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Caenorhabditis elegans che-5 is allelic to *gcy-22*

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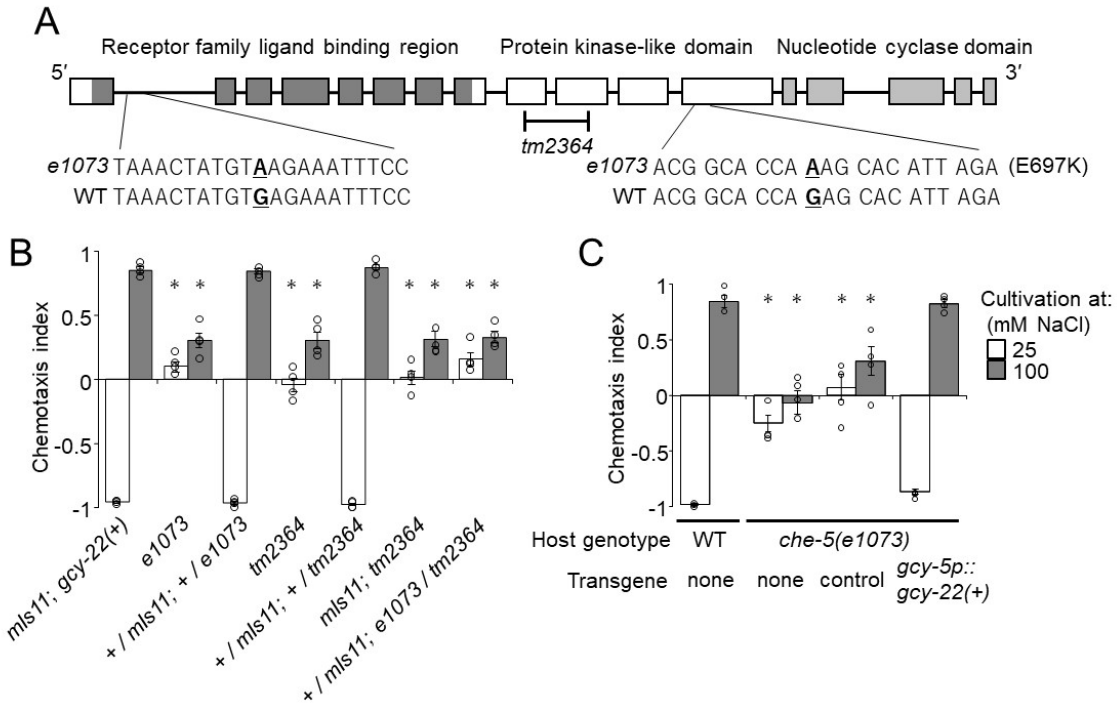

Figure 1. *che-5(e1073)* carries mutations in *gcy-22* that are responsible for chemotaxis defects of the mutants: (A) Schematic diagram of *gcy-22a* gene structure. Boxes represent exons. Protein domains and the nucleotide substitutions found in *e1073* are indicated above and below the diagram, respectively. (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, $*p \leq 0.001$, compared to *mIs11*, 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, $*p \leq 0.01$, 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 avoid 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 **A**AG 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. In addition, CB1073 carried another nucleotide substitution within the first intron of *gcy-22*, TAAACTATGT**A**AGAAATTTCC, in which the adenine residue in bold letter was guanine in wild type (Fig. 1A). 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* at high NaCl-region) – (*N* at low NaCl-region)} / {(total *N*) – (*N* 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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