Receptor-type guanylate cyclase is required for carbon dioxide sensation by Caenorhabditis elegans

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1073/pnas.1017354108">http://dx.doi.org/10.1073/pnas.1017354108</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>National Academy of Sciences (U.S.)</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Thu Jun 22 16:30:07 EDT 2017</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/64949">http://hdl.handle.net/1721.1/64949</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
Receptor-type guanylate cyclase is required for carbon dioxide sensation by Caenorhabditis elegans


is both a critical regulator of animal physiology and an important sensory cue for many animals for host detection, food location, and mate finding. The free-living soil nematode Caenorhabditis elegans shows CO₂ avoidance behavior, which requires a pair of ciliated sensory neurons, the BAG neurons. Using in vivo calcium imaging, we show that CO₂ specifically activates the BAG neurons and that the CO₂-sensing function of BAG neurons requires TAX-2/TAX-4 cyclic nucleotide-gated ion channels and the receptor-type guanylate cyclase GCY-9. Our results delineate a molecular pathway for CO₂ sensing and suggest that activation of a receptor-type guanylate cyclase is an evolutionarily conserved mechanism by which animals detect environmental CO₂.

CO₂ is both a critical regulator of animal physiology and an important sensory cue for many animals for host detection, food location, and mate finding. The free-living soil nematode Caenorhabditis elegans shows CO₂ avoidance behavior, which requires a pair of ciliated sensory neurons, the BAG neurons. Using in vivo calcium imaging, we show that CO₂ specifically activates the BAG neurons and that the CO₂-sensing function of BAG neurons requires TAX-2/TAX-4 cyclic nucleotide-gated ion channels and the receptor-type guanylate cyclase GCY-9. Our results delineate a molecular pathway for CO₂ sensing and suggest that activation of a receptor-type guanylate cyclase is an evolutionarily conserved mechanism by which animals detect environmental CO₂.

The ability to detect and respond to changing concentrations of environmental CO₂ is widespread among animals and plays a critical role in locating food, finding hosts and mates, and avoiding danger (1–4). CO₂ exposure can also have profound physiological effects, including altered respiration, motility, fecundity, and emotional state (5–7). CO₂ is sensed as an aversive cue by many free-living animals, including humans (3, 6, 8, 9). By contrast, many parasites and disease vectors are attracted to CO₂, which serves as a sensory cue for host location (1, 10).

Nematodes constitute a large and highly diverse phylum that includes both free-living and parasitic species. Many parasitic nematodes, including some of the most devastating human- and plant-parasitic nematodes, are attracted to CO₂. By contrast, adults of the free-living species Caenorhabditis elegans are repelled by CO₂ (11–14). CO₂ avoidance by C. elegans requires a pair of head neurons called the BAG neurons (13), which also mediate responses to decreases in ambient oxygen levels (15). Whether the BAG neurons directly sense CO₂ is not known, and the signaling pathways that mediate CO₂ detection are poorly understood.

We show here that environmental CO₂ specifically activates the BAG neurons and that other neurons that drive avoidance behavior, suggesting that the BAG neurons are primary sensory neurons that detect CO₂. Prolonged CO₂ exposure causes desensitization of avoidance behavior of the BAG neurons themselves, indicating that behavioral adaptation to CO₂ occurs at the level of the BAG neurons. In addition, we show that the CO₂-evoked activity of the BAG neurons requires a cGMP signaling pathway consisting of the receptor guanylate cyclase GCY-9 and the cGMP-gated cation channel TAX-2/TAX-4. Insects detect CO₂ using a pair of gustatory receptors (16, 17), whereas some mammals detect CO₂ using the receptor-type guanylate cyclase, guanylate cyclase D (GC-D), and soluble adenylate cyclase (18–20). Our results show that the mechanism of CO₂ detection in C. elegans more closely resembles that of mammals than insects and suggest an evolutionarily ancient role for receptor-type guanylate cyclases in mediating environmental CO₂ detection by animals.

Results

BAG Neurons Are Activated by CO₂. BAG neurons are located in the head, extend ciliated dendrites to the tip of the nose (21), and are required for avoidance of CO₂ across concentrations (Figs. S1 and S2). To determine whether the BAG neurons respond to environmental CO₂, we monitored the activity of BAG neurons using the genetically encoded ratiometric calcium indicator cameleon (22). The gcy-33 promoter was used to drive expression of cameleon specifically in the BAG neurons (23). We first confirmed that animals expressing gcy-33::cameleon show normal CO₂ avoidance behavior (Fig. S3). We then imaged these animals and found that CO₂ exposure evoked rapid and reversible calcium transients in the cell bodies of BAG neurons (Fig. 1A and Fig. S4). By contrast, other sensory neurons known to mediate C. elegans avoidance behavior, the amphid neurons ASH, ADL, and AWB, did not respond to a 10% CO₂ stimulus, indicating that the CO₂ response of BAG neurons is cell type-specific (Fig. 1B). This result suggests that the BAG neurons are the primary sensory neurons that detect CO₂.

Prolonged CO₂ Exposure Desensitizes BAG Neurons. Prolonged exposure to many sensory stimuli results in behavioral adaptation, which can occur either in the primary sensory neuron or in circuitry downstream of the sensory neuron (24–28). We tested whether CO₂ avoidance behavior adapts to prolonged CO₂ exposure by exposing animals to 1%, 5%, or 10% CO₂ for either 1 or 5 min and then testing their ability to respond to 5% or 10% CO₂ in an acute avoidance assay. We found that prolonged exposure to either 5% or 10% CO₂ greatly reduced subsequent behavioral responses to the same concentration of CO₂, and responses to 10% CO₂ were significantly decreased after a 1-min
exposure to 5% CO₂ (Fig. 2 A and B). Thus, *C. elegans* displays behavioral adaptation in response to prolonged CO₂ exposure.

To test whether behavioral adaptation to 5% or 10% CO₂ occurs at the level of the BAG neurons, we recorded the calcium response of BAG neurons evoked by prolonged exposure to CO₂. We found that a 1-min exposure to either 5% or 10% CO₂ caused an initial increase in BAG neuron calcium, which then dropped to below-baseline levels (Fig. 2 C and D). This drop in BAG neuron calcium was the result of desensitization; a 1-min exposure to 5% CO₂ blocked the calcium response to a subsequent 10% CO₂ stimulus (Fig. 2E). These results show that the BAG neurons desensitize during prolonged exposure to CO₂ and suggest that behavioral adaptation to CO₂ occurs at the level of the BAG sensory neuron.

cGMP-Gated Channel TAX-2/TAX-4 Is Required for BAG Neuron Responses to CO₂. CO₂ avoidance behavior by *C. elegans* requires multiple signaling pathways, including a cGMP, a G protein, an insulin, and a TGF-β pathway (13, 14). To test whether any of these pathways are required for the CO₂-evoked calcium response of the BAG neurons, we measured CO₂-evoked calcium transients of BAG neurons in animals that are mutant for these pathways and behaviorally defective in acute CO₂ avoidance. We found that mutations in *tax-2* and *tax-4*, which encode subunits of a cGMP-gated cation channel (29), eliminated the CO₂ calcium response of BAG neurons, indicating a direct requirement for TAX-2/TAX-4 in the signal transduction cascade that leads to CO₂ detection (Fig. 3).

We also found that G-protein signaling modulated BAG neuron responses to CO₂ mutation of *rgs-3*, which encodes a regulator of G protein signaling (30), resulted in reduced BAG neuron responses to CO₂ (Fig. 3). To test whether *rgs-3* functions in BAG neurons to regulate CO₂ responses, we expressed *rgs-3* specifically in the BAG neurons of *rgs-3* mutants and found that this expression restored normal CO₂ avoidance behavior (Fig. S5A). Because regulator of G protein signaling (RGS) proteins negatively regulate G-protein signaling (31), our results indicate that a G-protein signaling pathway negatively regulates BAG neurons.

A number of genes that are required for behavioral avoidance of CO₂ (13) were not required for CO₂-evoked calcium responses of the BAG neurons. These genes include *npr-1*, which

---

**Fig. 1.** BAG neurons are activated by CO₂. (A Left) BAG neuron cell bodies respond to CO₂. RR₀ is the YFP to CFP ratio (R) divided by the average YFP to CFP ratio of the first 10 frames (R₀). (A Right) Dose-response curve for CO₂. ΔR/ΔR_max is the maximal ratio change caused by presentation of a given CO₂ stimulus normalized to the maximal ratio change measured (evoked by 10% CO₂). The white area around each trace represents the SEM; the lower black traces indicate the stimulus onset and duration (n = 5–18 animals for each condition or genotype). For all graphs, error bars represent SEMs.

**Fig. 2.** Prolonged CO₂ exposure desensitizes BAG neurons. (A and B) Acute CO₂ avoidance after either a (A) 1-min or (B) 5-min exposure to CO₂. For each treatment condition, the stimulus used during the preexposure is indicated followed by the stimulus used for the acute avoidance assay (n = 11–15 trials for each treatment). (C and D) Prolonged pulses of either (C) 10% or (D) 5% CO₂ results in BAG neuron desensitization (n = 7 animals for each condition). (E) Prior exposure to 5% CO₂ blocks the response to 10% CO₂ (n = 7 animals). The white area around each trace represents the SEM; the lower black traces indicate the stimulus onset and duration.
encodes a G protein-coupled receptor similar to the neuropeptide Y receptor, daf-11, which encodes a receptor guanylate cyclase, daf-2, which encodes an insulin receptor, and daf-7, which encodes a TGF-β receptor. The BAG neurons of these mutants displayed robust calcium responses to CO₂, suggesting that these genes act either downstream of or in parallel to the calcium signal or that they act to regulate CO₂ avoidance in cells other than BAG neurons (Fig. 3). daf-7 was reported to be expressed specifically in the amphid neurons ASI (32, 33), and expression of daf-7 specifically in ASI neurons rescued the defect in CO₂ avoidance of daf-7 mutants (Fig. S5B). Thus, daf-7 acts in cells other than the BAG neurons to regulate acute CO₂ avoidance. Ablation of the ASI neurons does not affect CO₂ avoidance behavior (Fig. S5C), indicating that, unlike the BAG neurons, ASI neurons are not required for CO₂ detection. We also tested whether two BAG neuron-specific guanylate cyclases, gcy-31 and gcy-33, are required for CO₂-evoked BAG neuron activity. gcy-31 and gcy-33 encode soluble guanylate cyclases that are required for BAG neuron responses to acute hypoxia (15). We found that the BAG neurons of gcy-33; gcy-31 double mutants showed normal CO₂-evoked activity and that gcy-33; gcy-31 mutants had normal behavioral responses to CO₂. Therefore, the CO₂- and O₂-sensing functions of the BAG neurons require distinct signaling pathways (Fig. 3 and Fig. S6).

Transcriptional Profiling of Embryonic BAG Neurons Identifies Signaling Molecules That Might Function in CO₂ Sensation. To identify signaling pathways that function in BAG neurons in CO₂ sensing, we performed transcriptional profiling of embryonic BAG neurons using Affymetrix C. elegans tiling arrays (Fig. S7). We identified 850 mRNA transcripts that were significantly enriched in the BAG neurons relative to the aggregate of all other embryonic cells (Dataset S1). The BAG neuron transcriptional profile is consistent with the role of BAG as a sensory neuron: the most highly enriched gene ontology terms include chemotaxis, response to external stimuli, response to chemical stimulus, and signaling [false discovery rate (FDR) ≤ 1%] (Fig. 4). BAG-enriched transcripts include a number of genes reported to be specifically expressed by BAG neurons (gcy-31, gcy-33, and flp-17) and genes with known roles in CO₂ sensing by BAG neurons (tax-4 and tax-2) (13, 15, 34).

Among the genes most highly enriched in the BAG neurons is gcy-9 (Fig. 4), which encodes a receptor-type guanylate cyclase (23). Nothing was previously known about the function of gcy-9, and reporter gene constructs for gcy-9 resulted in either no expression or variable nonneuronal expression (23, 35). We found that gcy-9 shows an approximately ninefold enrichment in BAG neurons relative to other embryonic cells, suggesting a role for gcy-9 in BAG neuron function.

**gcy-9 Is Required for BAG Neurons to Respond to CO₂.** We examined the behavioral response of gcy-9 mutants to CO₂ and found that gcy-9 mutants did not respond to CO₂ (Fig. S4 and Fig. S8A and B). To test whether gcy-9 is required for CO₂-evoked activity of BAG neurons, we imaged the BAG neurons of gcy-9 mutants. We found that the BAG neurons of gcy-9 mutants did not show CO₂-evoked calcium transients, showing that gcy-9 is necessary for BAG neuron responses to CO₂ (Fig. 5B). Expression of WT gcy-9 specifically in the BAG neurons of gcy-9 mutants rescued the defect in CO₂ avoidance behavior of gcy-9 mutants and partially rescued the defect in CO₂-evoked neural activity of the BAG neuron (Fig. 5C), showing that gcy-9 functions cell autonomously in CO₂ detection by the BAG neurons. Mutations in other guanylate cyclase genes did not affect acute CO₂ avoidance.
Thus, gcy-9 is specifically required for CO₂-evoked BAG neuron activity. Taken together, our results suggest that GCY-9 acts upstream of TAX-2/TAX-4 in BAG neurons to mediate CO₂ detection.

**Discussion**

Our data indicate that the BAG neurons are activated by CO₂ and are likely the primary sensory neurons that detect CO₂. BAG neurons are sensitive detectors of CO₂, responding to CO₂ concentrations as little as twofold above the ambient level of ∼0.04%. Behavioral adaptation to environmental CO₂ is also mediated by the BAG neurons, which desensitize during prolonged CO₂ stimuli.

The CO₂ avoidance circuit is modulated by input from other sensory neurons. Our data show that *daf-7* is required in ASI neurons for CO₂ avoidance behavior, suggesting that sensory cues detected by ASI regulate CO₂ avoidance. Because the BAG neurons of *daf-7* mutants respond normally to CO₂, it is possible that ASI acts on the neural circuit that mediates CO₂ avoidance downstream of the BAG neurons. This circuit remains to be defined. The primary synaptic output of the BAG neurons is onto five interneurons: the ventral cord interneurons AVA and AVE and the ring interneurons RIA, RIB, and RIG (21). In preliminary studies, we did not observe CO₂-evoked responses in the cell bodies of these interneurons. However, we cannot exclude the possibility that CO₂-evoked responses might be restricted to the processes of interneurons, which has been reported for interneurons in thermotaxis and chemotaxis circuits of *C. elegans* (36, 37).

It is also possible that the CO₂ avoidance circuit involves extrasynaptic signaling (for example, through neuropeptide secretion from BAG). Our analysis of BAG neuron transcripts identified more than a dozen neuropeptides that show enriched expression in the BAG neurons (Dataset S1). A role for neuropeptide signaling in the regulation of egg laying by the BAG neurons has been shown: the FMRF-amide like neuropeptide FLP-17 is secreted by the BAG neurons and acts on the G protein-coupled receptor EGL-6 on the HSN hermaphrodite-specific motor neurons to inhibit egg laying (34). FLP-17 peptides are not restricted to the processes of interneurons, which has been reported for interneurons in thermotaxis and chemotaxis circuits of *C. elegans* (36, 37).

How do BAG neurons detect CO₂? CO₂ detection by insects requires the gustatory receptors GR21a and GR63a, which seem
to act through a G-protein signaling pathway (16, 17, 38). By contrast, mammalian olfactory receptor neurons that respond to CO2 express the receptor-type guanylate cyclase GC-D (8), which is activated by the CO2 metabolite bicarbonate (20, 39). Because CO2 sensing by the BAG neurons of C. elegans requires the receptor-type guanylate cyclase GCY-9, we propose that GCY-9 might function as a receptor for CO2 or a metabolite of CO2. GCY-9 is one of a large number of C. elegans guanylate cyclases (Fig. S10), many of which are expressed in small subsets of sensory neurons and are required for specific chemosensory behaviors (23, 29). Although some GCY proteins seem to function as chemoreceptors, others have been proposed to function downstream of G protein-coupled receptors (23, 40–42). It is, therefore, also possible that GCY-9 acts downstream of a yet to be identified receptor for CO2.

GCY-9 orthologs are present in multiple nematode species, including the plant-parasitic nematodes Heterodera glycines and Meloidogyne incognita (43) and the human-parasitic nematode Brugia malayi (44). The requirement for GCY-9 in mediating a behavioral response to CO2 and the importance of CO2 as a host-seeking cue for many parasitic nematodes raise the possibility that compounds that block GCY-9 activity might be useful in the development of strategies for nematode control. The possibility that compounds that block GCY-9 activity might be useful in the development of strategies for nematode control.

Materials and Methods

Standard techniques are listed in SI Materials and Methods.

Behavioral Assay for Acute CO2 Avoidance. CO2 assays were performed as previously described (13). Briefly, for each assay, about 10–20 L4 hermaphrodites were placed on 5-cm assay plates overnight and tested as young adults. Plates consisted of nematode growth medium (NGM) agar seeded with a thin lawn of Escherichia coli OP50 bacteria. Gases were medical grade certified mixes (Air Liquid) consisting of the indicated CO2 concentration, 10% O2, and the remaining percentage of N2. A concentration of 10% CO2 was used unless otherwise indicated. Two 50-ml syringes were filled with gas, one with CO2 and one without CO2. The mouths of the syringes were connected to tubes attached to Pasteur pipettes, and gases were pumped through the Pasteur pipettes using a syringe pump at a rate of 1.5 ml/min. Worms were exposed to gases by placing the tip of the Pasteur pipette near the head of a forward-moving worm, and a response was scored if the worm reversed within 4 s. An avoidance index was then calculated by subtracting the fraction of animals that reversed in response to the air control from the fraction of animals that reversed in response to the CO2 (Fig. S2A).

Behavioral Assay for Adaptation to CO2. For each assay, about five L4 hermaphrodites were placed on 9-cm assay plates overnight and tested as young adults. Plates consisted of NGM agar seeded with a thin lawn of OP50 in the center of the plate. Gas was pumped into the plate through a hole on one side of the lid at a rate of 1 L/min; a hole on the other side allowed gas to escape. Worms were preexposed to gas for either 1 or 5 min and then tested immediately in an acute avoidance assay.

Image Analysis. The mean pixel value of a background region of interest was subtracted from the mean pixel value of a region of interest that circumscribed the specimen. A correction factor, which we measured in images of samples that express only CFP, was applied to the YFP channel to compensate for bleed through of CFP emissions into the YFP channel (YFP adjusted = YFP – 0.86 × CFP). YFP to CFP ratios were normalized to the average value of the first 10 frames (1 s), and a boxcar filter of 5 frames (0.5 s) was applied to the time series.

Generating a Gene Expression Profile of Embryonic BAG Neurons. A synchronized population of nls2422[gcy-33::GFP] hermaphrodites was treated with hypochlorite to release embryos. Embryos were dissociated with chitinase as previously described (45, 46). GFP-labeled BAG neurons were isolated from the freshly dissociated suspension of embryonic cells by FACS; sorted cells were confirmed to be >80% BAG neurons by direct inspection in a Fluorescence microscope. A reference population comprised of all viable embryonic cells was also collected by FACS. Dead cells were marked with propidium iodide and excluded from these preparations. Sorting was performed with a Becton-Dickinson FACSAria (75-μm nozzle; ~15,000 events/s). Approximately 30,000 cells were obtained per sort. Cells were sorted directly into TRIzol LS for extraction with phenol chloroform. RNA was precipitated with iodoacetamide and concentration was evaluated using an Agilent Bioanalyzer. RNA (1–2 ng) was amplified with the WT-Ovation Pico RNA Amplification System (NuGEN). Double-stranded (ds) cDNA was generated with a WT-Ovation exon module (NuGEN), and it was fragmented and labeled with an Encore Biotin module (NuGEN) for application to the C. elegans Affymetrix 1.0R whole-genome tiling array; ds cDNA targets were used for hybridization, because all probes on the Affymetrix 1.0R array match a single DNA strand, whereas 10 target sites are derived from both strands. Tiling array results were obtained from three independent replicates (inter Pearson correlations ≥ 0.89). Methods for tiling array analysis are briefly summarized here. A more detailed de-
scription will be reported elsewhere. Unique PM (Perfect Match) probes from exonic regions of gene models were selected to generate a probe set for use with the modENCODE Base (WS1919). Intensity values were quantile-normalized, and probe-specific effects were reduced by Robust Multichip Analysis (RMA) (47). An empirical null model of background expression was derived from intergene probes, and genes with intensity values exceeding this threshold at ≤5% FDR were scored as significantly expressed genes (EGs). Transcripts that were significantly elevated in BAG neurons were identified by comparison with a reference dataset obtained from all viable embryonic cells. Differentially expressed genes were estimated using a linear model and Bayes-modified t statistic (48, 49); 850 transcripts with FDR ≤10% and 1.5-fold elevated vs. the reference dataset were scored as significantly enriched in BAG neurons (Dataset S1). Gene ontology analysis was performed with the gene ontology (GO) enrichment analysis widget on the modENCODE intermine website (http://intermine.modencode.org). The 850 enriched BAG transcripts were reviewed in WormBase on July 5, 2010; 464 transcripts were annotated with GO terms and compared with all genes for enrichment using the hypergeometric test with FDR ≤1%. All transcripts annotated with enriched GO terms are listed in Dataset S2.

Statistical Analysis. Statistical tests were performed using GraphPad Instat. Statistical comparisons were made using a one-way ANOVA with Dunnett’s posttest, except that Fig. 1A used a paired t test and Fig. 5A used an unpaired t test. For Fig. 1C, the dose-response curve shows the least squares fit of an Hill equation to the data points, with a K_0 of 0.9% CO₂ and a Hill coefficient of 1.6 (for all graphs, ***p < 0.001, **p < 0.01, and *p < 0.05). The GCY dendrogram was generated using ClustalW. The gcy-9 intron-exon structure was generated using Exon-Intron Graphic Maker by Nikhil Bhatia (http://www.wormweb.org/exonintron).

ACKNOWLEDGMENTS. We thank Araví Samuel, Chris Gabel, and Harrison Gabel (Harvard University, Cambridge, MA); Cori Bargmann (Rockefeller University, New York); Leon Avery (University of Texas Southwestern Medical Center, Dallas); Ikue Morii and Katsunori Kuhara (Nagoya University, Nagoya, Japan); Denise Ferrey (SUNY Buffalo, Buffalo, NY); Larry Salkoff (Washington University School of Medicine, St. Louis); Shohei Mitani (Tokyo Women's Medical University School of Medicine, Tokyo); Anne Hart (Brown University, Providence, RI); and the Caenorhabditis Genetics Center for strains and reagents. We thank the Caenorhabditis Genetics Center, the Oregon Health and Science University Cell Bank, and Shared GEF Research Samples (National Institutes of Health, Bethesda, MD) for providing reagents. We thank the Howard Hughes Medical Institute for financial support. This work was supported by a Helen Hay Whitney postdoctoral fellowship (to E.A.H.), a National Institutes of Health Pathway to Independence award (to E.A.H.), National Institutes of Health Grants U01 HG004263 (to D.M.M.), R01 NS26115 (to D.M.M.), and R01 GM24663 (to H.R.), the Howard Hughes Medical Institute (P.W.S.), a Whitehead Fellowship for Junior Faculty in Biomedical and Biological Sciences (to N.R.), and funds from the Helen L. and Martin S. Kimmel Center for Biology and Medicine (to N.R.).
