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Isolation of *Caenorhabditis elegans* Mutants Defective in Chemotaxis toward cAMP

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Abstract: Chemotactic behavior is essential for the survival of animals. However, the mechanism by which animals carry out chemotaxis is poorly understood. To explore the biochemical events underlying chemotaxis, we isolated *C. elegans* mutants that displayed abnormal chemotactic responses to cAMP, a strong attractant for *C. elegans*. Based on their responses to other chemoattractants, the mutant animals could be classified into five groups: (1) animals with defective chemotaxis to cAMP only; (2) animals with defective chemotaxis to both cAMP and cGMP; (3) animals with defective chemotaxis to water-soluble attractants; (4) animals with defective chemotaxis to both water-soluble and volatile attractants; and (5) animals with enhanced chemotactic responses. We expect that analyses of these mutants will help understand the molecular mechanisms underlying chemotaxis in *C. elegans*.

Key words: Chemotaxis, *C. elegans*, cAMP, animal behavior, mutant

Animal behavior is an outcome of a complex interplay of the nervous system. The nervous system consists of the sensory system, the motor system, and the integrating system that connects the sensory system and the motor system. The integrating system has evolved rapidly compared with the sensory or the motor system. The mammalian nervous system, especially the integrating system, is highly intricate and it is very difficult to understand the working mechanisms of the nervous system in molecular terms. Thus, it is often desirable to use an animal model with a relatively simple nervous system for the study of behavior.

Caenorhabditis elegans is a soil-dwelling animal with a size of 1 mm. The nervous system of an adult hermaphrodite contains only 302 neurons and the neuronal connections have been described by electron microscopic analysis (White et al., 1986). *C. elegans* is easily cultured and maintained in the laboratory by feeding them with bacterial cells (Brenner, 1974). Expression of genes in the live animals can be monitored by using GFP fusion technique (Chalfie et al., 1994), and specific gene knockdown is possible by RNA interference (Fire et al., 1998). Despite its simple nervous system, *C. elegans* displays a relatively diverse array of behaviors, including chemotaxis and thermotaxis (Bargmann, 1993; Mori and Ohshima, 1997). Together with convenient genetic and molecular biological techniques available, *C. elegans* could provide an excellent model system for the study of molecular neurobiology (Bargmann, 1998).

Chemotaxis is a chemical-seeking or -avoiding behavior that involves the sensory system, the integrating system, and the motor system. Animals recognize chemical signals from the environment with the chemosensory system, process the information through the integrating system, and move toward attractive chemicals or escape from repellent chemicals using the motor system. *C. elegans* has been known to chemotax to various attractants such as positive charges (Na^+, Li^+, and K^+ ions), negative charges (Cl^- and SO_4^{2-} ions), cyclic nucleotides (cAMP and cGMP), amino acids (lysine, histidine, and arginine), alcohols (1-butanol, 1-hexanol, and isoamyl alcohol), ketones (acetone and diacetyl), esters (ethyl acetate, isoamyl acetate, and ethyl butyrate), and pyrazines (pyrazine and 2-methyl pyrazine) (Ward, 1973; Bargmann et al., 1993; Jeong et al., 1996). Previous studies indicated that *C. elegans* chemotaxis is an excellent model to study behavior at the molecular level (Bargmann et al., 1993; Colbert and Bargmann, 1995; Troemel et al., 1995; Sengupta et al., 1996; Troemel et al., 1997).

In this study we isolated *C. elegans* mutants defective in chemotaxis to cAMP. These mutants could be classified into five groups according to their chemotactic responses to

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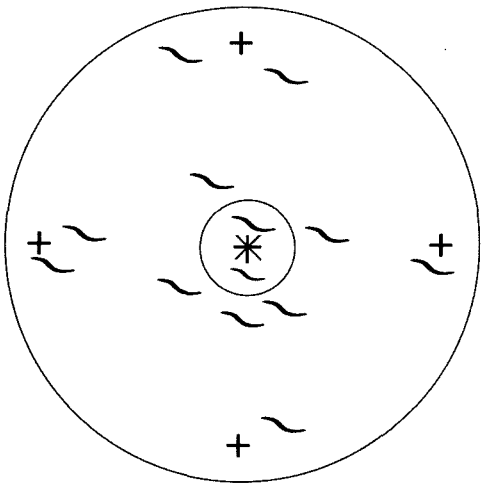

Fig. 1. Measurement of chemotaxis index. Two microliters of an attractant was applied at the center (asterisk) of a plate (3 cm diameter). After 3 h (5 min for isoamyl alcohol), worms were placed at four sites (crosses) and allowed to move toward the attractant. After 5 to 60 min of chemotaxis, worms present within a circle (0.6 cm diameter) at the center of the plate were counted and the chemotaxis index value was determined as described in Materials and Methods. Worms are not drawn to scale.

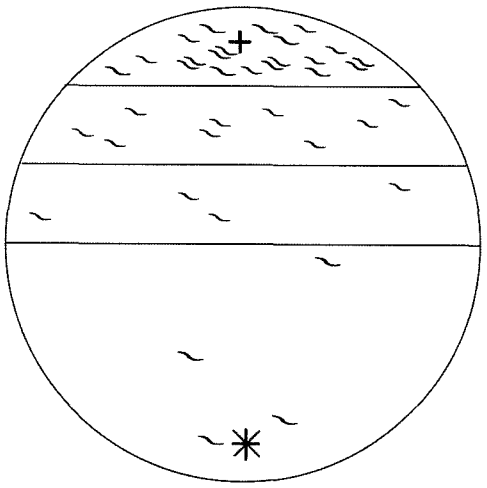

Fig. 2. Strategy for isolation of mutants defective in chemotaxis toward cAMP. Five microliters of cAMP solution (2 M) was applied at a pole (cross) in a plate (9 cm diameter). And three lines of cAMP solution (0.2 M) were drawn using a brush at intervals of 1.5 cm. Five microliters of CH_3COONa solution (0.5 M) was also applied at the opposite pole (asterisk) as a counter-attractant. Mutagenized worms were placed at the pole (cross) where 2 M of cAMP solution had been applied. After 20 to 30 min, worms that existed at the lower half of the plate were considered as putative mutants having a defect in chemotaxis to cAMP. Worms are not drawn to scale.

various kinds of attractants. The different groups of mutants support the idea that distinct subsets of genes control the chemotactic behavior of the animal at various levels.

MATERIALS AND METHODS

Strain

Wild-type nematodes used in this study were *C. elegans* variety Bristol, strain N2. Nematodes were grown and maintained as described previously (Brenner, 1974).

Chemotaxis assay

Chemotaxis was measured essentially as described by Ward (1973). Briefly, a gradient of an attractant was established in a 3-cm plate by applying the attractant solution at the center of the plate and allowing the attractant to diffuse (Fig. 1). Approximately one hundred worms were placed at four edges of the plate to test chemotaxis. After 5 to 60 min, worms that gathered within a 0.6-cm center circle in the plate were counted to determine the chemotaxis index.

Chemotaxis index (%) = (Number of worms in the center circle/Total number of worms) × 100

Isolation of *C. elegans* mutants defective in chemotaxis to cAMP

Wild-type worms were mutagenized with the treatment of 1% ethyl methanesulfonate (EMS) essentially as described by Dusenbery et al. (1975). Mutagenesis was done at 20°C for 4 to 6 h. F2 progeny of the mutagenized worms were tested for their ability to move toward cAMP. For screening of *cca* (chemotaxis to cAMP) mutants, cAMP solution (2 M) was applied at one edge of a 9-cm plate while CH_3COONa solution (0.5 M) was applied at the other edge of the plate as a counter-attractant (Fig. 2). About 500 mutagenized worms were placed at the spot where cAMP solution had been applied. Worms that moved away from the cAMP solution toward the CH_3COONa solution were considered as putative *cca* mutants. To help prevent the wild-type worms from moving toward the counter-attractant, three lines of cAMP solution (0.2 M) were drawn using a brush with an interval of 1.5 cm between the lines. Putative *cca* mutants were tested by chemotaxis assay as described above, and the worms that consistently showed the chemotaxis index value less than 30% for 0.5 M cAMP were isolated as *cca* mutants (the chemotaxis index value for the wild type was typically higher than 40% for 0.5 M cAMP). A mutant with enhanced chemotaxis to cAMP was isolated by chance and named as *cca-501*. No defect in movement or morphology was observed in the *cca* mutants.

RESULTS AND DISCUSSION

Chemotaxis of *C. elegans* toward cyclic nucleotides

Previous studies have shown that *C. elegans* can move toward many chemicals called attractants. These attractants include water-soluble chemicals (e.g., salts, amino acids, and cyclic nucleotides) and volatile chemicals (e.g., aldehydes, alcohols, and ketones) (Ward, 1973; Bargmann et al., 1993; Jeong et al., 1996). With a long-term goal to understand the molecular

mechanisms underlying chemotaxis, we isolated and characterized the mutants that were defective in chemotaxis to an attractant. The attractant we chose for this study was cAMP, which is found in virtually all living organisms. In many eukaryotes, cAMP serves as an intracellular second messenger for various extracellular signals. In *Dictyostelium*, cAMP is known to induce the aggregation of individual cells into a multicellular organism and regulate the developmental program (Strmecki et al., 2005).

First of all, we analyzed chemotactic responses of wild-type *C. elegans* to various concentrations of cyclic nucleotides, cAMP and cGMP. The worms showed little if any chemotaxis toward 1 mM cAMP, but displayed relatively strong chemotaxis in response to 10 mM cAMP (Fig. 3A). When 100 mM cAMP was treated, the animals exhibited a robust chemotactic response with the maximum chemotaxis index value reaching close to 60%. After 20 to 30 min exposure to 100 mM cAMP, the chemotaxis index values did not increase further (actually decreased) probably because the worms began to adapt to the attractant. Wild-type *C. elegans* showed stronger and faster chemotaxis to 500 mM cAMP than to 100 mM cAMP, but the maximum chemotaxis index values were similar. As in the case of 100 mM cAMP, the worms appeared to adapt to 500 mM cAMP after 20 to 30 min exposure. Wild-type *C. elegans* also showed a strong chemotactic response toward cGMP (Fig. 3B). The chemotactic behaviors of the worms toward various concentrations of cGMP were generally similar to those toward cAMP.

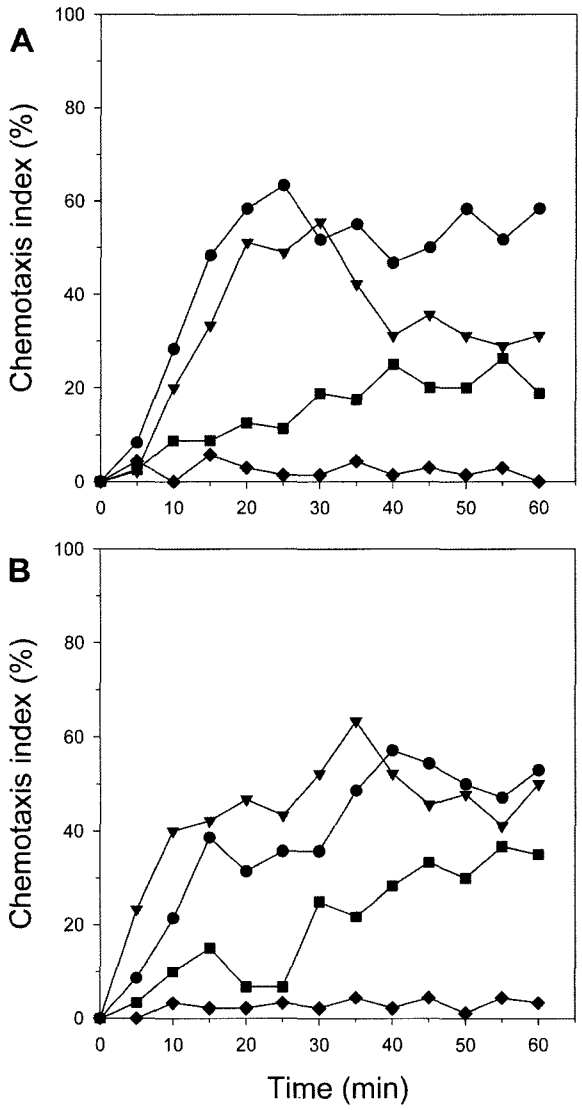

Fig. 3. Chemotactic behavior of *C. elegans* in response to cAMP (A) and cGMP (B). Cyclic nucleotide concentrations were 1 mM (diamonds), 10 mM (squares), 100 mM (triangles), and 500 mM (circles). The chemotaxis index values were obtained as described in Materials and Methods. Representative data from three independent experiments are shown.

Isolation and characterization of mutants defective in chemotaxis to cAMP

In an initial attempt to understand the molecular mechanisms underlying chemotaxis, we isolated mutants defective in chemotaxis toward cAMP. With the treatment of 1% EMS, nine *cca* (chemotaxis toward cAMP) mutants have been isolated. Based on their chemotactic behaviors, the mutants could be classified into the following five groups (Table 1).

(1) One mutant (*cca-101*) with defective chemotaxis to cAMP only (group 1)

This mutant exhibited defective chemotaxis to cAMP but retained the normal chemotactic response to other attractants, including cGMP. Chemotaxis to cGMP has been reported to be disturbed in a plate containing cAMP (that is, cGMP and cAMP are competing attractants for *C. elegans*) (Ward, 1973; Jeong et al., 1996), suggesting that chemotaxis signaling pathways triggered by cGMP and cAMP may share common components. The isolation of this mutant, however, indicates that the signaling pathways of cAMP and cGMP are not completely overlapping. And it can be postulated that this mutant has a defect in a component specific for the cAMP signaling pathway, possibly a cAMP receptor. Further characterization of this mutant should provide an important insight into the mechanism by which the cAMP information is processed in *C. elegans*.

(2) Three mutants (*cca-201*, *cca-202*, and *cca-203*) with defective chemotaxis to both cAMP and cGMP (group 2)

These mutants were defective in recognizing cyclic nucleotides (both cAMP and cGMP) but were able to respond to other kinds of attractants. It is highly likely that these mutants produce abnormal proteins specifically involved in the signaling of cyclic nucleotide information. Analysis of these mutants might reveal the components that play critical roles in the cyclic nucleotide signaling pathway.

Table 1. Comparison of chemotactic behaviors between *cca* mutants and the wild type

Mutant	cAMP	cGMP	Na^+	Cl^-	Lysine	Isoamyl alcohol
Wild type	46.4 ± 2.0[a]	51.0 ± 5.0	50.9 ± 3.0	51.6 ± 5.0	52.4 ± 1.2	60.0 ± 3.2
cca-101	15.2 ± 2.1	WT[b]	WT	WT	WT	WT
cca-201	19.4 ± 2.7	23.3 ± 4.3	WT	WT	WT	WT
cca-202	23.6 ± 6.1	26.8 ± 5.8	WT	WT	WT	WT
cca-203	27.2 ± 3.6	15.1 ± 1.5	WT	WT	WT	WT
cca-301	15.1 ± 2.0	22.6 ± 3.3	18.6 ± 0.6	15.0 ± 0.9	11.4 ± 1.0	WT
cca-401	2.7 ± 0.9	6.3 ± 1.2	2.1 ± 0.3	6.0 ± 0.3	5.8 ± 3.3	12.7 ± 6.7
cca-402	14.9 ± 2.5	11.3 ± 3.7	18.3 ± 0.6	19.8 ± 2.2	16.1 ± 4.9	28.3 ± 2.6
cca-403	21.8 ± 4.0	30.8 ± 2.6	35.8 ± 5.3	23.4 ± 5.1	29.2 ± 2.2	44.3 ± 6.0
cca-501	64.3 ± 2.6	75.3 ± 2.4	74.6 ± 4.4	76.9 ± 4.4	64.0 ± 3.1	72.0 ± 4.8

Mutants were tested for their chemotactic abilities toward various attractants. Chemical concentrations were 500 mM cAMP, 100 mM cGMP, 1 M sodium acetate (CH_3COONa), 1 M ammonium chloride (NH_4Cl), 1 M lysine, and 10% isoamyl alcohol (in ethanol). Highest chemotaxis index values displayed by each mutant are shown.
[a]Values (mean ± standard error) represent chemotaxis indexes (%). [b]The chemotaxis index values are similar to the wild type.

(3) One mutant (*cca-301*) with defective chemotaxis to water-soluble attractants (group 3)

In the nervous system, sensory information processing pathways for water-soluble and volatile chemicals are known to be distinct: water-soluble and volatile signals are processed by taste and olfactory neurons, respectively. Laser ablation experiments have shown that in *C. elegans* water-soluble and volatile chemicals are recognized by different subsets of sensory neurons (Bargmann and Horvitz, 1991; Bargmann et al., 1993).

As shown in Table 1, *cca-301* mutant displayed reduced chemotaxis to water-soluble attractants but normal chemotaxis to isoamyl alcohol, a volatile attractant. These results suggest that this mutant lacks a signaling component that is specifically involved in the chemotactic response to water-soluble chemicals.

(4) Three mutants (*cca-401*, *cca-402*, and *cca-403*) with defective chemotaxis to both water-soluble and volatile attractants (group 4)

These mutants exhibited reduced chemotaxis to both water-soluble and volatile attractants. It seems likely that these mutants cannot produce key signaling molecules that are needed for normal chemotaxis. For instance, these mutants may have a defect in the integrating system that coordinates the general chemotactic behavior.

(5) One mutant (*cca-501*) with enhanced chemotaxis to attractants (group 5)

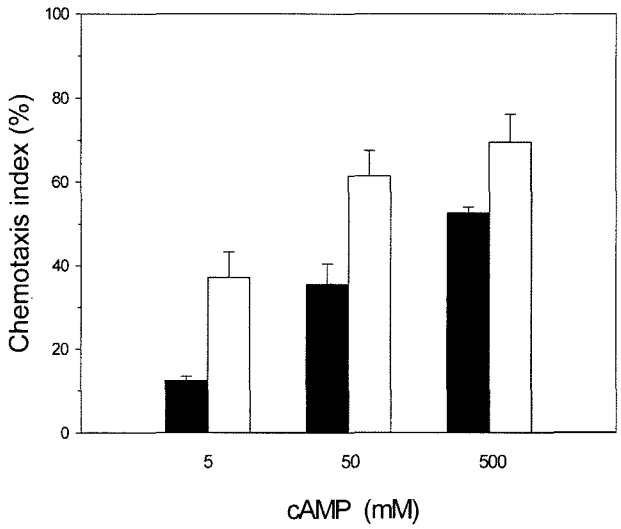

Fig. 4. Enhanced chemotactic response of *cca-501* mutant to cAMP. The mutant (gray bars) showed stronger chemotaxis than the wild type (black bars) in response to various concentrations of cAMP (5 mM, 50 mM, and 500 mM). Data are presented as mean ± SE (n = 3).

This mutant differed from other kinds of mutants in that it exhibited stronger chemotaxis than the wild type (Table 1). This mutant showed enhanced responses to both water-soluble and volatile attractants as well as cAMP. The maximum chemotaxis index values of the wild-type worms to water-soluble attractants were around 50%, rarely over 60%, but the values of *cca-501* mutant were often greater than 60%, sometimes close to 80%.

The mechanism of this enhanced chemotactic behavior is unknown at present. One possibility for this enhanced chemotaxis is that this mutant may not synthesize a key component for adaptation process, which desensitizes the worms to the existing concentration of attractants. As a result, the mutant worms could stay longer at the site of highest concentration of attractants. In fact, Matsuki et al. (2006) reported that *goa-1*, a mutant with defective olfactory adaptation, exhibited enhanced chemotaxis to the odorant benzaldehyde.

We further characterized the chemotactic behavior of *cca-501* mutant by applying various concentrations of cAMP. As shown in Fig. 4, the mutant displayed stronger responses than the wild type to all cAMP concentrations tested. The chemotaxis index values of the mutant were 37.1 ± 5.98 ($n = 3$) at 5 mM cAMP, 61.4 ± 5.95 ($n = 3$) at 50 mM cAMP, and 69.5 ± 6.60 ($n = 3$) at 500 mM cAMP. On the other hand, the chemotaxis index values of the wild type were 12.4 ± 0.98 ($n = 3$) at 5 mM cAMP, 35.5 ± 4.91 ($n = 3$) at 50 mM cAMP, and 52.6 ± 1.32 ($n = 3$) at 500 mM cAMP. Thus, even at lower concentrations of cAMP, the mutant showed significantly enhanced chemotactic responses.

Overall, our results indicate that a complex network of signaling molecules is working for effective *C. elegans* chemotactic behavior. We expect that future studies on the *cca* mutants isolated in this work will help understand the molecular mechanisms underlying chemotaxis.

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