Acute carbon dioxide avoidance in *Caenorhabditis elegans*

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Carbon dioxide is produced as a by-product of cellular respiration by all aerobic organisms and thus serves for many animals as an important indicator of food, mates, and predators. However, whether free-living terrestrial nematodes such as *Caenorhabditis elegans* respond to CO_2 was unclear. We have demonstrated that adult *C. elegans* display an acute avoidance response upon exposure to CO_2 that is characterized by the cessation of forward movement and the rapid initiation of backward movement. This response is mediated by a cGMP signaling pathway that includes the cGMP-gated heteromeric channel TAX-2/TAX-4. CO_2 avoidance is modulated by multiple signaling molecules, including the neuropeptide Y receptor NPR-1 and the calcineurin subunits TAX-6 and CNB-1. Nutritional status also modulates CO_2 responsiveness via the insulin and TGFβ signaling pathways. CO_2 response is mediated by a neural circuit that includes the BAG neurons, a pair of sensory neurons of previously unknown function. TAX-2/TAX-4 function in the BAG neurons to mediate acute CO_2 avoidance. Our results demonstrate that *C. elegans* senses and responds to CO_2 using multiple signaling pathways and a neural network that includes the BAG neurons and that this response is modulated by the physiological state of the worm.

behavioral genetics | cGMP signaling | chemosensation

Carbon dioxide is a critical sensory cue for organisms as diverse as humans (1), rodents (2, 3), insects (4, 5), and bacteria (6). The ability to rapidly detect and respond to changing CO_2 concentrations is important for survival: high CO_2 concentrations cause hypoxia, anesthetization, and death. CO_2 perception may be particularly important for soil-dwelling organisms such as *Caenorhabditis elegans*, because bacteria-rich soil microenvironments are often subject to large fluctuations in CO_2 and O_2 levels (7). However, despite the fundamental importance of CO_2 for all living organisms, remarkably little is known about how CO_2 evokes behavioral responses in animals.

Many mammals, including humans, are capable of detecting CO_2. Humans detect CO_2 as a trigeminal stimulus, and different humans show different sensitivities to CO_2 (1). Mice detect and avoid near-atmospheric concentrations of CO_2 via a subset of olfactory sensory neurons that project to the necklace glomeruli in the olfactory bulb (2). Many insects also detect CO_2. For example, *Drosophila* is repelled by CO_2 via a single class of olfactory receptor neurons on the antennae (8), whereas mosquitoes are attracted to CO_2 via olfactory receptor neurons on the maxillary palps (9).

The phylum Nematoda contains both free-living and parasitic species, some of which have been shown to respond to CO_2. Many animal-parasitic nematodes, including the human-parasitic nematodes *Ancylostoma duodenale* and *Necator americanus*, are attracted to CO_2; for these nematodes, CO_2 exhaled by their hosts serves as a host-localization and host-invasion cue (10, 11). Similarly, many plant-parasitic nematodes use CO_2 emission by plant roots for host localization (12, 13). Some species of free-living marine nematodes are also attracted to CO_2, which is used as a sensory cue to locate the decaying animal and plant carcasses that serve as their food sources (14). However, nothing is known about the mechanisms underlying CO_2 perception in these nematodes, and whether free-living terrestrial nematodes respond to CO_2 was unclear (10, 15, 16).

The well studied model *C. elegans* is a free-living terrestrial nematode that navigates through its environment using a combination of chemosensory, thermosensory, and mechanosensory cues. The nervous system of the *C. elegans* hermaphrodite consists of 302 neurons, >10% of which are chemosensory (17, 18). The perception of volatile and water-soluble chemicals is mediated primarily by 11 pairs of chemosensory neurons that extend ciliated dendrites into the paired amphid sensilla of the head (17). *C. elegans* also senses O_2 via two ciliated and two unciliated sensory neurons that extend dendrites into the pseudocoelomic body fluid (17, 19).

We demonstrate that *C. elegans* display an acute avoidance response to CO_2. We then identify signaling pathways that affect CO_2 avoidance by conducting a screen of existing mutants with neurosensory defects. We find that CO_2 avoidance is mediated by cGMP signaling and requires the receptor guanylyl cyclase DAF-11 and the cGMP-gated channel TAX-2/TAX-4. CO_2 response is modulated by multiple neuronal regulatory molecules. Nutrient deprivation decreases CO_2 avoidance, and this response is mediated by the insulin and TGFβ pathways. Finally, acute CO_2 avoidance is mediated primarily by the paired BAG neurons of the head, and cGMP signaling is required in the BAG neurons to mediate CO_2 avoidance.

Results

An Acute Carbon Dioxide Response in *C. elegans*. To determine whether *C. elegans* responds to CO_2, we developed a CO_2 avoidance assay based on the osmotic avoidance assay (20, 21). Specifically, the head of a forward-moving worm is exposed to an air stream containing CO_2, and a response is scored if the worm reverses direction within 4 seconds [Fig. 1*A* and supporting information (SI) Movies S1 and S2]. Reversals are characteristic of avoidance responses in *C. elegans*: exposure to 1-octanol, hyperosmolarity, and nose touch elicit rapid reversals (17, 22, 23).

Wild-type N2 worms respond to CO_2; for example, 76% reverse in response to an air stream containing 10% CO_2, whereas only 23% reverse when the air stream does not contain CO_2 (Table S1). We calculated an avoidance index (a.i.) for CO_2 by subtracting the fraction of worms that reversed in response to an air stream that does not contain CO_2 from the fraction that reversed in response to an air stream containing CO_2; N2 worms show an a.i. of 0.53 in response to 10% CO_2 (Fig. 1*B*).

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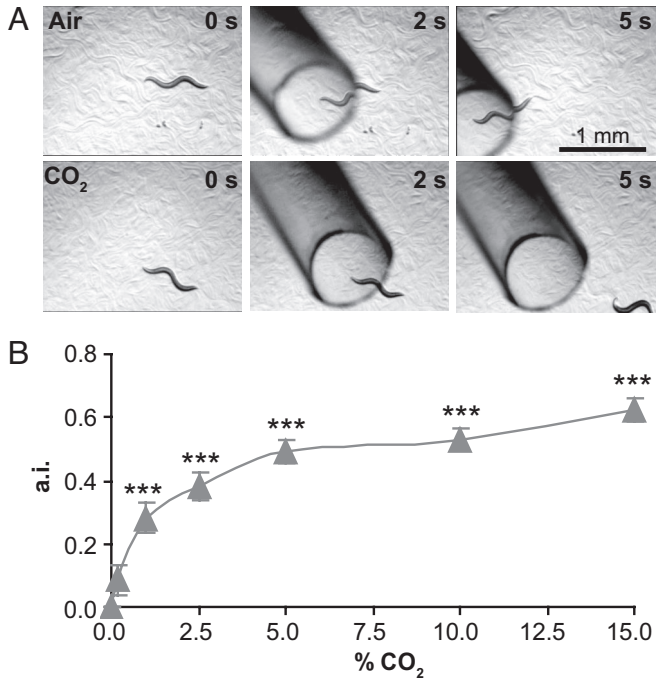

Fig. 1. Acute CO_2 avoidance in *C. elegans*. (*A*) When the head of a forward-moving N2 worm is exposed to an air mixture, the worm continues forward locomotion (*Upper*). When the worm is exposed to 10% CO_2, the worm halts forward locomotion and rapidly reverses (*Lower*). (*B*) The response of N2 worms to CO_2 is dose-dependent. ***, $P < 0.001$. $n = 22–44$ trials. For all graphs, error bars represent SEM.

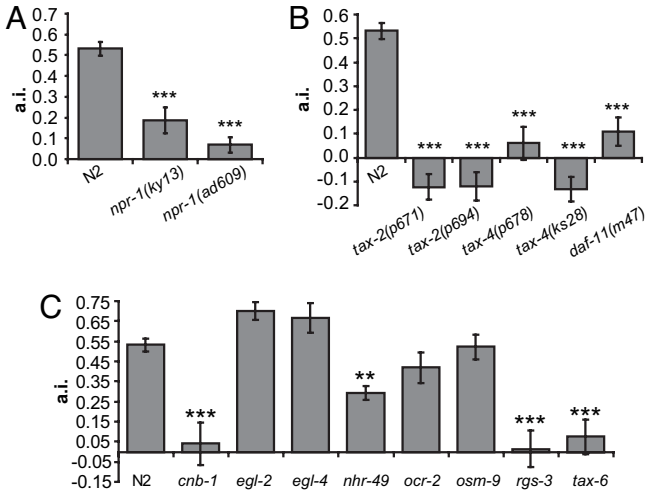

Fig. 2. Signaling pathways that affect acute CO_2 avoidance. (*A*) NPR-1 modulates CO_2 response. *npr-1(ky13)* hypomorphs show greatly reduced CO_2 avoidance, and *npr-1(ad609)* null mutants show essentially no CO_2 avoidance. ***, $P < 0.001$. $n = 12–44$ trials. For all graphs, error bars represent SEM. (*B*) cGMP signaling is required for CO_2 response. Mutants of *tax-2*, *tax-4*, and *daf-11* do not show acute CO_2 avoidance. ***, $P < 0.001$. $n = 11–44$ trials. (*C*) The *cnb-1*, *rgs-3*, and *tax-6* mutants do not show acute CO_2 avoidance, whereas the *nhr-49* mutant shows reduced CO_2 avoidance. **, $P < 0.01$; ***, $P < 0.001$. $n = 11–44$ trials. Data for N2 are from Fig. 1.

C. elegans responds to CO_2 in a dose-dependent manner (Fig. 1*B*). The response to 1% CO_2 was significantly different from the response to 0% CO_2, demonstrating that *C. elegans* can detect CO_2 concentrations as low as 1% above ambient levels (Fig. 1*B*). The average atmospheric concentration of CO_2 is ≈0.04%. A CO_2 concentration of 10% was used for subsequent experiments because of the robust response to this concentration.

Acute CO_2 Avoidance Varies Among Strains of *C. elegans* and Species of Free-Living Nematodes. We next asked whether CO_2 aversion is conserved across six strains of *C. elegans* (24). Strains N2, CB3191, and TR389 show robust CO_2 avoidance, whereas strains AB1, CB4853, and CB4856 are essentially unresponsive to CO_2 in this assay (Fig. S1*A*). This suggests that CO_2 avoidance is a rapidly evolving behavior. Different strains of *C. elegans* have been isolated from diverse ecological niches (25, 26), raising the possibility that this behavior may be advantageous in only some niches.

We then examined CO_2 avoidance in five phylogenetically and ecologically diverse species of nematodes from the order Rhabditida (27). *C. elegans* and *Pristionchus pacificus* show robust CO_2 avoidance, whereas *Caenorhabditis briggsae*, *Caenorhabditis* species 3, and *Panagrellus redivivus* show little or no CO_2 avoidance (Fig. S1*B*). Thus, acute CO_2 avoidance is found in some but not all free-living nematode species. However, species that do not avoid CO_2 in our assay may exhibit CO_2 responses under conditions not tested here.

The Neuropeptide Y Receptor NPR-1 Modulates CO_2 Avoidance. Different wild isolates of *C. elegans* show polymorphic feeding behavior: some are solitary feeders that disperse on bacterial lawns, and others are social feeders that aggregate into clumps at the edge of the bacterial lawn (28). The three CO_2-sensitive strains of *C. elegans* are solitary feeders, whereas the three CO_2-insensitive strains are social feeders (Fig. S1*A*) (28). Thus, solitary feeding correlates with CO_2 avoidance, suggesting that both behaviors may be subject to common regulatory mechanisms.

Feeding behavior is modulated by the neuropeptide Y receptor gene *npr-1*: loss-of-function mutations in *npr-1* can convert a solitary strain into a social strain (28). We therefore investigated whether *npr-1* also modulates acute CO_2 avoidance. A hypomorphic mutation in *npr-1* in an N2 background results in reduced CO_2 avoidance, and a null mutation eliminates acute CO_2 avoidance (Fig. 2*A*). Thus, *npr-1* modulates CO_2 avoidance.

Differences in feeding behavior among strains appear to be due to differences in O_2 response, and *npr-1* mutants display altered O_2 preference in the presence of food (29). The fact that O_2 and CO_2 responses are both modulated by NPR-1 raises the possibility that the same receptor proteins confer responses to both gases. O_2 receptors in *C. elegans* comprise a family of soluble guanylyl cyclases (sGCs) (29, 30). However, sGC mutants respond normally to CO_2 (Fig. S2*A*). Moreover, mutation of the transcription factor AHR-1, which regulates expression of the sGC genes, does not affect CO_2 avoidance (Fig. S2*A*). Thus, CO_2 and O_2 response are conferred by different receptors despite being subject to the same neuromodulatory control by *npr-1*.

Acute CO_2 Avoidance Is Mediated by cGMP Signaling. To identify the signaling pathways that mediate CO_2 avoidance, we screened candidate mutants that had been previously isolated and that display a wide variety of defects in neuronal development and function. TAX-2 and TAX-4 are subunits of a cGMP-gated channel required for normal chemosensory and thermosensory responses (17). We found that mutations in *tax-2* and *tax-4* eliminate acute CO_2 avoidance (Fig. 2*B*). Also, mutation of the receptor guanylyl cyclase DAF-11 eliminates CO_2 response (Fig. 2*B*). Thus, a cGMP signaling pathway(s) that includes DAF-11, TAX-2, and TAX-4 is required for acute CO_2 avoidance.

Multiple Signaling Molecules Modulate Acute CO_2 Avoidance. Our screen of candidate genes also identified a number of additional

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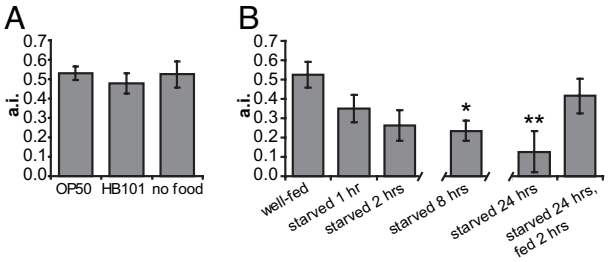

Fig. 3. The response to CO_2 is modulated by starvation. (*A*) Avoidance of CO_2 is not modulated by the presence of food. No significant differences were observed between worms tested on OP50, the *Escherichia coli* strain typically used as a food source for *C. elegans*; HB101, a different strain of *E. coli*; or off food. $n = 12–44$ trials. For all graphs, error bars represent SEM. The data for N2 tested on OP50 are from Fig. 1. (*B*) Starvation results in a reversible decrease in CO_2 avoidance. *, $P < 0.05$; **, $P < 0.01$. $n = 7–13$ trials. As a control for the 24-h time point off food, N2 worms were left on assay plates for an additional 24 h and then tested on food; no difference was observed between these worms and worms left on assay plates overnight and then tested.

signaling molecules that modulate CO_2 avoidance: *tax-6*, *cnb-1*, *rgs-3*, and *nhr-49*. The calcineurin subunits TAX-6 and CNB-1 are required for CO_2 avoidance: *tax-6* and *cnb-1* mutants are unresponsive to CO_2 in our assay (Fig. 2*C*). These results suggest that CO_2 response requires calcium signaling.

rgs-3 mutants fail to avoid CO_2 (Fig. 2*C*). RGS-3 is a regulator of G protein signaling that is expressed in nine types of sensory neurons: ASH, ADL, AWB, AWC, ASI, ASJ, ASK, PHA, and PHB (31). The fact that *rgs-3* mutants do not show acute CO_2 avoidance suggests that CO_2 response is modulated by signaling through G proteins acting in one or more of the neurons that express *rgs-3*. Animals with mutations in individual G protein subunits, as well as *gpa-1 gpa-2 gpa-3* triple mutants, responded normally to CO_2 (Fig. S2*B*), suggesting that multiple G proteins act redundantly to regulate CO_2 response.

Mutation of the nuclear hormone receptor gene *nhr-49* results in reduced CO_2 avoidance (Fig. 2*C*). NHR-49 regulates transcriptionally the response to starvation, including expression of fat and energy metabolism genes (32). Thus, acute CO_2 avoidance and the starvation response share a common regulatory mechanism involving *nhr-49*.

Mutants defective in the synthesis and reception of nonessential excitatory neurotransmitters, as well as mutants defective in neuropeptide synthesis and secretion, respond normally to CO_2 (Fig. S2 *C* and *D*). CO_2 avoidance may be mediated by both neuromodulators and neurotransmitters or by multiple neuromodulators or neurotransmitters acting in parallel.

Starvation Modulates CO_2 Sensitivity in *C. elegans*. The regulation of CO_2 response by NHR-49 raised the possibility that starvation affects CO_2 avoidance. We therefore first asked whether CO_2 avoidance is affected by the presence of bacterial food. Many sensory behaviors in *C. elegans* are affected by food, including O_2 aerotaxis and 1-octanol avoidance (29, 33). However, worms show equally robust CO_2 avoidance in the presence and absence of food (Fig. 3*A*). We then asked whether CO_2 avoidance is modulated by starvation. Well fed worms tested off food showed an a.i. of 0.53; however, after 24 h of food deprivation the a.i. was reduced to 0.13 (Fig. 3*B*). When animals deprived of food for 24 h were placed back on food for 2 h, the a.i. was restored to the level of well fed animals (Fig. 3*B*). Thus, CO_2 response is reversibly modulated by nutritional status.

The Insulin and TGFβ Pathways Modulate CO_2 Avoidance. The insulin and TGFβ pathways are key regulators of starvation in *C. elegans* (34). Given that starvation reduces CO_2 avoidance, we asked

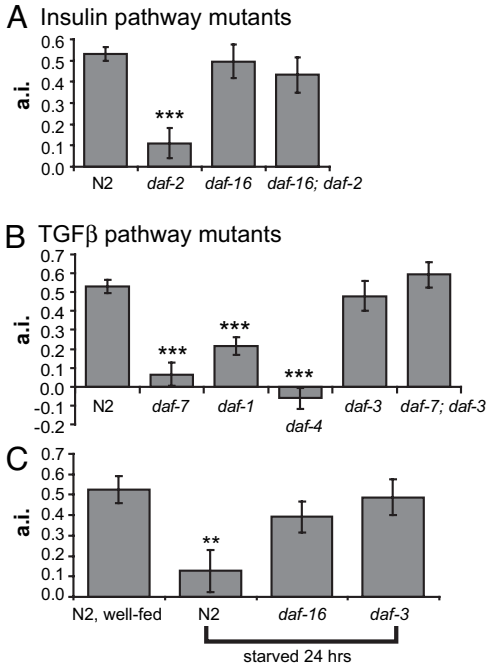

Fig. 4. Insulin and TGFβ signaling modulate CO_2 response. (*A*) *daf-2(e1370)* mutants do not respond to CO_2. This defect is rescued by mutation of *daf-16*. ***, $P < 0.001$. $n = 12–44$ trials. For all graphs, error bars represent SEM. (*B*) The TGFβ pathway mutants *daf-7*, *daf-1*, and *daf-4* show little or no CO_2 avoidance. The *daf-7* phenotype is rescued by mutation of *daf-3*. ***, $P < 0.001$. $n = 12–44$ trials. Data for N2 are from Fig. 1. (*C*) Epistatic interactions between insulin and TGFβ signaling and starvation. A 24-h starvation decreases acute CO_2 avoidance. The *daf-16(mgDf47)* and *daf-3(e1376)* mutations restore CO_2 response to starved animals, indicating that starvation acts via the insulin and TGFβ pathways. **, $P < 0.01$. $n = 7–15$ trials. Error bars represent SEM. Data for N2 are from Fig. 3.

whether insulin and TGFβ signaling might also affect CO_2 response. Mutation of the insulin receptor DAF-2 eliminates CO_2 avoidance, and mutation of the forkhead transcription factor DAF-16 suppresses the *daf-2* phenotype (Fig. 4*A*). Thus, DAF-16 acts downstream of DAF-2 in the regulation of CO_2 response. Also, different alleles of *daf-2* confer different CO_2 sensitivities (Fig. S3*A*). The strength of the different *daf-2* alleles with respect to CO_2 avoidance correlates with the strength of the alleles with respect to hypoxia resistance (Fig. S3*B*) (35) but not lifespan and dauer formation (36, 37), suggesting that CO_2 avoidance and hypoxia resistance may be subject to similar mechanisms of regulation by *daf-2*.

The TGFβ pathway also mediates acute CO_2 avoidance: mutations in the TGFβ ligand DAF-7, as well as the TGFβ receptors DAF-1 and DAF-4, show severely reduced CO_2 avoidance (Fig. 4*B*). The *daf-7* phenotype is rescued by mutation of the SMAD gene *daf-3*, demonstrating that DAF-3 acts downstream of DAF-7 in the regulation of CO_2 avoidance (Fig. 4*B*). DAF-7 is thought to be expressed specifically in the ASI chemosensory neurons (38). However, ablation of the ASI neurons did not affect CO_2 avoidance (data not shown). This may be because *daf-7* expression in ASI is required for CO_2 response only transiently during early development or because *daf-7* is expressed at low levels in other cells required for CO_2 response.

To investigate the epistatic relationship between starvation and insulin and TGFβ signaling, we examined whether starved *daf-16* and *daf-3* mutants respond to CO_2. In contrast to wild type, *daf-16* and *daf-3* mutants that had been starved for 24 h responded normally to CO_2 (Fig. 4*C*). Thus *daf-16* and *daf-3* rescue the CO_2 response defect of starved worms. Starvation

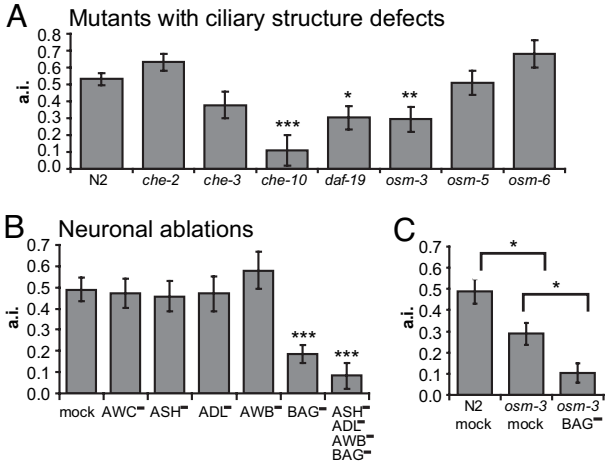

Fig. 5. CO_2 response is mediated primarily by the BAG neurons. (*A*) Among mutants with defects in ciliary structure, *osm-3* and *daf-19* mutants show reduced CO_2 avoidance, whereas *che-10* mutants show essentially no CO_2 avoidance. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. $n = 11$–44 trials. For all graphs, error bars represent SEM. Data for N2 are from Fig. 1. (*B*) BAG-ablated animals show greatly reduced CO_2 avoidance, whereas mock-, AWC-, ASH-, ADL-, and AWB-ablated animals respond normally to CO_2. No significant difference was observed between BAG-ablated animals and animals in which ASH, ADL, AWB, and BAG neurons were ablated. ***, $P < 0.001$. $n = 13$–36 animals for each condition. (*C*) *osm-3* mock-ablated animals show reduced CO_2 avoidance compared with N2 mock-ablated animals, and *osm-3* BAG-ablated animals show reduced CO_2 avoidance compared with *osm-3* mock-ablated animals. Data for N2 mock-ablated are from *B*. No significant difference was observed between N2 BAG-ablated and *osm-3* BAG-ablated animals. *, $P < 0.05$. $n = 22$–33 animals for each condition.

therefore modulates CO_2 response via the insulin and TGFβ pathways.

CO_2 Response Is Mediated by a Neural Circuit That Includes the BAG Neurons. To gain insight into the neural circuitry underlying CO_2 perception, we examined the CO_2 sensitivity of mutants with sensory neuron defects. We first tested mutations that affect the development of ciliated sensory neurons. *osm-3* and *daf-19* mutants showed reduced CO_2 response, whereas a *che-10* mutant was essentially unresponsive to CO_2 (Fig. 5*A*). *osm-3* encodes a kinesin subunit required for normal formation of amphid cilia, and *daf-19* encodes an RFX transcription factor required for the formation of all sensory cilia (39). These mutants implicate ciliated sensory neurons in CO_2 avoidance. A *che-10* mutation causes degeneration of amphid and phasmid neurons; however, the IL1, OLQ, and BAG neurons are also affected (40). These results suggest that one or more of the amphid or phasmid neurons, as well as one or more of IL1, OLQ, and BAG, play a role in CO_2 perception. Mutations that affect development of specific subsets of sensory neurons do not affect acute CO_2 avoidance (Fig. S4).

We also tested the *tax-2* allele *tax-2(p694)*, a *cis*-regulatory mutation that disrupts *tax-2* expression in the AQR, AFD, ASE, and BAG neurons (19). *tax-2(p694)* mutants do not respond to CO_2 in our assay (Fig. 2*B*). Because mutations and transgenes that compromise the function of AQR, AFD, and ASE respond normally to CO_2 (Fig. 6 and Fig. S4), *tax-2* is likely required in the BAG neurons (Fig. S5) for acute CO_2 avoidance.

To further test the role of the BAG neurons in CO_2 response, we ablated them using a laser microbeam and measured the ability of ablated worms to respond to CO_2. As a control, we ablated the AWC olfactory neurons, which mediate attraction (17). Ablation of the BAG neurons resulted in greatly reduced CO_2 avoidance (Fig. 5*B*). By contrast, mock-ablated and AWC-ablated animals responded normally to CO_2 (Fig. 5*B*). Therefore, the BAG neurons are important components of the neural circuit that mediates CO_2 response.

The fact that CO_2 response is severely reduced but not completely eliminated in BAG-ablated animals suggests that other sensory neurons play a role in acute CO_2 avoidance. In an attempt to identify these neurons, we ablated the ASH, ADL, and AWB neurons, which mediate olfactory repulsion (17). However, ablation of these neuron pairs individually did not affect acute CO_2 avoidance, and ablation of the ASH, ADL, AWB, and BAG neurons in the same animal resulted in a response that was not significantly different from the response of BAG-ablated animals (Fig. 5*B*). Thus ASH, ADL, and AWB play at most a minor role in acute CO_2 avoidance.

We then ablated the BAG neurons in *osm-3* mutants. We found that the response of mock-ablated *osm-3* mutants is reduced compared with mock-ablated wild-type animals, and the response of BAG-ablated *osm-3* mutants is further reduced (Fig. 5*C*). These results suggest that, in addition to BAG, one or more of the ciliated sensory neurons affected by the *osm-3* mutation play a role in CO_2 avoidance.

We note that acute CO_2 avoidance could be either a chemosensory or a nociceptive response. However, chemical nociception is mediated primarily by the ASH neurons (17), and ASH-ablated animals respond normally to CO_2 (Fig. 5*B*). Thus, acute CO_2 avoidance is likely to be a chemosensory response.

Finally, we generated dose–response curves for four mutants that showed defective CO_2 avoidance when tested with 10% CO_2: *osm-3* and *nhr-49*, which showed reduced avoidance of 10% CO_2; and *tax-4* and *npr-1*, which failed to avoid 10% CO_2. We found that the CO_2 response of all four mutants is defective across a broad range of concentrations (Fig. S6). These results suggest that acute CO_2 avoidance is mediated by the same signaling mechanisms across concentrations.

cGMP Signaling Is Required in the BAG Neurons for Acute CO_2 Avoidance. *tax-2* and *tax-4* are coexpressed in 12 neurons: AWC, AFD, ASE, ASG, ASJ, ASI, AWB, ASK, BAG, AQR, PQR, and URX (41). To identify the neuron(s) in which TAX-2/TAX-4 is required for acute CO_2 avoidance, we performed a series of cell-specific rescue experiments with *tax-4*. We first tested whether CO_2 avoidance requires *tax-4* expression in AQR, PQR, and URX because *tax-4* is required in these neurons for normal O_2 response (19, 29, 42). We found that *tax-4* mutants in which *tax-4* is specifically rescued in AQR, PQR, and URX (42) do not respond to CO_2 (Fig. 6*A*). Moreover, animals containing a genetic ablation of AQR, PQR, and URX (42) respond normally to CO_2 (Fig. 6*A*). Thus, AQR, PQR, and URX do not mediate acute CO_2 avoidance.

We then examined *tax-4* mutants containing an *odr-4*::*tax-4* transgene, in which *tax-4* is expressed in AWA, AWB, AWC, ADF, ASG, ASH, ASI, ASJ, ASK, ADL, PHA, and PHB (19). These worms show essentially no CO_2 avoidance (Fig. 6*B*). However, expression of *tax-4* in these neurons as well as AQR, PQR, URX, AFD, and BAG using the *odr-4*, *gcy-8*, *gcy-32*, and *gcy-33* promoters (19) is sufficient to rescue the CO_2 response defect of *tax-4* mutants (Fig. 6*B*). Given that ablation of the BAG neurons results in greatly reduced CO_2 avoidance, we then asked whether *tax-4* expression in the BAG neurons is sufficient for CO_2 avoidance by expressing *tax-4* under the control of only the *gcy-33* promoter. We found that *tax-4* expression in BAG rescues the CO_2 response defect of *tax-4* mutants (Fig. 6*B*). Thus, a cGMP signaling pathway involving TAX-2/TAX-4 operates within the BAG neurons to mediate acute CO_2 avoidance.

Discussion

We have found that *C. elegans* exhibits acute avoidance of CO_2. This response requires a cGMP signaling pathway acting within

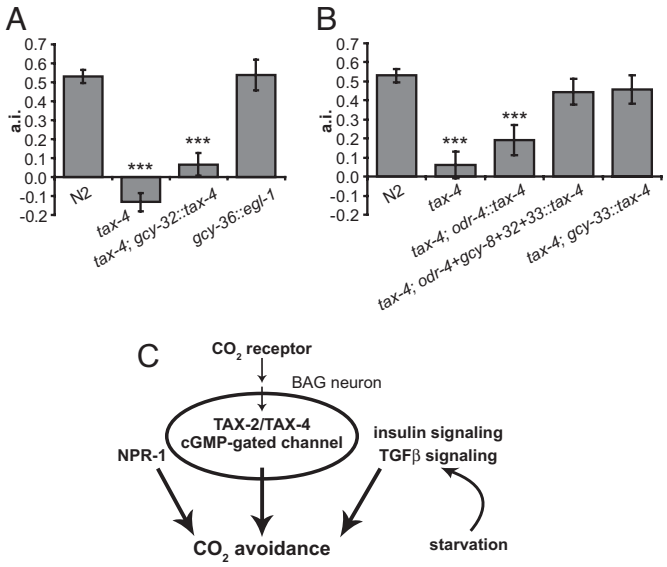

Fig. 6. cGMP signaling is required in the BAG neurons for acute CO_2 avoidance. (*A*) The URX, AQR, and PQR neurons are not required for acute CO_2 avoidance. Animals containing a *gcy-32*::*tax-4* transgene in the *tax-4(ks28)* mutant background, in which *tax-4* is expressed specifically in URX, AQR, and PQR (42), do not respond to CO_2. Animals containing a *gcy-36*::*egl-1* transgene, which kills URX, AQR, and PQR (42), respond normally to CO_2. ***, $P < 0.001$. $n = 11$–44 trials. For all graphs, error bars represent SEM. (*B*) *tax-4* is required in the BAG neurons for acute CO_2 avoidance. Animals containing an *odr-4*::*tax-4* transgene in the *tax-4(p678)* mutant background (19), in which *tax-4* is expressed in 12 sensory neurons, do not respond to CO_2. Animals containing *odr-4*+*gcy-8*+*gcy-32*+*gcy-33*::*tax-4* transgenes in the *tax-4(p678)* mutant background (19), in which *tax-4* is expressed in 17 neurons including BAG, respond normally to CO_2. Animals containing a *gcy-33*::*tax-4* transgene in the *tax-4(p678)* mutant background, in which *tax-4* is expressed specifically in the BAG neurons, also respond normally to CO_2. ***, $P < 0.001$. $n = 12$–44 trials. Data for N2 are from Fig. 1, and data for *tax-4* mutants are from Fig. 2. (*C*) A model for acute CO_2 avoidance in *C. elegans*. CO_2 avoidance is mediated by a cGMP signaling pathway involving TAX-2/TAX-4 acting within the BAG neurons. This response is modulated by NPR-1. CO_2 response is decreased by starvation, which acts via the insulin and TGFβ pathways.

the BAG neurons and is modulated by additional neuronal regulatory molecules as well as by insulin and TGFβ signaling (Fig. 6*C*).

Acute CO_2 avoidance is exhibited by some but not all wild isolates of *C. elegans* and some but not all species of free-living terrestrial nematodes. Thus, acute CO_2 avoidance is a rapidly evolving behavior that has either arisen or been lost multiple times during the course of nematode evolution, raising the possibility that it is advantageous only under some ecological conditions.

Starved worms show reduced CO_2 avoidance, and this effect is mediated by insulin and TGFβ signaling. The fact that CO_2 response is reduced by starvation contrasts with most olfactory responses in *C. elegans*, which are enhanced by starvation (17), presumably so as to maximize the worm's chance of finding food. The starvation-induced decrease in CO_2 avoidance may offer a similar ecological advantage: In nature, *C. elegans* presumably encounters CO_2 emitted by both bacterial food and predators. Under conditions of starvation it may be beneficial to down-regulate CO_2 avoidance so as to maximize the probability of encountering food, even if this incurs an increased risk of predation.

The sensitivity of CO_2 response to nutritional status is not universal among animals. For example, starved larvae of the bloodsucking insect *Triatoma infestans* respond as robustly to CO_2 as well fed larvae, even after 60 days of starvation (43). By contrast, many mosquitoes use CO_2 as their primary host-seeking cue, and host-seeking behavior is greatly reduced after a blood meal (44). Thus, CO_2 response may be subject to different regulatory mechanisms in organisms with different life cycles and behavioral repertoires.

Acute CO_2 avoidance is mediated primarily by the BAG neurons, and cGMP signaling mediated by TAX-2/TAX-4 is required in BAG for acute CO_2 avoidance. The BAG neurons are ciliated neurons of previously unknown function located in the head but not associated with the amphid sensillum (18). These neurons may sense CO_2 directly via one or more CO_2 receptors, or they may be indirect modulators of CO_2 response. It will be interesting to determine whether the BAG neurons also modulate O_2 response and also to identify additional signaling components that operate within the BAG neurons. Of the signaling molecules identified in this study as mediators of CO_2 avoidance, only TAX-2/TAX-4 are known to be expressed in the BAG neurons. In particular, expression of the guanylyl cyclase DAF-11 has not been observed in the BAG neurons (45), raising the possibility that the effect of *daf-11* on CO_2 response is indirect and that a different guanylyl cyclase acts upstream of TAX-2/TAX-4 in the BAG neurons to mediate CO_2 avoidance.

A CO_2 receptor in *C. elegans* has not yet been identified. In *Drosophila*, CO_2 avoidance is mediated by two members of the gustatory receptor (Gr) family of serpentine receptors, Gr21a and Gr63a (46–48), which are expressed in a single class of olfactory neurons on the fly antenna (5, 8, 46, 47). *C. elegans* does not contain orthologs of Gr21a and Gr63a, and thus *Drosophila* and *C. elegans* use different receptors for CO_2 detection.

The CO_2 avoidance we observed in some species of free-living terrestrial nematodes contrasts with the attraction to CO_2 exhibited by many parasitic and free-living marine nematodes (10–14, 49). Our study provides a foundation for investigations into how the CO_2 response network may have evolved in nematodes with very different life cycles and ecological niches.

Materials and Methods

Standard techniques are listed in the *SI Methods*.

Population Assay for Acute CO_2 Avoidance. For each assay, ≈10–30 *C. elegans* L4 hermaphrodites were placed onto assay plates overnight and tested as young adults. Assay plates consisted of NGM agar plates containing a thin lawn of OP50 bacteria grown for 1–2 days at room temperature. Gases were medical-grade certified mixtures (Air Liquide) of 0%, 0.2%, 1%, 2.5%, 5%, 10%, or 15% CO_2; 10% O_2; and the balance N_2. An O_2 concentration of 10% was chosen to closely approximate the preferred O_2 concentration of *C. elegans* (29). Ten percent CO_2 was used for all experiments unless otherwise indicated. For the avoidance assay, two 50-ml syringes were filled with gas, one with and one without CO_2. The mouth of the syringes were connected to tubes attached to Pasteur pipettes, and gases were pumped through the Pasteur pipettes by using a syringe pump (PHD 2000; Harvard Apparatus) at a rate of 1.5 ml/min. Individual worms were exposed to gases by placing the tip of the Pasteur pipette near the head of a forward-moving worm. A response was scored if the worm initiated backward movement within 4 seconds. The gas mixture to which each plate was exposed was alternated such that half of the plates were exposed to air and half were exposed to CO_2. Gases were delivered blindly, and worms were tested blindly. Each plate was considered one trial. Plates were assayed one to two times with at least 1 h between trials, except that worms tested off food were tested only once. An a.i. for each genotype was calculated by subtracting the fraction of worms that reversed in response to air from the fraction that reversed in response to CO_2. For assays involving other species, both males and females of dioecious species were tested. Values obtained for each genotype or treatment are listed in Table S1.

Single-Worm Assay for CO_2 Avoidance. For each assay, individual L4 or young adult hermaphrodites were placed onto assay plates overnight. Worms were tested as described above, except that each worm was tested 15 times with >2 min between trials. No adaptation was observed during the course of these experiments. For each worm, an a.i. was calculated by subtracting the fraction of trials the worm reversed in response to air from the fraction of trials the worm reversed in response to CO_2. The a.i. for each genotype or treatment was

calculated as the mean a.i. for each worm of the same genotype or treatment. Values obtained for each genotype or treatment are listed in Table S2. References for mutant strains are listed in Table S3.

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