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Roles of MAP Kinase Cascades in *Caenorhabditis elegans*

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Mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases that are activated by diverse stimuli such as growth factors, cytokines, neurotransmitters and various cellular stresses. MAPK cascades are generally present as three-component modules, consisting of MAPKKK, MAPKK and MAPK. The precise molecular mechanisms by which these MAPK cascades transmit signals is an area of intense research, and our evolving understanding of these signal cascades has been facilitated in great part by genetic analyses in model organisms. One organism that has been commonly used for genetic manipulation and physiological characterization is the nematode *Caenorhabditis elegans*. Genes sequenced in the *C. elegans* genome project have furthered the identification of components involved in several MAPK pathways. Genetic and biochemical studies on these components have shed light on the physiological roles of MAPK cascades in the control of cell fate decision, neuronal function and immunity in *C. elegans*.

Key words: *C. elegans*, MAP kinase, neuronal function, signal transduction, stress response.

Mitogen-activated protein kinase (MAPK) signal transduction pathways are evolutionarily conserved in eukaryotic cells and transduce signals in response to a variety of extracellular stimuli. Each pathway is composed of three classes of protein kinases: MAPK, MAPK kinase (MAPKK) and MAPK kinase kinase (MAPKKK) (*1*). Within the MAPK superfamily, three subgroups have been identified: ERK, JNK, and p38. Within the MAPKK superfamily, several groups have also been distinguished, such as MEK1/2, MKK3/6, MKK4, and MKK7. MEK1/2 have been shown to activate the ERK subgroup, MKK4 can activate both the JNK and p38 subgroups, MKK7 is specific for the JNK subgroup, and MKK3/6 act solely as activators of the p38 subgroup. These MAPKK superfamily members are activated in turn by members of the MAPKKK superfamily, such as Raf, MEKK, ASK, TAK1, MLK, TAO and COT.

The best characterized MAPK cascade consists of Raf, MEK and ERK, and is regulated by Ras. Ras, in turn, is activated by growth factors that signal through receptor protein tyrosine kinases. Thus, the Raf-MEK-ERK cascade constitutes an integral component of various growth-promoting pathways (*1*). Details of the ERK-type MAPK pathway in multicellular organisms have been elucidated through the genetic analyses of *Drosophila* and *C. elegans*, which have proven to be very useful genetic models to study signaling cascades (*2*, *3*). In *C. elegans*, the ERK pathway has been shown to consist of LIN-45 (MAPKKK), MEK-2 (MAPKK) and MPK-1 (MAPK), and this pathway plays an important role in hypodermal developments, meiotic progression, protein degradation and odorant notiception (*3–5*). In contrast to the ERK MAPK pathway, the physiological roles of the JNK and p38 pathways in the normal development and function of organisms are less well understood, although these pathways have been implicated in the responses to inflammation and some types of stress (*1*). The completion of the *C. elegans* genome sequence provides a new tool with which to decipher the molecular nature of these MAPK pathways. In this review, we will focus on recent progress in understanding the physiological and developmental roles of the MAPK cascades in *C. elegans*.

Components of the JNK and p38 pathways in *C. elegans*

Analysis of *C. elegans* genome data suggested that this organism possesses many MAPKs, MAPKKs and MAPKKKs (*6*). Components involved in the JNK and p38 MAP kinase cascades are summarized in Fig. 1. There are three JNK homologs in *C. elegans*: *jnk-1* (corresponding to gene B0478.1), *kgb-1* (corresponding to gene T07A9.3) and *kgb-2* (corresponding to gene ZC416.4). *C. elegans* contains three p38 homologs, *pmk-1* (corresponding to gene B0218.3), *pmk-2* (corresponding to gene F42G8.3) and *pmk-3* (corresponding to gene F42G8.4). These *pmk* genes are contiguous on chromosome IV and comprise an operon with the gene order *pmk-3-pmk-2-pmk-1*.

Among the MAPKKs identified, JKK-1 (corresponding to gene F35C8.3) and MEK-1 (corresponding to gene K08A8.1) belong to the MKK7 subgroup, and SEK-1 (corresponding to gene R03G5.2) belongs to the MKK3/6 subgroup (*7*). MKK-4 (corresponding to gene F42G10.2), VZC374L.1 and ZC449.3 have similarities to MKK4. F35C8.1 and F35C8.2 have very weak similarities to MKK3.

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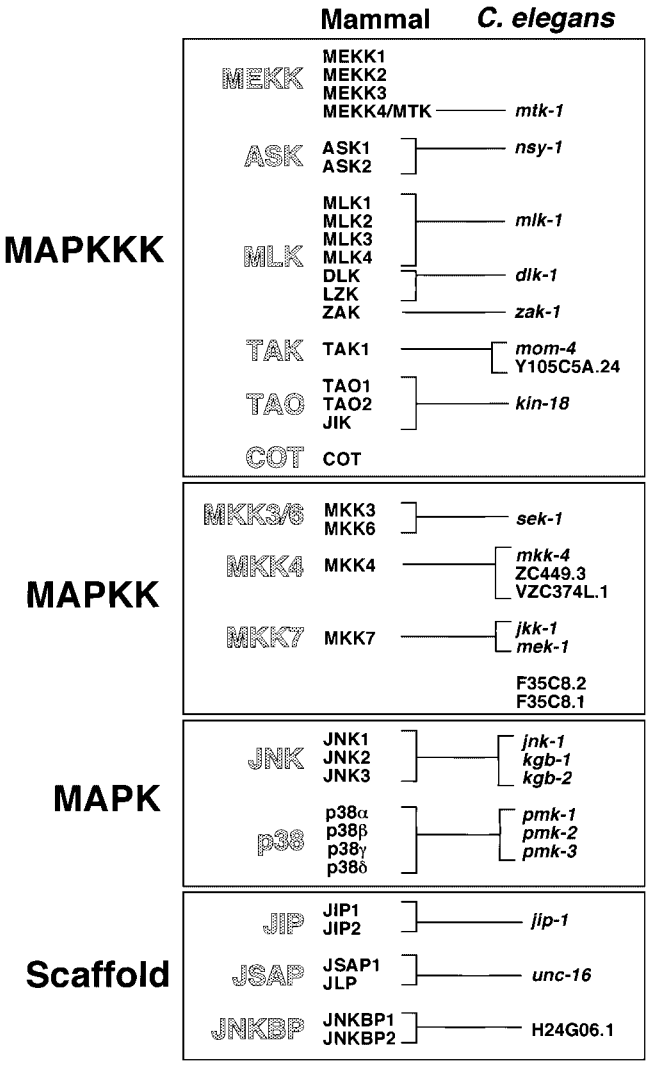

Fig. 1. **List of components involved in JNK and p38 MAPK cascades in mammal and *C. elegans*.** Each MAPKKK, MAPKK, MAPK and scaffold protein groups are each enclosed by lines. Subgroups in each group are indicated by gray characters. Components of the JNK and p38 MAPK cascades in mammal and corresponding homologs in *C. elegans* are linked by lines.

Members of the MAPKKK superfamily, such as MEKK, ASK, TAK1, MLK, TAO and COT, are known to act as activators of the JNK and p38 MAPK cascades in mammals. The *C. elegans* genome contains an MEKK4/MTK homolog (*mtk-1*: corresponding to gene B0414.7), an MLK homolog (*mlk-1*: corresponding to gene K11D12.10), an ASK homolog (*nsy-1*: corresponding to gene F59A6.1), a DLK/LZK homolog (*dlk-1*: corresponding to gene F33E2.2), a ZAK homolog (*zak-1*: corresponding to gene R13F6.6), a TAO/JIK homolog (*kin-18*: corresponding to gene T17E9.1) and two TAK1 homologs (*mom-4*: corresponding to gene F52F12.3 and Y105C5A.24). Obvious homologs of MEKK1, MEKK2, MEKK3 and COT are not found.

In mammals, JIP1/2, JSAP1/JLP and JNKBP appear to function as scaffold proteins in the JNK signaling pathway (*1*). Examination of the *C. elegans* genome project database reveals that it contains homologs of JIP1/2, JSAP1/JLP and JNKBP, called *jip-1* (corresponding to gene F56D12.4), *unc-16* (ZK1098.10) and H24G06.1, respectively.

Role of the JNK-1 signaling pathway in *C. elegans*

A *C. elegans* homolog of JNK, JNK-1, and its activator JKK-1 (MAPKK) were identified using a yeast mutant defective in the HOG1 MAPK cascade, which causes a high-osmolarity sensitive growth phenotype (*7*). In *C. elegans*, expressions of both *jkk-1* and *jnk-1* are neuron-specific. In addition, JNK-1 activity was observed to be abolished in *jkk-1* mutants, suggesting that JKK-1 functions upstream of JNK-1 in the same pathway to activate its kinase activity in neurons (*8*). Both *jkk-1* and *jnk-1* null mutant animals exhibit uncoordinated locomotion phenotype. Selective expression of *jkk-1* in the D-type motor neurons rescued the movement defect of *jkk-1* mutant animals. Expression of JKK-1 or JNK-1 from a conditional promoter in adults was also found to rescue the movement defect, indicating that they are involved in the continued functioning of neurons. These results suggest that the JKK-1-JNK-1 pathway modulates coordinated movement in *C. elegans* as a result of its function in type D motor neurons.

In mammals, JSAP1 was identified as a scaffold protein that binds to JNK. Subsequent studies demonstrated that JSAP1 also interacts with components of the JNK pathway, such as MLK and MKK7. In *C. elegans*, a JSAP1 homolog UNC-16 was found to associate with JKK-1 and JNK-1 (*8*). However, loss-of-function *unc-16* mutation had little effect on endogenous JNK-1 activity, suggesting that UNC-16 may not be required for the activation of the JKK-1-JNK-1 pathway. It is still possible that UNC-16 serves to foster assembly of the JNK-1 module, thereby facilitating the efficient activation of JNK within a restrictive region of the cell.

unc-16 mutants were isolated in a screen for mislocalized synaptic vesicle (SV) components in DD motor neurons (*8*). In wild-type animals, SV components such as synaptobrevin localize at presynaptic regions in axons but not in dendritic regions. In *unc-16* mutants, the synaptobrevin homolog SNB-1 is mislocalized to dendritic regions. Mutations in *jnk-1* and *jkk-1* exhibit similar mislocalization phenotypes in the DD motor neurons, suggesting that both JKK-1-JNK-1 signaling and UNC-16 scaffold protein are involved in the control of SV component localization. Interestingly, UNC-16 was found to interact with KLC-2, a kinesin light chain of conventional kinesin (unpublished data). Consistent with this interaction, a loss-of-function mutant of *unc-116*, which encodes a kinesin heavy chain, displayed phenotypes similar to those of *unc-16* (*8*). Furthermore, localization of the UNC-16 protein in neurons was disturbed both in *unc-116* (*8*) and *klc-2* mutants (unpublished result). These results suggest that conventional kinesin regulates UNC-16 localization and that UNC-16 regulates the localization of SV components through its associations with JNK-signaling components.

Roles of other JNK signaling pathways in *C. elegans*

MEK-1 is one of several MKK7 homologs found in *C. elegans*. Deletion of *mek-1* did not affect coordinated locomotion, but rather resulted in hypersensitivity to heavy metals and starvation (*9*). Evidence from another group

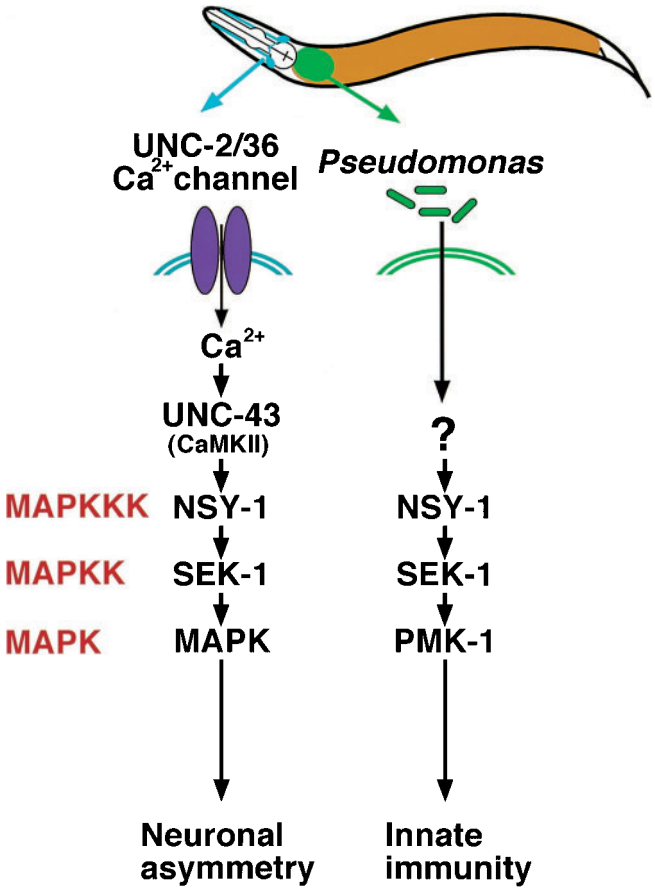

Fig. 2. **NSY-1- SEK-1 MAPK cascades in *C. elegans*.** In AWC neurons, the NSY-1-SEK-1-MAPK cascade is activated by Ca^{2+} signaling through CaMKII and establishes the asymmetric cell fate decision. In the intestine, the NSY-1-SEK-1-PMK-1 cascade is activated by and protects the worm from pathogen.

has suggested that the *jnk-1* mutant also exhibits hypersensitivity to heavy metals, in the same way that *mek-1* mutants do (*10*). However, our attempts to corroborate these findings have been unsuccessful (unpublished data). The relationship between MEK-1 and other components of the JNK cascade remains to be determined.

Recently, a JNK-like kinase KGB-1 was identified as a protein that binds to GLH-1, a germline-specific RNA helicase involved in germline development (*11*). Disruption of KGB-1 results in a temperature-sensitive, sterile phenotype, characterized by the absence of mature oocytes and the presence of trapped, immature endoreplicated oocytes. These results suggest that KGB-1 is required for germline development at high temperature. However, the relationship between KGB-1 and GLH-1 remains obscure, because the phenotype observed in *kgb-1* mutants is different from that observed in animals treated with *glh-1* RNAi. Further analysis is required to clarify the role of KGB-1.

The p38 signaling pathway in *C. elegans*

C. elegans contains three p38 homologs, PMK-1, PMK-2 and PMK-3. The PMK-1, PMK-2 and PMK-3 proteins can phosphorylate ATF-2 *in vitro* (*12*), similar to mammalian p38. PMK-1 was shown to complement the osmotic-sensitive phenotype of the yeast *hog1* disruption (*7*). Using the yeast HOG1 MAPK pathway, an upstream activator of both JNK-1 and PMK-1 was isolated and designated as *sek-1* (*13*). When expressed in mammalian cells, SEK-1 was found to phosphorylate and activate p38. The upstream MAPKKK of SEK-1 is NSY-1, an ASK homolog (Fig. 2) (*13*, *14*). Endogenous PMK-1 activity was greatly reduced in *nsy-1* or *sek-1* loss-of-function mutant animals (*15*). In addition, the SEK-1 activity was greatly reduced in *nsy-1* mutants (*13*). These results suggest that NSY-1, SEK-1 and PMK-1 constitute a kinase cascade in *C. elegans*.

The *nsy-1* (*n*euronal *sy*mmetry-1) gene was identified as a mutation that affected asymmetric olfactory neuron fate (*14*). Most neurons in *C. elegans* exist as bilaterally symmetric, morphologically similar pairs. However, the two AWC olfactory neurons interact with each other to determine their respective distinct fates. One outcome of this differentiation is the asymmetric expression of the candidate odorant receptor STR-2, which is expressed either in the left or right AWC neuron, but never in both. However, *nsy-1* mutants were observed to express STR-2 in both AWC neurons, disrupting this AWC asymmetry (*14*, *16*). Similar to *nsy-1*, *sek-1* null mutant animals also were defective in asymmetric AWC cell fate (*13*). Thus, the NSY-1-SEK-1-MAPK cascade is involved in determining asymmetric cell fates in AWC olfactory neurons. It is still unknown whether PMK-1 is involved in this pathway.

The mechanism by which asymmetric AWC cell fate is determined involves axon contact during development and Ca^{2+} signaling (*16*). Ca^{2+} enters through the UNC-2 and UNC-36 voltage-gated Ca^{2+} channels and stimulates UNC-43 Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity, leading to the inhibition of STR-2 expression in one of the two AWC neurons. In *unc-43* loss-of-function mutants, STR-2 is expressed in both AWC neurons, whereas in *unc-43* gain-of-function mutants, STR-2 is not expressed in either neuron. Genetic and biochemical analyses showed that UNC-43 CaMKII functions as a positive mediator of the NSY-1-SEK-1-MAPK cascade regulating AWC asymmetry. When co-expressed in mammalian cells, UNC-43 was found to associate with NSY-1 (*14*). The phenotype seen in the gain-of-function *unc-43* mutants was suppressed when this mutation was combined with *nsy-1* or *sek-1* loss-of-function mutations (*13*, *14*). The loss-of-function *unc-43* mutation was found to cause a decrease in SEK-1 kinase activity in worms (*13*). Thus, the NSY-1-SEK-1-MAPK cascade is activated by Ca^{2+} signaling through CaMKII, and establishes the asymmetric cell fate decision during neuronal development.

Recent analysis has revealed that the NSY-1-SEK-1-PMK-1 pathway is also involved in innate immunity in *C. elegans* (*15*). *Pseudomonas aeruginosa*, a pathogen against many organisms including human, proliferates in intestinal cells and kills the infected worms. The *nsy-1* and *sek-1* mutant worms exhibited an enhanced susceptibility to pathogen (Esp) phenotype and died faster than wild-type worms in the presence of pathogen. The phenotype of *sek-1* mutants can be rescued by expression of a SEK-1 transgene in the intestine. *pmk-1* RNAi worms were also reported to exhibit an Esp phenotype and path-

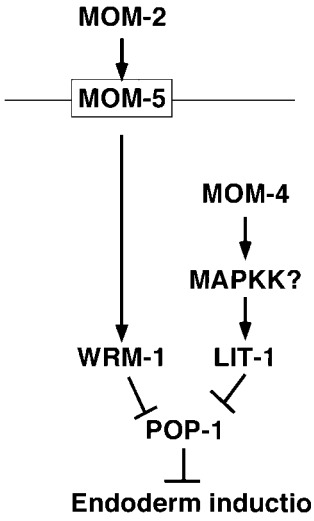

Fig. 3. **Wnt and the MOM-4-LIT-1 cascades in early *C. elegans* development.**

ogen infection caused an increase in endogenous PMK-1 activity. These results suggest that the NSY-1-SEK-1-PMK-1 pathway functions in an innate immunity system in the intestine that protects worms from pathogen infection. Interestingly, *unc-43* loss-of-function mutant did not exhibit this enhanced susceptibility. This suggests that the NSY-1-SEK-1-PMK-1 cascade is regulated by different upstream signals than the Ca^{2+}-CaMKII signaling involved in the innate immunity system.

The TAK1 signaling pathway in *C. elegans*

The mammalian TAK1 MAPKKK was identified as a kinase that is activated by TGF-β (*17*). Subsequent work has further demonstrated its involvement in other signaling pathways. Notably, TAK1 has been shown to signal through the JNK and p38 MAPKs, as well as IκB kinase (*18*). TAK1 binds to and is activated by TAB1 (*19*). Examination of the *C. elegans* genome database has revealed that this organism contains homologs of TAK1 and TAB1, called *mom-4* and *tap-1* (corresponding to gene C44H4.5), respectively. Similar to mammalian TAK1 and TAB1, MOM-4 was shown to phosphorylate MKK6, and TAP-1 was shown to associate with MOM-4 and promote its kinase activity (*20*, *21*).

In *C. elegans*, the production of the endoderm occurs at the four-cell stage of embryonic development, when signaling from the posterior-most blastomere, called P2, polarizes a neighboring blastomere, called EMS. Polarization results in the EMS producing two daughter cells, one called E that produces endoderm, and another called MS that produces mesoderm (*22*). Genetic studies suggest that a Wnt signaling pathway, consisting of MOM-1/Porcupine, MOM-2/Wingless, MOM-5/Frizzled, WRM-1/β-catenin and POP-1/ TCF/LEF, regulates this process. In addition, MOM-4, which is related to the mammalian TAK1, and LIT-1, a MAPK-like protein similar to the *Drosophila* Nemo and mammalian NLK protein kinases, also participate (*20*). Activation of LIT-1 appears to require a conserved motif analogous to the site required for activation and phosphorylation by MAPKKs. It was thus postulated that MOM-4 and LIT-1 constitute a novel MAPK cascade (Fig. 3). However, it is not known which protein kinase functions as a MAPKK between MOM-4 and LIT-1. MOM-4 and LIT-1 were genetically shown to act as negative upstream regulators of POP-1. Furthermore, *mom-4* mutants and Wnt pathway mutants exhibit genetic synergy. These observations suggest that the MOM-4-LIT-1 MAPK pathway functions in parallel with Wnt signaling by downregulating POP-1 activity, thereby specifying anterior-posterior cell fate differences during *C. elegans* development. It will be interesting to identify the upstream signals that activate the MOM-4-LIT-1 pathway.

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