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Genetic Control of Temperature Preference in the Nematode *Caenorhabditis elegans*

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ABSTRACT

Animals modify behavioral outputs in response to environmental changes. *C. elegans* exhibits thermotaxis, where well-fed animals show attraction to their cultivation temperature on a thermal gradient without food. We show here that feeding-state-dependent modulation of thermotaxis is a powerful behavioral paradigm for elucidating the mechanism underlying neural plasticity, learning, and memory in higher animals. Starved experience alone could induce aversive response to cultivation temperature. Changing both cultivation temperature and feeding state simultaneously evoked transient attraction to or aversion to the previous cultivation temperature: recultivation of starved animals with food immediately induced attraction to the temperature associated with starvation, although the animals eventually exhibited thermotaxis to the new temperature associated with food. These results suggest that the change in feeding state quickly stimulates the switch between attraction and aversion for the temperature in memory and that the acquisition of new temperature memory establishes more slowly. We isolated *aho* (*a*bnormal *h*unger *o*rientation) mutants that are defective in starvation-induced cultivation-temperature avoidance. Some *aho* mutants responded normally to changes in feeding state with respect to locomotory activity, implying that the primary thermosensation followed by temperature memory formation remains normal and the modulatory aspect of thermotaxis is specifically impaired in these mutants.

ANIMALS can modulate behavioral responses by sustained training and retain memory for a certain time. Learning and memory have been successfully investigated using model organisms such as Aplysia, Drosophila, and mice. Various learning paradigms have been developed in each model and analyzed behaviorally, physiologically, and pharmacologically (Mayford and Kandel 1999). From the standpoint of genetics, the development of conditioning assays in Drosophila made it possible to identify and manipulate genes involved in learning and memory (Waddell and Quinn 2001). On the basis of olfactory associative conditioning, Drosophila learning mutants were isolated. For example, the molecular and genetic analysis of *dunce* and *rutabaga* mutants revealed that cAMP-specific phosphodiesterase and Ca^{2+}/calmodulin-stimulated adenylyl cyclase are essential for establishment of learning and memory (Chen *et al.* 1986; Qiu *et al.* 1991; Levin *et al.* 1992).

The nematode *Caenorhabditis elegans* is well suited to a behavioral genetic approach to dissecting the molecular mechanism of learning and memory because of its accessible genetics (Brenner 1974), its stereotyped behavioral responses, and the ease of controlling experimental conditions in the laboratory. *C. elegans* is also ideal for the study of the cellular basis of learning and memory because of our complete anatomical knowledge of its nervous system, consisting of 302 identifiable neurons with all neuronal connections known (White *et al.* 1986). *C. elegans* can respond to various environmental cues, such as taste, smell, touch, and temperature, to ensure its own survival and reproduction in the natural environment. In addition to these primary behavioral responses, neural plasticity, learning, and memory can be observed in *C. elegans* despite its simple nervous system. Recently, various studies aiming to understand these complex mechanisms have been carried out on olfactory adaptation, nonassociative learning in mechanical tap response, and locomotory rate modulated by feeding state (Rankin *et al.* 1990; Colbert and Bargmann 1995; Sawin *et al.* 2000).

Hedgecock and Russell (1975) reported and defined thermotaxis of *C. elegans*. Well-fed animals migrated to their cultivation temperature and then moved isothermally, temperature preference of well-fed animals changed a few hours after cultivation temperature was shifted, and animals dispersed from their cultivation

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temperature after cultivation under starved and overcrowded conditions. These phenomena implicate two types of neural modulation in thermotaxis. First, animals store the temperature information after exposure to a certain temperature for a few hours. Second, animals somehow associate the cultivation temperature with the environmental condition, either pleasant or unpleasant, and drastically change the response to the temperature. Neural-circuit-regulating thermotaxis under well-fed conditions was proposed by laser-killing experiments (Mori and Ohshima 1995). It is highly likely that some of the neurons in the neural circuit are also required for neural modulations of thermotaxis.

In this study, we have demonstrated that *C. elegans* changes temperature preference in response to cultivation temperature and feeding states and that the memory formations for temperature and for feeding state are discrete processes. We also found that, as in the case of pharyngeal pumping and egg laying, exogenous serotonin and octopamine can mimic a well-fed and a starved state, respectively, in thermotaxis. We isolated several *aho* mutants, some of which are probably able to recognize the feeding state normally but are defective in reversal of temperature preference. We believe that some of these *aho* mutants are the novel example of thermotaxis-defective mutants, in which thermotactic neural modulation by the feeding state is specifically damaged. It is thus likely that the molecular and cellular analysis of *aho* genes should shed light on understanding mechanisms for neural plasticity, learning, and memory.

MATERIALS AND METHODS

Strains and genetics: The techniques used for culturing and handling *C. elegans* were essentially as described by Brenner (1974). The following strains were used in this work: wild-type *C. elegans* variety Bristol strain (N2), CB1111 *cat-1(e1111)*X, CB1141 *cat-4(e1141)*V, MT7988 *bas-1(ad446)*III, GR1321 *tph-1 (mg280)*II, MT2426 *goa-1(n1134)*I, *aho-1(nj5), aho-2(nj32), aho-3 (nj15), nj22, nj23, nj24, nj26, nj27, nj28, nj30, nj31*, wild-type *C. elegans* variety Hawaiian strain (CB4856), CB61 *dpy-5(e61)*I, CB51 *unc-13(e51)*I, CB3297 *vab-9(e1744)*II; *him-5(e1490)*V, CB189 *unc-32(e189)*III, CB185 *lon-1(e185)*III, CB49 *unc-8(e49)*IV, CB270 *unc-42(e270)*V, CB678 *lon-2(e678)*X, and CB151 *unc-3 (e151)*X.

Thermotaxis assay: A radial temperature-gradient assay was performed using a 9-cm agar plate without *Escherichia coli* (OP50), a food source, and a vial containing frozen acetic acid, as described by Mori and Ohshima (1995) with some modification. *C. elegans* was usually cultured at 20° from egg to L4 larval stage, and then L4 larvae, ~40 animals/6-cm NGM plate or ~120 animals/9-cm NGM plates, were cultured for 1 day under uncrowded conditions at designated temperature. In this study, we used a 45-ml glass vial (catalog no. 224833, Wheaton) to maintain a stable temperature gradient, and animals were allowed to move freely for 50 min on an assay plate. After the assay, animals were categorized into four groups, "17," "20," "25," and "17/25," on the basis of the tracks left on the assay plates (see legend of Figure 1, B and C, for detailed classification of thermotactic phenotype). Thermotaxis was evaluated using "fraction of 17," which is the fraction of animals categorized as "17," or "fraction of 25," which is the fraction of animals categorized as "25." The evaluation of thermotactic responses was made in a completely blind manner: an individual, who had done the experiment, and at least an additional individual with a career in thermotaxis assay for >3 years, who had not done the experiments and had not been told the experimental design, blindly evaluated the track left on each assay plate and categorized it into one of four groups. Then, the evaluations that had been individually made were put together and the final classification of each assay was determined by at least two individuals. The statistically significant differences of thermotaxis behavior were determined by using ANOVA for repeated measures.

Cultivation-temperature-shift assay: Well-fed naive animals were cultured with fresh food for 1 day under uncrowded conditions at designated temperatures of 17° or 25°. For cultivation-temperature-shift assay, well-fed naive animals were washed three times with NG buffer (0. 3% NaCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM, pH 6.0, potassium phosphate/liter) and transferred to conditioning plates (2% agar, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM, pH 6.0, potassium phosphate) at designated temperatures of 17° or 25°. The number of animals placed on a conditioning plate was restricted to 20 animals/6-cm plate or 120 animals/9-cm plate to assure cultivating animals under uncrowded conditions. Conditioning plates with fresh food or without food were preincubated at designated temperature overnight until the thermotaxis assays were conducted. Thermotaxis of cultivation-temperature-shifted animals was assayed at designated times after transfer (see Figures 2, 4, and 5 for details of time-course assays). To investigate the thermotactic responses of each time point after temperature shift, thermotaxis of animals after shifting the temperature was assayed at each time point in a 1-day cultivation-temperature-shift experiment, which was repeated over more than a few days.

Feeding-state-shift assay: Naive animals were prepared using the same procedure as described for well-fed naive animals in *Cultivation-temperature-shift assay* above. For food-deprived animals, well-fed naive animals were washed three times with NG buffer and transferred to conditioning plates without food. The number of animals placed on a conditioning plate was restricted to 120/9-cm plate to cultivate animals under uncrowded conditions. Conditioning plates were preincubated at designated temperatures overnight until the thermotaxis assays were conducted. To prevent animals from swimming off the agar, conditioning plates contained a high-osmolarity ring of 8 M glycerol at the periphery (Culotti and Russell 1978). The 8-M glycerol ring did not affect thermotaxis behavior (data not shown). Thermotaxis of food-deprived animals was assayed at designated time after transfer (see Figures 3–5, 7, and 8 for details of starvation time). For the experiment on recultivation with food, starved animals were transferred to conditioning plates with food as described by Colbert and Bargmann (1997). The number of animals was restricted to 20/6-cm conditioning plate to cultivate the animals under uncrowded conditions. Food drops were spotted on conditioning plates and incubated at 17° or 25° before transfer for at least a few hours. About 10 animals recultivated with food were assayed every 10 min after transfer. Thermotaxis of conditioned animals was continuously assayed in a day, and thermotaxis assays were repeated over more than a few days.

Pharmacological treatment: For serotonin-treated animals, well-fed animals grown at 25° were cultivated on serotonin plates (10 mM serotonin, 2% agar, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM, pH 6.0, potassium phosphate) without food at 25° for 2 hr. For octopamine-treated animals, well-fed animals grown at 25° were cultivated on octopamine plates (20 mg/ml octopamine, 2% agar, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM, pH 6.0, potassium phosphate) with food at 25° for 2 hr.

Mutant screen: Wild-type (N2) hermaphrodites at L4 stage were mutagenized by ethyl methanesulfonate, and *aho* mutant candidates were isolated from F_2 progeny (Brenner 1974). About 5700 genome-F_2 progeny grown at 25° were transferred to conditioning plates without food under uncrowded conditions for ~2–4 hr. These starved animals were then placed at 22.5°–23.5° on a Sephadex gel slurry (Sephadex G-200, superfine, Sigma, St. Louis), which had a linear temperature gradient from ~20° to 25° (Hedgecock and Russell 1975). After 1 hr, animals migrating at ~25° were picked to individual plates. Thermotaxis of these *aho* candidates was retested at well-fed and food-deprived states at least five times after conditioning at 17° or 25° to exclude athermotactic and thermophilic mutants from *aho* mutant candidates, and we selected as *aho* mutants strains that exhibit normal thermotaxis in a well-fed state but with abnormal cultivation-temperature avoidance in a starved state.

Locomotory rate assay: The procedure for the locomotory rate assay using a bacterial lawn was essentially according to Sawin *et al.* (2000) with some modifications. The 6-cm assay plates (2% agar, 1 mm $CaCl_2$, 1 mm $MgSO_4$, 25 mm, pH 6.0, potassium phosphate) with food were prepared before a few hours by spreading an OP50-enriched solution, a much concentrated overnight-cultured OP50, as described previously (Colbert and Bargmann 1997). OP50 was spread in a square with ~3 cm. Food-deprived animals grown at 17° or 25° were left in conditioning plates under uncrowded conditions for 2.5 hr or 30 min, respectively. Two minutes after transfer to assay plate, the number of body bends was counted for 10 animals for each strain.

Genetic mapping of *aho-1(nj5)*, *aho-2(nj32)*, *aho-3(nj15)*, *nj26*: All mutations were outcrossed by N2 at least two times before mapping and were mapped using the classical genetic method and the snip-SNPs method, a rapid gene mapping method using a high-density polymorphism map. *aho-1(nj5)* mutation was mapped to linkage groups using the following visibly marked tester strains: *dpy-5(e61)*I, *unc-13(e51)*I, *vab-9(e1744)*II; *him-5 (e1490)*V, *unc-32(e189)*III, *lon-1(e185)*III, *unc-8(e49)*IV, *unc-42(e270)*V, and *lon-2(e678)*X. *aho-2(nj32)*, *aho-3(nj15)*, and *nj26* mutations were mapped to linkage groups using the snip-SNPs method, a procedure essentially as described by Wicks *et al.* (2001). In addition, *nj26* mutation was also mapped using *unc-3(e151)*X.

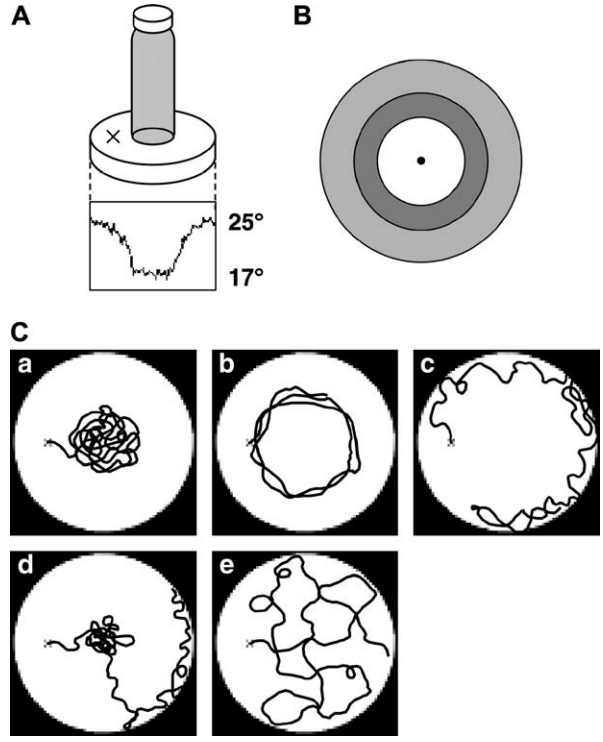

Figure 1.—Radial temperature-gradient assay. (A) Radial temperature-gradient assay was performed using a 9-cm agar plate and a vial containing frozen acetic acid. Thermograph (TVS-610, Nippon Avionics) showed the surface temperature of the plate contacting a glass vial. The stable radial temperature-gradient ranging from ~17° to 25° was established for at least 50 min on the agar surface. One or two animals were placed on the agar at ~22°, indicated by a cross on the assay plate. (B) The assay plate was divided into three areas: (1) an area at a distance of 0–2 cm from the center of the assay plate (open area), (2) an area at a distance of 2–3 cm from the center of the assay plate (dark shading), and (3) an area at a distance of 3–4.5 cm from the center of the assay plate (light shading). (C) Animal tracks were categorized into four groups after thermotaxis assay. Typical tracks of each category are shown in a–e. (a) Animals that moved on the open area, possibly at ~17°–20°, were classified as "17." (b) Animals that moved on the dark shaded area, possibly ~20°, were classified as "20." (c) Animals that moved on the light shaded area, possibly at ~20°–25°, were classified as "25." (d) Animals that moved back and forth between 17° and 25° were classified as "17/25." (e) Animals that moved almost randomly on the plate were also classified as "17/25," which stands for athermotactic phenotype.

RESULTS

Modulation of temperature preference by cultivation temperature and feeding state: Hedgecock and Russell (1975) reported that well-fed animals migrate to their cultivation temperature and move isothermally and that this thermotaxis behavior is modified by two kinds of experience. First, behavioral acclimation to a new cultivation temperature requires a few hours, after cultivation temperature of well-fed animals was shifted. Second, starved and overcrowded conditions make animals disperse from their cultivation temperature. In this study, we mostly analyzed thermotaxis of wild-type animals individually by changing condition of cultivation temperature and/or feeding state, whereas Hedgecock and Russell (1975) analyzed thermotaxis of population of animals mainly using a liner temperature-gradient assay. In an individual assay, a stable radial temperature gradient, ranging from 17° to 25°, was established in a 9-cm agar plate without food (Figure 1A), and thermotaxis of individual animals was evaluated by categorizing animal tracks into four groups (Figure 1, B and C). Most well-fed animals grown at 17°, 20°, and 25° migrated to their cultivation temperature in an individual assay with a radial temperature gradient (Table 1 and Figure 10A) as observed in a population assay with a linear temperature gradient (Hedgecock and Russell 1975; data not shown).

We shifted the cultivation temperature of animals

TABLE 1

Phenotypic distributions of well-fed wild-type animals

Cultivation temperature	Fraction of:			
	17	20	25	17/25
17°	0.80	0.02	0.07	0.11
20°	0.10	0.74	0.05	0.11
25°	0.15	0.01	0.75	0.09

Most well-fed wild-type animals migrated to their cultivation temperatures, 17°, 20°, and 25°, on the radial temperature-gradient assay. Animals' tracks were categorized into four groups after thermotaxis assay (see legend of Figure 1, B and C, for detail classification of the thermotaxis phenotype). Thermotaxis was evaluated using "fraction of 17," "fraction of 20," "fraction of 25," and "fraction of 17/25." About 10–20 animals were examined at each assay in 42 (17°), 17 (20°), and 34 (25°) trials.

with a well-fed state and analyzed the time course of behavioral response to a new cultivation temperature using an individual radial temperature-gradient assay (Figure 2A). After the cultivation temperature was shifted from 17° to 25° for a few hours, most well-fed animals migrated to 25°, although naive animals that had been cultivated continuously at 17° seldom migrated to 25° (Figure 2B). After the cultivation temperature was shifted from 25° to 17° for a few hours, most well-fed animals migrated to 17°, although naive animals continuously cultivated at 25° seldom migrated to 17° (Figure 2C). These results suggest that memory formation for a new cultivation temperature takes a few hours for well-fed animals and the speed of memory formation appears to be independent of cultivation temperature.

Hedgecock and Russell (1975) observed that animals from very old plates (>30% dauer larvae) as well as animals grown at high density in liquid culture dispersed from their cultivation temperature. To start illuminating more precisely the environmental parameters that influence thermotactic responses, we first investigated the effect of food deprivation on thermotaxis (Figure 3A). When well-fed animals preconditioned to migrate to 17° were cultivated without food at the same temperature (17°), they seldom migrated to the cultivation temperature after experiencing food deprivation for 3 hr (Figure 3B). When well-fed animals preconditioned to migrate to 25° were cultivated without food at the same temperature (25°), they seldom migrated to the cultivation temperature after experiencing food deprivation for 30 min (Figure 3C). The evaluation of individual thermotactic responses at various time points revealed that starvation induces avoidance and not simple dispersion from the temperature associated with a food-deprived condition (Figure 3, B and C). For example, most 17°-grown animals migrated to 25° while they hardly migrated to 17°, and most 25°-grown animals migrated to 17° while they hardly migrated to 25° (Figure 3, B and C). These results suggest that food deprivation alone can modulate ther-

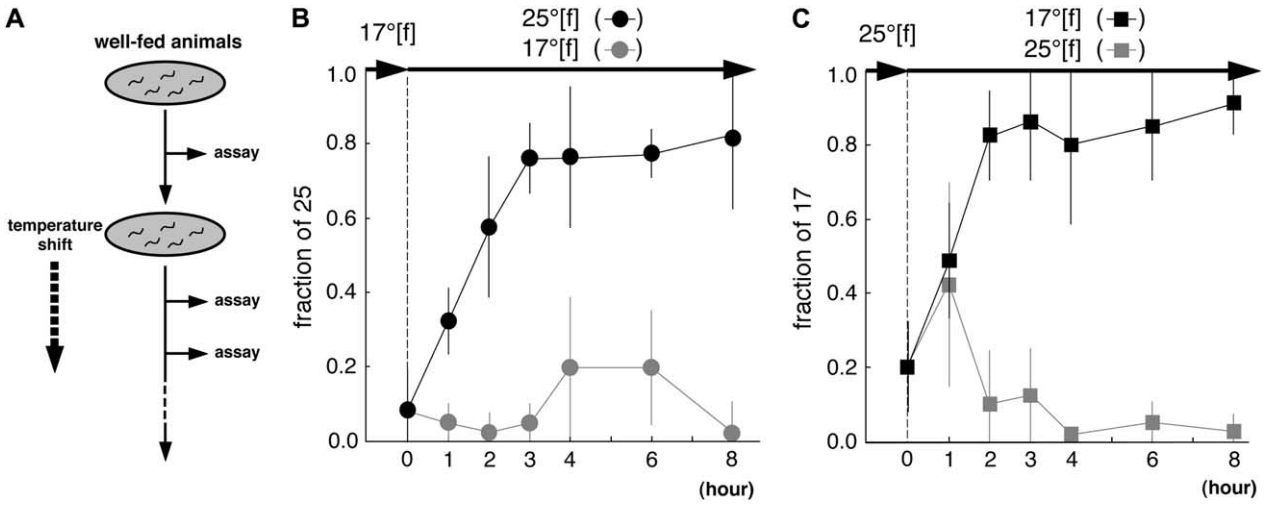

Figure 2.—The memory formation to a new temperature takes a few hours for well-fed animals in the radial temperature-gradient assay. (A) Experimental procedure for cultivation-temperature-shifted assay at the well-fed state. The solid ellipsoid indicates a conditioning plate with food. The thermotaxis behavior of naive and temperature-shifted animals was assayed at designated times after cultivation-temperature shift. (B) Cultivation temperature was shifted at the well-fed state from 17° to 25°. Animals temperature shifted after 1 hr had significant differences from naive animals ($P < 0.01$), and animals temperature-shifted after >2 hr had significant differences ($P < 0.0001$). (C) Cultivation temperature was shifted at the well-fed state from 25° to 17°. Animals temperature shifted after 1 hr had significant differences from naive animals ($P < 0.01$), and animals temperature shifted after >2 hr had significant differences ($P < 0.0001$). Thermotaxis was evaluated using "fraction of 17," which includes the class "17," and "fraction of 25," which includes the class "25." Top arrows indicate cultivation temperature and feeding state; "f" indicates cultivation with food. About 20 animals were examined at each time point in four trials. Error bar indicates SD.

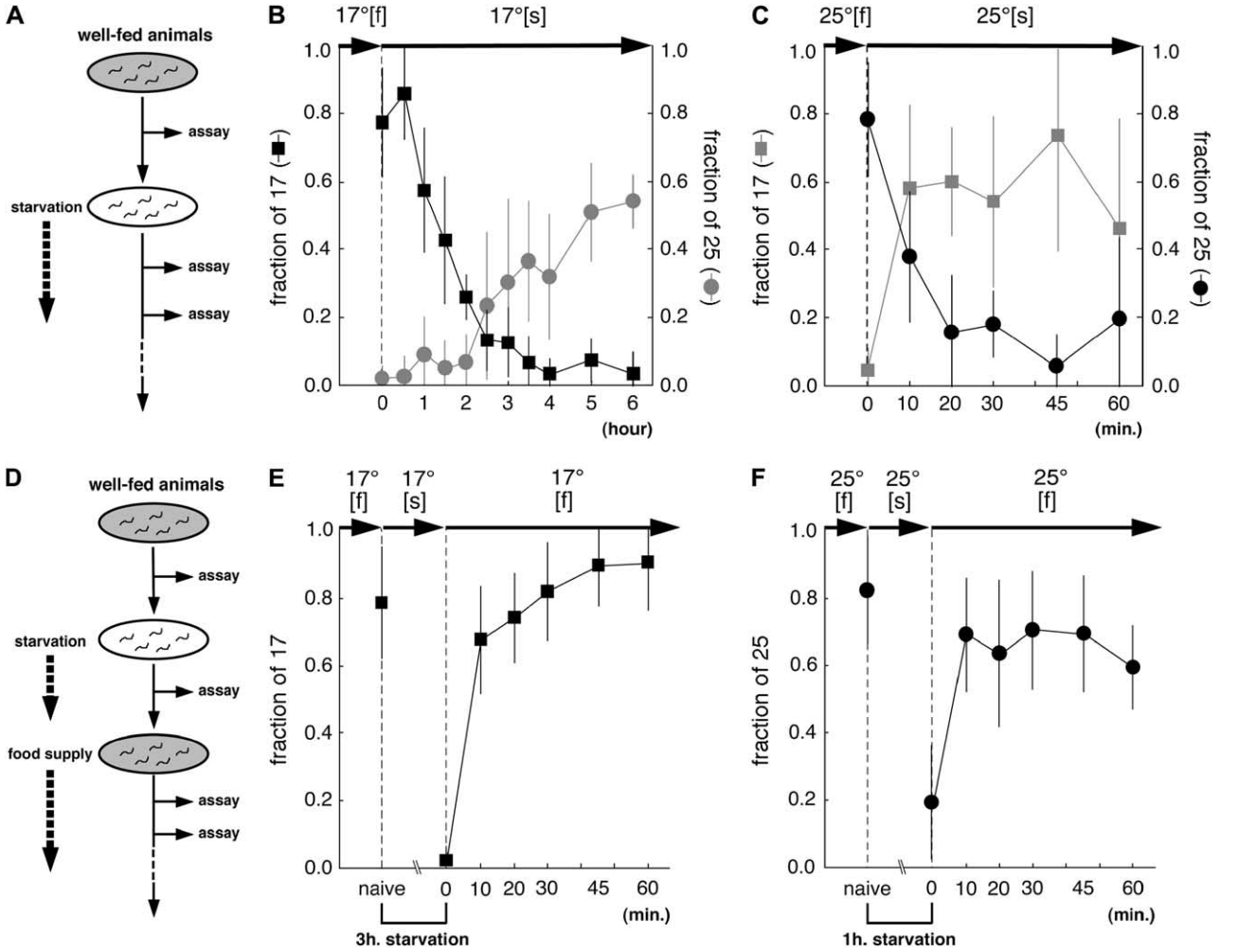

FIGURE 3.—Feeding states solely modulated thermotaxis behavior. (A and D) Experimental procedure for feeding-state-shifted assay at the same cultivation temperature. An ellipsoid indicates conditioning plate: a solid ellipsoid indicates cultivation with food and an open ellipsoid indicates cultivation without food. (B) Well-fed animals were transferred to conditioning plate without food at 17°. About 20 animals were examined at each time point in six trials. Animals food deprived for 1 hr had significant differences from naive animals ($P < 0.01$), and animals starved for >1.5 hr had significant differences ($P < 0.0001$). (C) Well-fed animals were transferred to conditioning plate without food at 25°. About 10 animals were examined at each time point in seven trials. Animals food deprived for >10 min had significant differences from naive animals ($P < 0.0001$). (E and F) Starved animals were recultivated with food at the same cultivation temperature (17° or 25°). About 10 animals were examined at each time point in 10 (17°) and 7 (25°) trials. Animals recultivated with food for >10 min had significant differences from starved animals ($P < 0.0001$). (B, C, E, and F) Thermotaxis was evaluated using "fraction of 17," which includes the class "17" and "fraction of 25," which includes the class "25." A square indicates fraction of 17 and a circle indicates fraction of 25. Top arrows indicate cultivation temperature and feeding state; "f" indicates cultivation with food and "s" cultivation under food-deprived conditions. Error bar indicates SD.

motaxis and the speed of the modulation depends on cultivation temperature.

We next examined the effect of food supply on thermotactic response of the animals that had been conditioned to avoid cultivation temperature (Figure 3D). The starved animals cultivated at either 17° or 25° immediately recovered from the starved state and migrated to their cultivation temperature after recultivation with food for only 10 min (Figure 3, E and F). These results suggest that the transition from a starved to a well-fed state is quickly triggered by food and that, in contrast to the transition from a well-fed to a starved state, this neural modulation is independent of cultivation temperature.

Distinct memory formations for temperature and feeding state: We showed that application of food to starved animals instantaneously triggered the switch from an aversive to an attractive response to cultivation temperature. To investigate this quick transition in more detail, we changed simultaneously two parameters, cultivation temperature and feeding state. We first shifted cultivation temperature at the time when starved animals were

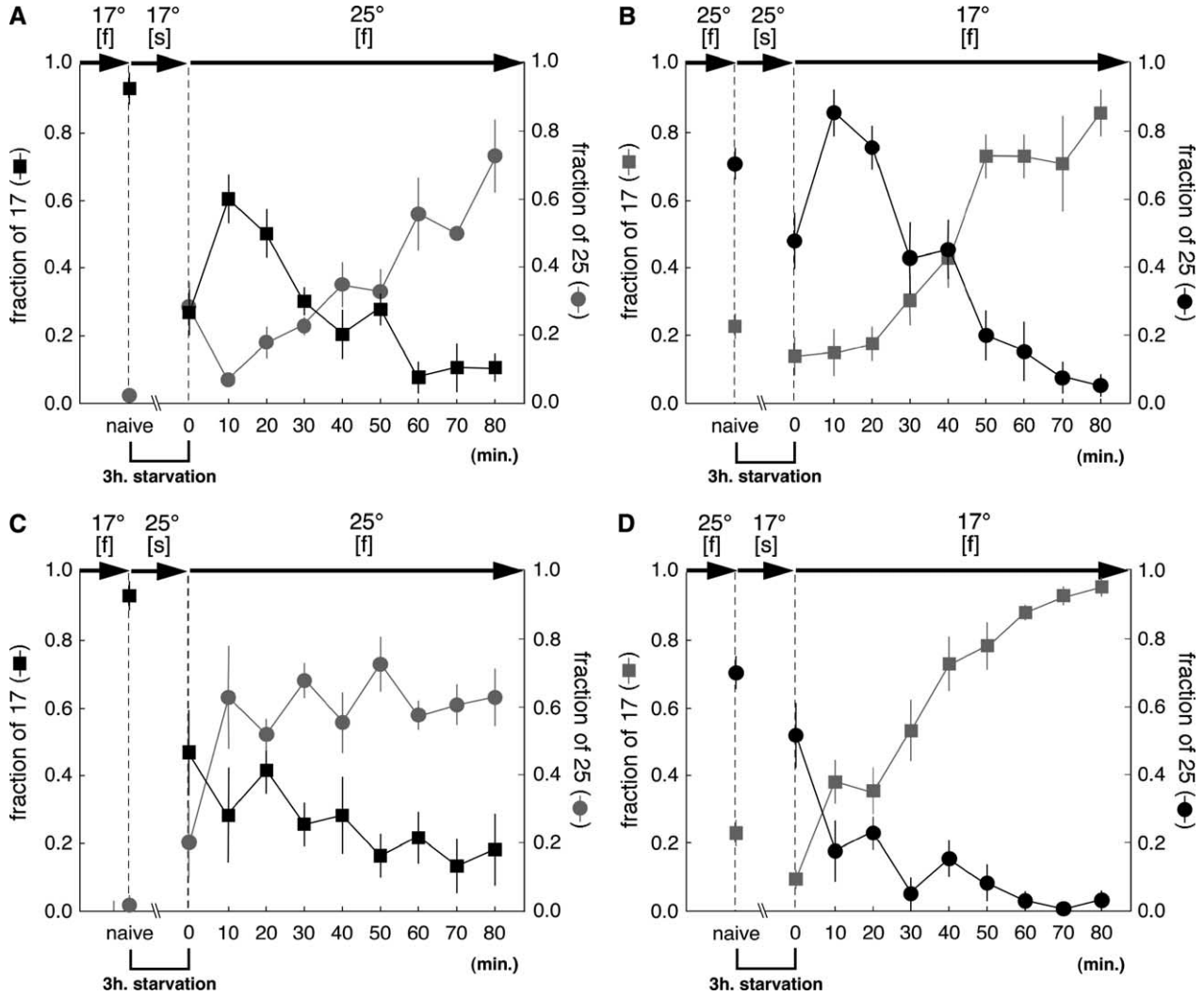

FIGURE 4.—The memory formation processes for temperature and feeding states are biologically discrete. (A) Animals conditioned to be starved at 17° for 3 hr were recultivated with food at 25°. (B) Animals conditioned to be starved at 25° for 3 hr were recultivated with food at 17°. (C) Animals conditioned to be well fed at 17° were cultivated without food at 25° for 3 hr and then recultivated with food at 25°. (D) Animals conditioned to be well fed at 25° were cultivated without food at 17° for 3 hr and then recultivated with food at 17°. (A–D) Thermotaxis was evaluated using "fraction of 17," which includes the class "17," and "fraction of 25," which includes the class "25." A square indicates fraction of 17 and a circle indicates fraction of 25. About 10 animals were examined at each time point in four trials. Top arrows indicate cultivation temperature and feeding state; "f" indicates cultivation with food and "s" cultivation under food-deprived conditions. Error bar indicates SEM.

provided food. When starved animals preconditioned to avoid 17° were exposed to food at 25° for 10 min, most animals migrated to the former cultivation temperature of 17°, the temperature that used to act as an aversive signal for the animals (Figure 4A). Likewise, when animals that had been starved at 25° were recultivated with food at 17°, most animals initially migrated to 25°, the noxious temperature associated with food-deprived experience (Figure 4B). In similar temperature/feeding state shift experiments, we next shifted cultivation temperature at the time when well-fed animals experienced starvation prior to recultivation with food. When well-fed animals cultivated at 17° were transferred to conditioning plates without food for 3 hr at 25° and then recultivated with food at 25°, the conditioned animals seldom migrated to 17° and mostly migrated to 25° after 10 min of recultivation with food (Figure 4C). When well-fed animals cultivated at 25° were transferred to conditioning plates without food at 17° for 3 hr and then recultivated with food at 17°, the conditioned animals seldom migrated to 25° and a fraction of animals migrating to 17° started to increase immediately after recultivation with food (Figure 4D). In this latter shift experiment, a fraction of animals migrating to 17° increased even faster after recultivation with food when animals were cultivated at 17° under food-deprived conditions for 4 instead of 3 hr (data not shown). Despite the initial and transient attractive response to the previous aversive temperature, the fraction of animals that migrated to 25° or 17°, a new temperature associated with food signal, gradually increased (Figure 4, A and B). These results suggest that food intake quickly causes

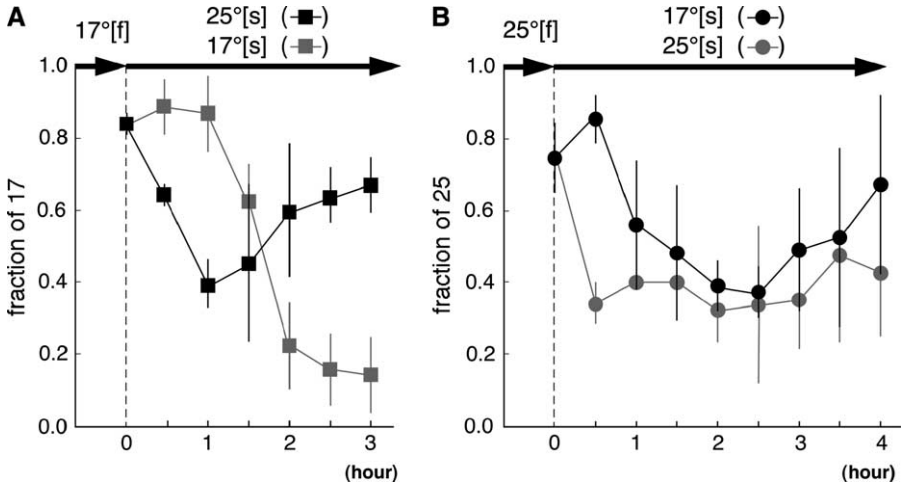

FIGURE 5.—(A) Animals conditioned to be well fed at 17° were cultivated without food. (B) Animals conditioned to be well fed at 25° were cultivated without food. (A and B) About 20 animals were examined at each time point in four trials. Top arrows are cultivation temperature and feeding state; "f" indicates cultivation with food and "s" cultivation under food-deprived conditions. Error bar indicates SEM.

the transition of thermotactic response from aversion to attraction to the temperature in memory. Interestingly, starved animals started to prefer the new attractive temperature associated with food much faster than well-fed animals (Figures 2 and 4), which suggests that starvation accelerates the formation of new temperature memory.

We also shifted cultivation temperature when well-fed animals were cultivated under food-deprived condition (Figure 5). After well-fed animals that had been preconditioned to migrate to 17° were cultivated without food at 25°, they first behaved to avoid 17°, the former attractive temperature, and after 1 hr of starvation the fraction of animals migrating to 17° started to increase (Figure 5A). In the converse temperature/feeding state shift experiment, where well-fed animals preconditioned to migrate to 25° were transferred to plates without food and cultivated at 17°, the conditioned animals first avoided 25°, and after a few hours of starvation the fraction of animals migrating to 25° started to increase (Figure 5B). These results suggest that food deprivation stimulates the transition of thermotactic responses from attraction to aversion to direct avoidance from the temperature in the animals' memory.

On the basis of the results obtained in this study, we propose a conceptual model for thermotaxis (Figure 6). First, both temperature and feeding state, indicative of environmental condition, are sensed independently (reception). Second, a prolonged exposure to the same condition leads the animals to store thermal information and feeding state independently (learning and memory), although these two parameters, temperature memory and feeding-state memory, interact with each other. For example, the speed of inducing cultivation-temperature avoidance depends on cultivation temperature, and starvation accelerates the formation of new temperature memory. Third, on a temperature gradient, the animals assess the changes in thermal information relative to the stored thermal and feeding-state memories (assessment). Finally, the animals thus migrate to either a colder or a warmer area and move isothermally around the region of interest as a final decision (movement). We suggest that thermotaxis is a kind of associative learning, an ideal behavioral paradigm advantageous to investigating neural plasticity at molecular and cellular levels.

Serotonin and octopamine can mimic a well-fed or a food-deprived state, respectively, in thermotaxis: Our results demonstrated that feeding condition is an important trigger to stimulate the transition between aversive and attractive responses to temperature in animals' memory. Feeding state is also known to modulate egg laying, pharyngeal pumping, and locomotion in *C. elegans*. The previous pharmacological analysis of these behaviors showed that exogenous serotonin and octopamine can mimic well-fed and food-deprived states, respectively (HORVITZ *et al.* 1982; TRENT *et al.* 1983; AVERY and HORVITZ 1990; Y. SHIBATA and S. TAKAGI, personal communication). Thus, serotonin and octopamine are likely to act as neurotransmitter or neurohormone to regulate feeding states antagonistically in *C. elegans*.

We addressed whether these biogenic amines are also involved in neural modulation of thermotaxis. When well-fed animals preconditioned to migrate to 25° were

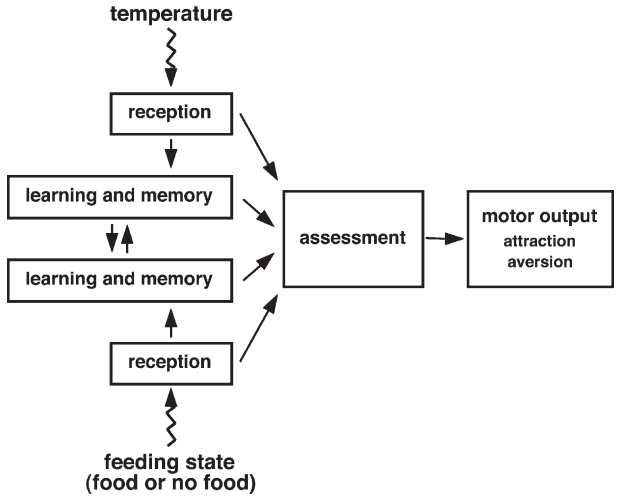

FIGURE 6.—A conceptual model for thermotaxis.

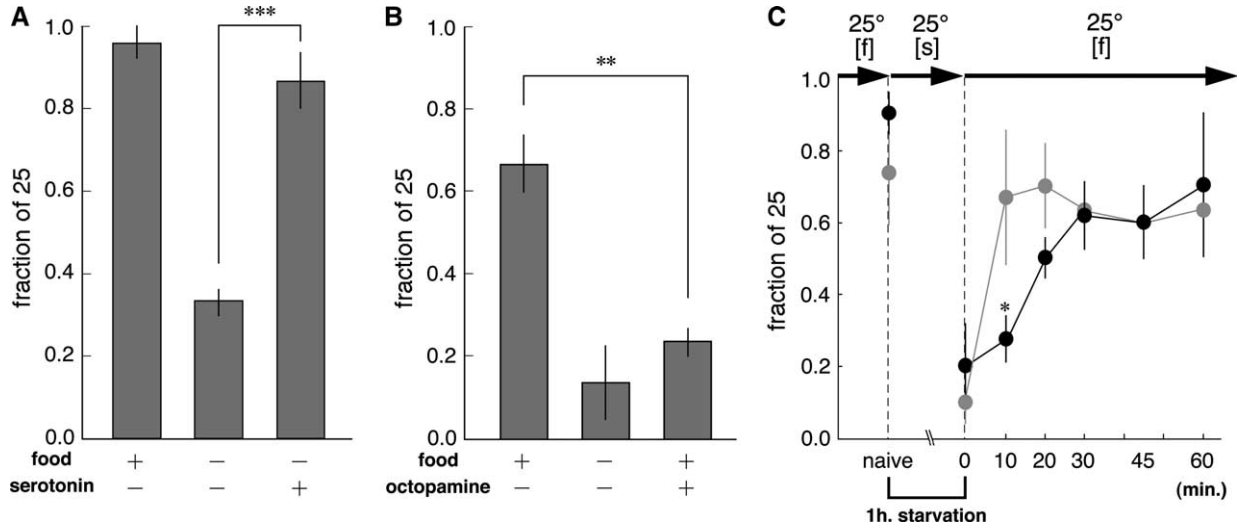

FIGURE 7.—Exogenous serotonin and octopamine can mimic well-fed or food-deprived states, respectively, in thermotaxis. (A and B) Well-fed animals conditioned to migrate to 25° were transferred onto plates with or without drug and with or without food for 2 hr. About 10 animals were examined in three trials. (C) The delayed recovery from starved state in *cat-1(e1111)* mutants cultivated at 25°. The solid line indicates the result of *cat-1(e1111)* and the shaded line indicates the result of wild type. Top arrows indicate cultivation temperature and feeding state; "f" indicates cultivation with food and "s" cultivation under food-deprived conditions. About 10 animals were examined at each period in three trials. (A–C) Error bar indicates SEM. The statistically significant differences of thermotaxis behavior of drug-treated animals from non-drug-treated animals or thermotaxis behavior of wild-type animals from *cat-1(e1111)* mutants were determined by using ANOVA for repeated measures. Single, double, and triple asterisks indicate statistically significant differences, $P < 0.02$, $P < 0.01$, and $P < 0.002$, respectively.

placed on food-deprived plates containing serotonin for 2 hr, most of these serotonin-treated animals migrated to 25° on a thermal gradient, regardless of food-deprived experience (Figure 7A). We also examined the effect of exogenous octopamine on thermotactic behavior. When well-fed animals preconditioned to migrate to 25° were placed on food plates containing octopamine for 2 hr, most octopamine-treated animals moved away from 25°, although they were physically fed with real food (Figure 7B). Consistent with the previous report on other behaviors, our results showed that exogenous serotonin and octopamine can also mimic a well-fed and a food-deprived state, respectively, in thermotaxis.

In *C. elegans*, serotonin is thought to be transported into secreted vesicles by CAT-1, a vesicular monoamine transporter, which is required for neurotransmitter release at synaptic terminal and has serotonin transport activity in mammalian cells (DUERR *et al.* 1999). Serotonin is absent from neuronal processes but present in cell bodies in *cat-1(e1111)* mutants, which are abnormal in egg laying, pharyngeal pumping, locomotion, and male mating (SULSTON *et al.* 1975; DUERR *et al.* 1999). Interestingly, we found that *cat-1(e1111)* mutants that had been preconditioned to avoid 25° exhibited a significant delay in the recovery from the starved state (Figure 7C). This result suggests that monoamines transported by CAT-1 act as feeding signals in thermotaxis.

Serotonin is synthesized from tryptophan via two steps. In mutants defective in the biosynthesis pathway, *cat-4(e1141)*, *bas-1(ad446)*, and *tph-1(mg280)*, the endogenous serotonin level appears to be reduced (SULSTON *et al.* 1975; LOER and KENYON 1993; SZE *et al.* 2000). We analyzed these mutants and found that they are essentially normal in all aspects of thermotactic responses tested, although *tph-1(mg280)* mutants cultivated at 25° were too sick to test any aspects of thermotactic responses (data not shown). Two possibilities explain the normal responses of these serotonin mutants. Serotonin is not necessary but sufficient to convey feeding signal, and other neurotransmitters or neurohormones transported by CAT-1 act as additional feeding signals. Alternatively, endogenous serotonin is still synthesized at low levels in these mutants and the small amount of serotonin is sufficient to work as a feeding signal. Previous immunostaining analysis showed that the endogenous serotonin was undetectable in *cat-4(e1141)*, *bas-1 (ad446)*, and *tph-1(mg280)* mutants (DESAI *et al.* 1988; LOER and KENYON 1993; SZE *et al.* 2000), although a report suggests that at least the *cat-4(e1141)* mutation did not lead to a complete loss of serotonin (RANGANATHAN *et al.* 2001).

Isolation of mutants defective in modulation of thermotaxis by cultivation temperature and feeding state: We performed a genetic screen to identify genes and cells required for feeding-state-dependent modulation of thermotaxis (Figure 8A). We mutagenized a wild-type strain and sought animals that were defective in cultivation-temperature avoidance. Among those animal lines, we sought mutant strains that exhibited normal thermotaxis after cultivation under well-fed conditions. It is highly

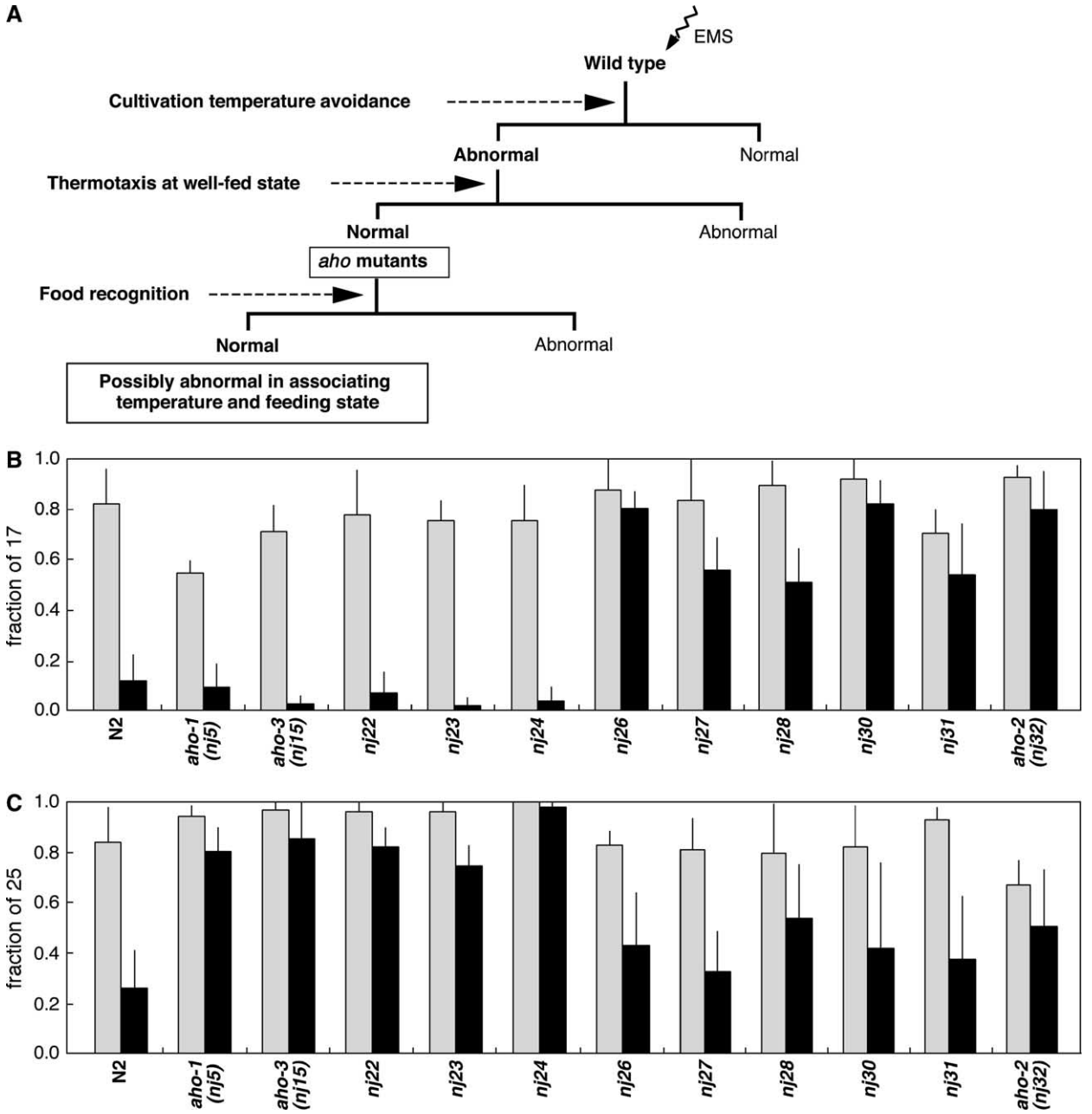

FIGURE 8.—A screen for mutants defective in modulation of thermotaxis affected by feeding states. (A) An experimental strategy to obtain mutants defective in neural modulation of thermotaxis by feeding states. (B) Results of 17°-thermotaxis assay for *aho* mutants. Shaded bars indicate results of well-fed animals and solid bars indicate results of food-deprived animals for 3 hr. (C) Results of 25°-thermotaxis assay for *aho* mutants. Shaded bars indicate results of well-fed animals and solid bars indicate results of food-deprived animals for 1 hr. (B and C) About 12 well-fed animals or 20 food-deprived animals were examined in at least three trials. Error bar indicates SD.

possible that the primary thermosensation followed by temperature storage remains normal in these mutants, designated *aho* (*a*bnormal *h*unger *o*rientation). Finally, we examined whether *aho* mutants were able to recognize change of feeding state normally, since in theory the failure to respond to the feeding state *per se* could cause the Aho phenotype, defective in cultivation-temperature avoidance.

Fifteen *aho* mutants were isolated among ~5700 genomes screened. Of 11 mutants, *aho-1(nj5)* was a dominant mutation and the others were recessive mutations (data not shown). These 11 *aho* mutants were defective in cultivation-temperature avoidance compared to wild-type animals (Figure 8, B and C). Statistical significances of aberrant thermotactic responses of *aho* mutants at starved state were evaluated by ANOVA for repeated measures. Statistical analysis was applied independently between wild type and each *aho* mutant, although Figure

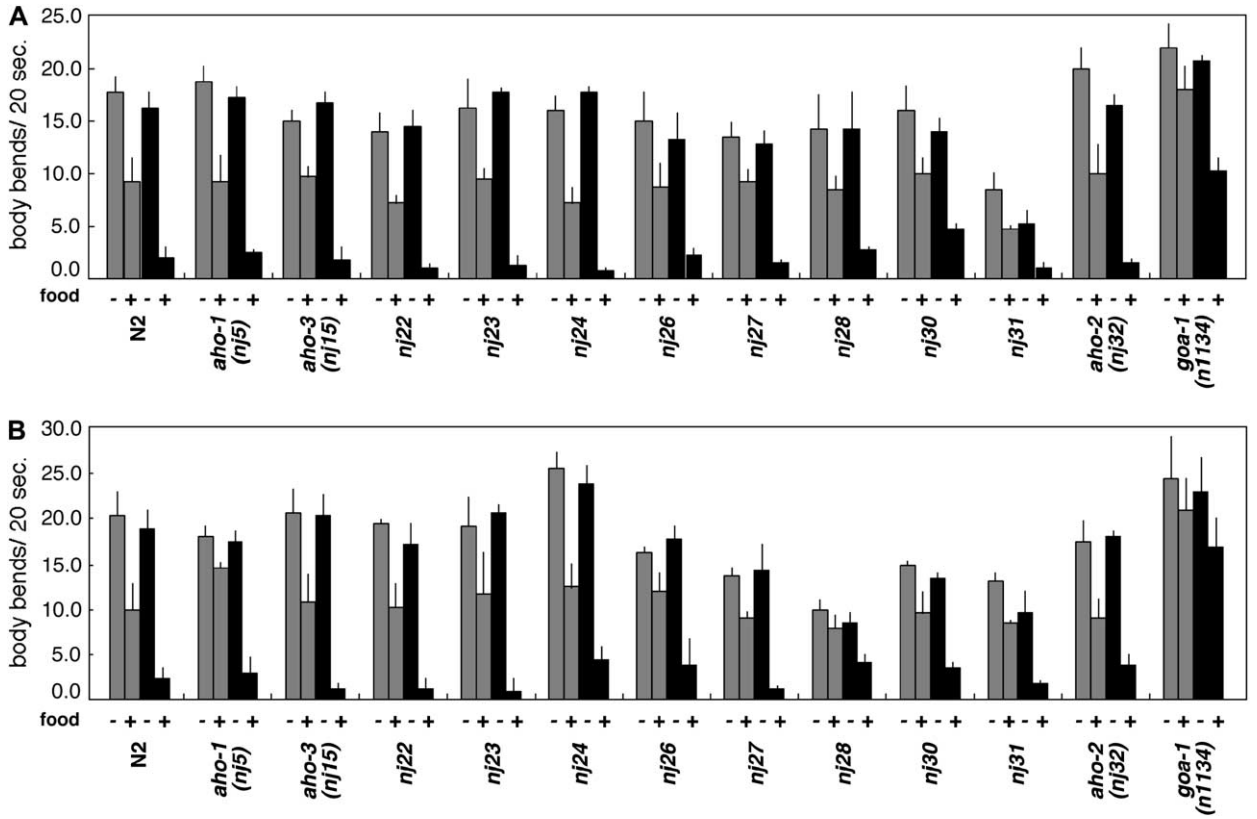

FIGURE 9.—Locomotory rate assay of *aho* mutants. (A) Modulation of locomotory rate in *aho* mutants cultivated at 17°. Starved animals were conditioned by cultivation under food-deprived conditions for 2.5 hr. (B) Modulation of locomotory rate in *aho* mutants cultivated at 25°. Starved animals were conditioned by cultivation under food-deprived conditions for 30 min. (A and B) Shaded bars indicate results of well-fed animals that were transferred to assay plates without and with food. Solid bars indicate results of starved animals that were transferred to assay plates without and with food. Plus and minus signs indicate locomotory assay plate with food or without food: "+," assay plates with food; "−," assay plates without food. The *goa-1(n1134)* mutant showed hyperactive locomotion (MENDEL *et al.* 1995; SEGALAT *et al.* 1995) and was used as a negative control. About 10 animals were examined in at least three trials. Error bar indicates SD.

8, B and C, shows the averages of all thermotaxis assays for each strain. Cultivated at 17°, *nj26, nj30*, and *aho-2 (nj32)* had significant differences ($P < 0.0002$), *nj28* and *nj31* had significant differences ($P < 0.004$), and *nj27* had significant differences ($P < 0.03$). Cultivated at 25°, *aho-1(nj5), aho-3(nj15)*, and *nj22* had significant differences ($P < 0.0005$), *nj23* and *nj24* had significant differences ($P < 0.001$), *nj28* had significant differences ($P < 0.07$), and *aho-2(nj32)* had significant differences ($P < 0.08$). Although the thermotactic abnormalities of *nj28* and *aho-2(nj32)* mutants cultivated at 25° were statistically not as significant as other *aho* mutants, both mutants showed apparently impaired responses to starvation. Most of *aho* mutants seem to be classified into three groups. The first group exhibits Aho after cultivation at both 17° and 25° (*i.e., aho-2(nj32)*), the second group exhibits Aho at 17° (*i.e., nj26*), and the third group exhibits Aho at 25° (*i.e., aho-3(nj15)*).

To assess whether food recognition is normal or not in these *aho* mutants, we tested the feeding-state modulation of locomotory activity. Well-fed wild-type animals move more slowly in plates with food than in plates without food (basal slowing response) and starved animals move even more slowly in plates with food than well-fed animals in plates with food (enhanced slowing response; SAWIN *et al.* 2000). Some *aho* mutants exhibited nearly the same responses as wild type in locomotory rate assay (Figure 9). Our results suggest that these *aho* mutants are normal in food recognition and defective in modulation of thermotaxis by cultivation temperature and feeding state, although we cannot rule out the possibility that a yet-to-be-known food recognition pathway, which is not reflected in change in locomotory activity, could be abnormal in these *aho* mutants. In addition, it is likely that *aho* mutants that exhibited abnormality in locomotory assay this time will come to exhibit a normal response of change of feeding state, if these are outcrossed several times with wild-type N2 strain.

Molecular dissection and behavioral analysis of *aho* mutants: To understand mechanisms for thermotactic neural modulation and to gain insights into the nature of the defects, we tried to investigate *aho* mutants in detail and analyzed the other sensory behaviors.

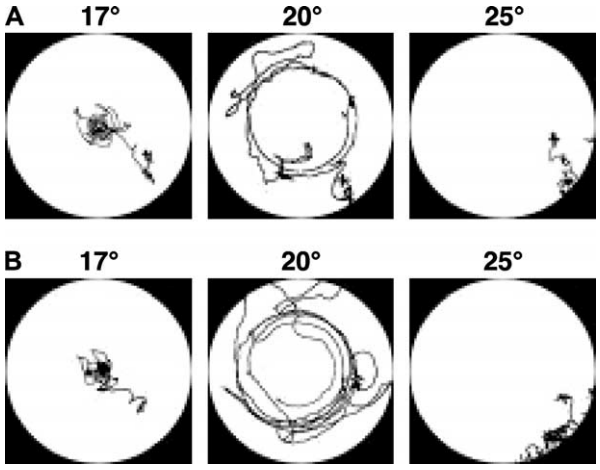

FIGURE 10.—*aho-2(nj32)* mutant exhibits normal thermotaxis at the well-fed state. (A) Typical tracks of well-fed wild-type animals cultivated at 17°, 20°, and 25°. (B) Typical tracks of well-fed *aho-2(nj32)* mutants cultivated at 17°, 20°, and 25°.

The *aho-2(nj32)* mutant exhibited normal thermotaxis at the well-fed state (Figure 10), but was defective in reversal of temperature preference modulated by starvation at 17° and 25° (Figure 11). *aho-2(nj32)* was normal in chemotaxis to Na^+ ion, avoidance of Cu^{2+} ion, and olfaction (diacetyl, isoamyl alcohol, benzaldehyde, and trimithylthiazole; data not shown). According to mapping results using snip-SNPs method, *nj32* mapped to the region near the center of chromosome IV (Figure 12).

The *aho-1(nj5)* mutant exhibited Aho at 25°. *aho-1(nj5)* was normal in olfactory adaptation (diacetyl, pyrazine, isoamyl alcohol, and benzaldehyde), pharyngeal pumping, and defecation, and *aho-1(nj5)* had a slight defect of chemotaxis to NaCl (data not shown). According to linkage analysis using visible markers, *aho-1(nj5)* was linked to the region between the left and the center of chromosome I (Figure 12).

The *aho-3(nj15)* mutant exhibited Aho at 25°. *aho-3 (nj15)* was normal in chemotaxis to NaCl, avoidance of Cu^{2+} ion, and olfaction (diacetyl, pyrazine, isoamyl alcohol, and benzaldehyde), and *aho-3(nj15)* was defective in male mating behavior (data not shown). Using snip-SNPs method, *aho-3(nj15)* was mapped near the center of chromosome I (Figure 12).

The *nj26* exhibited Aho at 17°. *nj26* was normal in olfaction (diacetyl and benzaldehyde) and slightly abnormal in chemotaxis to Na^+ ion (data not shown). Using the classical genetic method and snip-SNPs method, *nj26* was mapped to the right of chromosome X (Figure 12).

DISCUSSION

Neural modulation by cultivation temperature and feeding state in thermotactic responses: HEDGECOCK and RUSSELL (1975) reported that *C. elegans* cultured under very harsh conditions, such as cultivation in very old culture plates or in high density, dispersed from their cultivation temperature. Under these conditions, starvation, high density of dauer pheromone, and other unpleasant environmental factors are all likely to cause dispersion from cultivation temperature. In this study, we focused on food condition and showed that feeding state alone could be an important cue to modulation of behavioral responses to environmental temperature. These results suggest that *C. elegans* can associate cultivation temperature with feeding-state information and modify behavioral outputs in response to environmental changes.

After animals that had been preconditioned to migrate to cultivation temperature were cultivated under food-deprived and uncrowded conditions, they showed the complete reversal of temperature preference, from attractive to aversive response to the cultivation temperature (Figure 3, B and C). We also found that avoidance of the cultivation temperature turned into attraction to the temperature immediately after recultivation with food (Figure 3, E and F). Intriguingly, the transition from attractive to aversive thermotactic response stimulated by food deprivation occurred much more slowly at low (17°) temperature than at high (25°) temperature, although the time required for the transition from aversive to attractive response by food supply was similar at both temperatures. It is plausible to suppose that different mechanisms participate in feeding-state-dependent thermotactic modulation at low and high cultivation temperatures.

Our conditioning experiments with shifting temperature and feeding state simultaneously suggest that the memory formation processes for temperature and for feeding states occur independently but both processes are closely related and hardly separable with one another (Figures 4 and 5). Recultivation of starved animals with food first exhibited thermotaxis to the cultivation temperature that used to be an aversive cue (Figure 4, A and B). Further, food deprivation of well-fed animals initially evoked avoidance of the temperature that used to be an attractive cue (Figure 5, A and B). These results suggest that the switch between attraction and aversion stimulated by the change in feeding state establishes much more quickly than the formation of a new temperature memory. Our results also revealed that starvation experience accelerated the acquisition of a new temperature memory (Figures 2 and 4) and that the exposure to a new temperature influenced the time required for the transition from attractive to aversive response to cultivation temperature (Figure 3, B and C; Figure 5, A and B). These results implicate the close interaction between temperature memory and feeding-state memory processes (Figure 6).

RYU and SAMUEL (2002) reported that *C. elegans* migrate to lower temperature but not to higher than cultivation temperature within several minutes after temporal temperature change. However, analyses using special

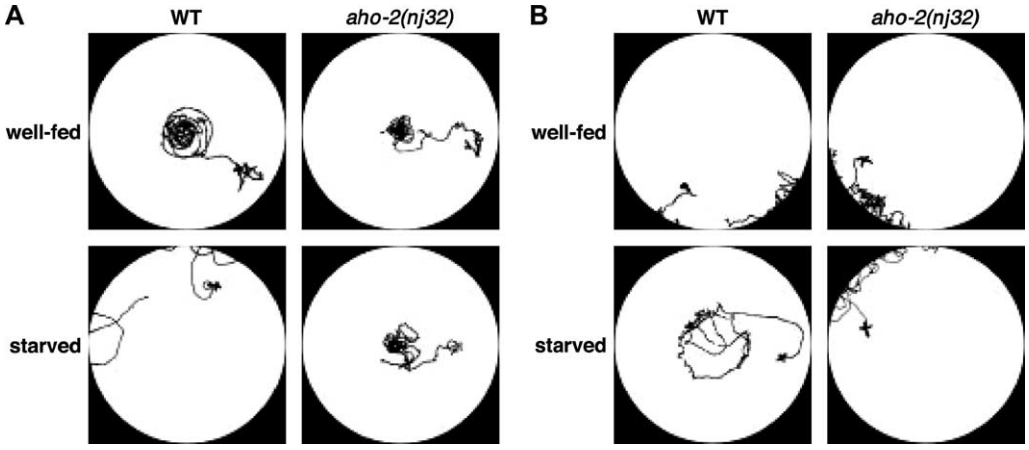

FIGURE 11.—*aho-2(nj32)* mutant defective in cultivation-temperature avoidance induced by starvation. (A) Typical tracks of wild-type animals and *aho-2(nj32)* mutants cultivated at 17°. Starved animals were conditioned by cultivation under food-deprived conditions for 3 hr. (B) Typical tracks of wild-type animals and *aho-2 (nj32)* mutants cultivated at 25°. Starved animals were conditioned by cultivation under food-deprived conditions for 1 hr.

(linear and radial) temperature-gradient assays indicate that animals do migrate to their cultivation temperature by moving up no less than down on a thermal gradient (HEDGECOCK and RUSSELL 1975; MORI and OHSHIMA 1995; KOMATSU *et al.* 1996; KUHARA *et al.* 2002). In addition, another study using temporal thermal gradients in different ways suggests that animals respond to up and down temporal thermal changes (ZARIWALA *et al.* 2003). At least two possibilities explain the different results between our observations and those of RYU and SAMUEL (2002). The difference in duration of assay time may affect evaluation of thermotaxis if both studies observed the same phenomenon. We allowed animals to move freely for 50 min, while Ryu and Samuel observed animals for only several minutes. Alternatively, the different results may reflect the fact that it is harder to observe migration up the gradient of the animals cultivated at higher temperature, as compared to observing migration down the gradient of the animals cultivated at lower temperature. For example, animals cultivated at 25° started to avoid 25° for only 10 min of starvation (Figure 3A). Thus, it is highly possible that animals cultivated at higher temperature started to starve during experimental manipulation and did not migrate to cultivation temperature. It is important to make clear the biological link between our results and the different conclusions deduced from these behavioral studies.

Feeding-state signals in thermotaxis: *C. elegans* eats bacteria as its food source. It is difficult to determine what *C. elegans* recognizes as a feeding signal. Bacteria are a composite stimulus that perhaps consists of taste, smell, touch, and so forth. Also, upon food intake the stimulation of an animal's metabolism likely takes place in addition to the activation of food-sensing signaling. We showed that exogenous serotonin and octopamine mimicked a well-fed and a starved state in thermotaxis, respectively, as in other behaviors such as egg laying, pharyngeal pumping, and locomotion (Figure 7, A and B). These results suggest that serotonin and octopamine act as feeding and food-deprived signals, respectively, to regulate temperature preference.

Importantly, CAT-1, a vesicular monoamine transporter with high affinity to serotonin, is required for the recovery from a starved state to a well-fed state at 25° (Figure 7C). One plausible scenario is that serotonin is released at a synaptic terminal as neurotransmitters or neurohormones as *C. elegans* eats food, although other components might act as feeding signals redundantly. Although CAT-1 seems to be a sole *v*esicular *m*ono*a*mine *t*ransporter (VMAT) in *C. elegans*, the *cat-1(e1111)* null mutant has no severe defects such as lethality and sterility (DUERR *et al.* 1999). We found that the *cat-1(e1111)* mutant exhibited normal reversal of thermotaxis at 17° and no severe defects other than the delay in thermotactic recovery at 25° (data not shown). It is possible that CAT-1 as well as other VMATs yet to be identified may act redundantly and that other VMATs substitute the role of CAT-1 in the *cat-1(e1111)* mutant.

The mechanism of modulation of thermotactic responses by feeding state: Neural plasticity is probably concerned with sensory transduction and synaptic or hormonal modulation in sensory neurons and interneurons. HEN-1, a secretory protein with an LDL receptor motif, is known to regulate sensory processing non-cell-autonomously in the mature neuronal circuit and the *hen-1* mutant exhibits Aho phenotype at both 17° and 25° (ISHIHARA *et al.* 2002). This result suggests that the neuroendocrine system may be important for modulation of thermotaxis. ISHIHARA *et al.* (2002) also reported that AIY interneurons are necessary for integration of two sensory signals and the expression of HEN-1 in other neurons is insufficient for substituting its function in AIY neurons (ISHIHARA *et al.* 2002). We anticipate that AIY is also crucial for the modulation of thermotaxis. It is conceivable that the synapses connecting with AIY act essentially in two different mechanisms, primary thermosensation followed by temperature memory formation and feeding-state-dependent neural plasticity. Consistent with this hypothesis, some, if not all, thermotaxis-defective mutants isolated to date are also defective in neural modulation by feeding states. For example, mutations in *ttx-3*, a LIM homeodomain-containing transcription factor expressed in AIY, lead not only to a cryophilic phenotype on a thermal gradient,

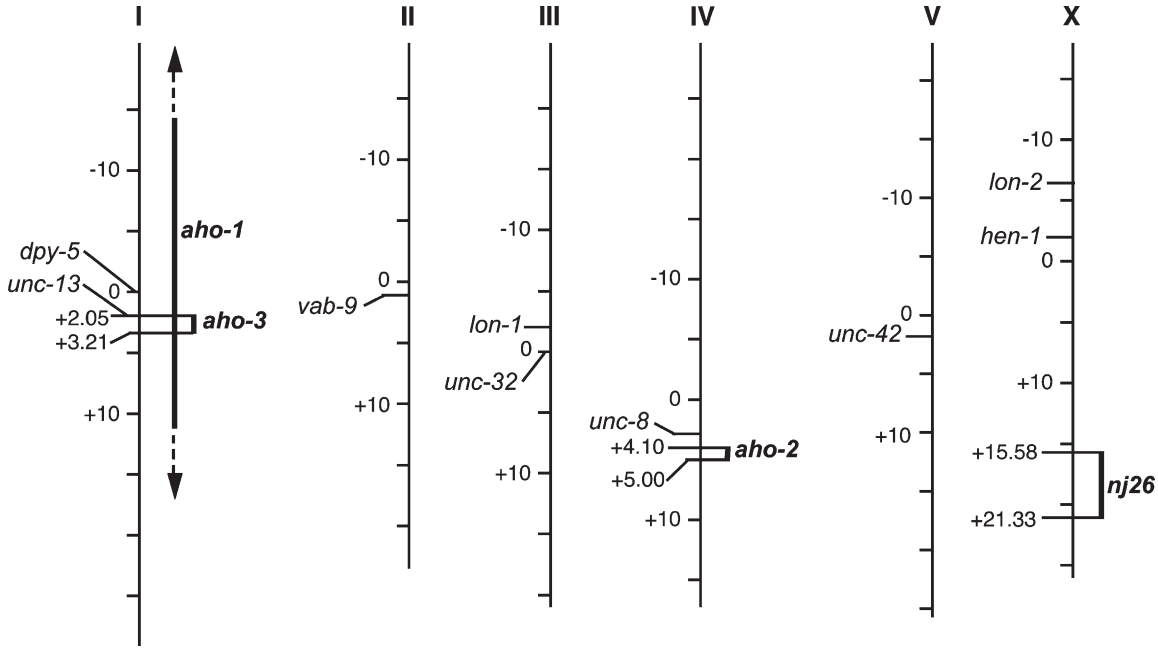

FIGURE 12.—The map position for each *aho* gene. Linkage groups are designated by roman numerals and X for the sex chromosome. The *hen-1* mutant also exhibits Aho phenotype at 17° and 25° (ISHIHARA *et al.* 2002).

but also to a defect in integration of two sensory signals (HOBERT *et al.* 1997; ISHIHARA *et al.* 2002).

To identify genes and cells required for modulation of thermotaxis by feeding state, we sought mutants that are defective in cultivation-temperature avoidance. *aho* mutants exhibit normal thermotaxis at a well-fed state and some of them responded to the change of feeding states normally, namely they exhibited normal sensorimotor responses in thermosensation and food recognition. They are the first example of novel thermotaxis-defective mutants in which modulation of thermotaxis by feeding state is specifically impaired.

aho mutants are classified into three groups: the first group exhibits Aho at both 17° and 25°, the second group exhibits Aho at 17°, and the third group exhibits Aho at 25°. This result predicts two possibilities. First, there are at least two mechanisms, a lower-temperature mechanism and a higher-temperature mechanism, for starvation-induced temperature avoidance. For example, in the second group of *aho* mutants, only the lower-temperature mechanism is damaged and the higher-temperature mechanism remains normal. Alternatively, *aho* mutants classified into the second and the third groups are simply temperature-sensitive and cold-sensitive mutants, respectively. We favor the former possibility, because prominent differences in time required for the transition from well-fed to starved state were observed in a cultivation-temperature-dependent manner (Figure 3, B and C). The analysis of other behaviors such as olfaction, habituation, and other learning paradigms for *aho* mutants would help to distinguish these possibilities.

What do *aho* genes encode? If the underlying mechanism for this paradigm is shared by a mechanism of learning and memory in higher animals, it is likely that some *aho* genes encode molecules already known to be important for learning and memory such as cyclic-nucleotide signaling pathway, neurotransmission, and cell adhesion. There are many candidates for *aho* genes in the *C. elegans* genome, for example, protein kinase A, cAMP response element-binding protein, and neuropeptides (BARGMANN 1998). It is also highly possible to identify novel molecules, leading to the discovery of new features to understand neural plasticity. We believe that the further dissection of *aho* genes will give us great insight and will clarify the mechanism of neural modulation in thermotaxis, which should lead to understanding a mechanism of learning and memory in mammals.

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LITERATURE CITED

AVERY, L., and H. R. HORVITZ, 1990 Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. J. Exp. Zool. **253:** 263–270.

BARGMANN, C. I., 1998 Neurobiology of the *Caenorhabditis elegans* genome. Science **282:** 2028–2033.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Chen, C. N., S. Denome and R. L. Davis, 1986 Molecular analysis of cDNA clones and the corresponding genomic coding sequences of the *Drosophila dunce*+ gene, the structural gene for cAMP phosphodiesterase. Proc. Natl. Acad. Sci. USA **83:** 9313–9317.

Colbert, H. A., and C. I. Bargmann, 1995 Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. Neuron **14:** 803–812.

Colbert, H. A., and C. I. Bargmann, 1997 Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*. Learn. Mem. **4:** 179–191.

Culotti, J. G., and R. L. Russell, 1978 Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics **90:** 243–256.

Desai, C., G. Garriga, S. L. McIntire and H. R. Horvitz, 1988 A genetic pathway for the development of the *Caenorhabditis elegans* HSN motor neurons. Nature **336:** 638–646.

Duerr, J. S., D. L. Frisby, J. Gaskin, A. Duke, K. Asermely *et al.*, 1999 The *cat-1* gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. J. Neurosci. **19:** 72–84.

Hedgecock, E. M., and R. L. Russell, 1975 Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **72:** 4061–4065.

Hobert, O., I. Mori, Y. Yamashita, H. Honda, Y. Ohshima *et al.*, 1997 Regulation of interneuron function in the *C. elegans* thermoregulatory pathway by the *ttx-3* LIM homeobox gene. Neuron **19:** 345–357.

Horvitz, H. R., M. Chalfie, C. Trent, J. E. Sulston and P. D. Evans, 1982 Serotonin and octopamine in the nematode *Caenorhabditis elegans*. Science **216:** 1012–1014.

Ishihara, T., Y. Iino, A. Mohri, I. Mori, K. Gengyo-Ando *et al.*, 2002 HEN-1, a secretory protein with an LDL receptor motif, regulates sensory integration and learning in *Caenorhabditis elegans*. Cell **109:** 639–649.

Komatsu, H., I. Mori, J. S. Rhee, N. Akaike and Y. Ohshima, 1996 Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. Neuron **17:** 707–718.

Kuhara, A., H. Inada, I. Katsura and I. Mori, 2002 Negative regulation and gain control of sensory neurons by the *C. elegans* calcineurin TAX-6. Neuron **33:** 751–763.

Levin, L. R., P. L. Han, P. M. Hwang, P. G. Feinstein, R. L. Davis *et al.*, 1992 The *Drosophila* learning and memory gene *rutabaga* encodes a Ca2+/calmodulin-responsive adenylyl cyclase. Cell **68:** 479–489.

Loer, C. M., and C. J. Kenyon, 1993 Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. J. Neurosci. **13:** 5407–5417.

Mayford, M., and E. R. Kandel, 1999 Genetic approaches to memory storage. Trends Genet. **15:** 463–470.

Mendel, J. E., H. C. Korswagen, K. S. Liu, Y. M. Hajdu-Cronin, M. I. Simon *et al.*, 1995 Participation of the protein Go in multiple aspects of behavior in *C. elegans*. Science **267:** 1652–1655.

Mori, I., and Y. Ohshima, 1995 Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature **376:** 344–348.

Qiu, Y. H., C. N. Chen, T. Malone, L. Richter, S. K. Beckendorf *et al.*, 1991 Characterization of the memory gene *dunce* of *Drosophila melanogaster*. J. Mol. Biol. **222:** 553–565.

Ranganathan, R., E. R. Sawin, C. Trent and H. R. Horvitz, 2001 Mutations in the *Caenorhabditis elegans* serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. J. Neurosci. **21:** 5871–5884.

Rankin, C. H., C. D. Beck and C. M. Chiba, 1990 *Caenorhabditis elegans*: a new model system for the study of learning and memory. Behav. Brain Res. **37:** 89–92.

Ryu, W. S., and A. D. Samuel, 2002 Thermotaxis in *Caenorhabditis elegans* analyzed by measuring responses to defined thermal stimuli. J. Neurosci. **22:** 5727–5733.

Sawin, E. R., R. Ranganathan and H. R. Horvitz, 2000 *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron **26:** 619–631.

Segalat, L., D. A. Elkes and J. M. Kaplan, 1995 Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. Science **267:** 1648–1651.

Sulston, J., M. Dew and S. Brenner, 1975 Dopaminergic neurons in the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **163:** 215–226.

Sze, J. Y., M. Victor, C. Loer, Y. Shi and G. Ruvkun, 2000 Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. Nature **403:** 560–564.

Trent, C., N. Tsung and H. R. Horvitz, 1983 Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

Waddell, S., and W. G. Quinn, 2001 Flies, genes, and learning. Annu. Rev. Neurosci. **24:** 1283–1309.

White, J. G., E. Southgate, J. N. Thomson and S. Brenner, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **314:** 1–340.

Wicks, S. R., R. T. Yeh, W. R. Gish, R. H. Waterston and R. H. Plasterk, 2001 Rapid gene mapping in *Caenorhabditis elegans* using a high density polymorphism map. Nat. Genet. **28:** 160–164.

Zariwala, H. A., A. C. Miller, S. Faumont and S. R. Lockery, 2003 Step response analysis of thermotaxis in *Caenorhabditis elegans*. J. Neurosci. **23:** 4369–4377.

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