A Novel Role for the Zinc-Finger Transcription Factor EGL-46 in the Differentiation of Gas-Sensing Neurons in *Caenorhabditis elegans*

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ABSTRACT Oxygen (O_2) and carbon dioxide (CO_2) provoke distinct olfactory behaviors via specialized sensory neurons across metazoa. In the nematode *C. elegans*, the BAG sensory neurons are specialized to sense changes in both O_2 and CO_2 levels in the environment. The precise functionality of these neurons is specified by the coexpression of a membrane-bound receptor-type guanylyl cyclase GCY-9 that is required for responses to CO_2 upshifts and the soluble guanylyl cyclases GCY-31 and GCY-33 that mediate responses to downshifts in O_2. Expression of these gas-sensing molecules in the BAG neurons is partially, although not completely, controlled by ETS-5, an ETS-domain-containing transcription factor, and EGL-13, a Sox transcription factor. We report here the identification of EGL-46, a zinc-finger transcription factor, which regulates BAG gas-sensing fate in partially parallel pathways to ETS-5 and EGL-13. Thereby, three conserved transcription factors collaborate to ensure neuron type-specific identity features of the BAG gas-sensing neurons.

THE acquisition of specific neuronal fates is controlled by regulatory transcription factors that activate the expression of neuron type-specific terminal differentiation genes (Hobert 2008, 2011). Specific individual transcription factors may act to control the expression of the entire terminal gene battery of a specific neuron, or alternatively, multiple transcription factors may act in parallel to control distinct aspects of terminal fates (Hobert *et al.* 2010). The use of combinatorial transcription factors to control aspects of terminal fates may provide flexibility to polymodal neurons that need to rapidly respond to changes in a specific environmental cue. We study the BAG neurons in *Caenorhabditis elegans* that have a dual function where they are activated by separate mechanisms when carbon dioxide (CO_2) levels increase or when oxygen (O_2) levels decrease (Zimmer *et al.* 2009). The membrane-bound receptor-type guanylyl cyclase GCY-9 is expressed specifically in the BAG neurons to mediate CO_2 avoidance behavior (Hallem *et al.* 2011). Whereas the soluble guanylyl cyclases GCY-31 and GCY-33 are expressed in the BAG neurons to mediate behavioral responses when O_2 levels decrease (Zimmer *et al.* 2009). Previous studies have shown that the ETS domain-containing transcription factor ETS-5 is critical for CO_2-sensing neuron fate through direct regulation of GCY-9 expression in the BAG neurons (Guillermin *et al.* 2011; Brandt *et al.* 2012; Gramstrup Petersen *et al.* 2013). In addition, the Sox transcription factor EGL-13 is partially required for GCY-9 expression in the BAG neurons (Gramstrup Petersen *et al.* 2013). Loss of either of these transcription factors causes defects in CO_2 avoidance behavior (Guillermin *et al.* 2011; Brandt *et al.* 2012; Gramstrup Petersen *et al.* 2013). The O_2-sensing neuron fate of the BAG neurons, as assessed by expression of *gcy-31* and *gcy-33*, is also defective in *ets-5* and *egl-13* mutant animals (Gramstrup Petersen *et al.* 2013). However, the expression of these and other BAG terminal fate markers is not fully abrogated by concomitant loss of *ets-5* and *egl-13* (Gramstrup Petersen *et al.* 2013). These studies suggest that parallel genetic programs exist that regulate the specification of the O_2- and CO_2-sensing BAG neuronal fates.

To identify factors that potentially act in parallel to *ets-5* and *egl-13* in BAG specification, we performed a forward genetic screen, using a fluorescent reporter for *gcy-33* that is exclusively expressed in the BAG neurons. Using this approach, we identified two alleles of *egl-46*, which encodes a TFIIA-like zinc finger transcription factor, a gene previously

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shown to regulate multiple neuronal fate decisions in *C. elegans* (Wu *et al.* 2001; Wang *et al.* 2010; Feng *et al.* 2013). We found that *egl-46* regulates the expression of a subset of BAG-expressed terminal differentiation genes that include *gcy-9*, which is required for CO_2 avoidance responses, and *gcy-31* and *gcy-33*, which are required for responses to O_2 downshifts. In concordance with these molecular deficits, we found that *egl-46* mutant animals are defective in the ability to respond to O_2 downshifts and upshifts in CO_2, which are BAG-mediated behaviors.

We subsequently performed double-mutant analysis to better understand the role of *egl-46* in BAG neuron fate specification. We found that *egl-46* acts in parallel to *ets-5* and *egl-13* to regulate *gcy-31* and *gcy-33* expression, and thereby O_2-sensing fate, in the BAG neurons. In addition, we found that *egl-46* acts in a partially parallel pathway to that of *egl-13* to direct CO_2-sensing fate.

Taken together, we have identified the transcription factor EGL-46 as a regulator required for the expression of a subset of O_2 and CO_2 terminal differentiation genes in *C. elegans*. The EGL-46 ortholog in *Drosophila*, Nerfin-1, is also required for CO_2-sensing neuron fate specification (Cayirlioglu *et al.* 2008). In addition, the mouse ortholog Insm1 regulates the differentiation of cells in the olfactory epithelium that harbors CO_2-responding neurons (Sun *et al.* 2009; Rosenbaum *et al.* 2011). Therefore, our findings suggest a conserved function for this gene family.

Materials and Methods

Mutant and transgenic reporter strains

Worms were grown using standard conditions on NGM agar plates and maintained at 20° (Brenner 1974). A complete list of mutant and transgenic strains used for this study is detailed in Supporting Information, Table S1. Fusion PCR was used to generate egl-46 cDNA and genomic rescue elements (Hobert 2002). For expression analysis, the *egl-46* promoter was cloned into a dsRed2::NLS expression vector using the following oligonucleotides: HindIII site oAR39 AAAAAAGCTTtgatttttgccagaaatgttacg and BamHI site oAR38 TTTTGGATCCggccttctgaaatcaaaacg.

Forward genetic screen

In the forward genetic screen, the BAG reporter strain *gcy-33^{prom}*::*gfp*; *dop-3*::*rfp* was EMS mutagenized according to standard protocols (Flibotte *et al.* 2010). BAG cell fate mutants were isolated based on decreased expression of *gcy-33^{prom}*::*gfp*, using the automated COPAS biosorter platform (Doitsidou *et al.* 2008).

The genomic lesion of *rp13* was identified using the previously described, one-step whole-genome sequencing and SNP mapping strategy (Doitsidou *et al.* 2010). Subsequent analysis using MAQGene enabled genome-wide analysis of Hawaiian and N2 SNP distribution to identify a region without recombination (Bigelow *et al.* 2009). A complementation test between *rp13* and *rp15* revealed that these two mutants are alleles of the same gene and *rp15* was Sanger sequenced at the *egl-46* locus.

Fluorescence microscopy

Unless stated otherwise, L4/young adult animals were analyzed for BAG cell fate defects by mounting them on a glass slide with a 5% agarose pad, using 50 mM NaN_3 as an anesthetic. Expression analysis was conducted using an automated fluorescence microscope [Zeiss (Thornwood, NY) AXIO Imager M2] and images were acquired with the Zen software (Zeiss).

Neuronal scoring

The percentage of animals that expressed GFP in both BAG neurons was scored.

Behavioral assays

Wild-type and *egl-46* mutant animals were starved for 1 hr and then transferred to 14-cm NGM plates containing a 56 × 56-mm arena of Whatman filter paper soaked in 20 mM $CuCl_2$. Eighty to 120 animals were used in a single experiment. Each experimental condition was repeated four to six times. A custom-made transparent plexiglass chamber with a flow volume of 60 × 60 × 0.7 mm was placed onto the assay arena and animals were accustomed to a gas flow of 100 ml/min containing 21% (v/v) O_2 for 5 min. For O_2 experiments animals were stimulated for 6 min with 10% or 17% O_2 and 0% CO_2. For CO_2 experiments animals were stimulated for 6 min with 1% CO_2 and 21% O_2. In all conditions, the gas compositions were balanced with N_2. Gases were mixed by red-y gas mixing units (Vögtlin Instruments) and controlled by LabView software. Recordings were illuminated with flat red LED lights and made at three frames per second on a 4-megapixel CCD camera (Jai), using Streampix software (Norpix).

For movie analysis, MatLab-based image processing and tracking scripts were used as previously described (Ramot *et al.* 2008; Tsunozaki *et al.* 2008). The resulting trajectories were used to calculate instantaneous speed during continuous forward movements (1-sec binning). Omega turns were detected based on characteristic changes in object eccentricity and their frequency was calculated in 30-sec bins.

Generation of transgenic worms

Transgenic animals were obtained through microinjection (Mello *et al.* 1991). Constructs were injected into young adult hermaphrodites as complex arrays, using 1–15 ng·μl^{-1} of PCR product, 150 ng·μl^{-1} of digested bacterial DNA, and *elt-2^{prom}*::*gfp* (3–5 ng·μl^{-1}) as a co-injection marker.

Statistical analyses

Statistical analyses were performed in Prism 6. *egl-46* regulation of BAG terminal differentiation genes and rescue experiments were evaluated using one-way ANOVA with a Newman–Keuls multiple-comparison test. For analysis of behavioral O_2 and CO_2 responses a two-tailed *t*-test was used. Differences with a *P*-value <0.05 were considered significant.

Results

egl-46 mutants display specific defects in BAG neuronal fate determination

We previously showed that the conserved transcription factors ETS-5 and EGL-13 are important for the specification of O_2- and CO_2-sensing fate in the BAG neurons (Guillermin *et al.* 2011; Brandt *et al.* 2012; Gramstrup Petersen *et al.* 2013). However, expression of the O_2-sensing fate markers, GCY-31 and GCY-33, is not fully abrogated in *ets-5 egl-13* double-mutant animals, and the CO_2-sensing fate marker, GCY-9, is only partially affected in *egl-13* mutant animals (Gramstrup Petersen *et al.* 2013). This suggests that other molecules contribute to the regulation of O_2- and CO_2-sensing fate in the BAG neurons. To identify such factors, we performed a forward genetic screen, using a COPAS Biosorter (Doitsidou *et al.* 2008). As a starting strain we used an integrated reporter (*gcy-33^{prom}*::*gfp*), which is exclusively expressed in the BAG neurons (Figure S1). We isolated two alleles (*rp13* and *rp15*) that were both egg-laying defective (Egl) and exhibited partial loss of *gcy-33^{prom}*::*gfp* expression (Figure S1). Whole-genome sequencing of the *rp13* allele identified a 517-bp deletion in the *egl-46* gene (Figure 1A). Complementation tests between *rp13* and *rp15* indicated that they were allelic and subsequent Sanger sequencing of *rp15* revealed a point mutation in exon 2 of *egl-46* that introduces a premature STOP codon (Figure 1A). Animals carrying the previously isolated *egl-46(gk692)* deletion allele display a similar BAG phenotype to *rp13* (Figure S2), further corroborating a role for *egl-46* in BAG development.

To better understand the role *egl-46* plays in BAG specification we used fluorescent reporter strains to monitor expression of a battery of terminal differentiation genes expressed in the BAG neurons (Figure 1B). We analyzed the expression of guanylyl cyclases (*gcy-9*, *gcy-31*, and *gcy-33*) and Phe-Met-Arg-Phe-NH2 (FMRF-amide)-related peptides (*flp-13*, *flp-17*, and *flp-19*) (Figure 1B). Using the *rp13* allele, we found that loss of *egl-46* affected the expression of *gcy-9*, *gcy-31*, and *flp-19*, indicating that *egl-46* expression is required for both O_2 and CO_2 aspects of BAG cell fate (Figure 1B).

Previous expression analysis of *egl-46* did not report expression in the BAG neurons (Wu *et al.* 2001). We therefore generated a transcriptional reporter line in which nuclear localized dsRed2 protein was driven by the full-length 4.5-kb *egl-46* promoter to help with cellular identification (Figure 2A). The reporter confirmed the previously published expression pattern for *egl-46* in the FLP, HSN, and PVD neurons and other neurons in the head (data not shown). We used an *egl-13*::*gfp* reporter (expressed in BAG, URX, AQR, and PQR) to examine colocalization in the BAG neurons. We observed colocalization events in the BAG neurons at the L2 and L3 stages of larval development, suggesting that *egl-46* is expressed during a short temporal window in the BAG neurons, as has been observed in other neurons previously (Figure 2B) (Wu *et al.* 2001). We next tested whether *egl-46* coding sequences could rescue defects in

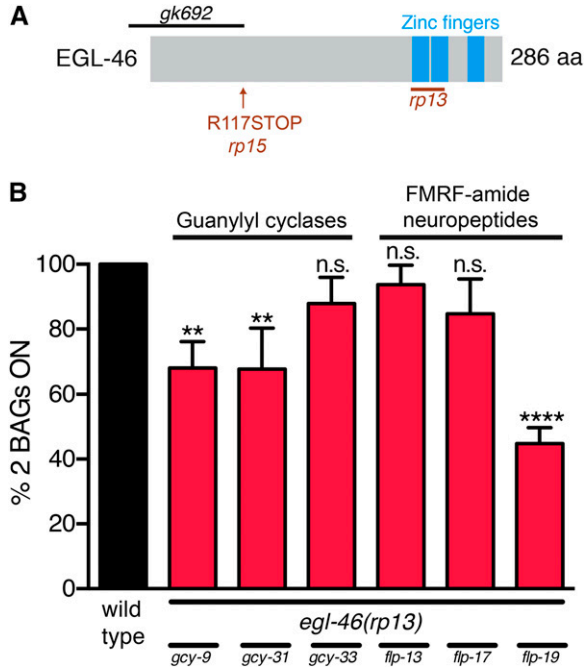

Figure 1 The identification of *egl-46* as a BAG neuronal fate specification gene. (A) Molecular identity of the *egl-46* alleles obtained from the forward genetic screen. *egl-46* alleles first described in this article are shown in red (*rp13* and *rp15*) and the previously described *gk692* allele is shown in black. The nature of the molecular lesions we isolated is as follows: *rp13* is a 517-bp out-of-frame deletion that removes the first two zinc fingers, and *rp15* is a C to T transition that converts an arginine to an opal stop codon early in the protein sequence. (B) Quantification of *rp13*-induced BAG defects in reporters for BAG neuronal fate. Loss of *egl-46* affects fluorescent reporter expression of the neuropeptide *flp-19* and the guanylyl cyclases, *gcy-9* and *gcy-31*. Black bar, BAG reporters in wild-type animals; red bars, reporters in *egl-46(rp13)* mutant animals. $n > 50$. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

BAG neuron expression by using the most strongly affected terminal fate marker, *flp-19^{prom}*::*gfp*. We found that both transgenic expression of *egl-46* cDNA under the control of the *egl-46* 4.5-kb promoter and an *egl-46^{prom}*::*EGL-46*::*dsRed2* translational fusion rescued the *flp-19^{prom}*::*gfp* loss of expression in *egl-46(gk692)* mutant animals (Figure 2C). These data, along with the presence of multiple independent *egl-46* mutant alleles, indicate that *egl-46* is required for BAG terminal fate specification.

egl-46 mutants are defective in O_2 and CO_2 sensing

egl-46 mutant animals exhibit reduction of reporter expression of *gcy-9*, *gcy-31*, and *flp-19* (Figure 1). Previous work has shown that loss-of-function mutations in *gcy-9* cause a defective CO_2 avoidance response (Hallem *et al.* 2011) and that loss of *gcy-31* or *gcy-33* causes a defect in the BAG-mediated response to O_2 downshifts (Zimmer *et al.* 2009). Therefore, we asked whether the effect on reporter expression for these BAG terminal differentiation genes in *egl-46* mutant animals has a consequence on these worm behaviors (Figure 3). We performed well-established behavioral paradigms to examine the importance of *egl-46*

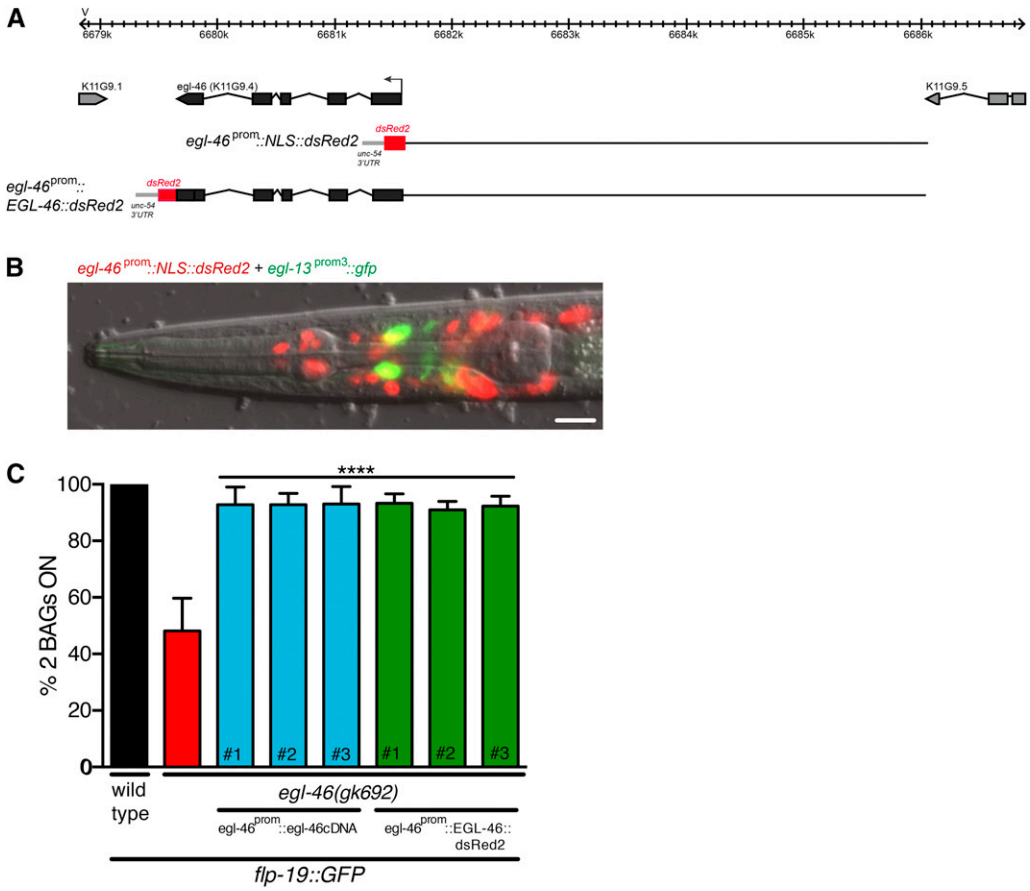

Figure 2 *egl-46* rescue of BAG neuronal fate defects. (A) Schematic representation of the *egl-46* genomic locus. The ATG codon is marked with an arrow and the exons are represented as black blocks. The *egl-46* translational reporter was constructed by driving *egl-46* genomic DNA with *dsRed2* coding sequence under the control of the 4.5-kb *egl-46* promoter. The transcriptional reporter was constructed using the 4.5-kb promoter to drive nuclear-localized dsRed2 (*NLS::dsRed2*). (B) The 4.5-kb *egl-46* promoter drives NLS::dsRed2 expression in multiple nuclei in the head. Colocalization (yellow) of NLS::dsRed2 was observed with cytoplasmic gfp driven by an *egl-13* promoter in the BAG neurons. Note that we only rarely observed colocalization with the BAG marker, suggesting that the *egl-46* promoter drives expression in the BAG neurons in a transient manner. Ventral view, anterior is to the left. Bar, 20 μm. (C) Transgenic expression of the *egl-46* cDNA or *egl-46* genomic sequence fused to dsRed2 under the control of the *egl-46* promoter rescues the *egl-46 (gk692)* mutant loss of *flp-19*^{prom}*::gfp* expression. $n > 50$. ****$P < 0.0001$. # refers to independent transgenic lines.

function on gas sensing. First, we tested whether *egl-46* mutant animals have defects in a CO_2-mediated behavior. Wild-type animals respond to increases in CO_2 concentrations by slowing their forward locomotion and changing direction by using an ω-turn (Bretscher *et al.* 2008). We found that *egl-46* mutant animals show a reduced response to 1% CO_2 with regard to change of speed and ω-turns (Figure 3, A and B).

Next we assayed the ability of *egl-46* mutant animals to respond to an O_2 downshift from 21% O_2 to 10% O_2 (BAG-mediated behavior) and O_2 upshift from 10% O_2 to 21% O_2 (URX-mediated behavior) (Zimmer *et al.* 2009) (Figure S3). Both of these O_2-triggered responses normally result in a reduction of locomotion speed and the performance of an ω-turn in response to an O_2 upshift (Zimmer *et al.* 2009). *egl-46* mutant animals exhibit a similar response compared to wild-type animals with regard to change of speed and ω-turns (Figure S3). We hypothesized that the partially penetrant defects in BAG terminal fate marker expression may cause defects in O_2 sensing to a less acute O_2 downshift. We therefore retested *egl-46* mutant animals in their responses to an O_2 downshift from 21% O_2 to 17% O_2 and an O_2 upshift from 17% O_2 to 21% O_2. Using these parameters we observed a defect in the ability of *egl-46* mutant animals to respond to an O_2 downshift but not to an O_2 upshift (Figure 3). Taken together, these data indicate that loss of *egl-46* causes defects in the response of *C. elegans* to CO_2 upshifts and O_2 downshifts, two BAG-controlled behaviors.

egl-46 acts in parallel to egl-13 and ets-5 to regulate O_2- and CO_2-sensing fate in the BAG neurons

Previous work has shown that *ets-5* and *egl-13* regulate specification of the BAG neurons. However, the expression of some BAG terminal fate markers, especially GCY-31 and GCY-33 that report O_2-sensing fate, is only partially affected by simultaneous loss of *ets-5* and *egl-13* (Gramstrup Petersen *et al.* 2013). In addition, the fully penetrant loss of GCY-9, a reporter for CO_2-sensing fate, in *ets-5* mutants is not observed in *egl-13* mutant animals (Gramstrup Petersen *et al.* 2013). We found that *egl-46* does not affect the expression of *ets-5* or *egl-13* reporters, suggesting that it acts in a parallel manner to these transcription factors in specifying BAG neuronal fate (data not shown). To examine how *egl-46* may regulate BAG O_2- and CO_2-sensing fate specification in parallel to *ets-5* and *egl-13*, we analyzed the expression of

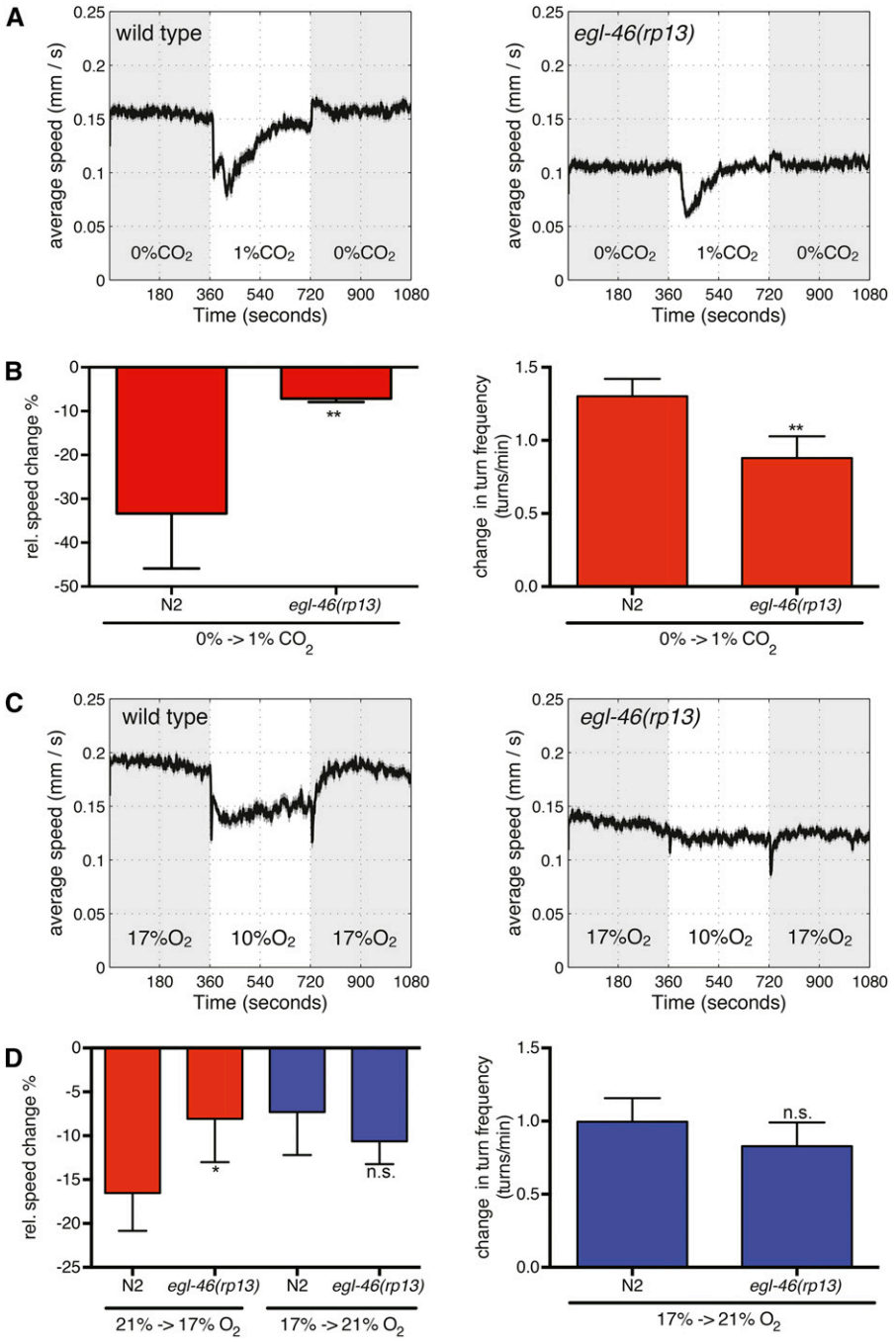

Figure 3 *egl-46* gas-sensing behavior analysis. (A) Locomotion speed of wild type (left) and *egl-46(rp13)* mutants (right) during CO_2 concentration shifts. Data presented are averages of multiple assays (four or more repetitions). CO_2 concentrations were switched between 0% and 1%. (B) Quantification of changes in relative speed (left) and ω-turn frequency (right) in response to a BAG-mediated upshift in CO_2. *egl-46(rp13)* mutant animals exhibit a reduced response. (C) Locomotion speed of wild type (left) and *egl-46(rp13)* mutants (right) during O_2 concentration shifts. Data presented are averages of multiple assays (four or more repetitions). O_2 concentrations were switched between 21% and 17%. (D) Quantification of changes in relative speed (left) and ω-turn frequency (right) in response to BAG-mediated downshift in O_2 (red) and URX-mediated O_2 upshift (blue). *egl-46(rp13)* mutant animals exhibit a decreased response to O_2 downshifts (BAG-mediated behavior) but exhibit a similar response to that of wild-type animals to O_2 upshifts (URX-mediated behavior). Each experimental condition was repeated a minimum four independent times with 80–120 animals in each assay. Statistical significance between wild-type and *egl-46(rp13)* animals was evaluated with a two-tailed *t*-test. *P*-values <0.05 were considered significant; $*P < 0.05$; $**P < 0.01$; n.s., not significantly different from wild-type controls.

gcy-31 and *gcy-33* (O_2 fate) and *gcy-9* (CO_2 fate) transgenic reporters. As previously reported, *gcy-31* and *gcy-33* reporter transgenes are partially affected in *ets-5 egl-13* double-mutant animals (Figure 4, A and B) (Gramstrup Petersen *et al.* 2013). Therefore, we constructed double- and triple-mutant combinations to decipher the role of *egl-46* (Figure 4, A and B). We found that *egl-46* acts in parallel to *ets-5* and *egl-13* to drive *gcy-31* and *gcy-33* expression (Figure 4, A and B). Loss of *egl-46* reduces the number of animals that express *gcy-31*^{prom}::*mCherry* and *gcy-33*^{prom}::*GCY-33*::*gfp* in both BAG neurons when combined with *ets-5* or *egl-13* mutations and when all three transcription factors are removed expression of these markers is abrogated (Figure 4, A and B). Next, we asked whether *egl-46* may act in parallel to *egl-13* to specify the CO_2 fate of the BAG neurons (we did not perform this analysis with *ets-5* as mutant animals are fully penetrant for loss of *gcy-9* expression) (Guillermin *et al.* 2011). We found that loss of *egl-46* reduces the number of animals that express *gcy-9*^{prom}::*dsRed2* when compared to the *egl-13* mutant, indicating that these genes act in parallel pathways to direct CO_2 fate of the BAG neurons (Figure 4C). Taken together, *egl-46* acts in parallel pathways to those of *ets-5* and *egl-13* to regulate the O_2- and CO_2-sensing fate of the BAG neurons (Figure 4D).

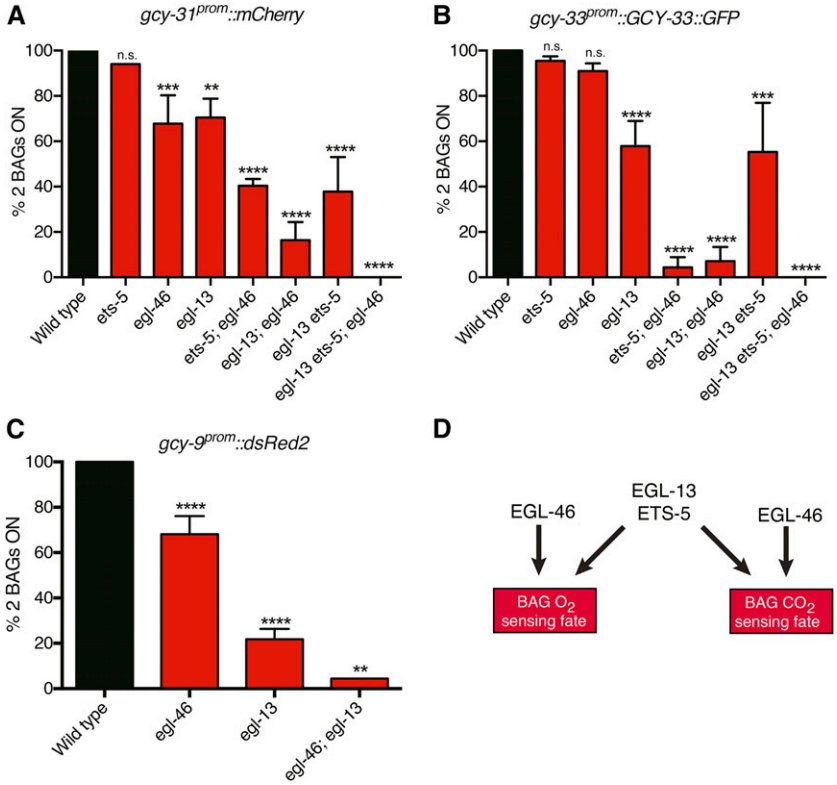

Figure 4 *egl-46* acts in parallel to *ets-5* and *egl-13* to direct gas-sensing fate of the BAG neurons. (A and B) Double-mutant analysis shows that EGL-46 acts in parallel to EGL-13 and ETS-5 to drive the *gcy-31*^{prom}::*mCherry* and *gcy-33*^{prom}::*GCY-33*::*GFP* transgenes in the BAG neurons. Expression of the *gcy-31*^{prom}::*mCherry* (A) and *gcy-33*^{prom}::*GCY-33*::*GFP* (B) fate markers in the BAG neurons is partially, if at all, affected in *egl-46(rp13)*, *ets-5(tm1734)*, or *egl-13 (ku194)* single-mutant animals. A synergistic interaction is observed, however, when *egl-46* is removed in either *egl-13* or *ets-5* mutants and when all three genes are deleted, expression of *gcy-31* and *gcy-33* in the BAG neurons is abrogated. $n > 50$. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (C) Double-mutant analysis shows that EGL-46 acts in parallel to EGL-13 to drive the *gcy-9*^{prom}::*dsRed2* transgenes in the BAG neurons. $n > 50$. $^{**}P < 0.01$, $^{****}P < 0.0001$. (D) Schematic showing the factors known to regulate BAG neuronal fate specification in *C. elegans*. We have shown that *egl-46* acts in parallel to *egl-13* and *ets*-5 to control O_2 and CO_2 sensory fate of the BAG neurons through the control of *gcy-9*, *gcy-31*, and *gcy-33* expression. Arrows denote regulatory activities.

Discussion

In this study, we show that the zinc-finger transcription factor EGL-46 is required for the correct expression of specific terminal differentiation genes in the gas-sensing BAG neurons. We found that loss of *egl-46* causes defects in the ability of worms to mount BAG-mediated behavioral responses to O_2 and CO_2. In addition, EGL-46 acts in a partially parallel pathway to that of EGL-13 and ETS-5 for the regulation of BAG terminal differentiation genes *gcy-9*, *gcy-31*, and *gcy-33*. In the absence of *ets-5* and *egl-13*, two previously identified genes required for expression of the terminal differentiation genes in the BAG neurons, *gcy-31* and *gcy-33* expression is only partially affected (Guillermin *et al.* 2011; Brandt *et al.* 2012; Gramstrup Petersen *et al.* 2013). Additionally, *gcy-9* is partially affected in *egl-13* mutant animals. We found that when *egl-46* is removed in *ets-5 egl-13* double-mutant animals, *gcy-31* and *gcy-33* expression in the BAG neurons is abrogated. Therefore, *egl-46* acts in a parallel pathway to that of *ets-5* and *egl-13* to regulate expression of O_2 cell fate markers in the BAG neurons. In addition, we found that *egl-46* acts in a parallel pathway to that of *egl-13* to drive gcy-9 expression, a reporter of CO_2-sensing fate in the BAG neurons.

The reason for an EGL-46-driven parallel pathway to ensure gas-sensing neuronal fate in the BAG neurons is unclear. Interestingly, however, in *Drosophila*, the *egl-46* ortholog Nerfin-1 is downregulated in maxillary palps by the *mir-279* microRNA to prevent CO_2 neuronal fate (Cayirlioglu *et al.* 2008; Hartl *et al.* 2011). In fact, overexpression of Nerfin-1 together with a second zinc-finger protein Escargot is sufficient to induce CO_2 neurons on the maxillary palps (Hartl *et al.* 2011). Therefore, the requirement for this family of zinc-finger transcription factors in the regulation of gas-sensing neuron specification is evolutionarily conserved.

Acknowledgments

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A Novel Role for the Zinc-Finger Transcription Factor EGL-46 in the Differentiation of Gas-Sensing Neurons in *Caenorhabditis elegans*

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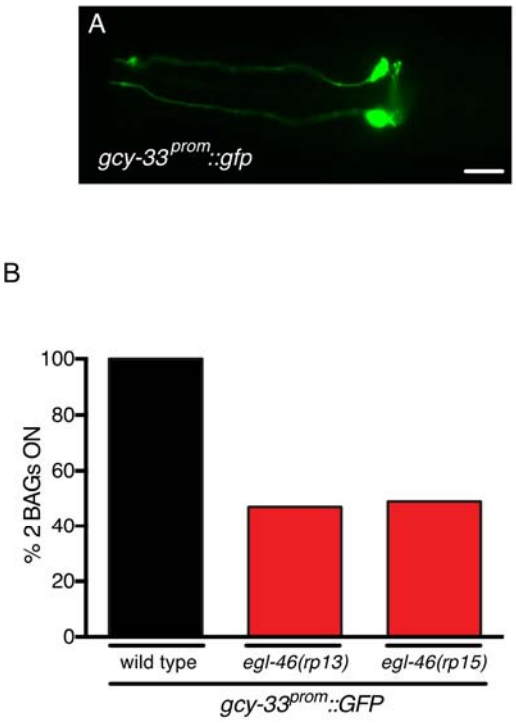

Figure S1 Identification of two *egl-46* alleles that affect *gcy-33^{prom}::gfp* expression

(A) Representative picture of an animal expressing a BAG reporter (*gcy-33^{prom}::gfp*) in wild type animals. Ventral view, anterior to the left. Scale bar, 20µm.

(B) Two mutant alleles of *egl-46* were isolated from an EMS forward mutagenesis screen using the BAG-specific transcriptional *gcy-33^{prom}::gfp* reporter. n>50 *P<0.05

Note that the translational *gcy-33* reporter called *gcy-33^{prom}::GCY-33::GFP* that we used in the rest of the study is minimally affected by loss of *egl-46* (Figure 4). This suggests that sequences included in the translational reporter transgene harbor binding sites for *ets-5* and *egl-13* that ensure expression in the BAG neurons.

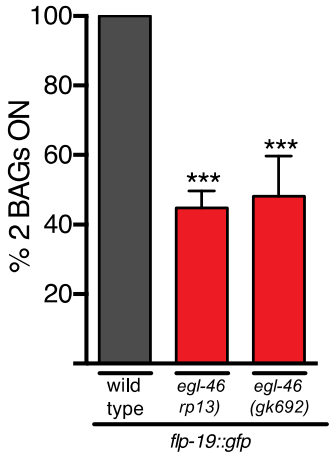

Figure S2 The *egl-46(gk692)* mutant phenocopies *rp13* for BAG neuronal fate specification defects

A previously isolated *egl-46(gk692)* deletion allele displays a similar loss of *flp-19::gfp* expression to *rp13*. n>50. ****P<0.0001.

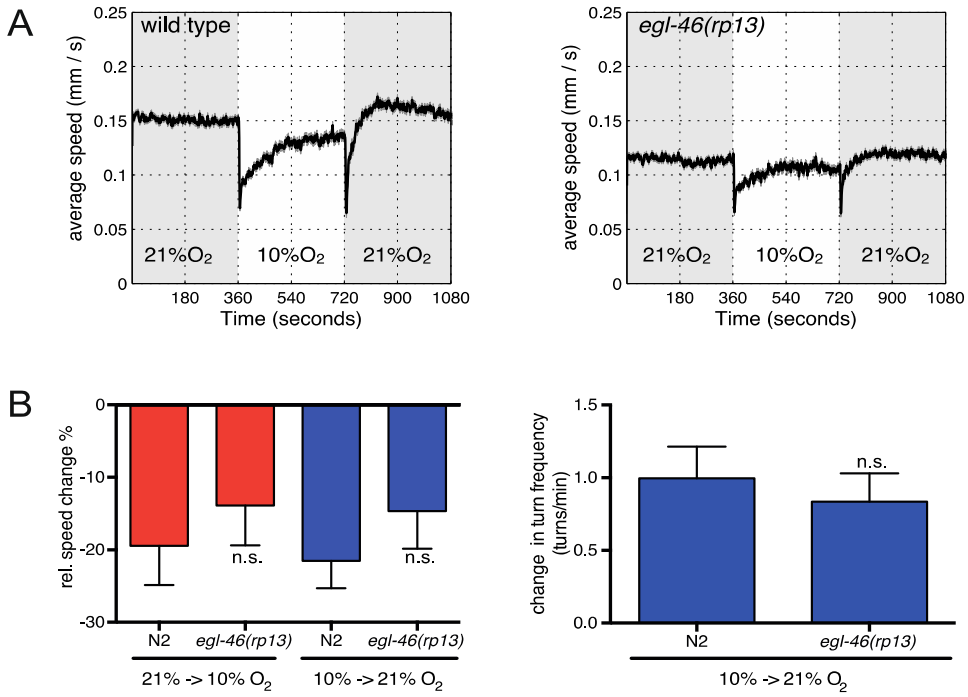

Figure S3 *egl-46* mutant animals are not defective in responding to large shifts in O_2

(A) Locomotion speed of wild type (left) and *egl-46(rp13)* mutants (right) during O_2 concentration shifts. Data presented are averages of multiple assays (≥3 repetitions). O_2 concentrations were switched between 21% and 10%.

(B) Quantification of changes in relative speed (left) and omega turn frequency (right) in response to BAG-mediated downshift in O_2 (red) and URX-mediated O_2 upshift (blue). Each experimental condition was repeated minimum four independent times with 80-120 animals in each assay. Statistical significance between wild type and *egl-46(rp13)* animals was evaluated with a two-tailed t-test. n.s. = not significantly different from wild type controls.

Table S1 List of strains used in this study

Genotype	Array number	Strain name	Plasmid used
Is[gcy-33^{prom}::GFP]; dop-3^{prom}::RFP	rpIs3	RJP22	-
egl-46(rp13); gcy-33^{prom}::GFP; dop-3::RFP	rpIs3	RJP147	-
egl-46(rp15); gcy-33^{prom}::GFP; dop-3::RPF	rpIs3	RJP149	-
gcy-9^{prom}::dsRed	wzIs112	FQ383	-
gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP296	Fusion PCR
Is[gcy-33^{prom}::GCY-33::GFP; rol-6]	rpIs7	RJP401	-
flp-13^{prom}::GFP	ynIs37	NY1037	-
flp-17^{prom}::GFP	ynIs64	RJP567	-
flp-19^{prom}::GFP; him-5(e1490)	ynIs34	RJP255	-
egl-46(rp13); gcy-9^{prom}::dsRed	wzIs112	RJP858	-
egl-46(rp13); gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP903	Fusion PCR
egl-46(rp13); gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP663	-
egl-46(rp13); flp-13^{prom}::GFP	ynIs37	RJP983	-
egl-46(rp13); flp-17^{prom}::GFP	ynIs64	RJP864	-
egl-46(rp13); flp-19^{prom}::GFP	ynIs34	RJP899	-
Is[egl-13^{prom}::GFP]; Ex[egl-46^{prom}::dsRed2::NLS; elt-2::GFP]	rpIs32; rpEx743	RJP1748	-
egl-46(gk692); flp-19^{prom}::GFP	ynIs34	RJP454	-
egl-46(gk692); flp-19^{prom}::GFP; Ex[egl-46^{prom}::EGL-46::dsRed2; elt-2::GFP]	rpEx723	RJP1706	Fusion PCR
	rpEx724	RJP1707	
	rpEx725	RJP1708	
egl-46(gk692); flp-19^{prom}::GFP; Ex[egl-46^{prom}::egl-46cDNA; elt-2::GFP]	rpEx726	RJP1709	Fusion PCR
	rpEx727	RJP1710	
	rpEx728	RJP1711	
N2 bristol	-	-	-
egl-46(rp13); him-8(e1489)	-	RJP898	-
egl-13(ku194); gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP361	-
ets-5(tm1734);gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP523	-
egl-13(ku194);ets-5(tm1734); gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP1263	-
egl-13(ku193);egl-46(rp13);gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP1485	-
ets-5(tm1734); egl-46(rp13); gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP1297	-
egl-13(ku194); ets-5(tm1734); egl-46(rp13); gcy-33^{prom}::GCY-33::GFP; rol-6	rpIs7	RJP1712	-
egl-13(ku194); gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP365	Fusion PCR
ets-5(tm1734);gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP1029	
egl-13(ku194);ets-5(tm1734);gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP1023	
egl-13(ku193);egl-46(rp13);gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP1781	
ets-5(tm1734); egl-46(rp13);gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP1782	
egl-13(ku194); ets-5(tm1734); egl-46(rp13);gcy-31^{prom1kb}::mCherry; elt-2^{prom}::GFP	rpEx100	RJP1783	
egl-13(ku194); gcy-9^{prom}::dsRed	wzIs112	RJP754	-
egl-46(rp13); egl-13(ku194); gcy-9^{prom}::dsRed	wzIs112	RJP1889	-