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Neuronal responses to stress and injury in *C. elegans*

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Abstract

The nervous system plays critical roles in the stress response. Animals can survive and function under harsh conditions, and resist and recover from injuries because neurons perceive and respond to various stressors through specific regulatory mechanisms. *Caenorhabditis elegans* has served as an excellent model to discover fundamental mechanisms underlying the neuronal response to stress. The basic physiological processes that *C. elegans* exhibits under stress conditions are similar to those observed in higher organisms. Many molecular pathways activated by environmental and cellular stresses are also conserved. In this review, we summarize major findings in examining neuronal responses to hypoxia, oxidative stress, osmotic stress, and traumatic injury. These studies from *C. elegans* have provided novel insights into our understanding of neuronal responses to stress at the molecular, cellular, and circuit levels.

Keywords

Stress response; Hypoxia; Oxidative stress; Osmotic stress; Traumatic injury; Axon regeneration; Neurodegeneration

1. Introduction

Neurons possess compartmentalized domains and establish cellular networks to detect stress from the environment and internal damage to the organism. Neurons process information and trigger protective responses to restore and maintain homeostasis of a cell and an organism. Inevitably, neurons are susceptible to damage caused by stress. For example, neurons require high amounts of oxygen to meet metabolic demands; low levels of oxygen (hypoxia) cause physiological damage to neurons (hypoxic injury), while high oxygen consumption results in excess production of reactive oxygen species (ROS), leading to oxidative injury to neurons. Furthermore, long processes such as axons and dendrites are at risk for localized injuries. A wide variety of stressors can cause neuronal dysfunction that

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may progress to irreversible damage and cell death. On the other hand, the susceptibility of neurons to stress is beneficial by allowing animals to respond rapidly to avoid or escape from harmful conditions (fast response). By minimizing the exposure to deleterious conditions, animals can be protected from irreversible damage or death. When the stress is chronic or unavoidable, neurons also play important roles in the adaptation of organisms to stress (slow response). Therefore, it is important to understand (1) how neurons perceive stress from the external environment or internal damage; (2) how neurons are affected by various stresses; (3) how neurons activate cellular pathways specific to the type of stress; and (4) how neuronal signaling helps organisms to establish responses for repair and survival.

Here, we review the recent studies of neuronal response from the model organism *C. elegans.* The adult hermaphrodite animal has 959 somatic cells, with defined anatomy and developmental lineage. The nervous system is composed of 302 neurons and the complete synaptic connectivity has been mapped at the ultrastructural level [1]. Despite its compact nervous system, *C. elegans* is capable of many complex behaviors, in addition to those displayed under standard culture conditions such as locomotion, foraging, feeding, egg-laying, and defecation [2]. Many sensory neurons have cilia with endings exposed to the external environment to facilitate the detection of environmental cues. The cilia express sensory receptors, predominantly a class of G protein-coupled receptors (GPCRs), and convert environmental stimuli into receptor potentials to stimulate release of neurotransmitters that trigger fast or neuromodulatory actions [3]. *C. elegans* can detect various environmental cues such as oxygen levels, temperature, and osmolarity, and display stereotyped responses by moving toward or away from the source of stimulation. Furthermore, *C. elegans* is greatly amenable to genetic dissection of the molecular and cellular mechanisms. Large-scale genetic screens that target stress resistant or sensitive animals have led to the identification of novel stress regulators. Importantly, over 60% of *C. elegans* genes have apparent human orthologs, and investigations of numerous genes also reveal their functional conservation in fundamental regulatory pathways [4].

Due to space limitation, here, we focus on the major findings in the area of hypoxia, oxidative stress, osmotic stress, and traumatic injury, with an emphasis on those relevant to neuronal responses. Readers are also recommended to consult several excellent reviews that cover other relevant topics, such as the neuro-immune communications [5, 6], and trans-cellular communication mechanisms in response to heat shock and unfolded protein responses (UPR) for the endoplasmic reticulum (ER) and mitochondria [7, 8].

2. Hypoxic stress

As an aerobic animal, *C. elegans* requires oxygen to live and has a preference for intermediate levels of oxygen (5–12%; ambient oxygen, 21%), avoiding both low (<4%) and high (>12%) levels of oxygen [9]. Such preference for lower oxygen concentrations could be linked with its natural habitat with food source of actively growing bacteria that consume oxygen more quickly, thereby creating a low oxygen environment [3]. Oxygen sensing is primarily mediated by the sensory neurons known as AQR, PQR, and URX (Fig. 1A). These neurons express the soluble guanylyl cyclase GCY-35 that binds directly to molecular

oxygen [9], and contribute to the avoidance of high levels of oxygen (hyperoxia). Activation of these neurons triggers a rapid response in fast neurotransmission and prompts animals to move away from high or low oxygen environment [10].

C. elegans neurons also respond to chronic hypoxic stress. To study chronic hypoxic stress response under laboratory conditions, *C. elegans* are placed in hypoxia chamber filled with low oxygen gas (0.1–1%) for a variable number of hours, and then are allowed to recover in ambient oxygen (21%). About half of adult animals die after 12 hours of hypoxia followed by a 24-hour recovery, and all die if exposed for 24 hours of hypoxia [11]. The damage to the organism under this hypoxic condition can be exacerbated if an adaptive response to hypoxia fails. Hypoxia activates a conserved pathway with the central regulator hypoxia inducible factor 1 (HIF-1) transcription factor [12], and HIF-1 is required for adaptation to hypoxia for worm to survive [13] (Fig. 1B). HIF-1 protein is maintained at low level in normoxia (21% oxygen), but strongly induced by hypoxia within 4 hours [12, 13], with maximum HIF-1 induction by 0.5% or lower oxygen concentration [12]. The induced HIF-1 protein persists over 24 hours, but disappears within minutes following reoxygenation [12]. By contrast, *hif-1* mRNA levels are not altered by hypoxia, indicating a post-transcriptional mechanism [13].

How is HIF-1 protein induced by hypoxia? Hypoxia is tightly linked to a high level of hydrogen sulfide (H_2S), which activates CYSL-1 cysteine synthase. CYSL-1 directly binds to EGL-9 dioxygenase and prevents EGL-9 from inhibiting and degrading HIF-1 [12]. In normoxia, CYSL-1 is inhibited or inactive, thus EGL-9 destabilizes and inactivates HIF-1. Importantly, CYSL-1 is predominantly expressed in the nervous system and drives activation of neuronal HIF-1 to modulate oxygen-dependent behavior [14]. Remarkably, this pathway is completely conserved in mammals. Mutations in the human homologs of HIF-1 (HIF-1α and HIF-2α) and EGL-9 (PHD2/EGLN1) have been found in populations living at high altitude with low oxygen levels [15]. Such genetic changes lead to increased HIF activity, allowing adaptation to the chronic hypoxia of high altitude. Thus, the molecular mechanism of HIF regulation and its role in oxygen homeostasis reflect evolutionary conservation for adaptation.

The neuronal response to hypoxia also triggers a dedicated circuit that contributes to the behavior modification of the organism (Fig. 2). Hypoxia activated HIF-1 induces serotonin expression through its transcriptional activity [16]. In normoxia, serotonin is robustly expressed in the NSM and ADF neurons, but is not normally expressed in the ASG sensory neurons. Upon chronic hypoxia at 1% oxygen, serotonin expression is strongly induced in ASG neurons, in addition to increased expression in the NSM and ADF neurons. While under normal culture conditions, ADF and ASG sensory neurons have a minor role in food sensing, and the gustatory cues are primarily detected by the ASE neurons [17]. Interestingly, the increased expression of serotonin in the ASG and ADF neurons enhances animal's gustatory behavior under hypoxia. This enhanced sensory detection is mediated by a GPCR SER-7 expressed in a M4 motor neuron in pharynx. This pharyngeal motor neuron, which normally functions to control pharyngeal muscle contraction, now relays the sensory information via an FMRFamide-related neuropeptide FLP-21 to AQR, PQR, and URX oxygen sensing neurons. This hypoxia-induced circuit has no detectable role under

normoxia. Such behavioral plasticity may help animals to escape from noxious conditions. In vertebrates, chronic intermediate hypoxia (10% oxygen for 14 days) also induces changes of serotonin activity in the brain, which is potentially linked to adaptive responses [18].

Chronic hypoxic conditions also cause severe defects in the developing *C. elegans* nervous system. In embryos and larvae cultured under chronic hypoxic conditions (1% oxygen), the axons of several motor neurons and interneurons aberrantly cross the ventral midline and the migratory path of the HSN motor neurons are compromised [19]. The neurodevelopmental defects under hypoxia is caused by HIF-1-dependent misregulation of specific axon guidance signaling pathway. However, it remains to be addressed whether altered neuronal outgrowth has a beneficial or detrimental role for long-term adaptation or survival in hypoxic condition.

Hypoxic conditions also affect the adult nervous system of *C. elegans*. Chronic hypoxia induces neuronal and myocyte injury, which leads to necrosis and contributes to the death of the organism [20]. The neuronal pathology of hypoxic injury includes axonal beading, tortuous axon, and large axonal gaps in mechanosensory neurons. These pathological features are similar to those found in traumatic and ischemic neuronal pathology in mammalian models [21, 22]. Therefore, further elucidation of the hypoxic injury mechanisms in *C. elegans* neurons can provide insights into how neurons can be protected or recover from hypoxic injury.

3. Oxidative stress

While oxygen is imperative for life, excess consumption can also pose significant risks. Reduction of oxygen to water by the mitochondrial electron transport chain reaction is necessary for energy production. However, this reaction also generates potentially harmful byproducts of oxygen, reactive oxygen species (ROS), which cause oxidative modifications to many cellular components such as lipids, proteins, and DNA. Cells normally have the ability to detoxify ROS or to repair damage caused by ROS. However, when ROS is over-produced or when the antioxidant defense system is damaged, oxidative stress occurs, leading to cellular dysfunction [23]. The nervous system is especially vulnerable to oxidative damage, in part because neurons have high demands for oxygen consumption and several neurotransmitters can be autoxidized [24].

C. elegans has been extensively used to understand the underlying mechanisms of oxidative stress in aging and neurodegeneration. Direct effects of ROS are often examined by administration of redox cycling compounds such as the herbicide paraquat, which generate ROS (superoxide) *in vivo*. When young adult worms are exposed to 100 mM paraquat, death occurs within 1–60 hours [25]. A key pathway responding to such treatment involves the SKN-1 protein, an Nrf family of transcriptional factor.

The Nrf family of transcription factors is a basic leucine zipper protein and plays a conserved role in response to oxidative stress. Both vertebrate NRF-2 and *C. elegans* SKN-1 are required for oxidative stress resistance [25–27]. *C. elegans* lacking SKN-1 are sensitive to oxidative stress and have shortened lifespan [25], while animals carrying constitutively activated SKN-1(gf) show defects in neuromuscular function [28]. There are three SKN-1

protein isoforms, SKN-1a, SKN-1b, and SKN-1c, which differ in their N-termini and display a differential expression pattern. SKN-1a appears to be expressed in the motor neurons; SKN-1b is in the ASI sensory neurons; and SKN-1c is in the intestine [25, 29, 30]. Many early studies revealed a role for intestinal SKN-1c in oxidative response. Under normal conditions, SKN-1c is present at very low levels in the intestine because of protein degradation involving a WD40 repeat protein WDR-23. In response to oxidative stress, SKN-1c accumulates in intestinal nuclei within 5 minutes [25]. This rapid posttranscriptional regulation of SKN-1 expression is under the control of a p38 mitogen-activated protein kinase (MAPK) pathway consisting of SEK-1/MAPKK and PMK-1/p38 MAPK [31]. Oxidative stress activates SEK-1 and PMK-1, which act to phosphorylate SKN-1, which in turn facilitates the nuclear localization of SKN-1 and inhibits protein degradation of SKN-1 by WDR-23 [32] (Fig. 1B). Animals lacking either SEK-1 or PMK-1 are sensitive to oxidative stress, whereas animals lacking WDR-23 are resistant, both of which effects are entirely mediated by SKN-1 [31, 32]. A recent study revealed a link between this intestinal SKN-1 pathway and neurons. Animals lacking WDR-23 display locomotion defects and are aldicarb resistant, suggesting a defective presynaptic function with decreased neurotransmission [28]. Both defects are abolished by eliminating *skn-1*, indicating that the neuronal effect of WDR-23 is mediated by SKN-1. Of note, the aldicarb resistance in *wdr-23* mutants is fully rescued by the expression of WDR-23 in the intestine but not in neurons, suggesting a non-autonomous role for WDR-23. Therefore, it is tempting to speculate that the WDR-23-SKN-1 pathway may likely induce diffusible endocrine signaling from intestine to the nervous system to reduce neurotransmission release.

SKN-1 also displays a cell-autonomous role in neurons in response to oxidative stress (Fig. 2). The Neuroligin family of synaptic cell adhesion molecules plays conserved roles in the development and function of synapses and are linked to autism in humans [33]. In *C. elegans*, a neuroligin homolog NLG-1 mediates a retrograde synaptic signal that inhibits neurotransmitter release at neuromuscular junctions (NMJs) [34]. The synaptic abundance of NLG-1 increases during oxidative stress due to transcriptional activation by SKN-1, and mutants lacking NLG-1 do not display SKN-1-mediated stress resistance [29, 35]. Together, this suggests that SKN-1 protects neurons or organisms from oxidative damage by reducing neurotransmission in two distinct ways: (1) intestinal SKN-1 may promote a cell non-autonomous endocrine signaling, and (2) neuronal SKN-1 regulates synaptic NLG-1 abundance.

How can the reduction of neurotransmission protect animals from oxidative damage? Most energy consumption in the nervous system occurs during synaptic transmission [36]. Reducing neurotransmission may help to relieve animals of this high energy demand, which in turn reduces ROS production and attenuates oxidative stress. Accumulating evidence in humans reveals oxidative stress impacts individuals with autism disorder [37]. The unexpected link between SKN-1 and NLG-1 implies a possible molecular correlation between oxidative stress and autism. Gaining further insights into the molecular and cellular regulation in autism may help find a new effective treatment for autism.

Oxidative stress is also thought to contribute to the development and progress of neurodegenerative diseases such as Parkinson’s disease, which is characterized by

progressive motor impairment attributed to progressive loss of dopaminergic neurons. Mutation in PINK1 is associated with early onset of familial Parkinson's disease [38], and PINK1 has been shown to protect against cell death induced by oxidative stress [39, 40]. In *C. elegans*, loss of the PINK1 homolog, PINK-1, results in oxidative stress sensitivity and neurite outgrowth defects [41, 42]. These data support cellular conservation in response to oxidative stress in neurodegeneration.

The majority of Parkinson's disease cases are sporadic (non-familial) and the disease can also be caused by environmental factors, such as pesticides, herbicides, metals, and pathogens. Indeed, there is a link between Parkinson's disease and the use of herbicides such as paraquat [43]. In *C. elegans*, exposure to paraquat causes dopaminergic neurodegeneration and mitochondrial DNA damage [44]. In addition, exposure of *C. elegans* to heavy metals such as manganese increases oxidative stress, leading to dopaminergic neuronal death [45]. Pathogens can also cause oxidative stress and dopaminergic neurodegeneration [46].

Oxidative stress can be used as an excellent tool to kill specific cells or to damage specific compartments of cell. In *C. elegans*, intracellular ROS generators, such as miniSOG and KillerRed, which generates ROS upon illumination of light, have been applied to kill a variety of neurons in freely moving animals [47, 48]. Using spatially restricted illumination, localized KillerRed activation in the axon triggers neuronal degeneration. In addition, targeting KillerRed to mitochondria results in organelle fragmentation without killing the cell [48].

4. Osmotic stress

Salt and water homeostasis is essential for all cellular life. Both hyper- and hypoosmotic environments can be harmful to the survival of an animal, thus all organisms, including *C. elegans*, have mechanisms to respond to osmotic environments. Osmotic avoidance (Osm) in worms has long been studied in the genetic dissection of sensory neuron development and function (Fig. 1A). Briefly, Osm behavior is quantified as the ratio of worms that cross a ring of high concentration NaCl (4 M) on solid medium within 30 minutes [49]. A sensory neurons, ASH, is essential for sensing high osmolarity, and another, ASE, for low osmolarity [17]. Genetic screening has generated many mutants defective in sensing high osmolarity [49–51]. Unlike wild-type worms, Osm mutants fail to avoid high osmolarity conditions [49] and many of the mutants cause defective development or function of ASH neurons [50, 51].

The second class of mutants isolated is osmotic stress resistant (Osr). Osr behavior is scored in two types of assays: in the acute motility assay, animals are observed in hypertonic medium (200–700 mM NaCl) over 10 minutes; in the chronic adaptation assay, the viability of animals is scored after a number of hours of hypertonic incubation and 24 hour recovery [52]. Wild-type animals exposed to hyperosmotic conditions rapidly lose body volume, motility, and gradually viability, whereas Osr mutants show no noticeable changes under similar conditions [52, 53]. *C. elegans* adapts to hyperosmotic stress by accumulating glycerol, and high osmolarity induces expression of glycerol biosynthetic enzymes, *gpdh-1*

and *gpdh-2* [54, 55]. Osr mutants have elevated basal levels of glycerol, which prevents acute water loss in high osmolarity environments [52, 53] (Fig. 1B). Hyperosmotic stress disrupts proteostasis and causes protein aggregation and misfolding [56, 57], this accumulation of damaged proteins functions as a signal to activate osmoprotective gene expression [55]. However, optimal survival in hyperosmotic conditions requires degradation of damaged proteins as well as translational inhibition [57].

Under acute osmotic shock, *C. elegans* engages a behavioral quiescent program, and the animals cease locomotion and feeding [58]. Interestingly, upon removal from osmotic shock, animals become even more quiescent such that both locomotion feeding remain suppressed for up to 1 hour after exposure (Fig. 2). This stress-induced quiescent state shares similar properties of sleep and is dependent on the sleep-inducing interneuron ALA [59]. The survival of animals defective for this quiescent behavior is impaired, demonstrating the benefit of stress-induced behavioral quiescence [58]. Intriguingly, sleep-like quiescence during molting cycle is impaired in animals lacking a conserved Notch signaling pathway that includes three Notch ligands, OSM-7, OSM-11 and LAG-2, and the LIN-12 Notch receptor [60]. In particular, OSM-11 regulates normal osmosensation and osmotic resistance, and its secretion appears to be diminished by osmotic stress. Taken together, one possible mechanism of adaptation to osmotic stress from environments or developmental changes is the sleep-like response, and indeed, some of the molecular regulators are involved in both stress response and sleep-like behavior [53, 60].

5. Traumatic injury

Traumatic injury often causes irreversible damage and debilitation. In the past decade, studies from *C. elegans* have made important contributions to the understanding of the cellular and molecular response to traumatic neuronal injury. Here, we will categorize the main findings in three types of injuries: neurotoxic stress, neuronal cytoskeletal stress, and neurite injury.

5-1. Neurotoxic stress

Traumatic injury to the brain or spinal cord can cause neurons to die, often as a result of a phenomenon known as excitotoxicity. Excitotoxicity, or the excessive stimulation by neurotransmitters such as glutamate, causes high levels of calcium to enter the cell and induces neuronal necrosis. Even if the primary mechanical insult may be localized or restricted to a small region, secondary neuronal death is often observed, partially due to excitotoxicity from overactivation of neurotransmitter receptors triggered by primary injury [61]. In humans, the secondary injury occurs within hours or days following the primary traumatic injury, and is reported to be a major contributing factor in progressive brain damage and death [62]. Therapeutic efforts have primarily concentrated on preventing or mitigating secondary injury. It will therefore be of significant medical relevance to understand the mechanisms of neuronal death by excitotoxicity.

In *C. elegans*, necrotic death of neurons due to excitotoxicity has long been studied either using genetic mutants with hyperactivated ion channels, such as *mec-4(d)*, or applying extrinsic stress such as extreme heat, osmotic or hypoxic shock on animals. *mec-4(d)*

mutants contain missense mutations in a sodium channel protein required for light touch sensation in the mechanosensory neurons [63]. To understand the molecular mechanisms underlying necrotic cell death, many genetic suppressors of *mec-4(d)*-induced necrosis have been identified (Fig. 3). Analyses of these suppressor mutations reveal a major role of conserved genes in controlling intracellular calcium levels [64]. Downregulation of genes involved in ER regulation of intracellular calcium blocks ER calcium release and suppresses necrotic neuronal death. Such genes include a calcium binding and ER storage protein CRT-1/calreticulin, an ER calcium binding chaperone CNX-1/calnexin; the inositol triphosphate receptor ITR-1 and the ryanodine receptor UNC-68, both of which release calcium from the ER [64].

In addition, regulators of endocytosis and intracellular trafficking have been identified to promote neuronal necrosis. Downregulation of a set of genes that function in synaptic exocytosis and endocytosis as well as synaptic vesicle trafficking, such as SNT-1/synaptotagmin, UNC-11/clathrin-adaptor AP180, UNC-26/synaptojanin, UNC-57/endophilin, UNC-104/kinesin-3, and UNC-116/kinesin-1, significantly suppresses neuronal death induced in *mec-4(d)* [65]. These genes may contribute to loss of plasma membrane integrity during necrosis.

Necrosis triggered by neurotoxic stress can cause secondary cell death. In *C. elegans*, hypoxic injury to a small group of neurons can induce widespread delayed secondary cell death and eventual organismal death [66]. One study tested the effects of hypoxia on a hypoxia-resistant *rars-1* mutant strain that only expresses the wild-type copy of *rars-1* in GABAergic motor neurons. *rars-1* encodes arginyl-tRNA synthetase. 24 hours post hypoxic exposure, all wild-type animals die, while the *rars-1* transgenic animals survive and exhibit normal behavior. However, these transgenic animals gradually die at 96 hours. Since animals lacking GABAergic neurons live and reproduce normally [67], this result suggests that hypoxia causes delayed cell-non-autonomous secondary injuries, which eventually kills the animal. The induction of this cell-non-autonomous injury does not appear to involve cell-cell contact [66]. Therefore, it will be of interest to identify the factors promoting secondary injury, which will provide insights into the discovery of potential therapeutic targets.

5-2. Cytoskeletal stress and disruption

Neurons have elaborate cytoskeletons to maintain their morphology and function, and hence are particularly susceptible to injury from cytoskeletal stress. Cytoskeletal disruption triggers a cascade of events including mitochondrial dysfunction and oxidative stress [68]. Emerging evidence suggests that mutations contributing to neurological diseases are associated with dysfunction of the cytoskeleton, which influences intracellular trafficking and acts as a scaffold for signaling molecules. In *C. elegans*, two major cytoskeletal stress/injury models are (1) the pharmacological and genetic perturbation of microtubules and (2) spectrin mutants (Fig. 3).

5-2-1 Microtubule stress—Microtubules are formed by the polymerization of α/β-tubulin heterodimers. In *C. elegans*, the axons of mechanosensory neurons are filled with

large-diameter microtubules made up of MEC-12 α-tubulin and MEC-7 β-tubulin. Microtubules can be disrupted by treatment with colchicine [69], a microtubule destabilizing drug that binds and sequesters tubulin to inhibit microtubule polymerization. High-dose colchicine effectively inhibits cell division, and inhibits neurite outgrowth in vertebrate neurons [70]. *C. elegans* cultured in 1 mM colchicine can live and reproduce, but show loss of touch sensitivity, resembling tubulin loss-of-function mutants [69, 71]. Recent studies show that colchicine-triggered microtubule stress causes two main defects in mechanosensory neurons, a loss of acetylated tubulin and a general reduction in protein expression [69, 72]. Interestingly, a forward genetic screen for mutants resistant to colchicine-induced protein reduction identified the DLK-1 MAPK cascade [72]. While the mechanisms of how the DLK-1 MAPK cascade is involved in this phenomenon are unknown, these observations support a role of DLK-1 as a sensor for microtubule stress. Furthermore, microtubule disruption triggers neuronal remodeling, such as synaptic branch retraction, through DLK-1 activation by a microtubule-associated RhoGEF RHGF-1 [73]. As described below, DLK-1 is also essential for axon regeneration after neurite injury.

5-2-2. Spectrin disruption—The spectrin cytoskeleton is composed of alpha and beta dimers, which form tetramers and stabilize the structure of the plasma membrane. Animals lacking β-spectrin/UNC-70 are severely impaired in development and growth, and their neurons display spontaneous axonal breakage, due to mechanical stress caused by movement [74]. Interestingly, the broken axons reform new growth cones and attempt to regenerate continuously throughout development and adulthood. This form of regeneration also requires DLK-1 and its downstream p38 MAPK. Upon loss of *dlk-1* in *unc-70* mutants, axons break but fail to regenerate [75, 76]. The spontaneous regenerating feature of *unc-70* formed the basis for a large-scale RNAi screen, which identified multiple signaling pathways involved in regeneration [75, 77] (see below for details).

5-3. Laser-assisted neurite injury

Laser-assisted neurite injury has recently become a powerful method to directly injure axons or dendrites of *C. elegans* neurons, and this technique has established *C. elegans* as a valuable model to study axon regeneration [78, 79]. Briefly, transgenic animals fluorescently marking single neurons are subjected to laser axotomy; then, the severed neurons are observed under a confocal microscope and the regrowing axons are analyzed. This laser axotomy technique enables *in vivo* live imaging at single axon resolution and genetic screens for factors involved in traumatic injury response in various types of neurons.

Laser severing of *C. elegans* axons causes severe axonal damage and induces rapid morphological responses including: (1) robust and error-prone axon regrowth from the proximal axon stump, (2) formation of new neurites from the soma, and (3) reconnection of the proximal axon by fusion with its distal axon fragment [80]. A single neuron can exhibit a combination of responses, and all three types of neuronal responses contribute to the restoration of the functional deficits caused by injury. For example, axon injury of motor neurons causes immediate locomotor defects, and axon regrowth correlates with recovery of locomotor functions [78].

What kind of insults do neurons sense from laser-induced injury? (see Fig. 3) Several studies have shown a rapid calcium influx following axon severing, which propagates bi-directionally from the injury site [81, 82]. Although increased calcium can result in neuronal cell death in some cases as discussed above, it can promote regeneration in surviving neurons [83]. Analyses using genetic and pharmacological manipulations show that the level of intracellular calcium correlates with the extent of axon regrowth [81]. Increased intracellular calcium levels are suggested to stimulate multiple signaling events including elevating the cAMP levels and activating signal transduction pathways.

Another rapid response after axonal injury involves the microtubule cytoskeleton. During the first few hours after laser surgery, the proximal axon stump begins to form short filopodial protrusions, which become a growth cone-like structure, following which the severed axon starts to extend [79]. Axonal cytoskeleton is required for the reformation and extension of growth cones. In a mature uninjured axon, the microtubule cytoskeleton is maintained in a stabilized state. However, traumatic axonal injury inevitably disrupts cytoskeletal structure and membrane integrity, and stable microtubules are converted into a dynamically growing state capable of supporting axon regrowth [84]. Growing microtubules can be tracked with GFP-tagged microtubule plus-end binding protein (EBP). Using *in vivo* live imaging of EBP-GFP, axon injury has been shown to result in increased numbers of growing microtubules at the injured axon tip and in the subsequent regrowing axon [84]. Numerous regulators controlling microtubule dynamics have been identified to play critical roles in *C. elegans* axon regeneration, including DLK-1, EBP-1, microtubule minus-end binding protein PTRN-1/patronin, KLP-7/kinesin-13, and tubulin posttranslational modifiers [84–87]. The overall findings are consistent with studies in mammals where microtubule stabilizing drugs such as paclitaxel (Taxol) and epothilone B have been shown to promote axon regeneration and improve motor function after spinal cord injury in mice [88–90].

What kinds of pathways respond to laser-induced injury? One of the first conserved signaling pathways identified in *C. elegans* as critical for axon regeneration is p38 MAPK cascade [75, 91]. DLK-1 is a MAPKKK that acts upstream in a kinase cascade consisting of MKK-4/MAPKK and PMK-3/p38 MAPK. The major downstream effector in this kinase cascade is a transcription factor CEBP-1/CCAAT-enhancer binding protein [91]. Another JNK MAPK cascade consisting of MLK-1/MAPKKK, MEK-1/MAPKK, and KGB-1/JNK kinase, with transcription factor FOS-1/c-Fos as the possible downstream effector, also acts in parallel to the DLK-1 cascade [76, 77]. These two kinase pathways cross-talk and are required to different degrees for axon regeneration in different types of neurons. While most regulators appear to act cell-autonomously to promote axon regrowth, evidence suggests that axon regeneration in *C. elegans* can be regulated cell-non-autonomously by growth factor signaling. A growth factor SVH-1 is secreted from a pair of ADL sensory neurons in the head and acts on its receptor SVH-2 in motor neurons and mechanosensory neurons to promote axon regrowth [92]. Although it is unknown whether SVH-1 expression and secretion are induced by injury, SVH-2 levels increase upon injury. Much of the regulation of axon regeneration has recently been discussed in an excellent review [93] and is not discussed further here.

In most laser axotomy paradigms, injury to axons does not lead to neuronal death, even when neurites fail to regenerate. Furthermore, the requirements for neuronal cell death also promote neuronal regeneration, which might appear contradictory (Fig. 4). Calcium surge is required for neurotoxicity, but on the other hand, it is considered to be beneficial for neuronal regeneration after injury. For example, the downregulation of CRT-1/calreticulin or of UNC-68/ryanodine receptor prevents neurons from dying [64], but also prevents certain neurons from regenerating [82]. The differential contributions of calcium to neuronal death and survival might reflect the nature or extent of injury. Neurotoxic stress may globally impact the neuronal cell body along with neuronal processes, while the precise injury occurs locally at axons; thus, the increased levels of calcium will not be comparable. Because the volume of the ER, the main intracellular calcium storage organelle, must be bigger in the soma compared to axons, the increased calcium levels must also be higher in neuronal cells in conditions of neurotoxic stress as compared to precise injury. In either type of neuronal injury, the initial calcium influx comes from extracellular calcium sources by plasma membrane depolarization. This initial calcium entry may provoke calcium-dependent calcium release from the ER, and this intracellular calcium surge contributes to either cell death or regeneration in injured neurons. If the levels of calcium are too high, neurons may turn on a death signal and undergo necrotic cell death; however, if the levels of calcium are not high enough to induce a death signal, they may instead turn on a pro-regenerative signal to promote neuronal regeneration and eventually restore their function. One result supporting this speculation is that axon injury close to the soma can often cause cell death [80]. This type of axon injury also tends to trigger new neurite outgrowth from the soma. Injury close to the soma may have a bigger effect, possibly via high levels of calcium, thus causing either new neurites from the soma or even death.

6. Conclusions and perspectives

Despite having a small number of cells, simple neural circuits, and short lifespan, *C. elegans* exhibit tremendous capacity to respond to stress and generate adaptive and protective mechanisms to defend against environmental threats and cellular damage. The awesome power of genetic analyses, together with modern tools in molecular, cellular and behavioral dissection, has enabled researchers to make pioneering discoveries of the genes and signaling pathways that are evolutionarily conserved in stress response. In closing, several principles emerge from these studies. First, cells respond to different forms of stress by inducing specific molecular pathways. Second, activation of these pathways leads to selective modifications of existing neural circuits or the engagement of latent ones. Third, inter-tissue interactions enable systemic responses and facilitate spreading of protective actions. Fourth, the behavioral outcome of the organismal or cellular responses to particular insults can be stereotypic or stochastic. While much is known about the developmental lineage and anatomical connections of *C. elegans*, our understanding of such cellular and behavioral plasticity remains in its infancy. The rapidly advancing technical tools and analytic methodologies will greatly aid researchers' ability to observe and dissect the nature of stress as well as intrinsic properties of the cell or network that respond to stress.

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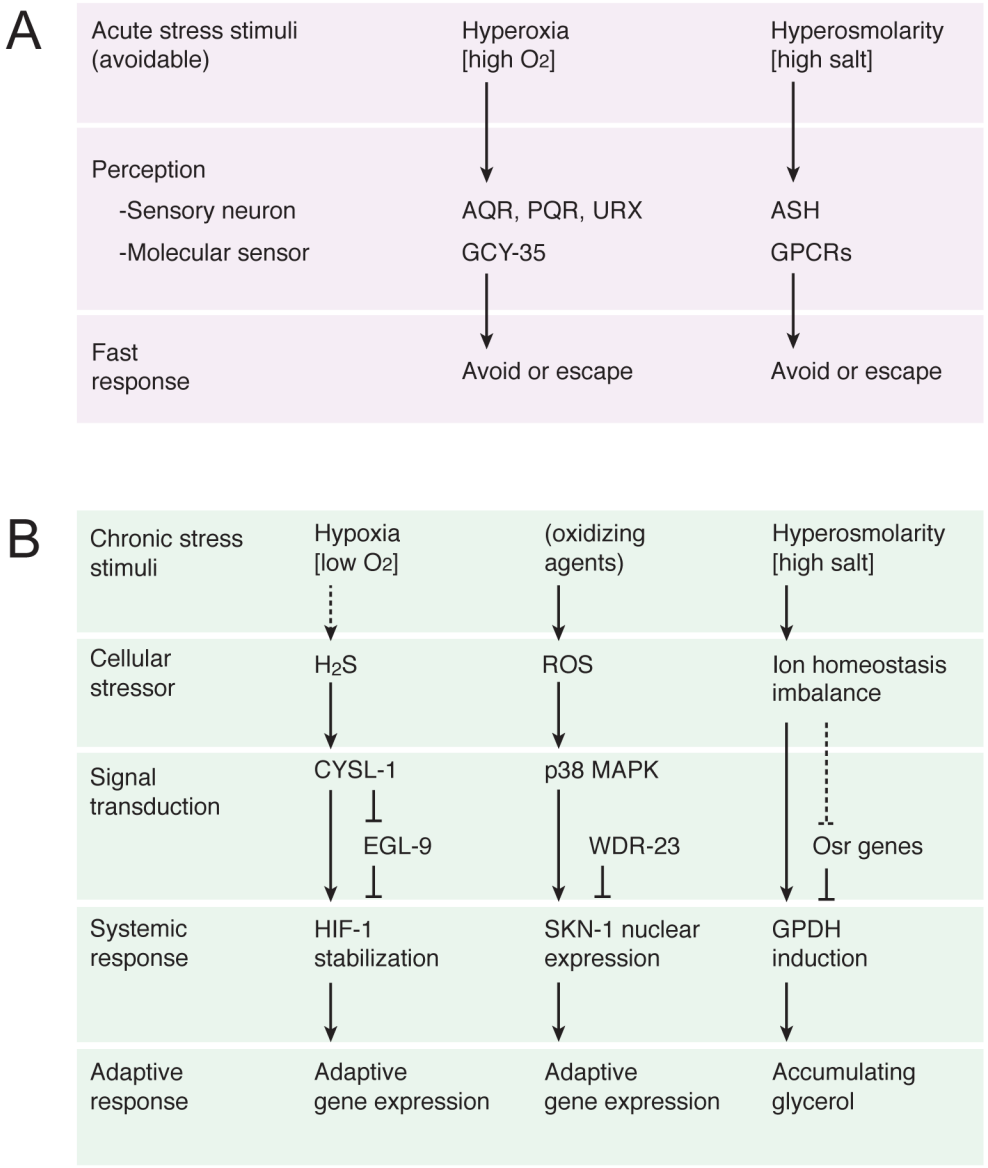

Fig. 1. *C. elegans* stress responses to acute or chronic stress stimuli

(A) Acute stress stimuli trigger fast response via the nervous system. Sensory neurons perceive environmental cues such as oxygen levels or osmolarity via their molecular sensors and prompt a behavioral response, such as moving away from the harmful environment. (B) Chronic stress stimuli trigger slow adaptive response. Accumulating cellular stressors induce specific molecular pathways that enable systemic response.

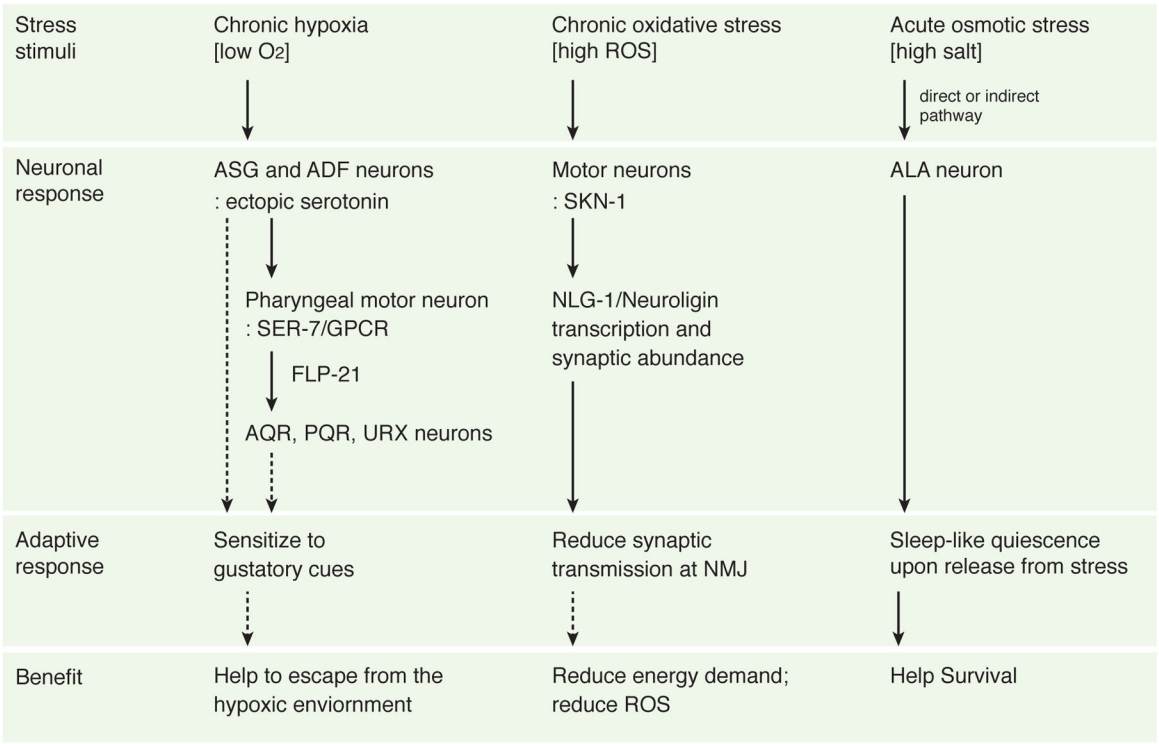

Fig. 2. *C. elegans* stress responses in the nervous system

Chronic hypoxia engages latent neural circuits and makes animals more sensitive to gustatory cues; chronic oxidative stress modulates neuronal gene expression (SKN-1 and NLG-1) to reduce synaptic transmission at NMJs. Upon removal from osmotic shock, *C. elegans* engages in a sleep-like quiescent behavior which is beneficial for survival.

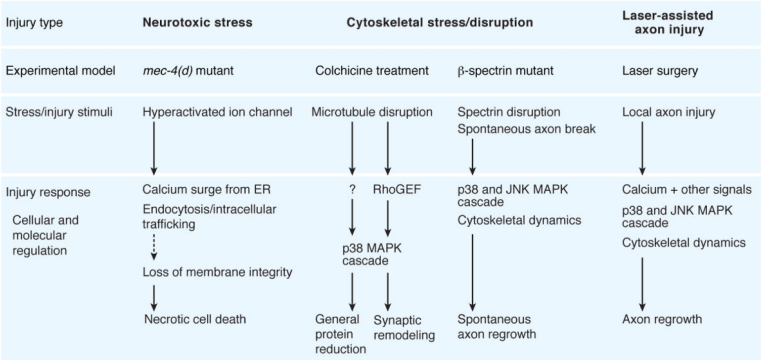

Fig. 3. Cellular and molecular responses to traumatic neuronal injury in *C. elegans*

In *C. elegans*, three types of neuronal injury models have been studied, including neurotoxic stress using *mec-4(d)* mutants, neuronal cytoskeletal stress/disruption using colchicine treatment or β-spectrin mutants, and laser-assisted axon injury.

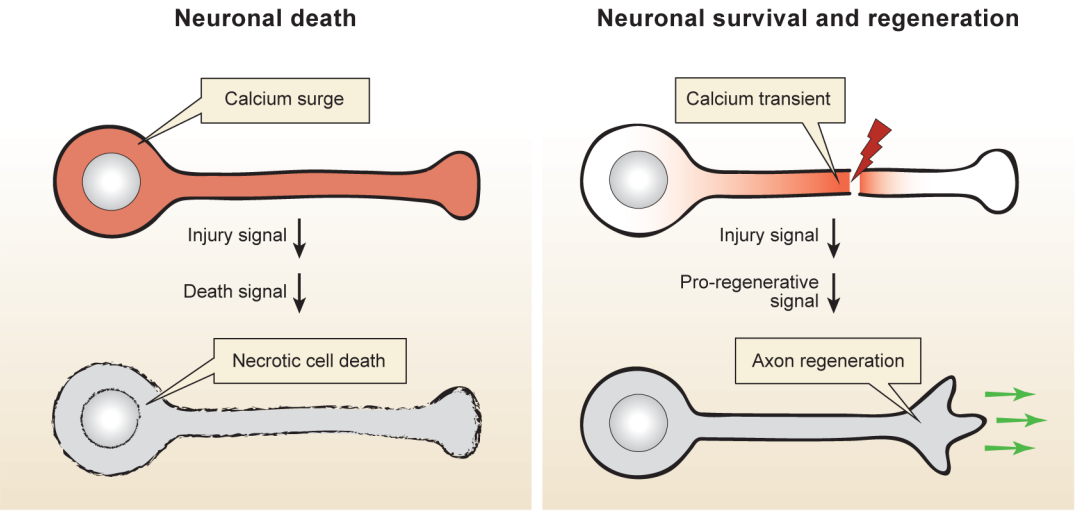

Fig. 4. Opposing neuronal responses to different types of injury

Injury at or near the soma of the neuron triggers a high calcium surge from the ER, which leads to neuronal death. Local neurite injury also triggers a calcium transient and this calcium signal spreads to the soma to promote neuronal regeneration. It is speculated that the increased levels of calcium or the duration of calcium stimulation might determine neuronal death or survival.