Neural circuit-dependent odor adaptation in *C. elegans* is regulated by the Ras-MAPK pathway

Takaaki Hirotsu[a] and Yuichi Iino*

Molecular Genetics Research Laboratory, The University of Tokyo, Tokyo 113-0033, Japan

The molecular machinery that mediates odor adaptation in the olfactory neurons is well documented in various animal species. However, types of adaptation that depend on neural circuits are mostly unexplored. We report here that the Ras-MAPK pathway is essential for such a type of odor adaptation, called early adaptation, in *C. elegans*. Early adaptation requires a pair of AIY interneurons, which receive synaptic inputs from olfactory neurons. Mutants of the Ras-MAPK pathway show defects in early adaptation. Continued exposure to an odorant causes activation of MAP kinase not only in the olfactory neurons, but also in the AIY interneurons. While activity of the Ras-MAPK pathway in the olfactory neurons is important for odor perception, its activity in the AIY interneurons is important for odor adaptation. Our results thus reveal a dual role of the Ras-MAPK pathway in sensory processing in the nervous system of *C. elegans*.

Introduction

Sensory systems can respond to an extraordinarily wide variety of qualities and strengths of environmental stimuli. To maintain their high sensitivity to changes in stimulus intensities and to ignore physiologically unimportant stimuli, animals often decrease their sensitivity to continuous stimuli. This phenomenon is generally referred to as adaptation, and is an intrinsic characteristic of sensory systems.

In the olfactory systems, adaptation is also commonly observed in a broad range of animals. Olfactory adaptation is generally considered to occur at multiple levels, that are often categorized into the peripheral level (occurring within sensory neurons) and the central level (occurring at higher-order relay neurons and interconnections between them) (Dalton 2000). Odor adaptation at the peripheral level has been intensively analyzed, mostly using isolated olfactory neurons from various species, and its molecular mechanisms are well documented. In vertebrate olfactory neurons, modulation by calcium/calmodulin of cyclic nucleotide-gated channels plays a major role in odor adaptation, which occurs within seconds (Munger *et al.* 2001). Modulation of adenylyl cyclase by calcium/calmodulin-dependent protein kinase II is also responsible for adaptation (Leinders-Zufall *et al.* 1999). On the other hand, carbon monoxide (CO) and cGMP mediate a slower form of adaptation (Zufall & Leinders-Zufall 2000).

In addition to the peripheral adaptation, the involvement of central adaptation (also called habituation), which depends on neural circuits, has been recognized through various observations, and is becoming a focus of interest in the context of olfactory information processing. In humans, the cognitive perception of odor adapts more rapidly than electrophysiological responses of olfactory receptor neurons (Hummel *et al.* 1996). In rats, electrophysiological studies have shown adaptation to occur at the connections between olfactory receptor neurons and second-order neurons in the main olfactory bulb (MOB), as well as at the connections between MOB projection neurons and third-order neurons such as those in the piriform cortex (PCX) (Potter & Chorover 1976; Wilson 1998). Lateral neural connections may play roles for adaptation in both MOB and PCX (Yokoi *et al.* 1995; Wilson 2003). Plasticity in both of these relay structures is implicated not only in sensory adaptation but also in the refinement of odor receptive fields and odor discrimination (Fletcher & Wilson 2003). In *Drosophila*, long-term odor exposure causes long-lasting adaptation (lasting for more than a week). This form of adaptation involves the reduction of both volume and the number of synapses in the antennal robes, which

Communicated by: Isao Katsura
**Correspondence*: E-mail: iino@gen.s.u-tokyo.ac.jp
[a]*Present address*: Department of Biology, Graduate School of Science, Kyushu University, Fukuoka 812-8581, Japan.

DOI: 10.1111/j.1365-2443.2005.00856.x

are the insect olfactory centers. A mutant impaired in the cAMP phosphodiesterase, *dunce*, is defective in this form of adaptation (Devaud *et al*. 2001). Despite these observations, the functioning of the neural circuitry and the underlying molecular mechanisms for odor adaptation are poorly understood due to the complexity of the structures in these organisms.

In order to analyze neural circuit-dependent odor adaptation at the molecular level, simple and genetically tractable model organisms are beneficial. The soil nematode *Caenorhabditis elegans* has a compact nervous system consisting of 302 neurons, whose connections have been fully documented (White *et al*. 1986), including the whole circuitry of the olfactory system. Outputs of the olfactory nervous system can be detected as chemotaxis behavior toward odorants (Bargmann *et al*. 1993). Based on the powerful genetics available in *C. elegans*, the functions of olfactory neurons and the molecular mechanisms of odor perception have been well documented in this organism. In AWC olfactory neurons, the sensory transduction pathway consists of GPCR odorant receptors, G proteins, the cGMP pathway, and cyclic nucleotide-gated channels (Bargmann & Kaplan 1998). The sensory transduction pathway is similar overall to those of higher organisms, thus providing a basis for using *C. elegans* as a model for understanding olfactory systems. In addition, the Ras-MAPK pathway is activated by odor stimuli in olfactory neurons (Hirotsu *et al*. 2000). The response of MAPK is also conserved in mammalian olfactory neurons (Watt & Storm 2001).

It is known that *C. elegans* shows odor adaptation as well at the behavioral level. After continuous exposure to an odorant for more than 30 min, *C. elegans* shows declined chemotaxis to that odorant (Colbert & Bargmann 1995). Impairment of this form of adaptation was reported for mutants in the *osm-9* gene, which encodes a TRPV ion channel, and overproduction of ODR-1, a guanylyl cyclase. The cyclic-GMP-dependent protein kinase EGL-4 is also important for adaptation. Phosphorylation of the beta subunit of the cyclic nucleotide gated channels TAX-2, which may be catalyzed by EGL-4, is essential for adaptation after 30 min of odor exposure, while the nuclear localization of EGL-4 is required for adaptation caused by odor exposure for one hour (L'etoile *et al*. 2002). On the other hand, calcineurin A subunit TAX-6 negatively regulates adaptation and acts antagonistically with OSM-9 (Kuhara *et al*. 2002). It has been suggested that these molecules act in the olfactory neurons, thereby altering the response of the olfactory neurons to odorants. However, neural circuit-dependent odor adaptation has not so far been reported in *C. elegans*.

In this study, we establish an assay system for analyzing neural circuit-dependent odor adaptation in *C. elegans*. This adaptation behavior requires AIY interneurons, which have synaptic inputs from olfactory neurons. The mutants of the Ras-MAPK pathway exhibit defects in early adaptation. MAP kinase is activated by odorant stimuli not only in the olfactory neurons, but also in the AIY interneurons. While activity of the Ras-MAPK pathway in the olfactory neurons is important for odor perception, its activity in the AIY interneurons is important for odor adaptation. These results reveal that the Ras-MAPK pathway regulates neural circuit-dependent adaptation and suggest a dual role of the pathway in olfactory sensory processing.

Results

Establishment of a novel assay system for odor adaptation, early adaptation

C. elegans exhibits chemotaxis to a set of odorants. Earlier studies showed that this response was decreased through adaptation after long pre-exposure to odorants, typically for an hour (Colbert & Bargmann 1995; Bernhard & Van Der Kooy 2000). We made several modifications to the assay protocols, including the procedure for odor pre-exposure and the plate layout for chemotaxis assays (see Experimental procedures). In this assay, we found that odor adaptation is observed after a very short pre-exposure to odorants: when worms were pre-exposed to odorants for 5 min, they showed greatly reduced chemotaxis to the same odorants at normally attractive concentrations (Fig. 1A, Supplementary Movie S1). This form of adaptation, early adaptation, has been observed for isoamyl alcohol, benzaldehyde (known to be sensed by AWC olfactory neurons, Bargmann *et al*. 1993), pyrazine and diacetyl (sensed by AWA). Pre-exposure to butanone, however, did not cause significant decrease in chemotaxis (data not shown). Extents of early adaptation depended on concentrations of both pre-exposed odorants and attractants on chemotaxis plates. We have observed that 10^{-4} dilution at pre-exposure is optimal for all odorants that cause early adaptation. On the other hand, optimal concentrations of attractants on chemotaxis plates varied between odorants, with adaptation to isoamyl alcohol and diacetyl being less pronounced at high attractant concentrations (Fig. 1A). Based on these observations, we fixed the assay conditions (see Experimental procedures). The decreased chemotaxis is unlikely to be due to a nonspecific effect of the pre-exposure treatment, since chemotaxis to NaCl (which is sensed by sensory neurons other than AWC and AWA)

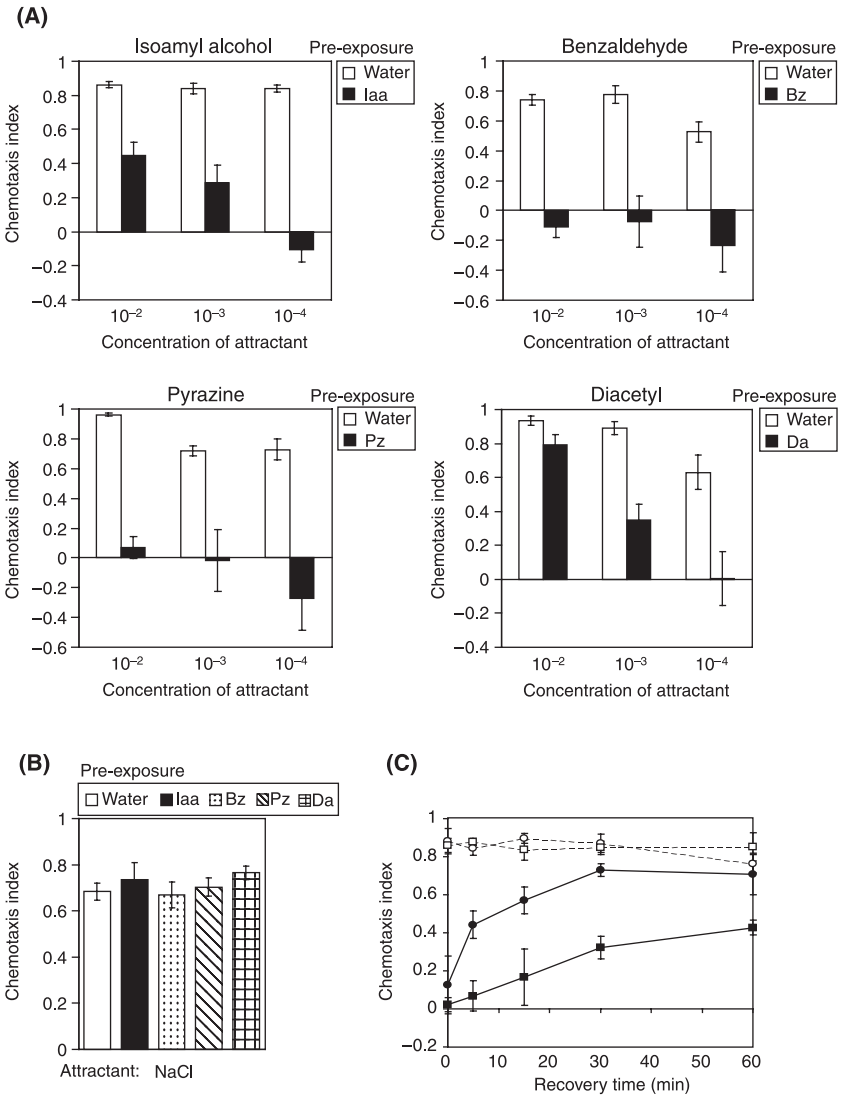


Figure 1 The early adaptation. (A) After pre-exposure to a 10^{-4} dilution of an odorant for 5 min, worms show a decrease in chemotaxis to the same odorant. □ chemotaxis after pre-exposure to water; ■ chemotaxis after pre-exposure to the odorants. Iaa, Bz, Pz and Da indicate isoamyl alcohol, benzaldehyde, pyrazine and diacetyl, respectively. The attractant dilutions are indicated at the bottom. (B) Chemotaxis to 100 mM NaCl after pre-exposure to water (open bar), isoamyl alcohol (filled bar), benzaldehyde (stippled bar), pyrazine (hatched bar) and diacetyl (cross-hatched bar). (C) Recovery from adaptation. Animals were pre-exposed to a 10^{-4} dilution of isoamyl alcohol for 5 min (●) or 30 min (■), allowed to recover in the absence of the odorant for different periods of time, and then tested for chemotaxis to a 10^{-4} dilution of isoamyl alcohol. Control animals which were exposed to water for 5 min (○) or 30 min (□) were also tested.

is not affected by pre-exposure to the odorants (Fig. 1B). The persistence of the adaptation is graded with the duration of the odor pre-exposure. After pre-exposure to the odorants for 5 min, 30 min are required for recovery, while the adaptation induced by a longer pre-exposure (30 min) requires a longer recovery time (Fig. 1C), suggesting that the underlying mechanisms may be different between the short and long exposures (see below).

Cross-adaptation occurs between odorants sensed by different olfactory neurons

Cross-adaptation is observed between the two AWC-sensed odorants, isoamyl alcohol and benzaldehyde, as previously reported for the conventional adaptation assay (Fig. 2A, filled and stippled bars) (Colbert & Bargmann 1995), and observed between two AWA-sensed odorants, pyrazine and diacetyl, where pyrazine exposure causes significant decrease in chemotaxis to diacetyl (Fig. 2B, hatched and cross-hatched bars). Unexpectedly, we found that cross adaptation occurs between AWC-sensed and AWA-sensed odorants in our assay. Five minutes of pre-exposure to AWA-sensed pyrazine and diacetyl caused a reduction in the chemotaxis to the AWC-sensed odorants, isoamyl alcohol and benzaldehyde (Fig. 2C, hatched and cross-hatched bars). Pre-exposure to AWC-sensed benzaldehyde also caused decreased chemotaxis to AWA-sensed pyrazine (Fig. 2D, stippled bars). No significant cross-adaptation was observed in other combinations of odorants. Although the basis for this limited occurrence of cross adaptation is currently unknown, cross-adaptation appears to occur

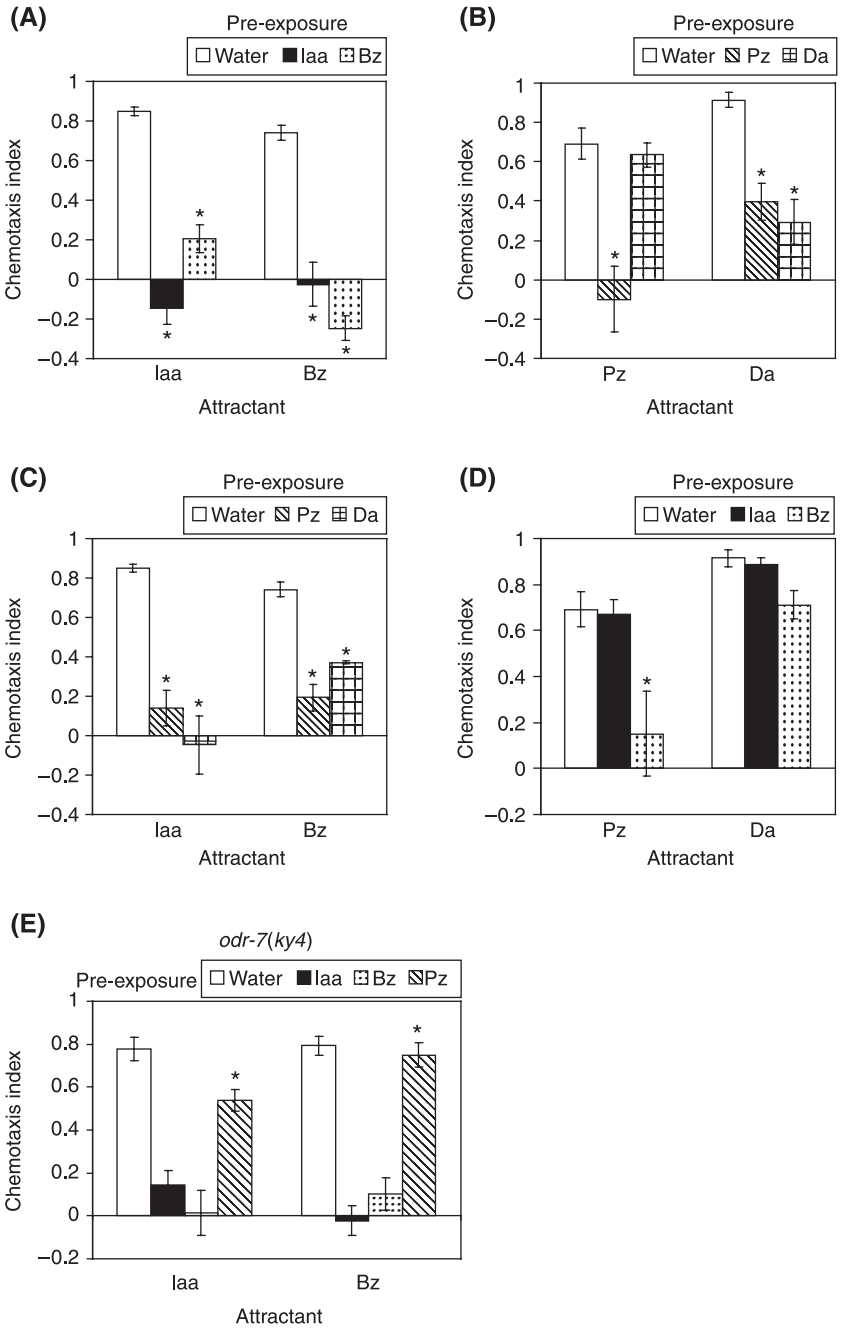


Figure 2 Cross-adaptation (A, B) Cross-adaptation between odorants sensed by the same olfactory neurons. Odorants used are (A) isoamyl alcohol and benzaldehyde, both sensed by AWC, (B) and pyrazine and diacetyl, both sensed by AWA. (C, D) Cross-adaptation between odorants sensed by different olfactory neurons. Chemotaxis to AWC-sensed odorants after pre-exposure to AWA-sensed odorants (C) and chemotaxis to AWA-sensed odorants after pre-exposure to AWC-sensed odorants (D). In (A–D), asterisks indicate the values that differ significantly ($P < 0.05$, *t*-test) from that of pre-exposure to water. (E) Cross-adaptation assay for *odr-7*(*ky4*) mutants. Pre-exposure odorants were water (open bars), isoamyl alcohol (filled bars), benzaldehyde (stippled bars) and pyrazine (hatched bars). ⋆the values that differ significantly ($P < 0.05$, *t*-test) from that of the wild-type.

only for chemotaxis mediated by olfactory neurons, because pre-exposure to odorants does not affect chemotaxis to NaCl.

These observations of cross-adaptation suggest the existence of a functional interplay between AWA and AWC olfactory neurons. However, because the animals are in direct contact with diluted odorants during the pre-exposure treatments, it is also possible that the odorants are sensed by neurons different from those used for chemotaxis. For instances, pyrazine might in fact be sensed by AWC during pre-exposure to cause adaptation of AWC. To test this possibility, we used *odr-7*(*ky4*) mutants, which do not have functional AWA neurons and exhibit severe defects in chemotaxis to AWA-sensed odorants including pyrazine, but show normal chemotaxis to AWC-sensed odorants (Sengupta *et al.* 1994). Cross-adaptation by pyrazine pre-exposure was specifically impaired in the *odr-7* mutant, indicating that

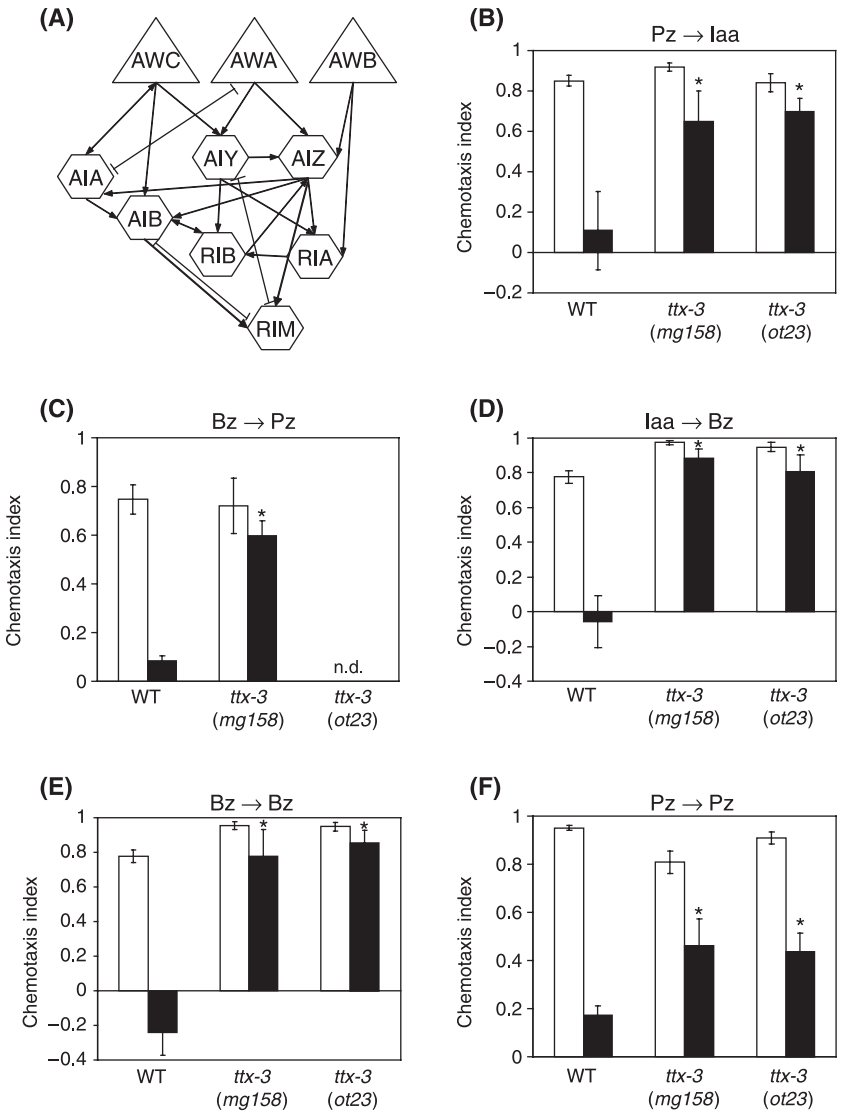


Figure 3 Adaptation defects of the *ttx-3* mutants. (A) A neural network for the olfactory system, extracted from the complete circuit (White *et al.* 1986). Triangles and hexagons represent sensory neurons and interneurons, respectively. Arrows indicate chemical synapses, and the bars with vertical lines at each end indicate gap junctions. (B–F) Early adaptation of wild-type animals, and *ttx-3*(*mg158*) and *ttx-3*(*ot23*) mutants. Open bars represent the control values of chemotaxis after pre-exposure (5 min) to water, and filled bars represent chemotaxis after pre-exposure (5 min) to an odorant. The odorants used for the pre-exposure and chemotaxis assays were: (B) pyrazine → isoamyl alcohol (chemotaxis to isoamyl alcohol after pyrazine adaptation); (C) benzaldehyde → pyrazine; (D) isoamyl alcohol → benzaldehyde; (E) benzaldehyde → benzaldehyde; (F) pyrazine → pyrazine. *values that differ significantly ($P < 0.05$, *t*-test) from that of the wild-type.

pyrazine was indeed sensed mostly by the AWA neurons (Fig. 2E, hatched bars; compare with Fig. 2C).

The AIY interneurons are essential for early adaptation

The observation of cross-adaptation led us to test the possible involvement of AIY interneurons, which receive synaptic inputs from both the AWA and AWC olfactory neurons and are known to be important for sensory processing (White *et al.* 1986; Mori & Ohshima 1995; Ishihara *et al.* 2002) (Fig. 3A). The *ttx-3* mutants have strong defects in the differentiation of AIY and weak defects in that of the AIA interneurons (Hobert *et al.* 1997; Altun-Gultekin *et al.* 2001). All the *ttx-3* mutants tested (*ks5*, *mg158*, *ot22*, *ot23*) exhibited severe defects in cross-adaptation between AWA-sensed and AWC-sensed odorants (pyrazine → isoamyl alcohol; Fig. 3B, benzaldehyde → pyrazine; Fig. 3C and data not shown). However, the defects are not restricted to such types of cross-adaptation, since the mutants also exhibited deficits in cross-adaptation between odorants sensed by same olfactory neurons and in adaptation to single odorants (Fig. 3D–F and data not shown). Therefore, the interneurons appear to be important for early adaptation. Although we cannot exclude the involvement of AIA for adaptation, lack of functional AIY neurons is probably responsible for the adaptation defect observed in the mutants, because of the results of AIY-specific rescue experiments described below.

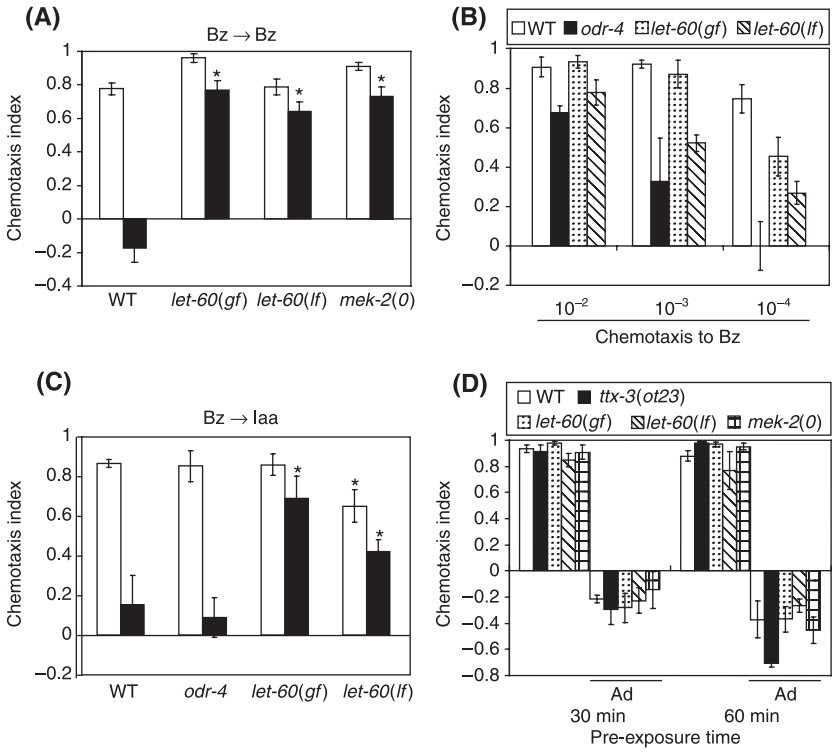


Figure 4 Early adaptation in the mutants of the Ras-MAPK pathway. (A) Adaptation to benzaldehyde in wild-type animals and mutants of the Ras-MAPK pathway. Open bars represent chemotaxis to benzaldehyde after exposure to water (5 min), and filled bars represent chemotaxis after exposure to benzaldehyde (5 min). Asterisks indicate the values that differ significantly ($P < 0.05$) from that of the wild-type. (B) Chemotaxis to benzaldehyde of untreated wild-type animals (open bars), and *odr-4*(*n2144*) (filled bars), *let-60*(*n1046*gf) (stippled bars) and *let-60*(*n2021*lf) (hatched bars) mutants. The dilutions of benzaldehyde used are indicated at the bottom. (C) Benzaldehyde → isoamyl alcohol cross-adaptation of wild-type animals, and *odr-4*(*n2144*), *let-60*(*n1046*gf) and *let-60*(*n2021*lf) mutants. Open bars represent chemotaxis to isoamyl alcohol after pre-exposure to water (5 min), and filled bars represent chemotaxis after pre-exposure to benzaldehyde (5 min). ⋆values that differ significantly ($P < 0.05$) from that of the wild-type. (D) Benzaldehyde adaptation of wild-type animals (open bars), and *ttx-3*(*ot23*) (filled bars), *let-60*(*n1046*gf) (stippled bars), *let-60*(*n2021*lf) (hatched bars) and *mek-2*(*n2678*) (cross-hatched bars) mutants after pre-exposure for 30 min and 1 h. The left data points in each set indicate chemotaxis after pre-exposure to water, and the right data points indicate chemotaxis after pre-exposure to benzaldehyde (marked as Ad). The pre-exposure times are indicated at the bottom.

In contrast to the severe defects in adaptation, *ttx-3* mutants do not show any deficits in naive chemotaxis to odorants under the conditions (Fig. 3B–F and data not shown). This observation is consistent with the previous report (Tsalik & Hobert 2003), in which the mutants showed defects in chemotaxis to low ('near-threshold') concentrations of odorants, but showed no defects at higher ('supra-threshold') concentrations, the same concentration range (including 10^{-2} dilution of benzaldehyde) as used in our adaptation assays.

Mutants of the Ras-MAPK pathway show defects in early adaptation

We have previously reported that mutants of the Ras-MAPK pathway exhibit weak defects in chemotaxis to low concentrations of odorants, and that exposure to an odorant causes activation of MAP kinase in the olfactory neurons (Supplementary Fig. S1 and Hirotsu *et al.* 2000). Because the Ras-MAPK signaling pathway is known to be versatile and seemed a good candidate for a mediator of the plasticity observed in the early adaptation assay, we tested the mutants in this pathway and found that they show large defects in the early adaptation assay (Fig. 4A and Supplementary Fig. S1). The mutants tested included the gain-of-function (gf) mutant of *let-60 ras*, *let-60*(*n1046*) (Beitel *et al.* 1990), the loss-of-function (lf) mutant of *let-60 ras*, *let-60*(*n2021*) (Han & Sternberg 1990), and the presumed null mutant *mek-2*(*n2678*), where *mek-2* encodes a MAP kinase kinase (Kornfeld *et al.* 1995; Wu *et al.* 1995). In contrast to the small or no defects of the mutants in chemotaxis at the odorant concentrations and plate

format used in the early adaptation assay, adaptation was severely impaired in the mutants in most odorant combinations (Fig. 4A and Supplementary Fig. S1).

To exclude the possibility that the apparent defects of the Ras-MAPK mutants in adaptation are due to reduced sensitivity to the odorants during pre-exposure, we assayed the *odr-4(n2144)* mutant for comparison. This mutant shows normal chemotaxis to isoamyl alcohol and concentration-dependent defects in chemotaxis to benzaldehyde (Dwyer *et al.* 1998). Therefore, by using the cross-adaptation paradigm (chemotaxis to isoamyl alcohol after pre-exposure to benzaldehyde), effect of reduced odorant sensitivity during pre-exposure could be assessed, and the effects could be compared between the *odr-4* and *let-60* mutants. The *odr-4* mutant showed similar or even weaker chemotaxis to benzaldehyde compared to the *let-60* mutants (Fig. 4B). However, unlike the *let-60* mutants, the *odr-4* mutants showed normal adaptation after pre-exposure to benzaldehyde (Fig. 4C). Therefore, the weak sensory defects of the *let-60* mutants cannot by themselves account for the deficits in adaptation. Thus, we infer that the Ras-MAPK pathway has a specific role in the plasticity of the odorant response, apart from its role in odor perception (Hirotsu *et al.* 2000).

In contrast to the early adaptation, the *let-60(gf)*, *let-60(lf)* and *mek-2(0)* mutants show normal adaptation after longer exposure to the odorants for 30 min or 1 h (Fig. 4D). This suggests that the Ras-MAPK pathway is mainly required for the early phase of adaptation. The *ttx-3* mutants also show a normal level of adaptation after long exposure to the odorants (Fig. 4D), in accordance with the presumption that adaptation to a long exposure to odorants (including both 'short-term' and 'long-term' components (L'etoile *et al.* 2002)) is caused by the molecular machinery in the olfactory neurons (Colbert & Bargmann 1995; L'etoile & Bargmann 2000; L'etoile *et al.* 2002). The *osm-9* mutant, which has defects in the conventional adaptation assay (Colbert & Bargmann 1995; Colbert *et al.* 1997), shows no defects in the early adaptation assay (data not shown), further implying that different types of adaptation depend on partially non-overlapping molecular mechanisms.

MAP kinase is activated by odorant stimulus in the AIY neurons

As mentioned above, MAP kinase is activated in the olfactory neurons by odor exposure, whereas the AIY interneurons appear to be important for early adaptation. A question therefore arises as to whether MAP kinase would be activated in the AIY interneurons by an olfactory stimulus as well. We therefore performed immunofluorescence with an antibody against activated (diphosphorylated) MAPK. After 5 min of application of a 10^{-4} dilution of isoamyl alcohol, the same treatment as that used for the adaptation assays, the activation of MAPK was detected in both AWC and AIY. The staining was observed in the cell bodies, nuclei and neural processes in both neurons (Fig. 5A,B). In contrast, phospho-MAPK staining in the salt-sensing ASE neuron did not increase above background by the same treatment, indicating that the activation of MAPK is not due to non-specific effect of odorant treatment (Fig. 5B). Staining of activated MAPK in AIY was eliminated in the *let-60(lf)* mutants (Fig. 5B), indicating that this activation of MAPK in AIY depends on LET-60 Ras. Application of other odorants, benzaldehyde or pyrazine, also caused activation of MAPK in AIY (Fig. 5B). These results are consistent with the general requirement of AIY interneurons for early adaptation and suggest the possibility that the Ras-MAPK pathway may function in AIY interneurons as well as in olfactory neurons.

Activity of *let-60 ras* in the AIY interneurons is required for adaptation

To determine whether the Ras-MAPK pathway functions in AIY interneurons or olfactory neurons in early adaptation, we tested whether the expression of the wild-type form of *let-60 ras* in AIY interneurons or AWC olfactory neurons could rescue the defects of the *let-60(lf)* mutants in adaptation to AWC-sensed odorants. As reported previously (Hirotsu *et al.* 2000), expression of *let-60(+)* in AWC (and two other neurons, AWB and I1) by the *gcy-10* promoter rescues the weak chemotaxis defects of the *let-60(lf)* mutant to AWC-sensed odorants (Fig. 6A). However, AIY-specific expression of *let-60(+)* by the cryptic *ttx-3* promoter did not significantly affect chemotaxis (Fig. 6A). In contrast, AIY-specific expression of *let-60(+)* rescued the adaptation defects of the *let-60(lf)* mutants in isoamyl alcohol adaptation, benzaldehyde adaptation, and cross-adaptation between these odorants, while AWC-specific expression did not appear to rescue (Fig. 6B,C and Supplementary Fig. S2). AIY-specific expression also rescued defects in cross-adaptation between AWA-sensed pyrazine and AWC-sensed odorants (Fig. 6D and data not shown). Expression of *let-60(+)* in AIY using *sra-11* promoter, which drives expression in AIY, AVB and a pharyngeal neuron (Troemel *et al.* 1995), also rescued the adaptation defects under all conditions (data not shown). Taken together, these results strongly suggest that the function of LET-60 in AIY interneurons is important for early adaptation.

Its function in sensory neurons, on the other hand, is required for efficient odor perception.

As another means to assess the influence of the weak chemotaxis defects of the *let-60*(*lf*) mutants, we tested the effect of AIY-specific expression of *let-60(+)* in *let-60*(*lf*) lines whose chemotaxis defect was rescued by AWC-specific expression of *let-60*(+) (Fig. 6A,E and Hirotsu *et al.* 2000). Compared to the expression of *let-60*(+) in AWC only, expression in AWC and AIY caused significant improvement of adaptation (Fig. 6E). This result reinforces the conclusion that the functions of the Ras-MAPK pathway in AIY are important for adaptation and its roles in adaptation can be separated from those in odor perception.

AIY neurons are known for several functions in the regulation of worm behaviors (Mori & Ohshima 1995; Ishihara *et al.* 2002; Tsalik & Hobert 2003). To see whether the Ras-MAPK pathway is required for all the functions of AIY neurons, we determined the reversal frequency of the *let-60* mutants. AIY neurons are known to act to reduce reversal frequency (Tsalik & Hobert 2003). In accordance, the *ttx-3* mutants showed enhanced reversal frequency. In contrast, neither the *let-60*(*gf*) nor *let-60*(*lf*) mutants showed this defect (Supplementary Fig. S3). These results suggest that the Ras-MAPK pathway is not required for all AIY functions but has some specialized roles, including those for odor adaptation.

Discussion

A novel assay system for neuronal circuit-dependent odor adaptation

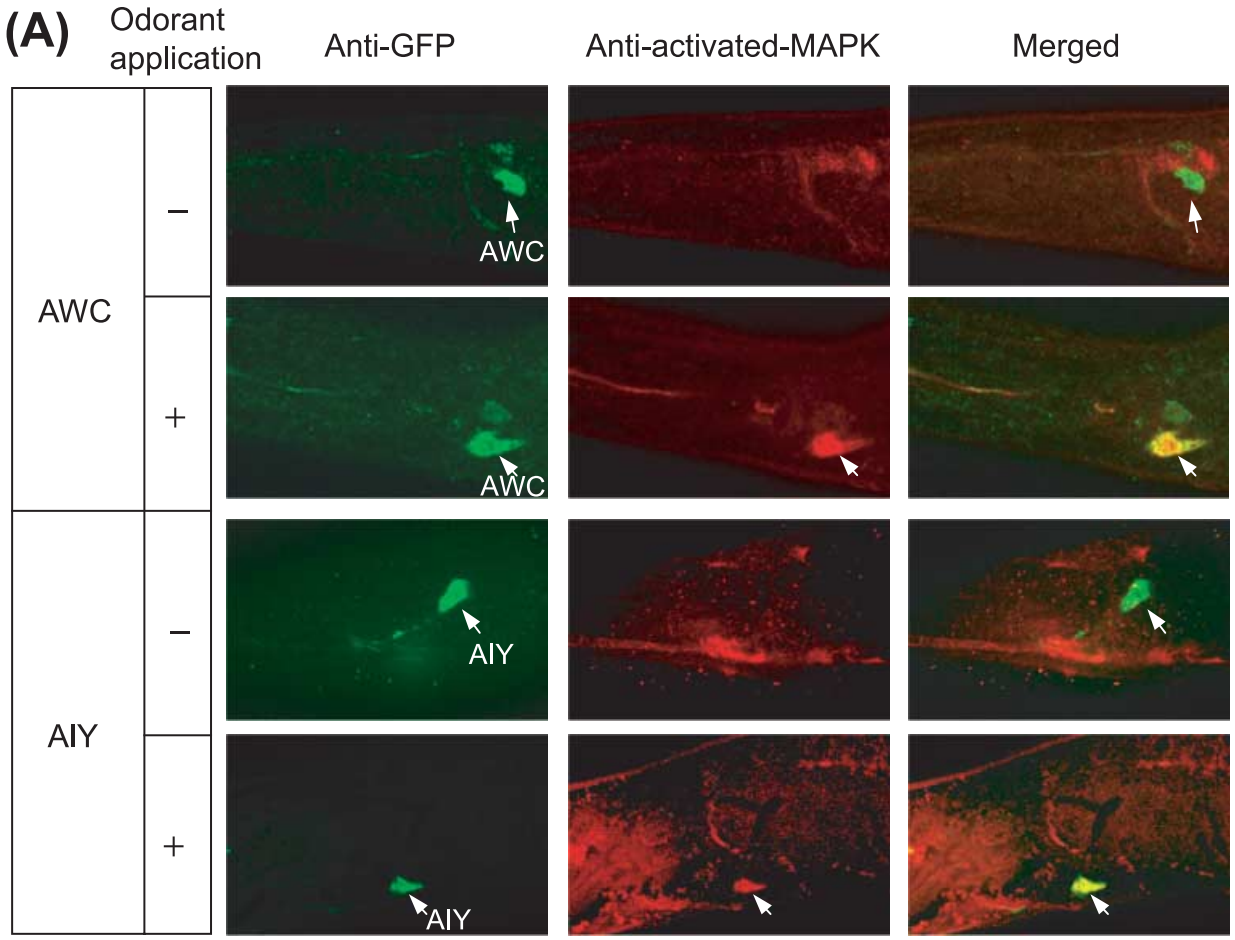
In this study, we established an assay system for odor adaptation in *C. elegans* that does not occur solely in olfactory neurons but depends on the function of AIY interneurons. We modified the conventional assay protocols for odor adaptation, which allowed us to detect a form of adaptation that occurs early. Modifications in the plate layout for chemotaxis were particularly essential for the early adaptation assay (See Experimental procedures). On the other hand, it was not essential to pre-expose the animals to the odorants in solution, because similar results were obtained by exposing the animals to vaporized odorants, as described in Colbert & Bargmann (1995). We speculate that our plate layout is more sensitive to odorant avoidance behaviors, which may have a significant contribution to early adaptation (see below). In fact, the layout is similar to that used previously for the long-range odorant avoidance assay (Troemel *et al.* 1997).

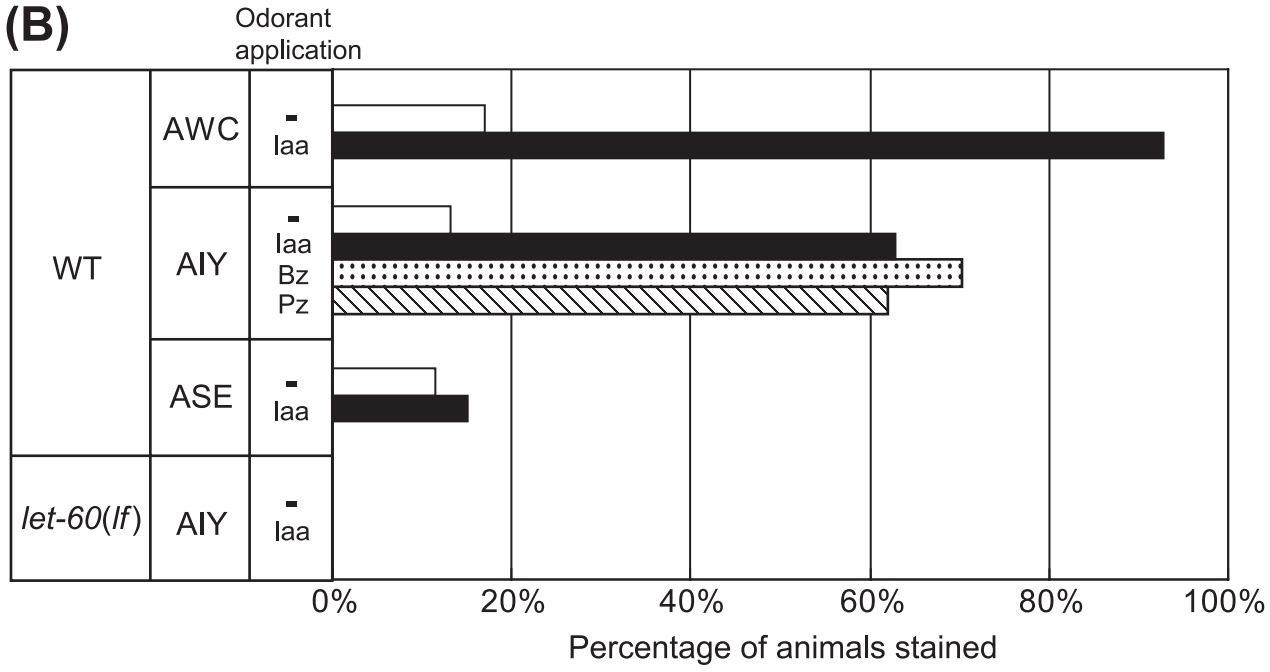
C. elegans, as in most other animals, can sense a wide range of concentrations of volatile odorants (about six orders of magnitude). To detect changing concentrations of odorants, animals probably need to dynamically fine-tune the sensitivity of the sensory system. Sensory adaptation that occurs in a time range of seconds is generally believed to contribute to such tuning mechanisms in sensory systems. Because early adaptation occurs faster than conventional odor adaptation, it might be manifestation of such a dynamic sensitivity-modulation mechanism.

In another view, the mechanisms of early adaptation may serve as a short-term memory necessary for sustained alteration of behaviors. Large amounts of undiluted attractive odorants often induce avoidance behaviors in chemotaxis assays (our unpublished data and Nuttley *et al.* 2001). In such cases, worms initially move towards the undiluted odorant, then switch the direction of locomotion near the odorant, and leave the odorant until they reach the opposite side of the assay plates. This long-range avoidance requires a memory that lasts for minutes, because worms continue to show the avoidance behavior throughout the assay once they experience a high concentration of odorants. Early adaptation might be a basis for such a behavioral switch from attraction to avoidance. Because avoidance of extreme high concentrations of odorants seems to be required for keeping away from the danger of organic solvents, early adaptation may be essential for the survival of worms.

Although the ethological significance of early adaptation remains unclear, the assay system will be useful for studies on neuronal functions. Several assay systems for analyzing behavioral plasticity in *C. elegans* are known. However, molecular mechanisms for the plasticity have been mostly uncharacterized (Hobert 2003). As an addition

Figure 5 Activation of MAPK after exposure to odorants. (A) Immunofluorescence images of anti-GFP staining (green) and anti-activated-MAPK staining (red), and the merged images (yellow indicates an overlap). Strains carrying *gcγ-10p*::*GFP* (top panels) or *ttx-3p*::*GFP* (bottom panels) (Hobert *et al.* 1997; Yu *et al.* 1997) that label AWC olfactory neurons and AIY interneurons, respectively, were used to facilitate cell identification. All images show the left lateral view of the head region of wild-type animals. '–' indicates animals fixed without odorant application. '+' indicates animals fixed after a 5 min application of a 10^{-4} dilution of isoamyl alcohol. (B) Proportions of wild-type animals showing staining with anti-activated-MAPK in AWC olfactory neurons, AIY interneurons or ASE sensory neuron and the *let-60*(*n2021*lf) mutants showing staining in AIY interneurons. Immunostaining was observed after exposure to water (open bars), a 10^{-4} dilution of isoamyl alcohol (filled bars), a 10^{-4} dilution of benzaldehyde (stippled bars) or a 10^{-4} dilution of pyrazine (hatched bars) for 5 min. Each bar represents a total of at least three independent experiments (wild-type: AWC; n = 28–71, AIY; n = 27–53, ASE; n = 20–26, and *let-60*(*lf*): AIY; n = 18–24).





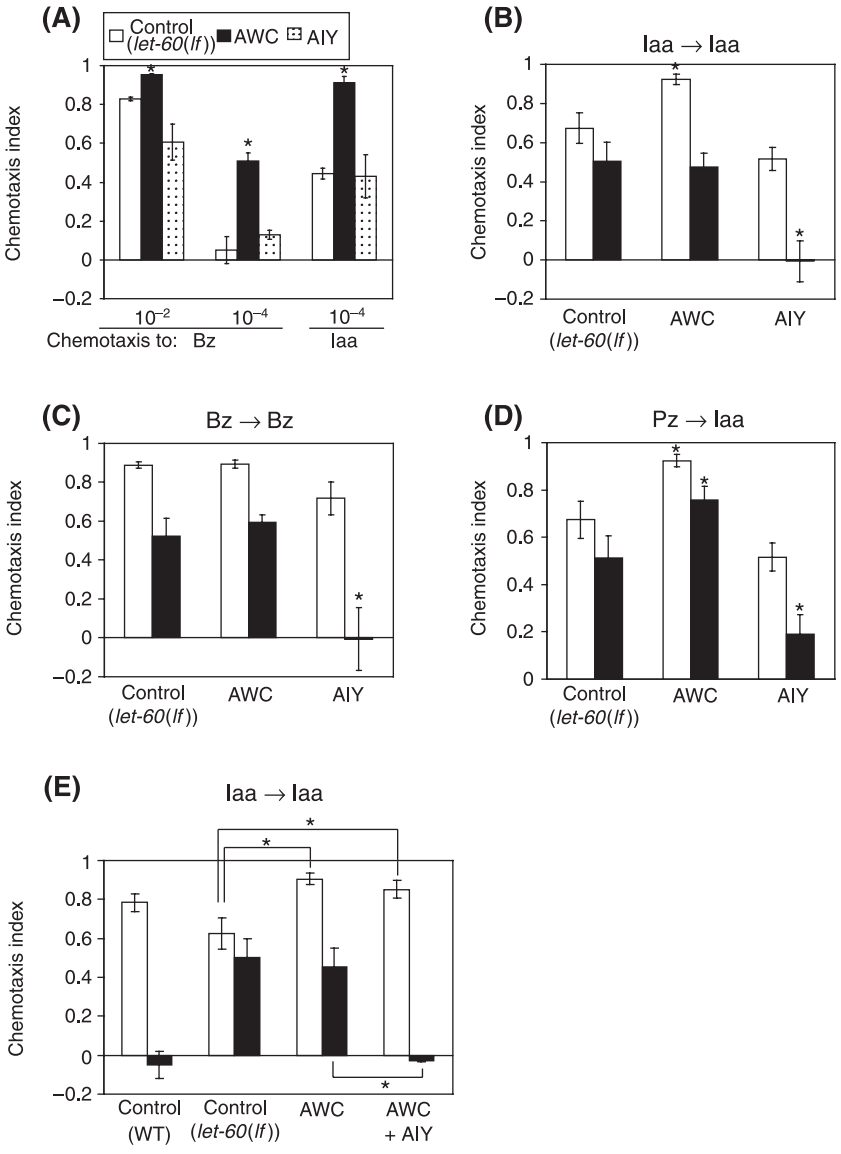


Figure 6 Cell-specific expression of *let-60*. (A) Chemotaxis to 10^{-2} or 10^{-4} dilutions of benzaldehyde and a 10^{-4} dilution of isoamyl alcohol of the *let-60*(*lf*) mutant carrying the transgenes. In (A–E), 'Control' indicates wild-type or the *let-60*(*n2021*lf) mutant carrying the transformation marker only. 'AWC' indicates the mutant carrying a *let-60*(+) transgene under the control of the *gcy-10* promoter, which drives expression in AWC, AWB and I1. 'AIY' indicates the mutant carrying *let-60*(+) under the control of the cryptic *ttx-3* promoter expressed exclusively in AIY (see Experimental procedures). (B–D) Early adaptation of the *let-60*(*lf*) mutant carrying the transgenes. Open bars represent chemotaxis after pre-exposure to water, and filled bars represent chemotaxis after pre-exposure to the odorant. The odorants used were: (B) isoamyl alcohol, (C) benzaldehyde and (D) pyrazine → isoamyl alcohol. *values that differ significantly ($P < 0.05$) from that of the control. (E) Isoamyl alcohol adaptation of wild-type control and the *let-60*(*lf*) mutant carrying the transgenes. Open bars represent chemotaxis after pre-exposure to water, and filled bars represent chemotaxis after pre-exposure to an odorant. *values differ significantly ($P < 0.05$).

to the repertoire of assay systems for behavioral plasticity, our new assay system provides an opportunity to analyze the molecular mechanisms of sensory adaptation that occur at the interneuron level.

AIY interneurons control early adaptation

Our results suggest that AIY interneurons are essential for early adaptation. These neurons are known to act to reduce reversal frequency of locomotion (Tsalik & Hobert 2003). In principle, this change in the basal locomotion behavior could affect chemotaxis. However, in the *ttx-3* mutants, which lack functional AIY neurons, efficient chemotaxis is still observed to high concentration of odorants used in our assays, suggesting that unbiased increase of reversal frequency does not itself impair chemotaxis (Fig. 3B–F, open bars, and data not shown). On the other hand, the *let-60*(*gf*) and *let-60*(*lf*) mutants, which have normal reversal frequency, show defects in early adaptation. These results suggest that apart from their functions in regulation of basal reversal frequencies, AIY interneurons have other important roles in regulation of chemotaxis after pre-exposure to odorants, and this function depends, at least partly, on the Ras-MAPK pathway.

As mentioned above, large amounts of undiluted attractive odorants often induce avoidance behaviors (our unpublished results and Nuttley *et al.* 2001), indicating that the same odorants can give both attraction and avoidance signals, depending on the concentration.

The behavioral output is probably determined by a balance between these opposing signals, and it is therefore conceivable that AIY regulates the balance of attraction and avoidance signals by inhibiting attraction signals and/or enhancing avoidance signals.

Both positive and negative regulations by AIY have been suggested in the neural circuit. There is suggestive evidence that AIZ interneurons, which are the most prominent synaptic targets of AIY (Fig. 3A and White *et al.* 1986), may be essential for attraction to odorants, and that modulation of AIZ functions can accelerate odor adaptation (Bargmann & Mori 1997; Sze & Ruvkun 2003). In the neural circuit for thermotaxis and regulation of reversal, removal of AIY and that of AIZ cause opposite effects, suggesting that AIY might be inhibitory to AIZ (Mori & Ohshima 1995; Tsalik & Hobert 2003; Wakabayashi *et al.* 2004; Gray *et al.* 2005).

The AIY interneurons also have other synaptic targets (White *et al.* 1986). These include RIA, RIB and RIM interneurons (Fig. 3A). Through analyses of locomotory behavior and the influence of odorants on that behavior, a negative relationship between AIY and AIZ and a positive relationship between AIY and RIM have been proposed (Gray *et al.* 2005; Tsalik & Hobert 2003). Although the roles for the interneurons RIA, RIB and RIM in chemoattraction or chemorepulsion to odorants have not been formally addressed so far, they are also good candidate components of the regulatory circuit for early adaptation, and could possibly modulate chemotaxis by regulating reversal and turning behaviors during chemotaxis.

It is also possible that neurons other than AIY have important functions at early steps of sensory processing, because cross-adaptation is not observed in all combinations of odorants (Fig. 2), and pyrazine adaptation is not completely impaired in *ttx-3* mutants (Fig. 3F). These observations possibly suggest that some neurons other than AIY may also act in parallel for early adaptation.

Various observations implicate regulation by interneurons in processing of olfactory signals in mammals. These include centrifugal inputs that inhibit excitation of mitral cells in the MOB (main olfactory bulb) (Potter & Chorover 1976); granule cells that convey lateral inhibition signals between mitral/tufted cells in the MOB (Yokoi *et al.* 1995); interglomerular connections by short axon cells and periglomerular cells that also mediate lateral inhibition (Aungst *et al.* 2003); and association fiber connections within the piriform cortex that may be important for higher-order refinement of odor information (Haberly 2001). These interneuron connections may also mediate experience-dependent modulation of sensory information (Wilson 2003), similar to the functions of AIY neurons in early adaptation in *C. elegans*.

The Ras-MAPK pathway plays key roles in early adaptation

Rescue experiments demonstrate that the functions of Ras in AIY interneurons are important for odor adaptation, while the functions of Ras in olfactory neurons are important for odor perception. These results suggest that the Ras-MAPK pathway plays dual roles in olfactory sensory processing in the nervous system of *C. elegans*.

In response to continued sensory inputs, MAPK becomes activated in AIY. The signals and molecules in AIY that mediate this activation are currently unknown. However, we speculate that calcium signaling plays important roles, because EGTA treatment impairs early adaptation (data not shown) and calcium signaling activates the Ras-MAPK pathway in olfactory neurons of *C. elegans* (Hirotsu *et al.* 2000). If this assumption is true, the calcium signaling may be mediated by calcium-dependent activators of Ras (Farnsworth *et al.* 1995), or UNC-43 CaMKII, as in rat hippocampal neurons (Zhu *et al.* 2002).

The downstream targets of MAPK important for odor adaptation may not be transcription factors since odor adaptation and recovery from it occurs in a relatively short time. In other organisms, MAPK is known to regulate synapsin I-actin interactions by phosphorylation of synapsin I (Jovanovic *et al.* 1996), to phosphorylate the A-type potassium channel Kv4.2 (Adams *et al.* 2000), to stimulate translation (Kelleher *et al.* 2004) and to regulate the insertion of AMPA receptors into postsynaptic sites during long-term potentiation (Zhu *et al.* 2002). The last mechanism may be relevant to early adaptation in *C. elegans*, because mutants affected in GLR-1, a homolog of non-NMDA type glutamate receptors with closet sequence similarity to the AMPA subtype, exhibit defects in early adaptation (our unpublished data).

An ideal system for *in vivo* analysis of neuronal plasticity

Accumulating evidence shows that Ras and MAP kinase (ERK1/2) play fundamental roles in neuronal plasticity, such as long-term potentiation (LTP), in both vertebrates and invertebrates. Ras and the MAPK cascade are activated downstream of several receptors or channels, such as NMDA receptors, muscarinic acetylcholine receptors, or voltage-gated Ca^{2+} channels. Activated MAPK then translocates to the nucleus, resulting in phosphorylation and activation of transcription factors such as CREB (reviewed in Adams & Sweatt 2002). This transcription-dependent pathway regulates the late phase of LTP (L-LTP), which is mediated by the *de novo* expression of genes (Patterson *et al.* 2001). Apart from

this scenario, the Ras-MAPK pathway is also involved in transcription-independent regulation of plasticity (Morozov *et al.* 2003), but few reports so far point to this function. Thus early adaptation may provide an ideal assay system for understanding the functions of the Ras-MAPK pathway in neural plasticity at the behavioral level. Based on the advantages of the simple neural circuits of *C. elegans* and powerful genetics, further studies of early adaptation should provide valuable insights in the general molecular mechanisms for behavioral and neural plasticity, as well as those for the processing of sensory information.

Experimental procedures

Strains and culture

Strains were maintained under standard conditions. The strains used were the following: N2(wild-type), *odr-7*(*ky4*), *odr-4*(*n2144*), *osm-9*(*ky10*), *ttx-3*(*ks5*), *ttx-3*(*mg158*), *ttx-3*(*ot22*), *ttx-3*(*ot23*), *let-60*(*n1046*), *let-60*(*n2021*), *mek-2*(*n2678*).

For the *let-60(n1046*gf*)* strain, we recently found that the standard strain, MT2124, carries a side mutation (Hirotsu *et al.* 2004). Therefore in this study we used the outcrossed strain JN130 for all the assays. This strain shows reduced chemotaxis to the two odorants tested, isoamyl alcohol and diacetyl, at low odorant concentrations, and shows better chemotaxis to isoamyl alcohol than MT2124 (Supplementary Fig. S4 and Hirotsu *et al.* 2000). We also outcrossed the MT4866 strain, the *let-60*(*n2021*lf) strain used in our previous study, and obtained the JN148 strain. This strain shows chemotaxis defects, the extents of which are comparable to the original MT4866 *let-60*(*n2021*lf) strain (Supplementary Fig. S4). Therefore, we used the MT4866 strain for all the assays.

Behavioral assays

Early adaptation assays were performed as described below. Animals grown on *E. coli* NA22 were collected in microfuge tubes and washed three times with basal buffer (5 mM potassium phosphate, 1 mM $CaCl_2$, 1 mM $MgSO_4$ and 0.5 g/L gelatin) and then 100 μL of 10^{-4} dilutions of odorants in water was added. For control animals, 100 μL of water was similarly added. After 5 min, 1 mL of basal buffer was added and centrifuged for 5 s at 1000 r.p.m. The animals that settled at the bottom were taken and about 50 animals were spotted at the center of 9 cm assay plates. Excess liquid was removed with Kimwipes, at the same time dispersing the animals along the midline of the plates (Supplementary Fig. S5). The number of animals spotted on a plate was critical, because when a greater number of animals were spotted, they tended to form clumps and failed to leave the origin. Residual odorants did not affect the chemotaxis, since animals which were pre-exposed to water and washed with buffer containing the odorants at the same concentration as above showed normal chemotaxis. Assay plates were made as previously described (Bargmann *et al.* 1993) with some modifications (Supplementary Fig. S5): 1 μL each of odorant and 1 M sodium azide were spotted on two points separated by about 2.5 cm at one end of the plates. Only sodium azide was similarly spotted on the other side. Odorant dilutions for the adaptation assays were: isoamyl alcohol, 10^{-4}; benzaldehyde, 10^{-2}; diacetyl, 10^{-3}; pyrazine, 10^{-2}, except for Fig. 2D and Supplementary Fig. S1H, where 10^{-4} dilutions were used. Thirty minutes after placing the animals at the center of the plates, the number of animals was counted and the chemotaxis index was calculated.

$$\text{The chemotaxis index} = (A - B)/(A + B)$$

where A was the number of animals on the odorant-spotted side of the plate and B was the number of the animals on the opposite side, while animals that remained within 0.5 cm of the midline were not counted to exclude immotile animals from consideration. These modifications in the plate layout for chemotaxis were particularly essential for the early adaptation assay. On the other hand, it was not essential to pre-expose the animals to the odorants in solution, because similar results were obtained by exposing the animals to vaporized odorants, in a manner similar to the conventional adaptation assay (Colbert & Bargmann 1995).

The chemotaxis to NaCl was assayed on a plate in which an NaCl gradient was formed for 15 h by placing an agar plug containing 100 mM NaCl on one end of a plate.

The recovery from adaptation was assessed by placing worms on blank chemotaxis plates without odorants after a pre-exposure treatment. Animals were then collected with buffer and spotted on chemotaxis assay plates.

Simple odorant chemotaxis assays (those shown in Figs 4B and 6A and Supplementary Fig. S1A-D) were performed on naive animals using the chemotaxis plates described above for adaptation assays, except for Supplementary Fig. S4. The animals were counted after 1 h. Note that this assay format is different from that used in our previous study (Hirotsu *et al.* 2000), causing minor discrepancies in chemotaxis indices between the two reports.

In Figs 1–4 and 6 and Supplementary Figs S1, S2 and S4, each set of data represents at least three independent assays. The error bars show the SEM.

Reversal assays

Reversal assays were performed as previously described (Tsalik & Hobert 2003). Reversal frequency was scored for 3 min at 25 °C.

Immunohistochemistry

For identification of the AWC, AIY or ASEL neurons, animals carrying the *gcy-10p*::*GFP*, *ttx-3p*::*GFP* or *gcy-7p*::*GFP* constructs, respectively, were used. After pre-exposure to odorants for 5 min, immunohistochemistry was performed as previously described (Hirotsu *et al.* 2000). Samples were observed by Zeiss Axioplan 2 epifluorescence microscope and Yokogawa CSU21 confocal unit.

Cell-specific expression of let-60

The construction of *gcy-10p*::*let-60* was previously described (Hirotsu *et al.* 2000). To drive expression in AIY, a genomic

fragment containing about 5 kb of the upstream regulatory region and the first two introns of *ttx-3* was used (Wenick & Hobert 2004). *let-60* ORF was placed downstream of this fragment so that the coding sequence of *let-60* was out of frame relative to that of *ttx-3*, and a strong translation-initiation signal AAAA was added immediately upstream of the *let-60* initiation codon. It has been reported that, although the *ttx-3* gene is expressed in several classes of neurons, this fragment drives expression only in AIY (Altun-Gultekin *et al.* 2001). To test whether the downstream gene is expressed in this frame-shift construct, *let-60* ORF was replaced with GFP and introduced into *C. elegans*. GFP fluorescence was observed in AIY neurons in this strain, indicating the expression of the downstream gene. *sra-11* promoter contained about 2.7 kb of upstream region. We confirmed with GFP that this region drives expression in AIY.

These constructs were injected with *myo-3p*::*GFP* as a transformation marker at a concentration of 50 ng/μL each. Germ-line transformation was performed as previously described (Mello *et al.* 1991).

Acknowledgements

We thank O. Hobert for *ttx-3* mutants; A. Fire for vectors; T. Ishihara for the *ttx-3p*::*GFP* transgenic strain and for helpful discussion; M. Tomioka for the *gcy-7p*::*GFP* transgenic strain; E. Nishida, N. L'Etoile, I. Maeda and members of our laboratory for useful advice and discussion; E. Tsalikü and O. Hobert for comments on the manuscript. All other strains were provided by the Caenorhabditis Genetic Center, which is funded by NIH National Center for Research Resources. This work was supported by the Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists (to T.H.).

Supplementary materials

The following material is available from:
http://www.blackwellpublishing.com/products/journals/suppmat/GTC/GTC856/GTC856sm.htm

Supplementary Move S1
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3
Supplementary Figure S4
Supplementary Figure S5

References

Adams, J.P., Anderson, A.E., Varga, A.W., *et al.* (2000) The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK. *J. Neurochem.* **75**, 2277–2287.

Adams, J.P. & Sweatt, J.D. (2002) Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annu. Rev. Pharmacol. Toxicol.* **42**, 135–163.

Altun-Gultekin, Z., Andachi, Y., Tsalik, E.L., Pilgrim, D., Kohara, Y. & Hobert, O. (2001) A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in *C. elegans*. *Development* **128**, 1951–1969.

Aungst, J.L., Heyward, P.M., Puche, A.C., *et al.* (2003) Centre-surround inhibition among olfactory bulb glomeruli. *Nature* **426**, 623–629.

Bargmann, C.I., Hartwieg, E. & Horvitz, H.R. (1993) Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* **74**, 515–527.

Bargmann, C.I. & Kaplan, J.M. (1998) Signal transduction in the *Caenorhabditis elegans* nervous system. *Annu. Rev. Neurosci.* **21**, 279–308.

Bargmann, C.I. & Mori, I. (1997) Chemotaxis and thermotaxis. In: *C. Elegans II* (eds D.L. Riddle, T. Blumenthal, B.J. Meyer & J.R. Priess), pp. 717–738. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Beitel, G.J., Clark, S.G. & Horvitz, H.R. (1990) *Caenorhabditis elegans* ras gene let-60 acts as a switch in the pathway of vulval induction. *Nature* **348**, 503–509.

Bernhard, N. & Van Der Kooy, D. (2000) A behavioral and genetic dissection of two forms of olfactory plasticity in *Caenorhabditis elegans*: adaptation and habituation. *Learn. Mem.* **7**, 199–212.

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

Colbert, H.A., Smith, T.L. & Bargmann, C.I. (1997) OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *J. Neurosci.* **17**, 8259–8269.

Dalton, P. (2000) Psychophysical and behavioral characteristics of olfactory adaptation. *Chem. Senses* **25**, 487–492.

Devaud, J.M., Acebes, A. & Ferrus, A. (2001) Odor exposure causes central adaptation and morphological changes in selected olfactory glomeruli in Drosophila. *J. Neurosci.* **21**, 6274–6282.

Dwyer, N.D., Troemel, E.R., Sengupta, P. & Bargmann, C.I. (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. *Cell* **93**, 455–466.

Farnsworth, C.L., Freshney, N.W., Rosen, L.B., Ghosh, A., Greenberg, M.E. & Feig, L.A. (1995) Calcium activation of Ras mediated by neuronal exchange factor Ras-GRF. *Nature* **376**, 524–527.

Fletcher, M.L. & Wilson, D.A. (2003) Olfactory bulb mitral-tufted cell plasticity: odorant-specific tuning reflects previous odorant exposure. *J. Neurosci.* **23**, 6946–6955.

Gray, J.M., Hill, J.J. & Bargmann, C.I. (2005) A circuit for navigation in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA*.

Haberly, L.B. (2001) Parallel-distributed processing in olfactory cortex: new insights from morphological and physiological analysis of neuronal circuitry. *Chem. Senses* **26**, 551–576.

Han, M. & Sternberg, P.W. (1990) *let-60*, a gene that specifies cell fates during *C. elegans* vulval induction, encodes a ras protein. *Cell* **63**, 921–931.

Hirotsu, T., Saeki, S., Yamamoto, M. & Iino, Y. (2000) The Ras-MAPK pathway is important for olfaction in *Caenorhabditis elegans*. *Nature* **404**, 289–293.

Hirotsu, T., Saeki, S., Yamamoto, M. & Iino, Y. (2004) Corrigendum: the Ras-MAPK pathway is important for olfaction in *Caenorhabditis elegans*. *Nature* **432**, 653.

Hobert, O. (2003) Behavioral plasticity in *C. elegans*: paradigms, circuits, genes. *J. Neurobiol.* **54**, 203–223.

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

Hummel, T., Knecht, M. & Kobal, G. (1996) Peripherally obtained electrophysiological responses to olfactory stimulation in man: electro-olfactograms exhibit a smaller degree of desensitization compared with subjective intensity estimates. *Brain Res.* **717**, 160–164.

Ishihara, T., Iino, Y., Mohri, A., *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.

Jovanovic, J.N., Benfenati, F., Siow, Y.L., *et al.* (1996) Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I–actin interactions. *Proc. Natl. Acad. Sci. USA* **93**, 3679–3683.

Kelleher, R.J., 3rd, Govindarajan, A., Jung, H.Y., Kang, H. & Tonegawa, S. (2004) Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell* **116**, 467–479.

Kornfeld, K., Guan, K.L. & Horvitz, H.R. (1995) The *Caenorhabditis elegans* gene mek-2 is required for vulval induction and encodes a protein similar to the protein kinase MEK. *Genes Dev.* **9**, 756–768.

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

L'etoile, N.D. & Bargmann, C.I. (2000) Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron* **25**, 575–586.

L'etoile, N.D., Coburn, C.M., Eastham, J., Kistler, A., Gallegos, G. & Bargmann, C.I. (2002) The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron* **36**, 1079–1089.

Leinders-Zufall, T., Ma, M. & Zufall, F. (1999) Impaired odor adaptation in olfactory receptor neurons after inhibition of Ca^{2+}/calmodulin kinase II. *J. Neurosci.* **19**, RC19.

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

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

Morozov, A., Muzzio, I.A., Bourtchouladze, R., *et al.* (2003) Rap1 couples cAMP signaling to a distinct pool of p42/44MAPK regulating excitability, synaptic plasticity, learning, and memory. *Neuron* **39**, 309–325.

Munger, S.D., Lane, A.P., Zhong, H., *et al.* (2001) Central role of the CNGA4 channel subunit in Ca^{2+}-calmodulin-dependent odor adaptation. *Science* **294**, 2172–2175.

Nuttley, W.M., Harbinder, S. & Van Der Kooy, D. (2001) Regulation of distinct attractive and aversive mechanisms mediating benzaldehyde chemotaxis in *Caenorhabditis elegans*. *Learn. Mem.* **8**, 170–181.

Patterson, S.L., Pittenger, C., Morozov, A., *et al.* (2001) Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* **32**, 123–140.

Potter, H. & Chorover, S.L. (1976) Response plasticity in hamster olfactory bulb: peripheral and central processes. *Brain Res.* **116**, 417–429.

Sengupta, P., Colbert, H.A. & Bargmann, C.I. (1994) The *C. elegans* gene odr-7 encodes an olfactory-specific member of the nuclear receptor superfamily. *Cell* **79**, 971–980.

Sze, J.Y. & Ruvkun, G. (2003) Activity of the *Caenorhabditis elegans* UNC-86 POU transcription factor modulates olfactory sensitivity. *Proc. Natl. Acad. Sci. USA* **100**, 9560–9565.

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A. & Bargmann, C.I. (1995) Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* **83**, 207–218.

Troemel, E.R., Kimmel, B.E. & Bargmann, C.I. (1997) Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* **91**, 161–169.

Tsalik, E.L. & Hobert, O. (2003) Functional mapping of neurons that control locomotory behavior in *Caenorhabditis elegans*. *J. Neurobiol.* **56**, 178–197.

Wakabayashi, T., Kitagawa, I. & Shingai, R. (2004) Neurons regulating the duration of forward locomotion in *Caenorhabditis elegans*. *Neurosci. Res.* **50**, 103–111.

Watt, W.C. & Storm, D.R. (2001) Odorants stimulate the ERK/mitogen-activated protein kinase pathway and activate cAMP-response element-mediated transcription in olfactory sensory neurons. *J. Biol. Chem.* **276**, 2047–2052.

Wenick, A.S. & Hobert, O. (2004) Genomic cis-regulatory architecture and trans-acting regulators of a single interneuron-specific gene battery in *C. elegans*. *Dev. Cell* **6**, 757–770.

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

Wilson, D.A. (1998) Synaptic correlates of odor habituation in the rat anterior piriform cortex. *J. Neurophysiol.* **80**, 998–1001.

Wilson, D.A. (2003) Rapid, experience-induced enhancement in odorant discrimination by anterior piriform cortex neurons. *J. Neurophysiol.* **90**, 65–72.

Wu, Y., Han, M. & Guan, K.L. (1995) MEK-2, a *Caenorhabditis elegans* MAP kinase kinase, functions in Ras-mediated vulval induction and other developmental events. *Genes Dev.* **9**, 742–755.

Yokoi, M., Mori, K. & Nakanishi, S. (1995) Refinement of odor molecule tuning by dendrodendritic synaptic inhibition in the olfactory bulb. *Proc. Natl. Acad. Sci. USA* **92**, 3371–3375.

Yu, S., Avery, L., Baude, E. & Garbers, D.L. (1997) Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. *Proc. Natl. Acad. Sci. USA* **94**, 3384–3387.

Zhu, J., Qin, Y., Zhao, M., Van Aelst, L. & Malinow, R. (2002) Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell* **110**, 443.

Zufall, F. & Leinders-Zufall, T. (2000) The cellular and molecular basis of odor adaptation. *Chem. Senses* **25**, 473–481.

Received: 20 January 2005
Accepted: 27 February 2005

