

Mutations in Chemosensory Cilia Cause Resistance to Paraquat in Nematode *Caenorhabditis elegans**

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The relationship between oxidative stress and longevity is a matter of concern in various organisms. We isolated mutants resistant to paraquat from nematode *Caenorhabditis elegans*. One mutant named *mev-4* was long-lived and showed cross-resistance to heat and Dyf phenotype (defective in dye filling). Genetic and sequence analysis revealed that *mev-4* had a nonsense mutation on the *che-11* gene, homologues of which are involved in formation of cilia and flagella in other organisms. The paraquat resistance was commonly observed in various Dyf mutants and did not depend on the *daf-16* gene, whereas the extension of life span did depend on it. Expression of antioxidant enzyme genes seemed normal. These results suggest that chemosensory neurons are a target of oxidative stress and influence longevity dependent on the *daf-16* signaling in *C. elegans*.

The life spans of animals are determined by both environmental and genetic parameters. Accumulating evidence in model organisms demonstrates the importance of genetic approaches with the findings that single gene mutations affect the life span in nematode *Caenorhabditis elegans*, fruit fly *Drosophila melanogaster*, and laboratory mice. The key to understanding longevity seems to lie in the network of cell maintenance systems that reduce accumulation of deleterious stresses. The life span of nematodes is controlled by the insulin-like signals from the nervous system (1–3). Such signals also seem to control life span of the fruit fly and mice (4–7). These results suggest that neuroendocrine pathways in the neurons constitute an important determinant of life span across phylogeny (8–11).

Various lines of evidence show that oxidative stress is a major damaging factor accelerating aging (12, 13). It is invoked by reactive oxygen species (ROS)¹ generated as chemical by-products of normal cellular metabolisms. Caloric restriction is shown to be beneficial in decreasing the production of ROS in metabolic pathways such as the mitochondrial electron transport system (13). Animals have evolved defense mechanisms

against ROS; antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase work to eradicate ROS as a first aid (14). However, much remains to be understood as regards the defense mechanisms in diverse tissues of vertebrates. To facilitate understanding of the mechanisms, *C. elegans* and *D. melanogaster* are frequently used as a multi-cellular model organism because powerful genetic analysis is possible.

In *C. elegans*, longevity is affected by particular genes involved in dauer larvae formation (15–17), stress resistance (18–24), mitochondrial function (25–28), caloric restriction (29), reproduction (30–32), sensory perception (33), neurosecretory function (34), and chromatin silencing (35). Although multiple factors seem to be involved in the longevity, there exist a positive relationship between the capacity to resist oxidative stress and the longevity (12). Nonetheless, the target of oxidative stress is yet to be understood even in this model organism. To address this question empirically, we isolated mutants of *C. elegans* with altered sensitivity to oxidative stress using paraquat (methyl viologen) as a selecting agent. In this study, we report that one of the paraquat-resistant mutants with extended life span is defective in the *che-11* gene that seems to be involved in the function of chemosensory cilia (33, 36). We also examined sensitivity to paraquat in various mutants of chemosensory perception.

EXPERIMENTAL PROCEDURES

Strains and Culture Conditions—The *C. elegans* strains used were obtained from the *C. elegans* Genetic Center. Worms were grown and maintained at 20 °C on NG plates seeded with *Escherichia coli* OP50 as a food source as described by Brenner (37) unless otherwise mentioned.

Mutant Isolation—L4 larvae of wild-type N2 were treated with 50 mM ethylmethane sulfonate for 4 h and cultured to bear F2 progenies. The worms were cultured singly on 96-well titer plates containing bacteria in liquid medium. These worms were allowed overnight to lay eggs, and mixtures of the eggs and hatched L1 larvae were transferred to fresh NG plates containing 0.4 mM paraquat. After incubation for 4–5 days, the plates were examined under a microscope to find adult worms. Eggs from these worms were prepared individually by treatment with alkaline hypochlorine as described previously (37) and placed on agar medium containing 0.4 mM paraquat to isolate worms that can grow under the selective conditions.

Genetic Analysis—Genetic crosses were performed as described previously (37). To assign a chromosome (LG V) to *mev-4* locus, its linkage with the following genetic markers were examined: *dpy-5(e61)* for LG (linking group) I, *rol-5(sc13)* for LG II, *dpy-1(e1)* for LG III, *unc-22(e66)* for LG IV, *dpy-11(e224)* for LG V, and *lon-2(e678)* for LG X. To regionally map *mev-4* locus on LG V, three factor-crosses were carried out with the following combinations of the markers: *dpy-11(e224) unc-42(e270)*, *rol-4(sc8) lin-25(n545)*, *him-5(e1467) unc-76(e911)*, *rol-4(sc8) unc-61(e228)*, *unc-42(e270) rol-4(sc8)*, *sma-1(e30) unc-76(e911)*, and *unc-42(e270) lon-3(e2175)*.

Analysis of Brood Size—The eggs were prepared and allowed to hatch by overnight incubation in S-basal buffer. Hatched L1 larvae

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¹ The abbreviations used are: ROS, reactive oxygen species; SOD, superoxide dismutase; FITC, fluorescein isothiocyanate; IFT, intraflagellar transport.

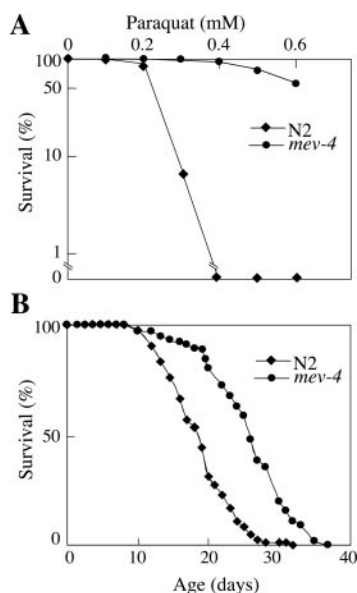


FIG. 1. Sensitivity to paraquat (A) and longevity (B) in mutant *mev-4* and the wild-type N2. In A, the eggs were incubated at 20 °C on medium containing various concentrations of paraquat for 4 days, and animals that reached adulthood were counted as described under "Experimental Procedures." In B, the animals remaining alive during cultured at 20 °C were counted as described under "Experimental Procedures."

were transferred to NG plates and incubated to develop to L3–L4 larvae. Individual worms were transferred to fresh plates every 24 h, and the numbers of laid eggs were scored until the worms ceased to lay eggs.

Assay of Sensitivities to Stresses—Sensitivity to paraquat was determined as described by Ishii *et al.* (38) with a slight modification. The eggs, instead of L1 larvae, were placed on growth medium containing various concentrations of paraquat. After incubation for 4–5 days, the number of adult worms was scored. Sensitivity to thermal stress was determined as described by Lithgow *et al.* (20). Briefly, L1 larvae were cultured on NG plates for 4 days. Then 50 adult worms were incubated at 36 °C on fresh NG plates, and their survival was examined at intervals. Similarly, 50 adult worms were placed on bacteria-free plates and exposed to UV light (40 J/m²) as described by Murakami and Johnson (39). Then they were transferred to NG plates and examined for their survival during subsequent culture at 20 °C.

Assay of Life Span—Life span was determined as described by Ishii *et al.* (38). L1 larvae were allowed to hatch by overnight incubation in S buffer and transferred to NG plates to develop to L4 larvae. A hundred of the L4 larvae were transferred to NG plates supplemented with 40 μ M 5-fluoro-2'-deoxyuridine to suppress the production of their progenies. The worms were examined daily, and dead worms, which did not move after we touched their heads with a platinum wire, were removed to count. The worms dead by desiccation at the walls of plates were excluded from the analysis. The worms on the plates contaminated by other microorganisms were also excluded from analysis because the life spans of the worms were affected by the concentration of dietary bacteria (40).

Staining of Chemosensory Neurons with FITC—Chemosensory neurons of worms were stained as described previously (41). Gravid adult worms were collected in S buffer, washed once, and incubated at 4 °C in S buffer containing 0.4 mg/ml of FITC for 4 h. The worms were fixed with formaldehyde after washing with S buffer, and fluorescence was observed under a fluorescence microscope.

Northern Blot Analysis—Total RNA samples were prepared from worms using the acid-guanidine-phenol-chloroform method as described previously (42). RNAs were resolved by formaldehyde gel electrophoresis and blotted onto nylon membrane (Biodyne). The membrane was hybridized with each cDNA probe labeled with [α -³²P]dCTP using random-primed DNA labeling kit (Amersham Biosciences) as described previously (43). After washing, the filter was exposed to x-ray film, and the signals were quantified by image analyzer BAS2000 (Fuji Film).

DNA Sequencing—Sequences of the *che-11* gene (C27A7.4) were amplified by PCR using *mev-4(qa5000)* and *che-11(e1810)* genomic DNA as

TABLE I
Survival after exposure to heat or UV light in *mev-4* and N2
n, number of individuals tested. The values are from two independent experiments done as described under "Experimental Procedures."

Experiment	Strain	Means \pm S.D.	Maximum	n
Heat	N2	3.9 \pm 0.1 h	6 h	50
	<i>mev-4</i>	6.6 \pm 0.2 h ^a	10 h	49
	N2	4.3 \pm 0.1 h	6 h	48
	<i>mev-4</i>	6.0 \pm 0.2 h ^a	9 h	50
UV	N2	3.1 \pm 0.3 days	10 days	46
	<i>mev-4</i>	2.3 \pm 0.1 days	4 days	48
	N2	2.4 \pm 0.2 days	7 days	40
	<i>mev-4</i>	2.4 \pm 0.3 days	10 days	39

^a The probability was <0.0001.

templates and primers: 5'-ATGGAGGAGTTTGTTCCTTATCC-3' and 5'-TTACGAAACATTTTGTCTCCGT-3'. PCR products were cut into several fragments with appropriate restriction endonucleases and cloned into a plasmid vector. Several clones obtained from each fragment were sequenced with a DNA sequencer LI-COR 2000 (LI-COR) as described previously (42).

RESULTS

Isolation and Characterization of *mev-4* Mutant—To isolate mutants with altered sensitivity to oxidative stress, we used paraquat as a selective agent. This herbicide is known to generate superoxide radicals in a living cell and confer oxidative stress in various organisms including *C. elegans* (38, 44). We screened a total of ~2,500 F2 worms born from the wild-type N2 worms that had been mutagenized with ethylmethane sulfonate and isolated several mutants capable of vigorously growing on the selective plate. Among them, we successfully characterized one mutant named *mev-4(qa5000)* after purifying its mutation by backcrossing five times to N2 worms.

This mutant was highly resistant to paraquat (Fig. 1A). For example, N2 worms hardly grew to adulthood in the presence of 0.3 mM paraquat, and none reached adulthood in the presence of 0.4 mM paraquat within 4 days. In contrast, all of the mutant worms grew normally under these conditions, and more than half of them grew to adulthood even in the presence of 0.6 mM paraquat. The mean and maximum life spans of *mev-4* were 30 and 20% longer on averages than those of N2, respectively, when cultured at 20 °C (Fig. 1B). When cultured at 26 °C, they were 15 and 23% longer than those of N2, respectively (data not shown).

Then we investigated sensitivity to thermal stress and UV light, both typical stress markers in *C. elegans*. When incubated at 36 °C, mean and maximum survival times were significantly longer in *mev-4* than in N2 (Table I). Then adult worms were irradiated with UV light and cultured until all died. The mean and maximum survival values were not significantly different between *mev-4* and N2 (Table I). We also examined fecundity in the mutant and N2 because the reproduction system is shown to affect longevity in *C. elegans* (30–32). N2 and *mev-4* laid 276 \pm 15 (n = 19) and 280 \pm 25 (n = 11) eggs, respectively, thereby showing no difference between these strains.

Characterization of the Mutated Gene in *mev-4*—The paraquat-resistant trait was genetically recessive and inherited to progenies in a Mendelian fashion. Linkage analysis revealed that the trait was concordant with the genetic marker *dpy-11* located on LG V. Therefore, we regionally mapped *mev-4* locus on LG V using three factor crosses with appropriate genetic markers. We obtained the following results: (*dpy-11 unc42*) (8/8) *mev-4*, *mev-4* (14/14) (*rol-4 lin-25 him-5 unc-76*), *mev-4* (4/4) (*rol-4 unc-61*), *unc-42* (15/18) *mev-4* (3/18) *rol-4*, *sma-1* (1/20) *mev-4* (19/20) *unc-76*, and (*unc-42 lon-3*) (29/29) *mev-4*. Thus, *mev-4* mutation was mapped to a region close to *lon-3*

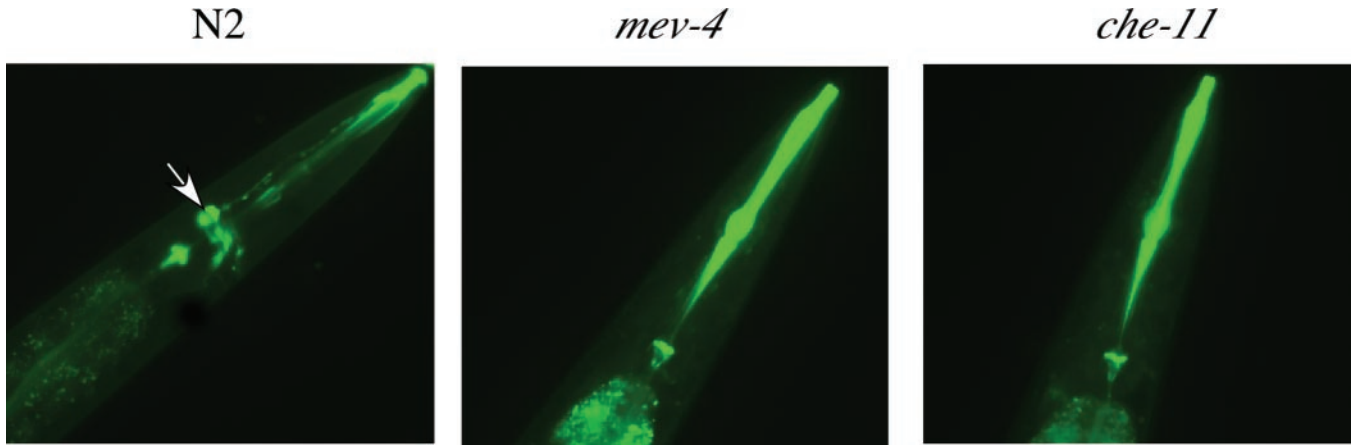


FIG. 2. **Staining of chemosensory neurons with FITC.** Gravid adult worms were incubated with FITC, and fluorescence was observed under a fluorescence microscope as described under "Experimental Procedures." An arrow in the wild-type N2 indicates stained chemosensory neurons in amphids.

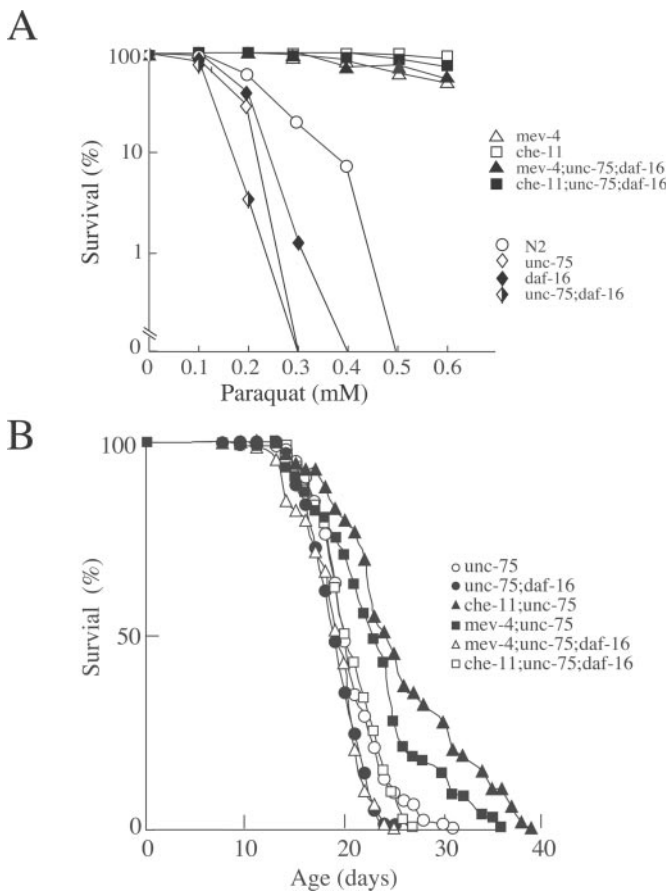


FIG. 3. **Effect of coexistence of a *daf-16* mutation on sensitivity to paraquat (A) and longevity (B) in *mev-4*.** Triple mutants were made by crossing *mev-4* or *che-11* to *daf-16(m26)* *unc-75(e950)* double mutant as described under "Experimental Procedures." Sensitivity to paraquat and longevity in the triple mutants and their related strains were determined as in Fig. 1.

locus. This region contains *che-11* locus. *che-11* exhibits a defect in chemosensory perception because of irregular sensory ciliary segments with the extension of the life span (33, 36). Therefore, we tested a possibility that *mev-4* is allelic to *che-11*. As expected, the chemosensory neurons of *mev-4(qa5000)* were not stained or very weakly stained with FITC similar to *che-11(e1810)*, whereas those of N2 were clearly stained (Fig. 2). Consistent with this finding, they showed a defect in osmotic avoidance (not shown). In addition, *che-11* was found to be

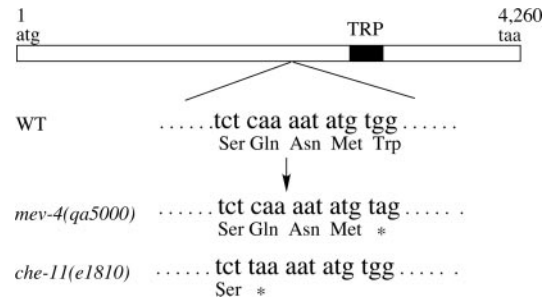


FIG. 4. **Schematic illustration of putative protein encoded by the *mev-4/che-11* gene.** An open reading frame of 4,260 bp encoding a putative protein of 1,419 amino acids is shown. The nonsense mutation was marked with an asterisk. WT, wild type.

resistant to paraquat as *mev-4* (Fig. 3A). We thus constructed worms (*rol-4 mev-4/che-11*) heterozygous for *mev-4* and *che-11* mutations and found that this heterozygote was as resistant to paraquat as *mev-4* (data not shown). These results suggest that the two mutants are identical.

Based on the above data, we sequenced the *che-11* gene (C27A7.4) that contains an open reading frame of 4,260 bp encoding a putative protein of 1,419 amino acids (Sanger Center web site and Ref. 45). The open reading frame of *mev-4* was found to have a transition from G to A at position 2,474, which results in conversion of 825Trp (TGG) to a stop codon (TAG). *che-11(e1810)* mutant also had a transition from C to T at position 2,464, resulting in the conversion of 822Gln (CAA) to a stop codon (TAA) (Fig. 4). This putative protein is homologous to human hypothetical protein KIAA0590 with 29.9% identity and 55.4% similarity and *Drosophila* protein CG11838 with 24.0% identity and 49.4% similarity. This protein is also homologous to IFT140 protein involved in intraflagellar transport (IFT) in *Chlamydomonas reinhardtii* (45). In *Chlamydomonas*, the biochemically identified IFT particle is composed of 16 polypeptides that can be classified into two complexes: complex A containing four polypeptides and complex B containing 12 polypeptides. IFT140 constitutes one of the complex A proteins with TRP motifs necessary for protein-protein interaction (46–48). Similar to *mev-4/che-11*, other ciliary mutants in *C. elegans* seem to have a defect in its possible IFT complex proteins (45). In addition, when CHE-11 protein was expressed using a *che-11::gfp* construct in *C. elegans*, GFP fluorescence was found in IFT particles in sensory neurons (45, 49). Taken together, we conclude that *mev-4* is defective in the function of chemosensory cilia.

TABLE II
Relevant properties in the mutants of chemosensory perception

Mutant	Paraquat resistance ^a	Lifespan ^b	FITC uptake	Genetic defect
N2	—	1.0	+	
<i>che-1(p679)</i>	—	ND	ND	ASE function
<i>che-2(e1033)</i>	++	1.4	—	Sensory cilia
<i>che-3(p801)</i>	+++	2.0	—	Sensory cilia
<i>che-5(e1073)</i>	—	ND	ND	
<i>che-6(e1126)</i>	—	ND	ND	ASE function
<i>che-7(e1128)</i>	—	ND	ND	
<i>che-9(e75)</i>	—	ND	ND	
<i>che-10(e1809)</i>	++	ND	—	Sensory cilia
<i>che-10(qa5011)^c</i>	++	1.2	—	Sensory cilia
<i>che-11(e1810)</i>	+++	1.5	—	Sensory cilia
<i>che-12(e1812)</i>	+++	ND	—	Sheath cells
<i>che-13(e1805)</i>	+++	1.3	—	Sensory cilia
<i>che-14(e1960)</i>	—	ND	—	Socket and sheath cells
<i>osm-1(p808)</i>	+++	1.4	—	Sensory cilia
<i>osm-3(p802)</i>	+++	1.6	—	Sensory cilia
<i>osm-5(p813)</i>	+++	2.2	—	Sensory cilia
<i>osm-6(p811)</i>	++	1.6	—	Sensory cilia
<i>daf-6(e1377)</i>	++	1.3	—	Socket and sheath cells
<i>daf-10(e1387)</i>	+++	1.6	—	Sensory cilia
<i>daf-19(m86)</i>	—	1.3	—	Sensory cilia
<i>mec-1(e1066)</i>	+	1.0	—	Fasciculation
<i>mec-8(e398)</i>	—	1.6	—	Fasciculation
<i>tax-2(p691)</i>	+	1.2	ND	Axon guidance
<i>tax-4(p678)</i>	—	1.9	ND	Axon guidance
<i>dyf-1(mn335)</i>	++	ND	—	
<i>dyf-2(m160)</i>	++	ND	—	
<i>dyf-2(m881)^d</i>	ND	ca.1.8	—	
<i>dyf-3(m185)</i>	++	ND	—	
<i>dyf-4(m158)</i>	++	ND	—	
<i>dyf-5(mn400)</i>	++	ND	—	
<i>dyf-6(m175)</i>	+++	ND	—	
<i>dyf-7(m537)</i>	+++	ND	—	
<i>dyf-8(m539)</i>	++	ND	—	
<i>dyf-9(n1513)</i>	+++	ND	—	
<i>dyf-10(e1383)</i>	+++	ND	—	
<i>dyf-11(mn392)</i>	+++	ND	—	
<i>dyf-12(sa127)</i>	++	ND	—	
<i>dyf-13(mn396)</i>	++	ND	—	

^a The phenotypes were determined as described under “Experimental Procedures.” ND, not determined. —, +, ++, and +++ denote that worms did not reach adulthood or did not on the fifth, fourth, and third days, respectively, during a 5-day incubation on medium containing paraquat.

^b Values relative to N2 (1.0) were shown using data (33) except for *che-10(qa5011)* and *dyf-2(m881)*.

^c The data of *mev-8(qa5011)* identical to *che-10*.

^d The data in Ref. 64.

Paraquat Sensitivity in Chemosensory Mutants—We tested sensitivity to paraquat in the following mutants in chemosensory perception (Table II) (36, 50–52). *daf-19* lacks all cilia. *che-2*, *che-13*, *osm-1*, *osm-5*, and *osm-6* have a deletion in the middle and/or distal segments of cilia. *che-3*, *che-11*, and *daf-10* have reduced or irregular cilial segments. *che-12*, *che-14*, and *daf-10* have a defect in the socket and/or sheath cells. *mec-1* and *mec-8* have a defect in amphid cilia fasciculation. *tax-2* and *tax-4* seem to have normal cilia but have a defect in AWC- and ASE-mediated chemotaxis and axon outgrowth (53, 54). *che-1* and *che-6* seem to have a defect in ASE sensory neuron function (54, 55). *dyf-1* to *dyf-13* can not uptake FITC, possibly because of chemosensory cilial dysfunction.

An outstanding finding is that most of the above mutants exhibited strong resistance to paraquat although *che-14*, *daf-19* and *mec-8* did not (Table II). *daf-19* grew very poorly under normal culture conditions, and *che-14* showed a weak activity to uptake FITC into chemosensory neurons. However, levels of the residual activity to uptake FITC did not clearly correlate with their resistance to paraquat, because one of such mutants *che-12(e1812)* (36) showed marked resistance to paraquat. Despite some ambiguities, the results demonstrate that a defect in chemosensory perception makes worms resistant to paraquat.

Effects of *daf-16* Pathway—Many mutants of *C. elegans* with extended life span are shown to depend on DAF-16 activity (17,

31–33, 35, 56, 57). The *daf-16* gene encodes a forkhead transcription factor and plays a key role in expression of genes involved in formation of dauer larvae and tolerance to various stresses (58, 59). In fact, extended life span, resistance to oxidative stress, and up-regulation of the *sod-3* gene in *daf-2* mutants are not observed on *daf-16* backgrounds (17, 60). We thus examined whether the phenotypes of *mev-4* were affected by coexistence of a *daf-16* mutation.

We crossed *mev-4(qa5000)* to *daf-16(m26) unc-75(e950)* double mutant to obtain the *mev-4 daf-16 unc-75* triple mutant. The triple mutant was extremely resistant to paraquat, whereas the *daf-16 unc-75* double mutant and the *daf-16* single mutant were significantly more sensitive to paraquat than N2 (Fig. 3A). Therefore, the paraquat-resistant trait in *mev-4* was not affected by the *daf-16* mutation. The same result was obtained in *che-11(e1810)*.

Then we examined life spans of the above mutants (Fig. 3B). The double mutant *mev-4 unc-75* showed significant extension of life span. However, the *mev-4 daf-16 unc-75* triple mutant showed a normal level of life span. Similar results were obtained in *che-11*. Therefore, the extension of life span in *mev-4* and *che-11* depend on the DAF-16 activity, demonstrating that the paraquat resistance can be uncoupled with the extension of life span.

Antioxidant Enzyme Genes—We examined mRNA levels for major antioxidant enzymes in *mev-4* and N2. The levels for

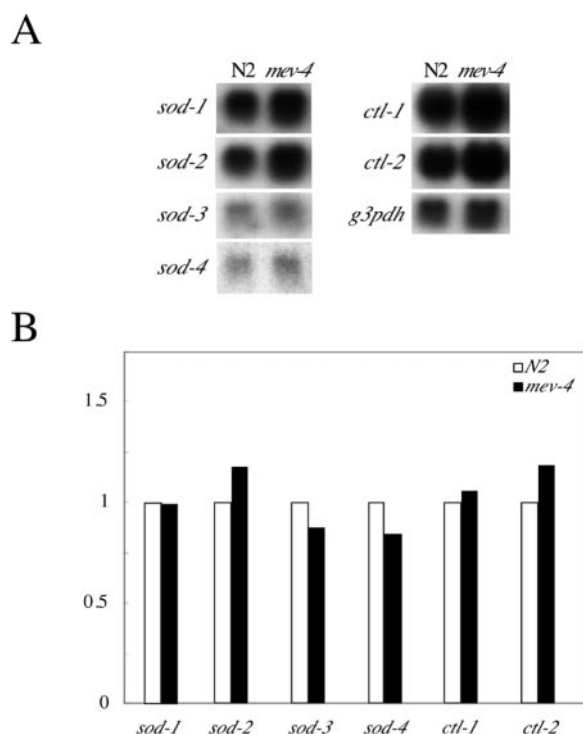


FIG. 5. Northern blot analysis of mRNA levels for antioxidant enzymes. Total RNA samples were subjected to Northern blot analysis (A), and signals were quantified by an image analyzer (B) as described under "Experimental Procedures."

cytosolic SOD (*sod-1*), two mitochondrial SODs (*sod-2* and *sod-3*), extracellular and membrane bound SODs (*sod-4*), cytosolic catalase (*ctl-1*), and peroxisomal catalase (*ctl-2*) were not significantly different between *mev-4* and N2 when normalized by that of *g3pdh* (Fig. 5). These results indicate that the paraquat-resistant trait in *mev-4* has not arisen by overexpression of such antioxidant genes.

DISCUSSION

C. elegans hermaphrodite animals have 302 neurons, of which 12 are chemosensory neurons in amphid. The chemosensory neurons sense environmental cues and chemical signals are processed and integrated with other signals to specify adult longevity (31, 33). The signals influence chemotaxis, thermotaxis, and dauer formation, but their roles in longevity are not well understood. Here we demonstrated that various mutations in chemosensory apparatus cause marked resistance to paraquat. These results suggest that neurons are direct targets of paraquat. Mutations in the *daf-2* insulin-like receptor (2) or the downstream *age-1* phosphoinositide 3-kinase genes (1) give rise to resistance to oxidative stress (19), UV (39), and heat (20). These phenotypes depend on DAF-16 activity (60, 61). When the *daf-2* signaling was specifically restored in neurons, muscle, or intestine in *daf-2* mutants to identify tissues where it regulates aging and metabolism (3), only neurons were able to specify wild-type life span. Enforced expression of SOD in motor neurons in *D. melanogaster* was also shown to extend the life span (62). These observations imply that oxidative stress acts in neurons to specify the life span.

Antioxidant enzymes (e.g. SODs and catalases) do not seem to be involved in the paraquat resistance in *mev-4* because they were not up-regulated. In *daf-2* mutants, the *sod-3* gene is up-regulated through activation of the DAF-16 activity (60). Therefore, *mev-4/che-11* might have acquired it through a mechanism not involving the *daf-2* signaling. A simple explanation is that neurons are highly sensitive to oxidative stress in

C. elegans, and these mutants become resistant to paraquat because of the inability to uptake paraquat into chemosensory neurons. Laser-assisted ablation of chemosensory neurons in amphid has revealed that they are not necessary for the post-embryonic viability of worms, and disruption of all of them leads to constitutive formation of dauer larvae (54, 63). When worms were cultured in the presence of paraquat for more than a week, the majority of them died instead of becoming dauer larvae. This implies that paraquat may damage the entire nervous system.

Many mutants in chemosensory cilia showed extended life spans dependent, at least in part, on the DAF-16 activity (33). DAF-16 seems to be activated in the ciliary structure mutants because it specifically accumulates in the nuclei of these mutants (61). The extended life span in *mev-4/che-11* can be explained in this context. On the contrary, the paraquat resistance did not depend on DAF-16, indicating that these two phenotypes in *mev-4/che-11* are uncoupled. Furthermore, resistance to heat shock did not always cause extension of life span in the worms. For instance, other mutants such as *mev-6(qa5006)* and *mev-7(qa5007)* were resistant to both paraquat and thermal stress, but they were not long-lived.² Therefore, paraquat or heat tolerance does not directly correlate with longevity, despite the fact that resistance to stress generally favors extended longevity in many organisms (64).

The mutants in chemosensory cilia show abnormalities not only in chemotaxis but also in dauer formation. In these mutants, their chemosensory cilia cannot sense outside signals, but their neurons can work normally. In these situations, worms may take actions as if they are under starved or unfavorable conditions because they may transmit to tissues those signals that there are no chemical attractants or foods. Thus, the absence of chemosensory signals may cause a caloric restriction state or a dauer-like state, although food intake and behaviors seem normal (33). If so, it is no wonder that a mutation in chemosensory cilia can affect metabolism, stress resistance, and longevity in *C. elegans*. Recently, it was reported that multiple genes involved in metabolism and stress resistance act downstream of DAF-16 and affect longevity in the nematode (65, 66).

Finally, most of the paraquat-resistant mutants showed a defect in chemosensory ciliary functions, but not all of them showed extension of life span. We examined the life spans in more than 50 paraquat-resistant mutants and found that ~20% of them were long-lived.² Revealing a signal from chemosensory neurons that causes extension of life span will be important for understanding aging in higher eukaryotes.

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