Integrative Biosciences 9: 107-111, 2005

Integrative **Biosciences**

http://www.ibiosci.or.kr

Analysis of the *Caenorhabditis elegans dlk-1* Gene Expression

Bumnoh Lee and Nam Jeong Cho*

School of Life Sciences, Chungbuk National University, Cheongju 361-763, Korea

Abstract: *C. elegans* DLK-1 has been reported to play an important role in synaptogenesis by shaping the structure of presynaptic terminal. In this study, we investigated the expression pattern and regulation of the *dlk-1* gene in *C. elegans*. To determine the expression pattern, we made a *dlk-1::gfp* fusion construct, named pPDdg1, which consisted of ~2.2 kb 5' upstream region, the first exon, the first intron, and a part of the second exon of the *dlk-1* gene. By microinjecting this construct into the worm, we observed that the DLK-1::GFP was expressed mainly in neurons. We next examined the regulatory elements of gene expression by deletion analysis of pPDdg1. Removal of a large portion of the 5' upstream region (Δ-361 to -2246) of the gene had little effect on the expression pattern, whereas deletion of the first intron led to elimination of the DLK-1::GFP expression in most of the neurons. Our results suggest that the first intron of the *C. elegans dlk-1* gene contains the regulatory element critical for gene expression.

Key words: DLK-1, dual leucine zipper-bearing kinase, *C. elegans*, GFP, gene expression

Eukaryotes have well-conserved biochemical pathways, termed mitogen-activated protein kinase (MAPK) pathways, which convert a variety of extracellular stimuli into intracellular responses. MAPK pathways are known to perform important roles in the control of cell growth, differentiation, and death (Chang and Karin, 2001). Typically, a MAPK pathway comprises three families of kinase enzymes, known as MAPKKK (MAP3K), MAPKK (MAP2K) and MAPK (Seger and Krebs, 1995; Garrington and Johnson, 1999). The MAPK pathway is activated by a phosphorylation cascade; that is, MAPK is phosphorylated by MAP2K and MAP2K is phosphorylated by MAP3K. Three prominent subfamilies of MAPK family, namely ERK, JNK, and p38 MAPK, have been extensively studied (Widmann et al., 1999; Pearson et al., 2001). In general, ERK is responsible for cell growth while JNK and p38 MAPK induce apoptosis and cell differentiation (Xia et al., 1995; Cross et al., 2000).

Mixed lineage kinases (MLKs) are members of a MAP3K family that activate JNK and p38 MAPK (Gallo and Johnson, 2002). MLKs are classified into three subfamilies, the MLKs, the dual leucine zipper-bearing kinases (DLKs), and zipper sterile-α-motif kinase (ZAK). Several members of the MLK family, including DLK, have been reported to be involved in neuronal cell death (Xu et al., 2001). A recent genetic study by Nakata et al. (2005) indicated that *C. elegans* DLK-1 might be involved in the shaping of presynaptic terminal. It was postulated that a MAPK pathway composed of DLK-1, MKK-4, and PMK-3 (p38 MAPK homolog) is critical for presynaptic development.

Amino acid sequence analysis indicates that the predicted structure of *C. elegans* DLK-1 is similar to that of mammalian DLK (Fig. 1). However, *C. elegans* DLK-1 differs from mammalian DLK in that it has only one

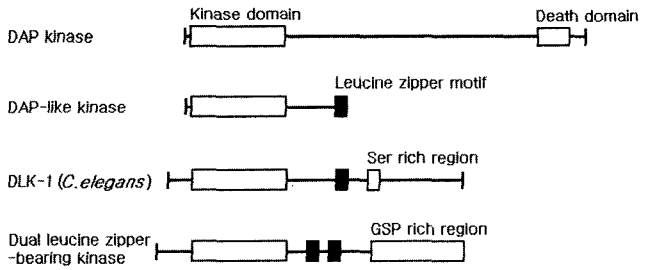

Fig. 1. Structural comparison between *C. elegans* DLK-1 and related proteins in mammals. DAP kinase, DAP-like kinase (Dlk/ZIP kinase), *C. elegans* DLK-1, and dual leucine zipper-bearing kinase (DLK) share a conserved kinase domain at their N-termini. DAP-like kinase (Dlk/ZIP kinase) and *C. elegans* DLK-1 contain one leucine zipper motif, whereas dual leucine zipper-bearing kinase (DLK) has two leucine zipper motifs. Gray box, kinase domain; black box, leucine zipper motif.

*To whom correspondence should be addressed.
Tel: 82-43-261-2310, Fax: 82-43-267-2306
E-mail: namjcho@chungbuk.ac.kr

leucine zipper motif instead of two. It is noteworthy that *C. elegans* DLK-1 is also structurally similar to death-associated protein (DAP)-like kinase (Dlk) or zipper-interacting protein (ZIP) kinase. Dlk/ZIP kinase is a serine/threonine kinase that belongs to the subfamily of DAP kinases (Kawai et al., 1998; Kogel et al., 1998). Both *C. elegans* DLK-1 and mammalian Dlk/ZIP kinase contain a kinase domain at their N-termini and a leucine zipper motif (Fig. 1). In mammals, Dlk/ZIP kinase has been implicated in regulation of apoptosis (Kawai et al., 1998).

In this study we investigated the expression pattern and regulation of the *C. elegans dlk-1* gene. We observed that the *C. elegans* DLK-1 was mainly expressed in neurons and found that the first intron, rather than the 5' upstream region, of the *dlk-1* gene might play a critical role in the regulation of gene expression.

MATERIALS AND METHODS

Strain

The wild-type strain N2 (Bristol) was used for experiments. Nematodes were grown and maintained as described previously (Brenner, 1974).

DLK-1::GFP fusion constructs

To examine the expression pattern of DLK-1, we constructed a *dlk-1::gfp* fusion gene between a portion of *dlk-1* and the green fluorescent protein (GFP) reporter gene. To obtain a portion of *dlk-1*, a cosmid DNA (C49G9; kindly provided by Audrey Fraser at Sanger Center, UK) containing *dlk-1* gene was amplified by PCR with primers DF1 and DR1. The PCR product, containing 2,246 bp of the 5' upstream region, the first exon (52 bp), the first intron (1,170 bp), and

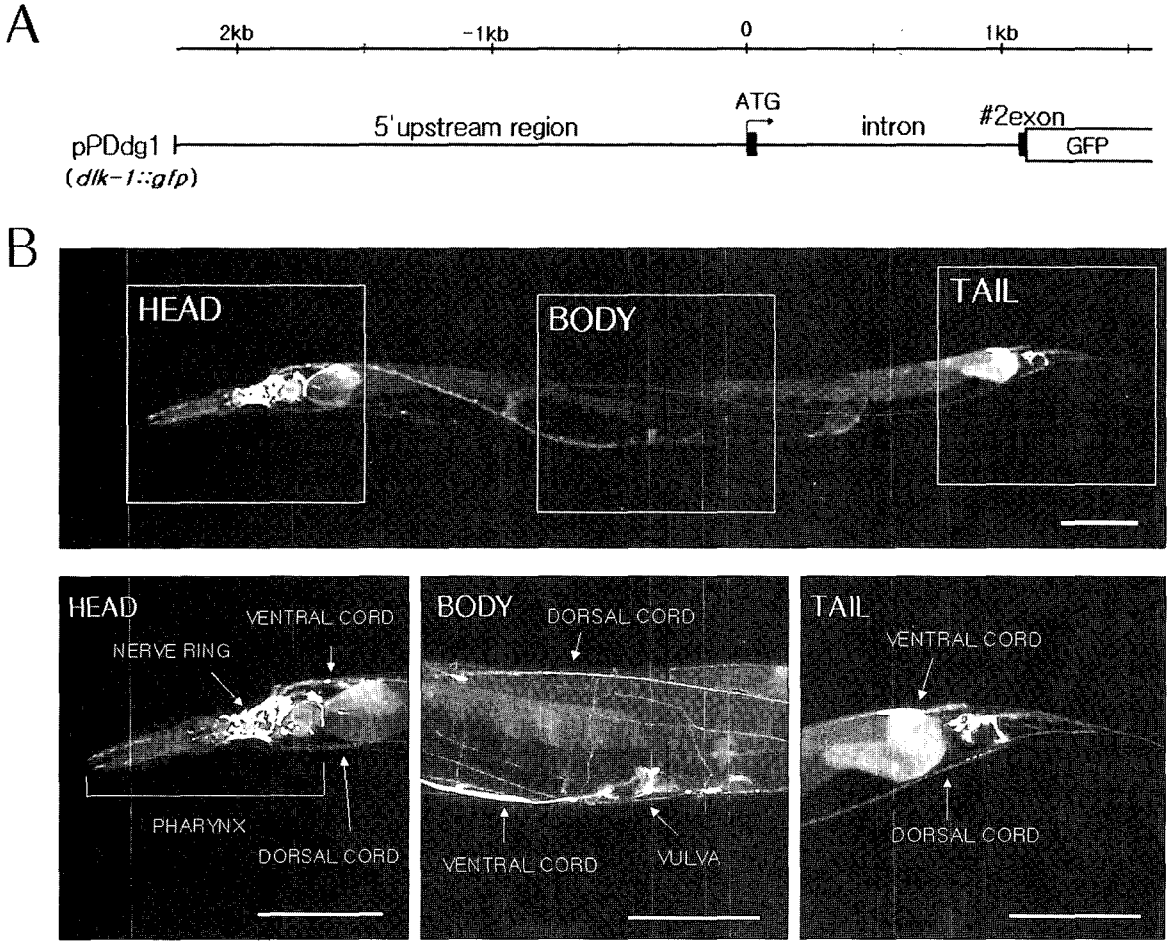

Fig. 2. *C. elegans* DLK-1 is mainly expressed in neurons. A, Genomic organization of *dlk-1::gfp* vector (pPDdg1). This plasmid contains ~2.2 kb 5' upstream region, the first exon, the first intron, and 16 codons of the second exon of the *dlk-1* gene. Black boxes represent exons. ATG indicates translation initiation codon. B, DLK-1::GFP was mainly expressed in neurons throughout the worm. Magnified views of head region (HEAD), body region (BODY), and tail region (TAIL) are shown in the lower panels. Scale bars=100 μm.

a part of the second exon (46 bp) of *dlk-1*, was digested with *Pst*I and then inserted into the *C. elegans* GFP expression vector pPD95.75 (kindly provided by Andrew Fire at Stanford University, USA). This construct was named pPDdg1 and the junction sequences were confirmed by DNA sequencing.

To obtain a 5' upstream region deletion construct, the pPDdg1 was partially digested with *Hin*dIII and self-ligated. A deletion construct (Δ-361 to -2246) was identified by restriction enzyme digestion and confirmed by DNA sequencing. This construct was named pPDdg2.

To obtain a first intron deletion construct, the pPDdg1 was amplified with primers DF1 and DR2. The PCR product, containing 2,246 bp of the 5' upstream region and 12 codons of the first exon of *dlk-1*, was digested with *Pst*I and then inserted into the pPD95.75. This construct was named pPDdg3.

Primers used are as follows: DF1, 5'-CTACTGCAG-CAGACAAGCTAAGCACTGT-3' (a *Pst*I site created is underlined); DR1, 5'-CTACTGCAGACTGGAGAGAAG TCGGAGGA-3' (a *Pst*I site created is underlined); DR2, 5'-ATCTGCAGAAG TCGAGAGTGGTTACCA-3' (a *Pst*I site is underlined). PCR was performed in a total volume of 50 μl containing 2.5 units of cloned Herculase DNA polymerase (Stratagene) or Ex Taq polymerase (Takara), 200 μM of each deoxynucleotide triphosphate, 40 pmol of each primer, and 300 ng of template DNA. PCR was done following the procedures recommended by the manufacturers.

Germline transformation

Transgenic nematodes were generated by gonadal injection of DNA plasmid constructs (100 ng/μl) together with the plasmid pRF4 (100 ng/μl) containing *rol-6* as a selectable marker into the wild-type N2 strain as described by Mello et al. (1991). Roller strains were selected and monitored for GFP expression.

C. elegans microscopy

Transgenic worms were mounted on a 5% agarose pad on a microscope slide wetted with 2~3 drops of 2.5 mM paraformaldehyde. A coverslip was applied onto the worms and the GFP expression pattern was determined by confocal microscopy (Lieca DM IRE2 with a TCS SP2 AOBS confocal system).

RESULTS AND DISCUSSION

C. elegans DLK-1 is mainly expressed in neurons

We used the GFP fusion technique to investigate the expression pattern of *C. elegans* DLK-1. From the C49G9 cosmid, we made a DNA clone comprising 5' upstream region (up to -2246 bp), the first exon, the first intron and a part of the second exon of the *dlk-1* gene by using PCR. We then inserted the DNA clone into the GFP vector pPD95.75.

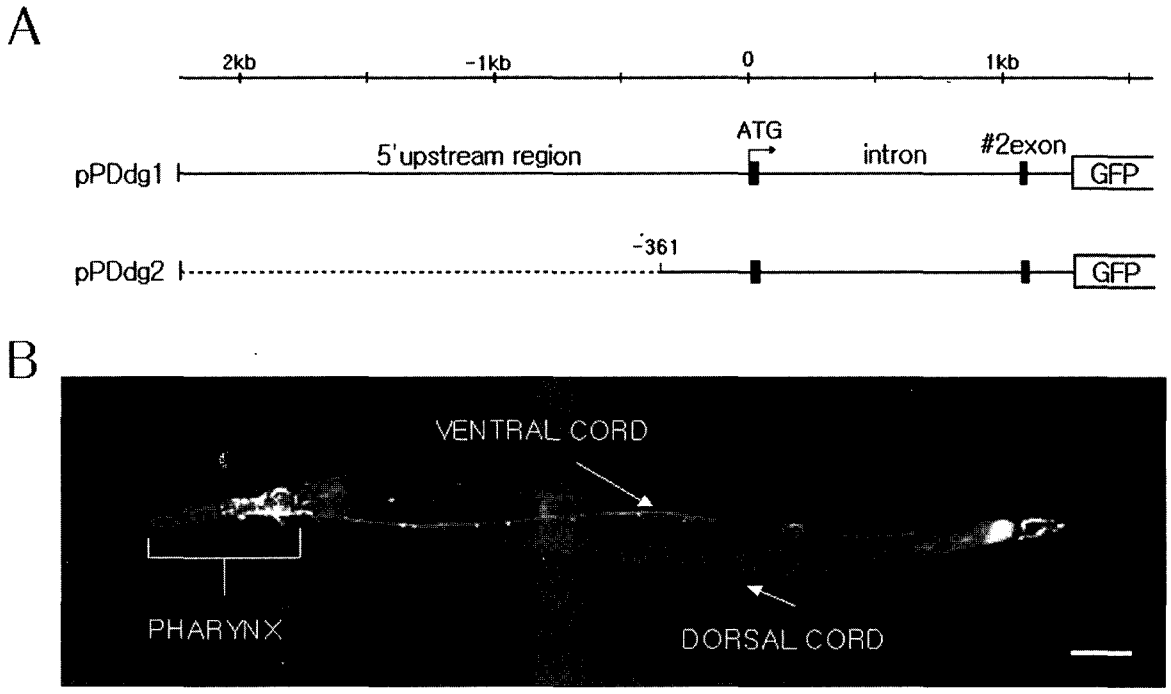

Fig. 3. Removal of 5' upstream region (Δ-361 to -2246) of the *dlk-1* gene exerts little effect on gene expression. A, Genomic organization of the 5' upstream region deletion construct (pPDdg2). Black boxes represent exons. ATG indicates translation initiation codon. B, GFP expression pattern of pPDdg2 was similar to that of pPDdg1 (shown in Fig. 1). Scale bar=100 μm.

This DLK-1::GFP construct was named pPDdg1 (Fig. 2A). After microinjecting pPDdg1 into worms, we determined the GFP expression pattern by confocal microscopy. As shown in Fig. 2B, GFP was mainly expressed in neurons throughout the worm. GFP was most strongly expressed in neurons surrounding the nerve ring. GFP was also observed in neurons along the ventral and dorsal cords. DLK-1::GFP appeared to be expressed in most if not all of the neurons, supporting the notion that DLK-1 is required for differentiation of presynaptic terminals (Nakata et al., 2005). In addition to the nervous system, DLK-1::GFP fluorescence was reproducibly seen in the cells of the tail region that might be associated with the digestive system (Fig. 2B).

5' upstream region (-361 to -2246) of the *dlk-1* gene has little effect on gene expression

To investigate the regulatory elements responsible for the *dlk-1* gene expression, we prepared a deletion construct of pPDdg1 (pPDdg2), which lacks a substantial portion of the 5' upstream region (Δ-361 to -2246) (Fig. 3A), by using

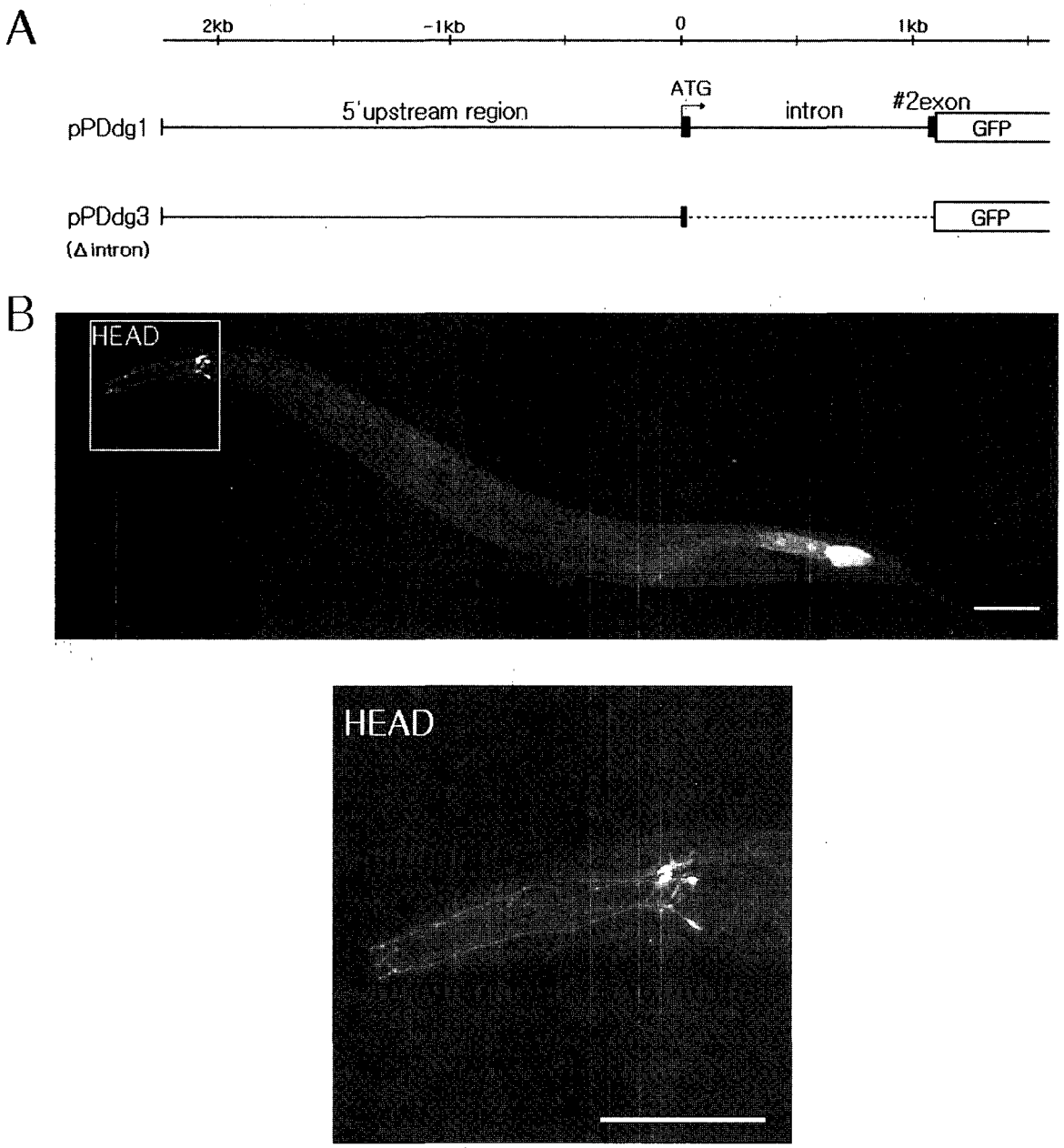

Fig. 4. Removal of first intron (Δintron) of the *dlk-1* gene substantially blocks gene expression. A, Genomic organization of the first intron deletion construct (pPDdg3). Black boxes represent exons. ATG indicates translation initiation codon. B, GFP expression was eliminated in most of the neurons. Only several neurons in the head region showed the GFP expression. A magnified view of head region (HEAD) is shown in the lower panel. Scale bars=100 μm.

PCR. We injected the deletion construct into *C. elegans* and analyzed the GFP expression pattern. As shown in Fig. 3B, the expression pattern of pPDdg2 was virtually identical to that of pPDdg1, implying that the critical regulatory element for the *dlk-1* gene expression does not reside in the deleted 5' upstream region (-361 to -2246).

First intron of the *dlk-1* gene appears to contain the critical element for gene expression

Previous studies have shown that in *C. elegans*, introns play important roles in the regulation of gene expression (Lee et al., 2000; Nam et al., 2002; Hwang and Lee, 2003). Thus, we tested if the first intron of the *dlk-1* gene is essential for gene expression. Again we used the PCR technique to obtain a deletion construct of pPDdg1 (pPDdg3), which lacks the last five codons of the first exon, the first intron and the second exon (Fig. 4A). Worms injected with this deletion construct did not express GFP in the nervous system, except for a few head neurons with ciliated endings (Fig. 4B). These results suggest that the first intron is critical for the regulation of the *dlk-1* gene expression, as it is unlikely that the exon sequences deleted in the pPDdg3 construct are involved in this process.

Comparative genomics between *C. elegans* and *C. briggsae*, which diverged from a common ancestor about 100 million years ago, can provide important insights into the regulatory elements in gene expression (Hwang and Lee, 2003; Stein et al., 2003). Nucleotide sequence comparison between *C. elegans* and *C. briggsae* reveals several conserved regions in the first intron of the *dlk-1* gene. We plan to analyze these regions and explore the regulatory mechanism of the *dlk-1* gene expression.

ACKNOWLEDGEMENTS

We would like to thank Dr. Audrey Fraser for providing the cosmid clone (C49G9) and Dr. Andrew Fire for providing the GFP vector (pPD95.75). This work was supported by Chungbuk National University Grant in 2004.

REFERENCES

Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71-94.

Chang L and Karin M (2001) Mammalian MAP kinase signaling cascades. *Nature* 410: 37-40.

Cross TG, Scheel-Toellner D, Henriquez NV, Deacon E, Salmon M, and Lord JM (2000) Serine/threonine protein kinases and apoptosis. *Exp Cell Res* 256: 34-41.

Gallo KA and Johnson SL (2002) Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nat Rev Mol Cell Biol* 3: 663-672.

Garrington TP and Johnson GL (1999) Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol* 11: 211-218.

Hwang SB and Lee J (2003) Neuron cell type-specific SNAP-25 expression driven by multiple regulatory elements in the nematode *Caenorhabditis elegans*. *J Mol Biol* 333: 237-247.

Kawai T, Matsumoto M, Takeda K, Sanjo H, and Akira S (1998) ZIP kinase, a novel serine/threonine kinase which mediates apoptosis. *Mol Cell Biol* 18: 1642-1651.

Kogel D, Plottner O, Landsberg G, Christian S, and Scheidtmann KH (1998) Cloning and characterization of Dlk, a novel serine/threonine kinase that is tightly associated with chromatin and phosphorylates core histones. *Oncogene* 17: 2645-2654.

Lee Y-S, Park Y-S, Nam S, Suh SJ, Lee J, Kaang B-K, and Cho NJ (2000) Characterization of GAR-2, a novel G protein-linked acetylcholine receptor from *Caenorhabditis elegans*. *J Neurochem* 75: 1800-1809.

Mello CC, Kramer JM, Stinchcomb D, and Ambros V (1991) Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J* 10: 3959-3970.

Nakata K, Abrams B, Grill B, Goncharov A, Huang X, Chisholm AD, and Jin Y (2005) Regulation of a DLK-1 and p38 MAP kinase pathway by the ubiquitin ligase RPM-1 is required for presynaptic development. *Cell* 120: 407-420.

Nam S, Jin Y-H, Li Q-L, Lee K-Y, Jeong G-B, Ito Y, Lee J, and Bae S-C (2002) Expression pattern, regulation, and biological role of runt domain transcription factor, *run*, in *Caenorhabditis elegans*. *Mol Cell Biol* 22: 547-554.

Pearson G, Robinson F, Beers Gibson T, Xu B-E, Karandikar M, Berman K, and Cobb MH (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 22: 153-183.

Seger R and Krebs EG (1995) The MAPK signaling cascade. *FASEB J* 9: 726-735.

Stein LD, Bao Z, Blasiar D, Blumenthal T et al. (2003) The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. *PLoS Biol* 1: 166-192.

Widmann C, Gibson S, Jarpe MB, and Johnson GL (1999) Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 79: 143-180.

Xia Z, Dickens M, Raingeaud J, Davis RJ, and Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* 270: 1326-1331.

Xu Z, Maroney AC, Dobrzanski P, Kukekov NV, and Greene LA (2001) The MLK family mediates c-Jun N-terminal kinase activation in neuronal apoptosis. *Mol Cell Biol* 21: 4713-4724.

[Received April 29, 2005; accepted June 20, 2005]