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Context Specificity of Stress-activated Mitogen-activated Protein (MAP) Kinase Signaling: The Story as Told by *Caenorhabditis elegans*[*]

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Stress-associated p38 and JNK mitogen-activated protein (MAP) kinase signaling cascades trigger specific cellular responses and are involved in multiple disease states. At the root of MAP kinase signaling complexity is the differential use of common components on a context-specific basis. The roundworm *Caenorhabditis elegans* was developed as a system to study genes required for development and nervous system function. The powerful genetics of *C. elegans* in combination with molecular and cellular dissections has led to a greater understanding of how p38 and JNK signaling affects many biological processes under normal and stress conditions. This review focuses on the studies revealing context specificity of different stress-activated MAPK components in *C. elegans*.

Sydney Brenner chose the roundworm *Caenorhabditis elegans* as a model biological system to address questions regarding development and neurobiology (1). Because of its stereotyped development, short life cycle, transparency, ease of genetic manipulation, and conservation of genes with higher mammals, *C. elegans* provides many advantages to characterize the function of genes. Studies over the past four decades using *C. elegans* have revealed fundamentally conserved principles underlying many biological processes (2–4). For example, isolation and characterization of uncoordinated mutants or drug-resistant mutants led to the discovery of genes and mechanisms functioning in the nervous system (1, 5). The evolution and intricacies of genetic screens in *C. elegans* have been reviewed in Ref. 6. Here we review the approaches used to uncover the *in vivo* roles of stress-activated MAP[3] kinases and discuss how these findings have advanced our understanding of stress responses in select cellular processes.

Stress-activated MAP Kinase Homologs in *C. elegans*

MAPKs act as central signaling hubs, transducing extracellular cues to control cellular processes such as proliferation, differentiation, apoptosis, and migration (7). Due to their multifaceted roles and importance in many cellular processes, their malfunctioning is associated with a wide array of human diseases, such as atherosclerosis and tumorigenesis (8, 9). MAPKs can be classified into three families; the ERK, p38, and JNK classes, originally defined based on kinase activity, target specificity and sequence homology. Early studies on p38 and JNK, mainly in mammalian cell lines, showed that they are predominantly activated by adverse stimuli such as osmotic stress, heat shock, or cytokines (7). This notion triggered a misnomer in the term "stress-activated MAP kinase." *In vivo* evidence in genetically tractable models did not support a solely stress-dependent role for JNK and p38, as null mutants generally resulted in severe developmental defects (10–13). It is now widely known that JNK and p38 regulate both normal and stress-associated signaling (7).

MAPK cascades consist of a sequential activation of kinase substrates(MAPKKK/MAPKK/MAPK)culminatinginthephosphorylation and activation of the terminal MAPK. The *C. elegans* genome encodes four JNK MAPK homologs, JNK-1 (67% identity to JNK1), KGB-1 (48%), KGB-2 (51%), and C49C3.10 (31%), which share a conserved TPY activation motif, and three p38 MAPK homologs, PMK-1 (64% identity to MAPK14), PMK-2 (56%), and PMK-3 (42%), which share the TGY or TQY activation motif (Fig. 1). The TQY motif is unique to nematodes and several plant species (14, 15). *C. elegans* homologs of stress MAPKKs include four groups, those similar to MKK7 (*mek-1*, *jkk-1*), MKK4 (*mkk-4*, *sek-3*, *sek-6*), MKK3/6 (*sek-1*), and MKK3 (*sek-4*, *sek-5*). Multiple homologs of the stress MAPKKK family are also found, including DLK/LZK (*dlk-1*), ASK1 (*nsy-1*), TAK1 (*mom-4*, *Y105C5A.24*), TAO (*kin-18*), MLK (*mlk-1*), and ZAK (*zak-1*) (16). The presence of p38 and JNK orthologs in *C. elegans* offers the opportunity to exploit the power of genetics to characterize these putative stress pathways *in vivo*. A general theme in stress-activated MAPK signaling is their context specificity, which is accomplished through integrating different components to activate distinct cellular responses (Figs. 2 and 3). The remarkable level of context specificity is somewhat paradoxical considering the broad expression pattern of the majority of p38 and JNK components (Fig. 1). Interestingly, the initial studies of stress-activated MAPK signaling in *C. elegans* came from observations of developmental and non-stress-related phenotypes (16).

A Not-so-stressful Beginning to Functional Studies of p38 and JNK in *C. elegans*

Locomotion and Feeding

The identification of *C. elegans* homologs of the JNK signaling pathway was born out of the need for an *in vivo* model to

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[3] The abbreviations used are: MAP, mitogen-activated protein; GLH, germline RNA helicase; IF, intermittent fasting; ROS, reactive oxygen species.

MAPKKK			MAPKK			MAPK		
C. elegans Gene Name (Chr)	Tissue Expression Pattern (Ref)	Orthologous Kinase in Mammals	*C. elegans* Gene Name (Chr)	Tissue Expression Pattern (Ref)	Orthologous Kinase in Mammals	*C. elegans* Gene Name (Chr)	Tissue Expression Pattern (Ref)	Orthologous Kinase in Mammals
dlk-1 (I)	Neurons, Pharynx (28)	*DLK, LZK*	*mkk-4 (X)*	Broad (28)	*MKK4*	*pmk-1 (IV)**	Broad (32)	*p38*
mlk-1 (V)	Broad (99)	*MLK1-4*	*sek-1 (X)*	Broad (31)	*MKK3/6*	*pmk-2 (IV)**	Neuronal (32)	*p38*
nsy-1 (II)	Broad (27)	*ASK1*	*mek-1 (X)*	Pharynx, intestine (21)	*MKK7*	*pmk-3 (IV)**	Broad (28)	*p38*
mom-4 (I)	ND	*TAK1*	*jkk-1 (X)*	Broad (19)	*MKK7*	*kgb-1 (IV)*	Broad (84)	*JNK*
Y105C5A.24 (IV)	ND	*TAK1*	*sek-3 (X)*	ND	*MKK4*	*kgb-2 (IV)*	ND	*JNK*
zak-1 (III)	ND	*ZAK*	*sek-4 (X)*	ND	*MKK3*	*jnk-1 (IV)*	Neuronal (19)	*JNK*
kin-18 (III)	Pharynx, Intestine (100)	*TAO*	*sek-5 (X)*	ND	*MKK3*	*C493C.10 (IV)*	ND	*JNK*
			sek-6 (X)	ND	*MKK4*			

FIGURE 1. **The core p38 and JNK signaling cassettes from worm to human.** The core MAPK signaling cassette consists of a MAPKKK/MAPKK/MAPK cascade. Expression patterns were obtained from published *in vivo* translational or transcriptional reporters. *Broad expression* is defined as reporters that display activity in three or more tissue types. Human orthologs are classified as defined in Ref. 98. *Chr* denotes the chromosome where the gene is located. *ND* denotes tissue expression that has not been described. *Ref* denotes the publication that published the reporter analysis (19, 21, 27, 28, 31, 32, 84, 99, 100). * denotes genes that exist in an operon.

Phenotype	Locomotion	Feeding	P-Granule Formation	Neuronal Differentiation	Synapse Development	Avoidance Behavior
Cell Type	Motor Neurons	Pharynx	Germ Cells	Olfactory Neurons	Motor Neurons	Sensory Neurons
Upstream Components	?	?	?	TIR-1, UNC-43	RPM-1	TOL-1
MAPKKK	?	?	?	NSY-1	DLK-1	MOM-4
MAPKK	JKK-1	MEK-1/SEK-1	?	SEK-1	MKK-4	MKK-4
MAPK	JNK-1	?	KGB-1	PMK-1/PMK-2	PMK-3	PMK-3
Downstream Components	?	?	?	?	MAK-2, CEBP-1	?

FIGURE 2. **Stress-activated MAPK components involved in non-stress-associated processes.** Redundancy in MAPK components is illustrated as MAPK/MAPK, whereas parallel pathways are denoted as MAPK, MAPK.

study the complexities of MAPK pathways. From an evolutionary perspective, the JNK and p38 signaling pathways are thought to have been derived from a duplication event in the ancestral yeast hyper-osmolarity Hog1p kinase pathway and *hog1* mutants are defective in osmotic sensing (17, 18). A *C. elegans* cDNA library was expressed in yeast *hog1* mutants with the reasoning that conserved components of the p38/JNK pathways would rescue osmo-regulatory defects (19). This strategy led to the identification of *jnk-1* and *jkk-1* as JNK and its upstream MAPKK, respectively. *jnk-1* and *jkk-1* are expressed in the nervous system (Fig. 1). Complete loss of function mutants of *jkk-1* and *jnk-1* exhibit normal growth and nervous system architecture but display movement defects, which was shown to be due to their function in GABAergic inhibitory motor neurons (19, 20). Another study identified *mek-1* based on homology to MKK7 (21). Animals overexpressing a phospho-mimetic activated form of MEK-1 displayed behavioral phenotypes such as pharyngeal pumping defects, uncoordinated movement, and defective egg laying. A *mek-1* deletion mutant was generated using transposon mutagenesis, and reported to enhance the feeding defects of several Eat mutants, which are defective in pharyngeal pumping (21). Recent analysis revealed that the original *mek-1(ks54)* mutant strain contains a linked mutation in *sek-1(qd127)*, a closely related MAPKK,[4] and this genetic background will be denoted as *mek-1(sek-1)* throughout the text. Although the precise roles of *mek-1*, *jnk-1*, and *jkk-1* in locomotion and feeding remain to be determined, these studies provide a glimpse of the function of the *C. elegans* JNK kinase pathway in the nervous system.

P Granule Formation in Germ Cell Proliferation

A developmental role for KGB-1 (kinase and GLH-binding) was identified in the germline of *C. elegans*. Germ cells and their precursors contain RNA-protein complexes termed P granules, which are important for fertility (22, 23). A key class of germline granule proteins is the germline RNA helicase (GLH) family (24). KGB-1 was initially identified in a yeast two-hybrid screen for GLH-binding proteins (25). KGB-1 can bind and phosphor-

[4] K. Reddy and D. Kim, personal communication.

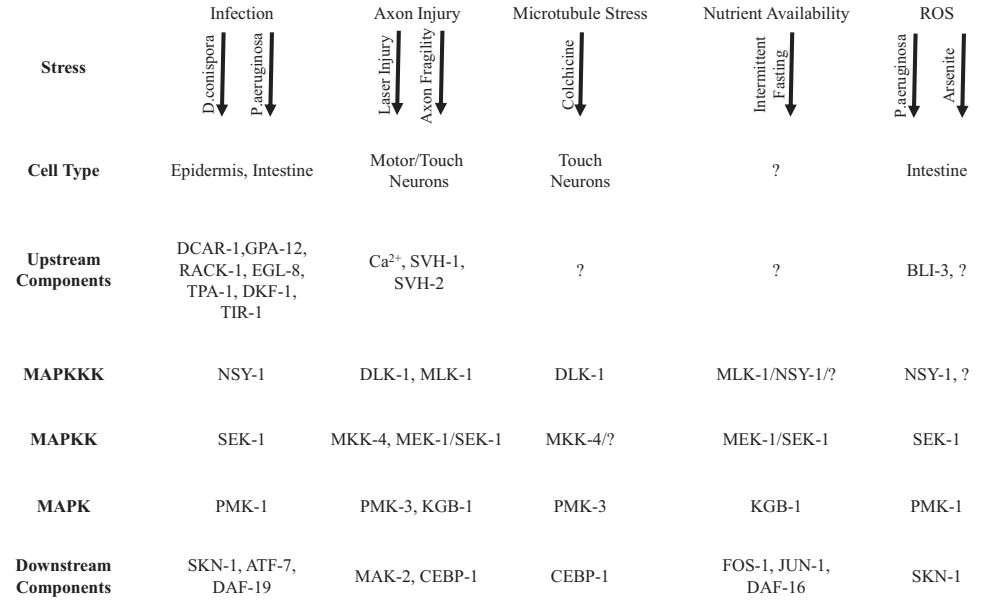

FIGURE 3. **Stress-activated MAPK components involved in stress-associated processes.** Redundancy in MAPK components is illustrated as MAPK/MAPK, whereas parallel pathways are denoted as MAPK, MAPK.

ylate GLH-1, which leads to degradation of phosphorylated GLH-1 (25, 26). A *kgb-1* null mutant, obtained by targeted deletion, showed extra germ cells, increased number of P granules, and temperature-sensitive sterility (25). RNAi-mediated knockdown of *glh-1* in *kgb-1* mutants partially rescued the P granule number and temperature-sensitive sterility (26). These studies suggest a model in which KGB-1 activity negatively regulates GLH-1 and the steady state level of P granules to maintain fertility. To date the specific MAPKKK or MAPKK upstream of KGB-1 in P granule formation remains to be determined.

Olfactory Neuron Specification

Roles for the p38 signaling pathway were first revealed from screens focusing on the developing nervous system (27, 28). One member of a pair of bilaterally symmetric AWC sensory neurons expresses the *str-2* odorant receptor in a stochastic manner (29, 30). A mutagenesis screen was designed to identify factors regulating AWC asymmetry. Analyses of mutants in which *str-2* was expressed in both AWC neurons revealed a loss of function allele for *nsy-1*, the homolog of the mammalian ASK1 MAPKKK (27). Based on a similar AWC phenotype, the MKK3/6 *sek-1* was subsequently placed downstream of *nsy-1* (31). Although these early observations suggested that a p38 MAPK might act downstream of *nsy-1* and *sek-1*, definitive evidence was only recently provided when an unexpected finding revealed functional redundancy of *pmk-1* and *pmk-2* (32). The three *pmk* genes are encoded by a single polycistronic transcript (operon), precluding the generation of double mutants by traditional genetic crosses. A strain with mutations in both *pmk-1* and *pmk-2* was serendipitously identified in a screen searching for suppressors of the *pmk-1* loss of function phenotype following bacterial infection (32, 33). Double mutants of *pmk-1* and *pmk-2* recapitulated the AWC phenotype of *sek-1* and *nsy-1* (32). These data provide a model where a NSY-1/SEK-1/PMK-1 and PMK-2 cascade acts during neuronal development to regulate AWC asymmetry (27, 32). Moreover, the activation of this p38 kinase cascade is regulated in part by calcium, via calmodulin kinase II, as well as the conserved protein TIR-1 (27, 31, 34). The mammalian ortholog of TIR-1, SARM1, has been studied for its vital importance during axon degeneration and has been shown to activate a MAPK cascade following axon injury (35). In addition, PMK-1 and PMK-2 were shown to act redundantly downstream of TIR-1/NSY-1/SEK-1 to induce TPH-1 expression in the ADF neuron following exposure to bacteria (32, 36, 37).

Synapse Development

PMK-3 and its upstream regulators were similarly defined in a neurodevelopmental context. The E3 ubiquitin ligase RPM-1 regulates synapse development such that *rpm-1* null mutants display disorganized synapses and reduction of synapse number (38, 39). To identify substrates for RPM-1, a large-scale suppressor screen took advantage of a synthetic paralysis when *rpm-1(lf)* was in combination with other synaptogenesis mutants (28). Mutant worms that suppressed the synthetic locomotor defect were isolated and shown to suppress the synapse defects of an *rpm-1* single mutant. Characterization of these suppressors led to the identification of loss of function mutations in *dlk-1*, *mkk-4*, and *pmk-3*. Genetic epistasis analyses further supported that these three kinases constitute a linear DLK-1/MKK-4/PMK-3 pathway. Moreover, cell biological and biochemical evidence showed that DLK-1 is a substrate of RPM-1 (28). Subsequent studies of other suppressor mutations led to the identification of two downstream targets, *mak*-2 and *cebp-1* (40). MAK-2 is the homolog of MAPKAPK2 (MK2), and acts downstream of PMK-3. CEBP-1 is a member of the C/EBP (CCAAT/enhancer-binding protein) transcription factor family, and represents a novel transcription factor downstream of MAK-2. During synapse development, the activity of this PMK-3 kinase cascade is controlled through ubiquitin-mediated degradation of DLK-1 by the RPM-1 E3 ubiquitin ligase.

Overall, these studies show that distinct p38 and JNK MAPK cascades regulate a diverse class of "normal" biological processes during development and nervous system function (Fig. 2). The third JNK homolog KGB-2 shows 84% identity with KGB-1. So far the only reported roles for KGB-2 are in excess carbon dioxide (hypercapnia)-induced fertility defects and a slight negative role in axon injury response (41, 42). Presently, little is known about the function of the fourth JNK homolog *C49C3.10*.

Uncovering Stress Pathways Involving p38 and JNK

The term "stress-response pathway" encompasses a wide range of cellular functions that protect an organism against harmful conditions. Types of stress include elevated temperature, toxin exposure, cellular damage, and infiltration by pathogens. Each type of stress has unique consequences and requires a different repertoire of subcellular signaling pathways to properly buffer their effects. Although the upstream and downstream components differ, and often the specific MAPK cascade varies, ultimately a MAPKKK/MAPKK/MAPK cascade is activated to trigger specific cellular and organismic responses (7). Studies in *C. elegans* have shed light on context specificity in stress-activated MAPK signaling (Fig. 2). Below, we will highlight how *C. elegans* has been used to characterize these MAPK-dependent cellular responses in infection, axon injury, and environmental stress.

Innate Immunity

Innate immunity, or host defense, is a mechanism that is conserved across evolution for a host organism's response to pathogens (43). The functional conservation of immune response in *C. elegans* has provided a powerful platform to rapidly screen genes and to dissect mechanisms regulating innate immunity (44, 45). These studies have led to the discovery of a myriad of novel signaling pathways associated with stress-activated MAPK activation and their downstream targets (46).

Bacterial Infection

It is fitting that the first investigation of innate immunity using genetic screening in *C. elegans* yielded the association of p38 signaling to a stress response (33). The human opportunistic pathogen *Pseudomonas aeruginosa* causes an intestinal infection and eventual death of the worm (47). This response offers a simple screening strategy to identify genes involved in protective mechanisms (33). Wild-type worms begin to succumb to infection at 34 h, and mutant worms with enhanced susceptibility to *P. aeruginosa* (Esp) die between 16 and 30 h. The death of the worms occurs after they become fertile adults, and thus progeny can be salvaged and propagated to establish strains. *esp-2* and *esp-8* mutations were found to affect *sek-1* and *nsy-1*, respectively. *pmk-1* mutants were also shown to exhibit an Esp phenotype, although not identified in the screen. Therefore following *P. aeruginosa* infection, a protective NSY-1/SEK-1/PMK-1 pathway is activated. As mentioned earlier, *pmk-1* and *pmk-2* function redundantly during olfactory neuronal development (32). However, double mutants of *pmk-1* and *pmk-2* did not exacerbate the *pmk-1* phenotype in the intestine following infection. Interestingly, *pmk-2* expression in the intestine is under negative regulation by a set of microRNAs via its 3′-UTR. Removing the 3′-UTR of *pmk-2* caused its expression in the intestine, which was sufficient to rescue the Esp phenotype of *pmk-1* mutants. The use of 3′-UTR elements to regulate tissue-specific expression of *pmk-2* provides a mechanism that endows context specificity to p38 paralogs. A recent study has revealed a similar regulatory theme utilizing 3′-UTR elements in cultured human cells (48).

Fungal Infection

The fungal pathogen *Drechmeria coniospora* infects *C. elegans* by adhering to and penetrating its cuticle, resulting in epidermal damage and ultimately organismal death (49). Studies of *D. coniospora* infection uncovered roles for the same p38 pathway used in the intestine following *P. aeruginosa* exposure (50). To identify the factors that initiate the epidermal innate immune cascade *in vivo*, a clever transcriptional reporter using green fluorescent protein driven by the promoter of the antimicrobial peptide (AMP) gene *nlp-29* was developed to detect immune activity upon *D. coniospora* infection. Several Nipi (no induction of peptide after *Drechmeria* infection) mutants were identified, and found to affect the entire NSY-1/SEK-1/PMK-1 cascade (50). Through a series of elegant genetic experiments, a G-protein-dependent cascade consisting of the α/β G-protein subunits of GPA-12/RACK1 via EGL-8/TPA-1/TIR-1 (phospholipase C, protein kinase C, and the Toll-1 interleukin receptor domain adaptor protein) was shown to act upstream of NSY-1/SEK-1/PMK-1 (51, 52). Following bacterial infection, the protein kinase D ortholog, *dkf-2*, was shown to act upstream of *tir-1* to activate PMK-1 (53). In a subsequent study, the G-protein-coupled receptor *dcar-1* and 4-hydroxyphenyllactic acid (HPLA) were identified as receptor and ligand upstream of GPA-12 activation following fungal infection (54). HPLA is generated by epidermal damage, rather than by the pathogen, providing a link between the similarities in MAPK signaling in wounding and infection (50, 55). These studies illustrate the regulation of the context specificity of the same p38 MAPK cascade in bacterial and fungal infection through different upstream components.

Axon Injury

Neurons are subject to many kinds of stress and damage throughout their lifetime. In the mammalian central nervous system, injured axons lack the ability to regenerate, largely due to a combination of inhibitory mechanisms involving both intrinsic and extrinsic factors (reviewed in Ref. 56). *C. elegans* neurons display a robust regeneration response following precise severing of single, stereotyped axons using a high-powered laser (57, 58). Although axon regeneration is observed in most neurons, the degree to which an axon regenerates is variable. To define molecular mechanisms underlying axon injury-induced stress, a large-scale axon regeneration screen using genetic mutants was performed, revealing multiple conserved pathways (59). Among them is the DLK-1/MKK-4/PMK-3 cascade, activation of which is necessary to initiate axonal regrowth (40, 60). As mentioned early, this p38 kinase cascade is tightly regulated by protein ubiquitination during synapse development (28). Axon injury triggers rapid and transient calcium influx

(61), which regulates DLK-1 activity via an auto-regulatory mechanism involving protein isoform binding (62). As described below, this same p38 kinase cascade has also been identified in studies of multiple neuronal insults.

Spontaneous Regrowth following Axon Fragility

In parallel to studies on laser-induced axon injury, p38 and JNK MAPK cascades were also implicated in regeneration by studies of *unc-70/β*-spectrin mutants (42, 63). Cytoskeletal defects in *unc-70* mutants make their axons, which frequently break upon mechanical stress caused by animal movement, fragile. The broken axons in *unc-70* mutants can spontaneously regrow (63). Taking advantage of the non-invasive nature of the axon breaks, a large-scale RNAi screen targeting conserved molecules was performed (42, 60). The initial studies revealed *dlk-1*, acting through *pmk-3*, to be required at the time of injury, where it regulates the formation of the growth cone, a specialized structure required for axon growth (60). Steered by these observations, studies of additional genes from this screen revealed the roles of a KGB-1/JNK kinase cascade, composed of *mlk-1*, *mek-1(sek-1)*, and *kgb-1*, in axon regeneration. Mutants in this JNK pathway showed impaired axon regrowth to variable degrees, with *kgb-1* being most severe (64). The dual phosphatase *vhp*-1 is a negative regulator of both p38 and JNK signaling (65, 66). Loss of function mutants of *vhp-1* showed improved regeneration (64). *pmk-3 vhp-1* double mutants displayed a modest improvement in regeneration when compared with *pmk-3* alone, suggesting that increasing *kgb-1* activity may compensate for loss of *pmk-3*. Conversely, *vhp-1 kgb-1* double mutants regenerated quite well, indicating that *pmk-3* appears to better compensate for the loss of *kgb-1* than vice versa. Thus, axon regeneration provides a specific context in which both p38 and JNK exert their effects, albeit p38 being the predominant MAPK.

Suppressors of vhp-1 Inform on Axon Injury

Null mutations in *vhp-1* cause larval lethality, which can be suppressed by null mutations in *mlk-1*, *mek-1*, *kgb-1*, *dlk-1*, or *pmk-3* (64, 65). A genome-wide RNAi screening approach was undertaken to identify suppressor *of vhp*-1 (Svh) genes (67, 68). *svh-1* encodes a predicted extracellular protein with a structure similar to that of the mammalian hepatocyte growth factor (HGF) and macrophage-stimulating protein (MSP). *svh-2* encodes a receptor tyrosine kinase homologous to the HGF and MSP receptors. Because *vhp-1* regulates MAP kinases in axon regeneration, the function of *svh-1* and *svh-2* during this process was examined (40, 60, 64). Loss of function mutants in either gene showed impaired regeneration capacity, resembling *mlk-1* mutants (40, 60, 68). SVH-2 could bind MLK-1 but not DLK-1 *in vitro* (68). *svh-2* mutants also showed a marked decrease in phospho-KGB-1. Thus, *svh-1* and *svh-2* likely provide a layer of specificity in controlling the KGB-1/JNK pathway, independently of PMK-3 in axon injury response. However, recent evidence suggests that there may be crosstalk between the KGB-1 and PMK-3 cascades, as the transcription of *svh-2* appears to be regulated by CEBP-1, a downstream factor of PMK-3 (69). The exact roles for transcriptional targets of CEBP-1 during axon regeneration have yet to be determined. Nonetheless, these studies implicate a growth factor as a potential intermediate between p38 and JNK kinase pathways during axon regrowth.

Cellular Response to Damage to the Microtubule Cytoskeleton

Microtubule dynamics plays an important role in axon regeneration in both *C. elegans* and vertebrate injury models (59, 70, 71). The touch neurons PLM and ALM are microtubule-rich and use specific microtubules to transmit mechanical stimuli (72). Treatment with colchicine, a drug that can cause microtubule depolymerization or damage to microtubule cytoskeleton, reduced overall gene expression in touch neurons (73). To dissect how microtubule disruption and gene expression intersect, an elegant screen using a short-lived GFP reporter was performed. Analyses of several Colchicine-resistant (Cre) mutants identified *dlk-1* and *cebp-1*. Loss of function of *pmk-3* showed a similar Cre phenotype, whereas *mkk-4* mutants exhibited a much milder phenotype when compared with *pmk-3* or *dlk-1*, pointing to possible redundancy at the MAPKK level (73). *C. elegans* MAPKK genes are clustered on the X chromosome, complicating examination of double mutant animals. Nonetheless, MAPKK redundancy may provide an interesting context specificity following colchicine treatment, contrasting axon regeneration, which is strongly dependent on *mkk-4* (40).

Environmental Stress

The day-to-day life of any organism is stressful with a near constant bombardment of environmental stresses ranging from toxins, to nutrient availability, to temperature. All of these stress paradigms have been studied extensively *in vivo* using *C. elegans* as a model and have greatly informed on the biology of these processes. Due to the vast literature on the subject of environmental stress and *C. elegans*, we will focus on stress-activated MAPK signaling in nutrient deprivation and oxidative stress.

Nutrient Deprivation

Long-term nutrient deprivation or malnutrition has a detrimental impact on organismal viability. Paradoxically, it has been shown that modest nutrient deprivation or dietary restriction increases lifespan in vertebrate and invertebrate models (74–76). In *C. elegans*, lifespan and diet intersect via the insulin signaling pathway, with mutants in genes such as the insulin receptor *daf-2* increasing lifespan (77–79). JNK signaling has also been shown to regulate longevity and interact with the insulin signaling pathway (80–82). Intermittent fasting (IF) is a paradigm involving transient exposure to nutrient sources and increases lifespan of wild-type *C. elegans* in an insulin signaling-dependent manner (83). Mutants in *kgb-1*, but not *kgb-2* or *jnk-1*, abrogated IF-mediated lifespan increase (84). This lifespan phenotype of *kgb-1* was independent of previously reported developmental/fertility phenotypes associated with P granule formation. Phosphorylation of KGB-1, which was abolished in *mek-1(sek-1)* mutants, was observed within 30 min of starvation and maintained for 9 h. Single mutants in the MAPKKK's *mlk-1* and *nsy-1* both exhibited partial suppression of IF-dependent lifespan extension; however, double mutants of *mlk-1* and *nsy-1*

did not completely recapitulate *kgb-1* mutants, suggesting additional upstream regulators. These observations support a model where following nutrient restriction by intermediate fasting, a KGB-1 cascade is required to mediate lifespan extension; however, regulation at the MAPKK and MAPKKK remains to be determined. A major factor in nutrient deprivation-induced changes in longevity is thought to stem from oxidative stress produced as a byproduct of metabolism (85, 86).

Oxidative Stress

Oxidative stress is the result of accumulation of reactive oxygen species (ROS), which in turn damage cellular constituents (87). The activation of JNK signaling was shown to occur under conditions of heavy metal stress (20, 21); however, the role of stress-activated MAPK signaling following treatment with compounds that induce ROS was unknown. Treatment of worms with arsenite, a toxic ROS-producing compound, induced a robust phosphorylation of PMK-1, induction of oxidative stress-responsive genes, and eventual lethality (88). The activation of PMK-1 following arsenite treatment was dependent on SEK-1 but independent of NSY-1, differing from the NSY-1/SEK-1/PMK-1 cascade used during infection and osmotic stress (33, 50, 89). Both *sek-1* and *pmk-1* mutants exhibited an enhanced sensitivity and lethality to arsenite treatment and prevented the induction of *gcs-1*, an oxidative stress reporter, in the intestine (88). *pmk-1* mutants were not as sensitive as *sek-1* mutants, suggesting a potential redundancy in MAPK usage. *gcs-1* expression is predominantly regulated by the transcription factor SKN-1, which translocates to the nucleus of intestinal cells following oxidative stress (90). SKN-1 can be phosphorylated by PMK-1 *in vitro*, and this phosphorylation is pivotal for its nuclear localization *in vivo*, as nuclear localization of SKN-1 was significantly impaired in *sek-1* and *pmk-1* mutants (88). Under another stress context, the nuclear localization of SKN-1 was shown to occur in an NSY-1/SEK-1/PMK-1-dependent manner in the intestine following bacterial infection (91). The upstream adaptor protein TIR-1 was dispensable for SKN-1 localization, which contrasts its requirement for organismal viability after *P. aeruginosa* exposure (52). In addition to SKN-1, following infection, PMK-1 was shown to phosphorylate and activate the transcription factor ATF-7 (92). These studies show that unique upstream components activating PMK-1 induce SKN-1 activation following toxin and bacterial exposure.

Emerging Roles for Stress-activated MAPK Signaling

The evolution of *C. elegans* as a model system has seen the analysis of complex behavioral and cell-specific processes. Recent studies have uncovered additional roles of stress-activated MAPK cascades in multiple behaviors and cellular function, with cell type- and behavior-dependent modification. For example, the avoidance of high CO_2 environments and pathogens was shown to be mediated by MOM-4/MKK-4/PMK-3 in the BAG neuron (93). In contrast, the aversive reaction to microbial exposure is mediated by a MLK-1/MEK-1(SEK-1)/KGB-1 pathway (94). Olfactory memory in *C. elegans* allows for the association of cues with positive or negative experiences. The loss of these memories was shown to proceed through UNC-43/TIR-1/NSY-1/SEK-1/JNK-1 (95). Interestingly, olfactory learning is mediated in part by the AWC neurons, which use a similar pathway during development (27, 96). Advances in microscopic techniques have also allowed for the study of subcellular processes and structures that were previously not possible in the worm. In a screen for mutants defective in dye filling, DLK-1/PMK-3 were identified to affect cilia length, via regulation of RAB-5 endosomes (97). The MAPKK(s) acting between DLK-1 and PMK-3 in this role remains to be identified.

Future Perspectives

Forty years out from the first published screen in *C. elegans* (1), the use of forward genetics remains a powerful approach to dissect biological processes at both the organismal and the cellular level. Specifically, the use of *C. elegans* models including infection, axon injury, and environmental stress has revealed stress-associated MAPK signaling components. However, there is still much to be understood about how these pathways truly function *in vivo*. A resounding theme in both non-stress and stress conditions is the context-specific nature for these pathways (Figs. 2 and 3). Recent studies have highlighted redundancy and crosstalk between p38 and JNK signaling components that may play an important role in regulating their context specificity (32, 64). Moreover, functions have yet to be revealed for several MAPK cascade components. For example, the roles of *kgb-2* have yet to be associated with any biological process, and it may function as a safeguard in the JNK cascade. CRISPR/Cas9-mediated genome editing (where CRISPR stands for clustered regularly-interspaced short palindromic repeat) can now be employed to manipulate potential redundant MAPKKs that all clustered on the X chromosome. Teasing apart unique cell type- and stress-specific aspects of MAPK signaling pathways will help inform on their overall roles, with hopes that *C. elegans* continues to serve as a platform to translate meaningful *in vivo* findings into relevant interventions for human conditions that exhibit de-regulated MAPK signaling.

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