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# Receptor Guanylyl Cyclases in Sensory Processing

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Invertebrate models have generated many new insights into transmembrane signaling by cell-surface receptors. This review focuses on receptor guanylyl cyclases (rGCs) and describes recent advances in understanding their roles in sensory processing in the nematode, *Caenorhabditis elegans*. A complete analysis of the *C. elegans* genome elucidated 27 rGCs, an unusually large number compared with mammalian genomes, which encode 7 rGCs. Most *C. elegans* rGCs are expressed in sensory neurons and play roles in sensory processing, including gustation, thermosensation, olfaction, and phototransduction, among others. Recent studies have found that by producing a second messenger, guanosine 3′,5′-cyclic monophosphate, some rGCs act as direct sensor molecules for ions and temperatures, while others relay signals from G protein-coupled receptors. Interestingly, genetic and biochemical analyses of rGCs provide the first example of an obligate heterodimeric rGC. Based on recent structural studies of rGCs in mammals and other organisms, molecular mechanisms underlying activation of rGCs are also discussed in this review.

**Keywords: behavior, *C. elegans*, dauer formation, gustation, olfaction, phototransduction, thermosensation**

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## INTRODUCTION

An intracellular second messenger, guanosine 3′,5′-cyclic monophosphate (cGMP), was first identified in rat urine (1). Since then, it has been demonstrated that cGMP participates in a wide range of physiological responses, including blood pressure regulation, phototransduction, olfaction, thermosensation, and synaptic plasticity (2, 3). cGMP is also important in the invertebrate nervous system. It is involved in ecdysis and foraging behaviors in insects, and in neuronal path finding and differentiation in insects and mollusks (4). In 1969, it was found that both the water-soluble and particulate fractions of tissue homogenates showed guanylyl cyclase (GC) (also called as guanyl cyclase or guanylate cyclase) activity (5–7). The enzyme activity differed in the two fractions (8–10) and was subsequently purified from both fractions (11, 12). The cDNAs of both were cloned (13–16), and GCs are classified into two groups: receptor guanylyl cyclase (rGC, also called membrane GC or receptor-type GC) and soluble GC (sGC) (2, 17). The soluble forms have been shown to exist as heterodimers, consisting of α and β subunits, and containing heme as a prosthetic group. Heterodimerization is required for their catalytic activity (18, 19), although β subunits have been reported to form active homodimers (20, 21). While sGC has a cytoplasmic heme-binding domain and a guanylyl cyclase domain (GCD), rGCs are type-1 transmembrane receptors, comprising of an extracellular domain (ECD), a transmembrane domain (TMD), and an intracellular domain (ICD), which consists of the protein kinase-homology domain [KHD; also called a protein kinase-like domain, or adenosine 5′-triphosphate (ATP)-regulatory module] and a GCD, separated by a ~50 residue linker region (**Figure 1A**). Apart from the overall structural differences, GCD monomers of sGCs and rGCs have the same protein fold as the mammalian adenylyl cyclase (AC) catalytic

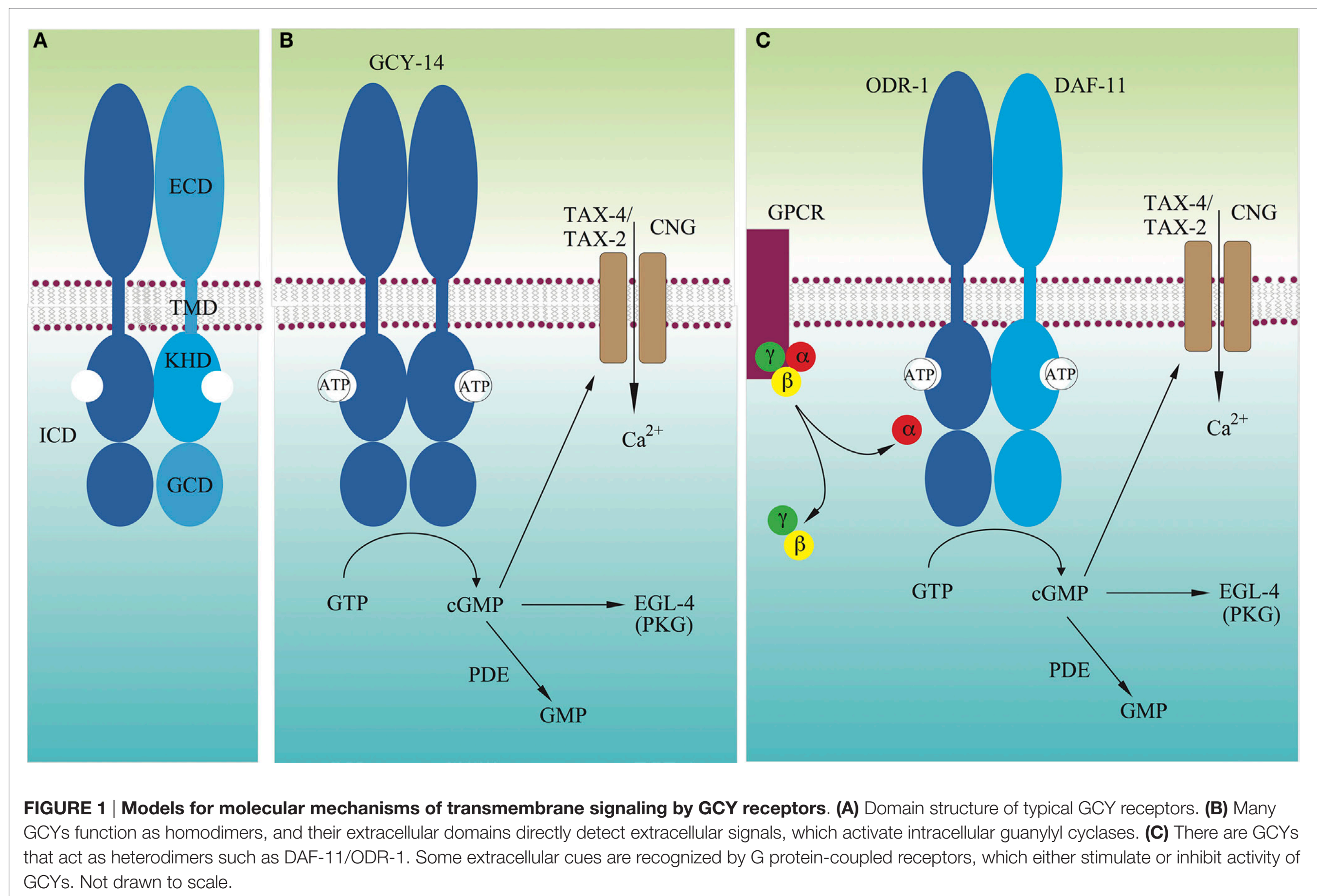


**FIGURE 1 | Models for molecular mechanisms of transmembrane signaling by GCY receptors. (A)** Domain structure of typical GCY receptors. **(B)** Many GCYs function as homodimers, and their extracellular domains directly detect extracellular signals, which activate intracellular guanylyl cyclases. **(C)** There are GCYs that act as heterodimers such as DAF-11/ODR-1. Some extracellular cues are recognized by G protein-coupled receptors, which either stimulate or inhibit activity of GCYs. Not drawn to scale.

domain (3). rGCs can be activated either by extracellular ligands, such as natriuretic peptides and uroguanylin in GC-A, GC-B, and GC-C, or by intracellular calcium-binding proteins, such as guanylyl cyclase-activating proteins (GCAPs) in GC-E (also known as RetGC-1) and GC-F (RetGC-2) (2, 17, 22).

The *Caenorhabditis elegans* genome encodes 27 rGCs (**Table 1**) (23–25). These numbers are unusually large, compared to other genomes. For example, mammalian and *Drosophila* genomes encode 7 and 6, respectively (4). Recent studies have elucidated physiological roles of rGCs in *C. elegans*, on which we focus in this review. Ectopic expression of rGCs in different neurons has shown that rGCs play roles as direct sensor molecules for alkaline pH, molecular $CO_2$, and temperature in sensory neurons (26–28). Many other rGCs are also involved in salt gustation, olfaction, pheromone detection, phototransduction, and body-size regulation. Based on recent structural studies of rGCs in mammals and other organisms, molecular mechanisms underlying activation of rGCs are also discussed. rGCs of other organisms have been reviewed elsewhere (2–4, 17, 22, 29).

## ROLES OF rGCs IN SENSORY PROCESSING

### Gustation

A bilaterally symmetric pair of *C. elegans* gustatory neurons, left ASE (ASEL) and right ASE (ASER) (refer to **Figure 2** for the positions of sensory neurons described in this review), senses a number of chemicals in a left/right asymmetric manner and coexpresses multiple rGCs, GCY-6 (Guanylyl CYclase), -7, -14, -19, -20, and -29, and GCY-1, -3, -4, -5, -19, -22, and -29, respectively (23). GCY-14 of ASEL is essential in sensing environmental alkaline pH (26). Ectopic expression of GCY-14 in other sensory neurons, ASG, ASI, and ASER, makes these neurons sensitive to alkaline pH. GCY-14 functions as a homodimer, like mammalian rGCs (**Figure 1B**). Histidine-174 of the GCY-14 ECD is required for the detection of alkaline pH. Deprotonation of this histidine residue by alkaline pH may cause conformational changes in the domain that activates intracellular GC. Activation of GCY-14 then opens cGMP-gated cation channels consisting of TAX-4 (abnormal chemoTAXis) (α) and TAX-2 (β) subunits (43, 44), resulting in $Ca^{2+}$ entry into ASEL. This $Ca^{2+}$ entry also involves EGL-4 (EGg Laying defective), a cGMP-dependent protein kinase (PKG) (45–47), TAX-6, a calcineurin A ortholog (48), and phosphodiesterases (PDEs) (30). A neuronal calcium sensor (NCS-1) (49), which is a calcium-binding protein related to vertebrate GCAPs and 74% identical to human frequenin (50), is not required for $Ca^{2+}$ entry, but enables its downstream signaling, since chemotaxis of *ncs-1* mutants to alkaline pH is deficient (26).

GCY-14 is also required for sensing increases in $Na^+$ or $Li^+$ concentrations (34) and is a direct sensor for an increase in NaCl concentrations (26). In contrast to alkaline pH sensation,

**TABLE 1 | Expression and function *of Caenorhabditis elegans* receptor guanylyl cyclases.**

| Receptor guanylyl cyclase (rGC) | Function | Reference | Cell/neuron expressing rGC | Reference |
|---|---|---|---|---|
| ODR-1 (GCY-10) | Olfaction, dauer formation, phototransduction | (30–32) | ASIL/R, ASJL/R, ASKL/R, AWBL/R, AWCL/R | (24, 31) |
| DAF-11 | Olfaction, dauer formation, phototransduction | (30, 31, 33) | ASIL/R, ASJL/R, ASKL/R, AWBL/R, AWCL/R | (33) |
| GCY-1 | $K^+$ | (34) | ASER, ASIL/R, PVT, URXL/R, AIYL/R, intestine | (23) |
| GCY-2 | | | ASIL/R, AWAL/R, RIAL/R, PVT | (23) |
| GCY-3 | | | ASER, ASIL/R, PVT | (23) |
| GCY-4 | $Br^-$, $I^-$ | (34) | ASER (biased) | (24) |
| GCY-5 | | | ASER | (24) |
| GCY-6 | $Mg^+$ | (34) | ASEL | (24, 35) |
| GCY-7 | | | ASEL, excretory canal cell (only in adults) | (23, 24, 36) |
| GCY-8 | Thermosensation | (28, 37) | AFDL/R | (24) |
| GCY-9 | Carbon dioxide | (27) | BAG | (23) |
| GCY-11 | | | Pharyngeal muscle | (23) |
| GCY-12 | Body size | (38) | ASE, AWC, ASJ, AUA, PHAL/R, PHB | (24, 38) |
| GCY-13 | | | RIML/R | (23) |
| GCY-14 | $Na^+$, $Li^+$, Alkaline pH | (26, 34) | ASEL (biased), AWCL/R (faint), PVT | (23) |
| GCY-15 | | | ASGL/R (faint) | (23) |
| GCY-17 (GCY-24) | | | PHAL/R | (23) |
| GCY-18 (GCY-26) | Thermosensation | (28, 39) | AFDL/R, AIML/R | (23) |
| GCY-19 | | | ASEL/R (faint), IL2, additional three sensory neurons (faint) | (23) |
| GCY-20 (GCY-16) | | | ASEL, AWCL/R (faint), excretory gland and canal cells | (23) |
| GCY-21 | | | ASGL/R, ADLL/R (faint) | (23) |
| GCY-22 | Nearly all salts, methionine | (34, 35, 40) | ASER | (36) |
| GCY-23 | Thermosensation | (28, 37) | AFDL/R | (23, 37) |
| GCY-25 | | | AQR, PQR, URXL/R | (23) |
| GCY-27 | | | ASKL/R, ASIL/R, ASJL/R | (23) |
| GCY-28 | Olfaction, behavioral choice | (41, 42) | Many neurons, muscle, hypodermis, somatic gonad, intestine | (23, 42) |
| GCY-29 | | | AFDL/R, ASEL/R, AWCL/R, AVKL/R, few other neurons (faint) | (23) |

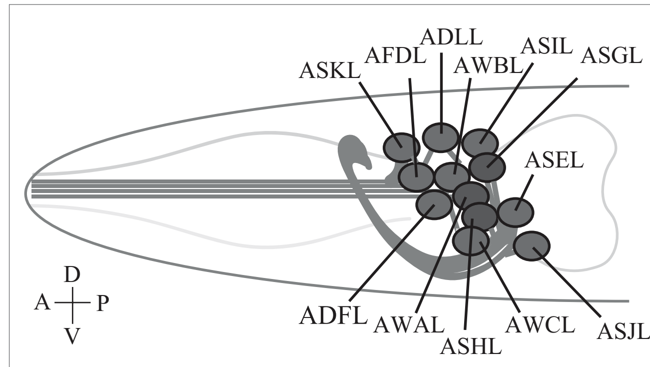


**FIGURE 2 | *Caenorhabditis elegans* sensory neurons**. A subset of *C. elegans* amphid sensory neurons described in this review consists of a pair of two bilaterally symmetric neurons. Each of the 12 pairs of neurons extends a dendrite to the tip of the nose, and an axon into the nerve ring, a nerve bundle where synaptic connections are made with other neurons including downstream interneurons (51). Only left-side neurons are shown. Not drawn to scale.

histidine-174 does not play a role in detecting increases in NaCl concentration, suggesting that other residues of the ECD are responsible for sensation. Disruption of the ASER-expressed rGC gene, *gcy-22*, results in broadly defective chemotaxis to nearly all salts sensed by ASER (27, 34, 40). *gcy-22* is also required for animal responses to the amino acid, methionine, which is primarily sensed by ASER (34). In contrast, disruption of other *gcy* genes results in highly salt-specific chemosensory defects (34). *gcy-1* mutant animals show markedly decreased responses to $K^+$, which is sensed by ASER. *gcy-4* shows chemotaxis defects on gradients of $Br^-$ and $I^-$. *GCY-4* and *GCY-22* may exist as homodimers and heterodimers, with each homodimer retaining residual function, since a *gcy-4;gcy-22* double mutant shows a stronger defective phenotype in chemotactic response to $Br^-$ and $I^-$ (34). Although all mammalian rGCs exist in a homodimeric form, but not in a heterodimeric form, it has been shown theoretically and experimentally that *C. elegans* rGCs, ODR-1 (ODoRant response abnormal) and DAF-11 (abnormal DAuer Formation), function as an obligate heterodimer (4, 26). *gcy-6* mutant animals show a dramatic decrease in their ability to respond to $Mg^+$ gradients, sensed by ASEL (34). However, except for GCY-14, it remains to be shown whether these GCYs are direct sensor molecules by ectopic expression of these rGCs in unrelated neurons.

## Thermosensation

*Caenorhabditis elegans* exhibits defined behavioral responses to thermal gradients, which evoke two distinct behaviors. The first is cryophilic movement, or migration toward cooler temperatures than the growth temperature. This is the dominant behavior when the ambient temperature exceeds the growth temperature and is achieved by means of a biased random walk (52–54). The second behavior, isothermal tracking, is observed when animals reach thermal zones that are within 2°C of the growth temperature (52, 54–57). These behaviors involve at

least three pairs of sensory neurons, AFD, AWC, and ASI (53, 56, 58, 59). AFD neurons, whose sensory dendrites terminate in a specialized ending that is composed of a primary cilium and an extensive array of microvilli (51, 60), depolarize and hyperpolarize upon warming and cooling, respectively, and temperatures warmer than the growth temperature drive cryophilic thermotactic behaviors (61). The thermosensory responses appear to be AFD cell-intrinsic properties, although surrounding glial cells tune thermal responses of AFD (62, 63).

At least eight genes are essential for thermotransduction by AFD: *gcy-8*, *gcy-18*, *gcy-23*, *tax-4*, *tax-2*, *pde-2*, and *ncs-1*. The three rGCs, GCY-8, GCY-18, and GCY-23, are exclusively expressed in AFD (37, 64), and participate in isothermal tracking (59, 65) and migration in linear thermal gradients (66). Recently, it has been shown by ectopic expression in diverse cell types that these rGCs are indeed direct thermosensor molecules, and that both the extracellular and ICDs are necessary for sensation (28). The TAX-4/TAX-2 cGMP-gated cation channel is also expressed in AFD and is essential for thermotaxis (37, 43, 44, 61). In AFD, *pde-2* and *pde-5*, but not *pde-1* or *pde-3*, are expressed, and *pde-2*, but not *pde-5*, mutations increase the threshold temperature for the activation of thermoreceptors and augment the thermoreceptor current lifetime (67). NCS-1 is also expressed in AFD and regulates behavioral responses to thermal gradients (49). As in *pde-2* mutants, loss of NCS-1 prolongs thermoreceptor currents and elevates their threshold. Unlike loss of PDE-2, however, loss of NCS-1 increases voltage-activated outward currents (67). These results suggest that cGMP concentrations and NCS-1 help set the threshold temperature of rGCs in AFD.

*Caenorhabditis elegans* AMsh glial cells ensheathe neuronal receptive endings of 12 neurons, including AFD. It has been recently shown how glia control shapes of neuronal receptive endings through inhibition of an rGC expressed on the neuronal surface (68). KCC-3, a $K^+/Cl^-$ cotransporter, localizes specifically to the glial microdomain surrounding AFD receptive ending microvilli, where it regulates $K^+$ and $Cl^-$ levels. $Cl^-$ ions act as a direct inhibitor of the GCY-8 rGC on the AFD receptive ending microvilli *via* $Cl^-$ binding to the receptor's ECD. GCY-8 has basal activity, which is inhibited by extracellular $Cl^-$ ions released from AMsh glial cells. Without the inhibition, an increased level of cGMP at the neuronal receptive ending promotes the disappearance of AFD receptive ending shapes. Similarly, a *pde-1 pde-5* double mutant displays a fully penetrant loss of AFD receptive endings. PDE-1 and PDE-5 are the only PDEs required for extension of AFD microvilli and appear to function redundantly. It appears that higher levels of cGMP at the AFD neuronal receptive ending antagonize WSP-1/Wiskott–Aldrich syndrome protein, an actin regulator that promotes microvillus formation through nucleation of actin filaments in the receptive ending (68). However, it remains to be clarified how cGMP antagonizes WSP-1 activity.

## Carbon Dioxide Detection

Carbon dioxide is detected by animals as an environmental cue that indicates the presence of food, predators, or mates, and as an internal cue that reflects internal metabolic state (69, 70). *C. elegans* has a pair of $CO_2$-sensing neurons, the BAG neurons, which mediate avoidance of $CO_2$ by adults and attraction to $CO_2$ by dauer larvae (71, 72). Adult *C. elegans* animals display an acute avoidance response upon exposure to $CO_2$ that is characterized by cessation of forward movement and rapid initiation of backward movement. This $CO_2$ avoidance is mediated by a cGMP signaling pathway that includes the cGMP-gated heteromeric channel TAX-4/TAX-2 (71). The GCY-9 rGC is responsible for $CO_2$ sensitivity of the BAG neurons (27). $CO_2$ avoidance behavior is modulated by multiple signaling molecules, including the neuropeptide Y receptor, NPR-1, and the calcineurin subunits, TAX-6 and CNB-1 (CalciNeurin B) (71). GCY-9 is a direct sensor for molecular $CO_2$, since the receptor ectopically expressed in AFD thermosensory neurons, which normally do not respond to $CO_2$, responded to $CO_2$ (27).

## Olfaction

*Caenorhabditis elegans* uses two pairs of ciliated olfactory neurons, AWA and AWC, to sense many volatile attractants (39). A pair of laterally symmetric AWC neurons, AWCL and AWCR, are functionally distinct from each other. The distinction between the two AWC neurons is random from animal to animal, but coordinated so that one neuron of each type is generated in each animal (73). $AWC^{on}$, which expresses the STR-2 (Seven Transmembrane Receptor) G protein-coupled receptor (GPCR), detects butanone, benzaldehyde, and isoamyl alcohol, whereas $AWC^{off}$, which does not express STR-2, detects 2,3-pentanedion, benzaldehyde, and isoamyl alcohol (74). Chemosensation in AWC is mediated by direct binding of odorants to GPCRs, which are exclusively localized to sensory cilia (75, 76). Intracellularly, these receptors are coupled to heterotrimeric G proteins, whose α subunit can positively or negatively regulate *C. elegans* chemotaxis to odorants (77). There are at least four Gα subunits expressed in AWC: ODR-3, GPA-2 (G Protein, Alpha subunit), GPA-3, and GPA-13 (77–79). Butanone signaling is mediated by two partly redundant Gα proteins, ODR-3 and GPA-2, whereas other odor responses in AWC are mediated by ODR-3, but not by GPA-2 (78). AWC utilizes cGMP as a second messenger, as well as the cGMP-gated cation channel TAX-4/TAX-2. ODR-1 and DAF-11 rGCs are both required for the animal's chemotaxis to all AWC-sensed odorants (31, 33, 39), and these rGCs form obligate heterodimers (**Figure 1C**), each monomer of which provides essential catalytic residues to form a single catalytic site (4, 26). The ECD of ODR-1 is dispensable for its olfactory function, indicating that the rGC does not act as a direct receptor for odorants sensed by AWC (31).

Genetically encoded calcium indicators have revealed that in the absence of odorants, the AWC exhibits high intracellular calcium levels, and upon odorant stimulation, intracellular calcium levels decrease, leading to hyperpolarization of the AWC neurons (80). This hyperpolarization can be explained by the decrease of cGMP levels followed by closing of TAX-4/TAX-2 channels. This mechanism shares many similarities with the light response in mammalian photoreceptor cells, where in the absence of light, calcium channels are open, exhibiting a "dark current." Upon photon binding, a cGMP PDE is activated, which reduces cGMP levels, closing cGMP-gated channels, and

thus hyperpolarizing the cell (81). Therefore, in AWC olfactory sensory processing in *C. elegans*, GPCRs act as sensor molecules, and upon stimulation, release their Gα subunits to regulate cGMP concentration in AWC cilia. Interestingly, *C. elegans* chemotaxis to odorants sensed by AWC is not affected by loss of the PDEs (82). Therefore, the released Gα may directly interact with the heterodimeric ODR-1/DAF-11 rGC to lower the GC activity (**Figure 1C**) (31).

The EGL-4 PKG is necessary for adaptation of the AWC chemosensory response (47, 83). Nuclear localization of EGL-4 is both necessary and sufficient to promote long-term adaptation (84) and is dependent on PDE activity (82). ODR-3, a Gα subunit, and the ability of EGL-4 to bind cGMP are both required for nuclear entry of EGL-4 after prolonged odorant exposure. Furthermore, loss of ODR-1 leads to constitutive entry of EGL-4 into the nucleus (83). Nuclear EGL-4 phosphorylates HPL-2 (Heterochromatin Protein Like), a heterochromatin-binding protein, and promotes the phosphorylated protein to bind to the *odr-1* locus in AWC in order to reduce *odr-1* mRNA levels in adapted animals. Concomitantly, the increased activity of an endo-siRNA pathway-targeted *odr-1* degrades the mRNA (85).

An attractive behavior mediated by $AWC^{on}$ requires the GCY-28 rGC, which acts in adults and localizes to the $AWC^{on}$ axon. Mutations in *gcy-28* lead to an avoidance behavior instead of the attractive behavior normally directed by the $AWC^{on}$ neuron. This behavioral reversal results from presynaptic changes in $AWC^{on}$ possibly through modification of $AWC^{on}$ excitability or synaptic release by GCY-28 (41). The *gcy-28* mutants also show an abnormal bias in the behavioral choice between two conflicting cues, the attractive odorant diacetyl sensed by AWA and the aversive stimulus $Cu^{2+}$ sensed by ASH/ADL, although their responses to each individual cue are similar to those in wild-type animals (42). GCY-28 regulates the neuronal activity of AIA interneurons, where the conflicting sensory cues from AWA and ASH/ADL sensory neurons seem to converge, by activation of CNG-1, a cyclic nucleotide-gated ion channel (42, 86).

## Dauer Formation

The environment determines whether *C. elegans* grows directly into adulthood, or whether it arrests at an alternative L3 larval stage to form a dauer larva (87). Dauer larvae are induced by harsh conditions, such as starvation and high population density, and can survive under severe conditions because of distinctive morphology, metabolism, and life span. When environmental conditions improve, dauer larvae reenter the reproductive cycle by molting into L4 larvae and subsequently into adulthood.

The dauer larva versus reproduction and growth decision is determined by at least two signaling cascades: the DAF-2 (insulin/IGF-1 receptor) (88) and the DAF-7 (TGFβ) pathways (89, 90). A decrease in either of the signals causes dauer arrest, indicating that both pathways are required for reproduction and growth. Downregulation of DAF-2 results in the activation and nuclear localization of DAF-16 (forkhead transcription factor) (91–93). The DAF-2 signaling pathway also regulates metabolism and aging. When DAF-2 signaling is decreased, life span is greatly extended (94, 95). *daf-7* encodes a member of the TGFβ superfamily that is a ligand for the parallel neuroendocrine pathway (89, 90). DAF-7 activates a heterodimeric receptor consisting of DAF-1 (TGFβ type I receptor) (96, 97) and DAF-4 (TGFβ type II receptor) (98).

Laser ablation of three sensory neurons, ADF, ASG, and ASI, causes dauer arrest, indicating that these neurons signal reproduction and growth (99). Mutations in *daf-11*, which is expressed in ciliated sensory neurons including ASI, ASJ, ASK, AWB, and AWC, cause dauer arrest (33). Moreover, cGMP-gated channels have also been implicated (43, 44, 100). Because dauer arrest caused by a loss-of-function mutation, *daf-11(lf)*, can be partially suppressed either by *daf-3(lf)* or *daf-16(lf)*, DAF-11 is likely to regulate both the DAF-2 and DAF-7 pathways (101, 102). Furthermore, dauer-inducing pheromone inhibits DAF-7 expression and promotes dauer arrest, and food activates DAF-7 expression (89, 90). *daf-7* gene expression is defective in *daf-11* mutants, and the constitutive dauer formation phenotype of *daf-11* mutants is suppressed by DAF-7 expression in ASI (103).

Two GPCRs, SRG-36 (Serpentine Receptor, class Gamma) and SRG-37, are strongly expressed in ASI neurons, where they localize to the sensory cilia, as receptors for the dauer pheromone, ascaroside ascr#5 (32, 104). A heterodimeric GPCR, DAF-37/DAF-38, is expressed in ASI and functions as a receptor for ascr#2 (105). *odr-1* mutants have reduced sensitivity to dauer pheromone, indicating a role in ascaroside perception (32). While *egl-4(lf)* mutations increase the propensity to form dauer larvae (106), *egl-4(gf)* mutations decrease that propensity (107).

Taken together, at high population density, concentrations of dauer pheromone ascarosides increase and activate GPCRs on the cilia of ASI and other neurons. Gα released from the receptors may interact with heterodimeric ODR-1/DAF-11 rGC to regulate GC activity.

## Phototransduction

*Caenorhabditis elegans* lives in darkness, but is able to sense blue or shorter wavelength of light (with maximal responsiveness to ultraviolet light), and engages in negative phototactic behavior (108). This negative phototaxis is important for survival because ultraviolet and blue light are toxic to the animal on the soil surface and requires *lite* (light-unresponsive) genes (108). Laser ablation of a combination of four ciliated neurons (ASJ, AWB, ASK, and ASH) led to a severe defect in the negative phototaxis from light (109).

*Caenorhabditis elegans* phototransduction requires LITE-1, a seven-transmembrane domain receptor-like protein that transduces light signals in ASJ *via* G protein signaling (30, 110). *C. elegans* Gα proteins, GOA-1 and GPA-3, have a redundant role in mediating phototransduction in ASJ. Downstream of the Gα proteins, the rGCs ODR-1 and DAF-11 are required for phototransduction in ASJ. cGMP-gated cation channels consisting of TAX-4/TAX-2 act downstream of ODR-1/DAF-11. In the *pde-1*, *-2*, and *-5* triple mutant, the photocurrent is markedly potentiated, with a current density about fivefold greater than that in wild-type animals. The photocurrent in *pde-1*, *-2*, *-3*,

and -*5* quadruple mutants exhibits very slow or no recovery after cessation of the light stimulus, consistent with a role for PDEs in downregulating cGMP levels. These results are reminiscent of vertebrate photoreceptor cells, where light-activated G proteins either inhibit PDEs (e.g., parietal eye photoreceptor cells) or stimulate them (e.g., rods and cones), increasing or decreasing cGMP levels and opening or closing cGMP-gated cation channels, respectively (30).

### Regulation of Body Size

Many mutants that lack normal sensory cilia exhibit small body size, suggesting that sensory cilia are involved in regulation of body size in *C. elegans* (111). To elucidate molecular mechanisms underlying sensory regulation of body size, a genetic screen for suppressors of the small body size of a cilium-defective mutant, *che-2* (abnormal CHEmotaxis), which is expressed in the cilia of most ciliated sensory neurons and which encodes a protein that contains G protein β-like WD-40 repeats has been carried out (112). Through the genetic screen, mutants defective in *daf-25*, *egl-4*, and *gcy-12* have been isolated as suppressors of *che-2* mutations. EGL-4 appears to regulate body size by functioning in sensory neurons, because EGL-4 expression in ASE (and AWC) sensory neurons is sufficient for the animal's growth to a normal body size (38, 45). *dbl-1* (DPP/BMP-Like) mutants have a small body size with low hypodermal ploidy. The DBL-1 (TGFβ) signaling pathway, which regulates hypodermal ploidy and consequently affects cell growth and body size (113), acts downstream of EGL-4 (45, 46). Indeed, *che-2* and *egl-4* mutants exhibit low and high ploidy of the hypodermal cells, respectively (114).

As described above, mutations in *gcy-12* suppress the small body size of the *che-2* mutant. As observed in *egl-4* mutants, *gcy-12* mutants showed increased body size, compared with wild-type animals (38). However, *gcy-12;egl-4* double mutants did not become larger than *egl-4* single mutants, indicating that *gcy-12* and *egl-4* act in the same pathway. The overexpression of GCY-12 in wild-type animals led to a small body size, presumably due to activation of EGL-4 kinase because GCY-12 overexpression did not cause body-size reduction in *egl-4* mutants (38). These results also suggest that GCY-12 acts upstream of EGL-4, most likely by synthesizing cGMP. However, the ECD of GCY-12 is not necessary for GCY-12 function in body-size regulation (38). The *pde-2* gene, which encodes a PDE is also required for normal body size, and *pde-2* mutations cause a small body-size phenotype. The *pde-2* mutation did not affect the body size of *egl-4* mutants, indicating PDE-2 acts upstream of EGL-4. The *gcy-12;pde-2* double mutants exhibited an intermediate body size relative to the two single mutants. These results indicate that PDE-2 hydrolyzes cGMP and negatively regulates EGL-4 kinase in regulating body size (38). A gain-of-function mutation of *egl-4* that produces a constitutively activated EGL-4 kinase, exhibited small body size, an opposite phenotype of a loss-of-function mutant of *egl-4* (115).

These results suggest the existence of other sensor molecules in addition to GCY-12 that detects extracellular signals. GCY-12 may be constitutively active in ASE. The extracellular signal may lower cGMP levels in ASE sensory neurons perhaps through activation of PDEs and consequently inhibits EGL-4 kinase. Abnormal activation of EGL-4 by an increased concentration of cGMP or by a mutation may upregulate the secretion of DBL-1 (also known as CET-1) or equivalent ligand molecules from ASE sensory neurons. An increase in DBL-1 concentrations may initiate the Sma/Mab signaling pathway in hypodermal cells, which regulates hypodermal ploidy and consequently affects cell growth and body size. In analogy to the role for EGL-4 in olfactory adaptation, in which loss of ODR-1 rGC leads to constitutive entry of EGL-4 into the nucleus of AWC, loss of GCY-12 rGC may also lead to entry of EGL-4 into the nucleus of ASE, where DBL-1 expression may be regulated.

## MECHANISM OF ACTIVATION OF rGCs

Like many other type-1 transmembrane receptors such as receptor tyrosine kinases and cytokine receptors (116, 117), rGCs have a homo- or heterodimeric structure in the absence of bound ligand (26, 118–120). The ECD of rGCs often encodes regions that recognize extracellular cues such as peptides or ions. Furthermore, the ECD of many rGCs, including GCY-8 and GC-A (also called NPRA or NPR-1), has the conserved $Cl^-$-binding structure motif, $S(x)_n$GPxC, near the dimer interface (68, 119, 120). In GCY-8, $Cl^-$ binding inhibits its rGC activity (68), while in GC-A, atrial natriuretic peptide (ANP) ligand binding to the receptor is $Cl^-$ concentration dependent (121). Therefore, it appears that $Cl^-$ ion binding to the ECD of rGCs induces conformational changes in these receptors.

Adenosine 5′-triphosphate is thought to bind to KHD and to augment GC stimulation by ligand binding (122, 123). The interaction between the KHD and ATP shows positive cooperativity, suggesting that binding of one ATP to one KHD of the dimer would facilitate binding of a second ATP to the other KHD (124). The KHD is normally phosphorylated (125, 126), and its dephosphorylation leads to receptor desensitization (126–128). The KHD of GC-A allosterically regulates both peptide binding to the ECD and activation of the GC in a bidirectional manner. ATP binding to the KHD of the GC-A dimer results in reduced ANP binding to the ECDs and enhanced GC activity (129–131). GC-A lacking the KHD is constitutively active (132). The KHD responds to ANP binding by adopting a conformation that allows direct ATP binding, resulting in release from GC activity repression (124). Therefore, it is clear that in the inactive form of rGC, the KHD exists in a stable dimeric form that inhibits its C-terminal GC activity (**Figure 1**). Stability of the KHD dimer may be affected by phosphorylation states of the domain. Upon ligand binding to the ECD of rGC, the KHD may assume a relatively flexible dimeric configuration, to which one molecule of ATP binds. This first ATP binding may induce configuration changes in the KHD dimer to increase its affinity for the second ATP molecule. Flexible structure of the KHD dimer may allow configuration changes in the GC dimer for activation. Configuration changes in the KHD dimer may also induce configurations of the ECD dimer, in which the ligand has low-affinity binding.

As described above, the KHD is connected to the GCD by a ~50 residue linker region in the receptor's ICD. Systematic mutational

and biochemical analyses of GC-C and GC-A have suggested an important role for the linker region in repressing the catalytic activity of the receptors in the absence of their ligands (133). Specific residues in the linker region seem to assist in repressing GC activity through its interaction with the GCD, where Gα may also interact with and activate (or inhibit) rGC.

Crystal structures of three catalytic domains of a sGC, Cyg12, from the green algae, *Chlamydomonas reinhardtii* (134), of a putative rGC, Cya2, from the cyanobacterium *Synechocystis PCC6803* (135), and of a human sGC α1 and β1 subunits, encoded by *GUCY1A3* and *GUCY1B3* genes, respectively (136), have recently been determined. Furthermore, GCs and ACs belong to the class III nucleotide cyclase family and share high sequence similarity (137). By structural comparison, the dimer structure of Cyg12 GCD is similar to the open, inactive conformation of the AC catalytic domain, to which it must be close in order to be catalytically active (134). On the other hand, the Cya2 GCD dimer is in a closed conformation that must open to bind the substrate GTP (135). Comparison of an "open" apo-AC structure (138) with a "closed" ligand-bound AC structure shows a 7° rotation of the monomer (139). This movement brings the catalytic residues on one subunit closer to the catalytic residues on the other subunit, thereby forming the catalytically competent active site. Comparing the open conformation of the *Chlamydomonas* structure with the closed conformation of the cyanobacteria homodimeric GCD shows a similar, 7°–8°, domain rotation (136, 140). The heterodimeric catalytic domains of the α and β subunits are in an inactive open conformation, but can be superposed onto an active AC by a structural transition involving a 26° rigid-body rotation of the α subunit (136). These structural studies suggest that the GCD subunit monomer of the inactive open structure of the dimer rotates to form an active closed structure. Such a flexible transition between open and closed structures may be essential for GTP binding to and the release of cGMP from the active site of the domain dimer.

As described above, rGCs exist as dimers, like many other type-1 transmembrane receptors (116, 117). ANP binding to GC-A induces a 40° rotation (or twist) of the receptor's TMD parallel to the plane of the membrane (141). The subunits of GCD dimers also rotate to form a catalytically active structure as described above (136). All these results suggest that upon ligand binding to the ECD of rGCs, the receptor subunits rotate to activate the GCD of the receptor dimer as previously proposed (140). In this transmembrane signaling process, the KHD may play a regulatory role by binding to the allosteric effector ATP and by varying its phosphorylation state. Prior to ligand binding, the KHD may exist as a stable dimer, like the inactive back-to-back homodimeric structure of the kinase domains of the epidermal growth factor receptor (142, 143). Like many other type-1 transmembrane receptors (117), ligand-induced rotation of the TMDs of rGCs may dissociate the dimeric KHD to adopt relatively flexible structures for ATP binding with positive cooperativity, and this flexibility may allow subunit rotation of the GCD dimer to form a closed, catalytically competent configuration. Such rGC rotational flexibility may explain how GCY-8, GCY-18, and GCY-23 function as thermosensors. The rotational flexibility is likely driven by thermal energy, and temperatures above the growth temperature may induce rotation of the receptor TMD for activation. The temperature thresholds may be determined by phosphorylation states of the receptor KHD. Configuration changes in the KHD dimer may also be induced through interaction with other proteins such as GCAPs and NCS-1 for the activation of rGCs without ligand binding to the receptor ECD, as shown in the ODR-1/DAF-11 heterodimer, GC-E and GC-F. In olfaction and dauer formation in *C. elegans*, GPCRs serve as sensor molecules and regulate cGMP concentrations without involvement of PDEs. Therefore, the Gα subunit of heterotrimeric G proteins may also directly interact with the KHD or the GCD of rGCs to regulate GC activity of the receptor (**Figure 1C**).

## CONCLUSION

The *C. elegans* genome encodes 27 rGCs, 6 of which are expressed in a single sensory neuron, ASEL. These rGCs are involved in diverse sensory processing including gustation, thermosensation, olfaction, pheromone sensation, and phototransduction. It has been shown that the homodimeric GCY-14 in ASEL senses alkaline pH, $Na^+$, and $Li^+$. Therefore, a single sensory neuron serves as a sensor for various environmental cues. Like all mammalian rGCs with a homodimeric structure, most *C. elegans* rGCs act as a homodimer. However, it is now clear that ODR-1 and DAF-11 have an obligate heterodimeric structure. Therefore, other GCYs, such as GCY-4 and GCY-22 in ASER, expressed in the same neuron may also function as heterodimers. It is also apparent that rGCs such as GCY-14, GCY-8, and GCY-9 serve as direct sensor molecules for alkaline pH, temperature, and molecular $CO_2$, respectively. As the ECD of ODR-1/DAF-11 and GCY-12 is dispensable, in contrast, these rGCs serve to relay signals from GPCRs, although they may be direct sensors for other environmental cues.

Unlike other type-1 transmembrane receptors, such as receptor tyrosine kinases, rGCs insert the KHD between the TMD and the GCD, which plays a key role in regulation of GC activity through phosphorylation and binding of ATP. Reminiscent of the kinase domain dimer of the epidermal growth factor receptor, this KHD may have a stable homodimeric structure prior to ligand binding to the ECD, in which stability may depend on phosphorylation levels of the domain, to prevent the GCD dimer from taking a flexible, active structure. Ligand binding to the ECD seems to induce rotation (or twist) of the TMD parallel to the plane of the membrane, which may subsequently dissociate the KHD dimer for activation of the GCD. The actual, detailed mechanism of rGC activation by ligand must await determination of the KHD structure, interaction between the linker region and the GCD, and ultimately the structure of full-length rGC with and without bound ligand.

## AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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