The *C. elegans* che-1 gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons

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SUMMARY

Chemotaxis to water-soluble chemicals such as NaCl is an important behavior of *C. elegans* when seeking food. ASE chemosensory neurons have a major role in this behavior. We show that che-1, defined by chemotaxis defects, encodes a zinc-finger protein similar to the GLASS transcription factor required for photoreceptor cell differentiation in *Drosophila*, and that che-1 is essential for specification and function of ASE neurons. Expression of a che-1::gfp fusion construct was predominant in ASE. In che-1 mutants, expression of genes characterizing ASE such as seven-transmembrane receptors, guanylate cyclases and a cyclic-nucleotide gated channel is lost. Ectopic expression of che-1 cDNA induced expression of ASE-specific marker genes, a dye-filling defect in neurons other than ASE and dauer formation.

Key words: che-1, Chemotaxis, Transcription factor, ASE neuron, *C. elegans*

INTRODUCTION

Animals develop a sophisticated nervous system with which to sense a variety of environmental stimuli and to produce suitable behavior that will ensure survival in their environment. The free-living soil nematode *C. elegans* can migrate toward the peak of a gradient of a number of water-soluble chemicals, including salts, amino acids, nucleotides and many kinds of volatile organic molecules (Bargmann et al., 1993; Bargmann and Horvitz, 1991a; Dusenbery, 1974; Ward, 1973). Many of these attractants are by-products of bacterial metabolism, and chemotaxis behavior seems important for the nematodes to find bacteria – its food in the natural environment. *C. elegans* has a rather simple nervous system with only 302 neurons in the adult hermaphrodite, and is amenable to genetic manipulation (Brenner, 1974; Wood and the Community of *C. elegans* Researchers, 1988). Chemotaxis of *C. elegans* is a good target for molecular genetic studies with the aim of understanding the development and function of the sensory nervous system of an animal.

A *C. elegans* hermaphrodite has 32 neurons of 14 types, which are thought to be chemosensory because they have ciliated endings exposed to the environment through an opening of the cuticle at the end of amphid, phasmid and inner labial sensory neurons (Ward et al., 1975; Ware et al., 1975; White et al., 1986). The amphid and phasmid neurons consist of a pair of similar neurons, each on the left and right sides of the animal. Functions of the chemosensory neurons have been determined by killing individual neurons with laser microbeam and analysis of the resultant behavioral responses (Bargmann et al., 1993; Bargmann and Horvitz, 1991a; Bargmann and Horvitz, 1991b; Kaplan and Horvitz, 1993; Troemel et al., 1995). Amphid neurons were found to be responsible for chemosensory behaviors, while phasmid neurons were reported recently to function as chemosensory cells that negatively modulate reversals to repellents (Hilliard et al., 2002). One pair of amphid neurons, the ASE neurons, were found to be uniquely important for chemotaxis to Na+, Cl–, cAMP and biotin: killing the ASE neurons greatly reduced chemotaxis to these chemicals (Bargmann and Horvitz, 1991a).

Several genes involved in the function of the ASE neurons for chemotaxis have been molecularly cloned. Among a number of che (chemotaxis-defective) and tax (chemotaxis abnormal) genes that have been identified in the studies of mutants that fail to respond to NaCl (Dusenbery et al., 1975; Lewis and Hodgkin, 1977), tax-2 and tax-4 encode cyclic nucleotide-gated channels (Coburn and Bargmann, 1996; Komatsu et al., 1996). *daf-11* gene, a mutation of which leads to the constitutive dauer larva phenotype, encodes a transmembrane guanylate cyclase (Birnby et al., 2000). Although no mutants are obtained, gcy-5, gcy-6 and gcy-7 encode putative transmembrane guanylate cyclases expressed only in the right side ASE (ASER) or the left side ASE (ASEL), and they are thought to function as chemoreceptors (Yu et al., 1997). *lim-6* encodes a LIM homeobox transcription factor expressed in ASE (Hobert et al., 1999). In *lim-6* mutants, gcy-5 is expressed in ASER in addition to ASER (Hobert et al., 1999), and the functional asymmetry of ASE for discriminating Na⁺ and Cl⁻ is lost (Pierce-Shimomura et al., 2001).
che-1 mutants were originally isolated as mutants defective in chemotaxis to NaCl. They also show chemotaxis defects to water-soluble attractants such as cAMP and biotin, but not to volatile odorants (Bargmann et al., 1993; Dusenbery, 1976; Dusenbery et al., 1975; Lewis and Hodgkin, 1977). che-1 mutants have no significant structural defects in ASE and the other chemosensory neurons of the amphid in electron micrographs (Lewis and Hodgkin, 1977). The che-1 gene is likely to affect the ASE function mediating chemotaxis. We show here that the che-1 gene encodes a C2H2-type zinc-finger protein similar to the GLASS transcription factor required for photoreceptor cell differentiation in Drosophila (Moses et al., 1989) and that che-1 is required for the identity of ASE neurons.

MATERIALS AND METHODS

Strains and genetics

Wild-type C. elegans is variety Bristol, strain N2. Worms were grown on NGM plates at 20°C using standard methods (Brenner, 1974). The following strains were used in this work.

PR672 che-1(p672)=tax-5[p672] I, PR674 che-1[p674] I, PR679 che-1[p679] I, PR680 che-1[p680] I, PR692 che-1[p692] I, PR696 che-1[p696] I, CB1034 che-1(e1034) I and BC700 sfm[4/bli-4(e937)] dpy-14(e188) I.

kyIs5[che-23::gfp, lin-15(+)] IV (Zellen et al., 1999) (Forrester et al., 1998) and NW1229 dpy-20; eves111[F25B3.3::gfp, dpy-20(+)]. OH811 nls[ggc-7::gfp], OH812 nls[14(6-6-6::gfp, rol-6(6)]. OH813 nls[ggc-5::gfp] (O. Hobert, personal communication).

Chemotaxis assay

Chemotaxis assays to NaCl were performed essentially as described (Dusenbery et al., 1975) but with some modification. A radial concentration gradient of NaCl was established by spotting 2 ml of 5 M NaCl to the center of a 9 cm plate containing 8 ml of agar medium (2% agar, 0.25% Tween 20, 10 mM HEPES (pH 7.2)), and leaving the plates at room temperature for 12-16 hours. For assays, the animals were placed on the surface of agar 1 cm distant from the perimeter and allowed to move freely for 1 hour.

Genetic mapping of che-1

bli-4(e937) dpy-14(e188) hermaphrodites were mated with che-1(696) males. F1 hermaphrodites were then placed on separate plates and allowed to self-fertilize for F2 progeny. Among the F2 animals, recombinant animals (Bli non-Dpy, Dpy non-Bli) were selected.

Cloning of che-1

General molecular biology manipulations were carried out using standard methods (Sambrook et al., 1989). Cosmid DNA was prepared from 200 ml of a liquid culture of E. coli at –0.2 of OD600 using QAFilter Plasmid Maxi Kit (Qiagen). PCR products for rescue experiments were amplified from wild-type N2 genomic DNA with a set of primers spanning the entire putative che-1 ORF and the product was directly sequenced.

Transgenic animals recognized by GFP expression were subjected to chemotaxis assays.

To identify molecular lesions in the che-1 alleles, the genomic sequence of che-1 was amplified from p672, p674, p679, p680, p692, p696 and e1034 mutants, and the products were directly sequenced.

Expression constructs and generation of transgenic animals

A che-1 promoter construct, pche-1::gfp, was prepared by amplifying 5.4 kb of che-1 upstream sequence of the predicted initiation codon from the wild-type-genome. A C-terminal tag construct, pche-1::gfp, was prepared by amplifying the 5.4 kb upstream sequence and the entire coding region of che-1 from the wild-type-genome. A Sphi site and a PlsI site engineered into the PCR primers were used to insert the amplified products into the GFP vector pPD95.77. Two internal GFP tag constructs, pche-1::gfp, were constructed by inserting gfp fragments amplified from pPD95.77 into frame in the BamHI site of the 5′/3′ site of che-1 HindIII, a che-1 gene subclone of 6.2 kb HindIII fragment from ZC130 cosm, in pHSG398 (a Cm + plasmid vector, Takara), respectively. For amplification by PCR, LA-PCR kit (TAKARA) was used. The coding region of che-1 and GFP originating from the PCR amplification in these constructs were verified by sequencing.

pFSE10.7::gfp and pR13H7.2::gfp transcriptional gfp fusions were constructed by ligating 4.5 kb and 4.3 kb of their upstream sequences amplified by PCR between the PstI and BamHI sites of pGFP-TT vector (Y. Jin, personal communication) and the SalI and BamHI sites of pPD95.75 vector (A, Fire, S. Xu, J. Ahnn and G. Seydoux, personal communication), respectively. ptax-2p::gfp was constructed by ligating 2.0 kb of the tax-2 upstream sequence amplified by PCR between the PstI and BamHI sites of pPD95.77. gcy-5::gfp, gcy-6::gfp and gey-7::gfp were gifts from D. Garbers (Yu et al., 1997).

pgpa-10p::che-1 and pgpa-14p::che-1 expression constructs were prepared by using pPD49.26 vector (A, Fire, S. Xu, J. Ahnn and G. Seydoux, personal communication) as a backbone. The 3 kb Sphi-Smal fragment of pgpa10-gfp or pgpa14-gfp (Murakami et al., 2001) containing an upstream sequence of gpa-10 or gpa-14 and a blunt-ended SacI-SacI fragment of che-1 cDNA containing a che-1 full-length cDNA were inserted between the Sphi and Smal sites and at the blunt-ended KpnI site of pPD49.26, respectively, through several subcloning steps.

Transgenic animals were obtained by germline transformation. These constructs were injected at 5-50 ng/µl without or with injection markers, pMTG25-4 (a fr-1::gfp expressed in the intestine) (Take-Uchi et al., 1998) at 50 ng/µl or pls44-gfp (a lin-44::gfp construct expressed in ASI) (Murakami et al., 2001) at 33-50 ng/µl into wild-type N2 or PR679 che-1[p679] animals.

Once the transformant lines had been established, the animals were tested for rescue of the che-1 defect or were observed under the fluorescent microscope for the GFP expression. Cells that expressed GFP were identified by positions mainly in L1 and L2 animals under Nomarski optics. The position of the cells in C. elegans has been described by Sulston et al. (Sulston et al., 1983) and White et al. (White et al., 1986).

Dye-filling assay

Dil stock solution was made by dissolving 2 mg Dil in 1 ml dimethly formamide, and stored at –20°C. Worms on a growth plate were washed off with M9 buffer into a test tube, washed at least twice with M9 buffer, and then suspended in 400 µl M9 buffer, to which 2 µl of
DIII solution was added. The tube was shielded from the light with aluminum foil and incubated for 2-3 hours at room temperature. After incubation, the animals were washed with M9 at least three times, put onto a growth plate and cultivated overnight. The animals were put on an agar pad containing 50 mM sodium azide and observed with a fluorescence microscope (AxioPhoto2, Zeiss).

**Dauer formation assay**
About 10 adult hermaphrodites were allowed to lay eggs for 12-24 hours on a 6 cm NGM agar plate with *E. coli* OP50. After the parental animals had been removed, plates were incubated for 2 days at 20°C. Then, dauer and non-dauer (L3 to adult) F1 progeny with or without the injection marker were counted, respectively.

**RESULTS**

**Genetic mapping and germline rescue of che-1**

*che-1(e1034)* had been mapped between *dpy-5* and *fer-1* on LG I (Lewis and Hodgkin, 1977; Ward and Miwa, 1978). We mapped *che-1(p696)* between *bli-4* and *dpy-14* using three factor crosses (Fig. 1A). From *bli-4 dpy-14* hermaphrodites, one out of five Bli non-Dpy recombinants and one out of two non-Bli Dpy recombinants segregated *che-1*. Cosmids located in this region were introduced into *che-1(p696)* mutant by germline transformation and the resulting transgenic animals were tested for chemotaxis to NaCl. C55B7 rescued the defect of the *che-1* mutant (Fig. 1B). The sequence of C55B7 had been determined by the *C. elegans* Sequence Consortium and 12 genes were predicted. Among PCR fragments amplified from various parts of the C55B7 and their restriction fragments, a fragment containing a single gene, C55B7.12, was identified to rescue the defect of the *che-1* mutant (Fig. 1C).

*che-1 encodes a zinc-finger protein like GLASS transcription factor*

The genomic sequence and the sequence of *che-1* cDNA amplified by RT-PCR representing the entire *che-1* coding region were determined. The genomic organization of *che-1* is shown in Fig. 2B. The cDNA has a single long open reading frame encoding a predicted protein product of 272 amino acids (Fig. 2A).

Comparison of the amino acid sequence of CHE-1 to other sequences in the databases indicated that CHE-1 has four C2H2 type zinc-finger motifs in the C-terminal that are most similar to those of GLASS, a transcription factor for photoreceptor differentiation in *Drosophila* (Moses et al., 1989). GLASS has five zinc-finger domains and the last three C-terminal zinc finger domains alone are necessary and sufficient for DNA binding (O’Neill et al., 1995). The first to the fourth zinc-finger domains of CHE-1 correspond to the second, third, fourth and fifth zinc-finger domains of GLASS, respectively. These combinations show higher similarities (71%, 71%, 68% and 57%) than any other combinations (Fig. 2C). These high similarities and the same order of corresponding zinc-finger domains along the primary structure indicate that *che-1* may be a *C. elegans* counterpart of *glass*. The other regions of CHE-1 apart from zinc-finger domains did not show a significant homology to any proteins or motifs.

The genomic DNA, including all exons and introns of six *che-1* alleles, was sequenced (Fig. 2A,B). The p672, p674, p679 and p680 mutations were found to be C to T transitions resulting in the conversion of Arg213 to a stop codon in the fifth exon of *che-1*. These mutations should result in a truncated protein with only the first zinc-finger domain intact but the second impaired, and the third and fourth lacking. The p692 and p696 mutations were G to A transitions resulting in the conversion of Gly263 to Arg in the last zinc-finger domain. This glycine is conserved between CHE-1 and GLASS. The e1034 mutation was a A to G transversion, resulting in the conversion of His268 to Arg in the last zinc-finger domain. This histidine is an invariant residue among the C2H2-type zinc fingers, and one of the four residues to bind a zinc ion. All these mutations led to alterations of CHE-1 in the last three zinc-finger domains, which are known to be essential for DNA binding in GLASS. These results show that the last three zinc-finger domains are essential for CHE-1 activity, or for the potential to bind to DNA.

*che-1 is expressed in the ASE chemosensory neurons*

To determine where *che-1* functions for chemotaxis behavior, four gfp fusion genes were constructed (Fig. 3A). Among them, only two fusions showed gfp expression, as observed in

![Diagram](image-url)
ASE chemosensory neurons and a few other neurons (Fig. 3B,C). The ASE was the only neuron class in which both of the fusion genes showed constant and strong gfp expression. The ASEs are the major neurons that mediate chemotaxis to water-soluble attractants such as Na⁺, Cl⁻, biotin and cAMP, which is affected by che-1 mutations (Bargmann and Horvitz, 1991a). These results suggest that che-1 functions in the ASE neuron for chemotaxis. Neither of the fusion genes rescued the chemotaxis defects of the che-1 mutants, although they have the entire coding region and the promoter included in the original genomic clone (pche-1-HindIII) that rescues chemotaxis defect (Fig. 3A,F). The GFP insertion may disrupt an unidentified functional domain or the GFP tag very close to the last zinc-finger domain may perturb the DNA binding activity of CHE-1. As these che-1::gfp constructs were expressed in the che-1 mutant (data not shown) as well as in the wild type animals, che-1 is not required for expression of itself.

che-1 mutations affect the identity of ASE

In the che-1 mutants, ASE neurons were found at the normal position and their morphology looked normal under DIC or fluorescent microscopes. In addition, it was reported that in animals with a che-1 mutation, e1034 or e1035, the cilia of ASE had no significant defect in ultrastructure except for a mild alteration in the patterning of cilia of ASE and other amphidial cells in the way their tips were bundled distally within the channel formed by the sheath cell opening to the outside of the worms (Lewis and Hodgkin, 1977). Based on these results, CHE-1 was expected to be a transcription factor mainly required for expression of genes involved in ASE-specific functions for chemotaxis to water-soluble attractants after morphological differentiation. To examine this idea further, expression of such genes was determined using gfp reporter constructs (Fig. 4; Table 1). Two putative seven transmembrane receptor genes, F55E10.7 and R13H7.2, were identified by the C. elegans Sequence Consortium. Among the amphid neurons, pF55E10.7::gfp transcriptional fusion construct was expressed in ASE, AFD and AWC, and pR13H7.2::gfp fusion was expressed in ASE, AFD and ASJ in wild-type animals (Fig. 4A,C). But in the che-1 mutant, the expression of both these genes was lost specifically in ASE neurons (Fig. 4B,D). gcy-5, gcy-6 and gcy-7, which are membrane-spanning guanylate cyclase genes expressed specifically on the left or right side ASE in wild-type animals (Yu et al., 1997) (Fig. 4E,G,I), lost their expression in the che-1 mutant (Fig. 4F,H,J). tax-2, a cyclic nucleotide-gated channel gene required for chemotaxis and thermotaxis (Coburn and Bargmann, 1996), also lost expression specifically in ASE neurons of the che-1 mutant (Fig. 4L). The
loss of tax-2 expression in the ASE neurons is thought to be sufficient to cause chemotaxis defects in the che-1 mutant. Expression of a homeobox gene ceh-23 (Wang et al., 1993) and a LIM-homeobox gene lim-6 (Hobert et al., 1999) was also lost specifically in ASE neurons (Fig. 4N,P). However, a pan-neuronal marker gene evIs111[che-1::gfp] (Hobert et al., 1999) was also expressed in the ASE neurons of the p692, p679, which was ectopically expressed using gpa-10 or gpa-14 promoters (Jansen et al., 1999). The gpa-10 promoter drives expression in ADF, ASI and ASJ amphid neurons, in ALN, CAN, LUA neurons and spermatheca. The gpa-14 promoter drives expression in ASI, ASJ, ASH, ASK amphid neurons, PHA, PHB, ADE, ALA, ADA, CAN, DVA, PVQ, RIA neurons, and in vulval muscles. gcy-5, 6 and 7::gfp reporters and a gpa-10p::che-1 or gpa-14::che-1 cDNA construct were injected into wild-type animals (Fig. 5B,D). As negative controls, promoter-only constructs, in which che-1 cDNA construct was introduced into OH811 and OH813 and 15 lines for the gpa-10p::che-1 construct, were injected with the gpa-10p::che-1 cDNA construct were injected into wild-type animals (Fig. 5B,D). As negative controls, promoter-only constructs, in which che-1 cDNA was removed from gpa-10p::che-1 cDNA or gpa-14::che-1 cDNA construct, were injected with the gcy::gfp reporters (Fig. 5A,C). In the transgenic animals with gpa-10p::che-1 cDNA (Fig. 5B), GFP fluorescence was observed not only in ASE but also constantly in ADF and occasionally in ASJ and ASI. In the transgenic animals with gpa-14p::che-1 cDNA, GFP fluorescence was observed constantly in PHA and PHB, and often or occasionally in ASI, ASJ, ASK, ADA, ADE and RIA (Fig. 5D). However, in transgenic animals with the promoter-only constructs, no ectopic gfp expression was observed out of 16 lines for the gpa-10 promoter-only construct and 15 lines for the gpa-14 promoter-only construct (Fig. 5A,C). Because, in these experiments, the che-1 cDNA expression construct and the GFP reporter constructs were in the same extrachromosomal array, it may be possible that the ectopic gfp expression was due to an artificial interaction of these constructs within the same extrachromosomal array. To examine this possibility, we used two integrated strains, OH811 otls3[gcy-7::gfp] and OH813 nts1[gcy-5::gfp]. gpa-10p::che-1 cDNA construct was introduced into OH811 animals as an extrachromosomal array. In this transgenic line, ectopic gfp expression was observed in cells likely to be

**Table 1. Expression of ASE markers in the wild type and a che-1 mutant**

| Reporter genes | Animals with GFP expression in ASE | Wild-type background | che-1(p679) background |
|----------------|-----------------------------------|----------------------|------------------------|
| Ex[F55E10.7::gfp] | #1 96 55 | #1 3 68 |
| Ex[R13H7.2::gfp] | #1 100 106 | #1 7 114 |
| Ex[gcy-5::gfp] | #1 98 101 | #1 0 140 |
| Ex[gcy-6::gfp] | #1 96 127 | #1 0 120 |
| Ex[gcy-7::gfp] | #2 98 100 #2 1 127 |
| Ex[tax-2::gfp] | #2 94 110 | #2 0 146 |
| kyIs5 [ceh-23::gfp] | #2 92 132 | #2 1* 137 |
| otls114[lim-6::gfp, rol-6(d)] | #2 100 75 | #2 0 85 |
| evIs111[F25B3.3::gfp] | 100 55 | 100 4 100 |

*Very faint.

**Table 2. Expression of ASE markers in three che-1 mutants and the wild type**

| Genotype | Transgene | Background | GFP expression in ASE (%) | n |
|----------|-----------|------------|---------------------------|---|
| che-1(e1034) flr-1::gfp | Ex[gcy-5::gfp, che-1(p679)] | 0 30 | 61 |
| che-1(e1034) flr-1::gfp | che-1(p679) | 0 80 | 0 80 |
| che-1(e1034) flr-1::gfp | che-1(p692) | 0 101 |
| gpa-14::che-1 cDNA | Ex[gcy-6::gfp, che-1(p679)] | 0 71 |
| che-1(e1034) flr-1::gfp | che-1(p679) | 0 85 |
| che-1(e1034) flr-1::gfp | che-1(p692) | 96 127 |

*These transgenes were transferred by genetic crosses. n, number of animals observed.

**Table 3. Dye-filling of ASE in the wild type and che-1 mutants**

| Genotype | DiI filling in ASE (%) | n |
|----------|------------------------|---|
| che-1(p679) | 88 40 |
| che-1(p674) | 87 31 |
| che-1(p696) | 80 35 |
| p679; Ex[che-1(+)]; line 1* | 3 28 |
| line 2 | 3 31 |

*This line is the same one shown in Fig. 3F.

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**Ectopic expression of che-1 induces misexpression of ASE-specific marker genes, dye-filling defect and dauer formation**

To determine whether che-1 is sufficient for ASE fate specification, che-1 cDNA was ectopically expressed using gpa-10 or gpa-14 promoters (Jansen et al., 1999). The gpa-10 promoter drives expression in ADF, ASI and ASJ amphid neurons, in ALN, CAN, LUA neurons and spermatheca. The gpa-14 promoter drives expression in ASI, ASJ, ASH, ASK amphid neurons, PHA, PHB, ADE, ALA, ADA, CAN, DVA, PVQ, RIA neurons, and in vulval muscles. gcy-5, 6 and 7::gfp reporters and a gpa-10p::che-1 or gpa-14::che-1 cDNA construct were injected into wild-type animals (Fig. 5B,D). As negative controls, promoter-only constructs, in which che-1 cDNA was removed from gpa-10p::che-1 cDNA or gpa-14::che-1 cDNA construct, were injected with the gcy::gfp reporters (Fig. 5A,C). In the transgenic animals with gpa-10p::che-1 cDNA (Fig. 5B), GFP fluorescence was observed not only in ASE but also constantly in ADF and occasionally in ASJ and ASI. In the transgenic animals with gpa-14p::che-1 cDNA, GFP fluorescence was observed constantly in PHA and PHB, and often or occasionally in ASI, ASJ, ASK, ADA, ADE and RIA (Fig. 5D). However, in transgenic animals with the promoter-only constructs, no ectopic gfp expression was observed out of 16 lines for the gpa-10 promoter-only construct and 15 lines for the gpa-14 promoter-only construct (Fig. 5A,C). Because, in these experiments, the che-1 cDNA expression construct and the GFP reporter constructs were in the same extrachromosomal array, it may be possible that the ectopic gfp expression was due to an artificial interaction of these constructs within the same extrachromosomal array. To examine this possibility, we used two integrated strains, OH811 otls3[gcy-7::gfp] and OH813 nts1[gcy-5::gfp]. gpa-10p::che-1 cDNA construct was introduced into OH811 animals as an extrachromosomal array. In this transgenic line, ectopic gfp expression was observed in cells likely to be
amphid neurons (six cells in 21% animals, five in 25%, four in 19%, three in 19%, two in 17% and one in 4% out of the 48 animals observed), although cell identification was impossible under Nomarski optics because of abnormal cell positions in OH811 strain. Next, the extrachromosomal array was transferred to the wild-type N2 strain, then to OH813. In the wild-type background, no ectopic gfp expression was observed, while in OH813 background, ectopic gfp expression was observed in the cells in which che-1 cDNA expression was expected to be driven by the gpa-10 promoter (Table 4). These

Fig. 3. Expression pattern of che-1 and rescue of chemotaxis defect by che-1 constructs. (A) A che-1 genomic clone and gfp fusion constructs. Transformed lines with extrachromosomal arrays of each of these constructs in the che-1(p679) mutant background were examined for rescue of chemotaxis as in Fig. 1. GFP expression pattern was examined in wild-type background. (B,C) Overlap of DIC and fluorescent images showing expression of pche-1::gfpBglII (B) or pche-1::gfpC (C) in wild-type L2 stage animals. Scale bars: 10 μm. (D-F) Tracks of animals showing chemotaxis behaviors on an agar plate with a concentration gradient of NaCl: (D) wild type, (E) che-1(p679), (F) che-1(p679); $	ext{Ex}[\text{che-1-HindIII (che-1+)}]$, plin44p-gfp]. Although the che-1 mutant failed to respond to the concentration gradient, the wild-type and the rescued animals migrated to the concentration peak at the center and remained there.

Table 4. Effect of ectopic che-1 expression on the expression of gcy-5::gfp

| Genotype | ADF | ASI | ASJ | ASE | n |
|----------|-----|-----|-----|-----|---|
| mIs1[gcy-5:gfp]; Ex[gpa-10p::che-1 cDNA, lin-44p::gfp] | 92 | 97 | 83 | 86 | 36 | 94 | 100 | 3 | 36 |
| ++/++; Ex[gpa-10p::che-1 cDNA, lin-44p::gfp] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 |
| mIs1[gcy-5::gfp] | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 |

R, right side cells; L, left side cells; n, number of animals observed.
The extrachromosomal array was transferred by genetic crosses.
results indicate that ectopic expression of che-1 is sufficient to confer ASE-specific gene expression on several classes of neurons.

Furthermore, to examine whether ectopic expression of che-1 cDNA provides another ASE property, namely inability to take up DiI, transgenic animals shown in Table 5 were observed for their Dil filling and gfp expression. As shown in Table 5, significant proportions of cells in which che-1 cDNA is expected to be expressed showed dye-filling defect, whereas almost all of these cells took up DiI when the promoter only (empty) constructs were introduced. Thus, che-1 cDNA expression changes the property of these cells not to take up DiI as is observed in ASE.

gpa-10 and gpa-14 promoters drive expression in ASI and ASG (gpa-10 promoter), and ASI (gpa-14 promoter) that are known to be involved in inhibition of dauer formation under favorable conditions (Bargmann and Horvitz, 1991b).

Therefore, we examined dauer formation of transgenic lines in which che-1 cDNA was ectopically expressed by each of these promoters (Table 6). In fact, a significant proportion of the animals formed dauer. This result suggests that the dauer-inhibiting function of ASI and ASG is perturbed by che-1 ectopic expression in these cells.

**DISCUSSION**

The che-1 gene was cloned and found to encode a C2H2 type zinc-finger protein. Mutations in the seven che-1 mutants were determined and all were nonsense or mis-sense mutations in the zinc-finger domains. Thus, zinc fingers are essential for the functions of CHE-1. CHE-1 is most similar to GLASS transcription factor of *Drosophila*. GLASS binds to a 27 bp sequence of the enhancer of a rhodopsin gene (Moses and

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**Fig. 4.** che-1 mutation affects expression of marker genes in ASE. Fluorescent images showing expression of F55E10.7::gfp (A,B), R13H7.2::gfp (C,D), gcy-5::gfp (E,F), gcy-6::gfp (G,H), gcy-7::gfp (I,J), tax-2::gfp (K,L), cheh-23::gfp (M,N), lim-6::gfp (O,P), F25B3.3::gfp (Q,R) and Dil staining (S,T). A,C,E,G,I,K,M,O,Q,S show expression in wild-type background; B,D,F,H,J,L,N,P,R,T expression in che-1(p679) background. Arrowheads indicate ASE neurons that have no gfp fluorescence. Animals were at L1 or L2 larval stages. Scale bars: 5 µm.
Rubin, 1991) and its zinc-finger domains are essential for this binding (O’Neill et al., 1995). These results suggest that CHE-1 binds to an enhancer sequences of some genes and activates their transcription. Expression of seven genes was lost in ASE neurons of che-1 mutants. These genes may be candidate targets of CHE-1. However, no putative CHE-1 binding sites conserved among the upstream regions of these genes or similar to the GLASS-binding site were found in comparison of the nucleotide sequences. CHE-1 may regulate these genes indirectly through activation of another transcription factor.

How does che-1 affects the ASE character? In che-1 mutants, expression of neuron subtype-specific gfp-marker genes that we examined were affected specifically in ASE but not in others. In addition, the number and position of ASE neurons, and ASJ, AUA, AWB and ADF, which are closely related to ASE in the cell lineage (Sulston et al., 1983), and the other amphid neurons were normal. These findings indicate that che-1 mutations do not cause alterations of the cell lineage. As ASE neurons of che-1 mutants took up DiI, it is possible that che-1 mutations alter ASE to another cell type which takes DiI, such as AWB, ASH, ASI, ASJ, ASK and ADL. However, ASH, ASI, ASJ and ADL can be excluded from such candidate cell types, because srg-8::gfp, tax-2::gfp and ceh-23::gfp expression were lost in ASE but not affected in ASH, ASI, ASJ and ADL in the che-1 mutant (ASI is not visible in Fig. 4L, and ADL and ASI are not visible in Fig. 4N, because they are out of the focus). In addition, ASK and AWB can be excluded, because srg-8::gfp (used as an ASK marker gene) (Troemel et al., 1995), lim-4::gfp (used as an AWB marker gene) (Baggiolini et al., 1999) and odr-3::gfp (used as an AWB and AWC marker gene) (Rouya et al., 1998) were not expressed in ASE of a che-1 mutant (Table 7). Furthermore, there is another reason for exclusion of AWB and ADL from such candidates. In the che-1 mutants: ASE has a single, long and slender cilium that is identical to that in wild type and very

### Table 5. Dye-filling defect induced by ectopic expression of che-1 cDNA

| Genotype | A=gpa-10p::che-1 cDNA (n=80) | A=gpa-10p::empty (n=200) |
|----------|-----------------------------|--------------------------|
|          | ASK | ADL | ASI | AWB | ASH | ASJ | ASK | ADL | ASI | AWB | ASH | ASJ |
| DiI+ GFP+ | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| DiI+ GFP– | 74  | 100 | 39  | 100 | 100 | 66  | 100 | 100 | 99.5| 100 | 100 | 100 |
| DiI– GFP+ | 10  | 0   | 13  | 0   | 0   | 19  | 0   | 0   | 0   | 0   | 0   | 0   |
| DiI– GFP– | 16  | 0   | 49  | 0   | 0   | 15  | 0   | 0   | 0.5 | 0   | 0   | 0   |

| Genotype | A=gpa-14p::che-1 cDNA (n=80) | A=gpa-14p::empty (n=200) |
|----------|-----------------------------|--------------------------|
|          | ASK | ADL | ASI | AWB | ASH | ASJ | ASK | ADL | ASI | AWB | ASH | ASJ |
| DiI+ GFP+ | 8   | 0   | 1   | 0   | 0   | 1   | 0   | 0   | 0   | 0   | 0   | 0   |
| DiI+ GFP– | 3   | 100 | 9   | 100 | 98  | 90  | 100 | 100 | 94  | 100 | 100 | 100 |
| DiI– GFP+ | 36  | 0   | 78  | 0   | 0   | 6   | 0   | 0   | 0   | 0   | 0   | 0   |
| DiI– GFP– | 55  | 0   | 13  | 0   | 3   | 3   | 0   | 7   | 0   | 0   | 0   | 0   |

### Table 6. Dauer formation induced by ectopic expression of che-1 cDNA

| Genotype | Proportion of dauers* |
|----------|-----------------------|
| A=gpa-10p::che-1 cDNA | 38.3±3.2% 349 10 |
| A=gpa-10p::empty | 0.0±0.0% 596 6 |
| A=gpa-14p::che-1 cDNA | 28.9±3.6% 563 10 |
| A=gpa-14p::empty | 0.7±0.3% 337 7 |
| +/+ (Wild type N2) | 0.0±0.0% 668 3 |

*Average and s.e.m.

### Table 7. Ectopic expression of cell markers in ASE in a che-1 mutant

| Genotype | Line | % of GFP expression |
|----------|------|---------------------|
| che-1(p679); Ex[srg-8::gfp, lin-44::gfp] | #1 | 0 100 (ASK) |
| che-1(p679); Ex[srg-8::gfp, lin-44::gfp] | #2 | 0 100 (ASK) |
| che-1(p679); Ex[srg-8::gfp, lin-44::gfp] | #3 | 0 100 (ASK) |
| che-1(p679); Ex[lm-4::gfp, lin-44::gfp] | #1 | 0 100 (AWB) |
| che-1(p679); Ex[lm-4::gfp, lin-44::gfp] | #2 | 0 100 (AWB) |
| che-1(p679); Ex[lm-4::gfp, lin-44::gfp] | #3 | 0 100 (AWB) |
| che-1(p679); kyIs37[odr-10::gfp] | 0 100 (AWA) |

*Average and s.e.m.

| Genotype | Line | % of GFP expression |
|----------|------|---------------------|
| che-1(p679); | #1 | 0 100 (ASK) |
| che-1(p679); | #2 | 0 100 (ASK) |
| che-1(p679); | #3 | 0 100 (ASK) |

*Average and s.e.m.

| Genotype | Line | % of GFP expression |
|----------|------|---------------------|
| che-1(p679); | #1 | 0 100 (AWB) |
| che-1(p679); | #2 | 0 100 (AWB) |
| che-1(p679); | #3 | 0 100 (AWB) |

*Average and s.e.m.

| Genotype | Line | % of GFP expression |
|----------|------|---------------------|
| che-1(p679); | #1 | 0 100 (AWA) |
| che-1(p679); | #2 | 0 100 (AWA) |
| che-1(p679); | #3 | 0 100 (AWA) |

*Average and s.e.m.

- n, number of cells observed in each class.
- Numbers are % of cells showing each phenotype.
- DiI+ and DiI– indicate cells dye filled and not, respectively.
- GFP+ and GFP– indicate cells with GFP fluorescence and without, respectively.

- n, number of animals in a plate; n, number of plates.
- *Average and s.e.m.
such as expression of cell-specific genes and functions. The mutations cause some morphological change in touch receptor neurons and AIY neurons, respectively. They encode LIM homeodomain proteins, which are required for expression of cell specific features in AW A olfactory neurons, AFD thermosensory neurons and AW B olfactory neurons, respectively (Sengupta et al., 1994; Sengupta et al., 1996; Sagasti et al., 1999; Satterlee et al., 2001). However, mutations in these genes brought about some expression of AW C olfactory neuron fate in AW A, AFD and AW B (Sagasti et al., 1999; Satterlee et al., 2001). This phenotype could be crucial for putting these genes into another category, which involves another hierarchy of cell specification process. Alternatively, if a default cell fate of AW A, AFD and AW B is AW C, odr-7, ttx-1 and lim-4 genes should be in the same category as che-1, mec-3 and ttx-3.

che-1 and glass genes were shown to encode highly homologous zinc-finger proteins and to be required for differentiation of ASE chemosensory neurons in Caenorhabditis elegans and the photoreceptor cell in Drosophila, respectively. This conservation in both structure and function suggests that che-1 and glass are homologous in evolution, and that ASE chemosensory neurons of C. elegans and the photoreceptor cells of Drosophila may be counterparts in the evolution of these two species. AFD thermosensory neurons in C. elegans are thought to have an evolutionary relationship to photoreceptor cells of a vertebrate (Satterlee et al., 2001). Therefore, ASE and AFD in C. elegans and photoreceptors cells in vertebrates and invertebrates might have a close relation in evolution.

To reveal developmental processes of ASE, che-1 is a good starting point from which to trace the process by asking how che-1 gene expression is regulated and what genes are targets of CHE-1.

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