Hydrogen sulfi de (H$_2$S) is common in the environment, and is also endogenously produced by animal cells. Although hydrogen sulfi de is often toxic, exposure to low levels of hydrogen sulfi de improves outcomes in a variety of mammalian models of ischemia-reperfusion injury. In *Caenorhabditis elegans*, the initial transcriptional response to hydrogen sulfi de depends on the *hif-1* transcription factor, and *hif-1* mutant animals die when exposed to hydrogen sulfi de. In this study, we use rescue experiments to identify tissues in which *hif-1* is required to survive exposure to hydrogen sulfi de. We find that expression of *hif-1* from the *unc-14* promoter is sufﬁcient to survive hydrogen sulfi de. Although *unc-14* is generally considered to be a pan-neuronal promoter, we show that it is active in many nonneuronal cells as well. Using other promoters, we show that pan-neuronal expression of *hif-1* is not sufﬁcient to survive exposure to hydrogen sulfi de. Our data suggest that *hif-1* is required in many different tissues to direct the essential response to hydrogen sulfi de.

*Caenorhabditis elegans* is an excellent system to deﬁne physiological responses to exogenous H$_2$S. In addition to powerful genetics, all cells are directly exposed to the gaseous environment (Shen and Powell-Coffman 2003). This feature allows for control of cellular H$_2$S exposure without confounding factors from physiological regulation of gas delivery. *C. elegans* grown in 50 ppm H$_2$S are long-lived, thermotolerant, and resistant to the hypoxia-induced disruption of proteostasis (Miller and Roth 2007; Fawcett et al. 2015). HIF-1 directs the transcriptional response to H$_2$S in *C. elegans* (Budde and Roth 2010; Miller et al. 2011). HIF-1 is a highly conserved transcription factor best known for regulating the transcriptional response to low oxygen (hypoxia) in metazoans (Semenza 2000, 2001). *C. elegans* *hif-1* mutant animals are viable and fertile in room air but die if exposed to hypoxia during embryogenesis (jiang et al. 2001; Nystul and Roth 2004). By contrast, exposure to low H$_2$S is lethal for *hif-1* mutant animals at all developmental stages (Budde and Roth 2010), and mutations in *hif-1* suppress protective effects of some mutations that confer tolerance to H$_2$S (Budde and Roth 2010; Livshits et al. 2017). Moreover, increasing the activity of HIF-1, by mutations in negative regulators VHL-1, EGL-9, or RHY-1, increases the tolerance of *C. elegans* to otherwise lethally high concentrations of H$_2$S (Budde and Roth 2010; Livshits et al. 2017). These observations indicate that HIF-1 has a central role in the organismal response to H$_2$S.

*Several studies have argued for neuronal-specific functions of HIF-1, although the *hif-1* promoter is active in most, if not all, cells, and HIF-1 protein is stabilized ubiquitously in *C. elegans* exposed to either hypoxia or H$_2$S (jiang et al. 2001; Budde and Roth 2010). Neuronal expression of *hif-1* in hypoxia is reported to be sufficient to prevent hypoxia-induced diapause and to increase lifespan through induction of intestinal expression of the flavin monoxygenase FMO-2 (Miller and Roth*
broadly expressed in nonneuronal cells. We show that it is considered a pan-neuronal promoter (Ogura 2009; Leiser et al. 2015). Furthermore, neuronal expression of the cysteine synthase-like protein CYSL-1 regulates the activity of HIF-1 to modulate behavioral responses to changes in oxygen availability (Ma et al. 2012). These data motivated us to determine whether neuronal HIF-1 activity is sufficient for C. elegans to survive exposure to H$_2$S. In this study, we used tissue-specific rescue of hif-1 to define the site of essential HIF-1 activity in low H$_2$S. We found that expression of hif-1 from the unc-14 promoter was sufficient for survival in H$_2$S. Although it is considered a pan-neuronal promoter (Ogura et al. 1997; Pocock and Hobert 2008), our data indicate that the unc-14 promoter is also broadly expressed in nonneuronal cells. We show that hif-1 expressed from the pan-neuronal rab-3 promoter is not sufficient for viability in H$_2$S. We further demonstrate that expression of hif-1 in muscle, hypodermis, and intestine is not sufficient for viability in low H$_2$S. Together, our data indicate that the activity of HIF-1 may be required in multiple tissues to coordinate the organismal response to H$_2$S.

**Materials and Methods**

### Strains

Strains were grown at room temperature on nematode growth media plates (NGM) seeded with the OP50 strain of *Escherichia coli* (Brenner 1974). All strains were derived from N2 (Bristol). Full genotypes of strains used in this study are shown in Table 1. To sequence the Punc-14::hif-1 junction of otls197, the region was amplified with forward primer oET479 (5’-GTTGTCACCACATGACATATG-3’) and reverse primer oET480 (5’-ACGAGGCGGTTCATG-3’). The oET479 primer was used for sequencing.

### Constructs and transgenes

All constructs were made using the multisite Gateway system (Invitrogen), where a promoter region, a gene region (*hif-1* cDNA or GFP), and a C-terminal 3’ untranslated region (UTR) were cloned into the destination vector pCFJ150 (Frokjær-Jensen et al. 2008). The *hif-1* A isoform was amplified from cDNA using forward primer oET467 (5’-GGGACAGTTTGTACAAAACGGCTCAATGGAGAAAATGGGAAATGGG-3’) and reverse primer oET469 (5’-AGGGACACTTTGGTACAAAGGACGTCCAGAGGATTTCCATGATGGGAAATGGGGATTTCCATG-3’). For the tissue-specific rescuing experiments, an operon GFP::H2B was included in the expression constructs downstream of the 3’ UTR (Frokjær-Jensen et al. 2012). This resulted in expression of untagged HIF-1 protein and histone H2B fused to GFP, which allowed for confirmation of promoter expression by monitoring GFP expression. The *unc-14* promoter (1425 bp upstream of the start codon) was amplified from genomic DNA using forward

---

**Table 1 Strains used in this study**

| Strain | Genotype | Source |
|--------|----------|--------|
| ZG31: hif-1[ia4] V | DLM25: hif-1[ia4] V; otls197 [Punc-14::hif-1P621A, Ptx:3::RFP] | I. Topalidou and D. L. Miller |
| DLM26: hif-1[ia4] V; otEx165 [Punc-120::hif-1P621A, Ptx:3::RFP] | XZ2085: hif-1[ia4] V, yakEx131 [eef-1A.1::hif-1cDNA, Prm-2::mCherry] | |
| XZ2086: hif-1[ia4] V, yakEx137 [Punc-14::hif-1P621A::YFP, Prm-2::mCherry] | XZ2086: hif-1[ia4] V, yakEx136 [Pvha-6::hif-1cDNA, Prm-2::mCherry] | |
| XZ2087: hif-1[ia4] V; yakEx142 [Punc-14::GFP, Prm-2::mCherry] | XZ2088: hif-1[ia4] V; yakEx143 [Pdpy-7::hif-1cDNA, Prm-2::mCherry] | |
| XZ2089: hif-1[ia4] V; yakEx144 [Punc-14::hif-1cDNA, Prm-2::mCherry] | XZ2097: hif-1[ia4] V, yakEx125 [Prab-3::hif-1cDNA, Prm-2::mCherry] | |
| XZ2098: hif-1[ia4] V; yakEx126 [Punc-17::hif-1cDNA, Prm-2::mCherry] | XZ2099: hif-1[ia4] V; yakEx125 [Prab-3::hif-1cDNA, Prm-2::mCherry] | |

**Table 2 Plasmids and constructs used in this study**

| Gateway entry clones | Gateway expression constructs |
|----------------------|-------------------------------|
| pCFJ326 | Punc-47::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pCFJ386 | Punc-17::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pCR110 | Punc-3::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pEG805 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET168 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET210 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pGH1 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pMHS22 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET187 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET188 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET171 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET172 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET182 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET187 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET188 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET212 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET213 | Pdpy-6::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
| pET216 | Pdpy-7::hif-1 cDNA::tbb-2 3’UTR::OPERON::GFP, Prm-150 |
primer oET520 (5'-GGGGACAACCTTTGTAGAAAGATGG GAGGCCAGCAGCTCTTGAG-3') and reverse primer oET507 (5'-GGGGACTGCTTTTTGTACAAAACTGGTTTGGTGAAGAA TTGAGG-3'). All plasmids constructed were verified by sequencing. Constructs used in this study are shown in Table 2. Extrachromosomal arrays were made by standard injection methods (Mello et al. 1991) with 10–15 ng/µl of the expression vector. At least two independent lines were isolated for each construct.

**H₂S atmospheres**

Construction of atmospheric chambers was as previously described (Miller and Roth 2007; Fawcett et al. 2012). In short, H₂S (5000 ppm with balance N₂) was diluted continuously with room air to a final concentration of 50 ppm. Final H₂S concentration was monitored using a custom-built H₂S detector containing a three-electrode electrochemical SureCell H₂S detector (Sixth Sense) as described (Miller and Roth 2007), calibrated with 100 ppm H₂S with balance N₂. Compressed gas mixtures were obtained from Airgas (Radnor, PA) and certified as standard to within 2% of the indicated concentration. H₂S atmospheres were maintained at 20°C.

**Survival assays**

20 to 40 L4 animals were picked to plates seeded with OP50. Plates were exposed to 50 ppm H₂S for 20–24 hr in a 20°C incubator, and then returned to room air to score viability. Death was defined as failure to move when probed with a platinum wire on the head or tail. Animals were scored 30 min after removal from H₂S, and plates with dead animals were reexamined after several hours to ensure animals had not reanimated.

**Imaging**

For imaging expression of GFP, larval stage 1 (L1) or first-day adult animals were mounted on 2% agarose pads and anesthetized with 50 mM sodium azide for 10 min before placing the cover slip. The images were obtained using a Nikon 80i wide-field compound microscope.

**Data availability**

Strains are available upon request and have been deposited at the Caenorhabditis Genetics Center (cgc.umn.edu). Plasmid constructs are available upon request.

**RESULTS AND DISCUSSION**

*C. elegans* requires *hif-1* to survive exposure to low H₂S (Budde and Roth 2010). To determine whether neuronal expression of *hif-1* was sufficient for survival in H₂S, we used transgenic *hif-1(ia4)* mutant animals that expressed *hif-1* from heterologous promoters. We first used the available *otsls197* transgene, which expresses *hif-1* from the putative pan-neuronal *unc-14* promoter (Pocock and Hobert 2008). We found that *hif-1(ia4); otsls197* animals survived exposure to 50 ppm H₂S (Figure 1A). This result suggests that neuronal expression of *hif-1*, from the *unc-14* promoter, is sufficient to survive exposure to H₂S.

To further dissect in which neuronal cell type(s) *HIF-1* activity was required to survive exposure to H₂S, we generated transgenic animals that expressed *hif-1* cDNA under the control of promoters active in specific neuronal subtypes. We found that expression in neither cholinergic neurons (*pun-17*) nor GABAergic neurons (*pun-47*) was sufficient to rescue the lethality of *hif-1(ia4)* mutant animals exposed to H₂S (Figure 1A). Curiously, we also observed that expression of *hif-1* cDNA from the pan-neuronal *rab-3* promoter did not rescue survival of the *hif-1(ia4)* mutant animals (Figure 1A). This was unexpected, as expression of *HIF-1* from the *unc-14* promoter (the *otsls197* transgene) was sufficient for survival in H₂S. We therefore pursued the source of this discrepancy.

We first sought to verify the molecular nature of the *otsls197* integrated transgene. We used PCR to amplify a region from the *unc-14*
promoter and the hif-1 coding region from the otIs197 transgenic animals. As expected, this reaction generated a single band of approximately 500 bp. However, when we sequenced the resulting PCR product, we discovered an insertion of an extra G immediately downstream of the ATG.

We generated transgenic strains expressing hif-1 under control of the unc-14 promoter using a Punc-14:hif-1a(P621A):YFP plasmid (Pocock and Hobert 2008), which we verified had had the expected hif-1a(P621A) sequence. We injected this plasmid into hif-1a::unc-14 animals (Pocock and Hobert 2008; Miller and Roth 2009; Ma et al. 2012; Leiser et al. 2015). The otIs197 transgene was constructed to express isoform A of hif-1, though there are six predicted isoforms (WormBase 2017). We noted that the ATG for isoform E is 21 bp downstream of the original ATG in the hif-1 cDNA. Thus, it could be that expression of the hif-1e isoform is the basis of the activity of the otIs197 transgene. Because our Prab-3:hif-1 transgene expressed the hif-1a isoform, it was possible that the differences we observed from otIs197 were due to the expression of different hif-1 isoforms. To test this possibility we created transgenic strains expressing hif-1a under control of the unc-14 promoter using a Punc-14:hif-1a(P621A):YFP plasmid (Pocock and Hobert 2008), which we verified had had the expected hif-1a(P621A) sequence. We injected this plasmid into hif-1a::unc-14 mutant animals to generate the yakEx137 transgene. If the rescue we observed in otIs197 was due to expression of hif1e rather than hif1a, then the animals expressing Punc-14:hif-1a(P621A):YFP would die in H2S. However, these animals survived exposure to H2S (Figure 1C), indicating that potential expression of different isoforms did not underlie differences in survival of exposure to H2S.

The HIF-1 protein expressed by the otIs197 transgene has a P621A mutation that prevents it from being hydroxylated and degraded by the proteasome (Pocock and Hobert 2008). By contrast, the constructs we generated produced wild-type HIF-1 protein. We did not expect this feature to be salient for our experiments, since HIF-1 protein is stabilized in H2S due to inhibition of the hydroxylation reaction (Buddle and Roth 2010; Ma et al. 2012). However, it is possible that constitutive stabilization of HIF-1 protein in neurons promotes survival in H2S. To evaluate this possibility, we cloned wild-type hif-1 cDNA under control of the unc-14 promoter, including 1.4 kb upstream of the transcription start site (Ogura et al. 1997). We found that hif-1::unc-14(hif-1a); Punc-14:hif-1(yakEx144) animals survived exposure to H2S, similar to hif-1a::unc-14(hif-1a); otIs197 animals (Figure 1C). We conclude that the P621A mutation in otIs197 does not underlie the difference in survival in H2S that we observed for animals expressing hif-1 from rab-3 and unc-14 promoters.

Given that the only other notable difference between the Prab-3:hif-1 and Punc-14:hif-1 constructs is the promoter elements, we hypothesized...
that differences between either the levels of expression from these promoters or the identity of the cells where these promoters are expressed should account for their different behavior. The transgenic constructs we generated all included an operon GFP::H2B downstream of the 3’ UTR (Frokjær-Jensen et al. 2012). This resulted in expression of untagged HIF-1 protein as well as GFP::H2B. We therefore visualized GFP expression to evaluate the expression levels and cellular patterns of promoter activity. As expected, GFP expression from adult hif-1(i4a); Prab-3::hif-1:: operon::GFP::H2B was exclusively in neurons (Figure 2A). However, when we imaged adult hif-1(i4a); Punc-14::hif-1::operon::GFP::H2B animals that had survived exposure to H2S, we observed GFP expression in neurons, as expected, but also in intestinal and hypodermal cells (Figure 2B). We saw similar expression in animals that had not been exposed to H2S. To corroborate this observation, we cloned the unc-14 promoter upstream of GFP and injected it into wild-type animals. We then imaged larvae (Figure 2C) and adult animals (Figure 2D) from three separate lines. We observed expression of GFP in numerous cells other than neurons including intestine, hypodermis, muscle, and the uterus. Every animal that we imaged had expression in at least one cell type other than neurons (n = 50).

Based on our understanding of Punc-14 expression and the fact that hif-1(i4a); Prab-3::hif-1 animals die when exposed to H2S (Figure 1A), we inferred that neuronal HIF-1 activity is not sufficient for survival in H2S. We therefore explored whether expression of hif-1 exclusively in nonneuronal tissues was sufficient for survival in H2S. For these experiments, we generated transgenes with hif-1 expressed under control of the unc-120 promoter, which is active in body-wall and vulval muscle; the dpy-7 promoter, which is active in hypodermis; the vha-6 promoter, which is active in intestine; and the ubiquitous cef-1A.1 promoter. We chose these promoters because they included many of the tissues that had unc-14-driven expression of GFP (Figure 2B). As shown in Figure 3, only the ubiquitously expressed cef-1A.1::hif-1 rescued the lethality of hif-1 (i4a) mutants exposed to H2S. Although we did not test all possible cell and tissue types, these data suggest that HIF-1 activity in a single tissue cannot support survival in H2S.

The fact that Punc-14::hif-1 was sufficient for survival in H2S (Figure 1A) suggests that activity of HIF-1 may not be required in all cells. Since we did not observe rescue when hif-1 was expressed in a single tissue, we made transgenic animals with expression of hif-1 in >1 tissue to determine whether we could find a minimal expression that was sufficient for survival in H2S. We found that even animals with hif-1 expression in neurons, hypodermis, and intestine—hif-1(i4a); yakEx146 [Prab-3::hif-1, Pvha-6::hif-1, Pdpy-7::hif-1]—did not survive exposure to H2S (Figure 3B). Together, our data suggest that HIF-1 activity is required in many tissues to coordinate the essential response to H2S. This could indicate that HIF-1 acts cell-autonomously to direct expression of many tissue-specific transcripts that are required to survive exposure to H2S.

Although it was reported that ots197 expresses hif-1 selectively in neurons (Pocock and Hobert 2008), our data show that the unc-14 promoter is more broadly expressed. In fact, others have reported nonneuronal expression of transgenes expressed under the control of the unc-14 promoter (Ogura et al. 1997; Wolkow et al. 2000; da Graca et al. 2004). However, the nonneuronal expression we have demonstrated is much more penetrant than has been previously acknowledged. This is an important consideration when interpreting the results of experiments using transgenes driven by unc-14, including hif-1 from ots197. Our data show that nonneuronal expression from the unc-14 promoter is significant, and that rescue by unc-14-driven transgenes is not sufficient to infer neuronal function of HIF-1 and, presumably, other proteins.

ACKNOWLEDGMENTS

We thank Michael Ailion (University of Washington) for sharing plasmids, discussing ideas, helping identify the different C. elegans tissues, and providing useful feedback on drafts of this manuscript. We are also grateful to Roger Pocock (Monash University) and Oliver Hobert (Columbia University) for sharing plasmids and strains. We thank Suzanne Hoppins (University of Washington) and Andrea Wills (University of Washington) for critical reading of the manuscript. Some strains were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health (NIH) Office of Research Infrastructure Programs (P40 OD010440). This work was supported by NIH grant R01 ES024958 to D.L.M. D.L.M. is a New Scholar in Aging of the Ellison Medical Foundation.

LITERATURE CITED

Bates, M. N., N. Garrett, and P. Shoemack, 2002 Investigation of health effects of hydrogen sulfide from a geothermal source. Arch. Environ. Health 57: 405–411.

Beauchamp, R. O., J. S. Bus, J. A. Popp, C. J. Boreiko, and D. A. Andjelkovich, 1984 A critical review of the literature on hydrogen sulfide toxicity. Crit. Rev. Toxicol. 13: 25–97.

Beaumont, M., M. Andriamihaja, A. Lan, N. Khodorova, M. Audebert et al., 2016 Detrimental effects for colonocytes of an increased exposure to luminal hydrogen sulfide: the adaptive response. Free Radic. Biol. Med. 93: 155–164.

Bos, E. M., H. Van Goor, J. A. Joles, M. Whiteman, and H. G. Leuvenink, 2015 Hydrogen sulfide: physiological properties and therapeutic potential in ischaemia. Br. J. Pharmacol. 172: 1479–1493.

Brenner, S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.

Budde, M. W., and M. B. Roth, 2010 Hydrogen sulfide increases hypoxia-inducible factor-1 activity independently of von Hippel-Lindau tumor suppressor-1 in C. elegans. Mol. Biol. Cell 21: 212–217.

da Graca, L. S., K. K. Zimmerman, M. C. Mitchell, M. Kozhan-Gorodetska, K. Sekiewicz et al., 2004 DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGF beta pathway to regulate C. elegans dauer development. Development 131: 435–446.

Eastman, C., H. R. Horvitz, and Y. Jin, 1999 Coordinated transcriptional regulation of the unc-25 glutamic acid decarboxylase and the unc-47 GABA vesicular transporter by the Caenorhabditis elegans UNC-30 homeodomain protein. J. Neurosci. 19: 6225–6234.

Fawcett, E. M., J. W. Horsman, and D. L. Miller, 2012 Creating defined gaseous environments to study the effects of hypoxia on C. elegans. J. Vis. Exp. 65: e4088.

Fawcett, E. M., J. M. Hoyt, J. K. Johnson, and D. L. Miller, 2015 Hypoxia disrupts prosenesis in Caenorhabditis elegans. Aging Cell 14: 92–101.

Frokjær-Jensen, C., M. W. Davis, C. E. Hopkins, B. J. Newman, J. M. Thummel et al., 2008 Single-copy insertion of transgenes in Caenorhabditis elegans. Nat. Genet. 40: 1375–1383.

Frokjær-Jensen, C., M. W. Davis, M. Ailion, and E. M. Jorgensen, 2012 Improved Mos-1-mediated transgenesis in C. elegans. Nat. Methods 9: 117–118.

Jiang, H., R. Guo, and J. A. Powell-Coffman, 2001 The Caenorhabditis elegans hif-1 gene encodes a bHLH-PAS protein that is required for adaptation to hypoxia. Proc. Natl. Acad. Sci. USA 98: 7916–7921.

Kilburn, K. H., and R. H. Warshaw, 1995 Hydrogen sulfide and reduced-sulfur gases adversely affect neurophysiological functions. Toxicol. Ind. Health 11: 185–197.

Leiser, S. F., H. Miller, R. Rossner, M. Fletcher, A. Leonard et al., 2015 Cell nonautonomous activation of flavin-containing monoxygenase promotes longevity and health span. Science 350: 1375–1378.
Li, L., P. Rose, and P. K. Moore, 2011 Hydrogen sulfide and cell signaling, Annu. Rev. Pharmacol. Toxicol. 51: 169–187.

Livshits, L., A. K. Chatterjee, N. Karbian, R. Abergel, Z. Abergel et al., 2017 Mechanisms of defense against products of cysteine catabolism in the nematode Caenorhabditis elegans. Free Radic. Biol. Med. 104: 346–359.

Ma, D. K., R. Vozdek, N. Bhatla, and H. R. Horvitz, 2012 CYSL-1 interacts with the O2-sensing hydroxylase EGL-9 to promote H2S-modulated hypoxia-induced behavioral plasticity in C. elegans. Neuron 73: 925–940.

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

Miller, D. L., and M. B. Roth, 2007 Hydrogen sulfide increases thermotolerance and lifespan in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 104: 20618–20622.

Miller, D. L., and M. B. Roth, 2009 C. elegans are protected from lethal hypoxia by an embryonic diapause. Curr. Biol. 19: 1233–1237.

Miller, D. L., M. W. Budde, and M. B. Roth, 2011 HIF-1 and SKN-1 coordinate the transcriptional response to hydrogen sulfide in Caenorhabditis elegans. PLoS One 6: e25476.

Nonet, M. L., J. E. Staunton, M. P. Kilgard, T. Fergestad, E. Hartwig et al., 1997 Caenorhabditis elegans rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. J. Neurosci. 17: 8061–8073.

Nystul, T. G., and M. B. Roth, 2004 Carbon monoxide-induced suspended animation protects against hypoxic damage in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA 101: 9133–9136.

Ogura, K., M. Shirakawa, T. M. Barnes, S. Hekimi, and Y. Ohshima, 1997 The UNC-14 protein required for axonal elongation and guidance in Caenorhabditis elegans interacts with the serine/threonine kinase UNC-51. Genes Dev. 11: 1801–1811.

Pocock, R., and O. Hobert, 2008 Oxygen levels affect axon guidance and neuronal migration in Caenorhabditis elegans. Nat. Neurosci. 11: 894–900.

Rand, J. B., J. S. Duerr, and D. L. Frisby, 2000 Neurogenetics of vesicular transporters in C. elegans. FASEB J. 14: 2414–2422.

Richardson, D. B., 1995 Respiratory effects of chronic hydrogen sulfide exposure. Am. J. Ind. Med. 28: 99–108.

Semenza, G. L., 2000 HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 88: 1474–1480.

Semenza, G. L., 2001 HIF-1, O2, and the 3 PHDs: how animal cells signal hypoxia to the nucleus. Cell 107: 1–3.

Shen, C., and J. A. Powell-Coffman, 2003 Genetic analysis of hypoxia signaling and response in C. elegans. Ann. N. Y. Acad. Sci. 995: 191–199.

Wang, R., 2012 Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol. Rev. 92: 791–896.

Wolkow, C. A., K. D. Kimura, M. S. Lee, and G. Ruvkun, 2000 Regulation of C. elegans life-span by insulinlike signaling in the nervous system. Science 290: 147–150.

WormBase, 2017 Release WS259. Available at: http://www.wormbase.org. Accessed: July 20, 2017.

Communicating editor: S. Lee