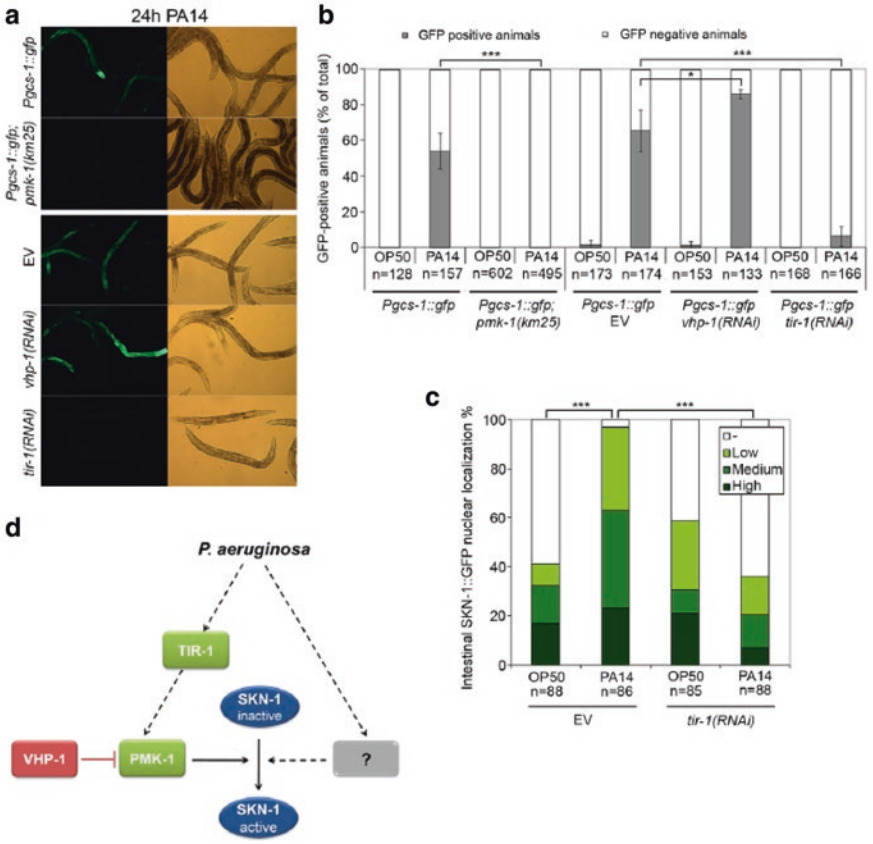

Fig. 4.11 The pathogen response-specific TIR-1 and p38 MAPK PMK-1 are required for SKN-1 activation upon *P. aeruginosa* infection [32]. (**a**) Representative epifluorescence microscopic images showing the expression of P*gcs-1::GFP* in *pmk-1*(*km25*) mutants as well as in the p38 MAPK phosphatase *vhp-1*(*RNAi*) and the Toll/IL-1 resistance (TIR) domain protein *tir-1(RNAi)* animals in response to *P. aeruginosa* infection. L3 larvae were exposed to PA14 for 24 h. Microscopic images are representatives from three independent experiments. (**b**) Quantification of reporter expression from the data shown on panel (**a**) completed with the data of control animals fed by OP50 for 24 h. (**c**) Quantification of SKN-1 nuclear translocation in *tir-1(RNAi)* L3 larvae upon 5 h PA14 exposure. (**d**) Suggested model of SKN-1 activation during *P. aeruginosa* infection. Upon exposure to PA14, the TIR-1/PMK-1 pathway is indispensable but insufficient to elicit SKN-1 transactivation. Solid arrows indicate a direct, while dashed arrows indicate an indirect/unknown connection. *EV* empty vector RNAi

4.2.7.3 MEK-1

mek-1 encodes a MAP kinase kinase (MAPKK). In nematodes, both the SEK-1 and the MEK-1 were essential for the PMK-1 activation (Fig. 4.12) [40]. Additionally, although RNAi knockdown of *pmk-1* did not affect the lifespan of *sek-1* mutant nematodes after pathogen infection, RNAi knockdown of *pmk-1* could further

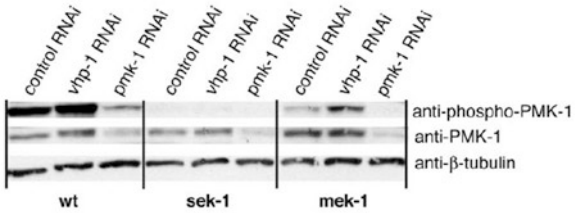

Fig. 4.12 Modulation of PMK-1 activation by MEK-1 and SEK-1 MAPKKs and VHP-1 MKP: correlation of pathogen susceptibility with levels of PMK-1 activation [40]. Immunoblot analysis of lysates derived from WT and *sek-1(km4)* and *mek-1(ks54)* mutants subjected to RNAi by feeding with bacterial strains expressing double-stranded RNA corresponding to the sequence of control (L4440 vector only), *pmk-1* (pDK177), or *vhp-1* (Ahringer 44D3) genes. Activated levels of PMK-1 were detected by using an Ab (anti-phospho-p38) specific for the doubly phosphorylated form of PMK-1.

reduce the lifespan of *mek-1* mutant nematodes after pathogen infection [40]. Therefore, both the MEK-1 and the signaling cascade of NSY-1-SEK-1 can act upstream of PMK-1 to regulate the response of nematodes to environmental toxicants or stresses in nematodes.

4.2.7.4 Mitochondrial Complex I

In nematodes, downregulating complex I gene *nuo-2* or *nduf-6* could cause a significant increase in p38MAPK phosphorylation (Fig. 4.13) [41]. Meanwhile, downregulation of at least five of the six tested mitochondrial complex I genes could upregulate four of the seven ATF-7-dependent genes induced by rotenone: *C17H12.8*, *F56D6.2*, and *M02F4.7* (C-type lectins) and *F49F1.6* (*mul-1*, a mucin-like protein) (Fig. 4.13) [41]. In contrast, RNAi-mediated downregulation of the respiratory chain complex assembly factor *oxa-1*, complex III, complex IV, and complex V ATP synthase subunits did not increase the levels of phosphorylated p38 MAPK (Fig. 4.13) [41]. These observations suggest that mitochondrial complex I dysfunction can act upstream of the p38MAPK signaling pathway to regulate the stress response in nematodes.

4.3 JNK Signaling Pathway

In nematodes, the c-Jun N-terminal kinase (JNK) signaling pathway mainly contains members of MEK-1, JKK-1, and JNK-1 [42]. *mek-1* and *jkk-1* encode MAP kinase kinases, homolog of human MKK-7a, and act as an activator of JNK. *jnk-1* encodes a serine/threonine kinase, homolog of human JNK, and acts as the sole member of the JNK subgroup of MAP kinase [42].

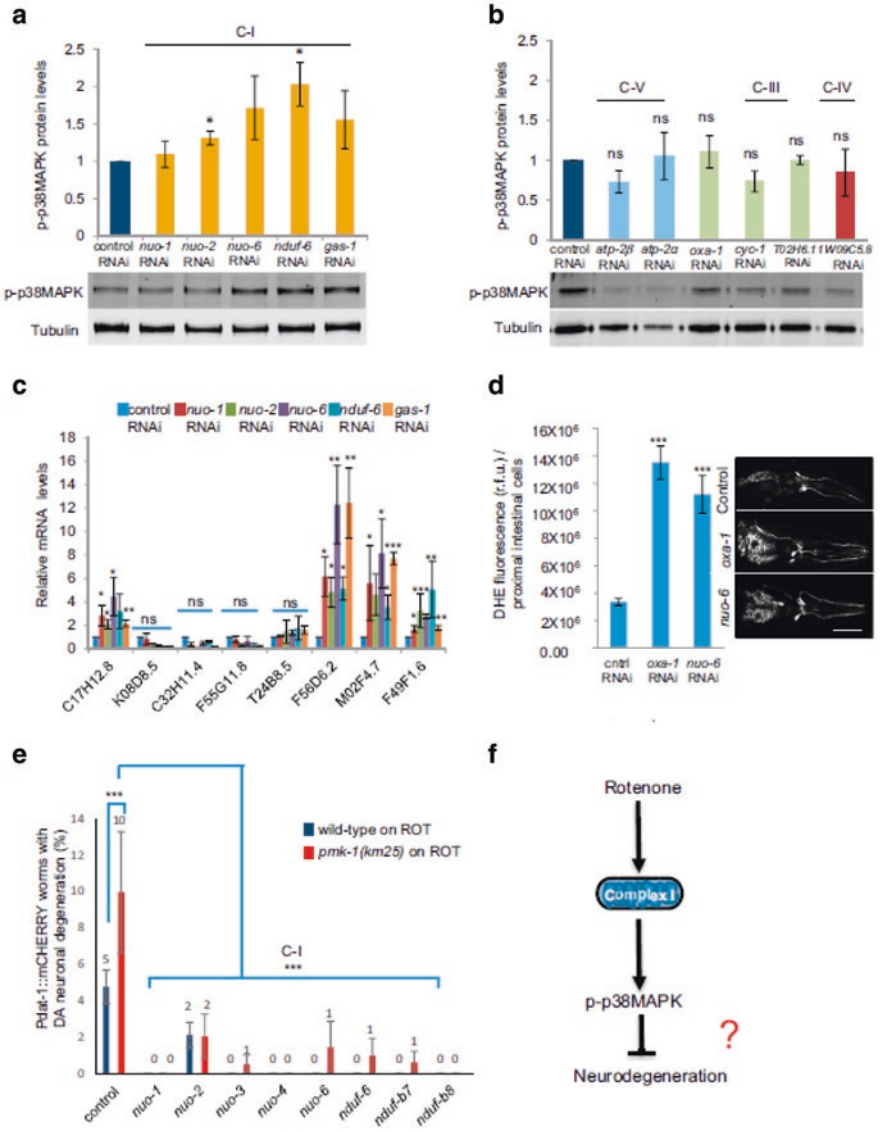

Fig. 4.13 Mitochondrial complex I dysfunction activates p38 MAPK/ATF-7 [41]. (**a**, **b**) Levels of phosphorylated p38MAPK following RNAi-mediated downregulation of complex I (C-I) subunits (**a**) and complex V (C-V), *oxa-1*, complex III (C-III), and complex IV (C-IV) subunits (**b**) relative to control RNAi-treated animals. Levels of phospho-p38MAPK (top lanes) were quantified relative to α-tubulin (bottom lanes). (**c**) mRNA levels of ATF-7-dependent innate immune genes in control animals and animals on complex I RNAi, determined by qRT-PCR. mRNA levels of the transcripts (x axis) were normalized to wild-type control levels. Actin is the internal control. (**d**) Levels of dihydroethidium (DHE) fluorescence quantitated in proximal intestinal cells of animals on control RNAi, complex I subunit *nuo-6* RNAi, and mitochondrial assembly factor *oxa-1* RNAi. Representative micrographs are of the anterior regions of animals. RFU, relative fluorescence units. Scale bar, 30 mm

4.3.1 Exposure to Environmental Toxicants or Stress Dysregulates the Expression of JNK MAPK Signal

With the GO as an example, after prolonged exposure, the transcriptional expressions of *mek-1*, *jkk-1*, and *jnk-1* were significantly decreased by GO exposure (10 mg/L) [43], suggesting that exposure to certain environmental toxicants or stress can dysregulate the expression of JNK MAPK signal in nematodes.

4.3.2 JNK MAPK Signaling Pathway Regulates the Toxicity of Environmental Toxicants or Stresses

In nematodes, mutation of *mek-1* or *jnk-1* could induce a susceptibility to Cd or Cu toxicity in inhibiting the survival [44, 45]. Besides this, mutation of *mek-1*, *jkk-1*, or *jnk-1* could also induce a susceptibility to GO toxicity in reducing brood size, in decreasing locomotion behavior, and in inducing intestinal ROS production (Fig. 4.14) [43], which suggests the involvement of JNK MAPK signaling pathway in the regulation of toxicity of environmental toxicants or stresses, such as GO exposure.

The genetic interaction analysis further indicated that the *jnk-1*(*gk7*)*;mek-1*(*ks54*) double mutant exhibited the similar GO toxicity in reducing brood size, in decreasing locomotion behavior, and in inducing intestinal ROS production to that in the *mek-1*(*ks54*) or the *jnk-1(gk7)* single mutant (Fig. 4.14) [43]. Meanwhile, the *jnk-1*(*gk7*)*;jkk-1*(*km2*) double mutant showed the similar GO toxicity in reducing brood size, in decreasing locomotion behavior, and in inducing intestinal ROS production to that in the *jkk-1*(*km2*) or the *jnk-1*(*gk7*) single mutant (Fig. 4.14) [43]. Additionally, the activated JNK-1 was detected only in neuronal cells, and JNK-1 was found to be controlled by the MAPK JKK-1 under the heat stress [46]. These results demonstrated that the MEK-1 and the JKK-1 may act genetically in the same pathway with JNK-1 in the regulation of toxicity of environmental toxicants or stresses in nematodes.

In nematodes, the JNK MAPK signaling pathway is regulated by MLK-1 MAPK kinase kinase (MAPKKK), MEK-1 MAPK kinase (MAPKK), and KGB-1 JNK-like MAPK [47]. Moreover, loss-of-function mutation of *shc-1* encoding a homolog of Shc was defective in activation of KGB-1 and caused the hypersensitivity to heavy metals [47]. Introduction of a mutation that perturbs binding to the PTB domain or the NPXY motif could abolish the function of SHC-1 or MLK-1 and the resistance to heavy metal stress [47]. These results imply that the SHC-1 can act as a scaffold to link MLK-1/MAPKKK to MEK-1/MAPKK activation in the KGB-1/MAPK signal transduction pathway (Fig. 4.15) [47].

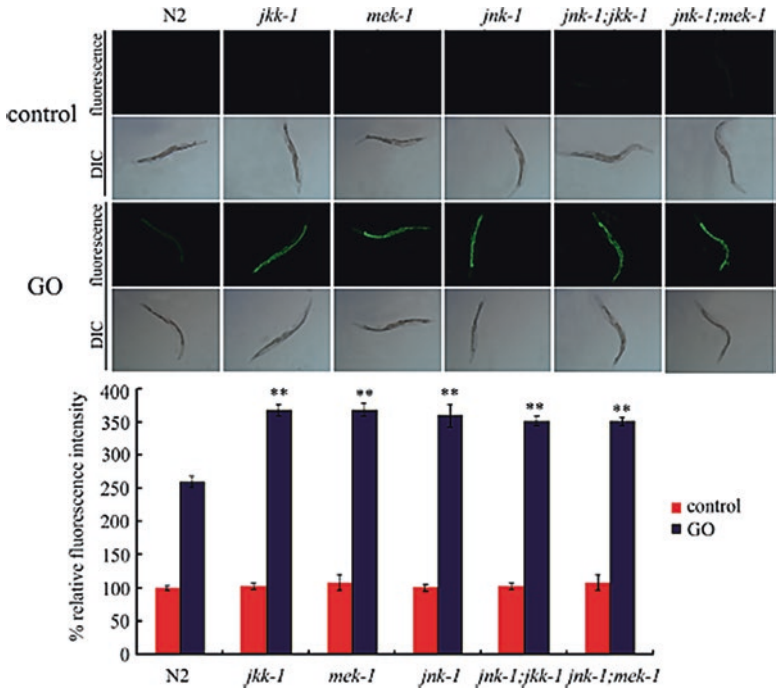

Fig. 4.14 Effects of mutations in the gene encoding JNK signaling pathways on intestinal ROS production in GO-exposed nematodes [43]. Bars represent the mean ± SEM $^{**}P < 0.01$ vs wild-type N2. GO (10 mg/L) was exposed from L1-larvae to adult day 1

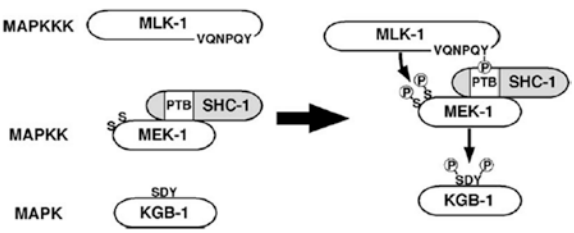

Fig. 4.15 Proposed model for SHC-1 function in the KGB-1 JNK signaling pathway [47]

4.4 ERK Signaling Pathway

4.4.1 Exposure to Environmental Toxicants or Stress Dysregulates the Expression of ERK MAPK Signal

In nematodes, the core ERK signaling pathway contains the *mek-2*-encoded MAPK kinase MEK and *mpk-1*-encoded ERK [48]. With GO as an example, although prolonged exposure to GO (1 mg/L) did not influence the expressions of *mpk-1* and *mek-1*, prolonged exposure to GO (10 or 100 mg/L) could significantly increase

both the transcriptional expression of *mpk-1* and the transcriptional expression of *mek-2* [49]. Therefore, exposure to environmental toxicants or stress can dysregulate the expression of ERK MAPK signal, and the increase in the expression of ERK signal may mediate a protection mechanism for nematodes in response to environmental toxicants or stresses, such as GO exposure.

4.4.2 *ERK MAPK Signaling Pathway Regulates the Toxicity of Environmental Toxicants or Stresses*

Using lifespan and intestinal ROS production as the toxicity assessment endpoints, mutation of *mpk-1* or *mek-2* resulted in the more severe reduction in lifespan and induction of intestinal ROS production in GO-exposed nematodes (Fig. 4.16) [49], suggesting that mutation of *mpk-1* or *mek-2* may induce a susceptibility to the toxicity of environmental toxicants or stresses.

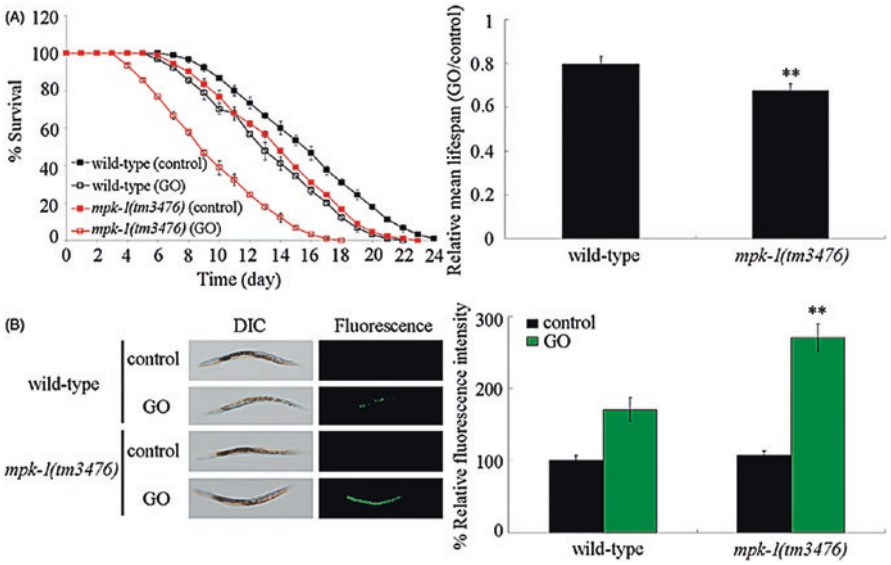

Fig. 4.16 Effects of *mpk-1* mutation on the GO toxicity [49]. (**a**) Effects of *mpk-1* mutation on the GO toxicity in reducing lifespan. (**b**) Effects of *mpk-1* mutation on the GO toxicity in inducing ROS production. Prolonged exposure was performed from L1-larvae to young adults. GO exposure concentration was 10 mg/L. Bars represent means ± SD. $^{**}p < 0.01$ vs wild type

4.4.3 *Signaling Cascade of ERK MAPK Signaling Pathway in the Regulation of Toxicity of Environmental Toxicants or Stresses*

Genetic interaction analysis demonstrated that mutation of *mpk-1* could significantly suppress the resistance of nematodes overexpressing the neuronal *mek-2* to GO toxicity in reducing lifespan and in inducing intestinal ROS production (Fig. 4.17) [49], suggesting that the MPK-1 acts downstream of the neuronal MEK-2 to regulate the toxicity of environmental toxicants or stresses, such as GO exposure.

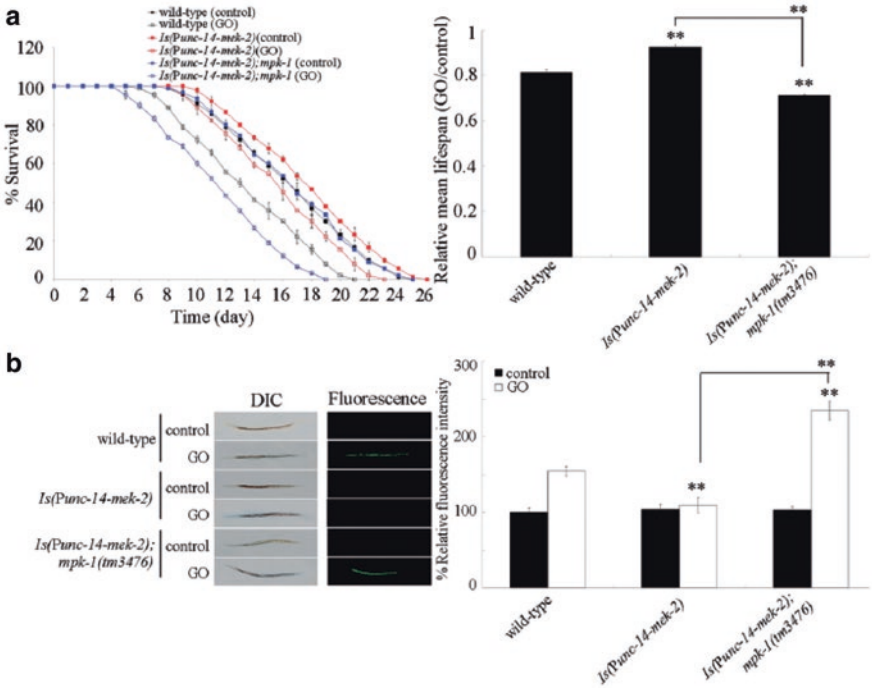

Fig. 4.17 Genetic interaction between MEK-2 and MPK-1 in the regulation of GO toxicity [49]. (**a**) Genetic interaction between MEK-2 and MPK-1 in the regulation of GO toxicity in reducing lifespan. (**b**) Genetic interaction between MEK-2 and MPK-1 in the regulation of GO toxicity in inducing ROS production. 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)

4.4.4 *Identification of Potential Downstream Targets for Neuronal MPK-1 in Regulating the Toxicity of Environmental Toxicants or Stresses*

In nematodes, MPK-1 is expressed in both the neurons and the germline. Expression of the *mpk-1* in the neurons could suppress the susceptibility of *mpk-1(tm3476)* mutant to GO toxicity in reducing lifespan and in inducing intestinal ROS production; however, germline-specific RNAi knockdown of *mpk-1* could not significantly induce a susceptibility to GO toxicity like the phenotypes observed in the *mpk-1(tm3476)* mutant [49]. Additionally, expression of the *mek-2* in the neurons could also significantly suppress the susceptibility of *mek-2(n1989)* mutant to GO toxicity in reducing lifespan and in inducing intestinal ROS production [49]. These results suggest that the neuronal signaling cascade of MEK-2-MPK-1 may play a crucial role in regulating the response of nematodes to GO exposure.

Moreover, it was found that mutation of *skn-1* could significantly suppress the resistance of nematodes overexpressing neuronal *mpk-1* to GO toxicity in reducing lifespan and in inducing intestinal ROS production (Fig. 4.18) [49], suggesting that the SKN-1 can act as a downstream target for neuronal MPK-1 to regulate the toxicity of environmental toxicants or stresses. In nematodes, *aex-3* encodes a guanine nucleotide exchange factor. It was further observed that mutation of *aex-3* could significantly suppress the resistance of nematodes overexpressing neuronal *mpk-1* to GO toxicity in reducing lifespan and in inducing intestinal ROS production (Fig. 4.18) [49], suggesting that the AEX-3 can act as another downstream target for neuronal MPK-1 to regulate the toxicity of environmental toxicants or stresses.

In nematodes, mutation of *aex-3* could further significantly suppress the resistance of nematodes overexpressing the neuronal *skn-1b* to GO toxicity in reducing lifespan and in inducing intestinal ROS production [49], suggesting the formation of neuronal signaling cascade of MEK-1-MPK-1-SKN-1b-AEX-3 in the regulation of toxicity of environmental toxicants or stresses in nematodes.

4.4.5 *Identification of Upstream Regulators for ERK MAPK Signaling Pathway in Regulating the Toxicity of Environmental Toxicants or Stresses*

In nematodes, *lin-45* encodes a Raf protein in ERK signaling pathway and acts upstream of the signaling cascade of MEK-1-MPK-1. It was found that mutation of *lin-45* induced a susceptibility to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior [50].

pkc-1 encodes a serine/threonine protein kinase C (PKC) protein. It was further observed that mutation of *lin-45* could noticeably inhibit the resistance of transgenic

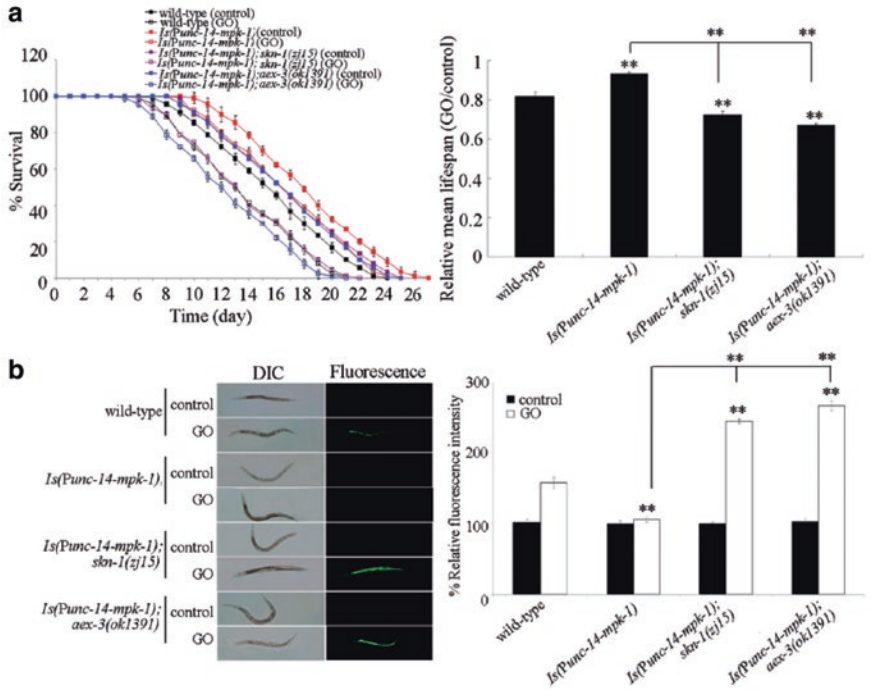

Fig. 4.18 Identification of potential downstream targets for neuronal MPK-1 in the regulation of response to GO exposure [49]. (**a**) Effect of *skn-1* or *aex-3* mutation on the resistance of nematodes overexpressing neuronal *mpk-1* to GO toxicity in reducing lifespan. (**b**) Effect of *skn-1* or *aex-3* mutation on the resistance of nematodes overexpressing neuronal *mpk-1* to GO toxicity in inducing ROS production. 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 wild type (if not specially indicated)

nematodes overexpressing neuronal PKC-1 to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior [50], suggesting LIN-45 acts downstream of neuronal PKC-1 to regulate the response to GO exposure.

In nematodes, *nlg-1* gene encodes a neuroligin, a postsynaptic cell adhesion protein. After GO (1 mg/L) exposure, mutation of *nlg-1* could significantly decrease the expression of *pkc-1* [50]. Moreover, mutation of *pkc-1* could obviously suppress the resistance of transgenic nematodes overexpressing neuronal NLG-1 to GO toxicity in inducing intestinal ROS production and in decreasing locomotion behavior [50], suggesting that PKC-1 can further act downstream of neuronal NLG-1 to regulate the response to GO exposure. Therefore, a signaling cascade of NLG-1-PKC-1-LIN-45 was identified to act upstream of ERK MAPK signaling pathway to regulate the toxicity of environmental toxicants or stresses in nematodes (Fig. 4.19) [50].

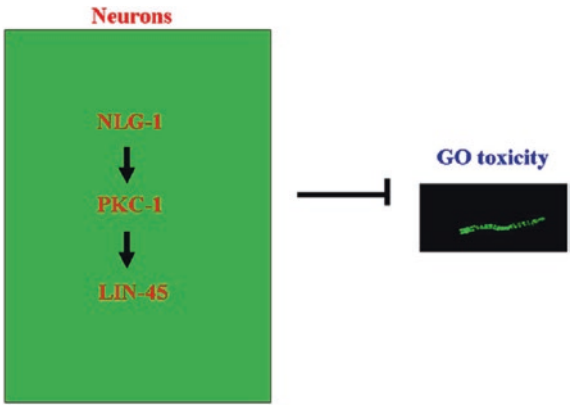

Fig. 4.19 A diagram showing the role of neuronal NLG-1-PKC-1-LIN-45 signaling cascade in the induction of GO toxicity in nematodes [50]

4.5 Perspectives

In this chapter, we introduced and discussed the involvement and contribution of three important MAPK signaling pathways (p38 MAPK, JNK MAPK, and ERK MAPK) in regulating the toxicity of environmental toxicants or stresses in nematodes. The introduced information here can largely reflect the crucial role of these three MAPK signaling pathways in the regulation of toxicity of environmental toxicants or stresses. So far, many more information for the involvement and the underlying molecular mechanisms of p38 MAPK signaling pathway in regulating the toxicity of environmental toxicants or stresses is available in nematodes. In contract to this, the available information for the involvement and the underlying molecular mechanisms of JNK MAPK or ERK MAPK signaling pathway in regulating the toxicity of environmental toxicants or stresses is relatively limited.

Moreover, there are at least two critical questions needed to be further clarified and elucidated. First of all, how these three MAPK signaling pathways transduce the extracellular cues into the cells through the intestinal barrier is still largely unknown. Additionally, the interactions among these three MAPK signaling pathways in different tissues to regulate the toxicity of environmental toxicants or stresses are still largely unclear. That is, what network may be formed among these three MAPK signaling pathways in the regulation of toxicity of environmental toxicants or stresses in nematodes?

References

1. Wang D-Y (2018) Nanotoxicology in *Caenorhabditis elegans*. Springer, Singapore
2. Matsukawa J, Matsuzawa A, Takeda K, Ichijo H (2004) The ASK1-MAP kinase cascades in mammalian stress response. J Biochem 136:261–265
3. Roux PP, Blenis J (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 68:320–344

4. Altman PL, Talbot JM (1987) Nutrition and metabolism in spaceflight. J Nutr 117:421–427
5. Smith SM, Zwart SR, Block G, Rice BL, Davis-Street JE (2005) The nutritional status of astronauts is altered after long-term space flight aboard the international Space Station. J Nutr 135:437–443
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. 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
8. Geim AK (2009) Graphene: status and prospects. Science 324:1530–1534
9. Bitounis D, Ali-Boucetta H, Hong BH, Min D, Kostarelos K (2013) Prospects and challenges of graphene in biomedical applications. Adv Mater 25:2258–2268
10. Yang K, Li Y, Tan X, Peng R, Liu Z (2013) Behavior and toxicity of graphene and its functionalized derivatives in biological systems. Small 9:1492–14503
11. 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
12. 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
13. 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
14. 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
15. 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
16. 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
17. 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
18. 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
19. Lim D, Roh JY, Eom HJ, Choi JY, Hyun J, Choi J (2012) Oxidative stress-related PMK-1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. Environ Toxicol Chem 31:585–592
20. Chatterjee N, Eom HJ, Choi J (2014) Effects of silver nanoparticles on oxidative DNA damage-repair as a function of p38 MAPK status: a comparative approach using human Jurkat T cells and the nematode *Caenorhabditis elegans*. Environ Mol Mutagen 55:122–133
21. 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
22. 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
23. 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
24. 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

25. Sun L-M, Liao K, Hong C-C, Wang D-Y (2017) Honokiol induces reactive oxygen species-mediated apoptosis in *Candida albicans* through mitochondrial dysfunction. PLoS ONE 12:e0172228
26. Sun L-M, Liao K, Wang D-Y (2017) Honokiol induces superoxide production by targeting mitochondrial respiratory chain complex I in *Candida albicans*. PLoS ONE 12:e0184003
27. Sun L-M, Liao K, Li Y-P, Zhao L, Liang S, Guo D, Hu J, Wang D-Y (2016) Synergy between PVP-coated silver nanoparticles and azole antifungal against drug-resistant *Candida albicans*. J Nanosci Nanotechnol 16:2325–2335
28. Sun L-M, Liao K, Liang S, Yu P-H, Wang D-Y (2015) Synergistic activity of magnolol with azoles and its possible antifungal mechanism against *Candida albicans*. J Appl Microbiol 118:826–838
29. Sun L-M, Zhi L-T, Shakoor S, Liao K, Wang D-Y (2016) microRNAs involved in the control of innate immunity in *Candida* infected *Caenorhabditis elegans*. Sci Rep 6:36036
30. 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
31. Blackwell TK, Steinbaugh MJ, Hourihan JM, Ewald CY (2015) SKN-1/Nrf, stress responses, and aging in *Caenorhabditis elegans*. Free Radic Biol Med 88:290–301
32. Papp D, Csermely P, Soti C (2012) A role for SKN-1/Nrf in pathogen resistance and immunosenescence in *Caenorhabditis elegans*. PLoS Pathog 8:e1002673
33. Yu Y-L, Sun L-M, 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
34. 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
35. 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
36. 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
37. Wu Q-L, Cao X-O, Yan D, Wang D-Y, Aballay A (2015) Genetic screen reveals link between maternal-effect sterile gene *mes-1* and *P. aeruginosa*-induced neurodegeneration in *C. elegans*. J Biol Chem 290:29231–29239
38. Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH (2006) p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. PLoS Genet 2:e183
39. van der Hoeven R, McCallum KC, Cruz MR, Garsin DA (2011) Ce-Duox1/BLI-3 generated reactive oxygen species trigger protective SKN-1 activity via p38 MAPK signaling during infection in *C. elegans*. PLoS Pathog 7:e1002453
40. Kim DH, Liberati NT, Mizuno T, Inoue H, Hisamoto N, Matsumoto K, Ausubel FM (2004) Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. Proc Natl Acad Sci USA 101:10990–10994
41. Chikka MR, Anbalagan C, Dvorak K, Dombeck K, Prahlad V (2016) The mitochondria-regulated immune pathway activated in the *C. elegans* intestine is neuroprotective. Cell Rep 16:2399–2414
42. Koga M, Zwaal R, Guan KL, Avery L, Ohshima Y (2000) A *Caenorhabditis elegans* MAP kinase kinase, MEK-1, is involved in stress responses. EMBO J 19:5148–5156
43. Zhao Y-L, Wu Q-L, Wang D-Y (2015) A microRNAs-mRNAs network involved in the control of graphene oxide toxicity in *Caenorhabditis elegans*. RSC Adv 5:92394–92405

44. Villanueva A, Lozano J, Morales A, Lin X, Deng X, Hengartner MO, Kolesnick RN (2001) *jkk-1* and *mek-1* regulate body movement coordination and response to heavy metals through *jnk-1* in *Caenorhabditis elegans*. EMBO J 20:5114–5128
45. Koga M, Zwaal R, Guan K, Avery L, Ohshima Y (2000) A *Caenorhabditis elegans* MAP kinase kinase, MEK-1, is involved in stress responses. EMBO J 19:5148–5156
46. Wolf M, Nunes F, Henkel A, Heinick A, Paul RJ (2018) 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
47. Mizuno T, Fujiki K, Sasakawa A, Hisamoto N, Matsumoto K (2008) Role of the *Caenorhabditis elegans* Shc adaptor protein in the c-Jun N-terminal kinase signaling pathway. Mol Cell Biol 28:7041–7049
48. Okuyama T, Inoue H, Ookuma S, Satoh T, Kano K, Honjoh S, Hisamoto N, Matsumoto K, Nishida E (2010) The ERK-MAPK pathway regulates longevity through SKN-1 and insulin-like signaling in *Caenorhabditis elegans*. J Biol Chem 285:30274–30281
49. 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
50. 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