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Short Review

Caenorhabditis elegans as a model for understanding ROS function in physiology and disease

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ABSTRACT

ROS (reactive oxygen species) are potentially damaging by-products of aerobic metabolism which, unchecked, can have detrimental effects on cell function. However, it is now widely accepted that, at physiological levels, certain ROS play important roles in cell signaling, acting as second messengers to regulate cell choices that contribute to the development, adaptation and survival of plants and animals. Despite important recent advances in the biochemical tools available to study redox-signaling, the molecular mechanisms underlying most of these responses remain poorly understood, particularly in multicellular organisms. As we will review here, *C. elegans* has emerged as a powerful animal model to elucidate these and other aspects of redox biology.

1. Introduction

With its many advantages as an experimental model, including transparency throughout its complete life cycle, easy maintenance of clonal populations, manipulation of genes, and neurons in the fully elucidated nervous system connectome [1], the nematode *Caenorhabditis elegans* has proven to be an ideal model to tackle biological questions in developmental and neurobiology [2–5]. Moreover, its short lifespan and easy manipulation of growth conditions has established *C. elegans* as a model for studying innate immunity [6] and aging [7]. In this context, *C. elegans* has been successfully used to recapitulate different human pathologies at the molecular level [8–10] and is rapidly consolidating as a model of choice in high-throughput approaches like drug screenings and therapeutic target identification [11]. Here we will focus on what we have learned about ROS functions in physiology and disease from *C. elegans* studies in which these, and additional tools specific to redox biology, have been applied and the prospects for further insight from *C. elegans* studies.

2. Physiological roles for ROS

Despite the increasing appreciation that endogenously generated ROS have important positive functions as signaling molecules and as weapons against pathogens, a lack of tools to measure or precisely manipulate *in vivo* ROS levels has made it difficult to discriminate between signaling and damaging responses, or investigate how low levels of ROS mediate responses which likely involve transient, reversible oxidation events. Therefore few examples of ROS-signaling mechanisms have been well worked out in terms of the downstream mechanisms. This is also the case in *C. elegans*. For instance, although recent work has shown that light leads to increases in endogenous H_2O_2 production that inhibits *C. elegans* feeding by a mechanism requiring the peroxiredoxin PRDX-2 and the gustatory receptors GUR-3 and LITE-1 in the I2 worm pharyngeal neuron, the precise details of the molecular mechanism are yet unclear [12]. Interestingly, low levels of H_2O_2 can also potentiate the function of the ASH neuron that mediates certain avoidance behaviours by promoting AKT-1-dependent phosphorylation of a sensory channel. Again, this role requires PRDX-2, as well as the activity of a conserved p38 MAP kinase pathway that is involved in many ROS responses, as is discussed in more detail below [13].

ROS have also demonstrated to function in reproduction. Major Sperm Proteins (MSP) are the most abundant proteins in *C. elegans* spermatozoa with both intracellular and extracellular functions [14]. Secreted MSPs bind to receptors in the surface of oocytes to induce their maturation via a signaling cascade that ultimately causes phos-

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phorylation of the MAPK, MPK-1. This process requires the phosphatase PTP-2 and ROS acting as a secondary messenger by a yet unknown mechanism that depends on Cu/Zn superoxide dismutase SOD-1 [15]. Interestingly, a member of the globin protein family termed GLB-12 has been recently found to work as a novel superoxide anion generator that coordinates with intra- and extracellular superoxide dismutases to regulate worm germline development [16]. Whether GLB-12 impinges on MPK-1 phosphorylation is currently unknown.

C. elegans cuticle, like mammalian skin, is a highly-structured extracellular matrix mainly composed of collagens (there are about 200 collagen-coding genes in *C. elegans*). The final step of collagen maturation involves tyrosine cross-linking within collagen polypeptides which confers the cuticle with its flexibility and strength [17]. H_2O_2 generated by the NADPH oxidase domain of the dual oxidase BLI-3 is used to generate the tyrosil radical that initiates the cross-linking of collagen moieties [18]. However, it is yet unclear whether the BLI-3 peroxidase domain is the functional peroxidase that uses H_2O_2 in the cross-linking mechanism as BLI-3 requires the formation of a complex with another peroxidase, MLT-7, along with the tretraspanin protein TSP-15 and the dual oxidase maturation factor DOXA-1 for efficient collagen cross-linking [19,20]. Furthermore, another hypodermal peroxidase SKPO-1 has also been suggested to participate in cuticle biosynthesis [21]. Interestingly, extensive disulphide cross-linking is also found within different components of the worm functional cuticle. In contrast to the tyrosine crosslinks, these disulphide bonds can be reversed. Indeed, reduction of these disulphides by either the thioredoxin or glutathione reductase systems is essential for efficient molting and larval survival [22]. Whether BLI-3 is also implicated in the disulphide cross-linking of cuticle components remains to be elucidated.

3. *In vivo* levels of ROS and changes in the redox environment

In trying to dissect the *in vivo* physiological roles of ROS, the development of redox sensors based on fluorescent probes has made a welcome addition to the tools available and started to provide important new insight in cell-based systems. The transparency of *C. elegans* along its embryonic and postembryonic stages offers an exceptional advantage for assessing the *in vivo* function of ROS in the context of a complete organism. Thus, expressing the H_2O_2 redox sensor HyPer under the control of an ubiquitous promoter, has demonstrated that H_2O_2 levels are higher during larval development but upon transition to reproductive stage peroxide levels considerably decrease, remaining low during the reproductive period and then increasing again as the animals age [23,24]. Interestingly, low levels of H_2O_2 during adulthood appear to correlate with long-lived worms while short-lived animals have high peroxide levels [24]. Remarkably, the Hyper probe has shown to be effective also at the single cell level as higher steady-state levels of H_2O_2 have been found in specific worm neurons, muscle cells or hypodermal syncytium [23]. On the other hand, the Grx1-roGFP1 and Grx1-roGFP2 probes have been successfully employed to determine the GSSG/2GSH ratio in worms, providing a way to determine *in vivo* the glutathione redox potential. Using these sensors, it has been found that the GSSG/2GSH ratio decreases during larval development [23] and that the glutathione redox potential varies among worm tissues [25]. Importantly, the use of the Grx1-roGFP1 sensor has challenged the prevalent view of glutathione being the major redox buffer in the cytosol, as the glutathione redox potential largely varies among individuals from an isogenic population. Instead, these new data indicate that the glutathione couple mediates the indirect effect of oxidants and reductants on the thiol-disulphide balance of cysteines in the proteome [25].

Despite these important advances, there remains room for improvements, with new generation redox sensors to overcome current limitations in the sensitivity of these probes and resolve problems with pH-dependency or interference from inherent *C. elegans* autofluorescence, particularly in the intestine [26].

4. Conserved ROS-protective systems are regulated by conserved ROS-activated signaling pathways and transcription factors

C. elegans express a host of ROS-detoxifying enzymes (Table 1). Moreover, the strong evolutionary pressure to increase ROS defences to deal with harmful increases in ROS and xenobiotic metabolites means that antioxidant response elements (ARE) are key regulatory promoter sequences driving increased expression of ROS defences and phase 2 detoxification systems from yeast to mammals (for reviews see [27–29]). In *C. elegans*, ARE-binding and ROS-induced increased expression of these genes, particularly in the intestine, is mediated by the SKN-1 transcription factor [30]. In addition, SKN-1 is also essential for mesendodermal specification [31]. Notably, SKN-1's ROS-protective function is shared by ARE-binding, AP-1-like transcription factors in unicellular fungi [27], suggesting that SKN-1's role in stress resistance pre-dates its role in embryogenesis [30]. Intriguingly, this implies that the mesendoderm may have originally evolved as a ROS-protective tissue, which makes sense as the intestine is generally the first tissue to encounter xenobiotics and pathogens. Indeed, intestinal ROS defences have been shown to afford important protection against exogenous stresses. For example, tissue-specific RNAi and transgenics approaches have demonstrated that expression of ROS-protective enzymes in the gut can be sufficient for *C. elegans* stress resistance [32,33]. Under non-stress conditions, SKN-1, like Nrf2, appears to be degraded by the ubiquitin-proteasome system [34] but upon oxidative stress, SKN-1 is activated via a conserved p38 MAP kinase signaling pathway involving sequential activation of NSY-1 (MAPKKK), SEK-1 (MAPKK) and PMK-1 (MAPK) that culminates in SKN-1 phosphorylation, nuclear accumulation and induction of stress protective genes [35]. Other transcriptional regulators are also important for ROS-induced gene expression and ROS-protective gene expression [36,37]. For example, the FOXO transcription factor DAF-16 is also important for ROS-protective gene expression. Although SKN-1 and DAF-16 have different target genes both are subject to similar regulatory mechanisms. For instance, DAF-16 activation also requires the stress-activated p38 MAPK pathway [38]. Moreover, importantly DAF-16 and SKN-1 are inhibited under normal growth conditions by phosphorylation on other residues by kinases in the insulin/insulin-like signaling pathway. This phosphorylation helps retain both transcription factors in the cytoplasm [39,40]. However, upon exposure to ROS producing chemicals or pathogens, this inhibition is relieved and DAF-16 and SKN-1 both translocate to the nucleus where they trigger a transcriptional program that includes the induction of several antioxidant and stress-protective enzymes [33,41,42]. Interestingly, induction of DAF-16 and SKN-1 transcriptional activity appears to be crucial not only to counteract harmful stresses but also for resistance to pathogenic infection and longevity [33,43,44]. Although it is still not well understood how ROS are sensed to lead to activation of SKN-1 and DAF-16, a recent report has shed some light on this process by identifying that ROS directly sulfenylate a cysteine residue within the IRE-1 kinase that, in turn, induces p38 MAP kinase-dependent activation of SKN-1 [45].

5. ROS in innate immunity and pathogen infection

Pathogen infection in mammals triggers the production of ROS, mainly by NADPH oxidases in macrophages, to kill the invading pathogen. Although *C. elegans* lacks specialised immune cells, nevertheless, in recent years, *C. elegans* has become established as a suitable model to study the molecular mechanisms involved in bacterial and fungal infections and counteracting innate immune host defence responses [46,47]. *C. elegans* produce ROS as a response to bacterial

Table 1
Expression and function of genes encoding ROS-producing or metabolizing enzymes as revealed by studies of loss of function mutants.

Gene	Function and cellular/subcellular localization	Mutant phenotype
sod-1	Superoxide anion to hydrogen peroxide. Ubiquitous/Cytoplasmic [93]	Accelerated wound healing [59] Increased or no effect on lifespan [66,67,93,94] Decreased resistance to oxidative stress-inducing agents [66,67,93,94]
sod-2	Superoxide anion to hydrogen peroxide. Mainly expressed in the head and tail regions/Mitochondrial [95]	Accelerated wound healing [59] Increased or no effect on lifespan [66,67,94,95] Decreased or no effect in resistance to oxidative stress-inducing agents [66,67,94,95]
sod-3	Superoxide anion to hydrogen peroxide Ubiquitous/Mitochondrial [95,96]	Accelerated wound healing [59] Increased or no effect on lifespan [66,67,94,95] Decreased, increased or no effect in resistance to oxidative stress-inducing agents [66,67,94,95]
sod-4	Superoxide anion to hydrogen peroxide Nervous system, intestine, somatic gonad/Extracellular and membrane bound [16,97,98]	Increased or no effect in resistance to oxidative stress-inducing agents [66,67,94]
sod-5	Superoxide anion to hydrogen peroxide. Amphid neurons/Cytoplasmic [66]	No phenotype reported
prdx-2	Hydrogen peroxide reduction to water Neurons, intestine and reproductive system/Cytoplasmic [32,98,99]	Decreased lifespan [32, 100, 101[a]] Increased resistance to arsenite and cadmium but increased sensitivity to hydrogen peroxide [32] Defective feeding response to light [12] Reduced insulin secretion [87] Defect in H2O2-induced potentiation of avoidance behaviour [13] Reduced fecundity and small size [32,99] Impaired PMK-1 activation by arsenite and metformin [32,77]
prdx-3	Hydrogen peroxide reduction to water Tissue distribution not determined/Putative mitochondrial	Decreased or no effect on lifespan (RNAi) [69,101][a] Paraquat sensitive (RNAi) [101][a] Reduced fitness (RNAi) [69][a]
prdx-6	Hydrogen peroxide reduction to water Tissue distribution not determined/Putative cytoplasmic	No phenotype reported
ctl-1	Hydrogen peroxide reduction to water Tissue distribution not determined/Cytoplasmic [102]	Desiccation sensitive [103]
ctl-2	Hydrogen peroxide reduction to water Tissue distribution not determined/Peroxisomal [102]	Decreased lifespan [68] Reduced egg-laying capacity [68]
ctl-3	Hydrogen peroxide reduction to water Pharynx and neurons/Subcellular localization not determined [68]	No phenotype reported
bli-3	Hydrogen peroxide production and reduction Hypodermis, intestine and pharynx/Subcellular localization not determined [18,104]	Increased pathogen susceptibility [104] Defective collagen cross-linking (RNAi) [18,20][a] Blistered and molting defects [20]
mlt-7	Hydrogen peroxide reduction Hypodermis/Subcellular localization not determined [20]	Defective collagen cross-linking (RNAi) [20][a] Larval arrest (associated to molting defects) and dumpy appearance [20]
skpo-1	Hydrogen peroxide reduction Hypodermis/Subcellular localization not determined [21]	Increased pathogen susceptibility [21] Decreased lifespan [21] Dumpy [21]
glb-12	Superoxide anion production Somatic reproductive system, vulva, head and tail neurons/Plasma membrane [16]	Reduced brood size and embryonic lethality [16] Abnormal germline development [16]

and fungal pathogens [33,48–51]. Although the essential role of the single *C. elegans* NADPH-oxidase family member, BLI-3, in cuticle formation makes it difficult to carry out conclusive experiments using mutants or chemical inhibitors, these ROS seem to be produced by this dual oxidase, independently of whether the route of infection is via epidermis or intestine [49–52]. Indeed, BLI-3 is also required for the enhanced generation of ROS mediated by increases in proline catabolism in *P. aeruginosa*-infected animals [51]. Interestingly, the host response elicited by *C. elegans* to avoid the deleterious effects of ROS appears to be pathogen-specific via induction of genes encoding ROS detoxifying enzymes, mainly regulated by either SKN-1 and/or DAF-16 transcription factors [42,48]. Thus, ROS-dependent activation of SKN-1 upon infection of the intestinal pathogens *P. aeruginosa* or *E. faecalis* is signalled via the conserved p38 MAP kinase signaling pathway (described above) [35,48] with the Toll/IL-1 receptor domain protein TIR-1 acting upstream in this pathway [53]. Similarly, in response to *E. faecalis* but not *P. aeruginosa* infection, DAF-16 is also activated in the intestine by BLI-3 generated ROS, triggering the expression of general ROS-detoxification enzymes such as catalases and superoxide dismutases [33]. Remarkably, the SKPO-1 peroxidase appears to partner BLI-3 in the hypodermis in the protection against *E. faecalis* [21]. As the transcriptional targets of SKN-1 and DAF-16 are not the same, a coordinated response involving both SKN-1 and DAF-16, and most likely other transcriptional regulators [36,54,55], is required to counteract the “friendly fire” associated with increased ROS produced by *C. elegans* to kill invading intestinal pathogens. In any case, the molecular mechanism by which ROS induce the SKN-1 and/or DAF-16 dependent anti-pathogenic responses in the intestine

remains elusive. Interestingly, DAF-16 has also been shown to mediate protection against infection by epidermal pathogens like the fungus *Drechmeria coniospora* or *Clonostachys rosea* [42]. In this case, infection triggers epidermal Ca^{2+} release that induces BLI-3 dependent ROS production that subsequently mediate DAF-16 activation in a cell autonomous manner [42]. In contrast to intestinal pathogens, no involvement of SKN-1-dependent responses to epidermal pathogen infection have so far been reported.

6. ROS in epidermal function and wound healing

All metazoa have evolved sophisticated mechanisms to counteract the damage generated by mechanical injury or wounding and ROS play an important role in this process. For instance, wounding in zebrafish generates an H_2O_2 gradient by the locally injured epidermal cells that attracts neutrophils to the wound site for healing [56]. ROS generated at the site of injury regulate signaling pathways also controls the reorganization dynamics of different cytoskeletal components required to closure the wound [57]. In worms, a functional cuticle and epidermis provides a first line of defence against environmental insults like external pathogens, mechanical damage or toxicants. Similar to cuticle-penetrating pathogens (see above), epidermal wounding by a needle or laser in *C. elegans* triggers a sustained rise in intracellular Ca^{2+} that eventually results in the local recruitment of F-actin filaments at the wound site to initiate the healing process that culminates within 2–4 h after wounding [58]. Remarkably, the worm model has provided a molecular mechanism for ROS involvement in the healing process; the rise in cytoplasmic Ca^{2+} provoking its uptake by mitochondria, hence causing an increase in ROS production that promotes wound repair by inhibiting RHO-1 GTPase which negatively regulates wound closure. This inhibition appears to require a specific cysteine residue within a conserved redox-sensitive motif present in Rho family GTPases [59]. As Rho GTPases are key regulators of actin dynamics and have been extensively implicated in wound repair [60], it is likely that a similar mechanism may also occur in higher eukaryotes.

7. ROS in aging

The oxidative stress theory of aging proposes that aging is a consequence of the progressive accumulation of oxidative damage caused by ROS generated during the lifetime of any organism [61]. While this was the prevalent view for many decades, seminal work in *C. elegans* challenged this notion as worm mutants with impaired mitochondrial respiration, like *clk-1* [62] or *isp-1* [63] were shown to have increased lifespan, despite increased ROS levels [64]. Indeed, diverse studies on genes encoding ROS-detoxifying enzymes have failed to conclusively demonstrate a direct link between ROS and ageing (Table 1) (reviewed in [65]). For instance, mutant worms lacking each of the five superoxide dismutase genes have normal or increased lifespan, depending on the studies performed [66,67]. Moreover, *C. elegans* lacking the cytosolic catalase *ctl-1* have normal lifespan, while loss of the peroxisomal catalase *ctl-2* or cytoplasmic peroxiredoxin *prdx-2* causes a progeric phenotype [32,68,100,101]. However, at least in the case of PRDX-2, this appears to be separate from any protection against ROS toxicity [32]. Exposure to antioxidant mimetics such as superoxide dismutase and catalase mimetics have not consistently increased *C. elegans* lifespan [70,71]. Overexpression of superoxide dismutase does increase lifespan but again, at least in the case of *sod-1*, this appears to be due to upregulation of other stress defences rather than a ROS-protective effect [66,72]. Furthermore lifespan extensions are also obtained when worms are exposed to ROS-generating chemicals such as paraquat or juglone at sublethal concentrations [64,73,74]. Together, these data support the notion that while increases in ROS-induced oxidative damage are associated with ageing, ROS do not initiate the ageing process and may instead modulate specific signaling pathways to promote longevity. Most of the candidate pathways for mediating these lifespan-extending effects of ROS are conserved in mammals, encouraging further work to elucidate the underlying mechanisms in *C. elegans*.

Many of the different genetic, physiological or pharmacological interventions that increase lifespan, including hypoxia, impaired insulin signaling, alteration of mitochondrial metabolism, germline removal, dietary restriction (DR) and metformin treatment cause increases in ROS production, mostly generated in mitochondria [75–78]. The DAF-16 and SKN-1 transcription factors [40], are required for the lifespan-extending effects of some of these treatments but the specific mechanisms by which ROS increase their activity are still being clarified. Some may be quite direct, for example, it is known that ROS generate a disulphide bond between transportin-1 and DAF-16 that is required for the later nuclear translocation and transcriptional activation resulting in longevity extension [79]. Furthermore, the recently uncovered sulfenylation of IRE-1 (see above) is another potential mechanism by which ROS may contribute to increases in longevity by promoting activation of SKN-1 by the p38 MAP kinase pathway [40,45].

In considering the roles of physiological levels of ROS in governing longevity (and other traits commented above), it is important to remember that *C. elegans* natural habitat contains much lower levels of oxygen than the ambient lab conditions under which most experiments are conducted. Indeed, *C. elegans* have developed a sophisticated oxygen-sensing system that regulates their behaviour to optimise the levels of oxygen to which they are exposed. Although this system is defective in the 'domesticated' lab N2 strain [80], analysis of this behaviour in natural isolates has revealed that *C. elegans* prefer 5–12% pO_2 over 21% ambient pO_2 [81]. Transient hypoxia (0.5% pO_2) has however been shown to increase longevity by inducing mitochondrial ROS that regulate the Ras-related small G protein RHEB-1 and TORC1 (Target of Rapamycin Complex) activating the intestinal GATA transcription factor ELT-2 and hence the expression of the omega-class glutathione S-transferase GSTO-1 [82]. Mitochondrial ROS have also been shown to promote longevity by activating the hypoxia-inducible factor 1, HIF-1, which, like its mammalian counterpart, is also stabilised under hypoxic conditions [83]. Interestingly, neuronal HIF-1 has recently been shown to promote longevity in a cell non-autonomous way by increasing the intestinal expression of a flavin monoxygenase that is also required for DR-induced increases in longevity [84]. Mitochondrial ROS also mediate the extended lifespan of worm mutants deficient in mitochondrial metabolism, but not other types of long-lived mutants, by specifically activating a mitochondrial-associated intrinsic apoptotic pathway [76]. Interestingly, rather than activating apoptosis, that depends on the BH3-only EGL-1 protein, mitochondrial ROS generated by these mutants induce a specific transcriptional program that leads to increased resistance and longevity. Although the underlying mechanism has not been yet clarified, it has been shown that this alternative pathway requires the BH3-only CED-13 protein [76]. Another intervention that increases *C. elegans* lifespan is the removal of its germline, which requires DAF-16 and the cytoskeletal adaptor protein KRI-1 [78]. Recent work demonstrates that loss of germ cells induces a KRI-1 dependent (but DAF-16 independent) production of ROS and H_2S, which are necessary for germline-deficient worms to increase their lifespan. Interestingly, the two redox species signal through different pathways, with ROS inducing an activation of the mitochondrial UPR response while H_2S activating the transsulfuration pathway which, in turn, promotes lifespan by activating SKN-1 transcription factor [78].

The antiglycemic drug metformin, commonly prescribed as treatment for type II diabetes mellitus, has been shown to have prolongevity effects in several organisms, including worms [72,77,85,86]. Mechanisms involving increased ROS and SKN-1 activity have been proposed, as well as those involving effects on the bacterial food source [72,77,85]. Although PRDX-2 is important for the effects of metformin and impaired insulin-signaling on lifespan, the elevated SKN-1 and DAF-16 activity and increased stress resistance of *C. elegans prdx-2*

mutants suggests that this reflects a so far uncharacterised role of PRDX-2 in mediating increases in longevity, rather than its role in mediating activation of PMK-1 by arsenite or metformin [32,77,87]. However, SKN-1 has been directly implicated in the lifespan extension caused by dietary restriction [88], in this case mediated by transient ROS signaling in ASI neurons [89]. *C. elegans* are also affected by feeding on different bacterial sources [90], with the L-proline catabolism gene *alh-6* and SKN-1 required to deal with potentially toxic increases in proline catabolism in animals fed B type *E. coli* strains, as they are in animals infected with *P. aeruginosa* [51,90,91]. Intriguingly, impairment of insulin signaling also causes transient ROS increases by promoting L-proline catabolism in mitochondria [75]. Thus activation of SKN-1 and DAF-16 may well be a common response to nutritional status and food quality cues.

The overall conclusion emerging from these studies seems to be that physiological levels of ROS have a primary role as signaling molecule extending *C. elegans* longevity and that, only when a certain threshold is breached, do ROS became detrimental, causing cellular damage that results in shorter lifespan.

8. Concluding remarks

Although this minireview is not comprehensive, as indicated by the studies above, *C. elegans* has already provided important insight into the roles of ROS in normal physiology but also in ageing and disease. The unique capacity to manipulate the diet will continue to allow *C. elegans* to provide a vital model for dissecting the influence of the microbiome on these processes. Genome-wide RNAi screens have already uncovered new genes and pathways influencing ROS responses [105-107]. Next generation sequencing has re-invigorated mutagenesis screens as powerful tools to identify both gain and loss of function mutants affecting these responses [92]. Genome-editing technology, tissue-specific RNAi and transgenics will enable further elucidation of how ROS signals are transduced and, importantly, facilitate the dissection of cell autonomous and non-autonomous functions of ROS signaling pathways. We anticipate that *C. elegans*, which provided the first completed animal genome sequence and neural connectome will go on to provide the first complete animal model of redox signal transduction.

Note added in proof

During the proof correction process, an article by Ewald et al. (C.Y. Ewald, J.M. Hourihan, M.S. Bland, C. Obieglo, I. Katic , L. E. Moronetti Mazzeo, J. Alcedo, T.K. Blackwell, N.E. Hynes, NADPH oxidase-mediated redox signaling promotes oxidative stress resistance and longevity through memo-1 in *C. elegans*, eLIFE 6 (2017) e19493) has reported that ROS produced by the BLI-3/NADPH oxidase promote stress resistance and longevity via SKN-1.

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