A Single Gene Target of an ETS-Family Transcription Factor Determines Neuronal CO₂-Chemosensitivity

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Abstract

Many animals possess neurons specialized for the detection of carbon dioxide (CO₂), which acts as a cue to elicit behavioral responses and is also an internally generated product of respiration that regulates animal physiology. In many organisms how such neurons detect CO₂ is poorly understood. We report here a mechanism that endows C. elegans neurons with the ability to detect CO₂. The ETS-5 transcription factor is necessary for the specification of CO₂-sensing BAG neurons. Expression of a single ETS-5 target gene, gcy-9, which encodes a receptor-type guanylate cyclase, is sufficient to bypass a requirement for ets-5 in CO₂-detection and transforms neurons into CO₂-sensing neurons. Because ETS-5 and GCY-9 are members of gene families that are conserved between nematodes and vertebrates, a similar mechanism might act in the specification of CO₂-sensing neurons in other phyla.

Introduction

CO₂-chemosensitive neurons are found in many animals. In vertebrates, CO₂-sensing neurons are critical regulators of respiration [1]. Their dysfunction is proposed to underlie disorders such as sudden infant death syndrome [2] and congenital hypoventilation syndrome [3]. CO₂ is also sensed by animals as an ethologically relevant environmental cue. For example, insects detect CO₂ in the contexts of host- and mate-finding and as an aversive odorant [4,5], and the rodent olfactory system contains neurons that can be activated by low concentrations of CO₂ [6,7]. Studies of the insect olfactory system have identified odorant receptors that mediate CO₂ sensation, indicating that CO₂ can act through cellular and molecular systems dedicated to its detection [4,8,9]. The molecular mechanisms that mediate CO₂ sensing by insect olfactory neurons are, however, unique to insects. How neurons of other organisms detect CO₂ is poorly understood.

To control internal concentrations of respiratory gases, the microscopic nematode C. elegans navigates to environments with preferred concentrations of oxygen and CO₂ [10,11,12,13,14,15]. Two anterior sensory neurons, the BAG neurons, detect environmental CO₂ and mediate a CO₂-avoidance behavior [15,16,17]. CO₂-sensing by BAG neurons requires cyclic nucleotide signaling; mutants that lack either TAX-2 or TAX-4 subunits of a cyclic nucleotide-gated ion channel are defective in behavioral and physiological responses to CO₂ [16,17], as are mutants that lack the receptor-type guanylate cyclase GCY-9 [17]. Because CO₂ activates the BAG neurons of C. elegans through a specific molecular pathway, their study offers the opportunity to understand the molecular basis of neuronal CO₂-sensing.

ETS-5, an ETS-domain-containing transcription factor, was recently shown to regulate expression of many BAG-neuron-specific genes [18]. Whether ETS-5 is required for BAG-neuron responses to CO₂, and, if so, how ETS-5 confers CO₂-chemosensitivity to BAG neurons is unknown. We show here that ets-5 mutants have defects in CO₂ sensory transduction, and that ETS-5 directly interacts in vitro and in vivo with elements in the promoter of gcy-9, which encodes a critical component of the CO₂ sensory transduction pathway. Strikingly, the requirement for ETS-5 in CO₂ sensing can be bypassed by forced expression of gcy-9. These data indicate that a mechanism by which ETS-5 specifies CO₂ chemosensitivity of BAG neurons is by direct regulation of the expression of GCY-9. GCY-9 in turn is sufficient to mediate neuronal responses to CO₂ and likely encodes a receptor for CO₂ or a CO₂ metabolite.

Results

One of ten ETS-family transcription factors encoded by the C. elegans genome is required for the specification of CO₂-sensing BAG neurons

To identify factors that specify BAG neurons, we analyzed promoters of genes that are expressed by BAG neurons. We hypothesized that identification of cis-regulatory elements required for expression of these terminal differentiation genes in BAG neurons would enable us to identify trans-acting factors required...
for BAG neuron differentiation. First we looked at the promoter of the BAG-neuron-specific neuropeptide gene *flp-17*. We dissected a 1460 bp *flp-17* promoter and found that a 138 bp promoter fragment was sufficient to drive expression in BAG neurons (Fig. S1). We noted that this minimal promoter contains three copies of a sequence motif predicted to bind ETS-family transcription factors. We then tested whether a promoter containing a single ETS-binding site is sufficient to drive gene expression in the BAG neurons. We observed that a 31 bp sequence containing a single ETS-binding site drove expression of a reporter transgene specifically in BAG neurons (Fig. 1A). These data indicated that one or more ETS-family transcription factors function to control BAG-cell fate specification.

Which of the ten ETS-family transcription factors encoded by the *C. elegans* genome regulates expression of BAG-neuron genes? We systematically analyzed the cell fate of CO2-chemosensitive BAG neurons in mutants for each ETS transcription factor. In wild-type animals, *Promflp-19::gfp* is expressed by the two BAG neurons, and also by five other paired neurons [19] (Fig. 1B,C). We found that nine ETS-gene mutants expressed the *Promflp-19::gfp* reporter in BAG neurons. *ets-5* mutants, however, failed to express *Promflp-19::gfp* in BAG neurons (Fig. 1B,C). Two independent *ets-5* alleles caused the same defect in BAG neuron expression, and a fosmid containing the genomic *ets-5* locus complemented this defect. (Fig. 1C and Fig. S2).

These results confirm the recently reported role for ETS-5 in BAG neuron development [18]. We noted, however, an additional role for ETS-5 in the specification of AWA sensory neurons, which failed to express a *Promflp-19::gfp* reporter transgene in *ets-5* mutants (Fig. 1B,C). A previous report indicated that *ets-5* is specifically expressed by the BAG neurons and suggested that ETS-5 functions only to specify BAG neurons but not other sensory neurons [18]. We determined the gene structure of *ets-5* by cloning an *ets-5* cDNA (Fig. S3), and we made an *ets-5::gfp* translational reporter transgene by inserting GFP coding sequences into the last exon of the *ets-5* genomic locus. We observed consistent expression of ETS-5::GFP in the nuclei of BAG neurons and fourteen other neuron nuclei, including amphid neurons likely to be AWAs (Fig. 2). ETS-5 is therefore required for specification of BAG neurons, but neither its expression nor its function is restricted to BAG neurons.

**ETS-5 is required for sensory transduction in CO2-sensing BAG neurons**

*ets-5* mutants are defective in an acute CO2 avoidance behavior [18], which we also observed (Fig. 3A). We monitored the locomotory behavior of animals exposed to a plume CO2-enriched artificial atmosphere and measured CO2-evoked reversals. The frequency of reversals by wild-type animals increased dramatically upon presentation of a CO2 stimulus (Fig. 3A). By contrast, the reversal frequency of *ets-5* mutants did not significantly change in response to presentation of a CO2 stimulus (Fig. 3A). Like BAG-ablated animals, the reversal frequency of *ets-5* mutants did not significantly increase in response to a CO2 stimulus (Fig. 3A). The CO2-avoidance defect of *ets-5* mutants was complemented by the *ets-5::gfp* transgene used to determine its cellular expression pattern (Fig. 3A). *ets-5* is therefore necessary for acute CO2 avoidance, a behavior that is driven specifically by BAG neurons. The effect of CO2 on reversal frequencies of the different strains was calculated as an avoidance index, showing that BAG-ablated animals and *ets-5* mutants were comparatively insensitive to CO2 (Fig. 3B).

![Figure 1. An ETS-family transcription factor is required for the specification of *C. elegans* CO2-chemosensitive BAG neurons.](http://www.plosone.org/figure/1)

(A) A 31 basepair DNA element comprising a single ETS-binding motif (top) drives expression of GFP specifically in the BAG chemosensitive neurons (bottom). (B) One of ten ETS-family transcription factors encoded by the *C. elegans* genome is required for specification of BAG neurons. Shown is percent of animals mutant for each of ten ETS-family transcription factors encoded by the *C. elegans* genome that are aeg/BL (green circles) and aeg/BR (open circles) for expression of a *Promflp-19::gfp* reporter transgene. N = number of animals scored. c We found one *lin-1(e1777)* mutant in which *Promfp-19::gfp* was not expressed in BAGR. (C) Fluorescence micrographs of *Promfp-19::gfp* expression in a wild-type animal, an *ets-5* mutant and an *ets-5* mutant carrying a wild-type copy of the *ets-5* locus in a fosmid-derived transgene. BAG/R neuron positions are marked by red circles and cells previously identified as AWAL/R [19] are marked by blue circles. The nerve ring is indicated by an arrowhead. The scale bar in lower panel is 20 μm. A: anterior, L: left. The *ets-5* mutant allele was *tm1734*. The *Promfp-19::gfp* transgene was *ynl334* and the *ets-5* rescuing transgene was *rpEx246*. doi:10.1371/journal.pone.0034014.g001
ETS-5 directly regulates the receptor-type guanylate cyclase gene \textit{gcy-9}.

The receptor-type cyclase \textit{GCY-9} is a critical component of the \textit{CO}_2 transduction pathway [17], and a \textit{Prom\_gcy-9::gfp} reporter transgene is not properly expressed by \textit{ets-5} mutants (Fig. 4A). Does ETS-5 directly regulate \textit{gcy-9}? We first sought to identify sequences in the \textit{gcy-9} promoter that are required for expression by BAG neurons of a \textit{Prom\_gcy-9::gfp} reporter. We noted that the ETS domain of ETS-5 is highly similar to the ETS domain of the vertebrate transcription factor Pet1 (Fig. 4B), which functions in the specification of neurons in the vertebrate midbrain [21,22,23]. Pet1 preferentially binds to a consensus sequence AC-CGGAAGTA [24], and the \textit{gcy-9} promoter contains a sequence that is almost identical to the reverse complement of this consensus sequence (Fig. 4C). Moreover, this putative ETS-binding site is highly conserved among nematode species, suggesting that it is functionally important (Figure 4C). This highly conserved ETS-site is adjacent to another putative ETS-binding site. We tested whether these presumptive ETS-binding sites are necessary for \textit{gcy-9} promoter function by introducing into the \textit{gcy-9} promoter a small deletion that removes them. We did not observe any activity of the mutant promoter in BAG neurons (Fig. 4D). We also introduced mutations in both ETS motifs, leaving the rest of the promoter intact. Like the deletion, these mutations abrogated expression of the reporter transgene, indicating that this motif is necessary for \textit{gcy-9} promoter function.

We next tested whether ETS-5 associates \textit{in vivo} with the \textit{gcy-9} promoter at the ETS sites using chromatin immunoprecipitation. ETS-5::GFP immunoprecipitates were enriched for sequences containing the ETS binding site from the \textit{gcy-9} promoter in comparison to precipitates prepared from extracts of non-transgenic animals, which did not express ETS-5::GFP (Fig. 5A). By contrast, sequences upstream of the ETS site were not significantly enriched in ETS-5::GFP immunoprecipitates. ETS-5, therefore, associates with a highly conserved and functionally important sequence element in the \textit{gcy-9} promoter. To determine whether ETS-5 directly interacts with this element, we performed electrophoretic mobility shift assays (EMSAs) using a synthetic 45 base-pair DNA duplex containing the ETS binding site and recombinant ETS-5 protein (Fig. 5B). Recombinant ETS-5 altered the mobility of the duplex, and incorporation of labeled duplex into the complex was efficiently blocked by an excess of unlabeled duplex but not by sequence-scrambled duplex (Fig. 5B). Taken together with the observation that these ETS-binding motifs are required for the function of the \textit{gcy-9} promoter (Fig. 4D), these data indicate that a direct interaction between ETS-5 and sequences in the \textit{gcy-9} promoter is required for expression of \textit{gcy-9} in BAG neurons.

Expression of \textit{gcy-9} bypasses a requirement for \textit{ets-5} in \textit{CO}_2-avoidance.

Are \textit{ets-5} mutants defective in \textit{CO}_2 sensing principally because they fail to express \textit{gcy-9}, or is \textit{gcy-9} one of many BAG-cell-specific genes that are regulated by ETS-5 and necessary for \textit{CO}_2 chemosensation? To answer this question, we used promoters that are not regulated by ETS-5 to express \textit{gcy-9} in neurons that (1) mediate an acute avoidance behavior similar to that triggered by BAG neurons and (2) like BAG neurons, use cGMP signaling. The \textit{gcy-36} promoter is active in the URX oxygen-sensing neurons [25], which drive reversals in response to a hyperoxic stimulus [11]. The \textit{gcy-18} promoter is specifically active in thermosensory AFD neurons [26], which also drive reversals [27]. Activity of neither the \textit{gcy-18} nor the \textit{gcy-36} promoter was affected by \textit{ets-5}.

To determine the effect of \textit{ets-5} mutation on the physiology of BAG neurons, we used the ratiometric calcium indicator cameleon YC3.60 [20] to monitor the responses of wild-type and mutant BAG neurons to \textit{CO}_2 stimuli. Wild-type BAG neurons showed robust responses to \textit{CO}_2 stimuli; the mean fractional change in the ratio of YFP to CFP emissions to a 10% \textit{CO}_2 stimulus was greater than 80% (Fig. 3C). By contrast, \textit{ets-5} mutant BAG neurons showed fractional ratio changes of less than 10% in response to the same \textit{CO}_2 stimulus (Fig. 3D). The BAG neurons of \textit{ets-5} mutants are, therefore, defective in sensory transduction at a point upstream of the generation of an intracellular calcium transient. We next tested whether the transduction defect of \textit{ets-5}-mutant BAG neurons might be caused by defects in the regulation of components of the \textit{CO}_2-transduction pathway.
mutation (Fig. 6A). We then tested whether these promoters driving expression of gcy-9 could rescue the CO2 avoidance defect of ets-5 mutants. Unlike non-transgenic ets-5 mutants, which failed to increase reversal frequency in response to a CO2 stimulus, ets-5 mutants carrying either a Promgcy-36::gcy-9 transgene or a Promgcy-18::gcy-9 transgene showed robust avoidance responses to CO2 (Fig. 6B). A requirement for ets-5 in CO2-avoidance behavior can therefore be bypassed by expressing the ETS-5 target gene gcy-9 in neurons that are specified by ets-5-independent mechanisms.

Figure 3. The BAG neurons of ets-5 mutants are defective in sensory transduction. (A) ets-5 mutants are defective in a BAG-neuron-dependent CO2 avoidance behavior. Plotted are the mean fractions of animals ± SEM that reversed during a four second exposure to either control atmosphere (0% CO2, 20% O2, balance N2) or CO2-enriched atmosphere (10% CO2, 20% O2, balance N2). Strains tested were: the wild-type strain N2, the BAG-ablated strain CX11697, the ets-5 mutant strain FX1734, which carries the tm1734 deletion allele of ets-5, and a derivative of FX1734 that carries the ets-5::gfp transgene wzIs80. N = 3–5 populations of 30–50 animals. (B) The effect of ets-5 mutation on CO2 avoidance behavior is comparable to that of BAG neuron ablation. An avoidance index was calculated by subtracting the fraction of animals in a population that reversed in response to exposure to control atmosphere from the fraction that reversed in response to CO2-enriched atmosphere. Plotted are the mean avoidance indices for each of the four strains tested ± SEM. P values were calculated by one-way ANOVA. N = 3–5 populations of 30–50 animals. (C) Wild-type BAG neurons show robust calcium responses to a CO2 stimulus. Wild-type animals carrying a Promgcy-9::cameleon transgene, which drives expression of cameleon specifically in BAG neurons, were immobilized and exposed to a 10 s pulse of 10% CO2. Plotted is the mean fractional ratio change in YFP/CFP emissions. The shaded area represents S.E.M. The cameleon expression transgene used was wzIs82. (D) The BAG neurons of ets-5 mutants show reduced calcium responses to a CO2 stimulus. Animals carrying a variant Promgcy-33::cameleon transgene, which drives ets-5-independent expression of cameleon in BAG neurons, were immobilized and exposed to a 10 s pulse of 10% CO2. Plotted is the mean fractional ratio change in YFP/CFP emissions. The shaded area represents S.E.M. The cameleon expression transgene used was wzEx56. doi:10.1371/journal.pone.0034014.g003
phase-locked to the CO2 stimulus (Fig. 6C). We performed the same measurements on wild-type and transgenic AFD thermo-sensory neurons (Fig. 6D). Under some experimental conditions, AFD neurons demonstrate a calcium response to the falling edge of a CO2 pulse [14] i.e. an ‘off’ response. Under our experimental conditions, wild-type AFD neurons showed little response to CO2.
channels [17], suggesting that CO₂-sensing by BAG neurons minimally requires a two-component transduction pathway: a CO₂-activated guanylate cyclase and a cGMP-gated ion channel. Could such a transduction pathway exist in other species? Mammalian genomes encode receptor-type cyclases related to GCY-9. These cyclases have been matched to diverse ligands [29] and are also known to be regulated by interactions with anions, including chloride [29] and bicarbonate [30,31,32,33]. Because vertebrate cyclases are modulated by the CO₂ metabolite bicarbonate, this mechanism of cyclase activation might lie at the heart of an evolutionarily conserved pathway for CO₂ detection.

Our data show that gcy-9 expression is a determinant of whether a C. elegans neuron is CO₂-chemosensitive. How is expression of gcy-9 restricted to the BAG chemosensors? ETS-5 is necessary for expression of gcy-9 but is also expressed by neurons that do not express gcy-9 (Fig. 2). ETS-5 must therefore cooperate with additional, cell-type-specific factors to permit the activation of the gcy-9 promoter specifically in BAG neurons. For example, overlapping expression of ETS-5 and a co-regulatory partner only in BAG neurons might drive cell-type-specific expression of GCY-9. Similar context-dependent neuronal specification events are known to require such 'combinatorial coding' [34]. Indeed, in other contexts, ETS-family transcription factors interact with co-regulatory transcription factors, including LIM-domain, homeodomain and JUN proteins [35,36,37]. Further studies will identify ETS-5 interacting partners that specifically drive expression of gcy-9 in BAG neurons and consequently determine their CO₂-chemosensitivity.

ETS-5 has the same domain structure as the vertebrate ETS protein Pet1, and its ETS domain, which mediates sequence-specific interactions with DNA [38], is almost identical to that of Pet1. Like ETS-5, Pet1 functions in the specification of neuronal cell fates [23]. Notably, among the neurons that are specified by Pet1 are serotonergic neurons, that are directly activated by CO₂ and have recently been shown to function in vivo in the respiratory CO₂ chemoreflex [21,22]. In addition to promoting a serotonergic identity, Pet1, like ETS-5, might regulate the expression of genes that constitute a molecular pathway dedicated to the detection of CO₂.

Materials and Methods

Strains used in this study

Strains used in this study are listed in Table S1. Strains were grown on NGM agar at 25°C on Escherichia coli OP50 except when used for chromatin immunoprecipitation experiments (see below). Transgenic animals were created according to Mello et al. [39]. Prom_gcy-9::dsRed coinjection marker was used at 5 ng ul⁻¹. Prom_rop-6::dsRed was used at 25 ng ul⁻¹. Prom_mop-122::dsRed was used at 30 ng ul⁻¹. The lin-15 rescuing plasmid pL15EK was used at 30 ng ul⁻¹. Prom_mop-122::dsRed was used at 3 ng ul⁻¹. rol-6 was used at 100 ng ul⁻¹. GFP, cameleon, dsRed and GCY-9 expression plasmids were injected at 100 ng ul⁻¹. In some cases, extrachromosomal transgenes were integrated using gamma radiation (5000 rads).

Plasmids

A complete list of plasmids used in this study is in Table S2.

Microscopy

Young adults were anaesthetized with 30 mM sodium azide and mounted on a 2% agarose pad made in M9 medium. Series of confocal images were obtained with a Zeiss LSM510 microscope.
Figure 6. Expression of the ETS-5 target gene *gcy-9* restores CO₂-chemosensitivity to *ets-5* mutants. (A) *gcy-36* and *gcy-18* promoters, which are active in oxygen-sensing and thermosensory neurons, respectively, are not regulated by ETS-5. Shown are lateral views of wild-type and *ets-5* mutant animals carrying either a *gcy-36* reporter, which is expressed by the oxygen-sensing URX neurons, or a *gcy-18* reporter, which is expressed by the thermosensory AFD neurons. A: anterior, V: ventral, scale bar is 20 μm. The Promₕₑₛ₃₆::cameleon transgene used was wzEx39 and the Promₕₑ₄₁₈::gfp transgene was wzEx40. (B) Expression of *gcy-9* in either the URX oxygen sensors or the AFD thermosensors rescues the behavioral defect of *ets-5* mutants. The left plot shows the mean fraction of animals ± SEM that reversed during a four second exposure to either control atmosphere (0% CO₂, 20% O₂, balance N₂) or CO₂-enriched atmosphere (10% CO₂, 20% O₂, balance N₂). The right plot shows the effect of CO₂ on the avoidance behavior of *ets-5* mutants with or without *gcy-9* expression.
and maximum-projection images were created using ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2011).

**Behavioral Assay for Acute CO2 Avoidance**

Behavioral assays were carried out essentially as described by Hallem and Sternberg [15]. Three or four trials of 30–50 animals were performed for each genotype. Animals were picked onto unseeded 6 cm nematode growth medium (NGM) agar plates. Gases were certified mixtures of 20% oxygen with or without 10% CO2, balance nitrogen (Airgas).

**Statistical Analysis**

Standard errors and P values were calculated using GraphPad Prism analysis software. P values were calculated from a one-way ANOVA analysis of avoidance indexes.

**Chromatin immunoprecipitation**

**Electrophoretic mobility shift assay**

Recombinant GST:ETS-5 was expressed in E. coli strain BL21 (DE3) purified using glutathione sepharose affinity chromatography (GE Healthcare). 1 µg of recombinant ETS-5 or 2 µg of GST was added to each reaction, which contained 0.1 pmol of 3'-biotin-labeled duplex probe. In some reactions, a molar excess of unlabeled competitor duplex (identical or sequence-scrambled) was included. Reactions were incubated at room temperature for 20 minutes and protein-DNA complexes were separated from unbound probe by electrophoresis in a 6% polyacrylamide gel in 0.5× TBE. Nucleic acids were then transferred to a nylon membrane (Amersham, GE Healthcare) and detected using HRP-coupled streptavidin and enhanced chemiluminescence (Pierce Protein Research Products).

The sequence of the biotinylated probe and the unlabeled competitor was:

5'-ATCCACATCCGATGGGCGCCCTTCCGGCATGATAA-GAACGTGATGGC-3'.

The sequence of the scrambled competitor was:

5'-CTGACCCCGTGTCGATAAGCAGTTACGTGCCATGAGATGGC-3'.

**in vivo calcium imaging**

Calcium imaging was performed essentially as previously described [17]. Young adults were immobilized with cyanacrylate veterinary glue (Surgi-Lock; Meridian Animal Health) on a cover glass coated with a 2% agarose pad made with 10 mM HEPES (pH 7.4). The cover glass was affixed to a custom-made air chamber. The specimen was illuminated with 435-nm excitation light and imaged using a 40× Nikon long-working distance objective (0.75 numerical aperture). The emission image was passed through a DV2 image splitter (Photometrics), and the CFP and YFP emission images were projected onto two halves of a cooled CCD camera (Andor). Images were acquired at 10 Hz, with exposure times between 10 and 50 ms. Gas perfusion was controlled by three-way valves (Numatics) driven by a custom-made valve controller unit. Excitation light, image acquisition, and hardware control were performed by the Live Acquisition software package (Till Photonics). Post-acquisition analysis of ratio plots was performed using custom Matlab scripts, which subtracted linear baseline drift from traces and applied a five-frame boxcar filter to the ratio time series. Custom certified gas mixes used for imaging were obtained from Airgas.

**Supporting Information**

**Figure S1 Deletion analysis of the flp-17 promoter.** (A) Genomic locus of the flp-17 gene with the location of predicted ETS binding sites shown as blue bars. mCherry protein was driven by the promoter elements indicated with black bars and expression in the BAG neurons was scored. The Prom9flp-17 is a 138 bp fragment that is sufficient to drive expression of mCherry protein
in the BAG neurons. N=30 animals/transgenic line. (B) Dorsal view of Prom9flp-17::mCherry expression in a wild-type animal. BAG neuron positions are marked by red circles. The scale bar is 20 μm.

**Figure S2** ets-5 mutants have defects in expression of multiple BAG-neuron genes. (A) An independently derived allele of ets-5 has BAG neurons that fail to express a Prom9flp-17::gfp transgene. The fraction of transgenic ets-5(tm1734) and ets-5(tm1734) animals that express GFP in BAG neurons is plotted next to the wild type (+) and ets-5(tm1734) mutants carrying rescuing transgenes derived from a fosmid that encompasses the ets-5 locus. N=20, # = independent transgenic lines. (B) Dorsal views of Prom9flp-17::gfp expression in a wild-type animal and an ets-5 mutant. Prom9flp-17::gfp expression was lost in the ets-5 mutant (bottom panel). BAG neuron positions are marked by red circles. The scale bar in lower panel is 20 μm. A: anterior, V: ventral. The ets-5 mutant allele was tm1734. The Prom9flp-17::gfp transgene was ynl37. (C) Lateral views of Prom9flp-17::gfp expression in a wild-type animal and an ets-5 mutant. Prom9flp-17::gfp expression was lost in the ets-5 mutant (bottom panel). BAG neuron position in the lower panel is marked by a red circle. The scale bar in lower panel is 20 μm. A: anterior, V: ventral. The ets-5 mutant allele was tm1734. The Prom9flp-17::gfp transgene was ynl64. (D) Lateral views of Prom9flp-17::gfp expression in a wild-type animal and an ets-5 mutant. Prom9flp-17::gfp expression was lost in the ets-5 mutant (bottom panel). BAG neuron position in the lower panel is marked by a red circle. The scale bar in lower panel is 20 μm. A: anterior, V: ventral. The ets-5 mutant allele was tm1734. The Prom9flp-17::gfp transgene was ynl64.

**Figure S3** Characterization of the ets-5 transcript. (A) Genomic organization of ets-5 coding sequences as determined by amplification of ets-5 cDNA using gene-specific and SL1 primers. cDNAs derived from SL1 trans-spliced messages contained 95 bases of 3′ non-coding sequence (white box) and were organized into five exons. brackets indicate regions deleted by the tm1734 and tm666 mutations. (B) Nucleotide sequence of ets-5 cDNA. The SL1 leader sequence is shown above the cDNA. Non-coding sequences are in lowercase and exons are denoted by text boxes alternately colored blue and green. (C) Predicted amino acid sequence of ETS-5. Sequences of the ETS homology domain are in white text on a black background.

**Table S1** Strains used in this study. Strain designations and complete genotypes of all the strains used in this study.

**Table S2** Plasmids used in this study. Complete descriptions of plasmids used in this study and the sequences of primers used for their construction.

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**Author Contributions**

Conceived and designed the experiments: JB VJ LAM RP NR. Performed the experiments: JB SAZ VJ LAM GJP RP NR. Analyzed the data: JB VJ LAM RP NR. Wrote the paper: JB RP NR.

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**References**

1. Spyer KM, Gourine AV (2009) Chemosensory pathways in the brainstem controlling cardiorespiratory activity. Philosophical Transactions of the Royal Society B: Biological Sciences 364: 2093-2102.

2. Juruena MF, Richerson GB, Dymecki SM, Darnall RA, Nattie EE (2009) The brainstem and serotonin in the sudden infant death syndrome. Annual review of physiology. 4: 571-590.

3. Amiel J, Lauter B, Amié-Bitach T, Trang H, de Pontual L, et al. (2003) Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat Genet 33: 459-461.

4. Suh GSB, Wong AM, Herger SC, Wang JW, Simon AF, et al. (2004) A single population of olfactory sensory neurons mediates an innate avoidance behaviour in Drosophila. Nature 431: 854-859.

5. Guerenstein PG, Hildebrand JD (2008) Roles and effects of environmental carbon dioxide in insect life. Annu Rev Entomol 53: 161-178.

6. Munger SD, Leinders-Zufall T, McDougall LM, Cockerham RE, Schmid A, et al. (2010) An Olfactory Subsystem that Detects Carbon Disulfide and Mediates Food-Related Social Learning. Current Biology. pp 1-7.

7. Hu J, Zhong C, Ding C, Chi Q, Walz A, et al. (2007) Detection of near-atmospheric concentrations of CO2 by an olfactory subsystem in the mouse. Science 317: 953-957.

8. Kwon KY, Babanakar A, Weiss LA, Carlson JR (2007) The molecular basis of CO2 reception in Drosophila. Proc Natl Acad Sci USA 104: 3574-3578.

9. Jones WD, Cayirlioglu P, Kadow IG, Vosshall LB (2007) Two chemosensory receptors together mediate carbon dioxide detection in Drosophila. Nature 445: 86-90.

10. McGrath PT, Rockman MV, Zimmer M, Jang H, Macskoc EZ, et al. (2009) Quantitative mapping of a digenetic behavioral trait implicates globin variation in C. elegans sensory behaviors. Neuron 61: 692-699.

11. Zimmer M, Gray JM, Pokala N, Chang AJ, Karowe DS, et al. (2009) Neurons detect increases and decreases in oxygen levels using distinct guanylate cyclase.

12. Gray JM, Karowe DS, Lu H, Chang AJ, Chang JS, et al. (2004) Oxygen sensation and social feeding mediated by C. elegans guanylate cyclase homologue. Nature 430: 317-322.
25. Cheung BH, Arellano-Carabajal F, Rybicki I, de Ronco M (2004) Soluble guanylate cyclases act in neurons exposed to the body fluid to promote C. elegans aggregation behavior. Curr Biol 14: 1105-1111.

26. Inada H, Iso H, Satterlee J, Sengupta P, Matsumoto K, et al. (2006) Identification of guanylyl cyclases that function in thermosensory neurons of Caenorhabditis elegans. Genetics 172: 2239-2252.

27. Ryu WS, Samuel AD (2006) Thermotaxis in Caenorhabditis elegans analyzed by measuring responses to defined thermal stimuli. J Neurosci 22: 5727-5733.

28. Wedel R, Garbers D (2003) The guanylyl cyclase family at Y2K. Annu Rev Physiol 65: 215-233.

29. van den Akker F, Zhang X, Miyagi M, Huo X, Misono KS, et al. (2000) Structure of the dimerized hormone-binding domain of a guanylyl-cyclase-coupled receptor. Nature 406: 101-104.

30. Chao Y-C, Chung C-J, Hsieh H-T, Lin C-C, Chen C-C, et al. (2010) Receptor guanylyl cyclase-G expressed in Gruneberg ganglion olfactory subsystem is activated by bicarbonate. The Biochemical journal.

31. Guo D, Zhang J, Huang X-Y (2009) Stimulation of guanylyl cyclase-D by bicarbonate. Biochemistry 48: 4417-4422.

32. Sun L, Wang H, Hu J, Han J, Matsunami H, et al. (2009) Guanylyl cyclase-D in the olfactory CO2 neurons is activated by bicarbonate. Proc Natl Acad Sci USA. pp 2041–2046.

33. Duda T, Sharma RK (2010) Distinct ONE-GC transduction modes and motifs of the odorants: Uroguanylin and CO2. Biochemical and Biophysical Research Communications 391: 1379-1384.

34. Shirasaki R, Pfaff SL, (2002) Transcriptional codes and the control of neuronal identity. Annu Rev Neurosci 25: 251-281.

35. Treier M, Bohmann D, Mlodzik M (1995) JUN cooperates with the ETS domain protein pointed to induce photoreceptor R7 fate in the Drosophila eye. Cell 83: 753-760.

36. Yuasa Y, Okabe M, Yoshikawa S, Tabuchi K, Xiong WC, et al. (2003) Drosophila homeodomain protein REPO controls glial differentiation by cooperating with ETS and BTB transcription factors. Development 130: 2419-2428.

37. Guo R, Sallis RE, Greenall A, Petit MM, Jansen E, et al. (2006) The LIM domain protein LPP is a coactivator for the ETS domain transcription factor PEA3. Mol Cell Biol 26: 4529-4538.

38. Karim FD, Urness LD, Thommel CS, Klemza MJ, McKercher SR, et al. (1990) The ETS-domain: a new DNA-binding motif that recognizes a purine-rich core DNA sequence. Genes Dev 4: 1451–1453.

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

40. Ercan S, Giresi PG, Whittle CM, Zhang X, Green RD, et al. (2007) X chromosome repression by localization of the C. elegans dosage compensation machinery to sites of transcription initiation. Nat Genet 39: 403–408.