DOI: 10.1534/genetics.105.050013

Identification of Guanylyl Cyclases That Function in Thermosensory Neurons of *Caenorhabditis elegans*

Hitoshi Inada,*,†,[1] Hiroko Ito,* John Satterlee,‡,[2] Piali Sengupta,‡ Kunihiro Matsumoto§,†, and Ikue Mori*,****

**Group of Molecular Neurobiology, §Group of Signal Transduction, Department of Molecular Biology, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan, †CREST, JST, Kawaguchi 332-0012, Japan, **Institute for Advanced Research, Nagoya University, Nagoya 464-8602, Japan and ‡Department of Biology, and National Center for Behavioral Genomics, Brandeis University, Waltham, Massachusetts 02454*

Manuscript received August 26, 2005
Accepted for publication January 9, 2006

ABSTRACT

The nematode *Caenorhabditis elegans* senses temperature primarily via the AFD thermosensory neurons in the head. The response to temperature can be observed as a behavior called thermotaxis on thermal gradients. It has been shown that a cyclic nucleotide-gated ion channel (CNG channel) plays a critical role in thermosensation in AFD. To further identify the thermosensory mechanisms in AFD, we attempted to identify components that function upstream of the CNG channel by a reverse genetic approach. Genetic and behavioral analyses showed that three members of a subfamily of *gcy* genes (*gcy-8*, *gcy-18*, and *gcy-23*) encoding guanylyl cyclases were essential for thermotaxis in *C. elegans*. Promoters of each gene drove reporter gene expression exclusively in the AFD neurons and, moreover, tagged proteins were localized to the sensory endings of AFD. Single mutants of each *gcy* gene showed almost normal thermotaxis. However, animals carrying double and triple mutations in these genes showed defective thermotaxis behavior. The abnormal phenotype of the *gcy* triple mutants was rescued by expression of any one of the three GCY proteins in the AFD neurons. These results suggest that three guanylyl cyclases function redundantly in the AFD neurons to mediate thermosensation by *C. elegans*.

TEMPERATURE is an extremely important environmental cue for all animals. Since most biochemical and physiological processes are very sensitive to temperature, it is critical that animals respond rapidly to changes in both external and internal temperature. Cold-blooded animals must seek the appropriate temperature to maintain active motility, whereas warm-blooded animals must maintain temperature homeostasis. Temperature may also be associated with feeding resources. Parasites recognize their hosts and snakes such as pit vipers appear to detect their prey by sensing body temperature (Campbell *et al.* 2002). These physiological and behavioral responses require a precise ability to sense ambient temperature. However, the molecular mechanisms for thermosensation remain poorly understood.

In mammals, temperature appears to be sensed by transient receptor potential (TRP) channels, each of which is activated by different temperature ranges (Patapoutian *et al.* 2003; Tominaga and Caterina 2004). The discovery of thermosensitive TRP channels in vertebrates and Drosophila was a milestone for elucidation of the molecular mechanisms required for thermosensation. However, it remains possible that other proteins are also involved in thermosensation. TREK-1, a two-pore domain K^+ channel in mammals, has been shown to open gradually and reversibly in response to heat (Maingret *et al.* 2000), whereas Na^+/K^+ ATPase, ATP-gated cation channel $P2X_3$ receptor, and Na^+ channels belonging to members of the degenerin/epithelial sodium channel family also have been reported to be involved in thermosensation (Pierau *et al.* 1974; Souslova *et al.* 2000; Askwith *et al.* 2001).

In the nonparasitic nematode *Caenorhabditis elegans*, the response to temperature may be observed as a behavior called thermotaxis (TTX; Hedgecock and Russell 1975). After cultivation at a uniform temperature with sufficient food, animals preferentially migrate to the cultivation temperature and move isothermally when placed on a thermal gradient without food. *C. elegans* sense their environmental temperature mainly via a pair of sensory neurons (AFD), which were identified by a series of laser ablation experiments (Mori and Ohshima 1995). A small number of molecules implicated in thermosensory signal transduction in AFD

Sequence data from this article have been deposited with the DDBJ/EMBL/GenBank Data Libraries under accession nos. AB201388, AB201389, and AB201390.

[1]*Corresponding author:* Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, Higashiyama 5-1, Myodaiji, Okazaki 444-8787, Japan. E-mail: hinada@nips.ac.jp

[2]*Present address:* NIDA, Genetics and Molecular Neurobiology Research Branch, Division of Neuroscience and Behavioral Research, 6001 Executive Blvd., Room 4264, MSC 9555, Bethesda, MD 20892-9555.

have been identified by analyses of mutants defective in thermotaxis. Of these, a calcium/calmodulin-dependent protein phosphatase, calcineurin, encoded by the *tax-6* gene, has been shown to negatively regulate the neuronal activity of AFD (Kuhara *et al.* 2002), whereas a calcium/calmodulin-dependent protein kinase I encoded by the *cmk-1* gene may modulate thermosensory responses (Satterlee *et al.* 2004). A cyclic nucleotide-gated ion channel (CNG channel) encoded by the *tax-2* and *tax-4* genes has been shown to be essential for thermotaxis (Coburn and Bargmann 1996; Komatsu *et al.* 1996). This CNG channel is expressed in AFD and shows a higher affinity to cGMP than to cAMP, suggesting that a guanylyl cyclase producing cGMP and a phosphodiesterase degrading cGMP are also likely to be involved in thermosensory signal transduction (Komatsu *et al.* 1996, 1999).

In this study, we use a reverse genetic approach to identify three *gcy* genes encoding *g*uanylyl *cy*clases and show that they play a role in thermosensation in *C. elegans*. These *gcy* genes, *gcy-8*, *gcy-18*, and *gcy-23*, form a subfamily and are expressed exclusively in the AFD neurons. Moreover, GFP-tagged GCY proteins are localized to the sensory endings of AFD, implicating these proteins in primary signal transduction. Single mutants of each of these *gcy* genes show normal thermotaxis, whereas double-mutant combinations for any pair of these *gcy* genes show a constitutitve cryophilic phenotype. *gcy-23 gcy-8 gcy-18* triple mutants show a cryophilic or athermotactic phenotype (no response to temperature) similar to the phenotype of AFD-killed wild-type animals. The *gcy* triple-mutant strain shows normal chemotaxis to odorants sensed by AWA and AWC olfactory neurons and to NaCl sensed mainly by ASE gustatory neurons. The thermotaxis mutant phenotype of the *gcy* triple-mutant strain was rescued by expression of any one of the three GCY proteins, suggesting that the GCY-8, -18, and -23 guanylyl cyclases function redundantly in *C. elegans* thermosensation.

MATERIALS AND METHODS

Strain: *C. elegans* was cultured using standard procedures (Brenner 1974). The following strains were used in this work: wild-type *C. elegans* variety Bristol (N2), *gcy-8 (oy44)* IV, *gcy-18 (nj38)* IV, *gcy-23 (nj37)* IV, and *tax-4 (p678)* III. Various multiple-mutant strains were constructed using standard genetic methods and verified by PCR.

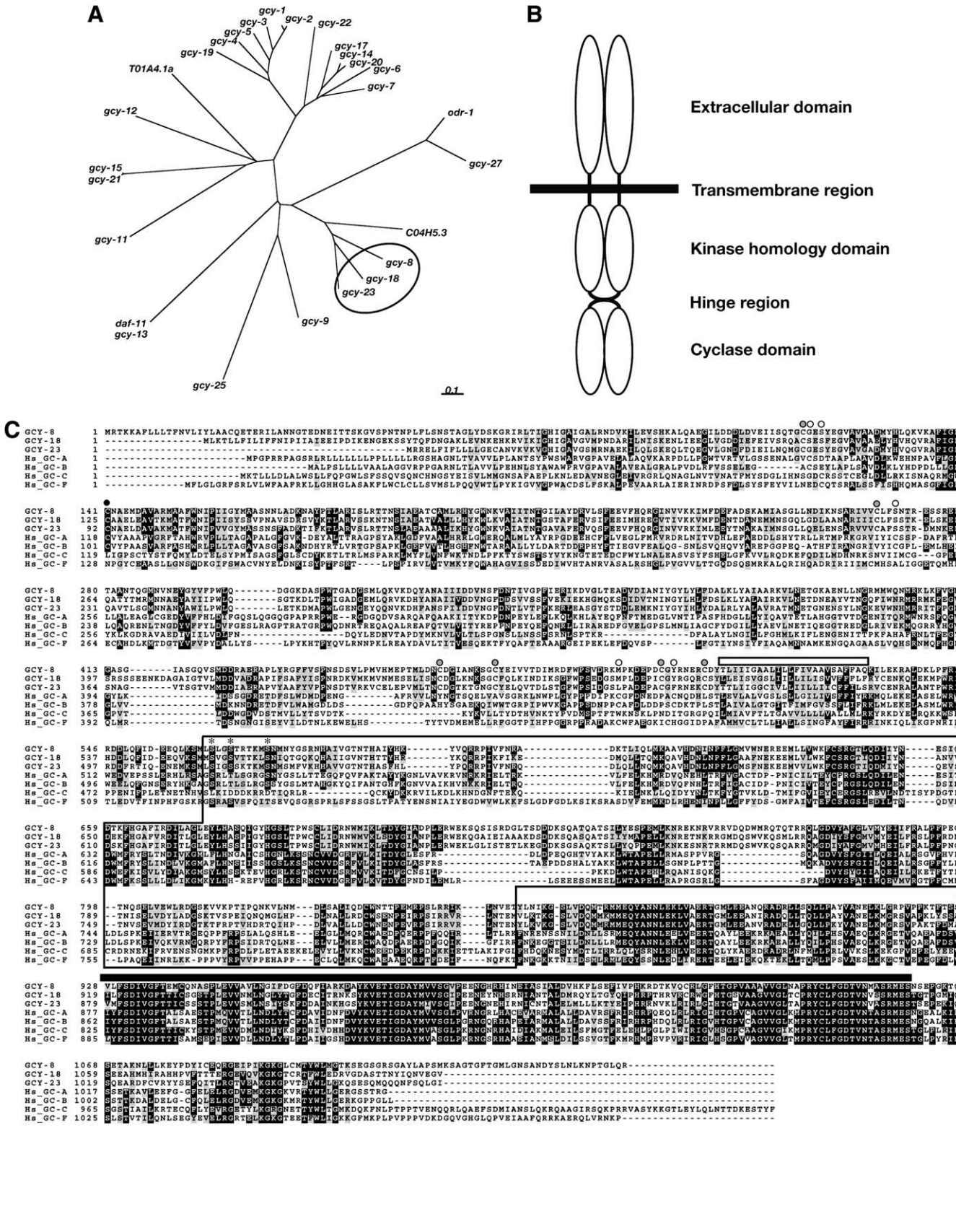
Isolation of deletion mutants: For isolation of *gcy-18 (nj38)* and *gcy-23 (nj37)*, a frozen deletion mutant library was constructed and ~2,400,000 genomes were screened by nested PCR. To induce deletion mutations, synchronized L4 larvae or young adults were treated with trimethylpsoralen (TMP) and UV light as described by Yandell *et al.* (1994) or Gengyo-Ando and Mitani (2000). Briefly, after incubation of 60 or 0.5 μg/ml TMP for 1 hr, the worms were irradiated with ~340 μW/cm² of UV light for 90 sec (~30,000 μJ) using a hand illuminator or ~1000 μW/cm² of UV light for ~5–10 sec (~5000–10,000 μJ) using a fluorescent microscope. Mutagenized worms were incubated on seeded NGM plates in the dark at 20° for 24 hr. F_1 eggs were collected and allowed to hatch for 24 hr on unseeded NGM plates. About 500 hatched larvae (~1000 genomes) were transferred onto a seeded 6-cm NGM plate and allowed to grow until bacterial lawns were consumed. One set of the library was composed of 96 plates (12 tubes in a row and 8 tubes in a column), and the standard size of the library consisted of 12 sets (~1,2000,000 genomes). After one generation, F_1 adults and F_2 larvae mixtures were collected with M9 buffer. Half of each plate was frozen for the deletion library and stored at −80°. The remaining half was treated for 8 hr at 60° with SDS worm lysis buffer, containing 50 mM Tris–HCl pH 8.0, 100 mM NaCl, 10 mM EDTA, 1% SDS, and 200 μg/ml of proteinase K. A fraction of worm lysates from each of a dozen tubes in a row were pooled, purified, and used in a first PCR screen for deletion mutants. The unpurified original tubes were stored at −20°.

Oligonucleotide primers used to detect deletions were designed using AcePrimer v1.0 (http://elegans.bcgsc.bc.ca/gko/aceprimer.shtml). PCR product sizes were ~3 kb. External-round PCRs were 20 μl in volume and contained 1 μl pooled DNA, 0.2 μM of each external-round primer, 50 μM of each dNTP, 2 mM $MgCl_2$, 1× standard PCR buffer, and 0.025 units/μl Taq polymerase (GIBCO, Gaithersburg, MD). Internal-round PCRs were 20 μl in volume and contained 1 μl external-round PCR reactant, 0.2 μM of each external-round primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 1× standard PCR buffer, and 0.025 units/μl Taq polymerase. External-round cycle conditions were 94° for 1 min, 35 cycles of 94° for 40 sec; 60° for 30 sec; 72° for 1 min; and 72° for 7 min. Internal-round cycle conditions were 94° for 1 min, 35 cycles of 94° for 40 sec; 60° for 30 sec; 72° for 2 min; and 72° for 7 min. For detection of the *gcy-18* deletion, primers ina122 ZK896.8-Ael and ina123 ZK896.8-Aer were used in external-round PCR and primers ina124 ZK896.8-Ail and ina125 ZK896.8-Air were used in internal-round PCR. For detection of the *gcy-23* deletion, primers ina118 T26C12.4-Ael and ina119 T26C12.4-Aer were

Figure 1.—*gcy-8*, *gcy-18*, and *gcy-23* form a subfamily of *gcy* genes and encode receptor-type guanylyl cyclases in *C. elegans*. (A) Phylogenetic tree of *C. elegans gcy* genes encoding receptor-type guanylyl cyclases. Sequences of *C. elegans* guanylyl cyclases (gene name or GenBank cosmid designation in italics) were compared using the ClustalW alignment program and the tree was constructed using TreeView. A subfamily consisting of *gcy-8*, *gcy-18*, and *gcy-23* is circled. (B) Protein domains of receptor-type guanylyl cyclases. Receptor-type guanylyl cyclases appear to form a homodimer or a homotetramer in mammals. Receptor-type guanylyl cyclases consist of three domains: an extracellular domain, a transmembrane region, and an intracellular region containing a kinase homology domain, a hinge region, and a cyclase domain. (C) Multiple alignment of amino acid sequences of three *C. elegans* guanylyl cyclases (GCY-8, GCY-18, and GCY-23) and four human guanylyl cyclases (Hs_GC-A, Hs_GC-B, Hs_GC-C, and Hs_GC-F). Conserved cysteine residues in both *C. elegans* and humans (solid circle), in *C. elegans* (shaded circle), and in humans (open circle) are indicated. The kinase homology domain is boxed. Conserved serine and threonine residues are indicated by an asterisk (*). The transmembrane region and cyclase domains are indicated by an open bar and a solid bar, respectively. DDBJ/EMBL/GenBank and Swiss-Prot accession numbers for sequences used in the alignment are AB201388, (GCY-8), AB201389 (GCY-18), AB201390 (GCY-23), P16066 (Hs_GC-A), P20594 (Hs_GC-B), P25092 (Hs_GC-C), and P51841 (Hs_GC-F).

used in external-round PCR and primers ina120 T26C12.4-Ail and ina121 T26C12.4-Air were used in internal-round PCR. Loading buffer was added to each reaction, and 10 μl of mixture was loaded into each lane of 1% agarose gels containing 10 μg/ml ethidium bromide. When positive signals were detected, worm lysates of the original 12 tubes in a row were purified and screened by nested PCR to identify a single tube containing the deletion mutant candidate. The identified tube was thawed and distributed into 96 NGM plates. DNA was extracted from each plate and screened by nested PCR. The screening cycle was repeated until a deletion mutant was isolated.

For isolation of *gcy-8 (oy44)*, ~1,000,000 genomes mutagenized with EMS were screened by nested PCR. The PCRs were



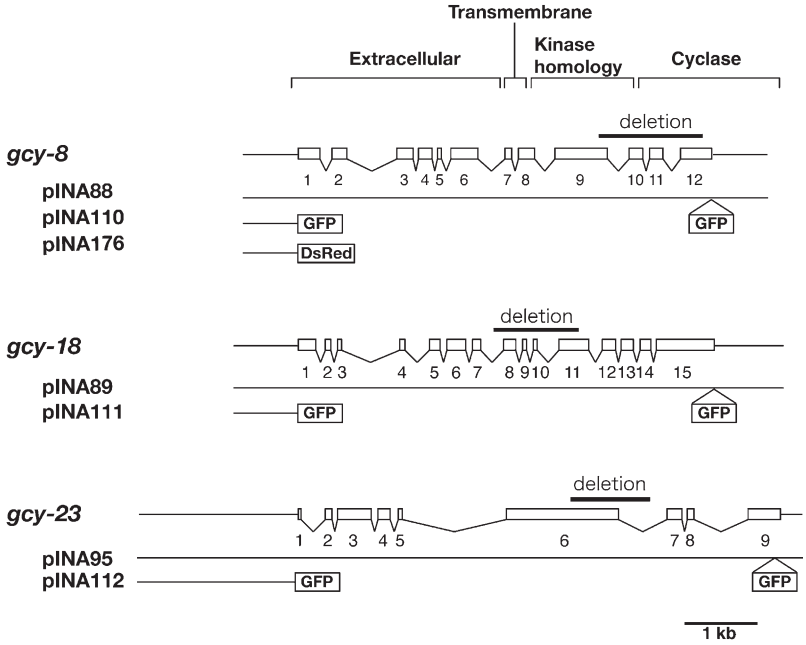


FIGURE 2.—Gene structures of *gcy-8*, *gcy-18*, and *gcy-23* genes and schematics of promoter fusions or full-length genomic fusions to reporter genes. Exons are boxed and numbered. Solid bar indicates a deleted region.

50 μl volume and contained 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% gelatin, 200 μM of each dNTPs, 1.2 μM of each forward and reverse primers, and 1 μl Taq polymerase. Cycle conditions were 30 cycles of 94° for 30 sec, 64° for 30 sec, and 72° for 1 min. Primers gcy8KF1 and gcy8KR1 were used in external-round PCR and primers gcy8KF2 and gcy8KR2 were used in internal-round PCR.

The isolated deletion mutants were outcrossed 10 times prior to use in behavioral assays.

Primers: For screening deletion mutants from libraries, the following primers were used:

For gcy-8,
gcy8KF1: 5′-ATGGGTCTTCCCACGAGAAAAC;
gcy8KR1: 5′-GAGAACATTGGATTCACGGACC;
gcy8KF2: 5′-AATCCCAAAGAAGCTGGCCTAC;
gcy8KR2: 5′-GACAGCTAGTACATGGGTGAGC;
For gcy-18,
ina122 ZK896.8-Ael: 5′-ATCGCTACAAACACCGGAAG;
ina123 ZK896.8-Aer: 5′-CCGATGAATAAAGCTTCGGA;
ina124 ZK896.8-Ail: 5′-TCGAAGAAATCATGCACAGG;
ina125 ZK896.8-Air: 5′-CGTAGGCTGGTAGGAGTTGG;
For gcy-23,
ina118 T26C12.4-Ael: 5′-AGACTCCATTCTCGAGGCAA;
ina119 T26C12.4-Aer: 5′-CGGTTTATGCGGCATTCTAT;
ina120 T26C12.4-Ail: 5′-ATAGGCAATAACGAGGTGCG;
ina121 T26C12.4-Air: 5′-AACTGGATTCTGGCCGTCTA.

Behavioral assays: Thermotaxis assays using a radial temperature gradient were performed essentially as described previously (MORI and OHSHIMA 1995). Thermotaxis of individual animals on a radial temperature gradient was evaluated using four phenotypic categories: animals that moved to the cold region (the center of the plate) were classified as "17," animals that moved to the 20° region were classified as "20," animals that moved to the warm region (the periphery) were classified as "25," and animals that moved to both cold and warm regions were classified as "17/25." Chemotaxis to volatile odorants was assayed according to previous reports (BARGMANN *et al.* 1993). Chemotaxis to NaCl was assayed according to KOMATSU *et al.* (1996). Animals that moved to the concentration peak of NaCl on the chemotaxis assay plate were evaluated as "+" and animals that were insensitive to the concentration gradient were evaluated as "−."

NGM plates containing 8-bromo-cGMP were prepared as described by BIRNBY *et al.* (2000). Briefly, plates (3.5 cm) were filled with 2 ml of NGM agar with 8-bromo-cGMP (Sigma, St. Louis) added to a given concentration. A stock of *Escherichia coli* OP50 in L broth was spread on the NGM plates, in which individual adult animals were placed, allowed to lay eggs at 20° for up to 12 hr, and then removed. These plates were placed at 20° for 3 days and the grown animals were used for TTX assay.

TTX plot analysis: Two values for the TTX plot were calculated as: (20 − 17/25) = (fraction of 20) − (fraction of 17/25) and (25 − 17) = (fraction of 25) − (fraction of 17), where fraction of 20 = number of animals classified as "20"/total number of animals in one trial, and so on. Statistical significance was tested by the pairwise test for multiple comparisons using Holm's method on the TTX plot values.

Molecular biology: Standard methods of general and *C. elegans* molecular biology were used (HOPE 1999; SAMBROOK and RUSSELL 2001). DNA constructs were made using pBluescript SK+ (Stratagene, La Jolla, CA). GFP and DsRed genes for genomic and promoter fusion constructs were amplified by PCR using pEGFP-N1 and pDsRed2 (CLONTECH, Mountain View, CA) as templates, respectively. High-fidelity PCR with Pfu DNA polymerase (Stratagene) was used for constructions and DNA sequences were confirmed.

The constructs of full-length *genomic gcy::GFP* gene fusion, pINA88 for *gcy-8*, pINA89 for *gcy-18*, and pINA95 for *gcy-23*, contain promoter regions, ORFs, and 3′-flanking regions of each *gcy* gene, respectively (see Figure 2). The stop codon of each *gcy* gene was replaced by GFP coding sequences.

The *gcy promoter::GFP* gene fusion constructs, pINA110, pINA111, and pINA112, were generated by PCR using pINA88, pINA89, and pINA95 as templates, respectively. The *gcy-8* promoter DsRed gene fusion construct, pINA176, was generated by replacement of GFP coding sequences in pINA110 by PCR-amplified DsRed sequences.

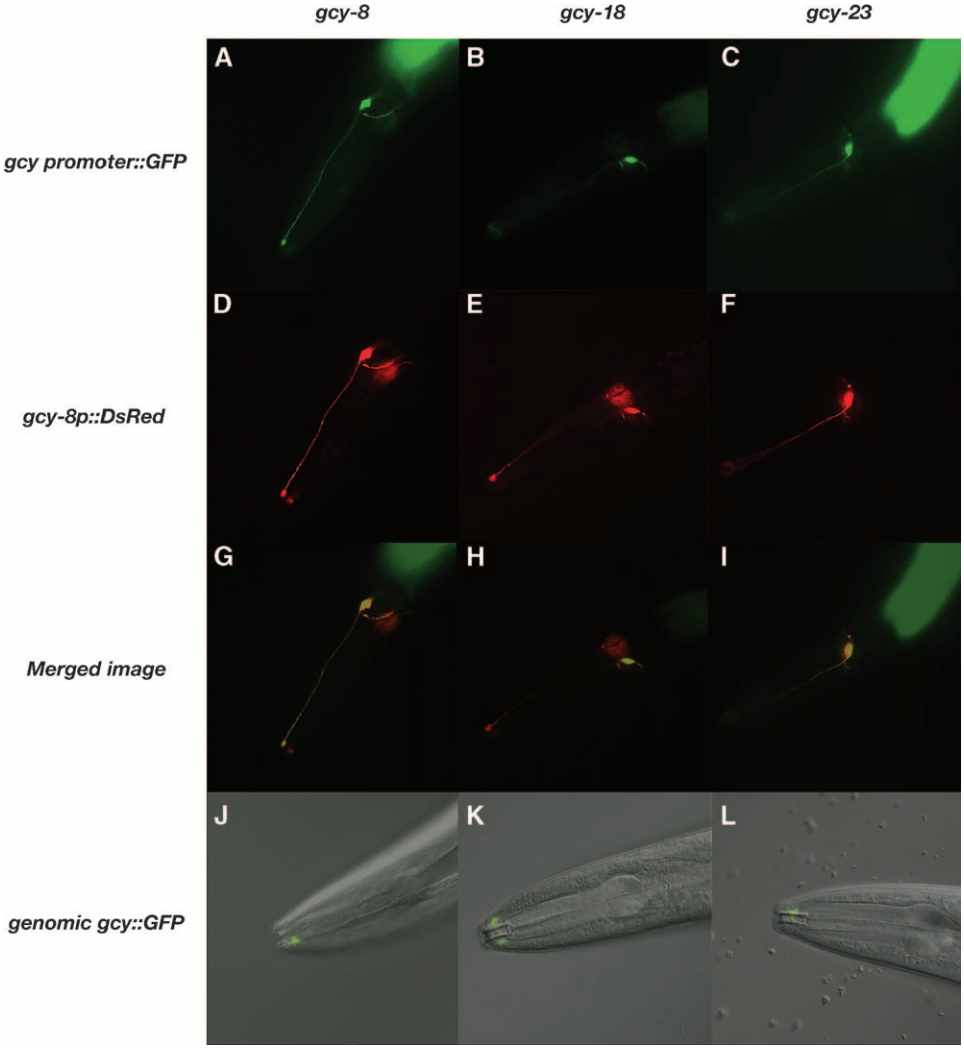


FIGURE 3.—Expression analysis of *gcy-8*, *gcy-18*, and *gcy-23* genes. (A–C) GFP fluorescence images of *gcy-8p::GFP* (A), *gcy-18p::GFP* (B), and *gcy-23p::GFP* (C) transgenes. (D–F) DsRed fluorescence images of *gcy-8p::DsRed* transgene as an AFD specific marker. (G–I) Merged images of top and middle. (J–L) Intracellular localization of products of *genomic gcy-8::GFP* fusion (J), *genomic gcy-18::GFP* fusion (K), and *genomic gcy-23::GFP* fusion (L).

Structures of *gcy-8*, *gcy-18*, and *gcy-23* genes were determined by sequencing of cDNAs of each *gcy* genes. The yk354a3 clone contained full-length *gcy-8* cDNA. Clones yk41f4 for *gcy-18* and yk1064a02 for *gcy-23* contained cDNAs lacking their 5′-ends. The 5′-end fragments of *gcy-18* and *gcy-23* cDNA were generated by BD SMART RACE cDNA amplification kit (CLONTECH) using *C. elegans* mRNA purified with a PolyATract mRNA isolation system (Promega, Madison, WI).

Transgenic animals: Germ-line transformation was performed by co-injecting test DNA and pKDK66 (*ges-1::GFP*) as an injection marker into the gonad of *gcy-23 gcy-8 gcy-18* or N2 animals (MELLO *et al.* 1991). Transgenic animals carrying *genomic gcy::GFP* genes were generated by co-injection of each construct at a concentration of 10 ng/μl, *ges-1::GFP* at a concentration of 50 ng/μl, and pBluescript SK+ at a concentration of 40 ng/μl. Transgenic animals carrying *gcy promoter::GFP* or *gcy-8p::DsRed* were generated by co-injection of each construct at a concentration of 50 ng/μl and *ges-1::GFP* at a concentration of 50 ng/μl. The *gcy-8p::DsRed* transgene was transferred by crossing to transgenic animals carrying *gcy promoter::GFP*. Transgenic animals were recognized with intestinal GFP fluorescence due to *ges-1::GFP* transformation marker expression. Multiple independent transgenic lines were established and examined for each transformation experiment. Cells expressing GFP and intracellular localization of chimera GCY/GFP proteins were identified and analyzed using a light microscope AxioPlan2 (Carl Zeiss, Thornwood, NY).

RESULTS

The *gcy-8*, *gcy-18*, and *gcy-23* guanylyl cyclase genes constitute a subfamily of guanylyl cyclase genes in *C. elegans*: In *C. elegans*, guanylyl cyclase genes (*gcy* genes) form a large gene family (YU *et al.* 1997). At least 26 *gcy* genes encoding receptor-type guanylyl cyclases and 7 *gcy* genes encoding soluble guanylyl cyclases are predicted from genome sequence analysis (CHEN *et al.* 2005). Phylogenetic analysis showed that the *gcy* genes encoding receptor-type guanylyl cyclases are divided into several subfamilies on the basis of amino acid sequences (Figure 1A). We focused on a subfamily that consists of the *gcy-8*, *gcy-18*, and *gcy-23* genes, because a previous report showed that the *gcy-8* gene was expressed specifically in AFD (YU *et al.* 1997). The three guanylyl cyclases encoded by these *gcy* genes showed high overall sequence homology (>55% identity and 73% similarity)

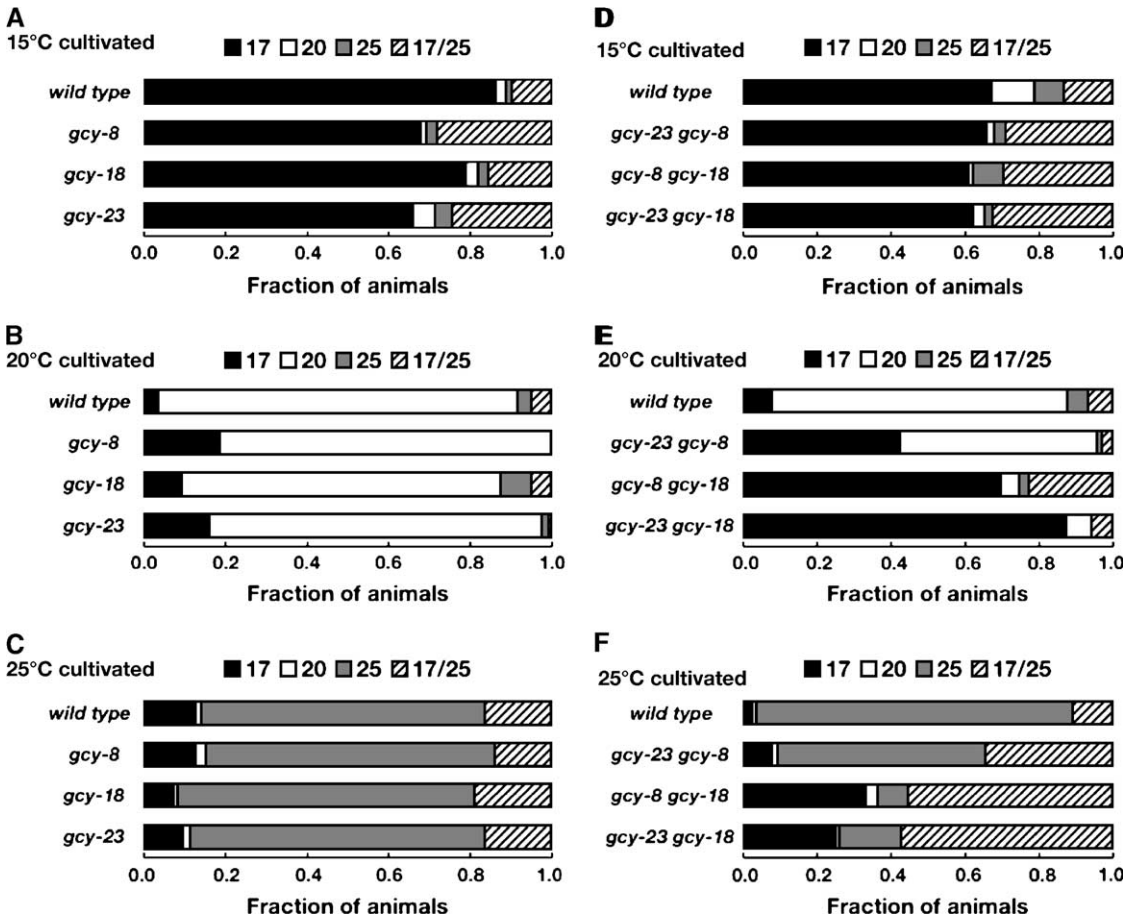


FIGURE 4.—Thermotaxis phenotypes of *gcy* single and double mutants cultivated at three different temperatures. (A–C) Thermotaxis phenotypes of wild-type animals and *gcy* single mutants cultivated at 15° (A), 20° (B), or 25° (C). (D–F) Thermotaxis phenotypes of wild-type animals and *gcy* double mutants cultivated at 15° (D), 20° (E), or 25° (F). For each genotype, 59–199 animals were assayed individually. Phenotypic categories are described in MATERIALS AND METHODS.

at the amino acid sequence level. Receptor-type guanylyl cyclases contain three domains: an extracellular domain, a transmembrane region, and an intracellular region containing a kinase homology domain, hinge region, and cyclase domain (Figure 1B). GCY-8, -18, and -23 showed little homology to human guanylyl cyclases in the extracellular domain (Figure 1C). At the N terminus and just before the transmembrane region in the extracellular domain, however, these guanylyl cyclases have seven conserved cysteine residues, which have been reported to form intrachain disulfide bridges in mammalian guanylyl cyclases. In contrast, the intracellular domain, especially the cyclase domain, showed high homology among all guanylyl cyclases (Figure 1C). Three serine and threonine residues that have been reported to be reversibly phosphorylated in mammalian guanylyl cyclases were also conserved in the kinase homology domains of the GCY-8, GCY-18, and GCY-23 guanylyl cyclases.

***gcy-8*, *-18*, and *-23* genes are expressed specifically in the AFD thermosensory neurons:** To determine whether these *gcy* genes are expressed in AFD, the expression of GFP reporter genes under the control of *gcy-8*, *gcy-18*, and *gcy-23* promoters was examined in wild-type animals (Figure 2). Expression in the AFD neurons was confirmed by colocalization with DsRed expression driven by the *gcy-8* promoter (Figure 3, A, D, and G), as reported previously (YU *et al.* 1997). Expression of both *gcy-18* and *gcy-23* genes was identical to that of the *gcy-8* gene (Figure 3, B and H, for *gcy-18*; Figure 3, C and I, for *gcy-23*), and no expression was observed in other cells. This result indicates that all three *gcy* genes are expressed specifically in AFD.

To determine the intracellular localization of the *gcy* gene products, full-length genomic *gcy* gene sequences fused to the GFP gene were injected into wild-type animals (Figure 2). GFP-tagged GCY-8, 18, and 23 were localized exclusively to the sensory endings of AFD (Figure 3, J–L). The TAX-4 CNG channel has also been shown to be localized to the AFD sensory endings, suggesting that these GCY proteins may function with the TAX-4 CNG channel to mediate thermosensation.

Strains doubly mutant for *gcy* genes show defects in thermotaxis: To examine whether these *gcy* genes

TABLE 1

Thermotaxis in *gcy* single mutants

Cultivation temperature	Strain	Phenotypic category 17	20	25	17/25	Total no. of animals
15°	Wild type	69	2	1	8	80
	gcy-8	53	1	2	22	78
	gcy-18	131	5	4	19	159
	gcy-23	106	8	7	39	160
20°	Wild type	2	52	2	3	59
	gcy-8	11	48	0	0	59*
	gcy-18	11	93	9	6	119
	gcy-23	19	97	2	1	119
25°	Wild type	10	1	55	13	79
	gcy-8	10	2	56	11	79
	gcy-18	12	1	116	30	159
	gcy-23	15	3	114	26	158

One animal was assayed per thermotaxis plate. The evaluation is described in MATERIALS AND METHODS. Statistical analysis by a chi-square test using a 2×4 contingency table was performed to compare the wild-type strain with each *gcy* strain. Each *gcy* strain showed no significant difference from the wild-type strain for thermotaxis, except for *gcy-8* cultivated at 20° ($*P < 0.01$).

TABLE 2

Thermotaxis in *gcy* double mutants

Cultivation temperature	Strain	Phenotypic category 17	20	25	17/25	Total no. of animals
15°	Wild type	101	17	12	20	150
	gcy-23 gcy-8	131	4	6	58	199**
	gcy-8 gcy-18	119	2	16	58	195**
	gcy-23 gcy-18	124	6	4	65	199**
20°	Wild type	7	71	5	6	89
	gcy-23 gcy-8	51	64	1	4	120**
	gcy-8 gcy-18	83	6	3	27	119**
	gcy-23 gcy-18	105	8	0	7	120**
25°	Wild type	3	1	103	13	120
	gcy-23 gcy-8	12	2	85	52	151**
	gcy-8 gcy-18	52	5	13	87	157**
	gcy-23 gcy-18	40	1	27	91	159**

One animal was assayed per thermotaxis plate. The evaluation is described in MATERIALS AND METHODS. Statistical analysis by a chi-square test using a 2×4 contingency table was performed to compare the wild-type strain with each gcy strain. Each *gcy* strain showed a significant difference from the wild-type strain for thermotaxis ($**P < 0.005$).

function in thermotaxis, deletion alleles were isolated (Figure 2). The isolated *gcy* deletion alleles are likely to be functional null alleles (see Figure 2). Thermotaxis behaviors of the *gcy* mutants were analyzed after growth at different cultivation temperatures. After cultivation at 15°, 20°, or 25°, the majority of wild-type animals migrated to their cultivation temperature on a radial thermal gradient. Animals singly mutant for one of these *gcy* genes also migrated to their cultivation temperature and showed nearly normal thermotaxis behavior (Figure 4, A–C, and Table 1). However, animals doubly mutant for any pair of these *gcy* genes showed different degrees of defects in thermotaxis behavior (Figure 4, D–F, and Table 2). When animals were cultivated at 15°, wild-type animals and *gcy* double mutants migrated to 15°, but the fraction of animals that showed athermotactic phenotype (classified as 17/25) was increased in any double *gcy* mutant combination (Figure 4D). When animals were cultivated at 20°, ~50% of *gcy-23 gcy-8* double mutants migrated to 20° but the remaining 50% migrated to colder temperatures. However, *gcy-8 gcy-18* and *gcy-23 gcy-18* double mutants cultivated at 20° migrated to colder temperatures and showed a severe cryophilic phenotype (Figure 4E). When animals were cultivated at 25°, *gcy-8 gcy-18* and *gcy-23 gcy-18* double mutants also showed a cryophilic phenotype, but the fraction of animals that showed athermotactic phenotype was increased in any double *gcy* mutant combination (Figure 4F). About half of *gcy-23 gcy-8* double-mutant animals that contain a wild-type *gcy-18* gene could migrate to the cultivation temperature when they were cultivated at 20° or 25°, suggesting that *gcy-18* functions as the primary guanylyl cyclase in these temperature ranges.

Animals triply mutant for the *gcy* genes show severe defects in thermotaxis but respond normally to odorants and NaCl: *gcy-23 gcy-8 gcy-18* triple mutants showed the severest cryophilic or athermotactic phenotype at any cultivation temperature (Figure 5A and Table 3). This phenotype of *gcy* triple-mutant animals is very similar to the phenotype of AFD-killed wild-type animals (Table 3), indicating that activity of AFD is almost abolished in the *gcy* triple mutants. However, significant differences were observed between the phenotype of *gcy* triple mutants and AFD(−) wild-type animals. The cryophilic behaviors of *gcy* triple mutants were completely suppressed by loss-of-function mutations in the *tax-4* gene. The thermotaxis behaviors of *tax-4 ; gcy-23 gcy-8 gcy-18* quadruple mutants showed no significant difference from those of *tax-4* mutants (Figure 5B and Table 4). This result is consistent with the hypothesis that these guanylyl cyclases function upstream of the TAX-4 CNG channel.

gcy triple mutants showed nearly normal chemotaxis toward odorants sensed by the AWA and AWC olfactory neurons (Figure 5C), as well as toward NaCl, which is sensed primarily by the ASE gustatory neuron (Figure 5D), suggesting that these *gcy* genes function specifically in thermotaxis.

TTX plot analysis of *gcy* mutants: As shown above, various degrees of defects in thermotaxis behaviors were

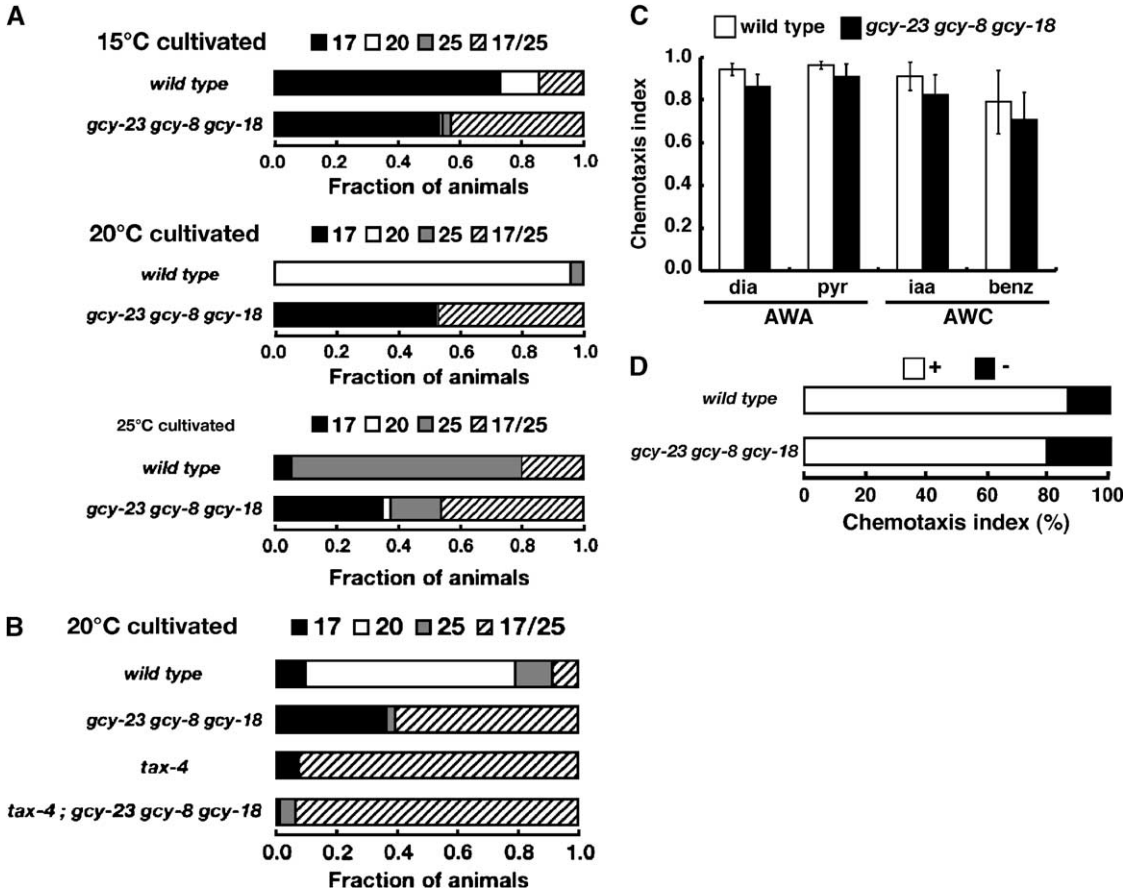


FIGURE 5.—Behavioral phenotypes of *gcy* triple mutants and *tax-4; gcy-23 gcy-8 gcy-18* quadruple mutants. (A) Thermotaxis phenotypes of wild-type animals and *gcy* triple mutants cultivated at different temperatures. Wild-type animals ($n = 90$) and *gcy* triple mutants ($n = 357$–359) were assayed individually. Phenotypic categories are described in MATERIALS AND METHODS. (B) Thermotaxis phenotypes of wild-type animals, *gcy* triple mutants, *tax-4* mutants, and *tax-4; gcy-23 gcy-8 gcy-18* quadruple mutants cultivated at 20°. For each genotype, 40–177 animals were assayed individually. Phenotypic categories are described in MATERIALS AND METHODS. (C) Chemotaxis phenotypes of wild-type animals and *gcy* triple mutants toward four volatile odorants: diacetyl (dia) and pyrazine (pyr) sensed by the AWA olfactory neurons and isoamyl alcohol (iaa) and benzaldehyde (benz) sensed by the AWC olfactory neurons. Each data point represents the average of 8–24 assays. The error bar indicates standard deviation. (D) Chemotaxis phenotypes of wild-type animals and *gcy* triple mutants toward NaCl. Wild-type animals ($n = 50$) and *gcy* triple mutants ($n = 100$) were assayed individually. *gcy* triple mutants showed no difference from wild type in a chi-square test.

observed in *gcy* double and triple mutants but not in *gcy* single mutants (Figures 4 and 5 and Tables 1–3). To analyze the thermotaxis behavioral defects in detail, we introduced a novel analysis—the TTX plot—for comprehensive statistical analysis (see MATERIALS AND METHODS).

When animals were cultivated at 15°, two *gcy* double-mutant strains (*gcy-8 gcy-18* and *gcy-23 gcy-18*) and the *gcy-23 gcy-8 gcy-18* triple-mutant strain showed significant behavioral differences compared with wild-type animals in the TTX plot value ($20 - 17/25$), whereas none of the *gcy* single mutants and *gcy-23 gcy-8* double-mutant combinations showed a significant difference (Figure 6A Table 5 and Table 6). No significant differences were observed among *gcy* single-, double-, and triple-mutant strains (Table 6).

When animals were cultivated at 20°, *gcy* single mutants showed no significant differences compared with wild-type animals in the TTX plot (Figure 6B, Table 5, and Table 6), whereas *gcy* double and triple mutants showed a significant difference. *gcy-23 gcy-8 gcy-18* triple mutants also showed a significant difference when compared with all strains with the exception of the *gcy-8 gcy-18* double mutants.

Wild-type animals and *gcy* mutants were clearly divided into two groups on the TTX plot when they were cultivated at 25° (Figure 6C). One group contained wild-type animals, *gcy* single mutants, and *gcy-23 gcy-8* double mutants, whereas another group contained the *gcy-8 gcy-18* and *gcy-23 gcy* -18 double mutants and *gcy-23 gcy-8 gcy-18* triple mutants. No significant differences were observed within each group (Table 5 and Table 6). These results of the TTX plot analysis also suggest the central role of *gcy-18* in thermosensation in animals cultivated at 20° or 25°.

Functional redundancy of the three *gcy* genes in thermotaxis: The defects in thermotaxis of *gcy* triple

TABLE 3

Thermotaxis in *gcy* triple mutants

Cultivation temperature	Strain	Phenotypic category 17	20	25	17/25	Total no. of animals
15°	Wild type	66	11	0	13	90
	gcy-23 gcy-8 gcy-18	190	4	10	153	357**
20°	Wild type	0	86	4	0	90
	gcy-23 gcy-8 gcy-18	184	2	3	170	359**
	Wild type (AFD−)	26	0	4	48	78[a]**
25°	Wild type	5	0	67	18	90
	gcy-23 gcy-8 gcy-18	124	9	59	165	357**

One animal was assayed per thermotaxis plate. The evaluation is described in MATERIALS AND METHODS. Statistical analysis by a chi-square test using a 2 × 4 contingency table was performed to compare the wild-type strain with each *gcy* strain. Each *gcy* strain showed a significant difference from the wild-type strain for thermotaxis (**$P < 0.005$).

[a] The value is the total number of assays in laser ablation result of AFD neurons derived from MORI and OHSHIMA (1995). *gcy* triple mutant strains show significant difference from the AFD-killed wild-type animals for thermotaxis (**$P < 0.005$).

mutants were rescued by introduction of *gcy* genomic fragments (Figure 7). Transgenic *gcy* triple mutants carrying the *gcy-8* genomic fragment showed a phenotype similar to that of *gcy-18 gcy-23* double mutants. Interestingly, a relatively large fraction of transgenic *gcy* triple mutants carrying a *gcy-23* genomic fragment showed normal thermotaxis when compared with *gcy-8 gcy-18* double mutants. Overexpression of *gcy* genes seemed to have a little effect on thermotaxis in wild-type animals. Wild-type animals overexpressing the *gcy-8* gene showed slight defects in thermotaxis, while wild-type animals overexpressing the *gcy-18* or *gcy-23* gene showed almost normal thermotaxis.

TABLE 4

Thermotaxis in quadruple mutants

Strain	Fraction 17	20	25	17/25	Total no. of animals
Wild type	17	123	22	15	177
gcy-23 gcy-8 gcy-18	36	0	3	60	99
tax-4	3	0	0	37	40
tax-4; gcy-23 gcy-8 gcy-18	2	0	7	131	140**

The animals were cultivated at 20°. One animal was assayed per thermotaxis plate. The evaluation is described in MATERIALS AND METHODS. Statistical analysis by a chi-square test using a 2 × 4 contingency table was performed to compare *gcy-23 gcy-8 gcy-18* and *tax-4 ; gcy-23 gcy-8 gcy-18* and *tax-4* and *tax-4; gcy-23 gcy-8 gcy-18* (**$P < 0.005$).

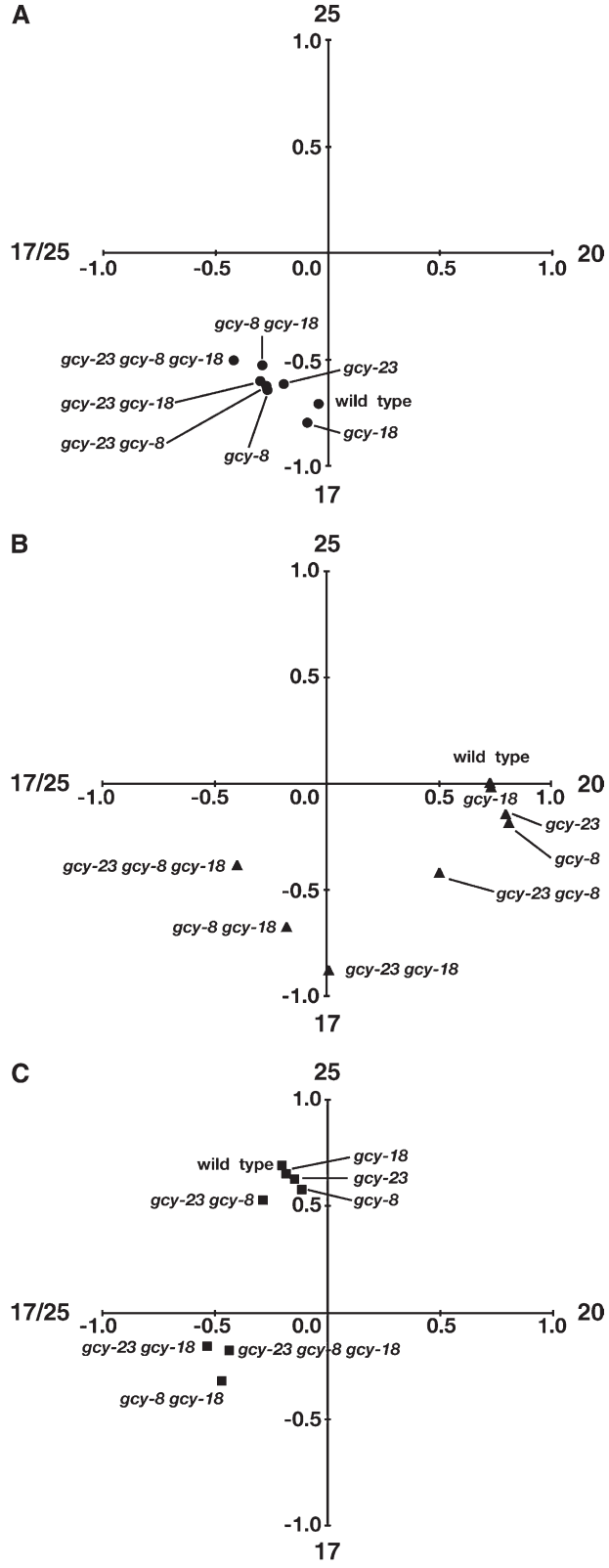


FIGURE 6.—TTX plot of wild-type animals and *gcy* mutants cultivated at three different temperatures. Wild-type animals and *gcy* mutants cultivated at 15° (A), 20° (B), or 25° (C) were assayed and the TTX plot values were calculated as described in MATERIALS AND METHODS . Horizontal axis indicates a value of (20 − 17/25). Vertical axis indicates a value of (25 − 17).

TABLE 5

TTX plot in *gcy* mutants

Cultivation temperature	Strain	TTX plot value (20 − 17/25)	TTX plot value (25 − 17)	No. of experiment
15°	Wild type	−0.04 ± 0.15	−0.71 ± 0.17	12
	gcy-8	−0.27 ± 0.11	−0.64 ± 0.17	4
	gcy-18	−0.09 ± 0.02	−0.80 ± 0.13	4
	gcy-23	−0.19 ± 0.08	−0.62 ± 0.04	4
	gcy-23 gcy-8	−0.27 ± 0.13	−0.63 ± 0.04	5
	gcy-8 gcy-18	−0.29 ± 0.11	−0.53 ± 0.06	5
	gcy-23 gcy-18	−0.30 ± 0.05	−0.60 ± 0.06	5
	gcy-23 gcy-8 gcy-18	−0.42 ± 0.09	−0.50 ± 0.11	3
20°	Wild type	0.73 ± 0.31	0.01 ± 0.06	9
	gcy-8	0.81 ± 0.06	−0.19 ± 0.06	3
	gcy-18	0.73 ± 0.15	−0.02 ± 0.04	3
	gcy-23	0.80 ± 0.04	−0.14 ± 0.07	3
	gcy-23 gcy-8	0.50 ± 0.28	−0.42 ± 0.29	3
	gcy-8 gcy-18	−0.18 ± 0.15	−0.67 ± 0.18	3
	gcy-23 gcy-18	0.01 ± 0.08	−0.88 ± 0.05	3
	gcy-23 gcy-8 gcy-18	−0.40 ± 0.16	−0.38 ± 0.22	3
25°	Wild type	−0.20 ± 0.15	0.69 ± 0.13	11
	gcy-8	−0.11 ± 0.10	0.58 ± 0.18	4
	gcy-18	−0.18 ± 0.08	0.65 ± 0.24	4
	gcy-23	−0.15 ± 0.07	0.62 ± 0.12	4
	gcy-23 gcy-8	−0.29 ± 0.12	0.52 ± 0.17	4
	gcy-8 gcy-18	−0.47 ± 0.14	−0.32 ± 0.02	4
	gcy-23 gcy-18	−0.53 ± 0.08	−0.16 ± 0.13	4
	gcy-23 gcy-8 gcy-18	−0.44 ± 0.08	−0.18 ± 0.16	3

The TTX plot values were calculated as described in MATERIALS AND METHODS. TTX plot values were shown as mean ± SD.

Although overexpression of *gcy* genes seems to have little effect on thermotaxis in wild-type animals, we assayed the effect of the membrane permeant cGMP analog 8-bromo-cGMP on thermotaxis to examine whether an appropriate concentration of intracellular cGMP is required for normal thermotaxis behavior (Figure 8). While ~60% of wild-type animals migrated to their cultivation temperature when they were cultivated without 8-bromo-cGMP, wild-type animals cultivated with 8-bromo-cGMP showed slight defects: ~35–60% of animals showed thermophilic (25) or athermotactic (17/25) phenotypes, possibly resulting from the high concentration of intracellular cGMP in AFD. This result and the phenotype of *gcy* triple mutants are consistent with the possibility that a low concentration of cGMP in AFD results in a cryophilic phenotype, whereas a high concentration of cGMP results in a thermophilic phenotype. The phenotype of the *gcy* triple mutant was not affected or rescued by the application of 8-bromo-cGMP at any concentration, suggesting that the appropriate regulation of intracellular cGMP concentration is essential for normal thermotaxis.

DISCUSSION

In *C. elegans*, cGMP plays a critical role in sensory signal transduction. For example, *tax-2* and *tax-4* genes encoding a cGMP-gated channel are required for various behaviors such as thermosensation, olfaction, and gustation (COBURN and BARGMANN 1996; KOMATSU *et al.* 1996). *odr-1* and *daf-11* genes encoding receptor-type guanylyl cyclases are also essential for olfaction in AWC (VOWELS and THOMAS 1994; BIRNBY *et al.* 2000; L'ETOILE and BARGMANN 2000). Recent studies have shown that a soluble guanylyl cyclase encoded by *gcy-35* functions as an oxygen sensor in some sensory neurons (GRAY *et al.* 2004; CHEUNG *et al.* 2005). In this study, we show that three *gcy* genes encoding receptor-type guanylyl cyclases are essential for thermotaxis in *C. elegans*.

Three guanylyl cyclases essential for thermotaxis in *C. elegans*: Receptor-type guanylyl cyclases have been studied extensively as receptors of peptide hormones (WEDEL and GARBERS 2001). In mammals, ligand-induced activity of guanylyl cyclases is regulated by phosphorylation and dephosphorylation of serine and threonine

TABLE 6

Statistical analysis among wild-type animals and *gcy* mutants in the TTX plot values (20 − 17/15) and (25 − 17)

		Statistical significance in the TTX values [(20 − 17/25) and (25 − 17)]						
Cultivation temperature	Strain	Wild type	*gcy-8*	*gcy-18*	*gcy-23*	*gcy-23 gcy-8*	*gcy-8 gcy-18*	*gcy-23 gcy-18*
15°	*gcy-8*	—						
	gcy-18	—	—					
	gcy-23	—	—	—				
	gcy-23 gcy-8	—	—	—	—			
	gcy-8 gcy-18	*, —	—	—	—	—		
	gcy-23 gcy-18	**, —	—	—	—	—	—	
	gcy-23 gcy-8 gcy-18	**, —	—	—	—	—	—	—
20°	*gcy-8*	—						
	gcy-18	—	—					
	gcy-23	—	—	—				
	gcy-23 gcy-8	—, **	—	—	—			
	gcy-8 gcy-18	**, **	**, **	**, **	**, **	—		
	gcy-23 gcy-18	**, **	**, **	**, **	**, **	—	—	
	gcy-23 gcy-8 gcy-18	**, *	**, —	**, —	**, —	**, —	—	—, **
25°	*gcy-8*	—						
	gcy-18	—	—					
	gcy-23	—	—	—				
	gcy-23 gcy-8	—	—	—	—			
	gcy-8 gcy-18	**, **	**, **	**, **	**, **	—, **		
	gcy-23 gcy-18	**, **	**, **	**, **	**, **	*, **	—	
	gcy-23 gcy-8 gcy-18	**, **	**, **	*, **	*, **	—, **	—	—

The pairwise test for multiple comparisons using Holm's method was performed on the TTX plot values in Table 5. * and ** indicate $P < 0.01$ and $P < 0.005$ in TTX plot values (20 − 17/25) or (25 − 17). —, no significance.

residues in the kinase homology domain (Potter and Garbers 1992, 1994; Potter and Hunter 1998). Phosphorylation of these residues is essential for activation of the guanylyl cyclase; dephosphorylation results in desensitization and a reduction in ligand-induced guanylyl cyclase activity. A subset of these serine and threonine residues was also conserved in the GCY-8, 18, and 23 guanylyl cyclases in *C. elegans*. Recently, TAX-6 calcineurin has been shown to be involved in thermosensation in AFD of *C. elegans* and the *tax-6* mutant showed a severe thermophilic (heat-seeking) phenotype resulting from hyperactivation of AFD (Kuhara *et al.* 2002). Activity of these three guanylyl cyclases might be regulated through dephosphorylation by TAX-6 calcineurin in *C. elegans*.

Why are three guanylyl cyclases required for thermotaxis? One possibility is that thermosensation requires a high level of cGMP concentration to maintain activity of the thermosensory neurons since temperature is a continuous stimulus unlike other chemical stimuli such as odorants. For continuous signal transduction, three guanylyl cyclases may be required to maintain high levels of intracellular cGMP concentration in AFD, as in mammalian retinal cells, which require the functions of two guanylyl cyclases (Shyjan *et al.* 1992; Lowe *et al.* 1995). Alternatively, since *C. elegans* can sense temperature changes with high resolution (∼0.05°) (Hedgecock and Russell 1975; Ryu and Samuel 2002), rapid synthesis of cGMP may be required to detect slight temperature changes. Application of the membrane permeant cGMP analog 8-bromo-cGMP (0.5, 1, or 5 mm) affected thermotaxis behaviors in wild-type animals, but did not rescue the abnormal phenotype of *gcy* triple mutants (Figure 8), suggesting that thermosensory signal transduction requires the accurate regulation of intracellular cGMP concentrations via the production or the degradation of cGMP. It is also possible that each guanylyl cyclase is involved in sensation of different ranges of temperature. In this case, the temperature ranges sensed by each guanylyl cyclase would overlap since *gcy* single mutants show normal thermotaxis when grown at a given cultivation temperature. The different phenotypes of *gcy* double mutants, in which only one guanylyl cyclase remains functional, may reflect temperature specificities of the guanylyl cyclases. The relatively weak defects of *gcy-23 gcy-8* mutants suggest that the GCY-18 guanylyl cyclase may be required for responses to a wider range of temperature than either the GCY-8 or the GCY-23 guanylyl cyclase. Considering that phenotypes of *gcy-8 gcy-18* and *gcy-23 gcy-18* mutants cultivated at 25° were similar to those of *gcy* triple mutants cultivated at 25°, GCY-8 and GCY-23 guanylyl cyclases might have a

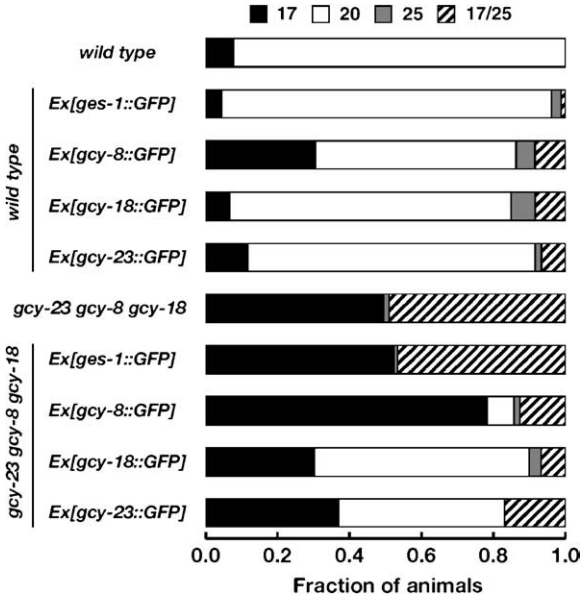


FIGURE 7.—Rescue experiments using full-length *gcy::GFP* fusions. Animals were cultivated at 20°. Transgenic animals carrying full-length *gcy::GFP* fusions, *Ex[gcy-8::GFP]*, *Ex[gcy-18::GFP]*, or *Ex[gcy-23::GFP]*, also carry the *ges-1::GFP* transgene. For each genotype, 59 ~ 359 animals were assayed individually. Phenotypic categories are described in MATERIALS AND METHODS.

minor contribution to thermotaxis in animals cultivated at 25°. Alternatively, each guanylyl cyclase might be involved in the learning of different temperatures. However, temperature-shift experiments (from 17° to 25° or from 25° to 17°) showed that *gcy* single mutants are able to learn a new temperature with a time course similar to that of wild-type animals (data not shown). Although results from the TTX assay, the TTX plot analysis, and the time-course assay strongly suggest that these three guanylyl cyclases function redundantly under normal conditions, it is possible that these guanylyl

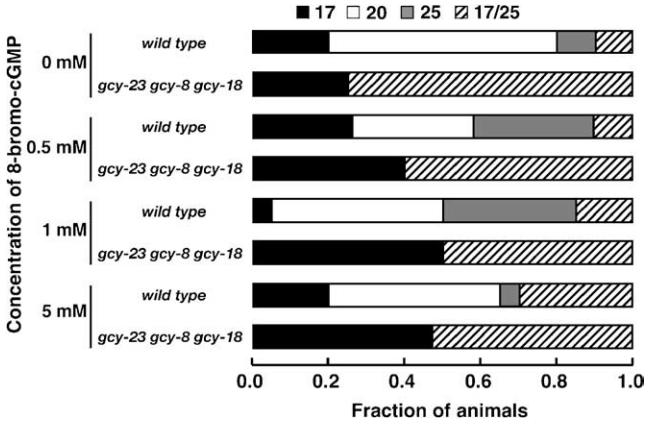


FIGURE 8.—Effect of application of 8-bromo-cGMP on thermotaxis behaviors of wild-type animals and *gcy* triple mutants. Animals were cultivated at 20°. For each condition, 19 or 20 animals were assayed individually. Phenotypic categories are described in MATERIALS AND METHODS.

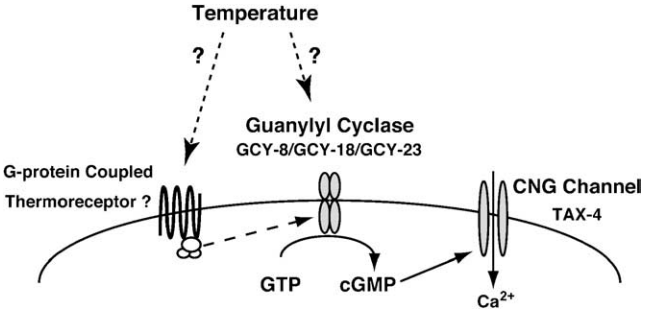


FIGURE 9.—A molecular model thermosensory signal transduction in AFD. Temperature sensed by a thermoreceptor leads to changes in intracellular cGMP concentration via the function of three guanylyl cyclases, GCY-8, GCY-18, and GCY-23. cGMP regulates activity of the TAX-4 CNG channel, resulting in changes in AFD membrane potential.

cyclases may have different activities or kinetics. A *gcy-8* genomic fragment rescued the phenotype of *gcy* triple mutants to a level similar to that of *gcy-23 gcy-18* double mutants in which only GCY-8 functions, but not to the level of wild-type animals. The relatively weak ability of the *gcy-8* genomic fragment to rescue the phenotype of the *gcy* triple mutants and the slight defects observed upon *gcy-8* overexpression in wild-type animals may suggest a modulatory role of GCY-8 in thermosensation. Receptor-type guanylyl cyclases appear to form homodimers or homotetramers in mammals. Combinatorial oligomerization between guanylyl cyclases with different features may contribute to sensitive regulation of signal transduction in thermosensation of *C. elegans*.

How many guanylyl cyclases are required in one sensory neuron? At least two guanylyl cyclases, ODR-1 and DAF-11, function in signal transduction in the AWC olfactory neurons (VOWELS and THOMAS 1994; BIRNBY *et al.* 2000; L'ETOILE and BARGMANN 2000). Expression of a few guanylyl cyclases was also reported in the ASE gustatory neurons (YU *et al.* 1997). Expression of a fourth *gcy* gene, *gcy-12*, was observed in the AFD thermosensory neurons (H. INADA, H. KOMATSU, M. KOSAKI and I. MORI, unpublished results). However, expression of the *gcy-12* gene was not restricted to the AFD neurons, but was observed in many sensory neurons, including the AWC olfactory neurons and the ASE gustatory neurons. Furthermore, the *gcy-12* single mutants and the *gcy-12; gcy-8* double mutants showed normal thermotaxis, suggesting that the GCY-12 guanylyl cyclase does not play a major role in AFD-mediated thermosensation. The presence of multiple *gcy* genes predicted by the *C. elegans* genome may reflect functional redundancy required for robustness of signal transduction pathways or, alternatively, may have specialized but as yet unidentified functions in individual neuron types.

Molecular mechanisms of signal transduction in the AFD thermosensory neuron: This study and previous studies on thermosensation in *C. elegans* lead to a possible

model of molecular mechanisms for thermosensory signal transduction shown in Figure 9. Temperature received by a thermoreceptor is transduced to changes in intracellular cGMP concentration via three guanylyl cyclases, GCY-8, GCY-18, and GCY-23, and transmitted to the TAX-4 CNG channel, resulting in a change of membrane potential of AFD.

What kind of molecule can function as a thermoreceptor? G-protein-coupled seven-transmembrane thermoreceptors (7TM receptor) might exist upstream of these guanylyl cyclases and the TAX-4 CNG channel (Komatsu *et al.* 1996; Roayaie *et al.* 1998; L'Etoile and Bargmann 2000). Recently, it was reported that a few genes encoding 7TM receptors are expressed in AFD (Colosimo *et al.* 2004). These 7TM receptors might function as thermoreceptors in AFD. However, it remains possible that the three guanylyl cyclases described in this study function as thermoreceptors. Biochemical analysis using a heterologous expression system has shown that activity of a *C. elegans* guanylyl cyclase, GCY-12, was clearly dependent on temperature (Yu *et al.* 1997). Biochemical characterization of GCY-8, GCY-18, and GCY-23 guanylyl cyclases may clarify whether these guanylyl cyclases function directly as thermoreceptors. Analysis of thermosensory signal transduction in *C. elegans* may reveal thermosensory mechanisms other than thermosensitive TRP channels.

We thank S. Mitani and Gengyo-Ando for technical advice on the preparation of the deletion mutant library by TMP/UV methods; C. Hunter, Y. Shi, and C. Li for construction of a nonfrozen library and *gcy-8* mutant screening; Y. Kohara for yk cDNA clones; the Caenorhabditis Genetic Center for cosmid clones; the *C. elegans* Sequence Consortium for updated *C. elegans* genome information; and members of the Mori laboratory for stimulating discussion. This work was supported by a research grant from Ministry of Education, Culture, Sports, Science and Technology (to I.M.) and by grants from the National Institutes of Health (PO1 NS44232 to P.S.; T32NS07292 and F32 GM20048 to J.S). I.M. is a Distinguished Scholar of Nagoya University.

LITERATURE CITED

Askwith, C. C., C. J. Benson, M. J. Welsh and P. M. Snyder, 2001 DEG/ENaC ion channels involved in sensory transduction are modulated by cold temperature. Proc. Natl. Acad. Sci. USA **98:** 6459–6463.

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

Birnby, D. A., E. M. Link, J. J. Vowels, H. Tian, P. L. Colacurcio *et al.*, 2000 A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. Genetics **155:** 85–104.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Campbell, A. L., R. R. Naik, L. Sowards and M. O. Stone, 2002 Biological infrared imaging and sensing. Micron **33:** 211–225.

Chen, N., T. W. Harris, I. Antoshechkin, C. Bastiani, T. Bieri *et al.*, 2005 WormBase: a comprehensive data resource for *Caenorhabditis* biology and genomics. Nucleic Acids Res. **33:** D383–D389.

Cheung, B. H., M. Cohen, C. Rogers, O. Albayram and M. de Bono, 2005 Experience-dependent modulation of *C. elegans* behavior by ambient oxygen. Curr. Biol. **15:** 905–917.

Coburn, C. M., and C. I. Bargmann, 1996 A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. Neuron **17:** 695–706.

Colosimo, M. E., A. Brown, S. Mukhopadhyay, C. Gabel, A. E. Lanjuin *et al.*, 2004 Identification of thermosensory and olfactory neuron-specific genes via expression profiling of single neuron types. Curr. Biol. **14:** 2245–2251.

Gengyo-Ando, K., and S. Mitani, 2000 Characterization of mutations induced by ethyl methanesulfonate, UV, and trimethylpsoralen in the nematode *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. **269:** 64–69.

Gray, J. M., D. S. Karow, H. Lu, A. J. Chang, J. S. Chang *et al.*, 2004 Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. Nature **430:** 317–322.

Hedgecock, E. M., and R. L. Russell, 1975 Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **72:** 4061–4065.

Hope, I., 1999 *C. elegans: A Practical Approach*. Oxford University Press, New York.

Komatsu, H., I. Mori, J. S. Rhee, N. Akaike and Y. Ohshima, 1996 Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. Neuron **17:** 707–718.

Komatsu, H., Y. H. Jin, N. L'Etoile, I. Mori, C. I. Bargmann *et al.*, 1999 Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of *Caenorhabditis elegans* in cultured cells. Brain Res. **821:** 160–168.

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

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

Lowe, D. G., A. M. Dizhoor, K. Liu, Q. Gu, M. Spencer *et al.*, 1995 Cloning and expression of a second photoreceptor-specific membrane retina guanylyl cyclase (RetGC), RetGC-2. Proc. Natl. Acad. Sci. USA **92:** 5535–5539.

Maingret, F., I. Lauritzen, A. J. Patel, C. Heurteaux, R. Reyes *et al.*, 2000 TREK-1 is a heat-activated background K(+) channel. EMBO J. **19:** 2483–2491.

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

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

Patapoutian, A., A. M. Peier, G. M. Story and V. Viswanath, 2003 ThermoTRP channels and beyond: mechanisms of temperature sensation. Nat. Rev. Neurosci. **4:** 529–539.

Pierau, F. K., P. Torrey and D. O. Carpenter, 1974 Mammalian cold receptor afferents: role of an electrogenic sodium pump in sensory transduction. Brain Res. **73:** 156–160.

Potter, L. R., and D. L. Garbers, 1992 Dephosphorylation of the guanylyl cyclase-A receptor causes desensitization. J. Biol. Chem. **267:** 14531–14534.

Potter, L. R., and D. L. Garbers, 1994 Protein kinase C-dependent desensitization of the atrial natriuretic peptide receptor is mediated by dephosphorylation. J. Biol. Chem. **269:** 14636–14642.

Potter, L. R., and T. Hunter, 1998 Phosphorylation of the kinase homology domain is essential for activation of the A-type natriuretic peptide receptor. Mol. Cell. Biol. **18:** 2164–2172.

Roayaie, K., J. G. Crump, A. Sagasti and C. I. Bargmann, 1998 The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron **20:** 55–67.

Ryu, W. S., and A. D. Samuel, 2002 Thermotaxis in *Caenorhabditis elegans* analyzed by measuring responses to defined thermal stimuli. J. Neurosci. **22:** 5727–5733.

Sambrook, J., and D. W. Russell, 2001 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Satterlee, J. S., W. S. Ryu and P. Sengupta, 2004 The CMK-1 CaMKI and the TAX-4 cyclic nucleotide-gated channel regulate thermosensory neuron gene expression and function in *C. elegans*. Curr. Biol. **14:** 62–68.

Shyjan, A. W., F. J. de Sauvage, N. A. Gillett, D. V. Goeddel and D. G. Lowe, 1992 Molecular cloning of a retina-specific membrane guanylyl cyclase. Neuron **9:** 727–737.

Souslova, V., P. Cesare, Y. Ding, A. N. Akopian, L. Stanfa *et al.*, 2000 Warm-coding deficits and aberrant inflammatory pain in mice lacking P2X3 receptors. Nature **407:** 1015–1017.

Tominaga, M., and M. J. Caterina, 2004 Thermosensation and pain. J. Neurobiol. **61:** 3–12.

Vowels, J. J., and J. H. Thomas, 1994 Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. Genetics **138:** 303–316.

Wedel, B., and D. Garbers, 2001 The guanylyl cyclase family at Y2K. Annu. Rev. Physiol. **63:** 215–233.

Yandell, M. D., L. G. Edgar and W. B. Wood, 1994 Trimethylpsoralen induces small deletion mutations in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **91:** 1381–1385.

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

Communicating editor: K. Kemphues