Caenorhabditis elegans che-5 is allelic to gcy-22
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Figure 1. che-5(e1073) carries mutations in gcy-22 that are responsible for chemotaxis defects of the mutants: (A) Schematic diagram of gcy-22a gene structure. Boxes represent exons. Protein domains and the nucleotide substitutions found in e1073 are indicated above and below the diagram, respectively. (B) Complementation test between che-5(e1073) and gcy-22(tm2364). Salt concentration chemotaxis was observed in e1073/tm2364 heterozygotes and their parental strains. e1073 failed to complement tm2364. n = 5, Mean±SEM, *p ≤ 0.001, compared to mls11, Dunnett’s test. (C) ASER-specific expression of gcy-22(+) rescued the chemotaxis defect of the che-5(e1073) mutants. n = 4 or 5, Mean±SEM, *p ≤ 0.01, compared to wild type, Dunnett’s test.

Description
Mechanisms of chemotactic behaviors have been of great interest in C. elegans neuroscience since the early days of its research (Ward 1973). Lewis and Hodgkin (1977) systematically isolated more than ten abnormal chemotaxis (che) mutants that showed defective chemotaxis to sodium (Na⁺) and chloride (Cl⁻) ions (Lewis and Hodgkin 1977), whose responsible genes have already been molecularly characterized except for che-5(e1073). We here show that che-5(e1073) is a missense allele of gcy-22, which encodes a receptor guanylyl cyclase (rGC) specifically expressed in the ASE-right (ASER) gustatory neuron and is essential for chemosensation through the neuron.

C. elegans is attracted to the NaCl concentration at which it has experienced with food, while avoiding the concentration at which it has experienced starvation. ASER plays a major role in food-associated salt concentration chemotaxis; input of salt information into ASER is required and sufficient for chemotaxis to the salt concentration associated with food (Kunitomo et al. 2013). ASE neurons, consisting of bilaterally symmetrical ASE-left (ASEL) and ASER, are the major sensory neuron for water-soluble attractants (Bargmann and Horvitz 1991). They respectively sense different sets of ions such as Na⁺ and Cl⁻ (Pierce-Shimomura et al. 2001; Suzuki et al. 2008; Ortiz et al. 2009). A cyclic GMP (cGMP) signaling pathway consisting of rGCs and TAX-4/TAX-2 cyclic nucleotide-gated ion channels mediates sensory transduction in ASE (Coburn and Bargmann 1996; Komatsu et al. 1996; Ortiz et al. 2009). ASEL and ASER express distinct sets of rGCs (Ortiz et al. 2006). Of these, gcy-22 is essential for ASER to respond to multiple ion species; therefore it is proposed as a common component of chemoreceptor complexes (Ortiz et al. 2009; Adachi et al. 2010; Smith et al. 2013). To further elucidate the mechanisms of chemosensation through ASER, we characterized as yet uncloned che-5. We focused on che-5 because CB1073 che-5(e1073) mutant, the unique strain/allele of the gene, showed...
chemotaxis defects characteristic of ASER-specific malfunction; a severe defect in attraction to Cl\(^-\), whereas relatively moderate defect in Na\(^+\) chemotaxis (Lewis and Hodgkin 1977).

We mapped the mutation of che-5(e1073) responsible for salt chemotaxis defect between genetic positions 24.52 (single nucleotide polymorphism (SNP): WBVar00240760) and 25.54 (SNP: WBVar00053592) on chromosome V by using SNPs between N2 and CB4856 (Wicks et al. 2001). The mapped region contained an ASER-specific chemotaxis gene, gcy-22 (genetic position: 25.28). This result was unexpected from the initial report that mapped e1073 on chromosome IV (Lewis and Hodgkin1977), but consistent with a recent observation in which whole-genome sequencing failed to identify a mutation corresponding to che-5(e1073) on chromosome IV (Smith et al. 2013).

gcy-22(tm2364) harbors a deletion in the middle of gcy-22 coding region that results in a frame shift and therefore is a putative null allele (Fig. 1A). Salt concentration chemotaxis of the animals heterozygous for e1073 and tm2364 showed that the two alleles failed to complement each other, indicating that these alleles affect the same locus (Fig. 1B). Nucleotide sequencing of the gcy-22 locus revealed that e1073 carried ACG GCA CCA AAG CAC ATT AGA in which the adenine residue in bold letter was guanine in wild type (Fig. 1A). This transition results in a missense change E697K in GCY-22 isoform a. The glutamate residue is located in the kinase-like domain and well conserved in rGC proteins. Furthermore, expression of a cDNA for gcy-22a in CB1073 in ASER-specific manner completely rescued the salt chemotaxis defect of the mutant (Fig. 1C). These results strongly indicate that che-5 is allelic to gcy-22 and the chemotaxis defect of e1073 is due to the mutations of gcy-22 locus.

**Methods**

*Request a detailed protocol*

Salt concentration chemotaxis was evaluated as described (Kunitomo et al. 2013). A chemotaxis index was calculated to quantify the behavior as follows. Chemotaxis index = \((N\text{ at high NaCl-region}) - (N\text{ at low NaCl-region})\) / \((\text{total } N - (N \text{ that did not move from the origin}))\), in which \(N\) represents number of animals. For complementation tests, males of PD4792 (mIs11 with gcy-22(+)) background) or JN2608 (mIs11 with gcy-22(tm2364) background) were mated with CB1073 hermaphrodites. F1 progenies were tested for salt concentration chemotaxis, and crossed progeny hermaphrodites that carried mIs11 were separately counted from self-progeny hermaphrodites that did not carry the marker. For rescue experiments, 5 ng/microL gcy-5p::gcy-22a construct was introduced into CB1073 with 15 ng/microL myo-3p::venus as a transformation marker.

**Reagents**

Strains. The JN strains are available upon request. Others are available at Caenorhabditis Genetic Center (CGC).

Bristol N2: wild type

CB4856: wild type

CB1073: che-5(e1073) V.

JN967: gcy-22(tm2364) V.

JN2606: che-5(e1073) V; peEx2606[myo-3p::venus].

JN2607: che-5(e1073) V; peEx2607[gcy-5p::gcy-22a myo-3p::venus].

JN2608: mIs11[myo-2p::GFP pes-10p::GFP gut-promoter::GFP] IV; gcy-22(tm2364) V.

PD4792: mIs11[myo-2p::GFP pes-10p::GFP gut-promoter::GFP] IV.

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**References**


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