Aerobic animals constantly monitor and adapt to changes in O₂ levels. The molecular mechanisms involved in sensing O₂ are, however, incompletely understood. Previous studies showed that a hexacoordinated globin called GLB-5 tunes the dynamic range of O₂-sensing neurons in natural C. elegans isolates, but is defective in the N2 lab reference strain (McGrath et al., 2009; Persson et al., 2009). GLB-5 enables a sharp behavioral switch when O₂ changes between 21 and 17%. Here, we show that GLB-5 also confers rapid behavioral and cellular recovery from exposure to hypoxia. Hypoxia reconfigures O₂-evoked Ca²⁺ responses in the URX O₂ sensors, and GLB-5 enables rapid recovery of these responses upon re-oxygenation. Forward genetic screens indicate that GLB-5’s effects on O₂ sensing require PDL-1, the C. elegans ortholog of mammalian PrBP/PDE6 protein. In mammals, PDE6 regulates the traffic and activity of prenylated proteins (Zhang et al., 2004; Norton et al., 2005). PDL-1 promotes localization of GCY-33 and GCY-35, atypical soluble guanylate cyclases that act as O₂ sensors, to the dendritic endings of URX and BAG neurons, where they colocalize with GLB-5. Both GCY-33 and GCY-35 are predicted to be prenylated. Dendritic localization is not essential for GCY-35 to function as an O₂ sensor, but disrupting pdl-1 alters the URX neuron’s O₂ response properties. Functional GLB-5 can restore dendritic localization of GCY-35 in pdl-1 mutants, suggesting GCY-33 and GLB-5 are in a complex. Our data suggest GLB-5 and the soluble guanylate cyclases operate in close proximity to sculpt O₂ responses.

Key words: C. elegans; globin; hypoxia; oxygen sensing; prenyl binding protein; soluble guanylate cyclase
globin domain that, like mammalian neuroglobin and cytoglobin, has a hexacoordinated heme Fe^{2+} (Persson et al., 2009; Hundahl et al., 2013). How hexacoordinated globins generally, and GLB-5 in particular, signal is poorly understood—one scenario is that they engage in ROS signaling.

O_{2}^- sensing enables natural C. elegans isolates to escape 21% O_{2}, accumulate where bacteria is thickest, usually at the lawn border, and to settle on food when O_{2} drops to 19% (Busch et al., 2012). During domestication the standard N2 (Bristol) laboratory strain lost these behaviors, due to a gain-of-function mutation in a neuropeptide receptor, npr-1 F215V, and a loss-of-function mutation in glb-5, glb-5 (McGrath et al., 2009; Weber et al., 2010).

Here, we explore the functions of GLB-5 neuroglobin further. Since globins are proposed to protect neurons against hypoxia, we ask whether a functional glb-5 allele from a Hawaiian wild strain, glb-5(Haw), alters C. elegans' responses to hypoxia/re-oxygenation stress. We show that without glb-5 hypoxia experience strongly attenuates both O_{2}^-evoked Ca^{2+} responses in URX neurons and bordering behavior. GLB-5(Haw)'s ability to influence O_{2} responses depends on a prenyl binding protein, PDL-1/PrBP, which promotes traffic of the soluble guanylate cyclases to dendritic endings. Our data suggest GLB-5 and the soluble guanylate cyclases operate in close proximity. However, dendritic localization is not essential for GCC-35/GCY36 to respond to O_{2} variation.

Materials and Methods

Strains

These include the following: AX204 glb-5(Bri) V; npr-1(ad609) X; AX1891 glb-5(Haw) V; npr-1(ad609) X; CB4856 Hawaiian wild strain; AX1198 gcy-35(ok769) I; npr-1(ad609) X; AX2363 gcy-33(ok232) V; npr-1(ad609) X; AX3450 pdl-1(db508) II; glb-5(Vaw) X; npr-1(ad609) X; AX3451 pdl-1(gk157) II; glb-5(Haw) V; npr-1(ad609) X; AX3452 pdl-1(gk157) II; glb-5(Bri) V; npr-1(ad609) X; AX1843 glb-5(Bri) V; npr-1(ad609) X; dbEx[Pglb-5:gcy-35::glb-5(Haw);pF15E11.1::GFP] X; dbEx[Pgcy-37::CYC2.60; glb-5(Bri) hif-1(ia4) V; npr-1(ad609) X; dbEx[Pgcy-37:gcy-35::HA GFP polycismCherry; pF15E11.1::GFP] X; dbEx[Pgcy-37::YC2.60; glb-5(Bri) hif-1(ia4) V; npr-1(ad609) X; dbEx[Gcy-37::YC2.60 + cRFP] V; EVG126 pdl-1(gk157) II; glb-5(Bri) V; npr-1(ad609) X; Ex[Pgcy-37::YC2.60 + cRFP] X; EVG127 gcy-33(ok232) V; npr-1(ad609) X; Ex[Pgcy-37::YC2.60 + cRFP] X; EVG128 gcy-35(n765) X; dbEx[Pgcy-37::gcy-35:HA GFP; polycismCherry] X; dbEx(Pgcy-37::gcy-35:HA GFP; polycismCherry + cRFP) X.

Behavioral assays

Bordering experiments were performed by transferring 40 L4 hermaphrodites (grown in 21% O_{2} at room temperature, RT) to a 5 cm NGM plate that had been seeded 2 d before with OP50 bacteria. Assay plates were put in 1% O_{2} (Coy hypoxia chamber; Coy Lab Products) RT for 3–18 h (as indicated), brought back to 21% O_{2}, and the percentage of animals bordering calculated after 30 min or longer, as indicated. The bordering index is the fraction of worms found on the bacterial lawn border divided by the total number of worms on the plate, multiplied by 100. To quantiﬁed speed 10–15 animals were picked onto a 3.5 cm Petri dish containing NGM agar that was seeded 2 d before with Escherichia coli OP50. We placed the Petri dish into a custom-designed Perspex chamber (48 × 17 mm; w × d × h) containing an inner circular cavity designed to ﬁt the 3.5 cm dish. Two metal pipes provided an inlet and an outlet into the chamber for gas supply. Gas was delivered via silicone tubing using a custom-built computer-controlled manifold. The manifold delivered a constant gas ﬂow of 7 ml/min via gas mass ﬂow controllers (FMA5510; Omega) from gas tanks containing deﬁned O_{2},N_{2}, mixtures (British Oxygen Company), and enabled gas switches to be made at deﬁned times. Behavior was recorded using a Grasshopper 2064M-C CCD camera (Point Gray Research) mounted onto a Leica dissection microscope using an appropriate C-mount adapter (Leica) at 2 frames/s. Videos were analyzed using Zentrokar, a custom software that tracks the center of mass of each animal and calculates instantaneous speed. The software is available on request.

Ethyl methanesulfonate mutagenesis screen

We mutagenized glb-5(Haw); npr-1 worms as described previously (Brenner, 1974). Two-hundred and ﬁfty mutagenized P_{r} hermaphrodites were divided into 50, 9 cm seeded plates. After 4 d, F1 animals (~6000 gravid hermaphrodites) were divided into 60, 9 cm seeded plates and allowed to lay eggs for 2 h. To kill most of the F1 worms without harming the eggs, 3 × 25 µl drops of chloroform were put on the lid of each plate. After 3 d, we transferred ~250 aggregating L4 worms from the glb-5(Bri) mutation was traced by examining the size of PCR fragments amplified with primers that flank the duplication in this allele (Persson et al., 2009). The npr-1(ad609) allele was followed by amplifying

Mapping

We mapped db805 to a 4 Mb genomic interval using single nucleotide polymorphisms (SNPs; Davis et al., 2005). To identify the gene disrupted by db805, we prepared amplification-free genomic libraries from db805 mutant DNA (Kozarewa et al., 2009; Weber et al., 2010) and sequenced them with the Illumina Genome Analyzer II. Sequence analysis was performed as described previously (Davis et al., 2005).

Molecular biology

General molecular manipulations followed standard protocols (Sambrook et al., 1989).

Genotyping.

The pdl-1(db805) allele was followed by amplifying and sequencing the interval flanking the splice-donor region of exon 3. The gk157 deletion was followed by PCR using primers that flank the deletion. The glb-5(Bri) mutation was traced by examining the size of PCR fragments amplified with primers that flank the duplication in this allele (Persson et al., 2009). The npr-1(ad609) allele was followed by amplifying

Gross et al. • GLOBIN-5-Dependent O_{2} Responses Are Regulated by PDL-1/PrBP J. Neurosci., December 10, 2014 • 34(50):16726 –16738 • 16727
the interval containing its associated DNA lesions, and using restriction enzyme digestion to distinguish wild-type and mutant alleles. pdl-1, gcy-35, and gcy-33 cDNA. We extracted total RNA from N2 worms by vortexing with TRIzol (Life Technologies) and acid-washed glass beads, and purifying using an RNaseasy Midi Kit (Qiagen). cDNAs were generated by RT-PCR unless otherwise mentioned (OneStep RT-PCR kit; Qiagen). cDNA of gcy-33 was amplified from a cDNA clone kindly provided by Y. Kohara. Oligonucleotides were designed using the sequences in Wormbase (http://www.wormbase.org/).

Transgenes. Microinjections were performed as described previously (Mello et al., 1991). Plasmids (2.5–50 ng/μl) were co-injected with fluorescent reporters, and multiple independent lines isolated. In brief, N2 genomic DNA was used to amplify the pdl-1 gene, including 3 kb of upstream sequences. The genomic fragment was cloned into pPFD95.75 (A. Fire, personal communication), and then modified by adding an outon-mCherry fragment to make a polyhistrionic expression vector, as described previously (Coates and de Bono, 2002; Persson et al., 2009). This expression construct was modified to create other expression constructs. To create a pdl-1 cDNA expression construct, the genomic part of the pdl-1 gene was replaced with pdl-1 cDNA. To create constructs that expressed pdl-1 in the BAG or AQR, PQR, and URX neurons, the promoter region of pdl-1 was replaced with the 3.3 kb and the unknown kb promoter of J7 and gcy-37, respectively. To express pdl-1 in the BAG, AQR, PQR, and URX neurons, the promoter region of pdl-1 was replaced with the 3.1 kb promoter of glb-5. To generate a functional pdl-1::GFP expression construct, the pdl-1 stop codon was deleted by meganuclease and sequences encoding GFP inserted instead of the outon-mCherry fragment to create an in-frame translational reporter gene. To create a functional gcy-35::gfp expression construct, we inserted the GFP reporter sequences after the codon encoding Ser671, thereby retaining intact N- and C-termini. Similarly, to create a functional gcy-33::gfp expression construct, we inserted the GFP reporter sequences after Ala900 to examine how hif-1 affected glb-5 responses to hypoxia, we injected a genomic copy of glb-5(Haw) into hif-1(ia4); npr-1(ad609) animals.

Imaging strains. To create imaging strains that expressed YC2.60 in the BAG or AQR, PQR, and URX neurons, cDNA for YC2.60 (Nagai et al., 2004) was cloned after the J7 and gcy-37 promoters, respectively. These constructs were injected into glb-5(Haw); npr-1(ad609) hermaphrodites, together with 50 ng/μl cc::RFP or F15E11.1:RFP/F15E11.1:GFP injection markers, following standard methods (Mello et al., 1991).

Cell-autonomous RNA interference Cell-specific knockdowns were generated as described previously (Esposito et al., 2007). To knockdown pdl-1 in the AQR, PQR, and URX neurons, sense and antisense RNA sequences of pdl-1 were expressed from the gcy-37 promoter. The sense and antisense PCR-fusion products were coinjected into pdl-1; npr-1(ad609) (Haw); npr-1(ad609) hermaphrodites, together with 25 ng/μl of the F15E11.1:GFP injection marker. Similarly, PCR fusion products of pgy-37::GFP (sense and antisense) were coinjected into pdl-1(gk157); glb-5(Haw); npr-1(ad609). Ex[Ppd1-1::pdl-1::GFP] hermaphrodites together with 25 ng/μl F15E11.1:RFP injection marker.

Y2H interaction assays We used the Matchmaker Gold Yeast two-hybrid system (Clontech Laboratories) to investigate protein interactions with PDL-1. In brief, pdl-1 cDNA was cloned into pGBK7, transformed into Y2HGold yeast cells, and grown on selective Trp plates (according to the manual from Clontech Laboratories). Positive and negative control vectors, pGBK7–53 and pGBK7-Lam, were transformed into Y2HGold yeast cells and grown under the same conditions. The cDNAs of gcy-35, gcy-33, ras-1, ras-2, evl-20, rob-3, rab-18, arl-3, and arl-13, and glb-5(Haw) were cloned into pGADT7, transformed into yeast strain Y187, and grown on selective -Leu plates. The negative control vector, pGAD7-T, was transformed into Y2HGold yeast cells and grown under the same conditions. Positive colonies from the -Trp and -Leu plates were mixed with a toothpick on a YPD plate, and grown at 30°C overnight. Y2HGold/Y187 diploid strains were streaked onto -Trp/-Leu double-selection plates and grown for 3–4 d at 30°C. Colonies were then grown overnight in liquid culture, after which 5 μl of diluted and nondiluted diploid suspensions were put on Trp/Leu/Aureobasidin A/X-α-Gal or -Trp/-Leu/-Adel/-His/Aureobasidin A/X-α-Gal plates. Colony growth was monitored after 3–4 d to detect protein interaction.

Ca<sup>2+</sup> imaging Ca<sup>2+</sup> imaging experiments were performed as described previously (Persson et al., 2009). Ca<sup>2+</sup> responses were recorded at 2 frames/s using a Zeiss Axio Observer D1 inverted epifluorescence microscope or an Olympus IX71SF3–5 inverted microscope equipped with a 40× C-Apochromat lens (Carl Zeiss) or UAPON40X Universal apochromatic water-immersion objective (Olympus) and Optosplit II beam splitter or DVM 2-channel imager (Cairn Research and Photometrics, respectively), Cascade II 1024 EMCCD camera, or Rolera EM-C2 (Photometrics and QImaging, respectively), and MetaMorph software (Molecular Devices). For imaging experiments, we glued the worms to agarose pads (2% agarose in M9) using Dermabond tissue adhesive (Closure Medical). Immobilized worms were trapped inside a 500 μm deep rectangular PDMS chamber. Alternatively, we anesthetized the worms using 10 mM levamisole and put them in a custom-made, airtight microfluidic chamber. Humidified gases were delivered to the microfluidic chambers using a PHD 2000 syringe pump (Harvard Apparatus) at a flow rate of 1 μl/min. Teflon valves, regulated by a ValveBank Controller (Automate Scientific), were used to rapidly switch between gas mixtures. Image analysis was performed using custom-written MATLAB software.

Confocal microscopy We mounted worms on 2% agarose pads (in M9 buffer supplemented with 10 mM sodium azide), and imaged them on a Zeiss LSM 510 laser scanning confocal microscope using a Plan-Apochromat 63× 1.4 NA objective (Zen operating software; Carl Zeiss). Image analysis was performed using ImageJ (Abramoff et al., 2004).

Results The neuroglobin GLB-5 regulates C. elegans responses to hypoxic exposure Transcription of many globin genes, including some C. elegans globins, is induced by hypoxia (Sun et al., 2001; Hoogewijs et al., 2007). This regulated expression is proposed to protect against hypoxia/re-oxygenation damage (Burmester and Hankeln, 2009). To investigate if GLB-5 altered C. elegans responses to hypoxia/re-oxygenation stress, we exposed animals bearing the functional glb-5(Haw) allele found in natural isolates or the loss-of-function glb-5(Bri) allele found in N2 Bristol lab strain, to 1% O<sub>2</sub>, returned them to 21% O<sub>2</sub>, and looked for differences in behavior. In our assays we used animals that also carried the npr-1 215F allele or the npr-1(ia4); npr-1(215F) functional null allele, since the npr-1 215V gain-of-function allele found in N2 suppresses the activity of some O<sub>2</sub>-sensing circuits (Gray et al., 2004; Cheung et al., 2005; Rockman and Kruglyak, 2009; Weber et al., 2010). We incubated the glb-5(Bri); npr-1(ad609) and glb-5(Haw); npr-1(ad609) animals (hereafter referred to as npr-1 and glb-5(Haw); npr-1 animals, respectively), and animals from the Hawaiian wild strain CB4856 (bearing the natural glb-5(Haw) and npr-1(215F) alleles) in 1% O<sub>2</sub> for 3, 6, 9, and 18 h, transferred them to 21% O<sub>2</sub>, and examined their behavior after 30 min. When grown in 21% O<sub>2</sub> animals from all three strains gather where the bacteria grow immediately at the lawn border, regardless of time in hypoxia (Gray et al., 2004). Upon exposure to 1% O<sub>2</sub> animals from each strain left the food and accumulated outside the bacteria. When returned to normoxia, glb-5(Haw); npr-1 and CB4856 animals immediately accumulated at the lawn border, regardless of time in hypoxia. In contrast, npr-1 animals incubated in hypoxia for 18 h showed little preference for the border when returned to 21% O<sub>2</sub> (Fig. 1B). The hypoxia-induced inhibition of bordering was reversed within 4 h of return to normoxia (Fig. 1C). Bordering reflects an O<sub>2</sub> preference, with [O<sub>2</sub>] ~13% in the thicker bacteria.
at the border, and \( \sim 17\% \) \( \text{O}_2 \) at the lawn center (Gray et al., 2004). These results suggest that GLB-5(Haw) signaling enables \( \text{C. elegans} \) exposed to hypoxia and returned to normoxia to remain sensitive to these \( \text{O}_2 \) differences and to border.

**\( \text{C. elegans} \) PDE66/PrBP prenyl binding protein is required for GLB-5 function**

How does GLB-5(Haw) alter \( \text{O}_2 \) sensing? To investigate this question we turned to forward genetics, and sought genes required for \( \text{glb-5} \) signaling. Originally, we identified the \( \text{glb-5(Haw)} \) allele because it enables natural isolates of \( \text{C. elegans} \) to switch from slow to rapid movement on a bacterial lawn when \( \text{O}_2 \) rises from 17 to 21\% (Persson et al., 2009). Although robust, this assay is too labor intensive for forward genetic screens. In contrast, identifying mutations that prevent \( \text{glb-5(Haw)} \); \( \text{npr-1} \) animals from bordering after hypoxia exposure is amenable to this approach. We mutagenized \( \text{glb-5(Haw)} \); \( \text{npr-1} \) animals and sought mutants that, after experiencing 1\% \( \text{O}_2 \), failed to border immediately after return to 21\% \( \text{O}_2 \). To exclude mutations that indiscriminately disrupted bordering, we selected lines that resumed bordering after recovering at 21\% \( \text{O}_2 \) for 4 h. We isolated four mutants that satisfied these criteria. By combining Snip SNP mapping with Illumina whole genome sequencing (Wicks et al., 2001; Sarin et al., 2008) we mapped one allele, \( \text{dbb805} \), to a 3.8 Mb genomic interval on chromosome II, and identified 10 mutations in this interval. Only one of these mutations altered the predicted open reading frame, disrupting a splice acceptor site in \( \text{pdl-1} \) (Fig. 1D). \( \text{pdl-1} \) encodes a 17 kDa protein orthologous to mammalian PDE6\( \delta \) (phosphodiesterase 6, \( \delta \)-subunit). PDL-1 and human PDE6\( \delta \) share 69\% amino acid identity at the protein level.

PDE6\( \delta \) was originally identified as a subunit of PDE6 from bovine retina (Gillespie et al., 1989). Biochemical studies showed that it is a prenyl binding protein (PrBP) that can extract prenylated proteins such as small GTPases (e.g., Ras) from membranes, sequestering them in the cytoplasm, and facilitating their traffic to different membrane compartments (Chandra et al., 2012). Mice lacking PDE6\( \delta \) are small but viable; they exhibit progressive cone–rod dystrophy and defective localization of prenylated rhodopsin kinase (GRK1) and PDE6 catalytic subunits to rod outer segments (Zhang et al., 2007).

To confirm the \( \text{pdl-1} \) phenotype, we obtained a deletion allele, \( \text{gk157} \), which removed most of the \( \text{pdl-1} \) coding region and \( \sim 0.5 \) kb of the upstream promoter region. \( \text{pdl-1(gk157)} \) delayed the recovery of bordering in \( \text{glb-5(Haw)} \); \( \text{npr-1} \) animals exposed to hypoxia more strongly than the \( \text{pdl-1(dbb805)} \) mutation (Fig. 2A). Since \( \text{pdl-1(gk157)} \) is probably a null allele, we used it in subsequent experiments, unless otherwise indicated.

**PDL-1 functions in the same neurons as GLB-5**

Where does PDL-1 function to modify GLB-5-dependent behaviors? \( \text{glb-5} \) is expressed in the AQR, PQR, URX, and BAG \( \text{O}_2 \)-sensing neurons. Previous work showed that selectively expressing a \( \text{glb-5(Haw)} \) transgene in AQR, PQR, and URX neurons confers on \( \text{npr-1} \) animals the sharply tuned \( \text{O}_2 \) responses of \( \text{glb-5(Haw)} \); \( \text{npr-1} \) animals (McGrath et al., 2009; Persson et al., 2009). To identify where PDL-1 is expressed, we generated a transgene in which genomic DNA for \( \text{pdl-1} \) was fused in-frame with a sequence-encoding GFP (Fig. 2B). Using behavioral assays, we showed that this transgene was fully functional (Fig. 2C,F). We then crossed it with a strain expressing a functional \( \text{pglb-5:(glb-5(Haw)::mCherry)} \) fusion construct, and looked for colocalization of green and red fluorescence in animals bearing both transgenes. \( \text{pdl-1} \) and \( \text{glb-5(Haw)} \) were coexpressed in the AQR, PQR, URX, and BAG neurons (Fig. 2B). \( \text{pdl-1} \) was also expressed in other neurons in the head and tail.

Is \( \text{pdl-1} \) generally required for \( \text{GLB-5(Haw)} \) signaling to modify \( \text{O}_2 \) sensing, or are its effects specific to the hypoxia paradigm? To address this, we asked whether \( \text{pdl-1} \) mutations affected the ability of normoxia-cultivated \( \text{glb-5(Haw)} \); \( \text{npr-1} \) animals to switch from rapid movement at 21\% \( \text{O}_2 \) to slow movement at 17\% \( \text{O}_2 \) (McGrath et al., 2009; Persson et al., 2009). Consistent
with a general role, pdl-1; glb-5(Haw); npr-1 animals moved much faster than glb-5(Haw); npr-1 animals at 17% O₂ (Fig. 2D).

These data suggest that GLB-5(Haw) effects on O₂ responses depend on correct trafficking of prenylated proteins. Countervirtually, disrupting pdl-1 also slightly but significantly reduced the rate of movement of npr-1 animals at 21% O₂ (Fig. 2E), suggesting PDL-1 can influence O₂ sensing independently of GLB-5, perhaps by affecting soluble guanylate cyclases. Does PDL-1 act in the same neurons as GLB-5 to regulate O₂ responses? Replacing the pdl-1 promoter with the glb-5 promoter enabled pdl-1; glb-5(Haw); npr-1 animals exposed to hypoxia to resume bordering rapidly after re-oxygenation, consistent with PDL-1 and GLB-5 functioning in the same cells (Fig. 2C). However, expressing pdl-1 specifically in the AQR, PQR, and URX neurons, by replacing the pdl-1 promoter with the gcy-37 promoter, or in BAG neurons, using the gcy-37 promoter, or only in BAG neurons, using the gcy-37 promoter, failed to rescue glb-5(Haw)-dependent responses to a switch from 21 to 17% O₂. In contrast, coexpressing the two transgenes rescued the behaviors (Fig. 2F).

To confirm our results, we made RNAi constructs under the control of the gcy-37 promoter that targeted pdl-1 or GFP sequences. We injected these into pdl-1; glb-5(Haw); npr-1 mutant animals that expressed a pdl-1(cDNA)-GFP fusion construct from the pdl-1 promoter. Whereas animals not expressing the RNAi constructs strongly reduced movement when switched from 21 to 17% O₂, animals expressing the RNAi transgenes did not (Fig. 2G). These data suggest that PDL-1 acts in AQR, PQR, URX, and BAG neurons to promote glb-5(Haw)-dependent O₂ responses in behavioral paradigms that operate at very different timescales.

**PDL-1/PrBP mediates localization of soluble guanylate cyclases to dendritic endings**

Mammalian PrBP/PDE6δ interacts with prenylated proteins and nonprenylated small GTPases and regulates their traffic (Hanzal-Bayer et al., 2002). GLB-5 is in neither of these categories, but three of the atypical soluble guanylate cyclases required for O₂ responses—GCY-35 and GCY-36 in AQR, PQR and URX neurons, and GCY-33 in BAG neurons—are predicted to have C-terminal -CAAX sequences, suggesting they are prenylated (Cheung et al., 2004, 2005; Gray et al., 2004; Persson et al., 2009;
Zimmer et al., 2009; Busch et al., 2012). These heme proteins are thought to directly bind O₂ and O₂ binding is thought to modulate their cGMP production and to change neural activity by gating cGMP ion channels (Coutu et al., 2013). The functional importance of the CAAX motif has been directly tested for the GCY-36 soluble guanylate cyclase. Mutating sequences encoding the CAAX motif to encode SAAX disrupted GCY-36 localization to dendritic endings and resulted in a nonfunctional gcy-36 gene (Cheung et al., 2004).

To examine if PDL-1 regulates trafficking of GCY-35 and GCY-33, we made transgenes that expressed functional fluorescently tagged versions of these proteins. Tagging GCY-35 N terminally or C terminally interfered with its biological activity. We recently tagged versions of these proteins. Tagging GCY-35 N terminally or C terminally interfered with its biological activity. We therefore inserted gfp cDNA into sequences encoding a predicted flexible loop in the GCY-35 C-terminal tail. Like GLB-5(Haw), GCY-35-GFP was enriched at the dendritic tips of URX neurons (Fig. 3A). Moreover, this transgene largely (although not completely) restored O₂ control of locomotor activity to gcy-35; npr-1 mutants (Fig. 3B). We compared the accumulation of GCY-35::GFP in the dendrite and cell body of URX neurons in gcy-35; npr-1 and gcy-35; pdl-1; npr-1 animals (Fig. 3C,D). While fluorescence in the cell body was very similar in the two genotypes, loss of pdl-1 essentially abolished GCY-35-GFP accumulation at the tip of URX dendrites. Thus, pdl-1 is required for correct localization of GCY-35 at dendritic endings. However, localization of GCY-35 to dendrites is not essential for O₂-evoked responses.

We also examined if prolonged exposure to hypoxia (18 h) altered levels and distribution of GCY-35 in the URX neurons. We incubated gcy-35; npr-1 and gcy-35; glb-5(Haw); npr-1 animals in 1% or 21% O₂ and quantified GCY-35-GFP fluorescence in dendrites (Fig. 3E). Exposure to hypoxia did not change GCY-35 levels in the dendrites of npr-1 animals or glb-5(Haw); npr-1 animals. We observed reduced cell body expression of GCY-35::GFP in glb-5(Haw); npr-1 animals following hypoxia, but this may reflect regulation of transcription from the gcy-35 promoter (Cheung et al., 2005).

To test if PDL-1 can directly interact with GCY-35, we performed yeast two-hybrid experiments (Y2H). We used PDL-1 as bait, mated it with a prey strain expressing GCY-35, and examined diploid growth on selective plates (Fig. 3F). The rapid growth of the pdl-1/gcy-35 diploids suggested that GCY-35 and PDL-1 physically interact. To determine whether this interaction required prenylation of GCY-35, we disrupted the CAAX prenylation motif by a cysteine-to-serine codon change. PDL-1 failed to interact with this mutant form of GCY-35. These results suggest that PDL-1, like its mammalian counterpart, is a prenyl binding protein involved in protein transport. More specifically, it is thought to directly bind O₂, and O₂ binding is thought to modulate the expression and/or stability of GLB-5(Haw).

Our expression studies and cell-specific rescue experiments suggested that pdl-1 functions not only in AQR, PQR, and URX but also in BAG sensory neurons (Fig. 2C,F). BAG neurons express GCY-33, a soluble guanylate cyclase that also has a C-terminal CAAX prenylation motif. GCY-33::GFP accumulated strongly at the dendritic endings of BAG (Fig. 3I). This dendritic fluorescence was reduced in pdl-1; gcy-33; npr-1 mutants although not abolished (Fig. 3I,J), suggesting that PDL-1 contributes to but is not solely responsible for localizing GCY-33 to BAG dendritic endings. Levels of GCY-33::GFP in the cell body were not altered in pdl-1 mutants (Fig. 3J).

To test if PDL-1 and GCY-33 can interact directly, we set up Y2H experiments, as described previously for GCY-35. GCY-33/ PDL-1-expressing diploids formed detectable but much smaller colonies than GCY-35/PDL-1 diploids, suggesting a weaker interaction. Consistent with this, when we tested the ability of PDLC-1/GCY-33 diploids to grow on a more restrictive medium, containing six selectable markers (-Trp/-Leu/-Ade/-His/Aureobasidin A/X-α-Gal), the PDL-1/GCY-33 diploids did not form colonies whereas the GCY-35/PDL-1 diploids did (Fig. 3F, button, right). Our results suggest that although GCY-33 localization in BAG dendritic endings is regulated by PDL-1, the interaction between PDL-1 and GCY-33 may involve additional proteins. Moreover, PDL-1-independent pathways exist to traffic GCY-33, since a small but significant amount of GCY-33 is found in BAG dendritic endings even in the absence of PDL-1 (Fig. 3I,J).

**O₂-evoked Ca²⁺ responses in URX in pdl-1 mutants**

To investigate how loss of pdl-1 and mislocalization of soluble guanylate cyclases altered the physiology of O₂-sensing neurons, we monitored O₂-evoked responses in these neurons using the Ca²⁺ sensor YC2.60 (Nagai et al., 2004). YC2.60 has higher Ca²⁺ affinity than the YC3.60 sensor we used previously, providing a better readout of intermediate Ca²⁺ levels (Persson et al., 2009; Busch et al., 2012). The ratiometric nature of YC Ca²⁺ sensors permits a steady-state level of Ca²⁺ to be compared across genotypes, which is informative since O₂-sensing neurons signal tonically (Persson et al., 2009; Busch et al., 2012). We began by examining animals grown at normoxia.

We compared Ca²⁺ responses evoked in URX by two O₂ profiles: 21%–17%–21% O₂ and 21%–7%–21% O₂. We included the 21%–7%–21% profile as a control because the neuronal activity of the O₂-sensing neurons and the speed of npr-1 animals at 7% O₂ is the same regardless of the glb-5 genotype (Persson et al., 2009). The 21%–17%–21% O₂ stimulus train probes the ability of GLB-5(Haw) to alter the dynamic range of the O₂ response (Fig. 4A, B; Persson et al., 2009). As expected, the glb-5(Haw) allele sharpened the tuning of URX: at 17% O₂, Ca²⁺ was significantly lower in glb-5(Haw); npr-1 animals compared with npr-1 animals, and was indistinguishable from Ca²⁺ at 7% O₂ (Fig. 4A, B). These results mirror the behavioral data: glb-5(Haw); npr-1 animals switch to slow movement at much higher O₂ levels than npr-1 animals (Persson et al., 2009). Despite the GCY-35 localization defects, and consistent with our behavioral observations, O₂ stimuli evoked robust Ca²⁺ responses in URX in both pdl-1; npr-1 and pdl-1; glb-5(Haw); npr-1 animals (Fig. 4C,D).

However, URX Ca²⁺ at 17% O₂ was significantly higher in pdl-1; glb-5(Haw); npr-1 compared with glb-5(Haw); npr-1 animals (Fig. 4E). This correlated with the greater locomotor activity of pdl-1; glb-5(Haw); npr-1 animals kept at 17% O₂ compared with glb-
Figure 3. PDL-1 regulates GCY-35 and GCY-33 localization. A, GCY-35::GFP accumulates at the dendritic tips of URX neurons. Scale bars: 10 μm. B, Expressing a gcy-35::GFP fusion protein in the AQR, POR, and URX neurons rescues gcy-35; npr-1 mutant phenotypes. Asterisks indicate significant differences from gcy-35; npr-1 animals at 21% O2. Kruskal–Wallis test with Dunn’s post-test. C, PDL-1 is required for GCY-35::GFP to accumulate at the dendritic tips of URX dendrites, but not in the cell body. Scale bars: 10 μm. D, Quantitation of GCY-35::GFP accumulation in dendrites and cell bodies of gcy-35; npr-1 and gcy-35; pdl-1; npr-1 animals; n ≥ 10, Mann–Whitney test. E, Hypoxia does not reduce GCY-35::GFP accumulation in the URX dendrites in gcy-35; npr-1 or gcy-35; glb-5(Haw); npr-1 animals; n = 16, Mann–Whitney test. F, Yeast two-hybrid assay. Diploid growth on selectable plates containing SD/-Leu/-Trp, X-Gal, and Aureobasidin A. Top, PDL-1 interacts strongly with GCY-35 but not ARL-3 or ARL-13; the interactions of SV40-T with p53 and lamin provide positive and negative controls, respectively. Mutating the CAAX prenylation motif to SAAX inhibits GCY-35–PDL-1 interaction. Middle and lower left, Colonies of diploids coexpressing PDL-1 with RAC-2 or GCY-33 were much smaller compared with PDL-1::GCY-35 colonies, indicating that the interaction between these proteins and PDL-1 is weak. Supporting this, PDL-1::GCY-33 diploids did not grow on a more restrictive media (-Trp/-Leu/-Ade/-His/Aureobasidin X-Gal; lower right). G, Disrupting PDL-1 reduces accumulation of mcherry-tagged GLB-5(Haw) in both dendrites and cell bodies. Scale bar, 10 μm. H, Quantification of GLB-5(Haw)::mCherry fluorescence in the dendritic ending and cell body of glb-5(Haw)-expressing head neurons in npr-1 and pdl-1; npr-1 animals. Data represent an average of at least 10 animals, Mann–Whitney test. I, PDL-1 regulates GCY-33::GFP localization in BAG neurons. Disrupting pdl-1 reduces accumulation of GCY-33::GFP at the tips of BAG dendrites (marked by arrows). Scale bar, 10 μm. J, Quantification of GCY-33::GFP fluorescence at the dendritic and cell body of BAG neurons in gcy-33; npr-1 and pdl-1; gcy-33; npr-1 animals. Data represent an average of at least 10 animals, Mann–Whitney test. *p < 0.05, **p < 0.01, and ***p < 0.001. Error bars represent SEM.
**GLOBIN-5-Dependent O₂ Responses Are Regulated by PDL-1/PYBP**

Gross et al., J. Neurosci., December 10, 2014 • 34(50):16726–16738 • 16733

5(Haw); npr-1 animals (Fig. 2D,F). Our results suggest that localizing the GCY-35 O₂ sensor to the dendritic endings of URX is not essential for transducing O₂ stimuli, but that PDL-1 activity modifies O₂-evoked Ca²⁺ signaling in URX.

**GLB-5 and PDL-1 modify O₂-evoked Ca²⁺ responses in BAG neurons**

We next investigated how the glb-5(Haw) allele and pdl-1 contributed to O₂-evoked Ca²⁺ responses in BAG neurons in normoxia-grown animals. Previous work showed that a fall in O₂ concentration evokes a rise in Ca²⁺ in BAG neurons (Zimmer et al., 2009). Our behavioral experiments suggested BAG neurons were part of the circuit that enables feeding glb-5(Haw); npr-1 animals to reduce their locomotor activity sharply when O₂ decreases from 21 to 17% (Fig. 2F). To monitor BAG Ca²⁺ changes evoked by a series of O₂ downsteps in well-fed animals in the absence of food. We could detect small but robust rises in Ca²⁺ when O₂ fell from 21 to 17%; as expected Ca²⁺ responses become bigger as we reduced O₂ levels further (Fig. 5A). Thus, in glb-5(Haw); npr-1 animals even small decreases in O₂ evoked an increase in BAG Ca²⁺.

The small size of the Ca²⁺ response evoked in BAG by a switch from 21 to 17% O₂ (Fig. 5A) precluded us from genetically dissecting this response. Instead, we studied responses evoked by a series of O₂ downsteps in well-fed animals in the absence of food. We could detect small but robust rises in Ca²⁺ when O₂ fell from 21 to 17%; as expected Ca²⁺ responses became bigger as we reduced O₂ levels further (Fig. 5A). Thus, in glb-5(Haw); npr-1 animals even small decreases in O₂ evoked an increase in BAG Ca²⁺.

To investigate how pdl-1 modifies BAG signaling, we first compared O₂-evoked responses in npr-1 and pdl-1; npr-1 mutants. Disrupting pdl-1 attenuated the rise in Ca²⁺ evoked by a switch from 21 to 7% O₂ (Fig. 5C). Unexpectedly, the presence of
GLB-5(Haw) compensated for the loss of PDL-1; C a²/H₁₁₀₀₁ responses evoked by a 21–7% O₂ stimulus in PDL-1; GLB-5(Haw); NPR-1 and GLB-5(Haw); NPR-1 animals were similar (Fig. 5B). Why? If the BAG response defect in PDL-1; NPR-1 animals reflected loss of GCY-33 localization to cilia, then GLB-5(Haw) should rescue not only the Ca²⁺/H₁₁₀₀₁ response of PDL-1; NPR-1 animals, but also the GCY-33 localization defect. This is in fact the case: GLB-5(Haw) restored GCY-33 accumulation at the dendritic endings of BAG to PDL-1; NPR-1 animals, without affecting GCY-33 levels in the cell body (Fig. 5D). Together, our results suggest that GCY-33 localization at the dendritic-endings of BAG is important for BAG O₂ sensing. Moreover, since GLB-5(Haw) is involved in GCY-33 trafficking to or retention in cilia, our data suggest that GLB-5 and GCY-33 are part of a complex in vivo.

Hypoxia modifies O₂-sensor physiology

Having explored how GLB-5(Haw) and PDL-1 activity shaped the O₂ responses of BAG and URX neurons in animals grown in normoxia, we explored how these genes influenced the physiology of O₂-sensing neurons after hypoxia experience. We kept animals in 1% O₂ for 18 h and then imaged O₂-evoked Ca²⁺/H₁₁₀₀₁ responses. For these experiments, individual worms were transferred from the hypoxia chamber and rapidly glued to agarose pads in a 21% O₂ environment. All experiments were done in the presence of food as described previously, and begun within 5 min from the time of transfer. For URX, we chose a 21%–17%–21% O₂ stimulus train to evoke neuronal activity, since it mimics the O₂ concentration difference between the bacterial lawn (17% O₂) and the surrounding agar (21% O₂) in the bordering assay plates. Ca²⁺/H₁₁₀₀₁ responses evoked by 21%–17%–21% O₂ stimulus trains in URX after 18 h incubation at 1% O₂, measured using YC².60. A, B, Prolonged hypoxia abolishes URX responsiveness to changes in O₂ in NPR-1 animals (A), but has much weaker effects on GLB-5(Haw); NPR-1 animals (B). C, Deleting pdl-1 reduced URX responsiveness of glb-5(Haw); NPR-1 animals in both normoxia and hypoxia cultivated animals, and led to higher Ca²⁺ levels at 17% O₂. D, Bar graph comparing tonic URX Ca²⁺ levels at 21% O₂ just after the switch from 17% O₂ to 21% O₂, in various genotypes; data taken from A–C (21%–17%–21% O₂ responses after 18 h in hypoxia); baseline normalized ratios. Asterisks indicate significant differences compared with glb-5(Haw); NPR-1. Mann–Whitney test, *p < 0.05. Error bars represent SEM. E, 21%–7%–21% O₂ stimulus trains evoked strong Ca²⁺ responses in BAG in NPR-1, glb-5(Haw); NPR-1, and pdl-1; glb-5(Haw); NPR-1 animals. F, The 21%–7%–21% O₂-evoked Ca²⁺ responses in BAG were smaller after hypoxia (for comparison see E). Asterisks indicate significant differences between Ca²⁺ level just after the shift from 21 to 7% O₂, at times indicated by the bars, Mann–Whitney test. Gray shading represents SEM. G, Disrupting hif-1 did not prevent the fast recovery of bordering behavior in glb-5(Haw); NPR-1 animals following exposure to hypoxia. Asterisks indicate significance for comparisons with GLB-5(Haw); NPR-1 animals. Kruskal–Wallis test with Dunn’s post-test, n ≥ 4, *p < 0.05, **p < 0.01, and ***p < 0.001. Error bars represent SEM.
GLB-5(Haw) suppresses behavioral reconfiguration by hypoxia in an HIF-1-independent mechanism

The reconfiguration of bordering behavior by hypoxia occurred over hours (Fig. 1B), suggesting that transcriptional regulation is involved. Since HIF-1 is a key factor in adaptation to hypoxia (Semenza and Wang, 1992; Jiang et al., 2001), we wondered if HIF-1 was involved. To test this, we examined the bordering behavior of hif-1(ia4); npr-1; pglb-5; glb-5(Haw) animals after hypoxia (Fig. 6G). hif-1(ia4) animals survived well in 24 h hypoxia, and upon exposure to 1% O2, left food and accumulated outside the bacteria. When returned to 21% O2, hif-1(ia4); npr-1; pglb-5; glb-5(Haw) animals rapidly resumed bordering behavior, like glb-5(Haw); npr-1 animals. These results suggest that HIF-1 is not required for GLB-5(Haw) to promote rapid recovery from hypoxia exposure, although we have not examined if it required for npr-1 animals to inhibit bordering following hypoxia experience.

GLB-5(Haw) enables URX neurons adapted to hypoxia to rapidly retone their O2 response set point upon return to normoxia

GLB-5(Haw) counteracts the effects of prolonged hypoxia on behavior and URX physiology after animals are returned to normoxia. We speculated that GLB-5 could do this in one of two ways: by preventing hypoxia from reconfiguring the physiology of URX or by enabling rapid recovery of URX response properties when animals exposed to prolonged hypoxia are returned to 21% O2. In the second model URX recovery would need to be fast, since glb-5(Haw); npr-1 animals showed substantial O2-evoked Ca2+ responses within 5 min of transfer from hypoxia to 21% O2 (Fig. 6B).

To differentiate between these possibilities we built an airtight microfluidic-imaging chamber that enabled us to measure URX response properties in hypoxia-grown animals without prior exposure to 21% O2. Since gluing animals in the hypoxia chamber was difficult, we immobilized animals using the cholinergic agonist levamisole (see Materials and Methods). We grew npr-1 and glb-5(Haw); npr-1 animals for 18 h in either 1% or 21% O2, transferred them to the imaging scope, and shifted them from 1 to 7% O2. To compare the effects of acute and prolonged hypoxia, we also exposed npr-1 and glb-5(Haw); npr-1 animals cultured at 21% O2 to a 21%–1%–7%–21% O2 stimulus train. Animals adapted to 1% O2 for 18 h exhibited large persistent increases in URX Ca2+ when shifted to 7% O2 (Fig. 7A, B), whereas animals exposed to 1% O2 for only 5 min exhibited a smaller, transient increase in Ca2+ to the same 7% O2 stimulus (Fig. 7C–E). The effects of prolonged hypoxia on URX activity after O2 levels were raised for the first time from 1 to 7% were independent of GLB-5(Haw) activity (Fig. 7A, B). This contrasts with the marked difference in O2-evoked Ca2+ responses in URX when hypoxia-treated npr-1 and glb-5(Haw); npr-1 animals were exposed to 21% O2 (Fig. 6A, B). These data suggest that hypoxia can program URX activity in both npr-1 and glb-5(Haw); npr-1 animals, but that recovery after further exposure to 21% O2 is much faster in glb-5(Haw); npr-1 than npr-1 animals.

Discussion

The neuroglobin GLB-5(Haw) enables fast behavioral recovery after exposure to hypoxia. At 21% O2 wild C. elegans rapidly accumulate where bacterial food is thickest. This foraging behavior, called bordering, is inhibited by prior hypoxia experience if glb-5 is defective. The effects of hypoxia exposure on subsequent behavior at 21% O2 build up gradually and plateau after 18 h in 1% O2. C. elegans relies on the URX O2-sensing neurons to accumulate where bacteria are thickest; these neurons are activated by a rise in O2. If GLB-5 is defective, prolonged hypoxia and re-oxygenation disrupts O2-evoked Ca2+ responses of URX neurons. A simple hypothesis is that GLB-5 mitigates the effects of hypoxia/re-oxygenation by controlling O2-evoked Ca2+ dynamics during this transition. The exact molecular mechanism by which this is achieved is unclear, but GLB-5, like many other hexacoordinated globins, oxidizes rapidly in 21% O2 (Persson et al., 2009; Yoon et al., 2010), making it a potential electron donor that signals by generating reactive oxygen species.

GLB-5(Haw)’s ability to promote rapid recovery of bordering after hypoxia experience, and to tune URX neurons to changes in O2 between 21 and 17%, depend on PDL-1, the C. elegans ortholog of the mammalian prenyl binding protein PDE6. PDL-1 is required in both the URX and BAG neurons to sustain these GLB-5(Haw)-dependent behaviors. BAG, like URX, is an O2 sensor, but is activated by a decrease instead of an increase in O2 due to the activity of the GGY-35/GGY-31 soluble guanylate cyclase (Zimmer et al., 2009). PDL-1 mediates GGY-35 and GGY-33 O2 sensor localization to the dendritic endings of URX and BAG neurons, respectively (Fig. 3C). Since GLB-5 is also localized to the dendritic endings of URX, one interpretation of our data is that physical proximity helps GLB-5 modify O2 sensory transduction by GGY-35/GGY-36. In BAG neurons GGY-33 and GLB-5 likely function in a complex, since GLB-5(Haw) can target GGY-33 to cilia in the absence of PDL-1. Although a simple model in which the effects of PDL-1 are mediated via localization of soluble guanylate cyclase is appealing, PDL-1 may play a more complex role involving trafficking of other prenylated proteins. For example, in mammals PDE6a traffics heterotrimeric guanine-nucleotide binding protein gamma subunits; the C. elegans orthologs of these genes, gpc-1 and gpc-2, are also predicted to be prenylated.

PDL-1 activity is required in BAG for GLB-5(Haw) to confer a strong behavioral response to switches between 21% and 17% O2 (Fig. 2F). Consistent with this, a 21%–17%–21% O2 stimulus
train evoked a significant rise and fall in Ca^{2+} levels in BAG in well fed glb-5(Haw); npr-1 animals (Fig. 5). Thus, the same small changes in ambient O2 concentration induce reciprocal O2 responses in URX and BAG, and act antagonistically to control locomotor activity: at 21% O2 high URX/low BAG activity promotes rapid movement, whereas at 17% O2 low URX/high BAG activity promotes slow movement (Figs. 4, 5). Previous work has already demonstrated reciprocal O2 responses in URX and BAG, but BAG responses in those studies were evoked at much lower O2 concentrations (Zimmer et al., 2009). Like URX, BAG neurons appear to have tonic signaling activity, since BAG Ca^{2+} levels remained elevated while O2 levels were reduced. Consistent with our data, previous work has shown that activating BAG induces animals to reduce locomotor activity (Zimmer et al., 2009).

PDE6δ is considered to be a promiscuous prenyl binding protein (Zhang et al., 2004). However, in our yeast two-hybrid assays PDL-1 did not interact with several well known prenylated proteins such as RAS-1 and RAB-18, or ARL2-like proteins such as ARL-3 and EVL-20 (Fig. 3F). Moreover, the interaction of PDL-1 with G CY-33 was significantly weaker than its interaction with GCY-35 in the same assay. One explanation is that expression of these prenylated proteins in yeast is variable. An alternative explanation is that the substrate specificity of PDL-1 for prenylated proteins is complex.

The neuronal functions of mammalian PDE6δ have been previously studied in rods and cones, where it regulates transport of phosphodiesterase PDE6, rhodopsin kinase, and Gt—all prenylated proteins—to the outer segment of these ciliated neurons (Li et al., 1998; Norton et al., 2005; Zhang et al., 2007; Luo et al., 2008). Like cone and rod photoreceptors, BAG has a ciliated dendritic ending, but URX is a nonciliated neuron (Ward et al., 1975). Thus, prenyl binding proteins can facilitate protein traffic to both ciliated and nonciliated dendritic endings. The localization of G CY-33 and G CY-35 interact with PDL-1, which facilitates their traffic to dendritic endings. There, they act together with GLB-5(Haw) to tune the dynamic range of O2 responses, and to rapidly retune O2-sensing properties after return to normoxia following prolonged hypoxia.
lings of URX and BAG are located close to the surface at the C. elegans nose tip, where O2 concentrations are likely to be higher than those found in the cell bodies, which lie buried inside the animal. Second, as highlighted above, components of the O2 sensory transduction machinery, including GLB-5 (Persson et al., 2009) and cGMP-gated channels (Arellano-Carbajal et al., 2011), are enriched at the dendritic endings of URX, facilitating compartmentalized signaling. Interestingly, although pdl-1 mutants have little detectable GCCY-35 at URX dendritic endings, they retain strong O2-evoked Ca2+ responses in these neurons, indicating that cGMP signal transduction can still function. This suggests that soluble guanylate cyclases and cGMP channels remain in close proximity in pdl-1 mutants.

Prolonged hypoxia reconfigures responses to O2 in other C. elegans behavioral paradigms, although the role of globins was not studied (Cheung et al., 2005; Chang and Bargmann, 2008; Ma et al., 2012). Our Ca2+ imaging experiments demonstrate that previous O2 experience can retune URX O2 responses (Fig. 7A, B). Interestingly, previous studies of HIF-1 activation showed that the HT22 mouse neuronal cell line and cardiofibroblasts can adjust their O2 sensitivity in response to prolonged incubation in 5% or 30% O2 (Roy et al., 2003; Khanna et al., 2006). Prolonged exposure of HT22 cells to 5% O2 attenuates subsequent HIF-1 activation by 0.5% O2. Moreover, HIF-1 activity increases when HT22 cells grown in 30% O2 are transferred to 20% O2. These studies suggest that in these cells the O2 balance point for HIF-1 induction is regulated by experience.

In summary, our results suggest that the GLB-5 neuroglobin confers on C. elegans O2 sensors the ability to retain their tuning properties following prolonged hypoxia and re-oxygenation. This mechanism allows for both behavioral and cellular activity adjustment following re-oxygenation. It would be interesting to test if mammalian neuroglobin regulates the threshold for O2 responses in glomus cells of the carotid body (Di Giulio et al., 2006, 2012). Our results also suggest that PDL-1, the C. elegans ortholog of PDEδ, facilitates traffic of prenylated GCCY-35 and GCCY-33 from the cell bodies of BAG and URX neurons to their ciliated and nonciliated dendritic endings, respectively. PDL-1 is highly conserved between C. elegans and humans, plays a major role in vision physiology, and spatially regulates K-RAS activity in human cells (Zhang et al., 2012; Zimmermann et al., 2013). Our studies establish a tractable in vivo system to study PDL-1 function in vivo, enabling its mechanisms of action to be explored further and potential small molecule inhibitors to be tested.

References
Abramoff MD, Magelhaes PJ, Ram SJ (2004) Image processing with ImageJ. Biophot Int 11:36–42.
Arellano-Carbajal F, Briseño-Roa L, Couto A, Cheung BH, Labouesse M, de Bono M (2011) Macoilin, a conserved nervous system-specific ER membrane protein that regulates neuronal excitability. PLoS Genet 7:e1001341.
Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77:71–94.
Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77:71–94.
Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77:71–94.
Burmeister T, Hankeln T (2009) What is the function of neuroglobin? J Exp Biol 212:1423–1428.
Bush KE, Laurent P, Soltesz Z, de Bono M (2012) Tone signaling from O2 sensors sets neural circuit activity and behavioral state. Nat Neurosci 15:581–591.
Chang AJ, Bargmann CI (2008) Hypoxia and the HIF-1 transcriptional pathway reorganize a neuronal circuit for oxygen-dependent behavior in Caenorhabditis elegans. Proc Natl Acad Sci U S A 105:7321–7326.
Cheung BH, Arellano-Carbajal F, Rybicki I, de Bono M (2004) Soluble guanylate cyclases act in neurons exposed to the body fluid to promote C. elegans aggregation behavior. Curr Biol 14:1105–1111.
Di Giulio C, Bianchi G, Caccio M, Artele I, Piccirilli M, Verratti V, Valerio R, Turriragga R (2006) Neuroglobin, a new oxygen binding protein is present in the carotid body and increases after chronic intermittent hypoxia. Adv Exp Med Biol 580:15–19; discussion 351–359.
Di Giulio C, Zara S, Cataldi A, Porzionario A, Pokorski M, De Caro R (2012) Human carotid body HIF and NGB expression during human development and aging. Adv Exp Med Biol 758:265–271.
Esposito G, Di Schiavi E, Bergamasco C, Bazzicalupo P (2007) Efficient and cell-specific knock-down of gene function in targeted C. elegans neurons. Gene 395:170–176.
Gillespie PG, Prusti RK, Apel ED, Beavo JA (1989) A soluble form of bovine rod photoreceptor phosphodiesterase has a novel 15-kDa subunit. J Biol Chem 264:12187–12193.
Gray JM, Karow DS, Lu H, Chang AJ, Chang JS, Ellis RE, Marletta MA, Bargmann CI (2004) Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Nature 430:317–322.
Hanzal-Bayer M, Renault L, Oversi P, Wittinghofer A, Hillig RC (2002) The complex of ArtZ-GTP and PDE delta: from structure to function. EMBO J 21:2095–2106.
Hoogewijs D, Geuens E, Dewilde S, Vierstraete A, Moens L, Vinogradov S, Vanhoutte PM (2007) Wide diversity in structure and expression profiles among members of the Caenorhabditis elegans globin protein family. BMC Genomics 8:356.
Hundahl CA, Kelsen J, Hay-Schnidt A (2013) Neuroglobin and Cytoglobin expression in the human brain. Brain Struct Function 218:603–609.
Jiang H, Guo R, Powell-Coffman JA (2001) The Caenorhabditis elegans hif-1 gene encodes a bHLH-PAS protein that is required for adaptation to hypoxia. Proc Natl Acad Sci U S A 98:7916–7921.
Koizumi K, Suyama M, Hara M, Yamanaka S, Koyama T, Soma T, Kiyama H, Hashimoto K, De Gruyter F, Matsuura M, Yamada K (2005) Tissue-specific gene expression mapping and assembly of C. elegans genes. BMC Genomics 6:118.
Kozarewa I, Ning Z, Quail MA, Sanders MJ, Berriman M, Turner DJ (2009) The Caenorhabditis elegans genome assembly and annotation. Nucleic Acids Res 37:519–531.
Kozarewa I, Ning Z, Quail MA, Sanders MJ, Berriman M, Turner DJ (2009) The Caenorhabditis elegans genome assembly and annotation. Nucleic Acids Res 37:519–531.
Kuo AO, Oda S, Nikolaev VO, Soltesz Z, de Bono M (2013) In vivo genetic dissection of O2-evoked cGMP dynamics in a Caenorhabditis elegans gas sensor. Proc Natl Acad Sci U S A 110:E3301–E3310.
Kuo AO, Oda S, Nikolaev VO, Soltesz Z, de Bono M (2013) In vivo genetic dissection of O2-evoked cGMP dynamics in a Caenorhabditis elegans gas sensor. Proc Natl Acad Sci U S A 110:E3301–E3310.
Kuo AO, Oda S, Nikolaev VO, Soltesz Z, de Bono M (2013) In vivo genetic dissection of O2-evoked cGMP dynamics in a Caenorhabditis elegans gas sensor. Proc Natl Acad Sci U S A 110:E3301–E3310.
Li N, Florio SK, Pettenati MJ, Rao PN, Beavo JA, Baehr W (1998) Characterization of human and mouse rod cGMP phosphodiesterase delta subunit (PDE6δ) and chromosomal localization of the human gene. Genomics 49:76–82.
Luo DG, Xue T, Yau KW (2008) How vision begins: an odyssey. Proc Natl Acad Sci U S A 105:9855–9862.
O2-sensing hydroxylase EGL-9 to promote H2S-modulated hypoxia-induced behavioral plasticity in C. elegans. Neuron 73:925–940. CrossRef Medline

McGrath PT, Rockman MV, Zimmer M, Jang H, Macosko EZ, Kruglyak L, Bargmann CI (2009) Quantitative mapping of a digenic behavioral trait implicates globin variation in C. elegans sensory behaviors. Neuron 61:692–699. CrossRef Medline

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. Medline

Nagai T, Yamada S, Tominaga T, Ichikawa M, Miyawaki A (2004) Expanded dynamic range of fluorescent indicators for Ca(2+)/H11001 by circularly permuted yellow fluorescent proteins. Proc Natl Acad Sci U S A 101:10554–10559. CrossRef Medline

Norton AW, Hosier S, Terew JM, Li N, Dhingra A, Vardi N, Baehr W, Cote RH (2005) Evaluation of the 17-kDa prenyl-binding protein as a regulatory protein for phototransduction in retinal photoreceptors. J Biol Chem 280:1248–1256. CrossRef Medline

Persson A, Gross E, Laurent P, Busch KE, Bretes H, de Bono M (2009) Natural variation in a neural globin tunes oxygen sensing in wild Caenorhabditis elegans. Nature 458:1030–1033. CrossRef Medline

Rockman MV, Kruglyak L (2009) Recombinational landscape and population genomics of Caenorhabditis elegans. PLoS Genet 5:e1000419. CrossRef Medline

Roy S, Khanna S, Wallace WA, Lappalainen J, Rink C, Cardounel AJ, Zweier JL, Sen CK (2003) Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. J Biol Chem 278:47129–47135. CrossRef Medline

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, Ed 2. New York: Cold Spring Harbor Laboratory.

Semenza GL, Wang GL (1992) A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 12:5447–5454. Medline

Sun Y, Jin K, Mao XO, Zhu Y, Greenberg DA (2001) Neuroglobin is up-regulated by and protects neurons from hypoxic-ischemic injury. Proc Natl Acad Sci U S A 98:15306–15311. CrossRef Medline

Van Voorhies WA, Ward S (2000) Broad oxygen tolerance in the nematode Caenorhabditis elegans. J Exp Biol 203:2467–2478. Medline

Ward IP (2008) Oxygen sensors in context. Biochim Biophys Acta 1777:1–14. CrossRef Medline

Ward S, Thomson N, White JG, Brenner S (1975) Electron microscopical reconstruction of the anterior sensory anatomy of the nematode Caenorhabditis elegans.2UU. J Comp Neurol 160:313–337. CrossRef Medline

Weber KP, De S, Kozarewa I, Turner DJ, Babu MM, de Bono M (2010) Whole genome sequencing highlights genetic changes associated with laboratory domestication of C. elegans. PLoS One 5:e13922. CrossRef Medline

Wicks SR, Yeh RT, Gish WR, Waterston RH, Plasterk RH (2001) Rapid gene mapping in Caenorhabditis elegans using a high density polymorphism map. Nat Genet 28:160–164. CrossRef Medline

Yoon J, Herzik MA Jr, Winter MB, Tran R, Olea C Jr, Marletta MA (2010) Structure and properties of a bis-histidyl ligated globin from Caenorhabditis elegans. Biochemistry 49:5662–5670. CrossRef Medline

Zhang H, Li S, Doan T, Rieke F, Detwiler PB, Frederick JM, Baehr W (2004) Photoreceptor cGMP phosphodiesterase delta subunit (PDE-delta) functions as a prenyl-binding protein. J Biol Chem 279:407–413. CrossRef Medline

Zhang H, Li S, Doan T, Rieke F, Detwiler PB, Frederick JM, Baehr W (2007) Deletion of PrBP/delta impedes transport of GRK1 and PDE6 catalytic subunits to photoreceptor outer segments. Proc Natl Acad Sci U S A 104:8857–8862. CrossRef Medline

Zhang H, Constantine R, Frederick JM, Baehr W (2012) The prenyl-binding protein PrBP/delta: a chaperone participating in intracellular trafficking. Vision Res 75:19–25. CrossRef Medline

Zimmer M, Gray JM, Pokala N, Chang AJ, Karow DS, Marletta MA, Hudson ML, Morton DB, Chronis N, Bargmann CI (2009) Neurons detect increases and decreases in oxygen levels using distinct guanylate cyclases. Neuron 61:865–879. CrossRef Medline

Zimmermann G, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M, Hahn SA, Triola G, Wittinghofer A, Bastiaens PI, Waldmann H (2013) Small molecule inhibition of the KRAS-PDEdelta interaction impairs oncogenic KRAS signalling. Nature 497:638–642. CrossRef Medline