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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 ASEL (Hobert et al., 1999). In *lim-6* mutants, *gcy-5* is expressed in ASEL 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 *sDf4/bli-4(e937) dpy-14(e188)* I.

kyIs5[ceh-23::gfp, lin-15(+)] IV (Zellen et al., 1999) (Forrester et al., 1998) and NW1229 *dpy-20; evIs111[F25B3.3::gfp, dpy-20(+)]*. OH811 *otIs3[gcy-7::gfp],* OH812 *otIs114[lim-6::gfp, rol-6(d)]* and OH813 *ntIs1[gcy-5::gfp]* (O. Hobert, personal communication).

kyIs37[odr-10::gfp] (Sengupta et al., 1996).

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 μl 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 periphery 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. Homozygotes, *bli-4* + or + *dpy-14*, derived from the recombinants were mated with *che-1* males, and the non-Bli non-Dpy progeny were subjected to chemotaxis assays.

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 QIAfilter Plasmid Maxi Kit (Qiagen). PCR products for rescue experiments were amplified from wild-type N2 genomic DNA with a high accuracy polymerase, LA Taq polymerase (Takara) and purified using QIAquick PCR purification kit (Qiagen).

Germline transformation (Mello et al., 1991) was carried out by co-injecting test DNA at a concentration of 5-50 ng/μl and marker DNA at a concentration of 5-100 ng/μl into the gonads. Rescue experiments for identification of the *che-1* gene were performed by injecting a cosmid or a PCR product at 5-10 ng/μl together with pTOCV01 GFP marker DNA (Oka et al., 1997) at 90-100 ng/μl into *che-1 (p696)*. 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.

che-1 cDNA was amplified from total RNA of a mixed-stage population of wild-type N2 by RT-PCR with a set of primers spanning the entire putative *che-1* ORF and the product was directly sequenced. The PCR product was cloned into pGEM-T vector so as to be pche-1cDNA. The sequence of the cDNA was verified by sequencing.

Expression constructs and generation of transgenic animals

A *che-1* promoter construct, pche-1p::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::gfpC, was prepared by amplifying the 5.4 kb upstream sequence and the entire coding region of *che-1* from the wild-type genome. A *Sph*I site and a *Pst*I 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*Bam*HI and pche-1::gfp*Bgl*II were constructed by inserting *gfp* fragments amplified from pPD95.77 in frame into the *Bam*HI site and the *Bgl*II site of pche-1*Hin*dIII, a *che-1* gene subclone of 6.2kb *Hin*dIII fragment from ZC130 cosmid in pHSG398 (a Cm^r 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.

pF55E10.7::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 *Pst*I and *Bam*HI sites of pGFP-TT vector (Y. Jin, personal communication) and the *Sal*I and *Bam*HI 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 *Pst*I and *Bam*HI sites of pPD95.77. gcy-5::GFP, gcy-6:: GFP and gcy-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 *Sph*I-*Sma*I fragment of pgpa10p-gfp or pgpa14p-gfp (Murakami et al., 2001) containing an upstream sequence of gpa-10 or gpa-14 and a blunt-ended *Sac*II-*Sac*I fragment of pche-1 cDNA containing a *che-1* full-length cDNA were inserted between the *Sph*I and *Sma*I sites and at the blunt-ended *Kpn*I 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 *flr-1::gfp* expressed in the intestine) (Take-Uchi et al., 1998) at 50 ng/μl or plin44-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 Normarski 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

DiI stock solution was made by dissolving 2 mg DiI 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

DiI 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).

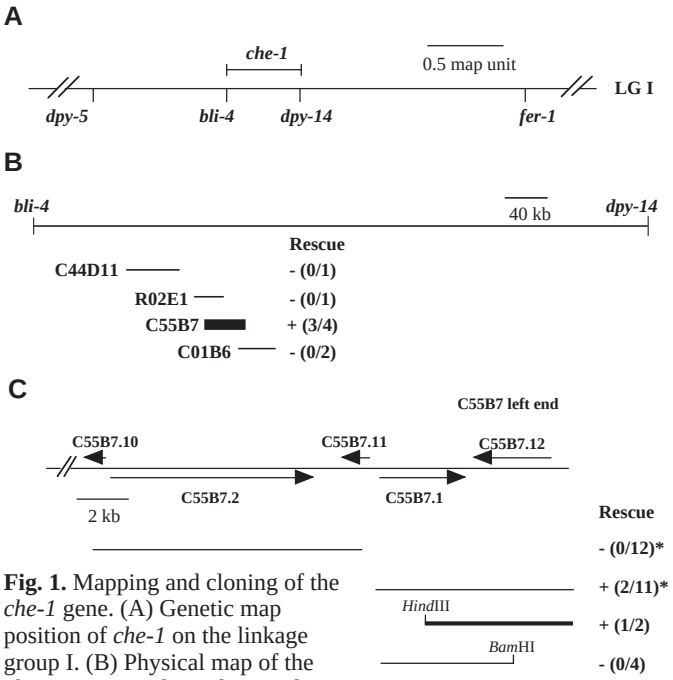

Fig. 1. Mapping and cloning of the *che-1* gene. (A) Genetic map position of *che-1* on the linkage group I. (B) Physical map of the *che-1* region. Indicated cosmids were tested for rescue of the defect in chemotaxis to NaCl of *che-1* mutants. Rescue results are shown as + (positive) or – (negative), and the number of transformed lines that rescued the *che-1* mutant phenotype as a fraction of the total number of independent lines tested are shown in parentheses. Each line was tested for chemotaxis at least with 20 individuals. When more than half of the animals show normal chemotaxis, the chemotaxis defect was regarded as being rescued. (C) Location of the *che-1* gene. The top line and arrows show C55B7 near the left end and predicted transcripts based on the genomic sequence. PCR products and restriction fragments of the products were tested for rescue of the *che-1(p696)* mutant phenotype and the results are shown as in B. *The number of F1 transient transformants that rescued the *che-1* mutant phenotype as a fraction of the total number of transient transformants tested.

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 Pro. 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

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-*Hin*dIII) 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

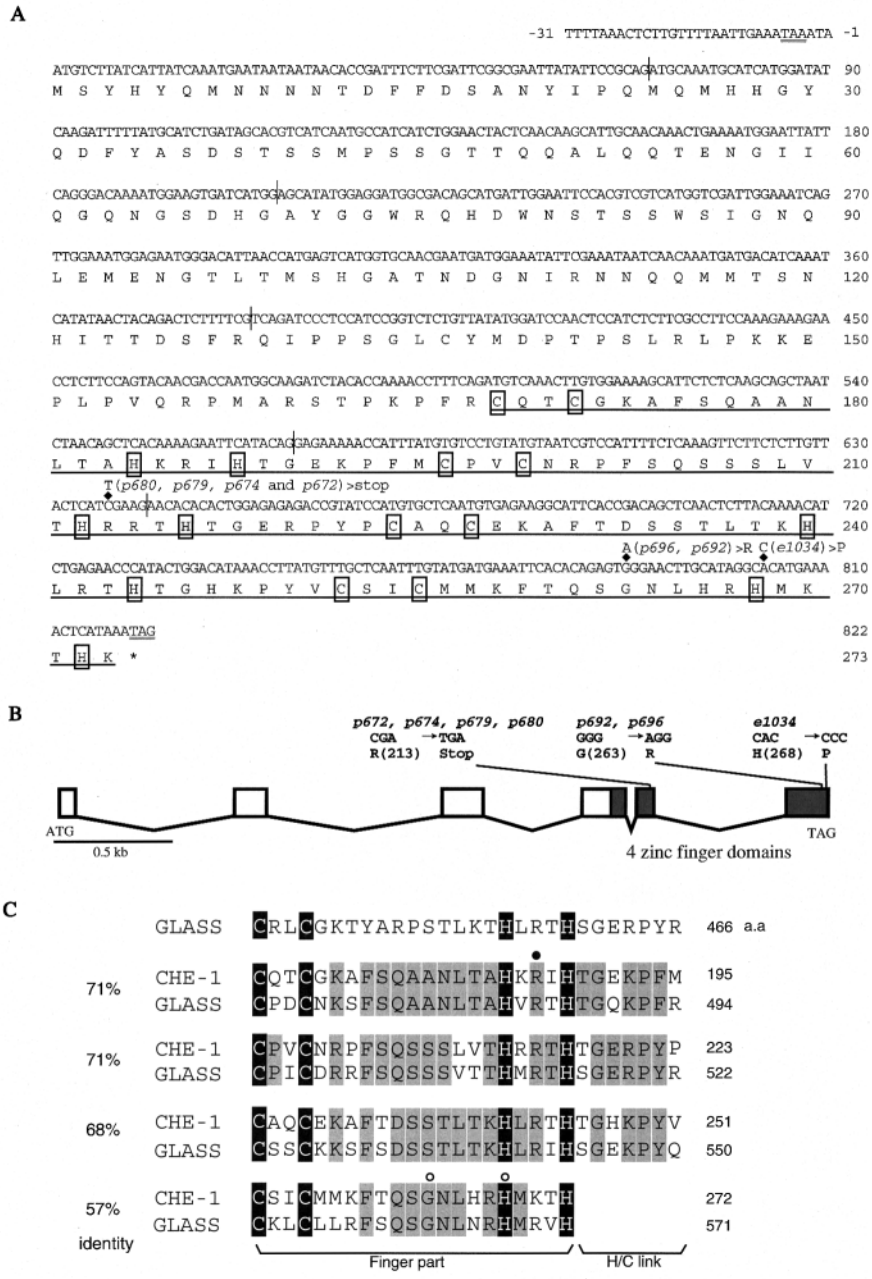

Fig. 2. Amino acid sequence of CHE-1. (A) Nucleotide sequence of the *che-1* cDNA and amino acid sequence of the predicted product (CHE-1). Splice junctions were determined by comparison with the genomic sequences and are marked with vertical lines. The putative termination codon is marked with double underlines and an asterisk. The zinc-finger region is underlined. The invariant cysteine and histidine residues of C2H2-type zinc-finger motifs are boxed. Mutation sites are indicated by diamonds with resultant nucleotide and amino acid substitutions. (B) Genomic organization of the *che-1* gene. Sequences encoding the zinc fingers are shaded. Seven *che-1* mutations are indicated along with their respective nucleotide and predicted amino acid alterations.
(C) Alignment of the zinc fingers of CHE-1 with the zinc fingers of the *Drosophila* protein GLASS (Moses et al., 1989). Identical residues are shaded gray. Conserved cysteine and histidine residues are highlighted. Mutation sites in CHE-1 are indicated by a black circle (nonsense) or white circles (mis-sense).

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

	Animals with GFP expression in ASE					
	Wild-type background			*che-1(p679)* background		
Reporter genes	Line	%	*n*	Line	%	*n*
Ex[F55E10.7::gfp]	#1	96	55	#1	3	68
	#2	90	58	#2	4	67
Ex[R13H7.2::gfp]	#1	100	106	#1	7	114
	#2	100	90	#2	3	91
Ex[gcy-5::gfp]	#1	98	101	#1	0	140
	#2	99	100	#2	0	120
Ex[gcy-6::gfp]	#1	96	127	#1	0	120
	#2	98	100	#2	1*	127
Ex[gcy-7::gfp]	#1	94	110	#1	0	146
	#2	92	132	#2	1*	137
Ex[tax-2::gfp]	#1	100	75	#1	0	120
	#2	100	70	#2	0	85
kyIs5 [ceh-23::gfp]		100	55		4	100
otIs114[lim-6::gfp, rol-6(d)]		100	100		0	100
evIs111[F25B3.3::gfp]		100	70		100	65

*Very faint.

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

Transgene	Background	GFP expression in ASE (%)	*n*
*Ex[gcy-5::gfp, flr-1::gfp]**	*che-1(p692)*	0	61
	che-1(e1034)	0	80
	che-1(p679)	0	80
	+/+	98	101
*Ex[gcy-6::gfp, flr-1::gfp]**	*che-1(p692)*	0	71
	che-1(e1034)	0	78
	che-1(p679)	0	85
	+/+	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*
Wild type	0	30
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.

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 F25B3.3 encoding a putative Ca^{2+}-regulated Ras nucleotide exchange factor (Altun-Gultekin et al., 2001), was expressed in the ASE neurons of the *che-1* mutant (Fig. 4R). These results are consistent with the idea outlined above.

We found three kinds of mutations among the seven *che-1* alleles, as described (Fig. 2B). *p692*, *e1034* and *p679*, which represent each of the three kinds of mutations were examined to see whether there are differences in effect on the expression of two ASE specific marker genes (*gcy-5* and *gcy-6*). We did not find any difference (Table 2). We did not find any difference in the defect of chemotaxis to NaCl among these alleles, either (data not shown). Therefore, these mutations are expected to lack the function of CHE-1 to the same extent.

Wild-type animals take up a fluorescent dye such as DiI into AWB, ASH, ASJ, ASK, ADL and ASI amphid neurons, PHA and PHB phasmid neurons, but not into ASE (Hedgecock et al., 1985; Starich et al., 1995). *che-1* mutants take DiI into ASE as well (Fig. 4S,T and Table 3). DiI is taken by the sensory cilia exposed to the outside through amphid or phasmid pores. But not all such cilia take up the dye in the wild type. Therefore, a specific mechanism should be involved in the dye filling, although little is known for such a mechanism. This unusual dye uptake into ASE in the *che-1* mutants suggests that a character of ASE cilia changed in the presence of a *che-1* mutation.

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, AVA, 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, AVA, 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 *otIs3[gcy-7::gfp]* and OH813 *ntIs1[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

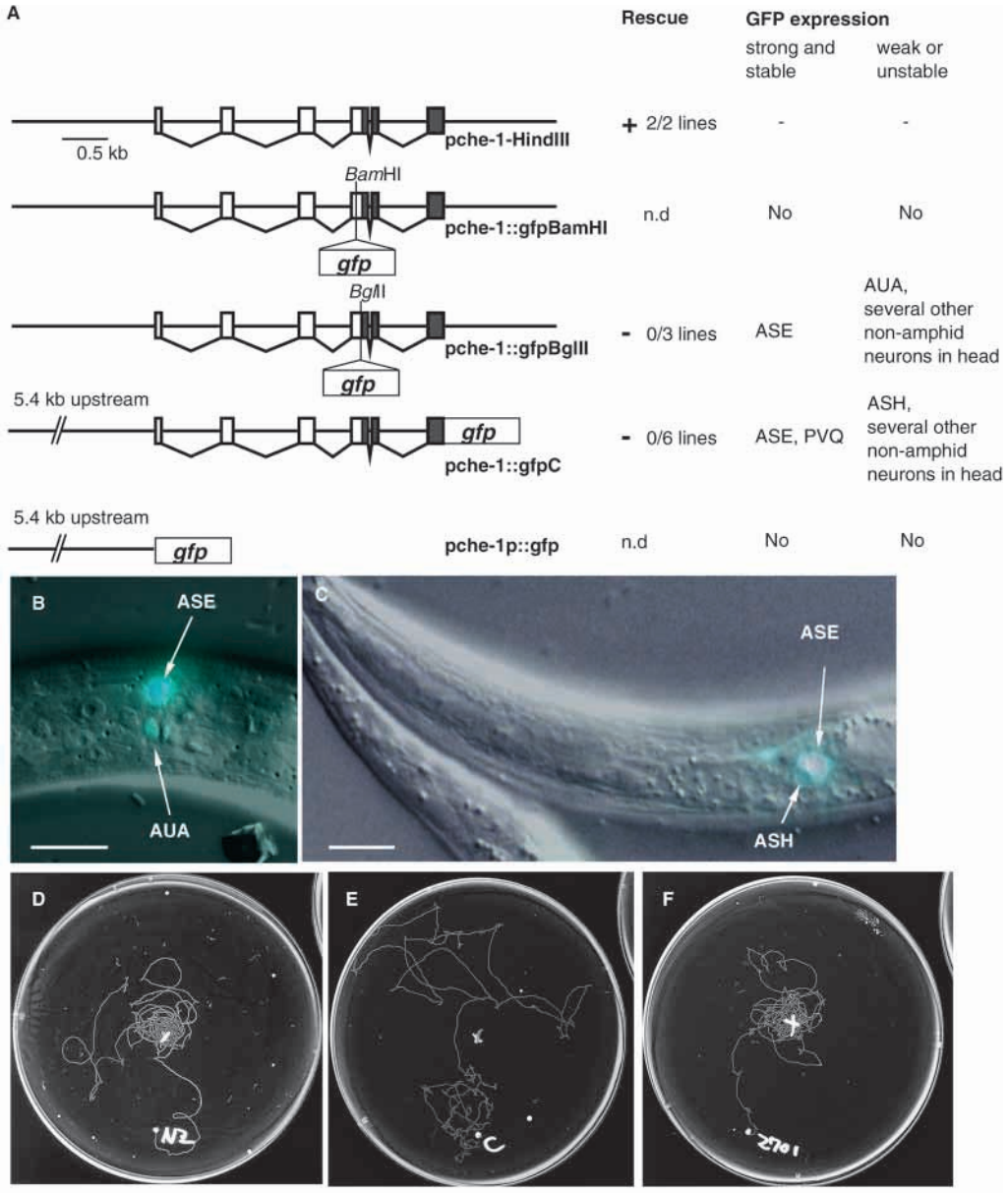

Fig. 3. Expression pattern of *che-1* and rescue of chemotaxis defect by *che-1* constructs. (A) A *che-1* genomic clone and *gfp* fusion constructs. Transformed lines with extrachromosomal arrays of each of these constructs in the *che-1(p679)* mutant background were examined for rescue of chemotaxis 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::gfp*Bgl*II (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); Ex[*pche-1-*Hin*dIII (*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*

	% of cells expressing GFP								
	ADF		ASI		ASJ		ASE		
Genotype	R	L	R	L	R	L	R	L	*n*
ntIs1[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	0	100
ntIs1[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.

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

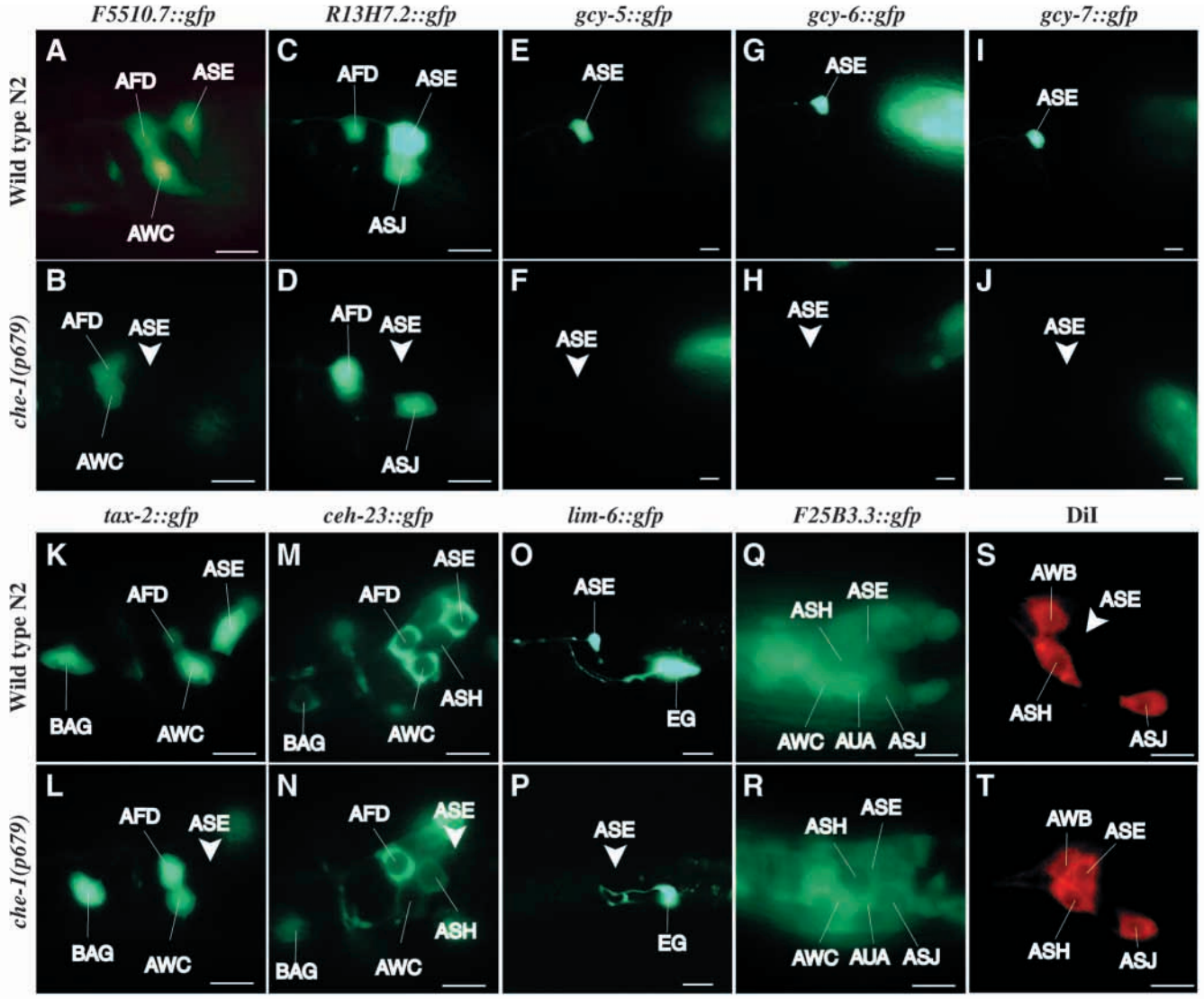

Fig. 4. *che-1* mutation affects expression of marker genes in ASE. Fluorescent images showing expression of *F55E10.7::gfp* (A,B), *R13H7.2::gfp* (C,D), *gcy-5::gfp* (E,F), *gcy-6::gfp* (G,H), *gcy-7::gfp* (I,J), *tax-2::gfp* (K,L), *ceh-23::gfp* (M,N), *lim-6::gfp* (O,P), *F25B3.3::gfp* (Q,R) and DiI 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.

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 dauers. 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

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

	Genotype: *+/+; Ex[A, gcy-5::gfp, gcy-6::gfp, gcy-7::gfp, lin-44p::gfp]*											
	A=*gpa-10p::che-1* cDNA (*n*=80)						A=*gpa-10p::empty* (*n*=200)					
Phenotype	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
Dil– 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
	A=*gpa-14p::che-1* cDNA (*n*=80)						A=*gpa-14p::empty* (*n*=200)					
Phenotype	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
Dil– GFP+	36	0	78	0	0	6	0	0	0	0	0	0
DiI– GFP–	55	0	13	0	3	3	0	0	7	0	0	0

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.

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

Genotype	Proportion of dauers*	N	*n*
+/+; Ex[A, gcy-5::gfp, gcy-6::gfp, gcy-7::gfp, lin-44p::gfp]			
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

N, Average of total number of animals in a plate; *n*, number of plates.
*Average and s.e.m.

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 *R13H7.2::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) (Sagasti et al., 1999) and *odr-3::gfp* (used as an AWB and AWC marker gene) (Roayaie 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 7. Ectopic expression of cell markers in ASE in a *che-1* mutant

		% of GFP expression		
Genotype	Line	ASE	Others	*n*
che-1(p679);	#1	0	100 (ASK)	50
Ex[srg-8::gfp, lin-44::gfp]	#2	0	100 (ASK)	55
	#3	0	100 (ASK)	60
che-1(p679);	#1	0	100 (AWB)	50
Ex[lim-4::gfp, lin-44::gfp]	#2	0	100 (AWB)	50
	#3	0	100 (AWB)	50
che-1(p679);	#1	0	100 (AWB, AWC)	50
Ex[odr-3::gfp, lin-44::gfp]	#2	0	100 (AWB, AWC)	50
	#3	0	100 (AWB, AWC)	50
che-1(p679); kyIs37[odr-10::gfp]		0	100 (AWA)	50

n, number of animals

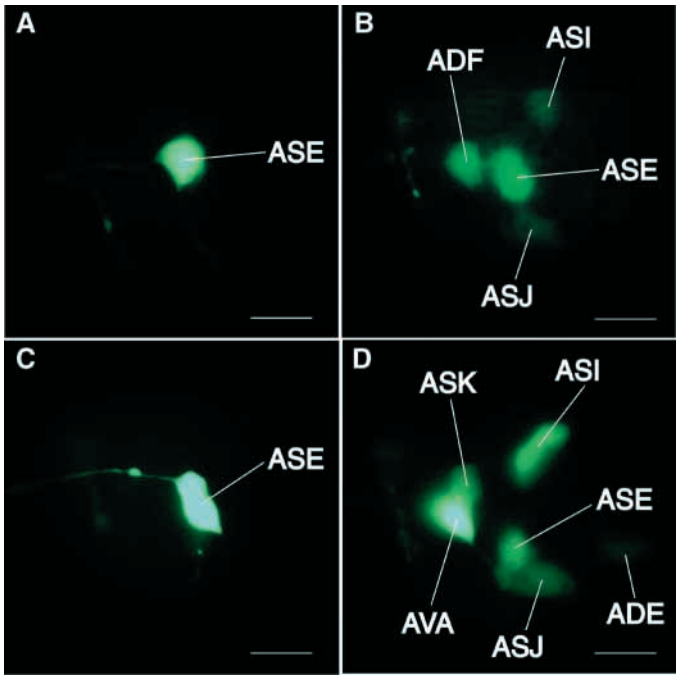

Fig. 5. Effect of ectopic expression of *che-1* cDNA on the expression of ASE marker genes. Fluorescent images showing expression of GFP in transformant lines carrying an extrachromosomal array *gpa-10* promoter-only construct (*gcy-5::gfp, gcy-6::gfp* and *gcy-7::gfp*) (A); *gpa-10p::che-1* cDNA (*gcy-5::gfp, gcy-6::gfp* and *gcy-7::gfp*) (B); *gpa-14* promoter-only construct (*gcy-5::gfp, gcy-6::gfp* and *gcy-7::gfp]* (C); or *gpa-14p::che-1* cDNA (*gcy-5::gfp, gcy-6::gfp and gcy-7::gfp*) (D) in the wild-type background. Animals were at L1 or L2 larval stages Scale bars: 5 μm.

different from those of AWB and ADL. An AWA marker *odr-10::gfp* was not expressed in ASE of a *che-1* mutant (Table 7). ADF does not take up DiI and its cilium morphology is different from that of ASE. Therefore, given the above discussion, it is difficult to consider that CHE-1 represses ASE fate and that *che-1* mutations cause a homeotic change of ASE to another cell type of amphid neurons. These results, together with the findings that *che-1* was expressed in ASE and that ectopic expression of CHE-1 was sufficient to confer ASE-specific gene expression onto several classes of neurons, indicate that *che-1* is a positive regulator capable of inducing a gene expression sequence in ASE neurons after their development to amphid neurons.

In development of a particular cell, the process may be divided into two parts, the first is responsible for determination of the basic cell fate and the second for terminal differentiation, such as expression of cell-specific genes and functions. *che-1* is likely to act at the point that links the first and second parts of the development of the ASE neurons, because a *che-1* mutation leads to loss of the second part. *mec-3* and *ttx-3* genes are likely to be in the same category, although *mec-3* and *ttx-3* mutations cause some morphological change in touch receptor neurons and AIY neurons, respectively. They encode LIM homeodomain proteins, which are required for expression of the final differentiated features in touch receptor neurons and AIY interneurons, respectively (Way and Chalfie, 1988; Mitani et al., 1993; Hobert et al., 1997; Altun-Gultekin et al., 2001). In this category, a mutation causes loss of identity of a specific neuron class without alteration of cell lineages. *odr-7, ttx-1* and *lim-4* may also be in this category, although they have an additional character. *odr-7, ttx-1* and *lim-4*, which encode a nuclear receptor superfamily member, an OTD/OTX homeodomain protein and a LIM homeodomain protein, are required for expression of cell specific features in AWA olfactory neurons, AFD thermosensory neurons and AWB 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 AWC olfactory neuron fate in AWA, AFD and AWB (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 AWA, AFD and AWB is AWC, *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 *C. 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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